

CRANFIELD UNIVERSITY

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THE PHYSICAL CHARACTERISATION AND COMPOSITION
OF ARCHAEOLOGICAL DENTAL CALCULUS

CRANFIELD DEFENCE AND SECURITY
POSTGRADUATE RESEARCH DEGREE

MARCH 2017

CRANFIELD UNIVERSITY

CRANFIELD FORENSIC INSTITUTE
CRANFIELD DEFENCE AND SECURITY

PhD Thesis

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THE PHYSICAL CHARACTERISATION AND COMPOSITION
OF ARCHAEOLOGICAL DENTAL CALCULUS

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March 2017

This thesis is submitted in partial fulfilment of the requirements for
the degree of PhD

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ABSTRACT

Dental calculus is a complex biological material that has been found to provide significant evidence of past population diet, health and habitual activity. It is composed of mineral phases, trace elements, organic species and can have inclusions such as starch granules and microfossils incorporated into its structure. This composition has been found to vary among individuals, although the reasons for this are poorly understood. Despite this, there is a wealth of knowledge that can be gained from analysing this biomineral, especially from archaeological remains. In past populations, the variables that affect composition, such as pharmaceuticals and diet are reduced compared to modern populations. As such the reliance on clinical studies that have investigated dental calculus from modern individuals, may be flawed when considering past populations.

The focus of this study was to provide insight about the variation in physical characterisation and composition of archaeological dental calculus. Despite there being an abundance of archaeological dental calculus research, this is the first large scale compositional study of specimens from three separate past populations. In addition, this research is the first study to adopt a non-destructive to destructive approach to archaeological dental calculus analysis. As well, it is the first application of nano-computed tomography to dental calculus from past populations.

Consequently, this study demonstrates the first evidence of accumulation layering that has been detected using non-destructive nano-computed tomography. Furthermore, this research has identified three types of layering in archaeological dental calculus. Due to these findings, it is expected that this research will impact the future of dental calculus analysis, especially when considering dental calculus as a method of mapping an individual's health, diet or lifestyle in the weeks or months prior to death.

The overall results of this thesis demonstrate that some aspects of the morphological, mineralogical and elemental analysis of archaeological dental calculus are inconsistent with clinical literature. The results have also shown that there are some differences between the dental calculus from different archaeological populations which can be related to post-mortem burial conditions.

ACKNOWLEDGEMENTS

I would like to thank Cranfield University for having me back as a research student. Huge thanks are due to Dr Sophie Beckett and Dr Nicholas Márquez-Grant for their supervision and enthusiasm for the project. Acknowledgements are due to Charlene Greenwood and Laura Wood for their previous work on dental calculus at Cranfield, which has provided a spring-board for this project. From Cranfield University, I would also like to thank Adrian Mustey for his continuous help with lab work, workshop requests and for checking in on me when it all got a bit much. I would also like to thank Dr Fiona Brock for her help with the ICP-MS, thank you for coming in on your own time to make sure my data was collected. To Dr Jon Painter, thank you for letting me analyse my ‘tooth gunk’ in your new SEM and for all the training and support in all my microscopy analysis. Thanks also to Prof. Keith Rogers for help with the computed tomography and X-ray diffraction data and again, for letting me put dental calculus in your D8 diffractometer! Special thanks also to Prof. Andrew Shortland for his valuable feedback and advice in my PhD review meetings. I would also like to thank all the other CFI staff who have helped me day to day, you have made Cranfield a great place to study. Especially Dr Sarah Morris for tea and Build-a-bear, which there is never a wrong time for!

In the wider Cranfield family, thank you to Dr Debra Carr for a friendly face and sound advice. To the DTC, particularly Bea Kingdon, thank you for help at moments of crisis and your support in my review meetings. In addition, I would like to thank Iain McKay in the Barrington Library for all his help with my inter-library loans, especially when I submitted so many at a time!

Regarding the archaeological collections in this study, I would like to thank Dr Dario Piombino-Mascali, the Order of the Capuchin Friars of Palermo and the Superintendent to the Cultural and Environment Heritage of Palermo for allowing me access to the Capuchin Catacombs. Additionally, I would like to thank the Consell Insular de Formentera and the Church of St. Francesc de Xavier for allowing access to individuals from Cementeri Vell. As well as Almudena García-Rubio for providing access and assistance to study the San Agustín sample.

Thanks, are also due to Debbie Martin and Andy Griffiths at Bristol Histology Unit. It took a long time to get the method right for sectioning the calculus but thank you for your patience and hard work. Thank you to Charlie Willis and Joanna Dunster for being the best office mates and friends. When I couldn't get past the worries and negativity myself, you were both there.

Finally, I would like to thank my family for their patience and support, which they give unconditionally with everything I undertake. To my mum, you have worked so hard for all three of us and I hope I can be as strong, clever and brave as you are. To Nanny, thank you for everything, you never think of yourself and have always supported me, even if you'd rather I'd had a proper job by now! Thank you to my Gaga, who has never wavered in his support and always made me feel like I can do anything. Thank you to Bex and Dan, I've been an awful big sister for the last four years, I promise to make it up to you both!

Lastly, special thanks go to my husband, Jay, who is always there for me through the good times and at my lowest. Thank you for letting me complete this, we've both had to sacrifice so much over the past few years but now we are stronger than ever.

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GLOSSARY

Accumulation plane	Dental calculus builds up from the tooth surface outwards. The ‘cross-section’ of dental calculus (referred to throughout this thesis) is the plane that cuts through a deposit perpendicularly to the tooth surface on which it is attached. This results in a surface that has dental calculus from the attachment surface (see deep surface) and the external surface (see superficial surface). This plane can be horizontal or vertical.
Acquired Pellicle	A thin layer of skin, membrane, or any other substance. The term ‘acquired pellicle’ is used in relation to the teeth for proteins and other macromolecules from the oral environment that are adsorbed onto the enamel surfaces of the teeth after eruption. This acquired pellicle is clearly distinguished from microbial biofilms of dental plaque (Hannig and Joiner 2006).
Alveolar bone	Bone of the upper and lower jaws that contains tooth sockets (dental alveoli).
Ante-mortem Tooth Loss (AMTL)	The loss of a tooth during life/before death.
Anterior (teeth)	The teeth at the front of the mouth (namely the incisors and canines in the maxilla and mandible).
Apex	The tip of a tooth root.
Buccal	Relating to the surface of a tooth closest to the cheeks (specifically for posterior teeth).
Calculus-Exhibitors	A term used in this thesis to mean individuals in archaeological remains that were recorded as having dental calculus present. This term was used to compare individuals with dental calculus to all individuals in the population.
Crown	The part of the tooth dentine which is covered by enamel (anatomical crown).
Deep Surface	In the context of this thesis and in relation to dental calculus deposits, this is the surface that would have been attached to the tooth surface, i.e. the contact surface; the attachment surface.
Distal	Relating to the surface of a tooth furthest from the midline.

Edentulism	A condition whereby an individual is lacking some or all teeth during life (partial or complete edentulism). An individual with the condition is described as edentulous.
Epithelial Cells	Cells that make up epithelium tissue, which covers the external surfaces of the body and lines ducts and cavities (excluding the blood and lymphatic vessels).
Exocrine fluid	The fluid that is secreted from glands into the digestive system of the body. These include the salivary glands, mucous glands, acid-secreting glands in the stomach and the pancreatic tissue and bile-secreting cells of the liver (Monkhouse 2007).
Exudate	A type of ascetic fluid that is protein-rich and composed of more cells and solid material than the transudate type (Curry et al. 2002).
Genus	A principle taxonomic category that ranks above species and below family, and is denoted by a capitalised Latin name.
Gingival Crevice (Sulcus)	The space between the inner aspect of the free gingiva and the tooth. It is normally 1-3 mm in depth and deepest interproximally. The gingiva is attached to the tooth at the base of the gingival crevice by the junctional epithelium beneath which is a band of connective tissue/free gingival fibres. The gingival crevice produces a serum exudate (gingival crevicular fluid) which alters when disease is present.
Gingival Crevicular Fluid (GFC)	The fluid secreted where the gingivae (gums) meet the teeth. There is an increased production of this fluid in inflamed gingivae.
Gingivae	The gums: the layer of dense connective tissue and overlying mucous membrane that covers the alveolar and necks of the teeth.
Gingival Location	Location relating to the gum-line in the mouth (see ‘supragingival’ and ‘subgingival’).
Gingivitis	The inflammation of the gums caused by plaque on the surfaces of the teeth at their necks. The gums are swollen and bleed easily. Chronic gingivitis is an early stage of periodontal disease but is reversible with good oral hygiene.
Growth-axis	Refers to the accumulation of dental calculus from the tooth’s surface outwards into the oral environment, perpendicularly out from the surface on which it is attached (see attachment plane) (Pérez et al. 2004).
Host Response	The reaction of a living system to the presence of a material (Williams 1987).

Labial	Relating to the surface of a tooth closest to the lips (specifically for anterior teeth).
Lingual	Surface of a tooth closest to the tongue.
Mandible	The lower jaw.
Maxilla	The upper jaw.
Mesial	Surface of a tooth closest to the midline.
Midline	An imaginary line runs from head to feet and divides the body into two halves (left and right), anatomically the 'sagittal plane' or 'median sagittal plane'.
Occlusal	Surface of the tooth, which contacts a tooth in the opposite jaw.
Parotid Glands	A pair of glands found in the subcutaneous tissue of the face. The parotids are the largest of the salivary glands and empty into the oral cavity opposite the upper second molar tooth via their ducts (see Figure 2.2.1 for diagram).
Periapical Cavity	A collection of granulation tissue at the apex of a tooth's root, caused by infection and death of the tooth pulp, depending on the size this can be a granuloma, cyst or abscess, (see Brickley and McKinley (2004)).
Periodontal Disease	Disease of the tissues that support and attach the teeth - gums, periodontal membrane, and alveolar bone. Periodontal disease includes gingivitis and the more advanced stage of periodontitis.
Periodontal Pocket	A pathological deepening of the gingival crevice (sulcus) produced by the destruction of the supporting tissues and the apical migration of the epithelial attachment. It provides an ideal protected environment for the continued growth of subgingival bacteria which release toxins that can damage the surrounding tissue and cementum.
Periodontitis	Advanced periodontal disease, which results in the formation of spaces between the gums and the teeth (periodontal pockets), the loss of some fibres that attach the tooth to the jaw, and the loss of bone.

Periodontology / Periodontal Research	The branch of dentistry concerned with the tissues that support and attach the teeth to the jaw: the gums, periodontal membrane, alveolar bone, and cementum.
Physiological fluid	Physiological fluids are biological fluids present, which allow the healthy or normal functioning of the body to occur.
Phytolith	A minute mineral particle formed inside a plant, in the context of dental calculus, a fossilised particle of plant tissue.
Post-mortem Tooth Loss (PMTL)	The loss of a tooth after death.
Posterior (teeth)	The teeth at the back of the mouth (namely the premolars and molars in the maxilla and mandible).
Root	Relating to the lower cementum portion of a tooth.
Saliva	The liquid secreted by the salivary glands and the mucous membrane of the mouth. Its principal constituents are water, mucus, buffers and enzymes (e.g. amylase). The functions of saliva are to keep the mouth moist, to aid swallowing of food, to minimise changes of acidity in the mouth, and to digest starch.
Salivary Gland	A gland that produces saliva. There are three pairs of salivary glands; the parotid glands, sublingual glands and submandibular glands (see Figure 2.2.1 for diagram). They are stimulated by reflex action, which can be initiated by the taste, smell, sight or thought of food.
Sensory Receptors	A cell or group of cells specialised to detect changes in the environment and trigger impulses in the sensory nervous system.
Subgingival	Below the gum-line (a gingival location).
Sublingual Glands	A pair of salivary glands situated in the lower part of the mouth, one on either side of the tongue. The sublingual glands are the smallest salivary glands; each gland has about 20 ducts, most of which open into the mouth directly above the gland (See Figure 2.2.1 for diagram).
Submandibular Glands	A pair of salivary glands situated below the parotid glands. Their ducts open in two papillae under the tongue on either side of the frenulum (also called submandibular glands) (See Figure 2.2.1 for diagram).

Superficial Surface	In relation to dental calculus deposits, this is the surface that would have been exposed to the oral environment when the deposits were attached to the tooth. This is also the surface that has been exposed to the burial or interment environment, i.e. the exposed surface.
Supragingival	Above the gum-line (a gingival location).
Starch	An odourless, tasteless white substance occurring widely in plant tissue and obtained chiefly from cereals and potatoes. It is a polysaccharide which functions as a carbohydrate store and is an important component of the human diet. They take the form of grains/granules and have different morphologies depending on the plant from which they come from.
Transudate	A type of ascetic fluid that is clear, colourless and low in protein. There are few cellular elements and the specific gravity is low (Curry et al. 2002).

Definitions compiled using Oxford Dictionary of Dentistry (2010); Oxford Concise Medical Dictionary (2010) and Concise Oxford English Dictionary (2011) unless otherwise stated.

Abbreviations

μ-CT	Micro-computed tomography
μ-XRD	Micro-beam X-ray diffraction
ABFO	American Board of Forensic Odontology
aDNA	Ancient DNA
AMTL	Ante-mortem tooth loss (see definitions)
B	Buccal (see definitions)
BSE	Back-scattered electrons
CC	Capuchin Catacombs, Sicily
CEJ	Cemento-enamel junction
CV	Cementerí Vell, Formentera

D	Distal (see definitions)
DCPD	Dicalcium phosphate (Common Mineral Name: Brushite; Chemical Formula: $\text{CaHPO}_4 \cdot 2\text{H}_2\text{O}$)
EDX	Energy-dispersive X-ray (analysis/spectroscopy)
GCF	Gingival crevicular fluid (see definitions)
HAp	Calcium hydroxyapatite/ Calcium hydroxylapatite (Chemical Formula: $\text{Ca}_5(\text{PO}_4)_3(\text{OH})$)
ICP-MS(Sol)	Inductively-coupled plasma mass spectrometry (solution)
La	Labial (see definitions)
Li	Lingual (see definitions)
M	Mesial (see definitions)
n-CT	Nano-computed tomography
O	Occlusal (see definitions)
OCP	Octacalcium phosphate (Chemical Formula: $\text{Ca}_8(\text{HPO}_4)_2(\text{PO}_4)_4 \cdot 5\text{H}_2\text{O}$)
PMTL	Post-mortem tooth loss (see definitions)
pXRD	Powder X-ray diffraction
SA	San Agustín, La Rioja
SEM	Scanning electron microscopy
SR-XRF	Synchrotron radiation X-ray fluorescence
WHT	Whitlockite /Magnesium-Whitlockite (β -tri-calcium phosphate; Chemical Formula: $\beta\text{-(Ca,Mg)}_3(\text{PO}_4)_2$)
XRD	X-ray diffraction
XRF	X-ray fluorescence

Units

Å	ångström
°	degree
°C	degrees centigrade
μA	microamps
μl	microlitre
μm	micrometre
cm	centimetre
cm⁻¹	wavenumber
cps	counts per second
g	gram
keV	kiloelectron volt
km²	square kilometres
I	intensity
m	metre
ml	millilitre
mm	millimetre
mm min⁻¹	millimetres per minute
ms	milliseconds
nm	nanometre
ppb	parts per billion
ppm	parts per million
ppt	parts per trillion
s	seconds

CHAPTER 1: INTRODUCTION

*Man develops two sets of teeth,
although the frequency of dental
disease in the civilised world
would make a third very welcome.*

- Don Brothwell 1981

Dental calculus is a complex biological material that has been found to provide significant evidence of past population diet, health and habitual activity. It is a hard, stony deposit that is found on the surfaces of teeth and persists after death. This substance is a product of biological mineralisation, similar to kidney, urinary and salivary calcifications; often called ‘stones’ (Khan 1992). These biological products form by mineralization of ions within the biological fluids that they occur (Jin and Yip 2002). Depending on the location and conditions in the body that the mineralization process occurs, the resulting biological substances have differing compositions and structures (Jin and Yip 2002).

Dental calculus is a widespread condition in populations across the world today. Most adults in the modern world will accumulate dental calculus, even with advances in preventative dentistry and the availability of oral hygiene products. As well as being prevalent in current society, the formation of dental calculus is not a modern condition. Historical sources have shown that dental concretions have affected humans throughout time, perhaps more so than today (Albucasis 1000 C.E. (*translated into English in 1973*); Fauchard 1728 (*translated into English in 1946*); Hunter 1803(1), (2)). In addition, archaeological observations have provided direct evidence of dental calculus on archaeological remains dating back as far back as Neanderthal times (Hardy et al. 2009; Preus et al 2011; Tao et al. 2015; Hardy et al. 2016).

With the physical material of this oral condition present in humans across time periods, it provides valuable research potential for past and present population studies. This thesis examines the bulk and cross-sectional¹ composition of archaeological dental calculus using a novel combination of techniques. In addition, this is the first study to utilize nano-

¹ See glossary for ‘accumulation plane’.

computed tomography in the investigation of archaeological dental calculus within a non-destructive to destructive methodology.

1.1 Basis for Research

1.1.1 Archaeological Dental Calculus Analysis

Research in recent years has expanded the potential of archaeological dental calculus for understanding the diet, health and habits of past populations. The recording in bioarchaeological studies utilises dental calculus presence and severity to make inferences about the diet and oral hygiene of a population² (Whittaker et al. 1998; Delgado-Darias et al. 2006; Valentin et al. 2006; Polo-Cerdá et al. 2007; Masotti et al. 2013; Nerlich et al. 2015). Alongside bioarchaeological studies of human remains, dental calculus research has advanced into the field of archaeological science, to involve analytical techniques.

The scientific methods that have been applied to archaeological dental calculus, include starch and microfossil analysis, isotopic and ancient DNA analysis and most recently, bacteriology and proteomics (Fox et al. 1996; Hardy et al. 2009; Preus et al. 2011; Hardy et al. 2012; Scott and Poulson 2012; Warinner et al. 2014; Warinner et al. 2015(1)). These continually advancing techniques have enhanced the potential for dental calculus deposits providing more specific evidence for past populations. They enable stronger conclusions to be made about food consumption, food preparation, habitual activity and health beyond the traditional recording of presence and severity.

Specifically, starch and microfossil analysis has enabled the identification of plant types from micro-remains trapped within calculi matrices. This has provided evidence of the use and consumption of plants, for instance, as reported by Wesolowski et al. (2010), *Ipomoea batatas* (sweet potato) and *Dioscorea* (yam) starch granules were identified

² Dental calculus is often assessed in conjunction with other dental pathology conditions and diseases for bioarchaeological studies. Additional conditions assessed are wear, dental caries, ante-mortem tooth-loss (AMTL), enamel hypoplasia, periapical cavities and periodontal disease (via alveolar bone remodeling).

from within dental calculus from individuals excavated from Brazilian sambaquis³. However, despite the specificity that can be achieved from identification of starch granules and microfossils, there are limitations for interpretation of dietary consumption. Firstly, definite identifications must be taken with caution, as without comparison to a reference source, results can only be deemed tentative or ‘consistent with’. In fact, Hardy et al. (2009) propose that no starch granule can be identified to species level and that determination of the plant genus is the most specific outcome of starch analysis. Further discussion about the starch and microfossils found in dental calculus section 2.4.4.

In contrast to starch and microfossil analysis, which has progressed steadily in the past 20 or so years, the isotopic analysis of dental calculus to investigate diet is more recent. Isotopic analysis of bone and teeth in archaeology has become widely used for palaeodietary analysis (Weiner 2010). However, Scott and Poulson (2012) investigated the use of dental calculus as a source material for carbon ($\delta^{13}\text{C}$) and nitrogen ($\delta^{15}\text{N}$) isotopes. While the results appeared positive when compared with historical literature regarding subsistence, only dental calculus was analysed with no comparison to bone or teeth samples from the same individuals.

A recent study, which did undertake comparative analysis, was that of Salazar-García et al. (2014) who concluded that dental calculus should not be used for isotopic analysis over other available sources (such as bone collagen, teeth, hair or nails). The study found that isotopic analysis of the carbon and nitrogen content of dental calculus was too variable, even within multiple specimens from one individual, for it to be reliable (see section 2.2.2.3 for site specificity of dental calculus and section 2.3.1 for influencing factors for calculus formation). Consequently, Salazar-García et al. (2014), suggested that microfossil analysis of dental calculus is preferential over isotopes when destructive analysis is performed. This was supported by a further study by Eerkens et al. (2014), who found that when the C/N ratio is over 12, the results from dental calculus are less reliable.

³ Sambaquis are shell-mounds found along the coasts of South America. Multiple theories exist as to their origin and use, however some sambaquis have been found to contain many burials while others contain none. The earliest sambaquis have been dated to around 7400 BP and the latest have been dated to around 700 BP (Wagner et al. 2011).

Another development has been in bacterial ancient DNA (aDNA) analysis. Preus et al. (2011) demonstrated that dental calculus can be a viable source of bacterial DNA. Not only does aDNA survive in dental calculus but due to the location and nature of dental calculus, it is unique (Preus et al. 2011). The bacterial content of dental calculus is indicative of bacteria in the oral environment, therefore if this environment changes or foreign microorganisms are introduced (such as from a disease), the dental calculus will reflect this (Preus 2011). Additionally, individuals from different countries have been found to have different oral bacteria, opening the potential that aDNA analysis of archaeological dental calculus could provide information regarding migration (Preus 1995).

In addition, the latest analysis to be applied to archaeological dental calculus is shotgun protein analysis Warinner et al. (2014). In that study, a protein unique to milk (β -lactoglobulin) was isolated and identified from samples of archaeological dental calculus (Warinner et al. 2014). From this data, not only was it possible to identify individuals who consumed milk, the samples were analysed from a region where previous isotopic analysis had concluded that dairy products had been in decline (Arneborg et al. 1999; Arneborg et al. 2011; Warinner et al. 2014). The protein analysis supported the isotopic evidence by finding β -lactoglobulin in the earlier remains and none in the latter (Warinner et al. 2014). Because of this research, the future development of proteomics applied to dental calculus looks to be a strong contender for a lead role in the study of palaeodietary evidence.

1.1.2 Modern Dental Calculus Analysis

The fields of dentistry and periodontal research⁴ have long been interested in the detection and formation of dental calculus to ultimately prevent its development in patients (Friskopp and Hammerström 1980; Mandel 1995; Poff et al. 1997; White 1997; Jin and Yip 2002; Dawes 2007). The clinical research is predominately achieved by analysis of dental plaque and the pathways of calcification, to understand dental calculus formation.

⁴ Periodontal research is a specific field of dentistry. From the Greek 'perio' meaning 'around', this field is concerned with the disease of the gingivae (gums), alveolar bone, cementum and periodontal ligament (Mueller 2016). These are the tissues and structures surrounding the teeth and are termed the 'periodontium' (Mueller 2016).

This focus on the mineral calcification and composition is often overlooked in archaeological science, which may be due to an assumption that the composition of dental calculus has not changed between past populations and populations today.

From dentistry research, the bulk mineral composition has been determined from modern samples (Hayashi 1993; Roberts-Harry and Clerehugh 2000; Jin and Yip 2002; Hayashizaki et al. 2008). It has been found that the mineral constituents are dependent on the oral environment in which they are formed (Friskopp and Isacsson 1984; Jin and Yip 2002; Wong and Sissons 2007). Therefore, it is reasonable to hypothesise that a change in oral environment will change the mineral composition of dental calculus. Furthermore, if changes in diet or health change the oral environment, these changes will affect the mineral composition.

Contemporary studies have generally established the formation and composition of dental calculus by determination of the conditions that cause calcification (Jin and Yip 2002; Wong and Sissons 2007). However, these studies have refrained from the additional step of relating dietary contributions to the necessary formation conditions. This is likely due to its complexity, especially considering, individual dietary variation particularly in modern times where worldwide trade is heavily relied on. Where researchers have investigated dietary influence on modern dental calculus composition, it has been through studies that have utilised animal proxies (Fitzgerald and McDaniel 1960; Luoma et al. 1975; Mann al. 1990). This is understandable due to animal diets being more consistent and controllable for the testing of variables. However, due to the human mouth having a vastly different chemistry and bacterial composition to animals, these data are potentially unrepresentative with regards to human dental calculus.

This thesis uses archaeological human populations which can have their own complications (such as diagenesis, see section 2.5.3). However, there are also benefits to analysing specimens taken from individuals who would have consumed simpler diets and not have be subject to the medication and oral hygiene products that modern populations are. The influencing factors that affect dental calculus are reduced, meaning that stronger conclusions can be made about the compositional differences seen between specimens and individuals.

1.1.3 Summary

Despite the vast amount of research that has been undertaken in dental calculus analysis in both archaeological and dentistry, there are still some gaps in knowledge. This primarily relates to features of clinical dental calculus that are assumed to be true about archaeological dental calculus. The mineral and elemental composition of archaeological dental calculus has been under-investigated. In addition, while accumulation layering has been demonstrated in clinical deposits, during compositional studies, it has not been identified in archaeological dental calculus.

The focus of this study was to provide insight about the variation in physical characterisation and composition of archaeological dental calculus. Despite there being an abundance of archaeological dental calculus research, this is the first large scale compositional study of specimens from three separate past populations. The overall aims of this thesis were to (1) to investigate if layering can be detected in archaeological dental calculus and whether the mineral and elemental composition is consistent through the accumulation plane (cross-section) of a deposit; (2) investigate whether dental calculus composition varies within and between archaeological populations; (3) investigate if there is a compositional influence on dental calculus from the presence of oral diseases and pathologies and (4) demonstrate whether modern dental calculus research accurately reflects the archaeological findings presented in this research.

To investigate these aims a novel approach was employed, from non-destructive to destructive analysis. Within this approach, this study presents the first use of nano-computed tomography to investigate the internal structure of dental calculus. Additionally, this is the first cross-sectional study of the mineral and elemental composition of archaeological dental calculus. Consequently, it is proposed that this research contributes to the fields of archaeology and dental calculus analysis in a previously unexplored area. The aims and hypotheses of this work are further outlined in Chapter 4.

1.2 Thesis Outline

This thesis is written over nine chapters. Moving on from the introduction, ‘Chapter 2: Dental Calculus’ consists of a comprehensive literature review of archaeological and dentistry sources pertaining to dental calculus composition and formation. In this review, the oral environment and conditions in which dental calculus accumulates is explained as well as influencing factors and timescales for accumulation. Additionally, the considerations, ethical and practical, for dental calculus analysis are discussed.

In ‘Chapter 3: Archaeological Material’, the populations from which dental calculus samples were taken for this research are described. Following this, ‘Chapter 4: Research Aims and Hypotheses’ fully outlines the focus of the work in this study. Then, ‘Chapter 5: Analytical Techniques’ is the rationale behind the methods of data acquisition. This chapter is not intended to fully explain the workings of the analytical techniques and equipment in detail. Instead the techniques are explained in terms of their contribution to the research aims. It is hoped that from this section of the thesis, it can be clearly understood why the chosen techniques were appropriate.

‘Chapter 6: Materials and Methods’ is two-part. Firstly, the materials section details the number of specimens analysed from each population for each technique. Secondly the methods sections describe the processes for sample preparation, data acquisition and data analysis for each technique. The results of this project are outlined in ‘Chapter 7: Results’ and the in-depth discussion and analysis of the results can be found in ‘Chapter 8: Discussion’. Finally, in ‘Chapter 9: Conclusions’ the thesis is rounded up in relation to the initial research aims. This chapter also includes how this work contributes to the field of dental calculus analysis and suggests areas that could be explored in future work.

All accompanying background information, recording forms, additional results tables are provided in a separate appendix document, *Supplementary Material*. The additional document also contains an appendix that relates to recording dental calculus, including a literature review, proposed quantitative method and the preliminary performance of this proposed method in the field. All raw data is archived at Cranfield Forensic Institute, Cranfield University, Shrivenham and is available on request.

CHAPTER 2: DENTAL CALCULUS

*Whence proceeds the calculus of the mouth?
Is it, as some have said, a secretion?
Is it a deposite, as has been repeated again
and again, of the saliva, as stated in every
medical work that has appeared for centuries?*

- Alexander Nasmyth 1842

2.1 Overview

Dental calculus (or dental tartar) is a hard deposit, which accumulates on the surface of the teeth. In Figure 2.1.1, dental calculus can be seen on the teeth of a dentistry patient *in vivo* (left) and on archaeological human remains (right). In the patient image (Figure 2.1.1 - left) the dental calculus has accumulated on and around the necks of the teeth and the gingivae can be seen (coated in salivary fluid). In contrast, with the archaeological remains (Figure 2.1.1 - right), the dental calculus is adhered to the teeth but the soft tissue of the gingivae has decomposed and is no longer present on the skeletonised remains.

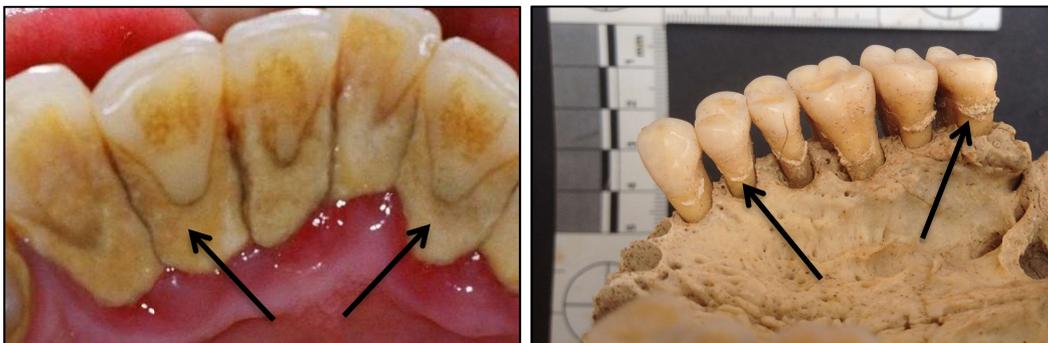


Figure 2.1.1 Photographs of dental calculus on the lingual surfaces of the anterior teeth in a clinical patient (left) and on the lingual surfaces of the posterior teeth on a skeletal maxilla (right) [Image credit: left: <http://www.intelligentdental.com/category/periodontics/> (Retrieved 26th June 2015); right: Photograph taken by author in June 2014, Formentera, Spain].

Dental calculus forms when dental plaque calcifies into a solid mineral deposit. In addition to the mineral phases that are formed during the calcification, the calculus is composed of bacterial cells, trapped microfossils and proteins. Additionally, the nature of the present minerals allows for the exchange and incorporation of alternative ions,

changing the trace elemental composition of the mineral phases. Both the formation and composition of dental calculus is explained in section 2.3 and 2.4 respectively.

This aim of this chapter within the context of the thesis was to examine the current and accepted knowledge regarding dental calculus from the fields of archaeology and dentistry. In doing so, critical evaluation of research publications that have contributed to both fields is included. The aim of critically analysing the relevant literature was to determine the breadth of research conducted as well as establish the areas that require more research to be conducted. Consequently, this chapter is a comprehensive literature review regarding dental calculus research to properly analyse how this thesis could contribute to the field. Additionally, when analysing dental calculus there are many practical considerations and these are explained section 2.5.

2.2 Fundamental Concepts

2.2.1 The Oral Environment

In the human mouth, the biological structures of the teeth, gingivae and oral soft tissue, interact with physiological fluids (Jones 2001). These fluids are saliva, gingival crevicular fluid and plaque fluid, which have buffering capacity and contain water, ions, amino acids and proteins (Roberts-Harry and Clerehugh 2000; de Almeida et al. 2008 Arabacı et al. 2015). Due to the importance of the oral environment in the formation of dental calculus, each of the physiological fluids, saliva, gingival crevicular fluid and plaque fluid will briefly be described in terms of function and composition. This will be paired with references to studies that have contributed to a better understanding of these fluids in relation to their role in dental calculus formation.

2.2.1.1 Saliva

Saliva is an exocrine fluid produced from three pairs of glands, the parotid, submaxillary and sublingual glands (Figure 2.2.1), and coats the oral tissues in the mouth (Holmberg and Hoffman 2014). Saliva is sterile when it is produced in the glands, however on

entering the mouth it picks up epithelial cells⁵ (that are shed from the soft tissues of the oral cavity) and picks up bacteria from the oral surfaces (Green and Furbee 1967; Bratthall and Carlsson 1989; Bell 2008).

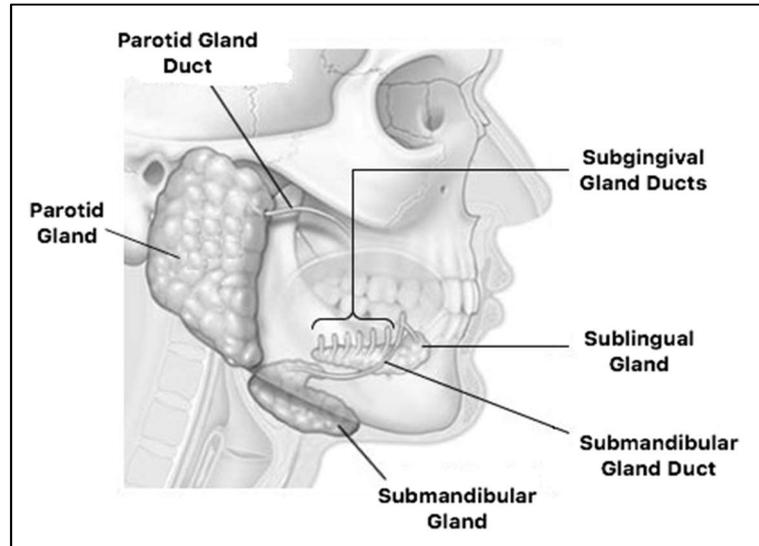


Figure 2.2.1 Diagram of the location of the salivary glands and salivary ducts in the human mouth [Adapted by author from <https://www.britannica.com/science/salivary-gland> (Retrieved 23rd September 2016)].

There are multiple roles of saliva including chemical degradation of food during chewing; assisting in swallowing, keeping oral tissue hydrated and healthy and, protecting oral tissue from irritant substances (Pedersen et al. 2002; de Almeida et al. 2008). Saliva also transports foreign bacteria from the mouth to the stomach acid to remove its potential harm to the teeth or surrounding tissue (Talaro and Talaro 1996). These multiple functions of saliva are supported by different components and these are summarised in Table 2.2.1 (Llena-Puy 2006).

Salivary fluid is primarily water (99%) but the remaining content is composed of inorganic and organic components (Humphrey and Williamson 2001; de Almeida et al. 2008). The identity and amount of inorganic and organic components produced by the

⁵ It is the epithelial cell content of saliva, which makes it a viable source of DNA, especially in forensic cases, for example from licked stamps or bite marks (Bell 2008).

salivary glands changes depending on which salivary function is required for the oral environment (Table 2.2.1) (Llena-Puy 2006; de Almeida et al. 2008). The composition of saliva adjusts based on a range of sensory receptors in the oral and nasal cavity or as a host response to certain species of bacteria (Ekström et al. 2012; Reddy 2014).

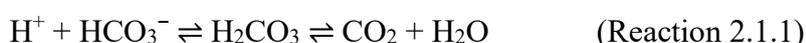
The salivary functions in Table 2.2.1 that have greatest relevance to dental calculus formation are; buffer capacity and tooth remineralisation. The main components in saliva, which are involved in the buffer capacity of saliva are the inorganic species, bicarbonate (HCO_3^-), calcium (Ca^{2+}) and phosphate (PO_4^{3-}). It is these ionic products that form dental calculus, when conditions are met and these mechanisms are described in section 2.3. However, these salivary components are primarily present, along with additional enzymes and proteins, to maintain the normal⁶ function of saliva at pH 6.5 to 7.5 (Humphrey and Williamson 2001).

Functions	Components
Lubrication	Mucins, Proline-rich Glycoproteins, Water
Anti-microbial action	Lysozyme, Lactoferrin, Lactoperoxides, Mucins, Cystins, Histatins, Immunoglobulins, Proline-rich Glycoproteins, IgA
Maintaining mucosa integrity	Mucins, Electrolytes, Water
Cleansing	Water
Buffer capacity and remineralisation	Bicarbonate, Phosphate, Calcium, Statherin, Proline-rich Anionic Proteins, Fluoride
Preparing food for swallowing	Water, Mucins
Digestion	Amylase, Lipase, Ribonucleases, Proteases, Water, Mucins
Taste	Water, Gustin
Phonation (Speech)	Water, Mucins

Table 2.2.1 Table showing the functions of saliva and the related components involved in facilitating those functions, shown in bold is the function, and its related components, involved in dental calculus formation [Reproduced from Llena-Puy (2006)].

⁶ The term ‘normal’ function assumes the mechanisms of pH regulation and tooth remineralization occur in the mouth and that the pH of the oral cavity can return to a healthy pH of between 6.5 and 7.5 between eating (Lagerlöf and Oliveby 1994; Humphrey and Williamson 2001).

There are three buffer systems in saliva, a protein buffer, a phosphate buffer and a bicarbonate buffer (Bardow et al. 2000; Humphrey and Williamson 2001; Chaeib and Lussi 2013). Of these, the bicarbonate buffer contributes the most regulation to oral pH when stimulated saliva⁷ is being produced and there are two buffer features that make this possible (Bardow et al. 2000; Humphrey and Williamson 2001). Firstly, this is a phase buffer meaning it converts dissolved bicarbonate (liquid solution) into carbon dioxide (gas) and vice versa (Reaction 2.1.1) (Bardow et al. 2000). Secondly, the partial pressure of carbon dioxide gas in saliva is much higher than in the atmosphere [$p\text{CO}_2$ (saliva) \approx 54 mmHg (estimated (Grøn and Messer 1965) $p\text{CO}_2$ (atmosphere) = 0.3 mmHg (Bardow et al. 2000)].



These features enable the buffer to ‘mop up’ acidic protons (which are formed as bacterial by-products) with bicarbonate that is formed in the salivary glands to ultimately produce carbon dioxide gas. The release of carbon dioxide is driven by the difference in partial pressures between the saliva and the atmosphere. Therefore, the more mixing of atmospheric air (i.e. during exercise or eating), containing a lower $p\text{CO}_2$ with the higher $p\text{CO}_2$, the more the buffer reaction will be driven to produce CO_2 gas to raise the salivary pH (Bardow et al. 2000). Consequently, this reaction needs a high flow rate of saliva to produce and distribute more bicarbonate, for the buffer reaction to occur. Additionally, this increased production of bicarbonate anions in stimulated saliva, causes it to be slightly higher pH than unstimulated saliva.

In contrast, the phosphate buffer capacity is maximised when the salivary flow rate is low so is most effective in unstimulated/resting saliva (Bardow et al. 2000; Humphrey and Williamson 2001). There are four phases of phosphate in saliva, phosphoric acid (H_3PO_4); dihydrogen phosphate (H_2PO_4^-); hydrogen phosphate (HPO_4^{2-}) and phosphate (PO_4^{3-}) (Bardow et al. 2008). The presence of these products is determined by pH as shown in Figure 2.2.2 and Reaction 2.1.2 (a-c).

⁷ Stimulated saliva is produced in response to autonomic receptors, which relay to the salivary glands that more saliva is required for the breakdown of foods during eating (Dodds et al. 2005; Bardow et al. 2008).

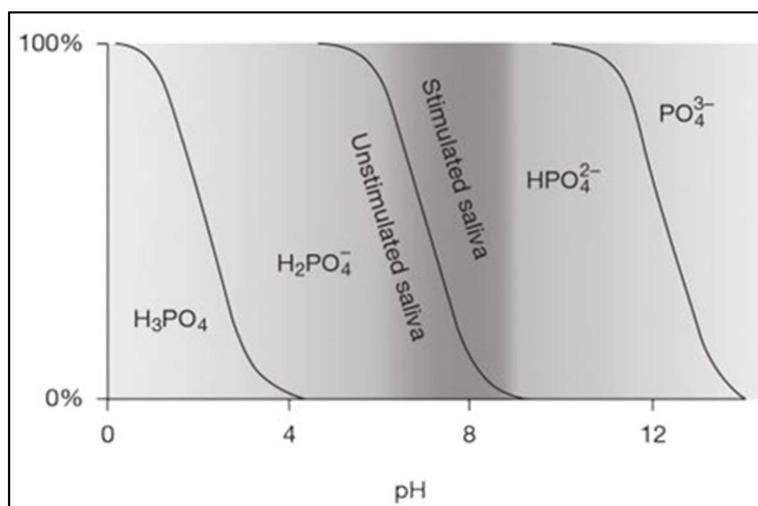
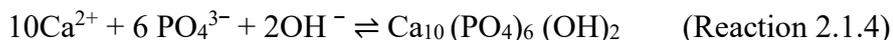


Figure 2.2.2 Graph showing the percentage of phosphate products in saliva as a function of pH. This graph shows that as salivary pH rises, protons are lost forming the more anionic phases of phosphate [Adapted by author from Bardow et al. (2008)].



At optimum pH (6.5-7.5), the phosphates present are dihydrogen phosphate (H₂PO₄⁻) and hydrogen phosphate (HPO₄²⁻) and these are the dominant products that are involved in the phosphate buffer that regulates pH in unstimulated saliva (Reaction 2.1.3) (Bardow et al. 2008). When saliva is stimulated, the flow rate and pH increases (due to the production of bicarbonate) and hydrogen phosphate (HPO₄²⁻) and phosphate (PO₄³⁻) constitute the phosphate phases (Bardow et al. 2000; Bardow et al. 2008). The higher the anionic charge on the phosphate, the more reactive it will be. The reactivity of the phosphate at high alkalinity makes it possible for the phosphate ions to precipitate out of solution to form calcium hydroxyapatite with calcium ions also in saliva (Reaction 2.1.4). It is this precipitation of calcium hydroxyapatite, combined with the calcification of dental plaque, which forms the mineral component of dental calculus. The calcification of dental plaque and the formation of dental calculus are further discussed in section 2.3.



Lastly, the protein buffer also contributes to the total buffering capacity of saliva and proteins that are present in saliva have a range of biological function (including catalysis of the formation of carbonic acid in the salivary bicarbonate buffer) (Bardow et al. 2000). A recent pilot study by Cheaib and Lussi (2013) has investigated the buffering capacity of a few salivary proteins, however the sample size was small (four healthy individuals) and more investigation is needed to understand the contribution of protein buffering in saliva.

The ionic products discussed above allow saliva to have an effective buffer capacity to regulate the optimum pH of the mouth to between 6.5 and 7.5 (Driessens and Verbeeck 1989; Sato 2002; Llana-Puy 2006; de Almeida et al. 2008). This pH regulation allows the saliva to assist in ensuring conditions are optimum for balancing remineralisation/demineralisation⁸ of tooth enamel (Hoyer et al. 1984; Lagerlöf and Oliveby 1994; Llana-Puy 2006; de Almeida et al. 2008). However, despite buffering capabilities, the pH of saliva is not always uniform around the mouth and this is due to the film characteristics of saliva (Dawes et al. 1989; Pedersen et al. 2002).

Saliva is a fluid that is always moving, with new saliva entering the mouth and older saliva being swallowed, therefore making it a ‘mobile film’ (Jin and Yip 2002). Clinical studies have investigated saliva *in vivo* and *in vitro*, with respect to salivary film thickness and velocity (Collins and Dawes 1987; Lagerlöf 1983; Dawes et al. 1989). The former, salivary film thickness, has been estimated to be between 0.07 and 0.1 mm, from calculations based on the surface area of the mouth and an estimation of the amount of saliva produced before and after an individual’s action of swallowing (Collins and Dawes 1987). While this value has been estimated, it has been accepted by later studies⁹ (Jin and

⁸ Dental carious lesions are caused by an imbalance in oral acidity, causing enamel and dentin demineralization (Tatevossian 1990; Hillson 2001). The saliva can reverse minimal effects of demineralization but this is over-ridden if there is a persistent acidic environment (Hoyer et al. 1984; Yip and To 2005).

⁹ Watanabe and Dawes (1990) replicated the same estimation methods for 5-year-old children and found that even though the surface area of the mouth was smaller, the salivary film thickness was of similar value (between 0.06 and 0.09 mm).

Yip 2002; Pedersen et al. 2002). Regarding dental calculus studies, the latter of these two film characteristics, salivary flow rate, is more important and will now be discussed.

Initial saliva research led to the investigation of the volume and average flow rate of saliva in the mouth (Lagerlöf 1983; Collins and Dawes 1987). However, a later study by Dawes et al. (1989) investigated whether the velocity of flow of saliva differs around the mouth. This research found that the velocity of salivary flow does differ around the oral cavity from 0.8 to 8 mm min⁻¹ (Dawes et al. 1989). The difference in flow rate determines the effectiveness of saliva to conduct two of its vital roles, to remove adhering plaque bacteria from oral surfaces and to regulate oral pH (Dawes et al. 1989; Pedersen et al. 2002).

In oral areas where the saliva flow rate is low, plaque bacteria is left for longer on dental surfaces (Dawes et al 1989). The slower movement of saliva out of the mouth enables the plaque bacteria to produce an increased concentration of acidic by-products¹⁰. The more acidic by-products produced by bacteria, the lower the pH of localised saliva in these areas (Dawes et al. 1989; Dawes 2007).

Differences in the normal flow rate of saliva around the mouth affect the localised pH of saliva. The flow rate and therefore the pH can also be altered by external factors. In the comprehensive literature survey by de Almeida et al. (2008), it is explained that there are many stimulation factors, which affect salivary flow rate including hydration of an individual; body posture; lighting, smoking, medication, age and more.

The physical and chemical characteristics of saliva that have been described are relevant to dental calculus formation and will form the basis of section 2.3.

2.2.1.2 Gingival Crevicular Fluid

Gingival crevicular fluid (GCF) is also produced in the oral cavity, however unlike saliva, GCF is found below the gingivae (Nield-Gehrig and Willmann 2007). In healthy oral tissue, the production of GCF is minimal and the role of the fluid is as a transudate that cleanses the subgingival crevice and maintains the attachment of the periodontal ligament (Jablonski 1982; Cappelli and Mobley 2008). In unhealthy oral tissues, which exhibit

¹⁰ Saliva removes these acidic by-products that are produced by acidogenic bacteria because they are harmful to dental enamel, causing demineralization (de Almeida et al. 2008).

gingival inflammation, gingival crevicular fluid is produced more readily and its role becomes that of an exudate to reduce inflammation (Jablonski 1982; Embery and Waddington 1994; Bernimoulin 2003; Cappelli and Mobley 2008).

The composition of gingival crevicular fluid is complicated and contains many organic molecules and electrolytes. The exact composition changes in response to the severity of the inflammation present (Rahnama et al. 2014). The important component that is relevant for dental calculus formation, is that GCF, like saliva, also contains calcium and phosphate ions but in higher concentrations (Little and Hazen 1964; Roberts-Harry and Clerehugh 2000). Additionally, GCF has a higher concentration of magnesium ions in its composition but a lower concentration of bicarbonate (Roberts-Harry and Clerehugh 2000).

A study by Bickel et al. (1985) determined that the standard pH of GCF is around 8.0, higher than that of saliva (pH 6.5-7.5). By measuring the loss of CO₂ from periodontal pockets, Bickel et al. (1985) confirmed the presence of a bicarbonate buffer in GCF, as in saliva. However, with lower concentrations of bicarbonate in GCF, it may be that the dominant buffering system in GCF is not the same as saliva.

There has been little research into the buffering capabilities of gingival crevicular fluid, probably due to the difficulty of collecting samples. Individuals who have increased production of GCF are exhibiting gingival or periodontal disease due to a lack of oral hygiene. Therefore, clinical research is often concerned with reducing inflammation and disease in patients rather than characterisation of the fluid produced, in terms of buffer capacity.

2.2.1.3 Dental Plaque

Dental plaque¹¹ is ‘an adherent gelatinous film’ (Fine 1988). Dental plaque is found as a thin biofilm on the oral surfaces and consists of abundant and diverse microorganism colonies (Marsh 2004). While in general a thin film is found across all oral surfaces,

¹¹ Dental plaque should not be confused with *materia alba* which is adherent food debris and dead epithelial cells that collects in spaces and grooves of the teeth when an individual has poor dental hygiene. This *materia alba* is loosely adherent to tooth surfaces and covers the dental plaque that is also present. It can be washed away, unlike dental plaque (Wirthlin and Armitage 2004).

dental plaque thickness is larger between the teeth (interproximally) and around the crevicular margins (Tatevossian 1990).

This biofilm consists of a complex and diverse mixture of multiple bacteria and collects on hard tissue (enamel) and soft tissue (gingivae) (Bernimoulin 2003; Marsh 2004). The varieties of bacteria that colonise to form dental plaque are interdependent and gain nutrients from the saliva or gingival crevicular fluid (GCF) depending on the location of the plaque (Bernimoulin 2003). The composition of dental plaque varies depending on the oral location of the accumulation because of the respective fluid that provides its nutrients (saliva or GCF) (Koparal and Tütüncü 2000; Wirthlin and Armitage 2004).

Dental plaque bacteria are constantly present in the oral environment and consistently form colonies on tooth surfaces. Upon oral hygiene intervention to remove these colonies, the initial stages of plaque deposition start again (Bernimoulin 2003). This begins with the coating of the dental surface with a film of saliva that contains the acquired pellicles that will form the base of the plaque colony (Bernimoulin 2003). After a couple of hours, plaque adhesion to this pellicle layer begins, and the colony starts to grow, which is irreversible without further physical interruption from oral hygiene methods (White 1997; Marsh 2004). Plaque accumulation starts supragingivally in the interproximal spaces and around the folds of the gingivae and with increased accumulation, spreads subgingivally (Bernimoulin 2003). An important factor of the sites where mature plaque colonization occurs is that they have some protection from external cleaning processes (Marsh 2004).

The bacteria colonies that form dental plaque include species of bacteria that can calcify to form dental calculus. The mineralization that can occur to calcify these colonies into dental calculus deposits, occurs because of these species of bacteria being exposed to an alkaline environment. The calcification of dental plaque bacteria is how dental calculus forms and is discussed in detail in section 2.3.2.

2.2.1.4 Plaque Fluid

In addition to saliva and gingival crevicular fluid, plaque fluid is also present in the oral cavity (Tatevossian 1990; Margolis and Moreno 1994; Duckworth and Huntington 2006). Dental plaque fluid is the water-based permeating liquid that provides nutrients to the bacterial colonies in dental plaque (Figure 2.2.3) (Tatevossian 1990). Plaque fluid

provides a medium, which exchanges proteins and ions between the constantly flowing saliva and the dental plaque microorganisms (Margolis and Moreno 1994). It therefore, has much the same composition as saliva but has been found to have an increased buffer capacity and can change pH depending on the time interval between food consumption (Margolis and Moreno 1994).

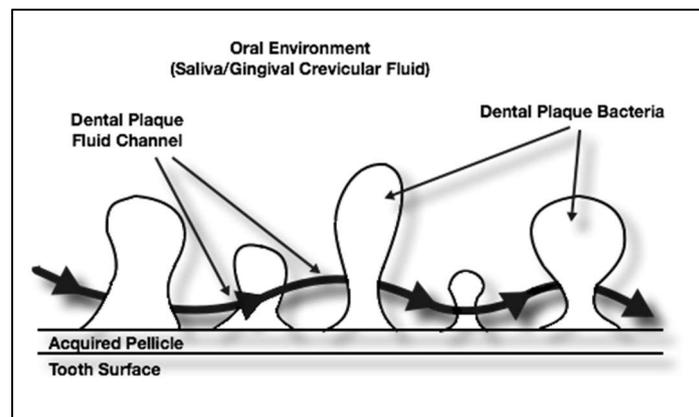


Figure 2.2.3 Diagram showing a dental plaque fluid channel in the oral environment. The channel provides nutrients to the bacterial colony adhere to a tooth surface [Image created by author using Paint 2 (version 5) (August 2015)].

2.2.2 Location of Dental Calculus Deposits

When dental plaque is left undisturbed (by a lack of oral hygiene), certain species of bacteria in the colony can calcify (Baehni and Takeuchi 2003; Wirthlin and Armitage 2004). The calcification of these bacteria is how dental calculus deposits form (this is further explained in section 2.3) (Bernimoulin 2003; Marsh 2004). Due to formation of dental calculus occurring in dental plaque, the location of calculus deposits is dependent on where the dental plaque has accumulated prior to mineralisation. Dental plaque can form on all hard surfaces in the oral cavity¹² and as such it is possible for dental calculus to also be found on all these surfaces (Fine 1988).

¹² These hard surfaces are primarily the enamel and cementum of the teeth; however also include materials utilized in dental work such as prosthetics, implants and restorative work (Bernimoulin 2003).

Dental calculus can be found both on enamel and cementum surfaces in the oral cavity however there are specific sites (on certain surfaces of certain teeth) that are favoured for calculus formation, this site specificity is explained below in section 2.2.2.3 (Hillson 1996). The gingival location (i.e. above the gum-line or below the gum-line) of dental calculus is an important distinction because the differing local environment can result in characteristic dental calculus compositional differences (Lieverse 1999; Roberts-Harry and Clerehugh 2000; Jin and Yip 2002).

2.2.2.1 Supragingival Calculus

Deposits that are present on the enamel surfaces are termed¹³ ‘supragingival calculus’ because of their position above (‘supra’) the gingival boundary (Hillson 1996). These accumulations form while being surrounded by saliva and they build up from the tooth surface outwards, perpendicular to the tooth surface (Figure 2.2.4). Supragingival calculus is generally reported to be yellow/white in colour (Poff et al. 1997; Roberts-Harry and Clerehugh 2000).

It is well-established knowledge that supragingival deposits are found to commonly form around teeth that have a proximity to the salivary ducts (Poff et al. 1997; Corbett and Dawes 1998; Lieverse 1999; Roberts-Harry and Clerehugh 2000; Wirthlin and Armitage 2004). These teeth are the mandibular incisors, which are close to the sublingual salivary gland and the mandibular and maxillary molars, which are close to the submandibular and parotid salivary glands respectively (Figure 2.2.1). There is also an aspect of site-specificity for surfaces of the teeth, and this is described in section 2.2.2.3.

¹³ Terms ‘supragingival’ and ‘subgingival’ are used by dental professionals (dentists, periodontists etc.) and anthropologists (osteologists, bioarchaeologists, dental anthropologists, palaeodontologists etc.) (Reddy 2014; Hillson 2001).

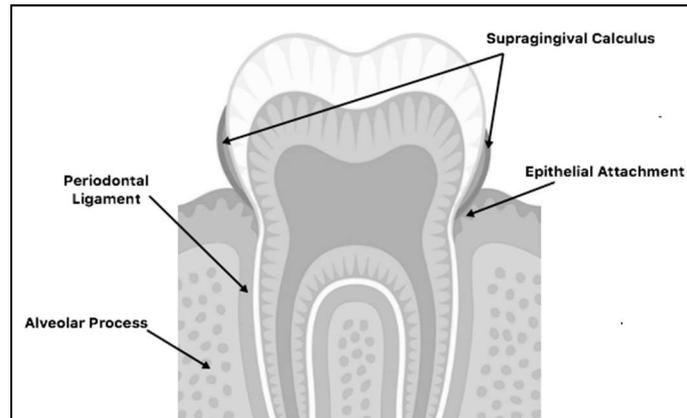


Figure 2.2.4 Diagram to illustrate the location of formation of supragingival dental calculus on a tooth *in vivo* [Adapted by author from <http://www.thedentalcheck.com/> (Retrieved 23rd September 2016)].

2.2.2.2 Subgingival Calculus

Dental calculus that forms on the cementum of the root of a tooth is termed ‘subgingival calculus’ because of its location below the gingival margin (Figure 2.2.5). In contrast to supragingival calculus, subgingival calculus requires a precursory condition to form. This condition is the ‘deepening of the gingival sulcus’ to cause the formation of a periodontal pocket¹⁴ (Waerhaug 1955; Reddy 2014).

Specifically, a pocket is formed when the periodontal tissue is detached from the tooth surface resulting in a loss of attachment of the epithelial wall. Periodontal pockets occur in individuals who have inflamed gingivae from gingivitis that is progressing into the more severe disease of periodontitis¹⁵ (Reddy 2014).

¹⁴ A healthy depth of the gingival sulcus is 3 mm and a depth larger than this is considered a pocket (Reddy 2014). There are different types of periodontal pocket, simple, complex/spiral, compound, suprabony and infrabony and all produce a gingival cavity from tissue destruction (Reddy 2014). As far as the author is aware, there has been no research into the types of periodontal pocket and the formation of subgingival calculus.

¹⁵ The inflammation of the gingival tissue weakens the attachment of the periodontal ligament, and connective tissue causing ‘pockets’ where the tissue is no longer connected to the tooth surface (Reddy 2014).

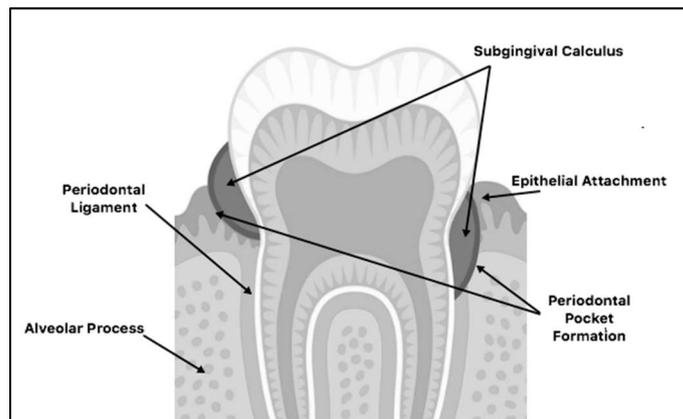


Figure 2.2.5 Diagram to illustrate the location of formation of subgingival dental calculus on a tooth *in vivo* [Adapted by author from <http://www.thedentalcheck.com/> (Retrieved 23rd September 2016)].

As described in section 2.2.1.2, the inflammation of gingival tissue causes an immune response that produces a higher volume of gingival crevicular fluid (Reddy 2014). If periodontal pockets are formed, they provide space for a greater quantity of gingival crevicular fluid to build up, rather than be dissipated into the oral cavity. Additionally, this pocket formation allows the accumulation of dental plaque on the tooth surfaces below the gingival margin. In healthy gingivae, these subgingival tooth surfaces would be protected from dental plaque accumulation.

From this subgingival plaque, subgingival calculus is formed within the gingival crevicular fluid onto the tooth surfaces in the periodontal pockets (Figure 2.2.5). Deposits of subgingival calculus are generally reported to be brown or black in colour and their composition differs compared to supragingival calculus (section 2.4) (Waerhaug 1955; Poff et al. 1997; Roberts-Harry and Clerehugh 2000).

2.2.2.3 Site-Specificity of Dental Calculus

As mentioned briefly above, calculus also has different site specificity for different teeth and tooth surfaces. It has been found from clinical patient surveys and examinations that there are differences in site preference for the two categories of dental calculus (Corbett and Dawes 1998; Dawes 2007). This can be difficult to confirm in archaeological populations because of post-mortem tooth loss and this is further discussed in section 2.5.

Subgingival calculus has been found to have no preference for any site within the mouth (Corbett and Dawes 1998; Dawes 2007). This is due to the precursory pocket formation not having any site specificity around the mouth. By contrast, supragingival deposits are found to commonly develop around teeth, and more specifically tooth surfaces, that have a proximity to the salivary ducts (Corbett and Dawes 1998; Dawes 2007). These are the lingual surfaces of the anterior teeth (incisors and canines) and the buccal surfaces of the posterior teeth (molars) (Dawes 2007; Hayashizaki et al. 2008).

An explanation for this supragingival specificity has been explained by the proximity of teeth, with a high prevalence for calculus deposition, to the salivary ducts (Poff et al. 1997; Lieveise 1999; Roberts-Harry and Clerehugh 2000; Wirthlin and Armitage 2004). The justification by some researchers is that, as saliva enters the mouth, it is at its greatest saturation of calcium phosphate ions and the first teeth that it reaches will be more susceptible to plaque mineralisation (Roberts-Harry and Clerehugh 2000; Wirthlin and Armitage 2004).

Dawes (2007) opposes this explanation based on plaque fluid research, which exhibits a higher saturation of calcium phosphate than saliva. This implies that the state of supersaturation of the saliva should not affect the already highly saturated plaque fluid. Instead research by Dawes (2007) indicates that the prevalence for calculus formation on the lower anterior teeth is due to plaque being thin, salivary flow being high and there being low concentrations of ingested sugar (Dawes 1993; Dawes 2007). All these factors cause plaque pH to stay high which is preferable for mineral formation (Dawes 2007).

Both the salivary gland and salivary flow theories make sense and are backed up by experimental evidence. However, it may be that these theories should be taken together, rather than separately. If the tooth surfaces are close to the salivary glands, they will be exposed to high rates of salivary flow. This will then cause the thin plaque colonies to more often be exposed to alkaline conditions with saliva that is supersaturated. The combination of having highly mobile saliva that contains supersaturated ionic species will promote calcification of plaque.

2.2.2.4 *Supra-/Sub- Considerations*

In either dentistry or bioarchaeology care must be taken during assignment of these supra- and sub- terms for calculus. The main consideration that must be accounted for is when an individual has gingival recession¹⁶ and the gum-line recedes below the level of the cemento-enamel junction (CEJ). This exposes the cementum and means that it is consequently no longer in a gingival crevicular fluid environment but a saliva one. This change in environment means that the assignment of subgingival to any calculus formed on the exposed cementum would be inaccurate.

In dentistry, when this condition occurs, and the gingivae is no longer at its normal height, the crown and the exposed cementum that are visible on inspection is termed the ‘clinical crown’ (Scheid and Weiss 2012). In addition, a ‘pseudo’ or gingival pocket is the deepening of the gingival sulcus without destruction of periodontal tissue and this type of pocket would not lead to the formation of subgingival dental calculus because the gingivae inflames to a level above the normal margin and there is no subgingival environment exposed (Reddy 2014).

In archaeological skeletal remains, however, there is no soft tissue remaining to measure the gingival height and consequently a ‘clinical crown’ height cannot be determined. It is therefore necessary to not only assign supra- or subgingival labels based on location but on characteristic (i.e. colour and deposit shape). As far as the author is aware this is not regularly undertaken in archaeological recording of dental calculus. However, this study will apply physical characterisation to collected specimens in a novel manner. This has the potential to determine if compositional changes could be identified by noting physical features of dental calculus. This could potentially contribute to past populations where destructive sampling is not permitted but visual observation is.

¹⁶ Lowering of the gum line (recession) can occur from improper tooth brushing; mild inflammation; disease (gingivitis/periodontitis); age-related gingival recession or following periodontal therapy (Wolf and Hassell 2006).

2.3 Formation of Dental Calculus

The research concerned with investigating the formation of dental calculus is found in dentistry. The experimentation required to investigate formation mechanisms involves the study of dental plaque and its involvement in calculus formation. The study of live dental plaque from archaeological material is simply not possible, even though ancient bacterial DNA analysis in dental calculus is currently developing (Preus et al. 2011; Warinner et al 2015(1)). Dental plaque consists of bacteria that require sustenance from salivary components, when the saliva is no longer produced (i.e. when a person is deceased) the live dental plaque cannot be sustained (Sharma 2007).

In patient-based studies, dental plaque accumulation can be monitored and samples can be retrieved to analyse the biological composition. Additionally, dental plaque in patients can be monitored for dental calculus formation. Within dentistry, this dental plaque research has allowed multiple complementary and competing theories to be suggested but the precise mechanism for dental calculus formation is not fully understood (Zander et al. 1960; Schroeder and Shanley 1969; Mandel 1987). Before discussing these proposed mechanisms for dental calculus formation, the propensity of an individual to form calculus and the factors that affect this formation are explained. Following the literature regarding the mechanisms of formation, the time interval for dental calculus accumulation is explained in section 2.3.3.

2.3.1 Influencing Factors for Dental Calculus Formation

Dental calculus formation requires two aspects, the accumulation of dental plaque bacteria and the presence of calcium and phosphate ions. Consequently, variables that promote one or other of these aspects, provided both parts are fulfilled overall; will influence the formation of dental calculus.

In terms of dental plaque presence, the largest contributing factor to calculus formation is a lack of oral hygiene (Gaare et al. 1990; White 1997; Hatipoglu et al. 2015). The physical process of cleaning the teeth removes plaque colonies and thus it cannot proceed to calcify and form dental calculus. Even for individuals who participate in regular oral hygiene, dental calculus can still form where plaque bacteria are missed during the cleaning process.

Once plaque is present on dental surfaces, it is the environmental conditions that influence formation. For dental calculus to form, an alkaline environment is required as this increases the amount of calcium and phosphate ions present (see section 2.2.1). With these ions supersaturated in the local environment, the plaque bacteria undergo calcification and thus dental calculus is formed (see section 2.3.2 for the calcification of dental plaque). Consequently, external factors that promote an alkaline oral environment or increase the concentration of calcium or phosphate in the environment increase the possibility of dental calculus forming from dental plaque.

In archaeology, there is a common assumption that diet is the greatest influence on the alkalinity of the oral environment (Lieverse 1999). This is understandable when considering that a major role of the mouth is to introduce and break down foods. It makes sense that eating foods that have a high pH would increase the pH of the mouth and therefore make it alkaline in nature. The continued consumption of such foods would repeatedly cause an alkaline environment, causing dental plaque calcification. However, it is a major role of saliva to balance pH changes in the mouth to return the pH to normal (6.5-7.5). Therefore, it may be that the limited time that food is held in the mouth would not contribute to alkalinity to the extent that calcification would occur.

As Lieverse (1999) explains, it is not the physical introduction of dietary proteins to the oral environment that is likely to influence dental calculus formation due to alkalinity. Instead it is the effect of protein consumption on the urea ($\text{CO}(\text{NH}_2)_2$) levels in blood during digestion that is the contributing factor. When urea concentrations are increased in blood, they are also increased in other physiological fluids (i.e. saliva) (Lieverse 1999). The dental plaque bacteria that are present in the mouth can metabolise the urea to form ammonia, which is basic (Lieverse 1999). The production of ammonia by these bacteria raises the pH of saliva, consequently increasing the concentration of calcium and phosphorus ions and creating the conditions needed for calculus formation (Lieverse 1999).

Another dietary contribution, which is perhaps more direct than protein consumption, involves the consumption of silicon-containing foods. Silicon has been suggested to promote calcium phosphate precipitation (Rølla et al. 1989). Additionally, and in line with this suggestion, individuals who consume foods that have high silicon concentration;

such as rice, have been found to be heavy calculus formers (Gaare 1990; White 1997; Jin and Yip 2002). Again, this may be due to an increase in silicon in physiological fluids, rather than direct contact with the silicon-containing foods.

The physical properties of diet may also influence dental calculus formation. The consumption of soft sticky foods, which collect around the teeth or on ready-formed rough dental calculus surfaces, may promote further calculus accumulation. While soft foods, have the potential to be caries-promoters, they may also increase calculus formation by providing nutrients and bacteria for plaque colonies. In contrast, hard and abrasive foods may restrict dental calculus promotion by disrupting the adhering layer of plaque or outermost layer of calculus during mastication.

Clinical studies have also suggested a positive association between the smoking of tobacco and increased dental calculus formation. A study by Pindborg (1949), examined the dentition of 5,690 Danish marines and reported the presence of calculus and gingivitis in relation to smoking habits. In this large-scale study, a statistical correlation was found between smoking and dental calculus accumulation (Pindborg 1949). A follow up paper by Kowalski (1971) confirmed the conclusions of Pindborg (1949) with further statistical analysis. In addition, Kowalski (1971) more clearly demonstrated that light smokers (10 grains¹⁷ or less) and heavy smokers (10 grains or more) are not statistically significant. Therefore, it is the participation of smoking that increases the calculus accumulation, not the amount smoked per day (Pindborg 1949; Kowalski 1971).

Archaeological habitual activity has also been linked to larger deposits of dental calculus being found on the dentition of individuals such as betel nut or coca leaf chewing (Klepinger et al. 1977; Lieverse 1999). Modern clinical studies have confirmed this observation through patient monitoring of known Sri Lankan and Taiwanese betel nut chewers (Anerud et al. 1991; Hsiao et al. 2015). Both clinical studies found higher amounts of calculus in individuals who chewed betel nut as a habitual activity. However, neither of these studies could separate the contribution of quid chewing from tobacco

¹⁷ A historical unit of mass (troy and avoirdupois systems) whereby one grain refers to the average weight of a seed of barley (1 grain = 0.0065 grams (approx.)) (Stephenson 2002).

smoking, as individuals studied participated in both activities (Anerud et al. 1991; Hsiao et al. 2015).

In addition to the above lifestyle factors, of foodstuff and habits such as smoking, it has been suggested that demographic factors also influence calculus accumulation. A study by Christersson et al. (1992) examined differences in age, sex and ethnicity in terms of dental plaque and calculus accumulation. The study group of 508 American individuals showed an increased risk for older individuals and males (Christersson et al. 1992). Additionally, the study concluded that individuals of African American ethnicity were also at higher risk for dental plaque/calculus accumulations (Christersson et al. 1992), however the proportion of individuals of different ethnicity in the study was unbalanced (94% White; 6% Black).

The demographic influence of age and sex in modern populations may be closely linked with oral hygiene and dental calculus accumulation. However, in terms of differences between male and female individuals, this is not consistent between studies. Studies by Buckley (1980) and Beiswanger et al. (1988) reported a difference between male and female patients, with male individuals exhibiting higher prevalence for dental calculus accumulation. Additionally, it has been found from a patient survey of young adults that women are more aware of good oral hygiene practice and attend regular dental check-ups, lowering the amount of dental calculus recorded on their teeth (Furuta et al. 2011). However, a large multi-population study by Beiswanger et al. (1988) did not find a statistical significance between the male and female patients studied.

In contrast to difference in sex, multiple studies have found an increase in dental calculus accumulation in older individuals (Beiswanger et al. 1988; Pilot et al. 1992; MacPherson 1995; Brown et al. 1996). There may be this correlation because once dental calculus has formed on dental surfaces, the nature of the calcification promotes further accumulation. If calculus deposits have initiated on tooth surfaces, factors associated with age such as mobility and health complications may contribute to the natural promotion of calculus accumulation on the rough surface of a formed deposit. The development of systemic conditions and the consumption of prescribed medications have also been found to influence dental calculus accumulation and this may skew the results for older individuals, who may suffer from these risk factors (White 1997; Jepsen et al. 2011).

The systemic conditions that have been found to affect dental calculus progression in individuals are primarily related to kidney disease and diabetes (Proctor et al. 2005; Kuo et al. 2008; Davidovich et al. 2009; Hatipoglu et al. 2015). This is potentially due to the ionic imbalances that occur during both the progression of these conditions and the treatment administered (Proctor et al. 2005; Kuo et al. 2008).

Additional factors have been suggested to influence calculus accumulation, such as the bacterial composition of dental plaque and the host response to plaque bacteria (Jepsen et al. 2011). While most influencing factors can be applied generally to individuals, these variables are specific to a person. Therefore, it should be noted that the broad influences described above should not be applied without some hesitation that other factors are present within a population or sample group.

2.3.2 Calcification of Dental Plaque

Once a bacterial colony of dental plaque has formed on a tooth surface it will be sustained by the saliva. Species of bacteria that have been identified within dental plaque are known to calcify when the right conditions are met (Wirthlin and Armitage 2004). These conditions include a high concentration of calcium and phosphates within the oral environment (Wirthlin and Armitage 2004).

The calcification of dental plaque is a complicated biological occurrence that is not completely understood. Before evaluating the possible pathways that have been theorised regarding the process of calcification. It is important to understand that not all bacterial species are calcifiable. Similarly, it has been found that not all oral bacteria calcify in the same way or at the same rate, when favourable conditions are present (Boyan et al. 1992). In addition, the species of bacteria that constitute an individual's dental plaque, is not consistent (Eilberg 1973). Rizzo et al. (1962) demonstrated that different species calcified in their cells, while others showed calcifications at the cell wall. However, whether these differences influence the overall process, is not known.

The time it takes for dental plaque to calcify is dependent on several factors. A study by Mislowsky and Mazzella (1974) found that dental plaque colonies do not begin to calcify until they have had several days of maturation. Consequently, when this dental plaque did

start to calcify, it did so at discrete points within the colony, creating crystal foci (Mislowsky and Mazzella 1974).

From *in vitro* and *in vivo* plaque studies, there have been four methods suggested as routes of dental plaque calcification. These are the Booster mechanism, the Epitactic concept, Transformation theory and the Inhibition theory (Mandel 1987; Lieverse 1999; Wirthlin and Armitage 2004). These methods of calcification are theoretical and based on knowledge of bacterial behaviour and mineralisation. In all these theories, the factor that allows the crystallisation to grow, is supersaturation of the saliva with calcium and phosphate ions (Jin and Yip 2002).

The Booster mechanism accounts for calcification of the bacteria in dental plaque by an increase in the pH of the saliva resulting in a rise in the concentration of calcium and phosphates in the local environment (Mandel 1987; Lieverse 1999; Wirthlin and Armitage 2004). An increase in alkalinity of the saliva has been found to occur by either loss of carbon dioxide (Schroeder and Shanley 1969; Mandel 1987) or metabolism of urea and amino acids to form ammonia (Margolis 1990). This increase forms a calcium and phosphate-rich medium for dental plaque and causes some species of the bacteria to mineralise (Wirthlin and Armitage 2004).

This mechanism implies that the initial mineralisation can start wherever the relevant bacteria are within the matrix of the plaque and there is salivary supersaturation of calcium ions and phosphates (Wirthlin and Armitage 2004). The mechanism suggests that once a crystal centre is formed and calcification starts, it spreads within the bacterial colony to form the deposit (Wirthlin and Armitage 2004).

The problem with the Booster mechanism as a theory is the initial mineralisation is not explained. The supersaturation concentrations of the saliva with calcium and phosphates has been found to not be sufficient to cause nucleation of the first crystal site (Lieverse 1999) but would be sufficient to facilitate crystal growth once initial nucleation had occurred (Mandel 1987).

The Epitactic concept is a more detailed theory of heterogeneous nucleation, which is where multiple sites within the plaque mineralise to form nuclei (Schroeder and Shanley 1969; Wirthlin and Armitage 2004). As stated above, the supersaturation concentrations

of the calcium and phosphates needs to be higher for nucleation to occur and the Epitactic concept theorises that this higher concentration occurs occasionally to keep a cycle of nucleation of different crystal centres (Schroeder and Shanley 1969; Lieverse 1999; Roberts-Harry and Clerehugh 2000). The concentration is therefore not always high enough to cause nucleation but can allow crystal growth between spikes of the highest alkalinity that initiate further new crystal formations. In the 'normal' concentration of supersaturation, these crystal nuclei can grow and join to form one body of crystallisation, which is the calculus deposit.

The Inhibition theory suggests that there are certain sites within the saliva and plaque matrix that do not crystallise due to the species that inhibit crystal formation (Mandel 1987). Some species, include proline rich proteins and pyrophosphate that stop mineralization by stabilising calcium (Lieverse 1999). Only when appropriate enzymes are present; can these inhibitor species be broken down and hydrolysed to allow mineralisation to proceed (Scheie 1989; Lieverse 1999).

The final theory of Transformation relies on the precipitation of precursor minerals of octacalcium phosphate and dicalcium phosphate dihydrate and subsequent formation of β -tricalcium phosphate and hydroxyapatite (Driessens and Verbeeck 1989; Mandel 1987; Hayashizaki et al. 2008; Rau et al. 2010). This precursor precipitation has been found to increase with the amount of silicon present (Damen and ten Cate 1992). The mineral content of dental calculus is further discussed in section 2.4.1.

There is still debate in the calculus research community regarding the Transformation concept and the presence of octacalcium phosphate (OCP) as a precursor to hydroxyapatite (HAp) in dental calculus (Kakei et al. 2000; Crane et al. 2006). Much of the debate comes from the identification of OCP peaks when using X-ray diffraction to identify the biominerals in calculus samples. The peak for OCP is very close to HAp and it is thought that original researchers were unable to determine the separate peaks due to resolution and therefore did not know of the presence of OCP (Driessens and Verbeeck 1989). A Raman micro-spectroscopy study by Crane et al. (2006) confirmed that there was 'OCP-like' mineral in their spectra but they could not confirm or deny that it was OCP.

While none of these theories give a definitive explanation of how dental plaque calcifies to form calculus deposits, they do note that the conditions required for the crystal initiation are an alkaline oral environment with salivary supersaturation of calcium and phosphates. The common similarity for calcification of plaque bacteria and formation of dental calculus in each theory, is the role of saliva. Without the nutrients from the saliva, the bacteria colonies in plaque could not be sustained and would not be present for calcification to occur. Equally the saliva also provides the medium in which the calcium and phosphate ions are introduced to the oral environment.

For archaeology, these theories have an important consequence that allows an assumption to be made. That is that dental calculus cannot be formed in the oral environment after death occurs. This is because there is only actively flowing saliva in living individuals and therefore calcification can only occur during life (Phee and Cowley 1975; Blatt et al. 2011). This means that dental calculus deposits in archaeological remains represent oral environment conditions in the weeks, months or maybe years, before death. The literature regarding time frames for dental calculus formation is further discussed in section 2.3.3.

2.3.2.1 Reversal Phenomenon

In addition to accumulation, and separate from deliberate calculus removal, deposits can decrease in size by a process called reversal phenomenon (Reddy 2014). This can occur as either physical abrasion of a deposit or chemical dissolution (Reddy 2014). The physical abrasion primarily comes from contact between the calculus surface and lips, cheeks and tongue but could also result from habitual activity using the mouth (Reddy 2014). Chemical dissolution of the calculus is also possible, when saliva pH levels decrease enough to form an acidic environment, thus dissolving the mineral component of the deposits.

For both these processes, there has been little research into the natural fluctuations in size of dental calculus in the oral environment. Considering dental literature implies that dental calculus can only be removed by intervention, it does not seem plausible that deposits can be entirely removed through these means alone. However, it is important to consider these points when analysing the microstructure of dental calculus. In this research, the cross-sectional composition of dental calculus has been analysed and

reversal phenomenon may or may not have influenced the mineral component of the material.

2.3.3 Time Interval for Dental Calculus Formation

A feature of dental calculus formation that is not often discussed in archaeological studies, is the time it takes for a dental calculus accumulation to build up. This has a few consequences regarding the analysis of deposits. Due to dental calculus being a condition that an individual can have without them suffering any discomfort, it may be present for several years before it becomes a problem to the individual (Scott and Poulson 2012). Equally a change in health may promote the rapid accumulation of calculus and there is a proportion of individual variation in formation (see section 2.3.1). Similarly, as described in section 2.3.2.1, dental calculus formation can decrease as well as increase due to environmental conditions in the mouth through reversal phenomenon. Therefore, it cannot be assumed that the deposits observed on archaeological material that have the same size, represent the same period of formation (Scott and Poulson 2012).

In several clinical studies, this feature of dental calculus has been investigated, however even with patients to study, difficulties arise in standardising formation conditions and calculus deposit measurement (Gaare et al. 1990; Tsuda and Arends 1993; Macpherson et al. 1995; Liu et al. 2001). Additionally, there are also two stages of dental calculus formation that can be investigated. Firstly, the time it takes for dental plaque to start to calcify and secondly the time it takes for the dental calculus deposit to increase in amount as further plaque is calcified (Mandel 1995). Both timescales have been found by clinicians to vary considerably among individuals (Mandel 1995). Additionally, as mentioned in section 2.2.2.3, dental calculus has site specificity for certain surfaces and so within the mouth, the rate of formation will vary regarding tooth type or surface as well as from person to person. As explained in section 2.3.1, the causes of variation amongst individuals are numerous. While most of these factors have been investigated in clinical studies they cannot be completely controlled or isolated from one another.

The studies that have focused on rates of formation fall into two categories; studies that have investigated histological progress of accumulation and studies that have investigated the rate of formation during the use of different oral health practices or products (Mandel

et al. 1957; Conroy and Sturzenburger 1968; Gaare et al. 1990; Tsuda and Arends 1993; Macpherson et al. 1995; Liu et al. 2001).

The former studies investigate the organisms and calcification of dental plaque cultures from patients (Mandel et al. 1957; Wasserman et al. 1958; Poff et al. 1997). The latter studies involve patients that exhibit dental calculus deposits who undergo a complete prophylaxis (scale and polish), to remove any adhering deposits. The patients are then advised to follow an oral health regime for a set period. Following this, the patients are examined for dental calculus formation (Gaare et al 1990; Macpherson et al. 1995). This is clearly a research format that cannot be achieved for archaeological remains and poses complications when applying the results to past populations.

In past populations, any oral hygiene methods employed would not have been sufficient to completely remove all traces of dental calculus from the teeth. The only point at which no calculus would be present, would be before any plaque bacteria had begun to calcify. For individuals that were unaware of the condition and had limited or no oral hygiene, this may have been as early as childhood. Even today with modern equipment, full removal is a difficult and a time-consuming process (Gaare et al. 1990). Additionally, in patient-based accumulation studies, it is only the difference in oral hygiene practice that has been considered in terms of calculus formation, other variables are not reported (Conroy and Struzenburger 1968; Gaare et al. 1990).

In the histology-based studies, there are also issues. For example, the specimens of plaque or calculus that are sampled, represent one point in an individual's lifetime (Mandel et al. 1957). This means that any changes to rate of calcification because of age, change in lifestyle or changes in health are not considered.

Despite these application difficulties, it is still useful to keep in mind the clinical literature. This states that dental plaque calcification begins between the 1st and 14th day of plaque formation (Mandel et al. 1957; Reddy 2014). After calcification begins, dental plaque can be 50% mineralised within two days and 60-90 % mineralised within 12 days (Mandel et al. 1957; Reddy 2014). It is reported that following the initial plaque calcification, further accumulation and mineralization occurs until the deposits reach a 'maximum level' which can be between 2.5 and 6 months, however there is no indication of how large a deposit this would be (Reddy 2014).

These timescales are very generalised and are based on a limited number of individuals. However, for archaeological studies and conclusions, it is important to consider the length of time of an individual's life, that dental calculus represents. Many archaeological studies do not examine the mineral component of dental calculus; therefore, they are unable to describe their results with a timescale in mind. Indeed, quite often results are applied to a person or population without mention that the conclusions are based on the calculus at the time of death rather than over a known period of an individual's life. For example, individual's that have died at a younger age, with large accumulations, the calculus may not be representative of life but of a health change that has resulted in death.

This research includes analysis of the mineral component of many dental calculus deposits of varying size. In conjunction with the other analyses performed, this will hopefully be able to contribute to understanding physical characteristics and compositional differences which may relate to the stages accumulation of dental calculus.

2.4 Composition of Dental Calculus Deposits

Dental calculus is a complex biological material, which has a composition that contains biomineral, inorganic and organic components. The location of the calcifications in the oral cavity also means that food and fibres that enter the mouth to be consumed or when the mouth is used as a 'third-hand', can also be trapped in the matrix of the calculus (Blatt et al. 2011). There has been found to be variation in dental calculus composition between individuals based on their health, diet and age amongst other factors (White 1997).

This section includes explanation of the composition of dental calculus, separated into mineral, elemental, organic and inclusion components. Most this information comes from clinical literature. However, the inclusion content and isotopic analysis is only found in the bioarchaeological literature. Each section contains the most up to date knowledge regarding calculus composition from all sources as well as subsections on specific studies from clinical and archaeological perspectives.

Despite the common reporting of the clinical literature of dental calculus composition to archaeological studies, this section aims to assess how much research has been performed

to verify this. In addition, this literature review will assist in the determination of gaps in archaeological knowledge, that may be filled by this study.

2.4.1 Mineral Composition

The term ‘biomineral’ is given to mineral structures that are formed in living organisms from environmental metal ions interacting with cells in an organism (Veis 2008). The biominerals that are formed in biological systems are diverse and are tailored to a function or role that is required by the host. However, in some cases biominerals form due to a set of conditions that favour formation, rather than because the formation is required for a function within the organism. This type of mineralization forms ‘pathological biominerals’ and the biominerals in dental calculus can be given this term. As explained in section 2.3.2, dental calculus forms through the calcification of plaque bacteria and this is an example of metal (calcium) ions interacting with bacterial (plaque) cells to form calcium-phosphate minerals.

Calcium-phosphate minerals in organisms are predominately apatitic in nature (LeGeros 1981). This means that in biological systems, the stoichiometric chemical formulas for the present biominerals are very generalised structures ((LeGeros 1981). The actual minerals that are present can be considered as highly impure due to the substitution of alternative ionic species within the mineral matrix. This incorporation of alternative ions can occur at formation, due to the presence of these species in the formation environment ((LeGeros 1981). Alternatively, ionic substitutions can occur within the minerals once they are formed. This can occur for a variety of reasons, for example ionic exchange to stabilise the structure due to a change in conditions (LeGeros 1981).

The alternative species that can be incorporated into a mineral structure are classed as foreign ions or impurities. While this is technically true in terms of the stoichiometric chemical formula, the incorporation of alternative ions is what makes apatites biologically adaptable. Dental calculus is composed of mineral phases of calcium phosphate that are highly substituted and consequently poorly crystalline. The high amount of substitution in dental calculus occurs because of the adaptive and changeable oral environment in which it forms. As explained in section 2.2.1, there are several ionic species present in both saliva and gingival crevicular fluid and some of these are available for the

substitution. The elemental composition of dental calculus is further discussed in section 2.4.2.

The stoichiometric chemical formulae of the four biominerals that have been identified in dental calculus are detailed in Table 2.4.1 (Driessens and Verbeeck 1989; Roberts-Harry and Clerehugh 2000; Jin and Yip 2002; Wirthlin and Armitage 2004). The researchers that have reported the presence of these four biominerals have all done so from a clinical perspective. After explaining the nature of these reported mineral phases, the literature for clinical and archaeological studies of the mineral composition of dental calculus is assessed.

To the author's knowledge, there has been no direct research focussing on diagenetic effects on dental calculus. Buckley et al. 2014 do report that they observed no diagenetic effects on starch granules extracted from archaeological dental calculus, however the mineral component was not considered in the study. This is an area that should be explored further in the future.

Chemical Name	Mineral Name	Abbreviation	Stoichiometric Chemical Formula
Calcium hydroxyapatite	Hydroxyapatite	HAp	$[\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2]$
Magnesium-containing β -tricalcium phosphate	Whitlockite	WHT	$[\text{Ca}, \text{Mg}]_3(\text{PO}_4)_2]$
Dicalcium phosphate dihydrate	Brushite	BRU	$[\text{CaHPO}_4 \cdot 2\text{H}_2\text{O}]$
Octacalcium phosphate	Octacalcium Phosphate	OCP	$[\text{Ca}_8\text{H}(\text{PO}_4)_3 \cdot 2\text{H}_2\text{O}]$

Table 2.4.1 Table showing the chemical and mineral names, abbreviations and stoichiometric chemical formulae of the mineral phases reported to be found in dental calculus.

Calcium hydroxyapatite (HAp) is a naturally occurring biomineral that as well as occurring in dental calculus, is also a mineral constituent in human bone and tooth enamel (LeGeros 1981). In addition to dental calculus, calcium hydroxyapatite has also been identified in other pathological calcifications such as urinary and renal stones and breast cancer calcifications (LeGeros 1981; Zelenkov et al. 2012; Cox and Morgan 2013). As

well as occurring biologically, this mineral occurs in geological specimens and it can also be produced synthetically (Smith 1994; Yeong et al. 2001).

The most common crystal structure of hydroxyapatite is hexagonal and belongs to space group $P6_3/m$ (LeGeros 1981; Mathew and Takagi 2001) (Figure 2.4.1). In its ideal form, calcium hydroxyapatite contains calcium, phosphate and hydroxyl ions and has the chemical formula of $Ca_{10}(PO_4)_6(OH)_2$. However, as described above, the nature of biological apatitic-minerals is that they can substitute alternative ions into their lattice. These substitutions change the stability and properties of the apatite because of the difference in ionic radii of the different ions that can be incorporated into the hexagonal phosphate matrix.

Hydroxyapatite is a thermodynamically stable calcium phosphate that precipitates in conditions of pH 7.0 – 8.0 (Kani et al. 1983). Due to the pH conditions of saliva being conducive for hydroxyapatite precipitation, it has been suggested that this mineral phase forms predominately in supragingival calculus, rather than subgingival calculus (Kani et al. 1983; Roberts-Harry and Clerehugh 2000). Additionally, it has been proposed that biological hydroxyapatite in dental calculus is formed by hydrolysis of the less stable phases of octacalcium phosphate and brushite (Barone and Nancollas 1978; Kani et al. 1983) (see below for detail on these precursor mineral phases).

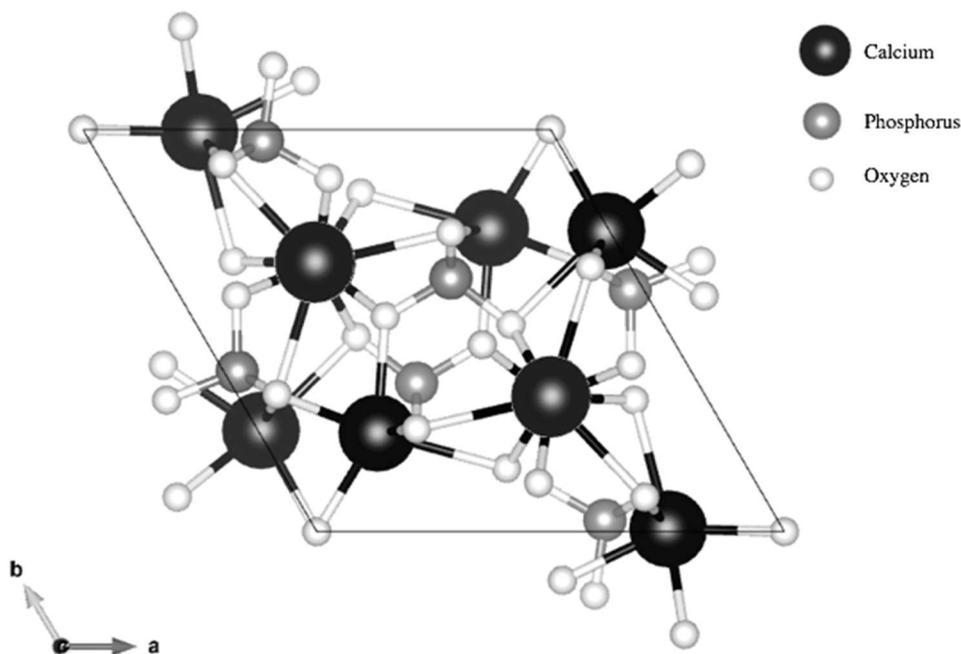


Figure 2.4.1 Model of the unit cell of the hexagonal crystal structure of stoichiometric calcium hydroxyapatite as viewed along the ‘c’ axis, for simplicity hydrogen atoms are not shown [Model compiled by author using VESTA version 3.3.8 (August 2016)].

Whitlockite (WHT) is the mineral term for the calcium orthophosphate, tricalcium phosphate, of chemical formula $\text{Ca}_3(\text{PO}_4)_2$ (Lagier and Baud 2003). This mineral is found in phosphate containing rocks and in biological calcifications (Tovborg Jensen and Rowles 1957; Gopal et al. 1974; Scotchford et al. 1995; Lagier and Baud 2003).

The mineral of whitlockite in biological deposits, including dental calculus, is technically ‘magnesium-whitlockite’, $(\text{Ca},\text{Mg})_3(\text{PO}_4)_2$ because the calcium ions are partially substituted for magnesium (Lagier and Baud 2003)¹⁸. In addition, it is the β -polymorph that is found in biological calcifications, as the α -polymorph forms at high temperature (around 1100°C) (Jillavenkatesa and Condrate 1998). The β -polymorph has a rhombohedral crystal structure and belongs to space group R_3C (Mathew and Takagi 2001; Zhou 2012) (Figure 2.4.2).

¹⁸ In line with literature sources regarding dental calculus, the term whitlockite is used in this thesis in relation to magnesium-whitlockite and abbreviated to WHT.

Whitlockite in dental calculus has been reported to form from the hydrolysis of brushite in alkaline conditions when magnesium, zinc or carbonate ions present (Kani et al. 1983). It has also been reported to be more abundant in subgingival calculus than supragingival calculus (Kani et al. 1983). This is due to the higher concentrations of the required ions (Mg^{2+} , Zn^{2+} , CO_3^{2-}) being present in gingival crevicular fluid as well as conditions of higher alkalinity due to ammonia production from dental plaque (see section 2.3.1).

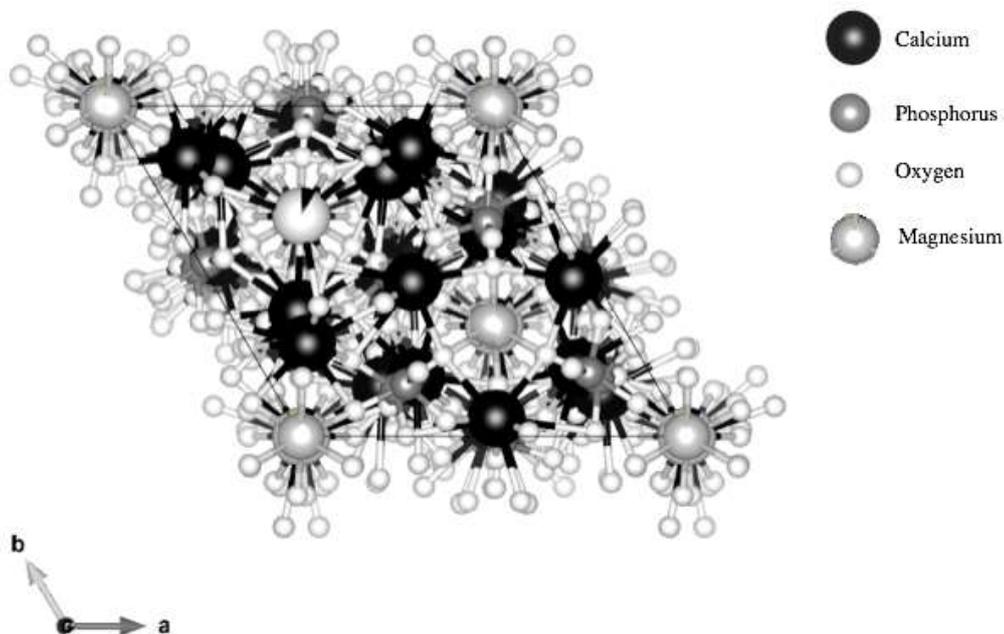


Figure 2.4.2 Model of the unit cell of the rhombohedral crystal structure of stoichiometric magnesium whitlockite as viewed along the 'c' axis, for simplicity hydrogen atoms are not shown [Model compiled by author using VESTA version 3.3.8 (August 2016)].

Brushite (BRU) is a calcium phosphate that has the stoichiometric chemical formula $CaHPO_4 \cdot 2H_2O$. The unit cell of brushite is monoclinic and belongs to space group $I2/a$ (Figure 2.4.3). In studies that have prepared synthetic brushite crystals, this mineral is found to precipitate in conditions of low pH and has been reported to hydrolyse into octacalcium phosphate, which is stable at higher pH conditions (Reaction 2.4.1) (Rowles 1964; Lundager Madsen 2008). In the presence of magnesium ions (Mg^{2+}), brushite has been found to transform directly into whitlockite (Lundager Madsen 2008) (Reaction 2.4.2).

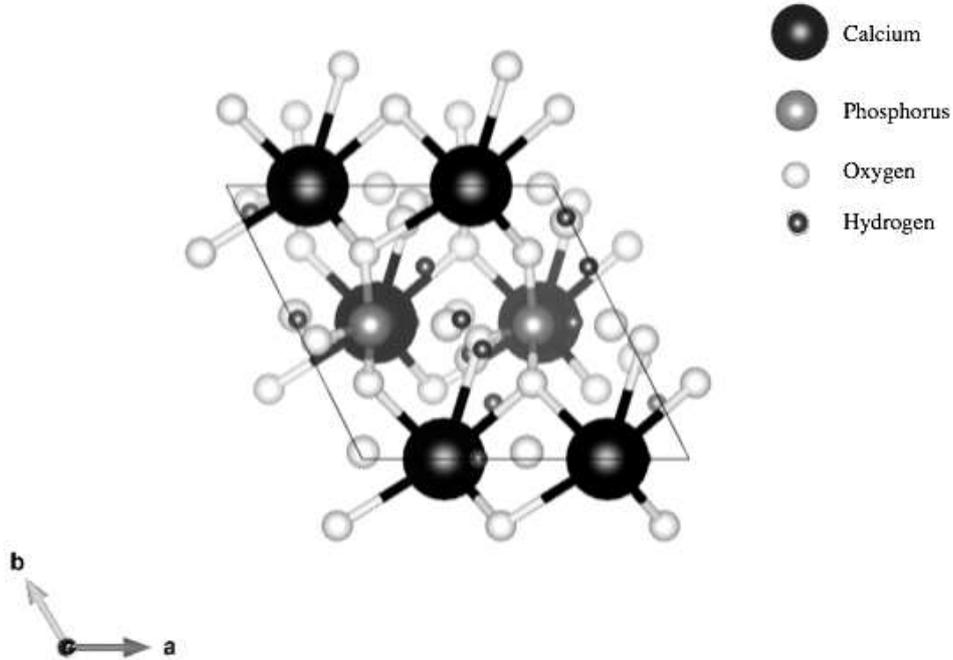
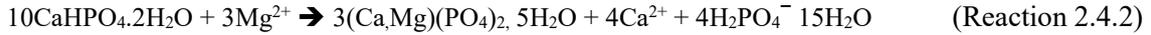
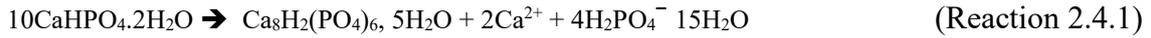


Figure 2.4.3 Model of the unit cell of the monoclinic crystal structure of stoichiometric brushite as viewed along the 'b' axis [Model compiled by author using VESTA version 3.3.8 (August 2016)].

Octacalcium phosphate (OCP) is a calcium phosphate mineral with the formula $\text{Ca}_8(\text{HPO}_4)_2(\text{PO}_4)_4 \cdot 5\text{H}_2\text{O}$ that has an apatite-type structure with a triclinic unit cell (Figure 2.4.4) (Mathew and Takagi 2001; Elliot 1994). This mineral is most commonly reported to be an initial precipitate in tooth and bone formation when in vitro studies are performed (Brecevic et al. 1972; Rau et al. 2010; Suzuki 2013). However, there is often difficulty in identifying OCP in specimens taken from subjects. The predominant method for identifying OCP is with X-ray diffraction, however the corresponding peaks are commonly obscured by peaks from other calcium phosphates present. In addition, an isolated peak that occurs between $4\text{--}5^\circ$ is usually at too low an angle for routine analysis to include (Rau et al. 2010). The route of OCP as a precursor has been suggested because when calcium ions are available, OCP is readily hydrolysed into the more stable calcium hydroxyapatite in Reactions 2.4.3 and 2.4.4.

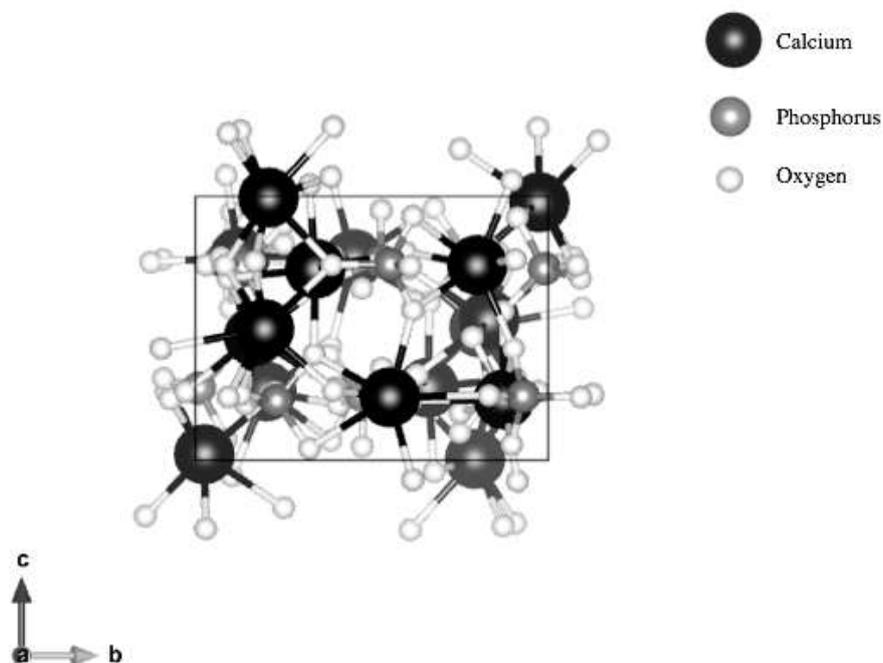


Figure 2.4.4 Model of the unit cell of the structure of stoichiometric octacalcium phosphate as viewed along the ‘a’ axis [Model compiled by author using VESTA version 3.3.8 (August 2016)].

2.4.1.1 Mineral Composition from Clinical Studies

As mentioned earlier, the dominant field that has investigated the mineral composition of dental calculus has been dentistry and the relevant publications are too numerous to cover for this thesis. This section covers the main papers that have contributed knowledge to the mineral composition of dental calculus.

In the paper, Tovborg Jensen and Danø (1954), it was outlined that dental calculus research of the time was primarily focused on ‘clinical, bacteriologic and histologic aspects of calculus’ and that the preceding research that had analysed dental calculus minerals had been during the 1930’s (Phillip 1935; Glock and Murray 1938). In these 1930’s studies, the X-ray diffraction technique was still in its early years and mixtures of minerals proved difficult to resolve (Tovborg Jensen and Danø 1954). However, the

earliest researchers did identify that calcium phosphates were present, and these included brushite, whitlockite, apatite and small amounts of aragonite (Tovborg Jensen and Danø 1954).

In the 52 human specimens analysed by Tovborg Jensen and Danø (1954), all contained apatite and 41 of the specimens contained whitlockite. Additionally, brushite was identified in seven specimens, which were all from the mandibular anterior teeth. Interestingly in this study, it was also found that amalgam (Ag_3Sn) could be identified in the calculus specimens from fillings that had corroded. Tovborg Jensen and Danø (1954) was the first large-scale study of clinical dental calculus mineral composition, however, X-ray diffraction at the time did not have the capability to determine mineral phase percentages. The authors also indicate at the end of the summary that there were peaks present for unidentified phases in two specimens (Tovborg Jensen and Danø 1954).

A later study by LeGeros (1974), used a newer diffraction method to analyse dental calculus minerals. This study aimed to apply the newly developed technique to more accurately build on the knowledge that had previously been gained from older X-ray diffraction research. A difficulty with hydroxyapatite and octacalcium phosphate, is that all but one of their diffraction peaks overlap. In earlier crystallographic techniques, this peak, for octacalcium phosphate was not easily identifiable due to its low angle position ($4.7^\circ 2\theta$) (LeGeros 1974). Therefore LeGeros (1974) aimed to determine the presence of either or both minerals, using the technological advances that had become available.

The analysis could identify all four of the expected minerals across the 35 specimens studied, although only three of these contained brushite (LeGeros 1974). In addition, at least one deposit of dental calculus was sectioned into 'inner' and 'outer' specimens, as the author had noticed layering during microscopy (Figure 2.4.5) (LeGeros 1974). Although 35 specimens were analysed, it was not clear if this process was done for all of them or indeed if these specimens were taken from 35 separate individuals. In the sectioned specimen, it was found that the 'inner' specimen contained whitlockite and apatite, whereas the 'outer' specimen contained apatite and octacalcium phosphate. The octacalcium phosphate was identified by the peak at $4.7^\circ 2\theta$.



Figure 2.4.5 Photograph of the layering noted as discontinuous mineralization in a clinical dental calculus specimen, as viewed at x100 magnification, with the original being reproduced at 67% [Reproduced from LeGeros (1974)].

LeGeros (1974) also semi-quantitatively estimated the amount of each mineral based on the peak heights of the specimens and the analysed standard powders. However, the author did not report these semi-quantitative determinations (LeGeros 1974). If they had been reported, they may have been reasonably tenuous, compared to the present-day algorithms used. This is because the standard powders were mixed using synthetic minerals and therefore did not fully represent the amount of substitution and variation that can occur in biological phases of these species.

In addition to the advances that the newer diffraction technique in LeGeros (1974) had provided, such as better peak resolution and phase determination, it also allowed for a shorter data acquisition time. The author also states that previous methods may account for the erroneous identification of additional calcium phosphate phases, such as monetite and calcite. LeGeros (1974) states that the human mouth is not conducive for forming these minerals and the publications that had previously reported these phases, may have falsely identified them due to the experimental limitations (Urbantschitsch 1933; Newesely 1961; LeGeros 1974).

A more recent study, Hayashizaki et al. (2008), aimed to progress the understanding of the mineral composition of dental calculus, in terms of how the phases differ around the mouth. The study used specimens taken from 66 male patients who had undergone a full dental clean within the 6 months prior to sampling. Therefore, the calculus was assumed

to have accumulated over no longer than this 6-month period, although this assumes the dental cleaning had been successful in full removal. The samples were removed from lingual surfaces of the anterior teeth and buccal surfaces of the posterior teeth, as these were the sites to be compared. However, due to the random sampling performed, this provided an uneven spread of 55 lingual anterior specimens and 11 buccal posterior specimens (Hayashizaki et al. 2008).

The authors could group the specimens into four types by the mineral phases identified¹⁹ and the percentage of specimens in each group was compared between the sites sampled (Hayashizaki et al. 2008). The study reported a significant difference ($p < 0.01$) in the crystal phases present between the lingual anterior and buccal posterior specimens sampled (Hayashizaki et al. 2008).

In all the clinical literature, it is widely accepted that dental calculus is composed of a combination of some or all of the four minerals described in the preceding section. A complication with analysing dental calculus from patients, particularly in recent decades, is the increased variables that can potentially affect composition. It is unknown whether modern oral hygiene products, prescription medication or even food additives, affect the minerals that are formed. It can therefore be beneficial to look to past populations, where these variables are minimised, to better understand mineral compositional differences around the mouth and between individuals.

2.4.1.2 Mineral Composition from Archaeological Studies

There have been limited archaeological studies that have analysed the mineral content of dental calculus. Instead focus has been on the starch and microfossil inclusions and more recently the organic, bacterial and protein content (Fox et al. 1996; Hardy et al. 2009; Preus et al. 2011; Hardy et al. 2012; Scott and Poulson 2012; Warinner et al. 2014; Warinner et al. 2015(1)). Consequently, there has been little research to determine if the mineral content of archaeological dental calculus is the same as clinical dental calculus. Therefore, this thesis has endeavoured to do exactly that.

¹⁹ Type I (apatite); Type II (apatite and octacalcium phosphate); Type III (apatite and whitlockite); Type IV (apatite, whitlockite and octacalcium phosphate).

Of the limited archaeological studies that have analysed the mineral composition, Klepinger et al. (1977) appears to be the first. This published study featured six specimens of dental calculus that were sampled from prehistoric Ecuadorean individuals and were analysed by X-ray diffraction. The analysis found that the deposits were composed of a poorly crystalline hydroxyapatite phase along with phases of quartz and calcium silicate (Klepinger et al. 1977). The researchers reported that no brushite or octacalcium phosphate could be detected in the specimens but that there was possibly magnesium whitlockite (Klepinger et al. 1977). The analysis was not able to distinguish the whitlockite peaks from the hydroxyapatite.

Klepinger et al. (1977) found that the presence of hydroxyapatite in the archaeological specimens indicated that the deposits were dental calculus in nature. However, they proposed that due to taphonomic degradation and post-mortem soil contamination the mineral composition of the specimens had been altered during burial. The study did not report the phase percentages of the identified minerals and there was no detailed comparison of mineral phases between specimens. It was however postulated that the silicate phase was potentially the result of groundwater minerals precipitating into the porous dental calculus structure (Klepinger et al. 1977).

More recently, powder X-ray diffraction has been performed, analysing a larger number of specimens (Greenwood 2009; Beckett 2010; Wood 2012). In these studies, the mineral phases were not only identified but the phase percentages were statistically analysed for differences in tooth or surface location of the deposits and for differences between male and female individuals.

In Greenwood (2009), calculus from nine male individuals that had been buried at Haslar Naval Hospital, Gosport, UK was sampled. In this study, it was proposed that the individuals were expected to have been enlisted in the Navy during the 18th Century²⁰, and therefore would have had a set diet through naval rationing. The powder X-ray

²⁰ The area of the Haslar Hospital burial ground in which the individuals were buried, was open between the years of 1753 and 1826. The burial ground was utilized for deceased patients of the Haslar Naval Hospital and to be patients, they would have had to have taken ill or be injured while serving in the Navy. Individuals would have been subject to rationing while on ships as well as while being treated in the Hospital (Greenwood 2009).

diffraction performed on the specimens found the dental calculus mineral phases of hydroxyapatite and whitlockite. In addition, and as in Klepinger et al. (1977), quartz was found that was contributed to soil contamination during burial. Also in line with Klepinger et al. (1977), no octacalcium phosphate or brushite was identified in any specimen.

By comparing the proportions of hydroxyapatite and whitlockite in the specimens, (Greenwood 2009) found that there were differences based on the tooth and surface from which the deposit was removed. The posterior teeth exhibited higher proportions of whitlockite compared to the anterior and the mesial and distal surfaces exhibited higher proportions of whitlockite compared to the lingual and buccal surfaces (Greenwood 2009). It was suggested that these differences might be due to local pH changes relating to carbohydrate consumption (Greenwood 2009). The salivary digestion of carbohydrates would decrease the local pH and therefore cause the preferential formation of brushite as a precursor rather than octacalcium phosphate (see section 2.4.1). The study also involved starch and microfossil analysis to confirm the consumption of carbohydrates (Greenwood 2009).

More recently, Wood (2012) carried out a similar study using specimens taken from five male, five female and two juvenile individuals from Anglo Saxon Norfolk²¹. The powder X-ray diffraction results identified the mineral phases of hydroxyapatite, whitlockite and quartz; the same phases as found in the study by Greenwood (2009) (Wood 2012). Regarding the proportions of mineral phases present, more whitlockite was found in female specimens compared to male specimens. This was hypothesised to be due to biochemical variation in magnesium concentrations between female and male individuals (Wood 2012).

Although limited in number, these archaeological studies have investigated the mineral composition of dental calculus and provided valuable insight. As they have been limited in their sample size, it is necessary that further work be done to broaden the insight into population and individual variation. It is intended that the research in this thesis will do

²¹ The individuals were excavated from the site of Sedgeford, Norfolk and this has been approximately dated to the 7th – 11th Century through burial archaeology (Wood 2012).

just that. By employing a larger sample size from a few populations with comparative and contrasting features, it is intended that further knowledge can be contributed to the mineral composition of archaeological dental calculus. Additionally, the mineral analysis will contribute to not only the bulk but also the cross-sectional variation in mineral composition to differentiate between dental calculus at the attachment surface and at the exposed surface.

2.4.2 Elemental Composition

As well as the major elements, that are found in the biominerals of dental calculus, namely calcium, phosphorus and oxygen, elemental incorporation can occur by mineralogical substitution or physical entrapment (Roberts-Harry and Clerehugh 2000; Abraham et al. 2007; Lazzati et al. 2015). In the preceding section, the nature of the apatite minerals found in dental calculus have been explained to be able to be structurally stable but elementally changeable. The possible interchangeable ionic species are detailed in Table 2.4.2.

Mineral Phase	Element/Ionic Species in Stoichiometric Formula	Possible Interchangeable Elements/Ionic Species
Hydroxyapatite/ Whitlockite	Ca ²⁺ (PO ₄) ³⁻	Na ⁺ , K ⁺ , Li ⁺ , Ba ²⁺ , Mg ²⁺ , Sr ²⁺ , Pb ²⁺ , Mn ²⁺ , Cd ²⁺ , Zn ²⁺ , Fe ²⁺ , Co ²⁺ , Ni ²⁺ , Al ³⁺ , Cr ³⁺ (CO ₃) ²⁻ , (P ₂ O ₇) ⁴⁻ , (HPO ₄) ²⁻ , (AlO ₄) ³⁻ , (CrO ₄) ³⁻
Hydroxyapatite	OH ⁻	F ⁻ , Cl ⁻

Table 2.4.2 Table showing the possible substitution species in calcium phosphate minerals and the stoichiometric species that they interchange for [Compiled from LeGeros (1981)].

2.4.2.1 Elemental Composition from Clinical Studies

The elemental analysis of clinical dental calculus has been investigated to determine the ‘normal’ distribution of elements, to further understand the role of elements in formation processes (Retief et al. 1972). Apart from the major elements of calcium and phosphorus that are often analysed in terms of the mineral composition (see section 2.4.1), different studies have focused on specific elements. Numerous early studies investigated the

concentration of a range of elements in calculus deposits, plaque and saliva using analytical techniques, as they were developed (Söremark and Samashl 1962; Dawes and Jenkins 1962; Little et al. 1963; Little and Hazen 1964; Grøn et al. 1967). The early studies of trace elemental composition were limited by the error associated with the measurements of small concentrations in the techniques of the time (Söremark and Samsahl 1962). In addition, depending on the analytical methods utilised, a different range of elements were detectable in different studies, however this may also be due to the variation in composition of dental calculus.

An in-depth study by McDougall (1985) analysed 20 thin sections of dental calculus from different individuals and found that the elemental composition was non-homogenous. In the specimens studied, the most frequently occurring minor elements were zinc and magnesium but the concentrations of these varied between locations in specimens and between individuals. This was also the case for the other minor elementals identified²².

More recent studies have used synchrotron radiation X-ray fluorescence (SR-XRF) to investigate trace element concentrations in clinical dental calculus (Sánchez et al. (2000); Pérez et al. 2004). The analysis by Sánchez et al. (2000) demonstrated that a variety of elements were present in the two dental calculus samples analysed. In addition, the study found that the elemental concentrations varied within the region of analysis, which was a square covering a cross-sectional surface of the deposits. In particular, in one specimen, the concentration of zinc decreased from one corner of the analysis region outwards into the rest of the deposit, as show in Figure 2.4.6 (Sánchez et al. 2000). This was explained by the presence of subgingival calculus having formed at the edge of the supragingival calculus. For this to occur, the deposit must have primarily accumulated supragingivally and as a periodontal pocket formed in the gingivae, the calculus further accumulated but in the medium of gingival crevicular fluid, rather than saliva. This was proposed to indicate the presence of early periodontitis.

²² Titanium, nickel, cobalt, arsenic, strontium, zirconium, molybdenum, cadmium and tin.

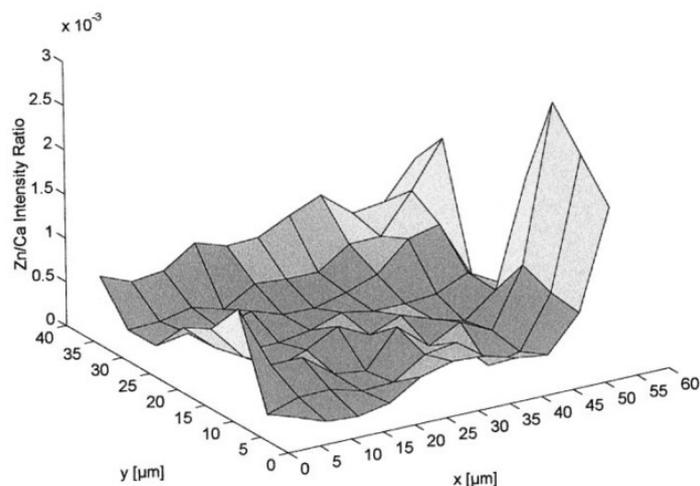


Figure 2.4.6 3-D Plot of the Zn/Ca ratio as measured from a region of the cross-section of dental calculus, as spike in Zn/Ca can be seen on one edge of the analysis region [Reproduced from Sánchez et al. (2000)].

Sánchez et al. (2000) were primarily concerned with the viability of synchrotron X-ray fluorescence for use in dental calculus studies and therefore the analysis of only two specimens limits the conclusions that can be drawn from the results. Despite this, the elements determined as present²³ in the specimens, were consistent with the earlier studies from the 1960s, although not all elements from the early studies were identified, such as gold, silver, lead and copper (Glock and Murray 1938; Söremark and Samsahl 1962).

An additional SR-XRF study by Pérez et al. 2004, also studied the concentrations of trace elements across the growth axis (from the tooth attachment surface to the oral surface) of four deposits. It was found that copper and zinc were present in higher concentrations near the attachment surface (Pérez et al. 2004). In this paper, the variation in zinc and copper has been attributed to the initial mineralisation process, whereby zinc ions are released by bacteria to inhibit calcium phosphate crystallisation, which is supported by other research into calculus formation (Pérez et al. 2004; Nancollas and Johnsson 1994; Jin and Yip 2002). Other than zinc and copper, there is little discussion on the other elements identified in the analysed specimens (Pérez et al. 2004). The authors note that

²³ Calcium, phosphorus, silicon, sulphur, chlorine, potassium, titanium, chromium, manganese, iron, zinc

more studies are required to understand the elemental composition of dental calculus, especially with consideration of individual factors such as eating habits, medicines and illness, which may also influence the elements present (Pérez et al. 2004).

In addition to elemental profiles for calculus, it is important to mention a study by Huang et al. (1997) regarding fluoride. This study investigated the fluoride profiles that could be obtained from dental calculus taken from patients from countries with different fluoride concentrations in drinking water (Huang et al. 1997). This research found that there was a correlation between the concentration of water fluoride and the fluoride concentrations in the calculus (Huang et al. 1997). The chemical fluoridation of water is a modern occurrence; however, this does demonstrate that elements from consumed fluids, do have an influence on dental calculus elemental composition. Consequently, elemental analysis of dental calculus from past populations provides results where the chemical treatment of food, water and the consumption of pharmaceuticals has less interference on the results.

2.4.2.2 Elemental Composition from Archaeological Studies

The amount of studies concerned with the elemental compositions of archaeological dental calculus are less numerous than clinical. This is potentially because archaeological studies refer to clinical literature for elemental composition, rather than investigating it separately.

A couple of studies that have performed elemental analysis on archaeological dental calculus have done so to compliment analysis by scanning electron microscopy (Charlier et al. 2010; Power et al. 2014). In these studies, the elemental compositions reported are consistent with the clinical literature. Both studies indicate that the most abundant elements are calcium and phosphorus with each study identifying a range of trace elements (Charlier et al. 2010; Power et al. 2014).

In Charlier et al. (2010), one specimen per individual, from five individuals from the Etruscan-Celtic necropolis of Monterenzio Vecchia in Bologna, Italy (5th – 3rd century B.C.E) was sampled from lingual surfaces of the mandibular posterior teeth. The five specimens were found to have a diverse elemental composition with each deposit containing variable trace elements. It was also noted that the most abundant trace elements were magnesium and zinc, as was also found in the analysis of clinical dental

calculus (McDougall 1985; Sánchez et al. 2000; Pérez et al. 2004). Although the elemental differences between the five specimens were not outlined in the paper, the trace elements identified were titanium, nickel, cobalt, arsenic, strontium, zirconium, cadmium, selenium and lead (Charlier et al. 2010).

Power et al. (2014) analysed six specimens of dental calculus from Camino del Molino, a Bronze Age Chalcolithic burial pit (3rd millennia B.C.E) by energy dispersive X-ray spectroscopy, as in Charlier et al. (2010). Again, the trace elemental concentrations of each specimen were not reported and it was not stated if all specimens came from the same or multiple individuals. However, the trace elements identified were consistent with previous studies of archaeological and clinical calculus (Power et al. 2014).

An alternative use of elemental analysis that has been performed relates to palaeodietary isotopic studies, although these are predominantly carried out on archaeological bone and other biomaterials, rather than dental calculus (Arnay-de-la-Rosa et al. 2009; Scott and Poulson 2012). A study that has investigated the potential for isotopic analysis to be performed on dental calculus has been Scott and Poulson (2012) who proposed dental calculus as an isotopic analysis alternative for when bone sampling is not permitted. The results of this study were promising with carbon and nitrogen isotope ratios found in the dental calculus specimens being comparable to bone collagen isotope values from literature sources (Scott and Poulson 2012). However, further studies by Salazar-García et al. (2014) and Eerkens et al. (2014) found that isotopic analysis of bone and dental calculus from the same individuals did not always produce consistent results. It has therefore been suggested that dental calculus should not be used as a proxy for bone for isotopic analysis, or the results should be at least taken with caution (Salazar-García et al. 2014; Eerkens et al. 2014).

The most recent elemental analysis of archaeological dental calculus has combined palaeodietary analysis with trace element identification. Lazzati et al. (2015) analysed two specimens of dental calculus from medieval Italy by solution inductively-coupled mass spectrometry. This study concluded that the results from both specimens gave similar results in terms of elemental composition and the most abundant elements were consistent with previous studies, although the trace elements identified were more numerous (see Lazzati et al. (2015) for full list).

Even though numerous elements were identified and the quantitative analysis was carried out for multi-elemental standard that used, only certain elements were of interest to the authors. These elements of interest were decided based on previous palaeodietary evidence from studies that applied isotopic and elemental analysis to bones and teeth (Lazzati et al. 2015) (see Table 2.4.3). This study uniquely reports the elemental compositions of dental calculus with consideration of the potential diet that may have been consumed.

Element	Suggested Related Sources
Zinc (Zn)	red meat, dairy products, nuts, legumes (lentils), crustaceans (crabs and shrimps), fish, egg yolk, oats and barley
Copper (Cu)	offal, red meat, fish, crustaceans, molluscs, legumes and nuts
Strontium (Sr)	vegetables, sea fish, shellfish, molluscs (related to high levels of zinc)
Manganese (Mn)	legumes, green vegetables, cereals, nuts (be careful because of clay)
Lead (Pb)	post-mortem contamination/drinking vessels/water pipes

Table 2.4.3 Table showing the palaeonutritional sources of trace elements that have been determined from isotopic and elemental analyses [Compiled from Lazzati et al. (2015)].

The archaeological elemental analysis of dental calculus, although not abundant in publications, has the potential to help clinicians understand clinical calculus compositions. In addition, the contribution that trace elements may provide for dietary analysis are intriguing, although further understanding of taphonomic ion exchange is required. In the above-mentioned studies, all the specimens that were analysed have been previously buried and no analysis of the surrounding soil was performed. This puts into question the true elemental composition of the calculus as it was during life. This research analyses archaeological dental calculus from mummified remains, where there has been no soil contact and therefore no burial contamination. It is intended that this will contribute to the compositional knowledge of archaeological dental calculus without taphonomic soil interference to the trace elemental composition.

2.4.3 Organic Content

The organic component of dental calculus is composed of amino acids, peptides, glycoproteins, proteins, carbohydrates and lipids (Mandel 1987; Hillson 1996; Lieverse 1999). The organic species present make up around 15-20% of the weight of calculus deposits (Lieverse 1999; Jin and Yip 2002) depending on the location that the calculus is formed (Jin and Yip 2002); subgingivally or supragingivally (see section 2.2.2). The percentage content of specific organic species has been published in detail by Jin and Yip (2002).

The organic components in dental calculus have been primarily studied through clinical research, which is understandable considering the degradation process associated with these species (Cooper and Hausman 2013). The research concerning the organic species in dental calculus has been performed to better understand their role in calculus formation as well as their potential link to other conditions and diseases (Slomiany et al. 1983; Kido et al. 1995; Weiner et al 2005; Wong and Sissons 2007).

The organic component of dental calculus is not examined in this research, however that is not to say that archaeological research is unable to contribute. Recent studies have used proteomics and ancient DNA (aDNA) techniques to gain valuable information from deposits of calculus on archaeological remains (Preus et al 2011; Warinner et al. 2014; Weyrich et al. 2014; Warinner et al. 2015(1); Warinner et al. 2015(2)). These studies have found that despite the degradation of organic components, dental calculus traps these species within its calcified structure, protecting it from degradation (Preus et al. 2011).

Preus et al. (2011) have been able to demonstrate that DNA can successfully be isolated from archaeological dental calculus from the Neolithic era. This finding demonstrates that as dental calculus forms in the mouth, the bacterial species in the oral cavity become entombed in the calcified material. Therefore, there is great potential in this analysis in terms of understanding diseases in past populations from the bacteria that was present at the time of calcification (Preus et al. 2011).

In addition, to palaeoepidemiology, the organic analysis of dental calculus has also been applied to gain dietary evidence. Warinner et al. (2014) have been able to isolate a protein linked to dairy consumption, preserved in ancient dental calculus. The study was able to

show that the presence of this protein in dental calculus was consistent with historical evidence for dairy-consuming populations; and its absence was related to non-dairy-consuming populations (Warinner et al. 2014). Furthermore, specimens from a Norse population where historical and isotopic evidence indicated a dietary shift from dairy to marine resources were analysed. The proteomic results supported the previous isotopic evidence because the dental calculus of earlier inhabitants of the Norse region in question contained the dairy protein (Warinner et al 2014). In contrast, the dental calculus from later inhabitants did not contain the protein, indicating that dairy products were no longer being consumed (Warinner et al 2014).

Dietary analysis of dental calculus has also been performed using organic components isolated using thermal-decomposition gas-chromatography mass-spectrometry (TD-GC-MS) (Buckley et al. 2014). However, rather than the organic components of the dental calculus itself, this study was focused on the organic species that could be isolated from trapped inclusions within the dental calculus matrix (Buckley et al. 2014).

2.4.4 Inclusions

Research in archaeological sciences has discovered plant microfossils that have been incorporated into the calculus matrix during formation (Fox et al. 1996; Boyadjian et al. 2007; Piperno and Dillehay 2008; Henry and Piperno 2008; Wesolowski et al. 2010; Mickleburgh and Pagán-Jiménez 2012; Chinique de Armas et al. 2015; Leonard et al. 2015; Wang et al. 2016). Plant microfossils that have been recovered from dental calculus can be phytoliths, starch granules, pollen grains or fungal spores (Juan-Tresserras et al. 1997; Henry et al. 2009; Henry et al. 2011; Afonso-Vargas et al. 2015).

As far as the author is aware and with extensive literature searching, there have been no studies to date that have extracted starch granules or microfossils from clinical dental calculus. However, there have been a few inclusion-based archaeological studies that have included dental calculus specimens from present day chimpanzees (Hardy et al. 2009; Power et al. 2014; Power et al. 2015(2)).

Phytoliths are the mineral remains of plant cells that have been absorbed during a plants life (Armitage 1975; Weiner 2010). When phytoliths are referenced in dental calculus analysis they are silica phytoliths because they are the most durable mineral remains and

keep the morphology of the cell long after the rest of the cell has decayed (Weiner 2010). Silica dissolved in the soil is absorbed by the plant and solidifies into the shapes of the cells that have absorbed it (Fox et al. 1996). The survivability of phytoliths means that they can be recovered from archaeological calculus. In addition, the shapes of the plant cells they have adopted, can be identified to a species (Dudgeon and Tromp 2014).

Phytoliths can become incorporated into dental calculus by any means where the mouth and teeth have contacted plants. This may be through subsistence, medicine or habitual activities (Henry and Piperno 2008; Hardy et al. 2012; Tromp and Dudgeon 2015). Therefore, caution should be taken when identifying plant remains in dental calculus not to immediately assume the plant in question was consumed for dietary purposes. For buried remains there is also the possibility that phytoliths from the soil have adhered to the calculus post-mortem (Fox et al. 1996).

Another type of inclusion, starch granules, store energy in plants and differ in size and shape between plants and depending on whether they are from roots, seeds or leaves (Henry et al. 2009). Dental calculus has been found to contain starch granules, indicating that the matrix protects the granules from both the enzymatic decomposition in the mouth and the burial environment (Weiner 2010). Again, caution must be taken when identifying starch granules from dental calculus because of the potential sources that starch could derive from. The presence of starch granules does not immediately indicate consumption of the plant that they derive from. Hardy et al. (2012) have identified starch granules from plants are believed to be medicinal because they have no nutritional value and a bitter taste, although Buck et al. (2016) have challenged this inference.

In addition to identifying the plants that starches have originated from, the state of the granules can also give an indication of the processes that have been carried out on the starch source. Henry et al. (2009) have shown the degradation of starch granules when subjected to different cooking methods to gain more information from starch found in dental calculus and other archaeological sources, such as on tools and in food containers. The paper presents microscopic images of contemporary starch granules after a variety of cooking activities such as boiling, baking, fermenting has been performed (Henry et al. 2009). With it being difficult to conclusively match plants from antiquity with starch

granules identified in archaeological dental calculus, an indication of preparation processes is also valuable to historical knowledge.

Pollen and fungal spores have also been identified in dental calculus with scanning electron microscopy (Power et al. 2014; Afonso-Vargas et al. 2015). There is the possibility of pollen identification becoming more widespread in dental calculus analysis, however the transient nature of pollen means that grains could be inhaled and thus not be representative of the local plants. In addition, pollen analysis is also performed on soils so therefore caution is needed when analysed dental calculus specimens that have been buried (Piperno 1998; Piperno and Holst 1998).

2.5 Considerations for Dental Calculus Analysis

When analysing dental calculus either in situ or as removed specimens, there are several factors to keep in mind, this section briefly outlines these considerations. To start, the ethics of dental calculus removal from archaeological remains is discussed. Subsequently, considerations that impact the presence and condition of dental calculus deposits in archaeology are outlined including dental calculus treatment during life (i.e. picking or scraping of the deposits), post-mortem damage and tooth loss in archaeological assemblages.

2.5.1 Ethics of Dental Calculus Sampling

To successfully determine ethical codes, it is vital to discuss whether dental calculus is part of the individual or archaeological debris. Most archaeological material in the UK is not covered by the Human Tissue Act legislation, due to its age. However, government publications, for example '*Guidance for the Care of Human Remains in Museums (2005)*' by the Department of Culture Media and Sport (DCMS) provide guidelines for sampling from archaeological human remains.

DCMS (2005) defines human remains as 'bodies or parts of bodies, of once living people'. This definition states that human remains include: "osteological material (whole or part skeletons, individual bones or fragments of bone and teeth), soft tissue including organs and skin, embryos and slide preparations of human tissue". Because dental

calculus is neither bone, tooth or soft tissue, it appears to be excluded from this definition of human remains.

The source of the DCMS (2005) definition is the Human Tissue Act (2004). In the case of Scotland, the Human Tissue (Scotland) Act (2006) applies. These define human material as consisting of; “including (or being derived) from human cells”. Included in the guidance, supplied by the Human Tissue Authority, a list of ‘relevant material’ can be found on their website. This includes bodily fluids, such as blood and bile, as well as tissue, such as bone marrow; due to the viability of these materials as sources of human DNA. As dental calculus is not composed of human DNA it is not included in this list of relevant material and is therefore not legally classified as human remains.

Recent developments in ancient DNA research have resulted in the isolation and replication of bacterial DNA from dental calculus (Preus et al. 2011; Warinner et al. 2014; Warinner 2015(1)), but this is not human DNA. Consequently, if only bacterial DNA (rather than human DNA) is present, then dental calculus is excluded from the ‘relevant material’ definition described above. However, a couple of studies have reported the identification of human nuclear and mitochondrial DNA from archaeological dental calculus (Kawano et al. 1995; Black et al. 2011). If dental calculus includes human DNA, then it would appear to be “relevant material,” and therefore should be categorised as human remains. However, following communication with the Human Tissue Authority (email communication on 09/12/15), *in vivo* calcifications (from deceased individuals who are covered by the Act) are not considered relevant material under the Human Tissue Act (2004) unless their study falls under one of the scheduled purposes. These purposes include research relating to disorders or function of the human body and obtaining scientific or medical information about a living or deceased person which may be relevant to any other person (for full list of scheduled purposes see Human Tissue Act 2004; Sch. 1, Pt. 1 and 2). With the development of the field of dental calculus analysis, this material assumes increased significance in disease studies. This may mean that the classification of archaeological dental calculus may also become aligned with bones and teeth in legal definitions.

Whether a researcher considers dental calculus as human remains or not, there are ethical concerns about destructive sampling. Dental calculus removal alters both the sample and

its context. It is important to document all sampling of human remains for future researchers as outlined in the British Association of Bioarchaeology and Osteoarchaeology (BABAO) Code of Practice (2010).

The DCMS (2005) guide acknowledges the potential to be gained from destructive analysis and balances this with recommendations to ensure best practice is followed. This includes a scientific justification for sampling as well as ensuring that appropriately qualified personnel perform the sampling. Other publications also support this, for example in Brickley and McKinley (2004), it is recommended that sampling of skeletal material should only occur when there are 'clearly defined reasons' to do so. Similarly, the BABAO Code of Ethics (2010) indicates that studies involving sampling 'should weigh the potential findings against resource availability and the amount of information that could be gained from such a study'. In addition, the BABAO Code of Ethics (2010) outlines that any remaining samples and the pertaining results are returned to the custodian of the human remains.

This thesis has endeavoured to sample dental calculus in an ethical manner as well as treating the remains with dignity and respect as per the standard ethical considerations that are to be expected of an anthropological study. For each population, comprehensive documentation of dental calculus deposits was carried out for each individual. In addition, a limited number of specimens were removed from the archaeological remains, to preserve deposits for future studies. These considerations are further discussed for each population in Chapter 3.

2.5.2 Dental Calculus Treatment

The presence of dental calculus is an indicator of insufficient oral hygiene, which has failed to consistently remove dental plaque from tooth surfaces. Once the calculus deposits have begun to accumulate, it is impossible to completely remove them without professional intervention. Microscopy studies have shown the intimate attachment of the crystals in dental calculus to the surface of enamel and cementum (Hayashi 1993; Rohanizadeh and LeGeros 2005).

2.5.2.1 Treatment Methods

For the prevention of dental calculus development, modern dentists constantly promote the benefits of proper tooth-brushing practice to keep oral tissues and teeth healthy but this is not a new concept (Gaare et al. 1990). Ancient Chinese and Arab populations used sticks and brushes throughout history but in the 18th Century by William Addis in Clerkenwald, England the first mass-produced bristled toothbrush for consumers was developed (Lucente 2006; Moyer and Everett 2010). Improvement on this came in the 20th Century with the invention of the nylon toothbrush that is still the basis for toothbrushes today (Lucente 2006; Moyer and Everett 2010; Hayasaki et al. 2014). Studies have proven the value of tooth brushing on gingival health with patient studies involving people who present calculus deposits (Gaare et al. 1990; Kressin et al. 2003; Bosma et al. 2008). One such study by Gaare et al. (1990) showed that even patients with calculus showed reduced gingival bleeding with regular and well-informed tooth brushing.

Additional prevention methods come from the use of toothpastes and mouthwashes. The dentistry industry has also spent vast amounts of time and money researching and developing dentifrices to be used in conjunction with manual tooth brushing (Jowett et al. 2013). There are several anti-calculus agents that have been found to either remove the bacteria involved in dental plaque, such as Triclosan; inhibit crystal formation within the bacteria, such as pyrophosphate or dissolve calcium-containing deposits, such as zinc salts (Jin and Yip 2002).

When a patient has developed dental calculus, using prevention method such as tooth brushing and mouthwash may increase gingival health but will not remove the calculus from tooth surfaces (Gaare et al. 1990). The recommended method to remove all calculus is through dental prophylaxis, which is a full removal of deposits from tooth surfaces by a dentist or dental hygienist. This process is termed ‘periodontal debridement’ and can involve manual or ultrasonic instruments to remove calcifications from enamel or cementum surfaces (Nield-Gehrig and Willmann 2007).

The methods used to remove calculus have developed alongside the increase in knowledge about its possible formation processes. The previous treatment of root planing to eliminate subgingival calculus was routine practice when it was believed the cementum

was involved in the formation of the calculus (Nield-Gehrig and Willmann 2007). This process irreparably damaged a patient's cementum and now the objectives of removal are to cause as little damage as possible to oral hard tissues (Nield-Gehrig and Willmann 2007). This has been a modern change in methodology for removal of calculus with increased technology to research formation and composition but past populations have had to suffer the condition and treat it with little to no information.

2.5.2.2 Evidence of Treatment in Archaeology Remains

In any dental calculus study, if possible, the review of dental care (particularly dental calculus knowledge) of a population is important. Firstly, it allows an understanding of the attitudes towards this dental condition and any treatment, if any that was adopted for calculus removal. This is particularly important when analysing a population's calculus prevalence. If there was knowledge on the practice of dental treatment in a past population, particularly regarding calculus, this would influence the presence of deposits seen on the teeth.

It is often the case that this type of historical information is not available or that no treatment was performed (Roberts and Manchester 2010). The nature of dental calculus being able to accumulate without causing pain or discomfort means that for most people it was likely ignored. In addition, if accumulations were considerable enough to cause irritation, they were probably picked at or scraped by the individuals themselves with implements to hand. It may have been that this was done as a sporadic event that removed the bulk of the deposit and the left it again to re-accumulate or as a regular scraping that became almost habitual.

While these potential dental calculus self-treatments can never be definitively known for an individual, they should be kept in mind. If dental calculus deposits may have been affected by treatment, the time interval that the deposit represents would be different. As described above, it has taken the development of modern technology to be able to fully remove dental calculus adhering to the teeth. Therefore, it is reasonable to assume that past populations, would never have been able to fully remove a dental calculus deposit fully. Therefore, some or all the calculus closest to the tooth may represent an earlier accumulation that has been reduced in size by intervention which then has a later accumulation built up on top (Figure 2.5.1).

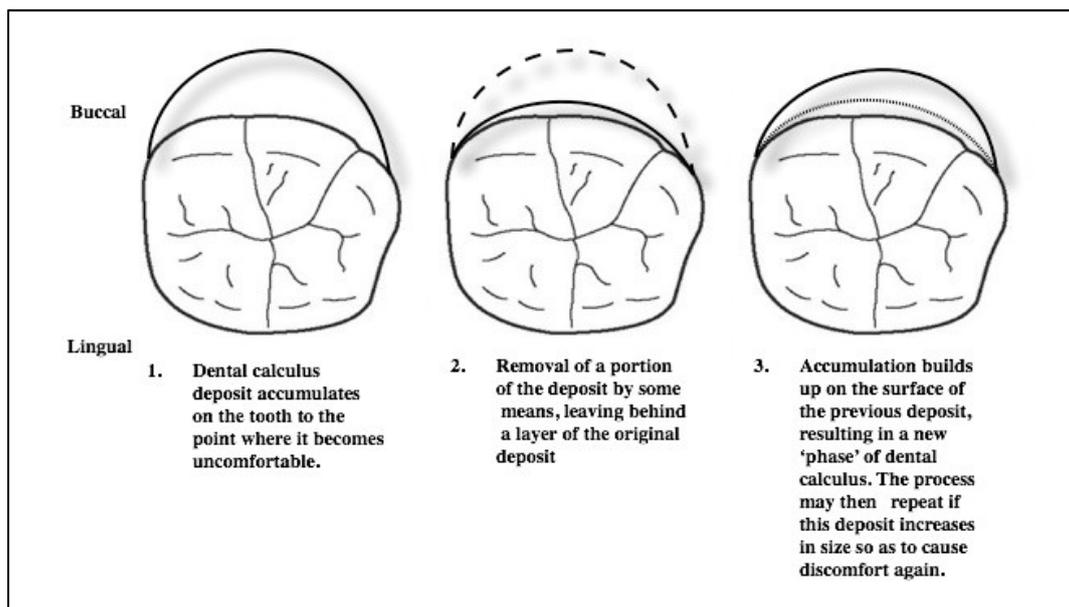


Figure 2.5.1 Diagrammatic representation of how dental calculus deposits may be affected by self-treatment in past populations, where full oral prophylaxis would not have been possible [Image created by author using Paint 2 (version 5) (December 2016)].

As this thesis is concerned with the cross-sectional composition, the consideration of dental calculus treatment is of importance. The analysis of layering in dental calculus may assist in determination of phases of treatment as well as a possible timeline of build-up and potential changes in health and diet.

2.5.3 Post-mortem Dental Calculus Damage

Unfortunately, post-mortem damage to archaeological dental calculus deposits must also be considered. In past archaeological procedure, this may have been intentional or accidental. Before the value of dental calculus was understood, the deposits were more than likely removed as debris to observe the obscured tooth surfaces on which they adhered. Now that significant work has been invested in the development of dental calculus analysis, the value of these accumulations is better understood and protected during archaeological and anthropological recording.

Despite this developed respect for archaeological dental calcifications, post-mortem damage can still occur accidentally. This can happen during the excavation process, handling of remains and storage and transport of remains. Post-mortem damage to

calculus deposits can often be observed from differences in colour in the deposit and irregular shaped breakages in the deposit (Figure 2.5.2).



Figure 2.5.2 Photograph of dental calculus deposits that have suffered from post-mortem damage, indicated by an irregular breakage pattern causing exposure of calculus lighter in colour [Image Credit: Almudena García-Rubio (Universidad Autónoma de Madrid)].

While post-mortem damage to deposits cannot be reversed when it comes to recording and analysing dental calculus, it should be noted. For this thesis, deposits that were observed as suffering from post-mortem damage were not sampled for analysis. This was to ensure that when analysing the bulk and cross-sectional composition of the calculus, the data was not erroneous due to missing material.

2.5.4 Tooth Loss

In addition to missing data from damage to dental calculus deposits, missing data can also occur from post-mortem tooth loss (PMTL). This is an inherent problem in any dental study of archaeological material. It means that in most populations, it may be the case that dental calculus is under reported in populations. The PMTL may mean that for some individuals, different numbers of teeth are present for observation.

In terms of dental calculus prevalence in a population, the statistics are also skewed by ante-mortem tooth loss (AMTL). This is particularly problematic in populations of older individuals where AMTL is more common. Because of this, the amount of dental calculus recorded on an archaeological population will again likely be under-represented. For example, in a population where large deposits may be accumulated through adult-hood,

these may not be observable on the remains if the individuals reached such an age where their dental condition proceeded through periodontal disease and resulted in ante-mortem tooth loss.

Due to both PMTL and AMTL, it is common to report dental diseases and condition as the number of teeth affected rather than the number of individuals affected. In this thesis, the number of teeth and the number of surfaces affected are reported, as well as the minimum number of individuals that the teeth and surfaces represent. In addition, the number of alveolar positions that could be observed as having missing teeth due to post-mortem and ante-mortem loss are reported. This helps to assess what proportion of missing data there is for the number of individuals recorded.

2.6 Chapter Summary

This chapter has demonstrated that dental calculus is a complex material that forms in the oral environment. Dental calculus is found in clinical dentistry in modern individuals as well as in archaeological populations. It forms because of the calcification of dental plaque colonies that accumulate on undisturbed dental surfaces. Consequently, dental calculus formation is promoted by poor oral hygiene however, other factors contribute to the conditions required, for example, diet, health, medication and habitual activities.

Dental calculus is composed of mineral phases, trace elements, organic species and can have inclusions such as starch granules and microfossils incorporated into its structure. This composition has been found to vary among individuals, although the reasons for this are poorly understood. In addition, there are several complications that make archaeological dental calculus analysis more complicated, such as tooth loss and post-mortem damage. Despite this, there is a wealth of knowledge that can be gained from analysing this biomineral, especially from past populations where the variables that affect composition, such as medical treatment are reduced compared to modern populations.

This chapter has also highlighted areas of archaeological dental calculus that are insufficient in comparison to clinical literature. As such the reliance on clinical studies that have investigated dental calculus from modern individuals, may be flawed when considering past populations. The research aims that have been formed following

examination of the literature are outlined in Chapter 4. The following chapter explains the archaeological materials that have been used in this research to achieve the largest compositional study of archaeological dental calculus to date.

CHAPTER 3: RESEARCH AIMS & HYPOTHESES

If these means of cure are of no avail, on account of the presence of hardened limosity (tartar), this must be removed by scraping it away with appropriate instruments.

- Guy de Chauliac 1363

3.1 Cross-Sectional Variation

Aim 1	Investigate whether layers can be detected in archaeological dental calculus and whether the mineral and elemental composition varies throughout an archaeological dental calculus deposit.
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This aim was set to determine if layering in archaeological dental calculus could be detected, as it has been in clinical specimens. These clinical studies have found that dental calculus begins to form in the oldest dental plaque on a tooth's surface and builds up in layers (LeGeros 1974; Hayashi 1993; White 1997; Roberts-Harry and Clerehugh 2000). Due to calculus formation and composition being dependant on the oral environment, a change in conditions should hypothetically be reflected in the layers of calculus. This has important implications for archaeological research because it indicates that dental calculus composition could be used a timeline for health, diet or habitual activity. Consequently, the following hypotheses were tested.

- 1(a) Archaeological dental calculus contains discrete layers that can be detected.
- 1(b) The mineral composition of dental calculus varies between the layers of archaeological dental calculus.
- 1(c) The elemental composition of dental calculus varies between the layers of archaeological dental calculus.

Hypothesis 1(a) was tested using the first application of nano-computed tomography in archaeological dental calculus research. This allowed non-destructive analysis of the removed specimens of calculus. Hypothesis 1(b) and 1(c) were tested using micro-beam X-ray diffraction and energy dispersive X-ray analysis respectively. This required sample

preparation in the form of microtome sectioning and resin embedding, to expose a cross-sectional surface of the dental calculus.

3.2 Inter/Intra- Population Variation

Aim 2: Investigate if dental calculus external morphology and composition varies within and between archaeological populations.

This aim was developed to investigate both the interpopulation and intrapopulation differences in archaeological dental calculus. As such three Mediterranean populations were investigated for this study (see Chapter 3) and the following hypotheses were formulated.

- 2(a) Within and between each population, the external morphologies of dental calculus deposits are consistent.
- 2(b) Within and between each population, the mineral composition is consistent.
- 2(c) Within and between each population, the elemental composition is consistent.
- 2(d) The burial/interment environment of archaeological human remains has no effect on the external morphology, mineral or elemental composition.

Hypothesis 2(a) was tested using optical microscopy, nano-computed tomography and scanning electron microscopy to investigate the external physical features of the specimens. Hypothesis 2(b) and 2(c) were tested using powder X-ray diffraction and ICP-MS(Sol) respectively. The final hypothesis, 2(d) was tested by comparison of the results from each population, due to each one having a different post-mortem environment (see section 3.5).

3.3 Evidence of Periodontal Health

Aim 3: Investigate if the mineral and elemental composition of archaeological dental calculus be linked to periodontal health in human remains.

The third aim of this thesis was concerned with concomitant oral pathologies that can be present in an individual. Despite carious lesions and dental calculus requiring different conditions to develop, it has been found that both can be present in the same individual (Little et al. 1960; de Sousa et al. 2013). This suggests that periapical cavities, which can develop because of carious lesions, can also develop when calculus is present on the teeth (Roberts and Manchester 2010). In addition, the pus produced from periapical cavities has been found to be acidic, which is contrary to the alkaline conditions required for calculus accumulation (Bowen 1994; Nekoofar et al. 2009; Costolonga and Herzberg 2014).

In addition, gingivitis and periodontal disease has been found to be exacerbated by the presence of dental plaque, which can build up readily on the rough surface of dental calculus (Baehni and Takeuchi 2003; Kamath et al. 2014). The progression of gingivitis can cause inflammation of the gums due to plaque build-up and bleeding and develop to where the periodontal attachment and alveolar bone are compromised, and the tooth becomes loose in the socket (Obiechina 2011).

In addition, the symptom of oral bleeding from these dental diseases may influence dental calculus composition. The presence of blood in the oral environment, introduces haem into the local environment around ready-formed or accumulating dental calculus deposits. This may cause iron to be more readily incorporated into the elemental composition of dental calculus.

The presence of concomitant oral pathologies and may have an influence on dental calculus mineral and elemental (specifically iron) composition due to the different oral conditions that they need to develop or that they cause once present. For archaeology, this may be useful in understand the periodontal and oral health of individuals, when there is missing dental data. Therefore, this aim was outlined to test the influence that these pathologies that have on calculus mineral and elemental composition and the following hypotheses were tested.

- 3(a) Dental calculus can be concomitantly present with other oral pathologies in archaeological remains.
- 3(b) Dental calculus mineral composition differs for individuals who also exhibit dental caries and/or periapical cavities.
- 3(c) The presence of iron in dental calculus is related to the periodontal health of the individual.

The first hypothesis, 3(a), was tested by recording not only the dental calculus, but the additional dental pathologies present in the individual from the three populations studied. These results were then compared to the mineralogical compositional analysis to test hypothesis 3(b). Finally, by combining ICP-MS(Sol) analysis with measurements of alveolar recession, hypothesis 3(c) was tested.

3.4 Clinical Vs. Archaeological Dental Calculus

Aim 4: Demonstrate whether archaeological dental calculus is different to contemporary dental calculus, regarding features such as site-specificity; mineral and elemental composition; and physical structure.

The final aim of this research was concerned with establishing the similarities or differences between archaeological and clinical dental calculus. The literature review in Chapter 2 has highlighted some large voids in the research of archaeological dental calculus, which up to now have been filled by clinical data. The reliance on modern dental calculus analysis has the potential to be flawed when considering past populations. In times where diet, medicine and dental treatment was simpler the dental calculus that accumulated may have formed differently to now.

Consequently, this aim was established to test clinical literature against the results of a large compositional study of archaeological dental calculus and the following hypotheses were tested.

- 4(a) The physical characteristics of dental calculus from clinical literature, such as colour, surface morphology and site-specificity are true for archaeological dental calculus.
- 4(b) Archaeological dental calculus mineral composition is the same as clinical dental calculus.
- 4(c) Archaeological dental calculus elemental composition is the same as clinical dental calculus.

Hypotheses 4(a)-(c) were tested by comparing the results of this study to clinical literature in the discussion chapter of the thesis (Chapter 8).

CHAPTER 4: ARCHAEOLOGICAL MATERIAL

While the remainder of the body is utterly decomposed and resolved into the elements, the teeth continue entire, their enamel white and perfect.

- David Brewster 1830

4.1 Overview

The collections described in this section represent three populations, from which dental calculus samples have been taken, specifically for this research (see Figure 3.1.1). Regarding this, it is an inherent factor of archaeological research that sample selection is reliant on both availability and permission. Furthermore, for the nature of this specific research, it was not only permission to inspect and record that was required but also permission to remove specimens of dental calculus.



Figure 4.1.1 Map showing the locations of the archaeological collections of the Capuchin Catacombs (star), Cementeri Vell (square) and San Agustín (triangle) in relation to each other [Adapted by author from Google Maps (Retrieved 23rd September 2016)].

The following organisations and individuals gave permission for the archaeological collections to be recorded and sampled in this research. The curator, Dr Dario Piombino-Mascali and The Order of the Capuchin Friars kindly granted access to the mummies of

the Capuchin Catacombs, Palermo; and The Department of Sicilian Cultural Heritage gratefully permitted sampling. Sonia Cardona Ferrer, Jaume Castelló Guash, Miguel Ángel Riera Planells, The Formentera Council for Heritage and Culture and the Roman Catholic Diocese of Ibiza kindly granted access and sampling for the individuals of Cementeri Vell, Formentera. Universidad Autónoma de Madrid and Almudena García-Rubio gratefully provided dental calculus specimens and osteological information for the individuals from The Chapel of San Agustín, La Rioja.

4.2 The Capuchin Catacombs, Palermo, Sicily

The Capuchin Catacombs are in Palermo, on the Italian island of Sicily. The Catacombs are resting place to the mummified remains of individuals from the 16th to 20th centuries. They are situated below the church of Santa Maria della Pace, which is home to members of the Order of the Capuchin Friars. According to cataloguing carried out by the curator, Dr Dario Piombino-Mascali, the Catacombs contain 1252 individuals and 600 wooden coffins (Piombino-Mascali et al. 2011). Some of the coffins do not contain remains, although the exact numbers of empty and occupied coffins are not known (Piombino-Mascali et al. 2011).

4.2.1 Sicily: Geography and Geology

Sicily (37°28'57" N 14°09'51" E) is situated in the Central Mediterranean and is the largest island in Italy. The waters around the island of Sicily consist of the Mediterranean Sea to the south, the Ionian Sea to the East and the Tyrrhenian Sea to the north. The island is located southwest of the Italy's 'toe' and the minimum distance between Sicily and the Italian mainland is two miles (Figure 3.2.1). To the south of the island are the coasts of Tunisia and Libya in North Africa and to the northwest is the Italian island of Sardinia. The coastline of Sicily is dotted with multiple smaller islands and archipelagos.



Figure 4.2.1 Map showing the location of the city of Palermo (star) on the island of Sicily, situated southwest of the Italian mainland [Adapted by author from Google Maps (Retrieved 23rd September 2016)].

The island of Sicily is approximately 25,400 km² and the highest point of the island comes from Mount Etna, which reaches over 3300 m (Chester et al. 1985; Nesto and Di Savino 2013). The island consists of mountainous, hilly and flat regions of land. There are several river systems across the island, the main ones being the River Salso, River Simeto and River Belice that vary in size depending on the time of year. The capital of Sicily is Palermo, which is a coastal city on the northern side of the island (Figure 3.2.2).

Sicily experiences a subtropical Mediterranean climate with hot dry summers that average 22-38 °C and winters that are cool and rainy (Nesto and Di Savino 2013). This climate is ideal for the cultivation of agricultural crops. In 2010, Sicily's top agricultural products included oranges, lemons, olive oil, grapes, artichokes, tomatoes and durum wheat (ISTAT 2010).

The geology of Sicily is extremely varied and can be contributed to both long-term island formation and current volcanic activity (Venturella et al. 2004). To almost over-simplify, areas of the island where agriculture tends to be, have soil that is calcareous from sedimentary formation of the land from marine organisms and land folding. However, near Mount Etna and around the mountain range in the northeast of the island, the soil is derived from volcanic ash and is therefore metamorphic and igneous in composition.

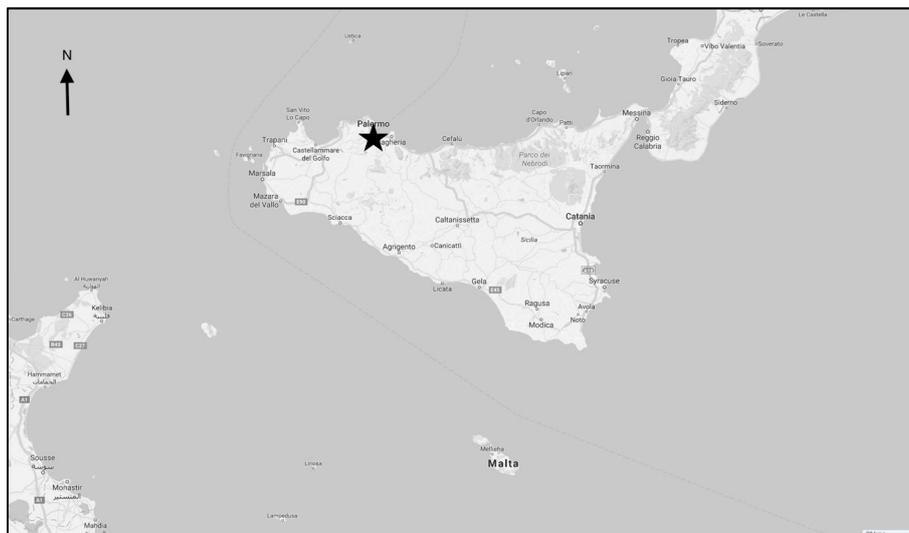


Figure 4.2.2 Map showing the location of the city of Palermo (star) and the distribution of other major towns and cities on the island of Sicily, situated southwest of the Italian mainland [Adapted by author from Google Maps (Retrieved 23rd September 2016)].

4.2.2 The Capuchin Catacombs: History

The Order of the Capuchin Friars was established during the sixteenth century as a catholic brotherhood in central Italy (M'Creie 1827). The Order expanded to different regions of Italy and a group of friars settled in Palermo in 1534 and were granted land (outside the then city walls) (Farella 1982; Piombino-Mascali 2015). With this land, permission was granted to build a convent adjacent to the existing Norman chapel, Santa Maria della Pace (Figure 3.2.3) (Piombino-Mascali et al. 2010; Piombino-Mascali 2015).

In the late sixteenth century, the Order commissioned the building of the convent as well as the restoration of the church (Farella 1982). During the process of refurbishment and expansion, a section of the existing burial ground was opened (Farella 1982; Piombino-Mascali 2015). In the burial pit, forty-five individuals of religious standing were found to have naturally mummified while buried (Farella 1982; Piombino-Mascali 2015). It was the opinion of the friars at the time that God had preserved the individuals as a sign, that even after death, their bodies were incorruptible (Farella 1982; Piombino-Mascali 2015).



Figure 4.2.3 Photograph of the main entrance to Santa Maria della Pace church in Palermo, Sicily as it currently stands. The building to the left of the church consists of part of the convent of the Order of the Capuchin Friars and the tourist entrance to the Capuchin Catacombs [Adapted by author from Google Maps (Retrieved 25th May 2016)].

The discovered forty individuals were relocated into a newly created room, under the altar in the church (Farella 1982; Piombino-Mascali 2015). This was the establishment of the underground resting place now known as the Capuchin Catacombs (Farella 1982). In 1599, a friar, by the name of Silvestro di Gubbio passed away and was the first person to be purposely interred in the subterranean crypt (Farella 1982). From then on, friars from Palermo were interred in this way, beneath the Church of Santa Maria della Pace (Farella 1982). A chapel was constructed underground and subsequent tunnels and rooms were expanded to make space for the increasing number of individuals who were laid to rest there (Farella 1982).

By 1823 the building works on the Catacombs had finished and the subterranean cemetery was an established resting place for not only friars but also local benefactors (Farella 1982). For these benefactors to be granted acceptance in this holy cemetery, a burial fee was introduced (Farella 1982). Because of this tariff, it is known that many non-clergy mummified in the Catacombs, were from the Palermo aristocracy (Farella 1982). The popularity of mummification increased during the eighteenth and nineteenth centuries, which led to a substantial increase in capacity (Farella 1982). During this time,

assignment of specific corridors for different groups of individuals was established (i.e. Women, Men, Priests, Professionals etc.) (Figure 3.2.4)²⁴ (Farella 1982).

In 1880, due to a prohibition regarding the drainage of fluids from a human cadaver, the mummification of individuals in the Catacombs was ceased (Farella 1982; Piombino-Mascoli 2015). Despite this, within Palermo, there was a continued reverence for the site. Some embalmed individuals in coffins were admitted to the Catacombs as a temporary resting place before burial (Piombino-Mascoli 2015). After the cessation, two examples of individuals that were permanently admitted to rest in the Catacombs were Giovanni Paterniti, United States Vice-consul (died 1911) and Rosalia Lombardo, known as “The Sicilian Sleeping Beauty” (died 1920) (Farella 1982; Piombino-Mascoli 2015). In addition to the increased capacity of the Catacombs from 1599 to 1823, there was a development in the process of mummification.

²⁴ Separate diagrams showing the layout and positions of the mummified remains in each corridor can be found in *Supplementary Material: Appendix C*.

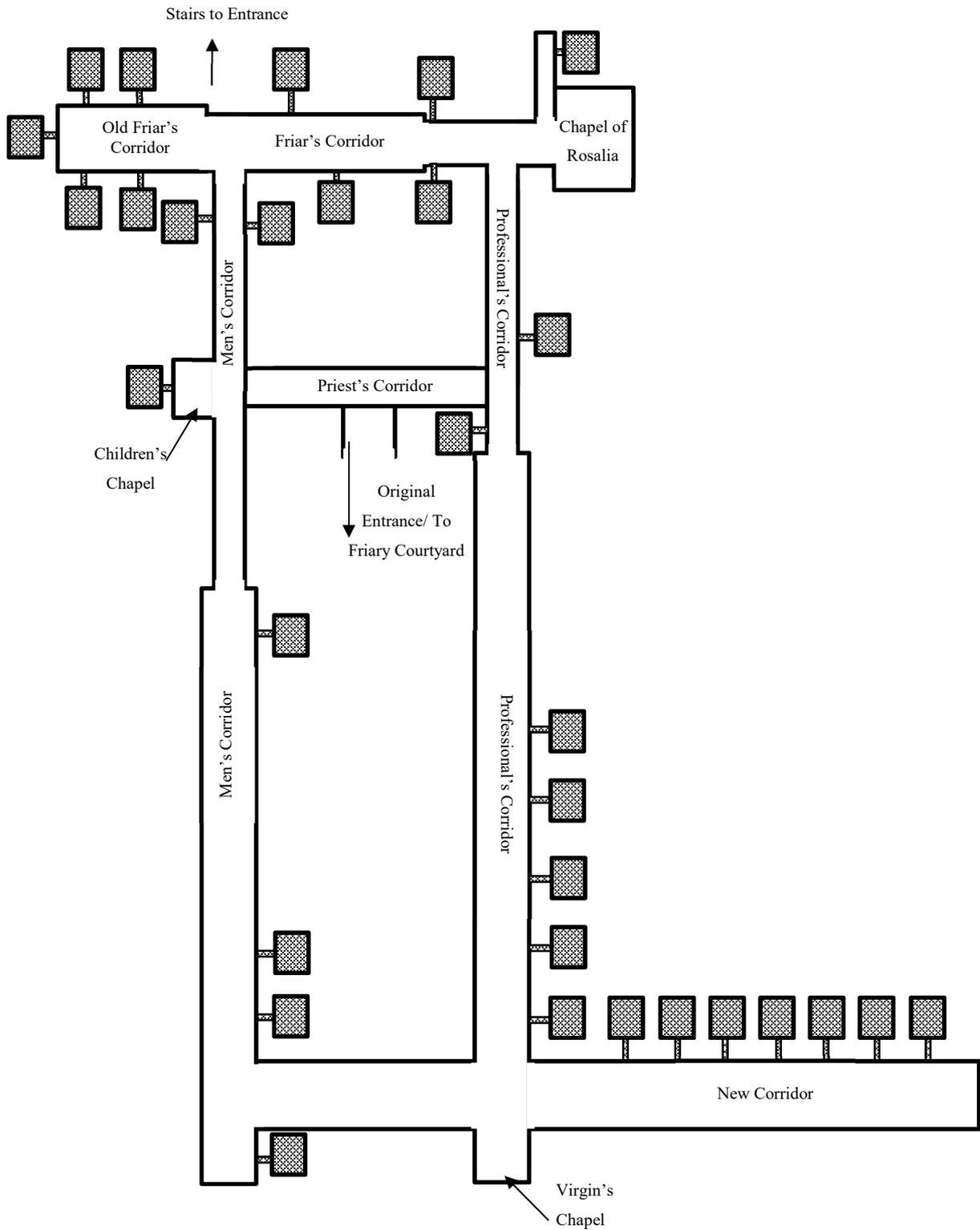


Figure 4.2.4 Overall plan of the Capuchin Catacombs (not to scale) [Compiled by author (November 2016)].

When the friars opened the burial ground in 1599 and found the previously buried individuals preserved, they explained the phenomenon with their faith. The process that caused this preservation was natural mummification caused by environmental factors. Sledzik and Micozzi (1996) describe three categories of mummification; natural, intentional and artificial. In the Capuchin Catacombs, all three types of mummified remains can be found (Piombino-Mascali et al. 2009; Piombino-Mascali 2015).

When remains are mummified naturally, there is no human influence on the process and it results from one or more factors, such as a dry, hot environment, a cold environment or an anaerobic environment (Sledzik and Micozzi 1996). The basis of the process is that bacterial purification²⁵ is outweighed by dehydration and desiccation of the tissue, thus stopping the digestion of the soft tissue and preserving it (Hayman and Oxenham 2016). For the Capuchin friars in Palermo the mummification of the forty-five individuals was likely caused by multiple individuals laid one top of one another in an underground tomb combined with dry burial conditions.

Over time, subsequent individuals that were laid to rest in the underground passages of the church were not mummified because of burial. Instead, deceased individuals were intentionally mummified in the underground rooms below Santa Maria della Pace. Intentional mummification involves the exploitation of conditions that cause natural mummification. In the Capuchin Catacombs, this involved the underground environment of dry air in combination with specially designed draining rooms to promote dehydration and desiccation of an individual's soft tissue.

The earliest individuals to be mummified were unclothed and placed within the tuff draining rooms shortly after death (Aufderheide 2003). In these rooms, corpses were placed on horizontal terracotta pipes to allow fluids to drain away from the body. The draining rooms were sealed for the period of about a year to allow the corpse to desiccate, after which the individuals were transferred above ground and washed with vinegar (Panzer et al. 2010; Musshoff et al. 2013; Piñar et al. 2014). The individuals were then dressed in clothes that were presumably left for them by their family. It has been found

²⁵ Bacterial putrefaction is the digestion of the soft tissue by the bacteria present in the human body (Tsokos 2005).

that for some individuals, straw, tow or wool was inserted into the chest cavity or clothing to shape the body where the dried soft tissue had not maintained its structure (Piombino-Mascali et al. 2012; Piñar et al. 2014).

As mummification became ever more popular for the wealthy residents of Palermo, during the 19th Century, it could be assumed that the intentional method of mummification became too slow. Artificial mummification was established where cadavers were injected with solutions of arsenic or mercury (Panzer et al. 2010). It is also reported by Piombino-Mascali et al. (2010) that during periods of disease epidemic in the city, bodies were treated with lime to promote mummification to speed up the process from death to interment in the Catacombs.

Two individuals to have been artificially mummified are the Vice-consul Paterniti and Rosalia Lombardo, the individuals interred after the closure of the Catacombs. The embalmer Alfredo Salafia, who is one of the earliest examples of embalmers who used a formaldehyde-based preservation solution, preserved both individuals (Piombino-Mascali et al. 2009).

4.2.3 The Capuchin Catacombs: Present Day

Today the Capuchin Catacombs are still under the care of the Order of the Capuchin Friars of Palermo. The church of Santa Maria della Pace is a catholic place of worship for the local community and the adjacent convent is still home to brothers of the Order. The Catacombs themselves are now open to visitors for a small fee (€3.00/May 2016).

The Catacombs became a popular tourist attraction in the 18th Century (D. Piombino-Mascali, per comms. May 2014). During this time, there were no walkways or fences to prevent visitors from touching the mummified individuals. Considering the dates on the graffiti, it seems that after World War II there was the unethical practice carried out by some visitors of taking teeth and finger bones and even skulls as souvenirs (D. Piombino-Mascali per comms. May 2014). It is also unclear how much disturbance there has been to the individuals in terms of relocation.

Since the late 1970's, the Catacombs have undergone changes to minimise the possibility of contact between the living and the deceased (Farella 1982). These additions may also have been implemented for health and safety reasons. Less than ten years ago, a glass

walkway with railings either side was installed in the oldest corridors, which lead into ramps or steps. In the remaining corridors, 7 ft. fences, which were installed during the second half of the 20th century, are in front of the wall niches and coffins that line the corridors (Farella 1982). There has, at some point, been the installation of electric lighting rather than candles that would have originally lit the underground cemetery. There is CCTV to enable viewing of the corridors, which is watched by the staff and friars in the ticket booth at the entrance. Additionally, a lift has been installed for anyone unable to descend from the entrance via the stairs.

In the corridors, mummified individuals are positioned in two ways, either hung vertically in wall niches or lying horizontally on shelves. Vertical wall niches are situated at multiple levels with many at floor height. The remains are secured in position by string or wire, hanging from a nail in the wall. Some individuals have vertical wooden stakes along their backs, supporting the weight of the remains in their standing position.

There are also coffins present in the corridors, many are stacked at floor height and others are on shelving. Most of the coffins are designed with lids that are secured closed, however others have been designed to allow viewing of the deceased. Where remains are visible, the coffin has either an open top or open side with some sides consisting of wire mesh, rather than being fully open (Figure 3.2.5). There are many glass-panelled coffins that have been designed contemporarily for loved ones to view the deceased; although some of these glass panels are broken or missing.



Figure 4.2.5 Photographs of stacked coffins in the Capuchin Catacombs showing open sides, with (right) and without (left) hexagonal mesh wire, for viewing the deceased [Photograph taken by author (May 2014)].

Mummified individuals in the Catacombs are dressed in clothing that is assumed to be contemporary to the time of their death. Friars typically are dressed in brown robes with loose rope belts and priests in vestments', some with headdresses. Individuals in the Men's Corridor are dressed in trousers and shirts some with jackets or hats/hoods. The women are dressed in ankle-length dresses some with shawls or head coverings, such as bonnets or headscarves. Many of the women have gloved hands and wear long socks/stockings and shoes. There may be the possibility that individuals have been redressed since their original placement in the Catacombs. In the past, this could have occurred by either by family members or caretakers (Farella 1982; D. Piombino-Mascali, per comms. May 2014).

The presence of clothing causes limitations when observing the remains themselves as most of the body is covered. Most clothing is in a degraded condition with dust covering it and environmental and insect damage destroying the integrity of the fabrics. Due to these conditions as well as offering respect to the individuals, the clothing is unable to be moved away to access any covered remains.

The most accessible body parts are the skull and hands; and in several robed individuals (i.e. friars), the lower extremities can be seen. On inspection of the skulls, there is a varying amount of soft tissue preservation between individuals (Figure 3.2.6). Some individuals have extensive soft tissue remaining over the face and neck, while others are partially or fully skeletonised. The extent of desiccated soft tissue over the anterior skull causes difficulties when inspecting the dentition of some individuals. Depending on the facial expression of the mummified remains as well as the internal soft tissue of the tongue, dental examination is not always possible. Some individuals are missing their mandibles, possibly due to the soft tissue degrading over time causing the mandible to become detached from the cranium.



Figure 4.2.6 Photographs of mummified individuals in the Capuchin Catacombs showing variable preservation of soft tissue on the skull. Left: individual shows extensive soft tissue covering the dentition. Right: individual shows a fully skeletonised skull allowing inspection of most of the dentition [Photographs taken by author (May 2014)].

4.2.4 Research Sampling

This unique collection provided several challenges for both recording and sampling. These challenges were encountered in both the environment that the remains were located and the nature of the remains themselves. Consequently, these challenges affected the sampling protocol that was undertaken.

Prior to recording, the corridors were inspected to determine which mummies could be safely accessed for recording and sampling. Due to a large proportion of the Catacombs having fencing to separate the remains from visitors, these areas were not accessible. Similarly remains that were displayed in wall niches above floor height were inaccessible and coffins that were stacked above head height were also too high to safely access. Consequently, there were 206 individuals who had accessible remains.

From this sample of 206 individuals, 126 individuals could be recorded. This was due to the nature of the remains. While many individuals had become skeletonised in exposed

areas such as the skull, hands and feet, a proportion of mummies had retained desiccated soft tissue that restricted dental recording (see section 3.2.4 and Figure 3.2.6).

As mentioned earlier, the mummies have suffered from post-mortem damage and vandalism, particularly the loss of anterior dentition. These teeth, which only have one root, are susceptible to post-mortem loss or disassociation from the alveolar socket. The added feature of to the upright positioning of many mummies in the Catacombs may have contributed to the upper anterior dentition falling from the skull over the years. The additional possibility of visitors removing teeth as souvenirs has resulted in few incisor or canine teeth remaining. The individual that tended to have these teeth in-situ, also had copious amounts of soft tissue, inhibiting recording of the teeth.

For the remains that were recorded and had dentition present, to observe the lingual dentition a torch and dental mirror was utilised. Consequently, it was decided that sampling calculus from these tooth surfaces would be too risky in terms of specimen loss. Considering the challenges posed, for individuals that exhibited dental calculus, the primary locations that were present and accessible for sampling were limited. Therefore, specimens were primarily removed from buccal surfaces of posterior teeth. Graphs, summarising the specimens collected in terms of dental quadrant, tooth and surface are included in section 6.2.

Additionally, due to the uniqueness of the collection and the potential for future dental calculus analysis advancement, it was agreed between the author, the curator and the *Sovrintendente Della Cultura e del Patrimonio*²⁶ that only individuals with more than one surface affected by dental calculus would be sampled. Additionally, any individuals sampled would not have all dental calculus removed with ideally only one specimen taken per mummy.

In addition to the articulated mummies that were recorded and sampled, a small group of disarticulated partially mummified skulls were included in the population sample. These were piled on the floor in the old friar's corridor and the exact origin of their disarticulation was unknown (Figure 3.2.7). It was explained by the curator that the

²⁶ Superintendent of Culture and Heritage of Sicily

shelving on which the skulls had been located, had suffered water damage and consequently, the skulls had been hurriedly moved to stop them falling. To afford these remains more dignity, during the fieldwork, these skulls were repositioned in a more respectful manner. With the permission of the curator, these skulls were included in the study. In total, eight of these crania and five mandibles were sampled for dental calculus.



Figure 4.2.7 Photographs of the disarticulated skulls and mandibles located in the Old Friar's corridor. Prior to recording, the remains were found piled on the floor where the wet newspaper can be seen. On a subsequent visit to the Catacombs in May 2016, this area had been fully cleaned and the skulls removed to another location [Photographs taken by author (May 2014)].

4.3 Cementeri Vell, Formentera, Spain

Cementeri Vell is in the village of St. Francesc on the small island of Formentera. From around 1757 Cementeri Vell was the only used cemetery on the island until the new and current cemetery was built in the mid 1930's (Ferrer 2004; Ferrer 2012). After the new cemetery was built, Cementeri Vell was gradually abandoned and was completely unused by 1940 (Ferrer; 2004; Ferrer 2012). The cemetery consists of five small chapels, unmarked graves and two ossuaries and since 2009 has been an approved cultural heritage site in Formentera.

4.3.1 Formentera: Geography and Geology

Formentera (38°41'50" N 1°27'11" E), along with Eivissa²⁷ and many other small rocks, make up the Pityusic Islands. This archipelago is part of the province of the Balearic Islands, which includes the Pityusic and Gymnesic Islands (Mallorca and Menorca). The islands are situated in the Western Mediterranean to the southeast of the Spanish mainland (Figure 3.3.1).



Figure 4.3.1 Map showing the location of the island of Formentera (square) amongst the Balearic Islands in the Western Mediterranean, off the southeast coast of Spain [Adapted by author from Google Maps (Retrieved 23rd September 2016)].

The island of Formentera is approximately 82 km² with a maximum height above sea level of approximately 200 m (Kuhbier 1984; Cuerda Barceló 1984). It consists of a long, narrow and flat portion of land that joins two hills (*La Mola* and *Cap de Barbaria*). The largest settlement, Sant Francesc-Xavier, is situated on the western half of the island, south of the port (*La Savina*) (Figure 3.3.2).

The island experiences a typical Mediterranean climate, which is greatly influenced by exposure of the land to the surrounding sea and wind (Vallès 1984). There are typically

²⁷ The inhabitants of the island speak both Spanish and Catalan, with the latter being preferred. In each language, the name of the island differs, with 'Ibiza' used in Spanish and 'Eivissa' used in Catalan.

long hot summers with a strong intensity of sunlight and subsequently high rates of evaporation. The island has no river network and rainwater is quickly filtered through the soil or evaporated (Vallès 1984; Rangheard 1984). The landmass that makes up Formentera is exclusively sedimentary, calcareous rock (Rangheard 1984).

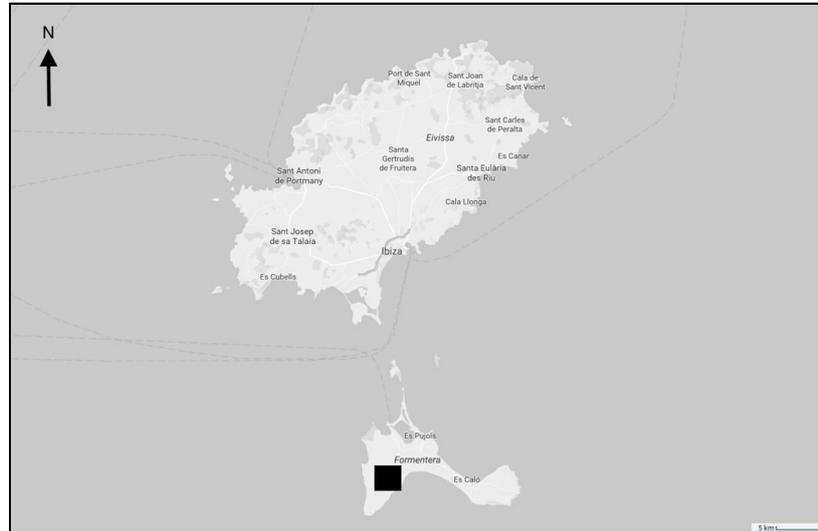


Figure 4.3.2 Map showing the location of the island of Formentera (square) in relation to its larger neighbour Eivissa, in the Western Mediterranean [Adapted by author from Google Maps (Retrieved 23rd September 2016)].



Figure 4.3.3 Map showing the location of Cementeri Vell in the village of Sant Francesc de Xavier on the island of Formentera, Spain [Adapted by author from Google Maps (Retrieved 23rd September 2016)].

4.3.2 Cementeri Vell: History

Prior to the commissioning of Cementeri Vell, the first centralised place of burial on Formentera was Tanca Vella chapel (Wilson 2014). This small structure was constructed to accommodate deceased island inhabitants, between the years of 1369 and 1757 (Wilson 2014). Between that time, the population size of the island increased and Tanca Vella chapel was no longer large enough to accept any more individuals.

After the opening of the newer Cementeri Vell, inhabitants from all over the island were buried in the cemetery (Wilson 2014). For few decades, this was the only designated burial site on the island and was linked to the newly constructed church of Sant Francesc Xavier (constructed 1726-1738) which is still used today by the local community (Wilson 2014).

In 1839, the cemetery was extended and the large pit ossuary, in the eastern corner was constructed (Ferrer 2004; Ferrer 2012). Consequently, previous burials were excavated and redeposited in the ossuary, to make burial plots available for more recently deceased individuals. It is assumed that this practice of burial rotation to the ossuary continued until the closure of the cemetery in 1940 (Ferrer 2012). The cemetery use was ceased due to the construction of a newer and larger cemetery, San Fernando which was opened in 1938 and is still in use today (Ferrer 2012, Wilson 2014).

4.3.3 Cementeri Vell: Present Day

Since its cessation of use in 1940, Cementeri Vell has suffered from environmental degradation and disrepair (Figure 3.3.4). In 2012, during building work to restore the buildings and walls, the large ossuary containing hundreds of skeletal elements was exposed in the eastern corner of the cemetery (Figure 3.3.5). To ensure that the skeletal remains were properly handled and protected from further exposure, the Consell Insular de Formentera²⁸ requested additional assistance from forensic anthropologists and archaeologists (Wilson 2014).

²⁸ The Island Council of Formentera, which is responsible for the heritage and cultural on Formentera.



Figure 4.3.4 Photograph showing the western boundary of Cementeri Vell, including three of the chapels of rest in their degraded state of wear [Photograph taken by author (June 2014)].

In May 2012, Dr Nicholas Márquez-Grant and a team were permitted by the Consell Insular de Formentera and the Roman Catholic Diocese of Ibiza to systematically collect, excavate and record the exposed human remains (Figure 3.3.5). This work was undertaken to discover the extent of the human remains present in the exposed ossuary and to collect anthropological information that could contribute to the past population knowledge of the island (Wilson 2014).

During the excavation, approximately a fifth of the ossuary was stratigraphically excavated and recorded, resulting in a large collection of disarticulated skeletal elements (Figure 3.3.6). The post-cranial bones were documented during the excavation to record the count of each element present, age and sex estimations, disease or infections present and trauma. At the close of the 2012 excavation season, the excavated remains were packaged and stored in one of the chapels of rest within the cemetery.



Figure 4.3.5 Photograph of the ossuary, which held the remains of the individuals, excavated. The bones still in the ossuary are exposed to the elements and are suffering preservation issues [Photograph taken by author (June 2014)].



Figure 4.3.6 Photograph of the ossuary, showing the section that was excavated, by Dr Márquez-Grant and team in May 2012 [Photograph taken by author (June 2014)].

In June 2014, a second season of fieldwork was organised to complete the documentation of the excavated human remains. During this time, the crania and dentition were recorded for minimum number of individuals, age and sex estimation, disease and pathology and

trauma. In addition, permission was granted to remove specimens of dental calculus for subsequent analysis at Cranfield Forensic Institute.

Since the two seasons of fieldwork, the excavated remains have now been replaced in the ossuary from which they were removed. In addition, a breathable membrane has been installed to cover the remains, to protect them from further environmental exposure. The cemetery has undergone the planned restoration to preserve the buildings and walls that had fallen into disrepair (Noudiari.es (2014)). Following this work, in April 2015, the cemetery was reopened to the public as a site of local heritage (Noudiari.es (2015)).

4.3.4 Research Sampling

The dental calculus samples from this collection were recorded and collected in June 2014, during overseas fieldwork at Cementeri Vell, St. Franscec. Permission to record and sample the remains was granted by the Consell Insular de Formentera and the Church of St. Francesc de Xavier. The individuals recorded and sampled were excavated from the ossuary in June 2012 by Dr Márquez-Grant and team and make up approximately 10% of the individuals contained in the ossuary.

The sample from this collection is based on the excavated remains from the ossuary and the remains were fragmented and commingled. Unlike the Capuchin Catacombs sample, there was no selection of individuals required due to accessibility. There were however protocols taken to ensure the number of individuals that were sampled was known based on the fragments/teeth present.

Samples were taken from crania, mandibles and loose teeth that could be determined to be separate individuals. The commingled remains from each context were laid out and checked to determine if fragments could be associated with each other. All crania with dental calculus present were recorded and sampled. Mandibles, whole or fragmented, with dental calculus, were only recorded if the recorded and sampled crania could be determined to be from a different individual. This was done by inspecting the mandibular condyles for association with mandibular fossa of the crania and occlusion of the upper and lower teeth.

To maximise the sample, loose teeth were recorded and sampled if they could be determined to be from separate individuals to the crania already sampled. Firstly, the

loose teeth were inspected for the presence of dental calculus and if deposits were present, were put to one side. These teeth were then identified according to tooth type, maxillary or mandibular and left or right.

Following the identification of the teeth, they were compared against skulls already recorded and sampled to determine if the loose tooth could potentially be from those individuals. For example, if there was a loose left maxillary first molar (UL M1), each cranium was checked to determine if that tooth was present or missing. Skulls with the same UL M1 tooth present were ruled out, as duplicate teeth would not be possible in the same individual. Skulls without an UL M1 present, had to be either confirmed to be the same individual as the loose tooth, or excluded as a different individual. This was done by carefully inserting the loose tooth into the relevant socket, if the alveolar bone was present to do so. Additional checks included comparing the wear and taphonomy of the loose tooth with the present socketed teeth of the skull.

When all the loose teeth had been checked against the recorded and sampled skulls, a final decision was made about which teeth were sampled. Even if all the loose teeth could be determined to be from different individuals from skulls in that zone, between them it was more difficult. To maximise the number of loose teeth sampled, multiples of the same teeth were used when present and the same criteria as the skull comparison were used. Adjacent teeth were checked for wear facets and wear patterns, tooth size and taphonomy were also used. This protocol allowed for the recording and sampled of sixteen extra teeth, which increased the number of dental calculus sampled by 90%.

4.4 The Chapel of San Agustín, La Rioja, Spain

The chapel of San Agustín is one of three chapels that were converted from the original naves of the church of San Millán. The chapel sites are in the historic monastic complex of Yuso in San Millán de la Cogolla which has been a World Heritage Site since 1997 (García-Rubio et al. 2014). The chapel is documented as being the resting place of the Benedictine monks that inhabited the monastery in the 17th and 18th centuries (García-Rubio et al. 2014).

4.4.1 La Rioja, Spain: Geography and Geology

La Rioja (42°17'50" N 2°36'0" W) is a province in Northern Spain that is bordered by Basque country to the northwest; the provinces of Navarra and Aragón to the north and east and the province of Castilla y León to the south and west. The province sits northeast of Spain's capital, Madrid and south west of the national border with France (Figure 3.4.1).



Figure 4.4.1 Map showing the location of the province of La Rioja (triangle) in Northern Spain [Adapted by author from Google Maps (Retrieved 23rd September 2016)].

The province of La Rioja is approximately 5 km² and consist of mountainous terrain to the south, highlands to the northwest and lowlands to the east and southeast (Figure 3.4.2). The mountain range in the south is named *La Tierra de Cameros* and has numerous river networks. Along the northern border is the River Ebro and it is along the river that most inhabitants are situated (FOLR 2016). The capital of La Rioja is Logroño, situated on the northern border of the province.

La Rioja experiences a continental Mediterranean climate with winter and spring months being cold and windy and summer and autumn months being moderately hot (FOLR 2016). This variable climate allows for the cultivation of produce such as wheat, barley, asparagus, peppers and vines, especially in the lowland regions (FOLR 2016). The province is also suitable for the grazing of livestock, with sheep being particularly suited

to the more mountainous areas (FOLR 2016). The soil geology of La Rioja is primarily calcareous-clay and the lowland clay has a high iron content which makes the soil red (Rabe 2015; Rojo-Smith 2016).

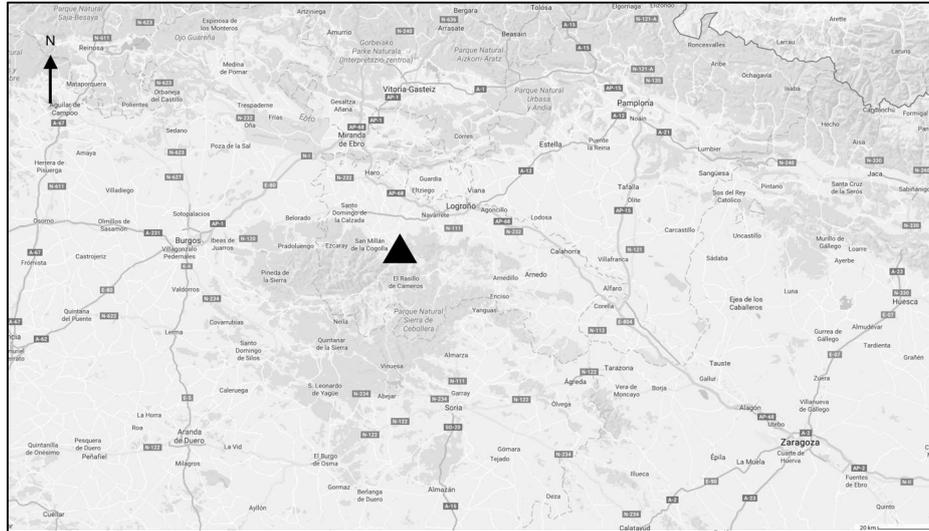


Figure 4.4.2 Map showing the location of the Chapel of San Agustín in the village of San Millán de la Cogolla (triangle) in the province of La Rioja, Spain [Adapted by author from Google Maps by author (Retrieved 23rd September 2016)].

4.4.2 The Chapel of San Agustín: History

San Millán de la Cogolla in the province of La Rioja has two monasteries, Yuso and Suso²⁹. The area became a popular pilgrimage destination because of the canonisation of the local Saint Emilianus who had lived at the original monastery, Suso, in the 6th century (UNESCO). Because of this, the larger monastery of Yuso was built in the 16th century and is still in use today by The Augustine Recollect friars (San Millán 2001). The Augustinian monks of the older Suso monastery lived an eremitic lifestyle, however the relocation of the Order to the larger Yuso monastery transformed the lifestyle of the monks to a cenobitic community and the monks adopted the rules of the Augustine Recollects (UNESCO). These rules were less strict than the traditional Augustinian Order and valued community with non-religious persons from the local communities

²⁹ Yuso means ‘upper’ and Suso means ‘lower’, which is in relation to the locations of the monasteries on the mountain that they are built.

(UNESCO). This would have potentially increased the variability in the monk's diets due to increased contact with local farmers and suppliers, also increasing the variation of dental pathologies present. In addition, this variation would potentially be seasonal due to crop availability which may decrease or increase the amount of dental calculus present depending on the nature of the foods consumed (see section 2.3 for formation influences).

The Church in the monastery Yuso was constructed with three naves and the two side naves were used as chapels (García-Rubio et al. 2014). The chapel of San Agustín, the left lateral nave, was used as a burial place for Benedictine friars from the monastery (SMYSA 2014) (see Figure 3.4.3). In a document from 1690, detailing the sale of the chapel to Fray Benito de Salazar, Bishop of Barcelona and former abbot of the monastery, there was burial space in the chapel that was to be reserved for Benito de Salazar and his nephews (SMYSA 2014). It is also detailed that the remaining burial space would be used for monks at the monastery (SMYSA 2014; García-Rubio et al. 2014). However, it is believed that the practice of interring friars in this chapel was already underway prior to this document, possibly from the time of construction of the church (SMYSA 2014).

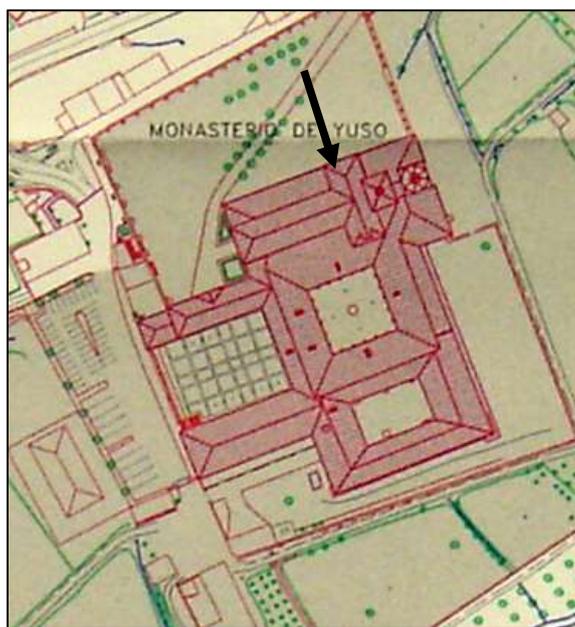


Figure 4.4.3 Map showing the location of the Chapel of San Agustín, as indicated by the arrow (black), within the church of the Yuso monastery in San Millán de la Cogolla [Adapted by author from http://whc.unesco.org/en/list/805/multiple=1&unique_number=951 (Retrieved 17th March 2017)].

4.4.3 The Chapel of San Agustín: Present Day

The monasteries of Yuso and Suso in San Millán de la Cogolla became a World Heritage Site on the 4th December 1997 (UNESCO). The monasteries are known as “cradle of the language” due to their history as the birthplace of the modern Spanish language (UNESCO; San Millán 2001). The human remains in this sample were excavated from one of the chapels, San Agustín, inside the Church of the monastery Yuso (Figure 3.4.4) (SMYSA 2014). This was carried out as part of a large restoration project between 2007-2010, funded by the Foundation San Millán, Fundación Caja Madrid and The Order of Augustinian Recollects (Morate et al. 2008).



Figure 4.4.4 Photograph of the church in the monastery of Yuso, San Millán de la Cogolla where the chapel of San Agustín is located [Image credit: http://lugaresconhistoria.com/monasterios-yusosuso/monasterio_de_yuso_horrapics (Retrieved 17th March 2017)].

The excavation was carried out throughout 2009 and in total fifty-eight tombs were documented within the chapel. Most of the graves were individual burials, however some also contained commingled remains, consisting of incomplete skeletons (Figure 3.4.5 and 3.4.6). Of the fifty-eight graves recorded, thirty-four which contained remains that were in a better state of preservation, were lifted and transferred to the Universidad Autónoma de Madrid where osteological analysis was conducted (SMYSA 2014). All the individuals analysed were estimated to be male, which coincides with the historical documentation that it was a burial place for monks (SMYSA 2014). In September 2014, Cranfield

University was given permission to analyse dental calculus specimens from this monastic population.



Figure 4.4.5 Photographs of the excavated burial in San Agustín. Most individuals were found in the supine position in an individual grave cut, however grave edges overlapped with adjacent burials [Image Credit: Almudena García-Rubio (Universidad Autónoma de Madrid)].



Figure 4.4.6 Photograph of an excavated commingled burial in San Agustín [Image Credit: Almudena García-Rubio (Universidad Autónoma de Madrid)].

4.4.4 Research Sampling

The dental calculus samples from this collection were recorded and collected in March 2015, by Almudena García-Rubio at the Universidad Autónoma de Madrid. Permission to record and sample the remains was granted by the Universidad Autónoma de Madrid, as custodians of the remains.

The sample used from this collection is based on the excavated remains from the chapel. The remains consisted of discrete burials and were reasonably complete with minimal taphonomic degradation. Individuals were sampled based on the presence of dental calculus, provided it had not been damaged post-mortem (see Figure 2.5.2). All photography, recording and sampling of this population was performed by Almudena García-Rubio. Samples were taken from tooth surfaces that exhibited the largest deposits, primarily the posterior buccal and anterior lingual surfaces.

4.5 Rationale for Dental Calculus Analysis

All three of the populations described above are from Mediterranean locations, with one mainland and two island populations. The San Agustín individuals represented a purely male monastic population (16th - 18th century), while Cementeri Vell included both male and female individuals who are assumed to be local inhabitants of Formentera and were not from a religious order (18th - 20th century)³⁰. Furthermore, the Capuchin Catacombs sample included both monastic male individuals and male and female civilian individuals from the upper classes of Palermo (16th - 18th century). Despite these time-period and demographic differences, the rationale for choosing these populations was based on the expected consistency of diet and oral health among the populations.

The individuals from the Capuchin Catacombs have been assumed to be local inhabitants from Palermo. Due to Sicily being a self-sufficient island, it was expected that they consumed a reasonably similar diet, whether monastic or upper-class civilian (Piombino-

³⁰ Although male and female individuals were identified using sex estimation criteria, this population had many unknown sex individuals. In addition, the individuals who were estimated as female, did not exhibit any dental calculus deposits (see section 7.2).

Mascali per comms. May 2014). This is the same for the civilian individuals from Cementeri Vell who would presumably have had access to the same produce from Formentera and its neighbour, Eivissa. In addition, the island nature of these populations, implies that the individuals would have had similar knowledge, practices and access to oral hygiene, medicine and social habits. In terms of the mainland population of San Agustín, these individuals were exclusively monastic and as such are assumed to have consumed a similar diet to each other.

In contrast, considerable differences between the populations were the burial environments that the remains (and consequently the dental calculus deposits) were subjected to. In the Capuchin Catacombs, individuals were mummified and displayed within the Catacombs without any soil burial occurring. Contrastingly, both the Cementeri Vell and San Agustín samples had been excavated, although one represents a commingled sample, with secondary deposition and the other represents single interments. The differences in post-mortem environment were of interest to this study.

Currently there is an absence of literature regarding the post-mortem changes in dental calculus morphology, mineral or elemental composition. As such, one of the research aims of this study was to determine differences between clinical calculus literature and archaeological calculus results in terms of physical characterisation, and composition (mineral and elemental) (see Chapter 4). The analysis of three types of archaeological populations, mummified, commingled with secondary deposition and individual interments, was important to be able to consider if there were differences between clinical and archaeological calculus. If only buried remains had been analysed, any compositional or physical differences between the specimens and the literature might have been only attributed to soil contact. As such, the inclusion of mummified remains from the Capuchin Catacombs, where burial had not occurred, allows for comparison to the buried archaeological remains, as well an archaeological to clinical comparison (see section 4.2).

4.6 Chapter Summary

This chapter has outlined the origins of the populations that were used for this research. It has also explained the sampling procedure undertaken and the rationale for choosing

these specific samples. As mentioned in the preceding section, the rationale was closely connected to the research aims of this study and these are outlined in the next chapter.

CHAPTER 5: ANALYTICAL TECHNIQUES

...the Newtonian method of deductive reasoning, combined with elaborate experimental verification, which has led to all the great triumphs of scientific research.

- William Carpenter 1874

5.1 Overview

This study has used a range of complementary analytical techniques to analyse the physical characteristics and composition of archaeological dental calculus. These were divided into three sub-groups, imaging, mineralogical and elemental techniques and were used in a non-destructive to destructive approach. The imaging techniques consisted of optical microscopy, scanning electron microscopy and the novel use of nano-computed tomography. Following this, the mineral composition was determined using powder X-ray diffraction and micro-beam X-ray diffraction. The elemental composition was investigated using energy-dispersive X-ray analysis during the SEM imaging as well as by solution inductively-coupled plasma mass spectrometry.

This chapter provides a brief overview of each technique and the includes references to the previous use of each technique in dental calculus analysis, from archaeology and dentistry. In addition, the limitations of each technique are considered in terms of data collection and analysis.

5.2 Imaging Techniques

5.2.1 Optical Microscopy

Optical microscopy relies on visible light and a series of lenses to allow the magnification of an object (Figure 5.2.1) (Sluder and Nordberg 2007; Murphy and Davidson 2012). Digital optical microscopy also allows the capture of images with an attached digital camera (Sluder and Nordberg 2007). The adjustment of the focal length of the lenses, allows the image of the object to be focused. In addition, the aperture can be adjusted to vary the amount of light being shone on the target.

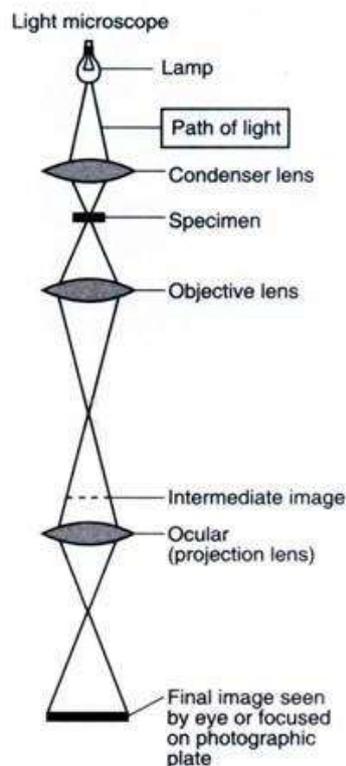


Figure 5.2.1 Diagram showing a simplified lens set-up in an optical microscopy that allows light to be focused to view specimen at a higher magnification [Image credit: <http://www.biologydiscussion.com/microscope/> (Retrieved 13th March 2017)].

In dental calculus analysis, this technique has predominantly been used to view microfossils that have been extracted, rather than viewing the calculus material itself (Charlier et al. 2010; Hardy et al. 2012; Lazzati et al. 2015; Power et al. 2015(1); Hardy et al. 2016). In this study, optical microscopy was used to better observe the surface features of archaeological dental calculus (see section 7.3.1). This application of optical microscopy has allowed the comparison of the physical characteristics of archaeological and clinical calculus (Roberts-Harry and Clerehugh 2000). In addition, the images gained from viewing each specimen under magnification were used as a reference for each specimen after sample preparation methods had been employed for further techniques.

5.2.2 Nano-Computed Tomography

Computed tomography uses X-ray radiation to map the density of an object (Abel et al. 2012). The technique involves taking numerous radiographs of an object from multiple angles, which are then reconstructed together to produce a 3-D rendering (Figure 5.2.2) (Abel et al. 2012). During the reconstruction, image artefacts and noise can be removed to make the images as clear as possible (Abel et al. 2012).

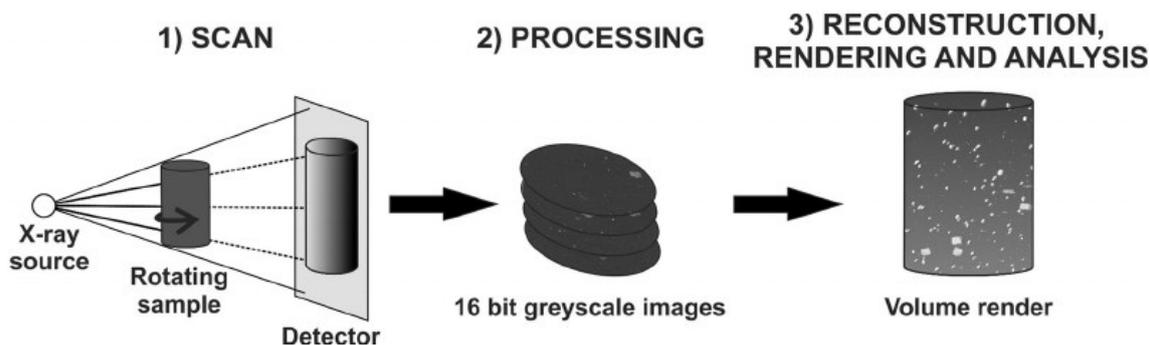


Figure 5.2.2 Diagram showing the simplified stages involved in micro-computed tomography [Image Credit: Cárdenes et al. (2016)].

Computed tomography (CT) has traditionally been applied to medical applications, including patient investigations and post-mortem examinations (Graser et al. 2008; Dreiseidler et al. 2010; Jeffery 2010). The development of micro-computed tomography has enabled high-resolution images to be collected from materials and objects, rather than the human body. This includes investigations into surgically removed biological calcifications, particularly kidney stones, as well as in the fields of material science and geology (Blaschko et al. 2013; Cordes et al. 2015; Kyle and Ketcham 2015; Thiemeyer et al. 2015; Cárdenes et al. 2016). Recently, micro-computed tomography has been applied to palaeontological and archaeological material to provide non-destructive imaging to human remains, fossils, pottery and artefacts (Abel et al. 2012; Miles et al. 2012; Panzer et al. 2012; Panzer et al. 2013; Brough et al. 2016; Sanger 2016).

During the development of the methods used in this research, micro-computed tomography was applied, however this did not provide sufficient resolution of the specimens. Consequently, nano-computed tomography was employed, which involved

changing the X-ray target from reflection to transmission. This decreased the focal spot size and increased the resolution of the images obtained.

In this study, nano-computed tomography was used to produce 3-D images of dental calculus samples to determine topographical surface features of whole specimens. Additionally, the 2-D images produced, allowed investigation of the internal structure of archaeological calculus deposits to determine whether accumulation layering could be detected. In addition, void algorithms were applied to the collected data to ascertain the distribution of pores throughout a deposit to determine if this might relate to accumulation layers.

5.2.3 Scanning Electron Microscopy

Scanning electron microscopy is a technique that uses a beam of electrons to produce topographic images of the surface of a material (Goldstein et al. 2003). When the electron beam bombards the sample, secondary electrons and back-scattered electrons are ejected and detected (Figure 5.2.3) (Goldstein et al. 2003). The changes in electron intensity detected across a surface, produce an image of the analysed sample (Goldstein et al. 2003). In this study, the specimens were not coated with a conductive layer, so that the specimens could be further analysed by μ -XRD (section 5.3.2). As the lack of a conductive coating decreases the number of secondary electrons produced, the SEM images in this study were produced by low-energy back-scattered electrons (BSE).

The use of back-scattered electrons (BSE) rather than secondary electrons allows the images obtained to indicate differences in composition across a sample (Goldstein et al. 2003). As the BSE are produced at a greater depth than secondary electrons, they are sensitive to the atomic mass of the nuclei they are produced from (Goldstein et al. 2003) (Figure 5.2.3). Consequently, the grey-scale image observed indicates compositional differences, with heavier element producing higher energy electrons and appearing brighter than lighter elements. A disadvantage with using (BSE) is that the resolution of the microscopy images is lower than for secondary electrons (Figure 5.2.3).

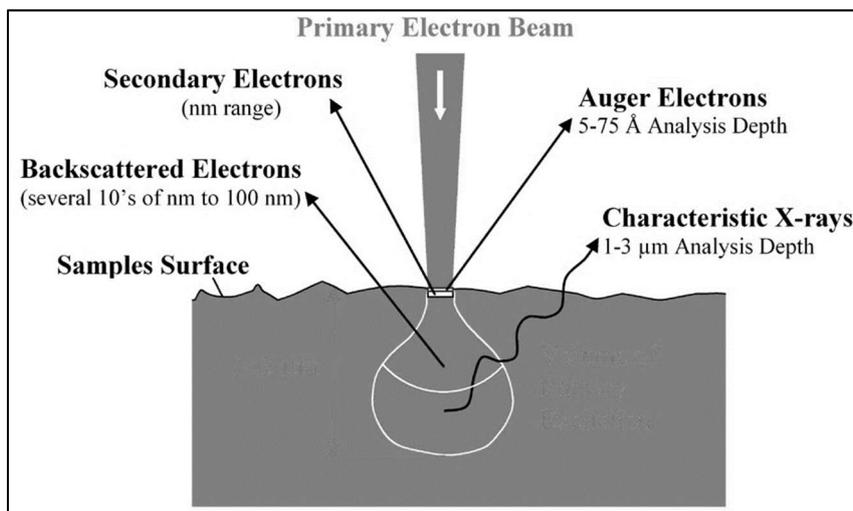


Figure 5.2.3 Diagram showing the secondary and back-scattered electrons produced during scanning electron microscopy. Also shown are the X-rays produced for EDX analysis (see section 5.4.2) and Auger electrons which are not relevant for this study [Image Credit: <https://www.surfgroup.be/semedx> (Retrieved 24th March 2017)].

In this study, SEM was employed to determine information about the sectioned samples, including voids and the shapes of mineral crystals. In addition, this technique provided elemental analysis from the complementary technique of energy dispersive X-ray analysis (see section 5.4.2). Also, due to the BSE-mode allowing composition changes to be observed, the SEM image of the cross-sectioned specimens were compared to the 3-D nano-computed tomography images to corroborate any observed density changes with compositional ones.

As well as mineral crystals, this technique allowed the possibility of observing dental calculus inclusions such as starch granules and microfossils, to determine if their presence contributes to the elemental composition determined by EDX. Unfortunately, due to the unforeseen damage that the sectioning process caused to the dental calculus cross-sections, most of the sought after topographical information, was not obtainable (see section 6.6.3).

5.3 Mineralogical Analysis Techniques

In this research, the mineral composition of dental calculus was determined by X-ray diffraction, as it has been in past studies (Tovborg Jensen and Danø 1954; LeGeros 1974; Klepinger et al. 1977; LeGeros 1981; Kani et al. 1983; Hayashizaki et al. 2008; Greenwood 2009; Wood 2012). Two methods were employed, powder X-ray diffraction and micro-beam X-ray diffraction, which could determine the bulk and cross-sectional mineral composition respectively.

Both techniques are based on the same principles, that diffraction occurs when X-rays are scattered by atoms or ions within a crystal (Jackson and Jackson 2004). The nature of a crystal means that the atoms are arranged in a regular array, which consists of a repeating unit cell (Atkins and de Paula 2010). The parallel distance between these unit cells is the inter-planar spacing (d-spacing) and can occur in three dimensions (h,k,l) (Atkins and de Paula 2010). When a beam of X-rays is targeted at a crystalline solid, the atoms vibrate with the same frequency as the incoming waves. If the X-ray waves are out of phase with each other after reflection by atoms in the crystal, destructive interference occurs and the X-rays cancelled out. However, if the X-ray waves are in phase with each other after reflection by atoms in the crystal, constructive interference occurs (Figure 5.2.1).

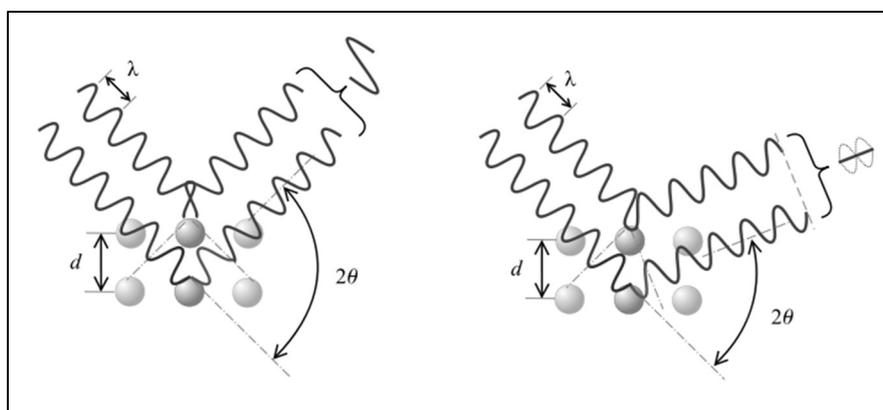


Figure 5.3.1 Diagram showing constructive interference of X-ray waves (left) and destructive interference of X-ray waves (right). The occurrence of constructive interference satisfies Bragg's Law [Image Credit: <http://www.ammrf.org.au/myscope/xrd/background/concepts/diffraction/> (Retrieved 19th March 2017)].

This constructive interference is required for a crystalline structure to satisfy Bragg's Law (Equation 5.2.1) (Jackson and Jackson 2004). Consequently, the relative intensities of the reflected X-rays can be measured and the inter-planar distances (d-spacing) calculated. The d-spacing of the three planes (h,k,l) can then be used to identify the arrangement of atoms in the crystal and consequently determine its structure (Atkins and de Paula 2010).

$$\lambda = 2d_{(h,k,l)} \sin\theta \quad \text{Equation (5.2.1)}$$

(λ = wavelength of the X-rays; d = d-spacing; θ = angle of incidence/diffraction)

X-Ray diffractometers consist of an X-ray tube, to produce the incident beam; a specimen stage where the material of interest is mounted; and an X-ray detector to measure the incident X-rays that have been successfully diffracted (Figure 5.2.2).

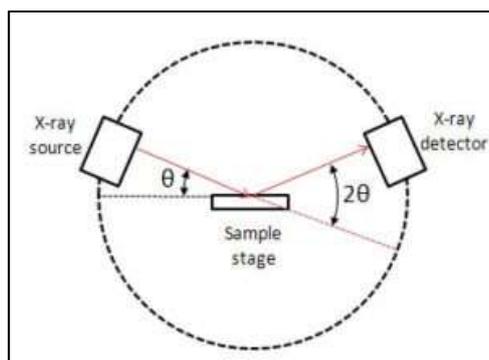


Figure 5.3.2 Diagram showing the components of an X-ray diffractometer, the source, stage and detector [Image Credit: Arora (2016)].

5.3.1 Powder X-ray Diffraction

Powder X-ray diffraction enables bulk mineral phase identification. Although this method is considered non-destructive, because the material can be recovered after analysis, the sample preparation does alter the state of the material. This preparation involves grinding the crystalline solid to a fine powder. This produces a greater surface area and increases the chance of X-rays interacting with crystallites in the correct orientation to produce the coherent interference required.

In the analysis of biological calcifications, X-ray diffraction suffers because of the poorly crystalline nature of the mineral phases. Additionally, the mineral phases can contain defects in the chemical structure (as described in section 2.4.1). These features cause the peaks in the diffractograms to be broad and overlap with each other. This can lead to difficulties in the quantification of the mineral phases.

Before any quantification can occur the qualitative analysis of the mineral phases that are represented within the data need to be identified. This is done using the position and relative intensity of the diffraction peaks. This is generally achieved by comparing the collected data against database records for verified crystalline materials. In this study comparison was carried out by searching the International Centre for Diffraction Data, Powder Diffraction File, using Crystallographica Search-Match v.2.1.1.1.

Following identification of the mineral phases, quantitative analysis of the data was performed to determine the percentages of each phase present. In this study, Rietveld full profile fitting method was employed within Bruker Topas software. The analysis involved using Rietveld fitting to quantitatively determine the percentage content of the analysed specimen. This was achieved by using standard diffraction patterns for each mineral phase and adjusting the mineralogical parameters (such as space group symmetry and lattice parameters) to the experimental data.

In this study, powder X-ray diffraction was applied to archaeological dental calculus analysis to gain mineral phase identifications and the percentage distribution of the present phases. In addition, the lattice parameters were recorded from the fitted Rietveld parameters to provide an indication of how substituted each mineral phase was likely to be. The specific methods used for this technique are detailed in section 6.8.1.

5.3.2 Micro-beam X-ray Diffraction

Micro-beam X-ray diffraction (μ -XRD) is a more sensitive technique than powder X-ray diffraction (pXRD) with longer data collection times and a more complicated experimental set-up. In this study, this technique was used to determine the in-situ mineral composition, rather than the bulk composition of archaeological dental calculus and as such the specimens did not require powdering. Instead, the specimens were embedded in resin and sectioned to expose the internal cross-section of the deposit. In μ -XRD, the

surface to be analysed is required to be as flat as possible, so that imperfections in the surface do not cause X-ray beam shadowing effects. Unfortunately, the resin embedding of the specimens causes its own problems, as resin is a highly crystalline material.

The μ -XRD data collected in this study was used in a similar manner to the pXRD data. The mineral phases were identified qualitatively and Rietveld whole pattern fitting was applied to determine the percentages of present phases. Details of the analysis conducted using this technique can be found in section 6.8.2.

5.4 Elemental Analysis Techniques

5.4.1 Inductively-Coupled Plasma Mass Spectrometry (Solution)

The bulk elemental analysis of the archaeological dental calculus specimens was analysed by inductively-coupled plasma mass spectrometry (solution) (ICP-MS(Sol)). This is a trace element analysis technique that can identify multiple elements and isotopes per analysis and has a low detection capability (parts per trillion) (Thomas 2013). The technique involves the sample being injected into a spray chamber to be mixed with argon, which acts a carrier gas (Figure 5.4.1). The mixed aerosol of sample and argon is then passed over a plasma torch to positively ionise the aerosol (Figure 5.4.1) (Thomas 2013). The ions produced are then directed into the mass spectrometer, which separates the ions by their mass-to-charge (m/z) ratio (de Hoffman and Stroobant 2007; Thomas 2013).

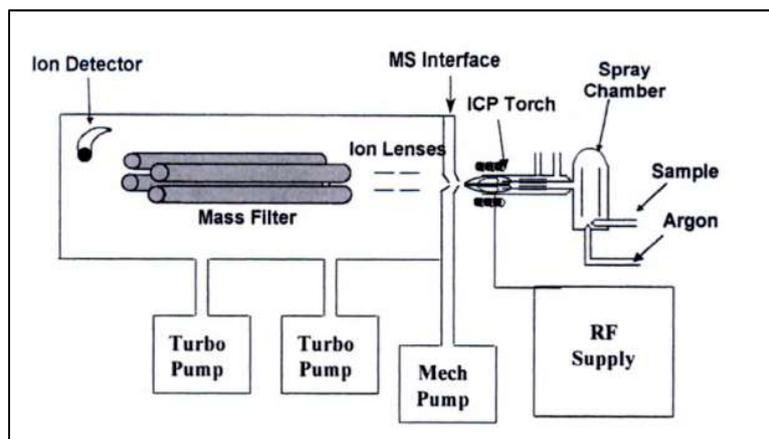


Figure 5.4.1 Diagram showing the basic components of an inductively-coupled plasma mass spectrometer [Image Credit: Thomas (2013)].

The ions are detected by the counts per second (cps) produced over an acquisition time. For each element, the cps is compared to data for standard solutions where the concentration (ppt, ppb or ppm) is known. For each analysis, fresh standard solutions must be prepared and analysed to ensure the concentrations determined for samples is as accurate as possible.

The use of laser-ablation ICP-MS (LA-ICP-MS) has been widely used in archaeological research in recent years (Dudgeon et al. 2016). However, this study employed the use of the solution-based version of this technique. This was primarily because EDX analysis was being utilised for the cross-sectional elemental analysis (see section 5.4.2) and ICP-MS(Sol) was employed to determine the bulk trace element composition. In addition, the sample preparation method that was required to prepare the ICP-MS solutions can be incorporated into starch and microfossil extraction techniques, by using the usually discarded supernatant (Hardy et al. 2009; Mickleburgh and Pagán-Jiménez 2012). Unfortunately, the research timescale and aims of this study did not afford the starch and microfossil analysis to be undertaken, however the extracted inclusions were retained for future work.

The first published study that has applied ICP-MS(Sol) to the analysis of archaeological dental calculus was Lazzati et al. (2015). This study combined ICP-MS(Sol) with phytolith analysis to determine palaeodietary evidence from two specimens of

archaeological dental calculus. However, the substitution of ions in the dental calculus mineral phases from the burial environment was not considered. As one of the aims in this study was to investigate population variation between different post-mortem conditions, this technique was employed to determine if the buried specimens had a different trace element composition to the mummified specimens. Therefore, a multi-element analysis was performed to identify trace elements in the archaeological dental calculus from the three populations.

In addition, a calcium phosphate analysis was performed to determine the amount of substitution in the mineral phases identified using pXRD (section 5.3.1). In stoichiometric calcium hydroxyapatite, the Ca/P is 1.67 and for β -tricalcium phosphate is 1.50. In specimens where the Ca/P ratio deviates from these stoichiometric values, it can be concluded that substitution of Ca^{2+} or $(\text{PO}_4)^{3-}$ has occurred. Further investigation of the elements present in the specimen can assist (in combination with the Ca/P ratio) can evaluate the likely substitutions that have occurred. This evaluation can allow inferences for the sources of the identified ions.

Finally, an iron analysis was performed to determine whether the iron concentration can be linked to the periodontal health of the individuals that the calculus had been formed in (see section 4.3). This analysis had to be performed separately to the multi-element analysis due to the interference that iron causes with other elements (May and Wiedmeyer 1998). The information regarding the sample preparation, data acquisition and data analysis for the use of this technique in this research is detailed in section 6.9.1.

5.4.2 Energy Dispersive X-ray Analysis

The cross-sectional elemental analysis of archaeological dental calculus in this study was carried out by energy-dispersive X-ray (EDX) analysis. This is a secondary technique that is performed during scanning electron microscopy (SEM) (see section 5.2.3).

During analysis by SEM, the surface of a sample is bombarded with an electron beam. As these electrons collide with the atoms in the sample material, inner-shell electrons are ejected (Garratt-Reed and Bell 2003) (Figure 5.4.2). This causes electrons from outer shells to fill these inner-shell gaps, which results in the release of a photon (Garratt-Reed and Bell 2003). The energy of the released photon, corresponds to the difference in energy

between the electron shells and to the atomic number of the element being bombarded (Garratt-Reed and Bell 2003). Consequently, the measurement of photon energies during bombardment by an electron beam can produce qualitative elemental data.

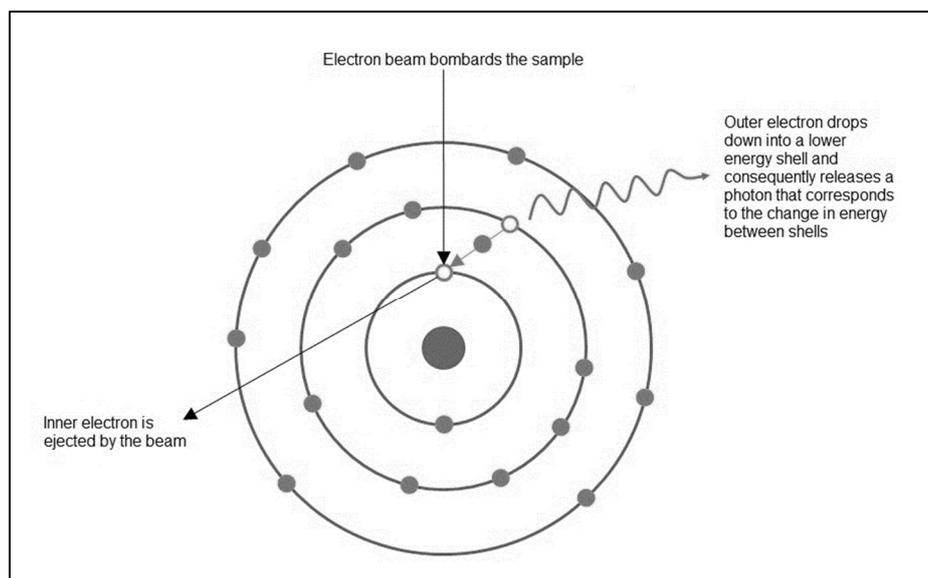


Figure 5.4.2 Diagram showing the photon release that occurs during energy dispersive X-ray analysis, which allows the elemental analysis of materials [Adapted by author from <http://www.umms.sav.sk/6522-en/mikrostrukturna-analyza/> (Retrieved 24th March 2017)].

Energy dispersive X-ray analysis has previously been applied to dental calculus analysis in archaeology (Charlier et al. 2010; Power et al. 2014; Lazzati et al. 2015; Power et al. 2015). This has mainly been applied microfossil analysis (extracted or in-situ) where EDX has been used to confirm the composition of the inclusions observed using SEM (Power et al. 2014; Lazzati et al. 2015; Power et al. 2015). However, Charlier et al. (2010) have utilised EDX to determine the elemental composition of archaeological dental calculus, which is how EDX was applied in this study.

This elemental technique was chosen to complement the scanning electron microscopy that was performed. Despite its qualitative nature, it can analyse light elements, such as carbon and oxygen, when other elemental techniques such as X-ray fluorescence, cannot. In this study, comparative analyses between the superficial, central and deep regions of the calculus were performed to determine if compositional changes can be detected in the

cross-section of dental calculus. The methods and analysis conditions used for this technique can be found in section 6.9.2.

5.5 Chapter Summary

This study employed a wide range of analytical techniques to analyse dental calculus. Imaging techniques of optical microscopy, nano-computed tomography and scanning electron microscopy were used to determine the morphology and structure of the specimens. Powder X-ray diffraction and micro-beam X-ray diffraction were used to determine the bulk and cross-sectional mineral composition. Finally, inductively-coupled plasma mass spectrometry and energy dispersive X-ray analysis were employed to determine the bulk and cross-sectional elemental composition.

Most of these techniques have been previously applied to dental calculus analysis, some predominantly through clinical studies and others in archaeology. However, this is the first application of nano-computed tomography to archaeological calculus research. Additionally, this study involved a non-destructive to destructive methodology to be able to perform multiple complimentary techniques on dental calculus specimens to maximise the information gained.

This chapter has provided some basic theory about each of the analytical techniques used. As well, the rationale behind using each technique has been outlined. The following chapter will explain the materials analysed and the specific methods that have been undertaken.

CHAPTER 6: MATERIALS AND METHODS

Scaling the teeth, that is, clearing them of the stony concretions which frequently collect about their necks, while nothing is scraped off but that adventitious substance is proper and useful.

- John Hunter 1803

6.1 Overview

This chapter consists of two parts. Firstly, the materials section details the three samples of dental calculus specimens that have been analysed. This includes the number of specimens that have been subjected to each stage of preparation and analysis. The remainder of the chapter outlines the methods employed to analyse said specimens. Information regarding the methods has been divided into subsections relating to recording and sampling; imaging; sectioning; and mineralogical and elemental analysis.

6.2 Materials

For this research, there were three populations from which dental calculus deposits were sampled; two populations from Spain and one from Italy. The information regarding the populations that were sampled for this research, are described in Chapter 3.

The Italian population, from the Capuchin Catacombs (CC), consisted primarily of mummified individuals (47 individuals sampled) with the addition of a sub-group of disarticulated crania and mandibles (8 crania and 5 mandibles). This sample of dental calculus specimens from mummified remains represents approximately 22% of the total individuals at floor height (or for open coffins, stacked on the floor) in accessible corridors. For more information regarding accessibility see section 3.2.4.

Of the two Spanish populations, Cementeri Vell (CV) consisted of secondarily deposited, disarticulated commingled skeletal remains (29 individuals sampled). Most specimens in this sample were taken from maxillary teeth (33/40, 83%) due to the low survivability of mandibles with intact dentition. Many specimens were sampled from loose teeth (19/40, 48%) after determination that they were not associated to crania that had already been

sampled (See section 3.3.4). This sample consisted of 10% of the population that was available for study from the previous excavation (See section 3.3.3). For statistics relating to the prevalence of dental calculus within the population, see section 7.2.2.

The second Spanish population, San Agustín (SA), consisted of 34 graves with 22 of these producing multiple sets of remains in varying states of completeness. From this population, 14 individuals were sampled for dental calculus deposits. Within this population were individuals with a large amount of calculus on multiple teeth and therefore, multiple specimens were taken per individual. For statistics relating to the prevalence of dental calculus within the population, see section 7.2.2.

In total 134 specimens from 98 individuals³¹ were sampled from three populations. Specimens were taken from both the maxillary teeth (79/134; 59%) and mandibular teeth (55/134; 41%) and specimens were taken from anterior teeth (33/134; 25%) and posterior teeth (101/134; 75%). Specimens were predominately taken from the buccal (95/134; 71%) and lingual (30/134; 22%) surfaces of the teeth because these are where calculus is most likely to accumulate (See section 2.2.2).

The following graphs (Figure 6.2.1 - 6.2.4) summarise the specimens that were collected regarding the jaw, tooth and surface from which they were taken for each population. The number of specimens analysed at each stage of the analytical process, is detailed in Table 6.2.1. The methods in Table 6.2.1, are described in the following sections of this chapter.

³¹ This is the minimum number of individuals (MNI) sampled across all three populations, see section 6.4.2.1 for individual population calculations of MNI.

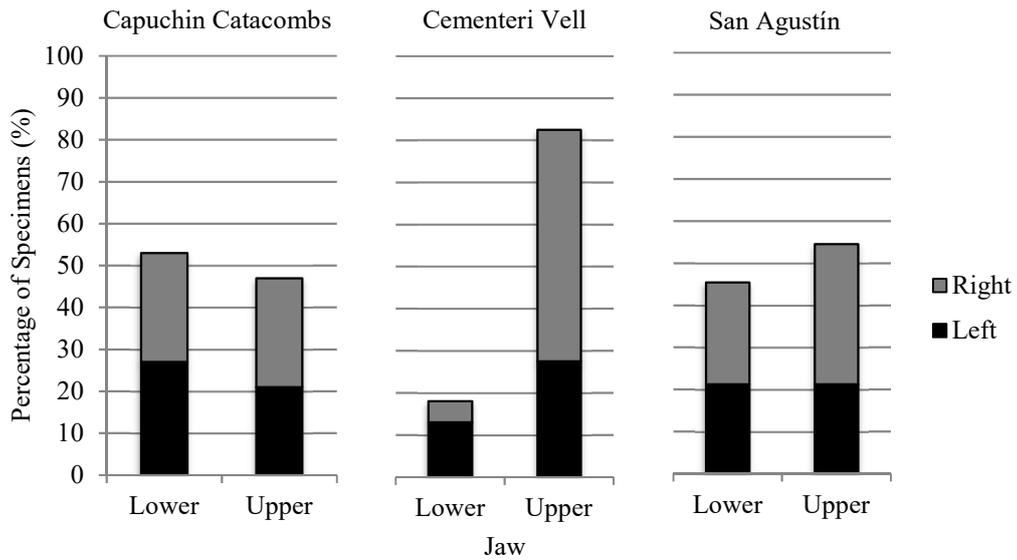


Figure 6.2.1 Graph showing the percentage distribution of specimens sampled from the dentition of each population displayed per jaw location (left/right and maxillary/mandibular) of the tooth from which they were taken.

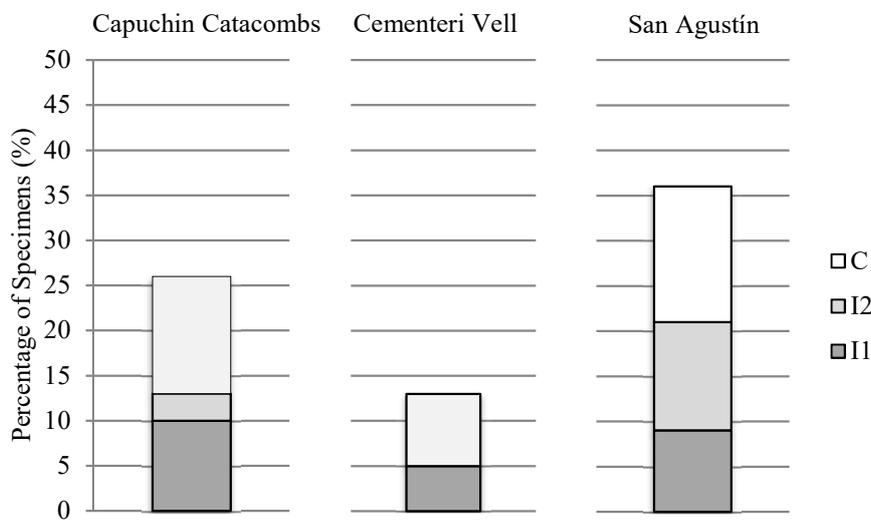


Figure 6.2.2 Graph showing the percentage distribution of specimens sampled from the anterior dentition of each population displayed per the tooth (first incisor, second incisor or canine) from which they were taken.

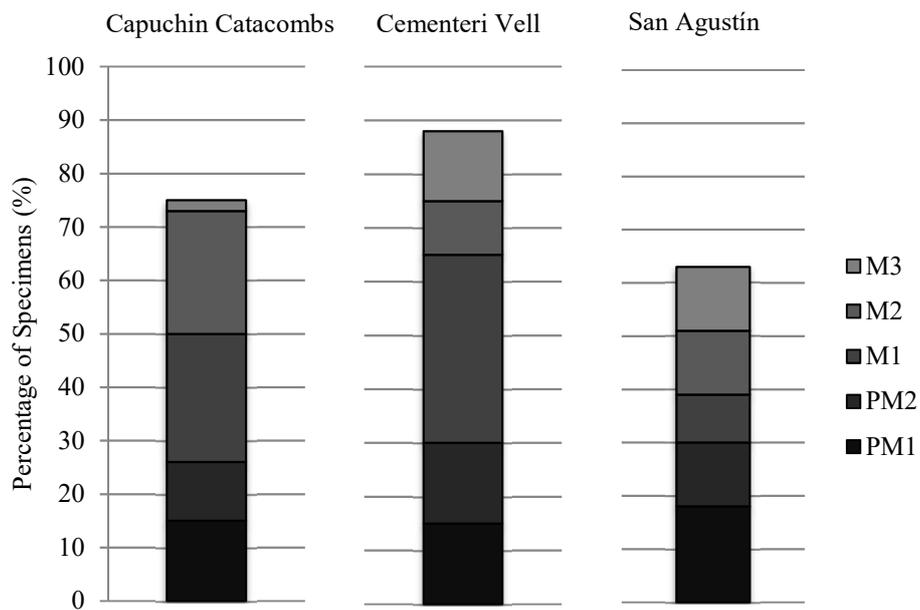


Figure 6.2.3 Graph showing the percentage distribution of specimens sampled from the posterior dentition of each population displayed per the tooth (premolars or molars) from which they were taken.

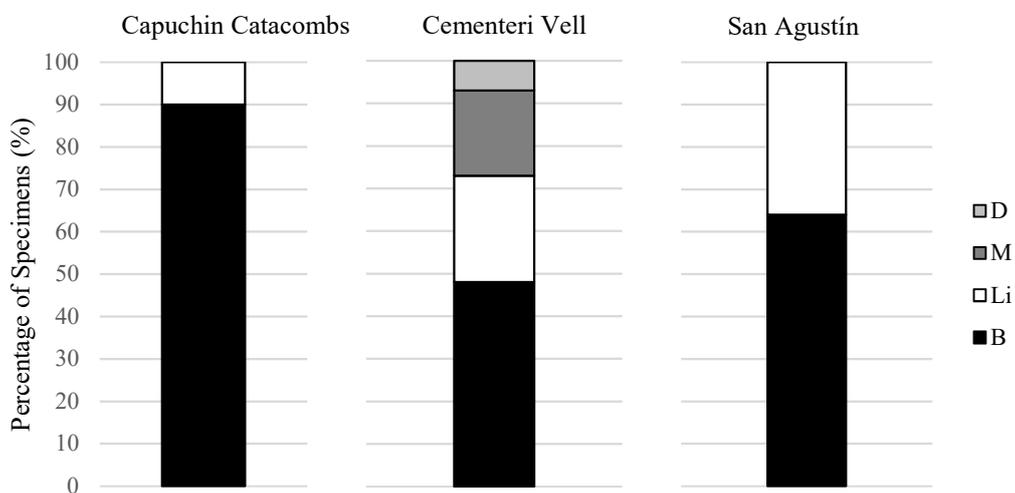


Figure 6.2.4 Graph showing the percentage distribution of specimens sampled each tooth surface for each population.

		Capuchin Catacombs, Sicily	Cementerí Vell, Formentera	San Agustín, La Rioja
MNI Sampled		55	29	14
Total No. of specimens collected [^] :		62	40	33
Imaging	Optical Microscopy	62	33	29
	Nano-Computed Tomography	40	19	14
	Scanning Electron Microscopy	31*	-	-
Sample Prep	Microtome Sectioning	31*	-	-
	Powdering	20	28	29
Mineralogical	Powder X-ray Diffraction	20	28	29
	Micro-beam X-ray Diffraction	2	-	-
Elemental	Energy Dispersive X-ray Analysis	31*	-	-
	Inductively- Coupled Plasma Mass Spectrometry	10	10	10

[^] This number of specimens collected, is the number of dental calculus deposits taken from different teeth even if from the same individual. It is not equal the number of individuals sampled and counts a specimen as one regardless of the amount of fragmentation.

*¶ Sectioned deposits were not all from separate individuals.

Table 6.2.1 Table detailing the number of specimens from each population analysed by each technique.

6.3 Methods: Overview

This is the first archaeological dental calculus study to include a range of complimentary techniques that collectively analyse the morphology and mineral and elemental composition of the specimens. Many studies have performed one process or method to gain evidential value from calcified dental deposits, but none have maximised the

potential by analysing these three key components from the same specimens. Additionally, many researchers in this field are provided samples that have already been collected. For this research, it was possible to have some control prior to specimen removal, thus incorporating the entire process into the study, from in-situ recording to sampling and non-destructive to destructive analysis.

6.4 Dental Recording

Prior to the sampling of dental calculus specimens for analysis, a review of published methods for recording dental calculus was carried out (see *Supplementary Material: Appendix B*). Within this review, publications that reported dental calculus analysis were examined to determine the recording protocols that were employed. It was found that many peer-reviewed papers regarding dental calculus analysis do not specify any pre-sampling recording of dental calculus. Considering that all dental calculus analysis requires some or all the deposit to be irreversibly removed from the dentition; and that most analysis carried out on removed deposits ‘consumes’ part or the entire specimen, it was felt that this stage was particularly important.

6.4.1 In-situ Recording

For all collections, the dentition was recorded using original dental recording forms. These forms were compiled with dental calculus recording and sampling as a focus but also included relevant information regarding the individual and present dental pathologies. The presence of dental calculus and pathological conditions were recorded in line with standard dental recording protocols (Buikstra and Ubelaker 1994; Brickley and McKinley 2004). Copies of all forms can be found in *Supplementary Material: Appendix D*.

In addition, the Cooper method, a novel quantitative scoring method was also included in the forms. Following the recording method review (see *Supplementary Material: Appendix B*), this periodontal method was adapted from the Volpe-Manhold Probe method (1965), for skeletal remains. It was included in these recording forms to test its feasibility in the field and in relation to the standard scoring methodology. The Cooper

method is explained briefly below; however initial performance results against the widely-accepted index method are included in *Supplementary Material: Appendix B*.

In the Capuchin Catacombs, the author recorded the dentition of accessible individuals. For the Cementeri Vell population, individuals that were sampled for dental calculus were recorded by the author using the above explained forms and methods. In addition, Emily Wilson (MSc Student) and Dr Nicholas Márquez-Grant (Fieldwork Co-ordinator) completed full skeletal recording (including dentition) of all commingled remains. A full osteological report from the Universidad Autónoma de Madrid was available for individuals from the San Agustín population (SMYSA 2014). Using the forms designed by the author, additional dental calculus scoring was performed by Almudena García-Rubio (PhD Researcher, Universidad Autónoma de Madrid).

6.4.1.1 Tooth Presence/Absence

The initial recording of the dentition documented the presence and absence of the teeth and the alveolar positions of the maxillae and mandibles observed. In the recording forms this was done in a visual symbol-based matrix, using symbols for the five most common presence or absence types, further info for example ‘in crypt’ was noted below (Figure 6.4.1). Although wear was not scored for this study, basic comments were noted regarding the amount of wear observed.

Teeth Present	UL	UR	LL	LR
	I1	I1	I1	I1
	I2	I2	I2	I2
	C	C	C	C
	PM1	PM1	PM1	PM1
	PM2	PM2	PM2	PM2
	M1	M1	M1	M1
	M2	M2	M2	M2
	M3	M3	M3	M3

- Present in occlusion
- Present not in occlusion (no associated alveolar bone)
- Present not in occlusion (associated alveolar bone)
- Present but unobservable
- Ante-Mortem Tooth Loss

Notes on Presence:

Comments on Wear:

Figure 6.4.1 Image of the section of paper recording forms where tooth presence/absence was recorded by visual symbols on a dental matrix.

For some teeth, predominantly in the Capuchin Catacombs, the teeth could be observed as present, but not observed for dental calculus or pathologies. This was mostly due to soft tissue of the lips and mouth obscuring the buccal, lingual, mesial and distal surfaces. For tooth absence, ante-mortem tooth loss (AMTL) was assigned to a tooth position if there were observable signs of partial or full resorption of the alveolar bone. Conversely, if a tooth was missing and these observations were not present, the tooth was noted as being lost post-mortem (PMTL).

On transference of the recording forms to Microsoft Excel for statistical analysis, the entries for tooth status was converted into numerical codes. This allowed for better data handling for the large data sets collected for each population. The assigned numerical code and related tooth status are detailed in Table 6.4.1.

1	Present, not in occlusion with associated alveolar bone
2	Present, not in occlusion with no associated alveolar bone
3	Present, in occlusion
4	Present, but unobservable (in crypt/soft tissue etc.)
5	Missing, no associated alveolar bone
6	Missing, with alveolar resorption (indicating AMTL)
7	Missing, no alveolar absorption
8	Missing, alveolar bone not observable (debris/ soft issue etc.)
9	Missing, congenital absence
10	Unobservable, possible congenital absence/ possibly unerupted

Table 6.4.1 Table showing the numerical tooth codes and related tooth status options, tooth presence and absence, for data compiled in Microsoft Excel.

6.4.1.2 Dental Calculus Scoring

Dental calculus deposits were scored by two methods. Firstly, the Brothwell (1981)/Buikstra and Ubelaker (1994) method and then the Cooper method (adapted from the Volpe–Manhold method (1965)) (see *Supplementary Material: Appendix B*).

Using the Brothwell (1981) method, each dental calculus deposit was assigned a score of 1-3 depending on the amount of calculus present as shown in Figure 6.4.2. Following the

standards by Buikstra and Ubelaker (1994), Brothwell (1981) scores were assigned to each surface of a tooth, rather than one score per tooth. If a dental calculus deposit exhibited signs of post-mortem damage, it was not scored as the full size of the deposit could not be determined (see section 2.5.3).

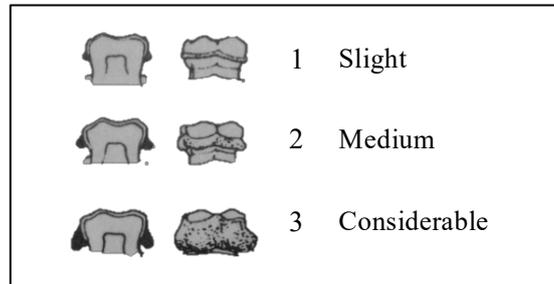


Figure 6.4.2 Illustration of the amounts of dental calculus per score in the Brothwell method (1981). [Adapted by author from Brothwell (1981), p. 155]

The scoring for the Cooper method used a UNC-15 periodontal probe, which is graduated in millimetres (see Figure 6.4.3). Dental calculus was scored per surface of a tooth, i.e. calculus on a buccal surface of a tooth would receive a score and calculus on the lingual surface of the same tooth would receive a separate score. Scores were not possible when the probe could not be held parallel to the tooth surface.

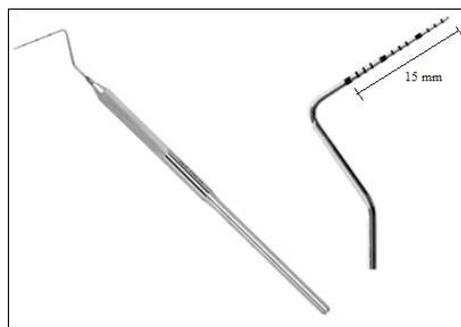


Figure 6.4.3 Photographic examples of UNC-15 periodontal probe utilised for the Cooper method of dental calculus scoring. Full probe (left); Close-up of measurement tip showing millimetre graduations (right) [Image Credit: Left: <http://www.jakobidental.com/Diagnostic/Periodontal-Probes/Periodontal-probe-UNC-15-Standard-handle-%23-30::1285.html>; Right: <http://perio basics.com/periodontal-instruments.html> (Retrieved 12th September 2015)].

When scoring a surface, the probe was carefully placed up to the tooth, ensuring that no contact was made that may cause damage to the calculus deposit. Using the graduations on the probe, the length of calculus in three planes was measured. When dental calculus was present below the cemento-enamel junction (CEJ), the measurements were taken in the same planar positions but the values were assigned negative scores. When a tooth could not be scored, for example, when impacted (not having erupted), the measurement was recorded as 'Unscored' (UN) (see *Supplementary Material: Appendix B* for diagram).

6.4.1.3 Oral Pathologies

In addition to comprehensive dental calculus recording, oral pathologies were also recorded. When designing the recording forms, tick boxes were implemented for the presence of carious lesions, periapical cavities, enamel hypoplasia and periodontal disease (Figure 6.4.4). However, when in the field, it was decided that this was too simplified for lesions and cavities and further information was recorded in the notes section.

Carious lesions were identified and scored by size according to Lukacs (1989). Additional information regarding the number of lesions and the teeth and surfaces affected, were noted on the forms. Where teeth were present but unobservable, carious lesions were noted as being unobservable and these teeth were not included in the percentages of teeth affected (see section 6.4.2.5).

Periapical cavities were identified as drainage sinuses in the alveolar bone and these were noted in accordance with Brickley and McKinley (2004). The tooth position and where the cavity was situated (buccal or lingual) were documented in the notes section of the recording form (Figure 6.4.4).

Enamel hypoplasia was recorded if observable, however the number of lines present was not. In the Capuchin Catacombs, enamel hypoplasia was not observable on most dentition due to the dust and debris that could not be cleaned from the teeth. As enamel hypoplasia is indicative of childhood malnutrition rather than related to adult health and diet, and following the difficulty in recording the condition, it was decided that this pathology would not be included in the dental statistics of the populations for this study.

Periodontal disease was identified by alveolar pitting combined with bone loss and root exposure as described in Roberts and Manchester (2010). Periodontal disease was recorded as present or absent for all tooth positions when an associated tooth was present, however the amount of recession was not scored from visual estimation. Instead, for Cementeri Vell, where comprehensive photographs with measurement scales were taken, alveolar recession measurements were taken using ImageJ software. Unfortunately, this could not be carried out for the Capuchin Catacombs or San Agustín (see section 6.4.3).

Pathologies Present:

Caries:

Enamel Cementum Dentine

Pinprick Small Medium Large

Lesions:

Periapical Granuloma Radicular Cyst Abscess

Enamel Hypoplasia

Periodontal Disease

Notes on Pathologies:

.....

.....

Figure 6.4.4 Image of the section of paper recording form used for indicating the presence of oral pathologies present on an individual.

6.4.2 Calculation of the Dental Statistics

The results of the dental recording were transferred into Microsoft Excel spreadsheets, one for each population. The data was then organised to be able to calculate several values to assess the dental condition and health of the populations. In this section, the methods used to calculate each value are described and the results for these values can be found in section 7.2. In each population, these values were calculated for all individuals and separately for the calculus-exhibitors (i.e. for the sub-group of individuals who had at least one dental calculus deposit present on their dentition). Each value was also calculated for sub-groups according to sex for all individuals and for the calculus-exhibitors.

6.4.2.1 Minimum Number of Individuals (MNI)

The minimum number of individuals (MNI) was calculated for each population using the dentition. This was determined to calculate further values pertaining to the expected number of teeth for the population samples recorded.

In each population, the overall minimum number of individuals (MNI_{TOT}) for the whole population sample and the minimum number of individuals with dental calculus (MNI_{CALC}) were calculated. This allowed calculation of the percentage of individuals that were recorded as having dental calculus per population ($I_{CALC}\%$).

$$I_{CALC}\% = MNI_{CALC} / MNI_{TOT} * 100$$

The method of calculation of MNI varied between populations because of the nature of the archaeological populations.

For the Capuchin Catacombs, MNI was reasonably straight forward as each mummy clearly represented an individual. Therefore, the MNI of the mummified remains was simply the number of individuals able to be recorded for dentition. In addition to mummified individuals, there were also several disarticulated skulls, crania and mandibles (both mummified and skeletonised). The MNI of this sub-group was calculated from the present number of crania, as these were more numerous than present mandibles. Therefore, the total MNI for the Capuchin Catacombs (MNI_{TOT}) was as follows:

$$MNI_{TOT} = \text{no. of mummified individuals recorded} + \text{no. of disarticulated crania}$$

Consequently, the minimum number of individuals that had been recorded as having at least one dental calculus deposit was calculated:

$$MNI_{CALC} = \text{no. of mummified individuals recorded with dental calculus} \\ + \text{no. of disarticulated crania recorded with dental calculus}$$

The Cementeri Vell MNI was more complicated due to the commingled nature of the remains. The assemblage of dentition included, partial and whole maxillae and mandibles as well as loose teeth with no associated alveolar bone (only permanent dentition was included for this study). For the whole population, the presence of loose teeth complicated the MNI calculation because it was not possible determine if loose teeth should be

associated with an alveolar fragment, especially when the tooth socket was missing. Therefore, initially it was determined whether there were more teeth than alveolar tooth positions observable, as the more numerous would be the basis for calculating MNI. This was found to be teeth rather than alveolar bone positions.

Following this, the permanent teeth were tabulated for presence per the dental quadrant that they occurred in. The most frequently occurring tooth was the upper left first molar (ULM1) with 61 occurring across all teeth recorded. In addition, the number of ULM1 alveolar bone tooth positions that were recorded as exhibiting ante-mortem tooth loss was calculated. By observing ante-mortem tooth loss at this position, it is assumed that the corresponding tooth would not be in the assemblage due to it having been lost prior to death. Therefore, individuals with AMTL at the ULM1 position are an additional contribution to the MNI along with the quantity of the most repeated tooth.

The MNI was calculated using the assumption that each individual had one upper left first molar (as is the case in standard adult dentition, see *Supplementary Material: Appendix A*). Therefore, the total MNI for the Cementeri Vell (MNI_{CV}) was as follows:

$$\text{MNI} = \text{number of upper left first molars present loose or in occlusion} + \text{number of upper left first molar tooth positions observable for AMTL}$$

For San Agustín, MNI was straight forward as each interment clearly represented an individual. There were interments that included additional remains (cranial and post-cranial), however the Universidad Autónoma de Madrid did not include these in the dental recording. Therefore, the MNI of the skeletal remains was simply the number of individuals recorded for dentition. Therefore, the total MNI for San Agustín (MNI_{SA}) was as follows:

$$\text{MNI} = \text{number of individuals recorded (by Universidad Autónoma de Madrid)}$$

6.4.2.2 Tooth Presence

For each population, the minimum number of individuals (MNI) was used to calculate how many teeth would be expected (T_{EXP}) if all teeth were accounted for. Additionally, from the calculated T_{EXP} , the total number of surfaces that would be expected (S_{EXP}) was calculated.

$$T_{EXP} = MNI \times 32$$

(where each individual was assumed to have a full set of adult dentition)

$$S_{EXP} = MNI \times (24+80)$$

(where 24 = number of anterior surfaces (two per tooth x 12 teeth per person) and 80 = number of posterior surfaces (four per tooth x 20 teeth per person), occlusal surfaces were not included)

The number of teeth that were observed as present (T_{OBS}) was calculated and the recording method for tooth presence is detailed in section 6.4.1.1. In addition, the potential number of surfaces that could have been observed (S_{POT}) for the present number of teeth (T_{OBS}) is included with the actual number of observable surfaces (S_{OBS}). These values are defined as follows:

$$T_{OBS} = \text{no. of teeth recorded as present}$$

(where the teeth recorded as present were in occlusion or loose)

$$S_{POT} = (\text{no. of anterior teeth present} \times 2) \\ + (\text{no. of posterior teeth present} \times 4)$$

$$S_{OBS} = S_{POT} - (\text{no. of surfaces not observable})$$

(where 'surfaces not observable' means for example surface that had suffered post-mortem damage, or were unobservable due to soft tissue)

The percentages of teeth ($T\%$) and surfaces ($S\%$) present were then calculated as follows:

$$T\% = T_{OBS} / T_{EXP} * 100$$

$$S\% = S_{OBS} / S_{POT} * 100$$

6.4.2.3 Tooth Loss

To determine the amount of tooth loss observed, the alveolar positions that were observable (P_{OBS}) were calculated.

$$P_{OBS} = \text{no. of observable alveolar tooth positions}$$

(where an observable alveolar tooth position was intact with no post-mortem damage)

Subsequently, positions with no associated tooth that were either recorded as post-mortem tooth loss (P_{PMTL}) or ante-mortem tooth loss (P_{AMTL}) were calculated.

$$P_{PMTL} = \text{no. of PMTL alveolar tooth positions}$$

$$P_{AMTL} = \text{no. of AMTL alveolar tooth positions}$$

These values were then used to calculate the percentages of each type of tooth loss, post-mortem ($P_P\%$) and ante-mortem ($P_A\%$).

$$P_P\% = P_{PMTL} / P_{OBS} * 100$$

$$P_A\% = P_{AMTL} / P_{OBS} * 100$$

The percentage of PMTL and AMTL was also calculated for each tooth position. This was done by dividing the number of alveolar positions affected by each type of tooth loss by the total number of observable cases for each tooth position.

6.4.2.4 Dental Calculus

The percentage of teeth with dental calculus ($T_{CALC}\%$) was calculated from the number of teeth recorded as having at least one surface with a dental calculus deposit (T_{CALC}).

$$T_{CALC} = \text{no. of teeth recorded as having dental calculus present on at least one tooth surface}$$

$$T_{CALC}\% = T_{CALC} / T_{OBS} * 100$$

(where T_{OBS} is the no. of teeth observable for dental calculus, which was not the same as the T_{OBS} in 6.4.2.2, as some teeth could be observed to be present but not observed for pathologies, i.e. when soft tissue obscured it or when carious lesions had destroyed the crown)

Similarly, the percentage of surfaces with dental calculus ($S_{CALC}\%$) was calculated from the number of surfaces recorded as having a dental calculus deposit (S_{CALC}).

$$S_{CALC} = \text{no. of surfaces recorded as having dental calculus present}$$

$$S_{CALC}\% = S_{CALC} / S_{OBS} * 100$$

(where S_{OBS} is the no. of observable surfaces (see section 6.4.2.2))

In each population, $T_{CALC}\%$ was also calculated for each tooth position. This was done by dividing the number of each tooth type with dental calculus by the total number of observable cases of each tooth type. These values were also calculated according to the

Brothwell score given to each deposit. The $S_{CALC}\%$ was also calculated for each tooth surface. This was done by dividing the number of each surface type with dental calculus by the total number of observable cases of each surface. These values were also calculated according to the Brothwell score given to each deposit.

6.4.2.5 Oral Pathologies

The number of teeth affected by dental caries (T_{CALC}) was used to calculate the percentage of observable teeth with the condition ($T_{CL}\%$). In this calculation, the number of observable teeth (T_{OBS}) was not equal to T_{OBS} in section 6.4.2.2, as not all teeth could be observed for carious lesions due to post-mortem damage.

T_{CL} = no. of teeth recorded as having at least one carious lesion

$$T_{CL}\% = T_{CL} / T_{OBS} * 100$$

The number of alveolar positions affected by periapical cavities (P_{PAC}) was used to calculate the percentage of observable positions with the condition ($P_{PAC}\%$). In this calculation, the number of observable positions (P_{OBS}) was not equal to P_{OBS} in section 6.4.2.3. In the Capuchin Catacombs, this was predominantly due to the presence of soft tissue obscuring the alveolar bone on the exterior and/or interior surfaces even if the socket could be observed for AMTL or PMTL. In the other populations, some positions were unobservable for cavities due to post-mortem damage.

P_{PAC} = no. of tooth positions recorded as having at least one periapical cavity

$$P_{PAC}\% = P_{PAC} / P_{OBS} * 100$$

The number and percentage of alveolar tooth positions with associated teeth that exhibited periodontal disease (P_{PD} , $P_{PD}\%$ respectively) were calculated.

P_{PD} = no. of alveolar positions with an associated tooth that exhibited periodontal disease

$$P_{PD}\% = P_{PD} / \text{no. of alveolar positions with associated teeth} * 100$$

6.4.3 In-situ Photography of Dental Calculus Deposits

Where possible, dental calculus deposits were photographed in-situ, prior to sampling. Images were taken of the occlusal view of the dentition and the medial and lateral views of the maxillae and mandibles, as shown in Figure 6.4.5. All photographs were taken with the inclusion of an ABFO³² No. 2 photomacrographic scale (graduated in millimetres). A polystyrene skull support-ring was utilised, when required, to assist in positioning disarticulated skeletal elements being photographed.

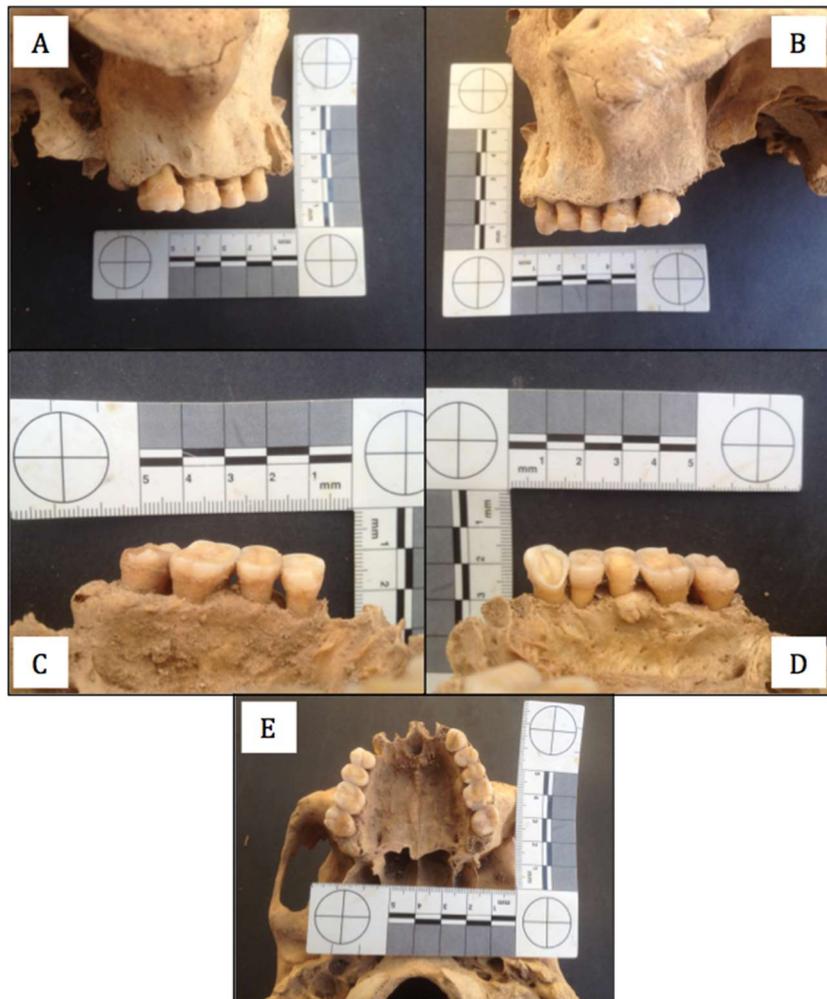


Figure 6.4.5 Example photographs taken of in-situ dental calculus deposits. A: lateral view of right side; B: lateral view of left side; C: medial view of left side; D: medial view of right side; E: inferior view of cranium, showing occlusal surfaces of the maxillary dentition [Images taken by author (June 2014)].

³² American Board of Forensic Odontology

In the Capuchin Catacombs, photography was not possible for all medial surfaces of the maxillae and mandibles. This was mainly due to many of the individuals having string or wire holding the mandible onto the cranium (in most cases via the mandibular condyles and zygomatic process of the temporal bone) as shown in Figure 6.4.6. However, other limitations also restricted photography including the height and positioning of the remains. There were also ethical considerations because photography in the Capuchin Catacombs is banned for tourists and visitors out of respect for the deceased. Therefore, it was decided that to comply with these restrictions, photographs would only be taken for research purposes when there were less visitors around, so as not to cause offence or encourage photography for leisure. For San Agustín, high resolution photographs were kindly taken by Almudena García-Rubio (PhD Researcher, Universidad Autónoma de Madrid), however a scale was not included in these images.

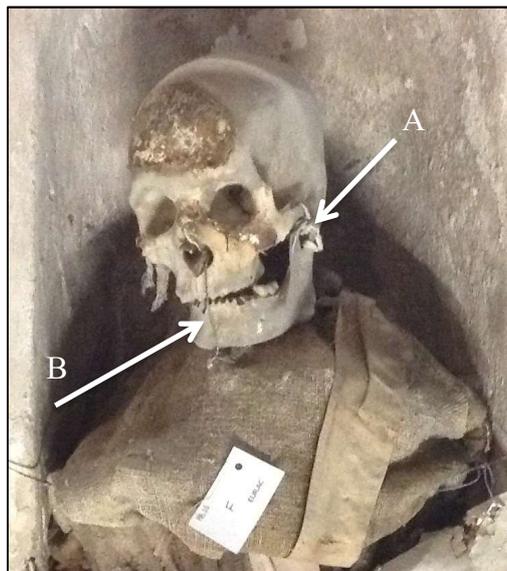


Figure 6.4.6 Photographic example of a mummified individual from the Capuchin Catacombs with string suspending the mandibular condyles from the zygomatic process of the temporal bone (A) and wire holding the mandible at the mandibular symphysis through the nasal cavity (B) [Image taken by author (May 2014)]

6.5 Dental Calculus Sampling

Once recording had been completed and relevant photographs had been taken, one or more deposits were chosen to sample. Primarily, specimens were chosen per the size of

the deposit and accessibility. Prior to removal, a sample bag was labelled with the remains reference, tooth and surface from which the specimen was taken.

6.5.1 Removal of Dental Calculus Specimens

For the mummified remains in the Capuchin Catacombs collection a filter paper cone was used due to the access to the remains and the height of the dentition. This cone was placed under the tooth with the dental calculus specimen so that the deposit could be caught in the cone easily. The Cementeri Vell collection was more accessible and a clean sample bag was placed under the tooth to catch the specimen removed.

A curved dental pick was used to pry the dental calculus of the tooth and either into the filter paper cone or onto the clean sample bag (Henry and Piperno 2008). When the deposit was not removed in one piece, the remaining adhering pieces were pried off from the tooth surface into the same filter paper cone or sample bag. The dental pick was cleaned and dried between each specimen, with either water or acetone, depending on availability.

The specimen was then carefully transferred into the pre-labelled sample bag and stored in a plastic lidded specimen container to prevent damage to the specimens during transit back to Cranfield University (Figure 6.5.1). This method was also followed for San Agustín sampling; however, the specimen removal was carried out by Almudena García-Rubio (Universidad Autónoma de Madrid). The San Agustín specimens were delivered to Cranfield by Miss García-Rubio in person.



Figure 6.5.1 Example of specimen packaging and storage. Each specimen was packaged in a pre-labelled sample bag and stored in sample pots to minimise specimen breakage [Image taken by author (September 2015)].

6.6 Dental Calculus Imaging

For each specimen of dental calculus collected, photographs were taken under magnification. These images form a catalogue of the specimens, as they were post-removal, before analysis or sectioning. With the handling of the specimens possibly causing breakage or loss and the inevitable destructive preparation and analysis methods, these images serve as an important reference collection. Additionally, these photographs allowed image analysis for specimens to determine physical characteristic trends among the specimens for each archaeological collection. The characteristics analysed using these images were texture, colour and shape. Subsequent imaging was carried out using nano-computed tomography of the specimens, prior to any sample preparation.

6.6.1 Optical Microscopy

The specimens collected from each tooth surface were recorded for their state (whole/fragmented) and where relevant, the number of fragments present. The term ‘whole’ was assigned to specimens where the dental calculus removed from a tooth surface, had resulted in one piece of calculus being collected. The term ‘fragmented’ was assigned to specimens where, upon removal the dental calculus from the tooth surface had produced more than one piece of calculus. These terms were assigned to the deposits at the start of analysis and it is possible that some deposits, which were whole at the time of removal, had become fragmentary in transit from the site of collection to Cranfield University, although packaging methods were taken to minimise this risk (see section 6.5.1).

For each specimen, the dental calculus was carefully removed from the sample bag with plastic forceps and placed on a clean microscope slide. The deposits were then viewed using an Olympus SZX10 stereomicroscope fitted with a Watec WAT-202D digital colour camera. A set of photographs for each specimen was taken. A set consisted of photographs of the superficial and deep surfaces of each piece of dental calculus, each photographed at two different aperture settings. The superficial surface of the dental calculus refers to the side, which, was furthest from the enamel surface, and deep refers to the side closest to the enamel. Photographs were taken at the highest magnification possible for the size of the piece of calculus and a set resulted in four photographs

(example of a set shown in Figure 6.6.1). An ABFO No. 2 photomacrographic scale (graduated in millimetres) was included in each photograph.

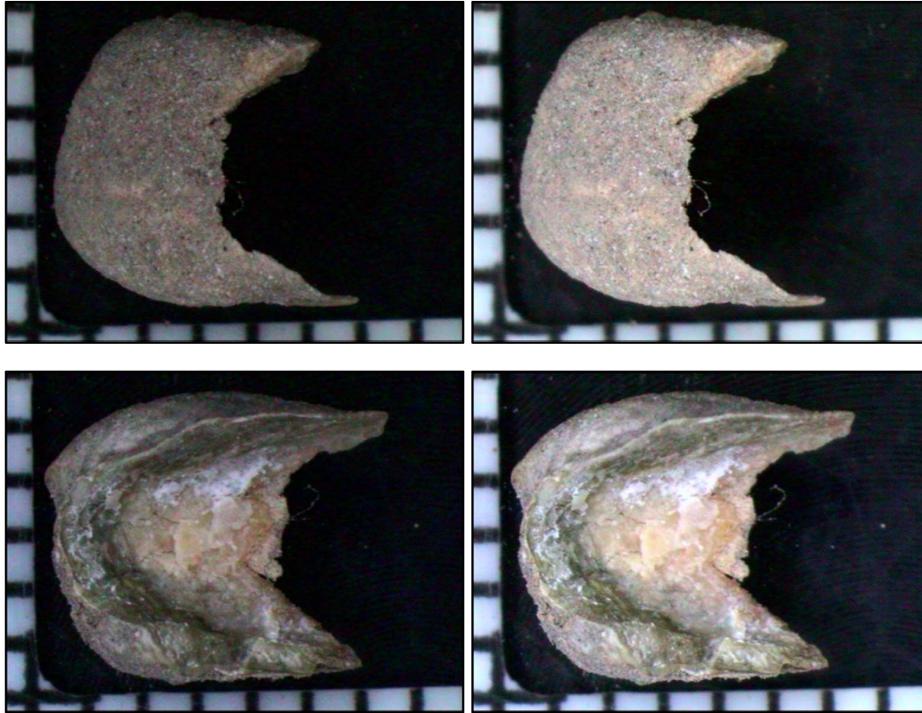


Figure 6.6.1 A set of microscope images of a whole specimen of dental calculus. Photographs show (clockwise from top left) the superficial side (smaller aperture); superficial side (larger aperture); deep side (larger aperture) and deep side (smaller aperture) (scale in millimetres) [Images taken by author (September 2014)].

For whole specimens, one set was taken due to there being one piece of dental calculus present. For fragmented specimens, a set of photographs was taken for each fragment of dental calculus present. Additionally, for fragmented specimens, a set was taken of all pieces of calculus at the same magnification. The magnification and aperture settings for each photograph were recorded with a designated image reference in Microsoft Excel 2010.

6.6.2 Nano-Computed Tomography

Data Acquisition

Prior to imaging by nano-computed tomography, specimens were weighed using a microbalance. For a fragmented specimen, each fragment was weighed and recorded separately and the fragments for that specimen were weighed together and recorded.

The acquisition of 3-D images of each piece of calculus was performed using a Nikon Metrology CT Scanner XT H 225 with a 180kV, 20W transmission target configuration (Figure 6.6.2). Due to the fragmentation of many of the specimens of dental calculus, with some fragments being very small and difficult to manipulate, a threshold of 0.002 g (2 mg) was applied.



Figure 6.6.2 Photograph of the Nikon Metrology CT Scanner XT H 225 in Cranfield Forensic Institute Analytical Laboratory [Image taken by author (September 2015)].

To mount the specimens, a low-density foam holder was made to allow multiple specimens to be set-up vertically for a batch of specimens to be run. The holder was inserted into a forceps-style sample stage and adjusted to align the mounted specimens as centrally as possible. The same method of mounting the specimens was used for all specimens (see Figure 6.6.3).

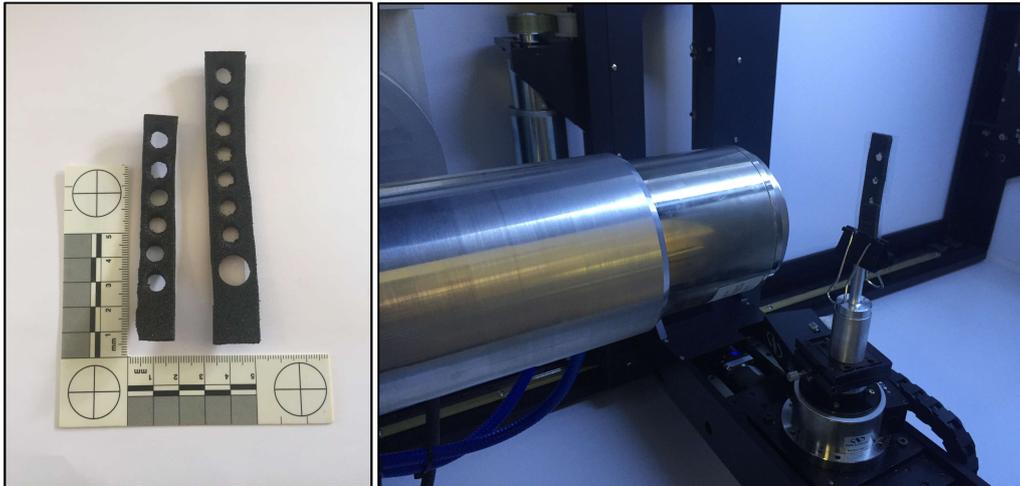


Figure 6.6.3 Photograph showing the low-density foam holder (left) and the holder with mounted specimens, held on the sample stage in front of the X-ray tube within the CT Scanner (right) [Image taken by author (September 2015)].

On acquisition of each computed tomography scan the specimen reference code, voltage, current, exposure time and number of projections were recorded in a Microsoft Excel spreadsheet; ‘Computed Tomography Log’. When acquiring nano-computed tomography images, set-up involved the following settings: 60 kV; 150 μ A; 1000 ms; 720 projections.

Data Reconstruction

Following acquisition and for all specimens, the data was manually reconstructed using 3-D Pro XTek Software. During this process, dual centres of rotation were found; where this could not be found automatically, they were determined using the ‘manual centre of rotation’ tool. Subsequently, beaming hardening reduction was set at ‘3’ and noise reduction was set at ‘1’. For each specimen, the centres of rotation; beam hardening and noise reduction was recorded in the ‘Computed Tomography Log’. Following this, the area of reconstruction was confirmed at 0° and 90° and the reconstructed file saved within the original data file.

Data Analysis

Using VGStudio Software (v. 2.2), the manually reconstructed data files were cropped to isolate the calculus specimen by removing excess air and the sample holder from the image file. The 3-D rendering of the specimen was registered so as the ‘front’ orientation had 2-D slices through the specimen from the superficial surface to the deep surface. Within the registration, the ‘right’ orientation had 2-D slices through the specimen from the mesial to distal edges of the specimen. Finally, the ‘top’ orientation had 2-D slices through the specimen from the top to the bottom of the specimen.

A manual surface determination was then applied and the volume of the determined specimen surface was acquired and noted in the ‘Computed Tomography Log’. The density histogram was adjusted so that a marker was placed at the peak of the specimen peak, to view the areas of the specimen with a higher density. Following this, defect detection algorithms within the software were run for both void detection and inclusion detection. The 2-D image slices that were exported from VGStudio were further viewed and analysed using ImageJ software.

During the analysis of the n-CT data, the specimens were categorised according to the density changes that could be observed (see section 7.3.2.1 for categories). The voxel sizes for each specimen were analysed to confirm that the data between batches was consistent. This ensured that the data regarding density changes across the specimens was not because of the differences in specimen set-up, particularly distance from the X-ray source. The statistics indicated there was no significant difference between the voxel sizes of the specimens that were assigned to each n-CT category (see *Supplementary Material: Appendix E.2.2*).

6.6.3 Scanning Electron Microscopy

The specimens that had been mounted in resin and sectioned by microtome were imaged using a Hitachi SU3500 scanning electron microscope with a Eucentric 5-Axis motorised stage (Figure 6.6.4) (see section 6.7 for specimen sectioning). Specimens were imaged using an electron beam of 30 kV at a pressure of 80 Pa in back-scattered electron (BSE) mode, which has a standard specimen resolution of 4.0 nm (see Hitachi Document HTD-E203P).



Figure 6.6.4 Photograph of the Hitachi SU3500 scanning electron microscope in Cranfield Forensic Institute Microscopy Suite [Image taken by author (November 2016)].

Specimens were attached to an SEM stub and levelled using Blu-tack to support the rounded resin block. The height of the surface to be imaged was then measured to adjust the sample stage to the correct height (Figure 6.6.5). The specimen chamber was closed and pumped down to vacuum.

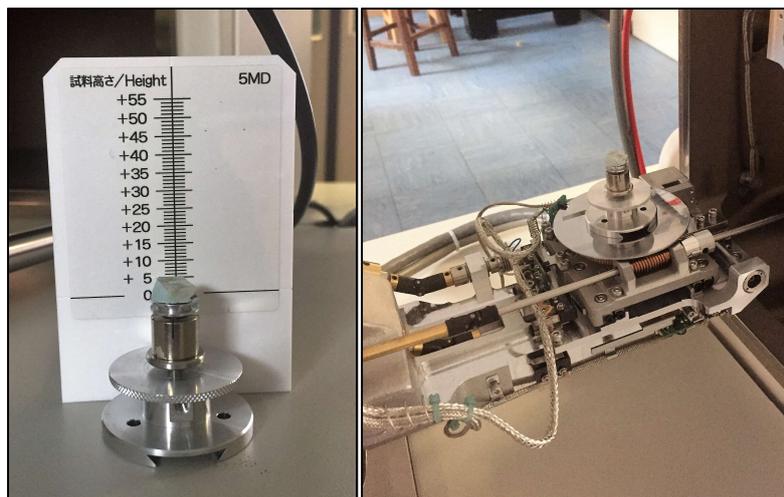


Figure 6.6.5 Photograph of the mounted sample (left) and the specimen stage in the scanning electron microscope (right) [Image taken by author (November 2016)].

An overall image was taken for each specimen and when the specimen was too large for one image, multiple overlapping images were taken to stitch together. Following this, the surfaces of the specimens were explored to determine if any features of interest could be identified. Features looked for included cracks and pores, inclusions and mineral crystals.

The scanning electron microscopy of cross-sectioned specimens of dental calculus unfortunately did not yield all the results desired. Due to the sectioning method, the diamond blade had left large striations that were visible on the exposed surface (Figure 6.6.6). The striations were orientated from the mesial to distal edges of the calculus, indicating the sectioning cuts were made from the superficial surface to the deep surface of the calculus. The presence of the blade striations on the cross-sections prevented the visualisation of any microfossils, starch granules or mineral crystal morphologies as the surface had been destructively altered during sectioning.

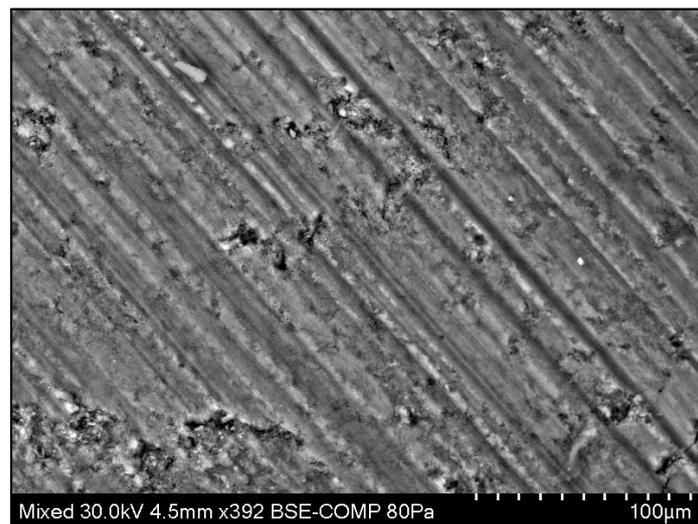


Figure 6.6.6 Scanning electron microscope image of the striations visible on the cross-sectioned surface of the dental calculus specimens from the diamond blade of the micro-slice.

6.6.3.1 Internal Fracturing

There were two types of fractures identifiable in the scanning electron microscopy analysis of sectioned specimens. Firstly, there were larger cracks that ran parallel to the deep or superficial surfaces and these cracks were approximately 5-10 µm wide and had varying lengths. Secondly there were smaller cracks, still with parallel edges, that radiated

out from both larger cracks and voids (Figure 6.6.7). These cracks were much narrower in width, approximately 1-2 μm , also with varying lengths.

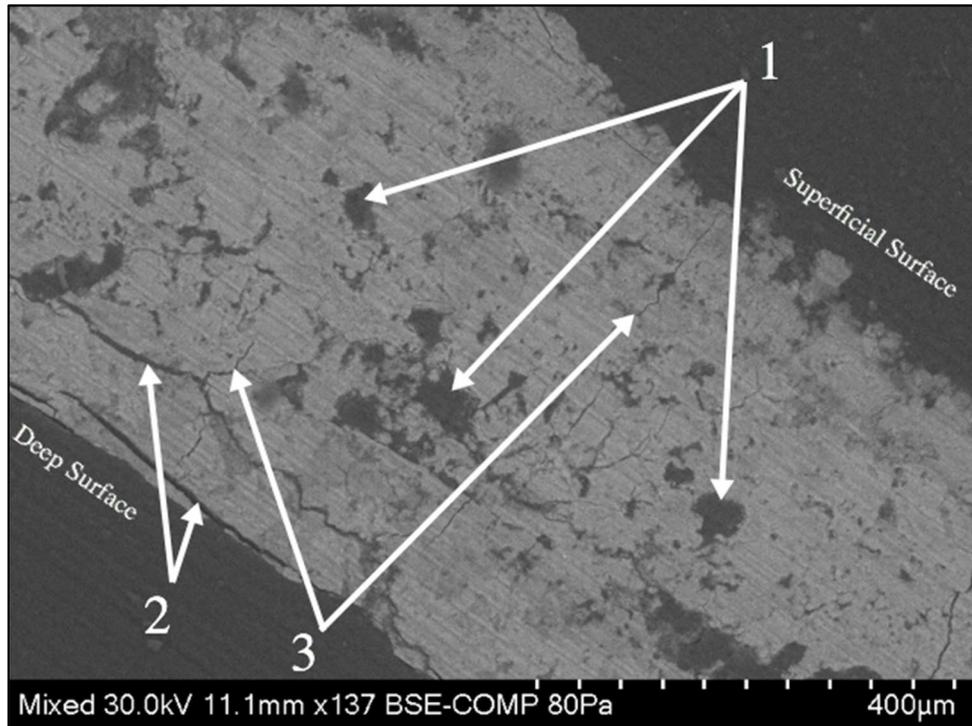


Figure 6.6.7 Scanning electron microscopy image of a specimen that shows the differences between voids and cracks observed in the dental calculus cross-sections; 1: voids (irregular edges); 2: larger cracks; 3: radiating cracks, (scale included).

The nano-computed tomography data was examined to determine if any of the fracturing could be determined to have been present in the whole specimens, before the sectioning method was applied. A difficulty with this was finding the exact location of the embedded cross-section in the n-CT data. Despite not being able to exactly compare cross-sectional slices, it was uncommon to find specimen cracks in the CT images. Figure 6.6.8 shows one of the CT slices where a possible crack can be observed and how this may relate to a crack observed in the SEM image.

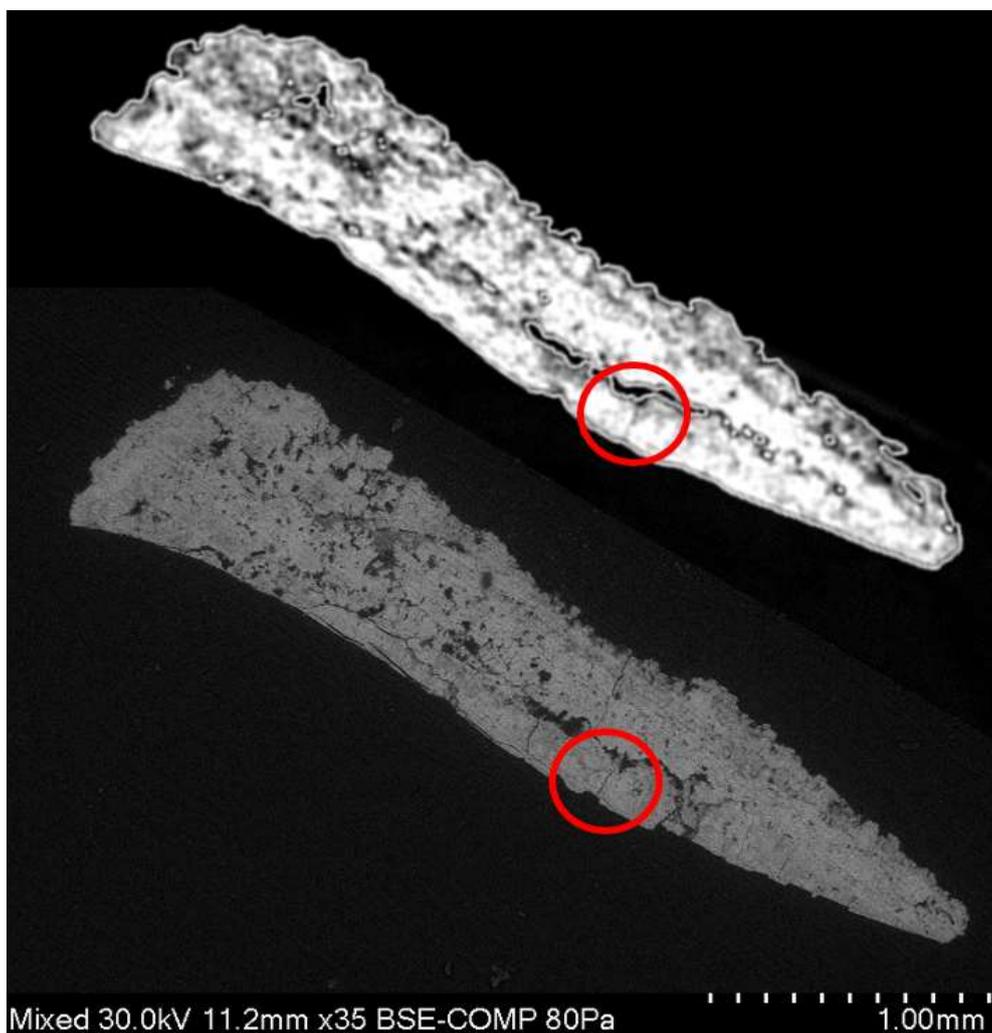


Figure 6.6.8 Images of a possible crack observed in the nano-CT data (top) and the corresponding observed crack seen in the SEM image (bottom) of the embedded cross-section of the same sample (the n-CT slice of the specimen is not an exact match to the cross-sectional surface exposed in the embedded specimen, but is as close as possible).

A couple of specimens exhibited cracks that extended through the specimen and into the resin (Figure 6.6.9). The extension of the crack into the resin indicated that they were not present before the specimens were embedded. Additionally, these cracks were not filled with resin, so it could be concluded that these most likely occurred, as the resin blocks, with embedded specimens, were unclamped from the micro-slice equipment.

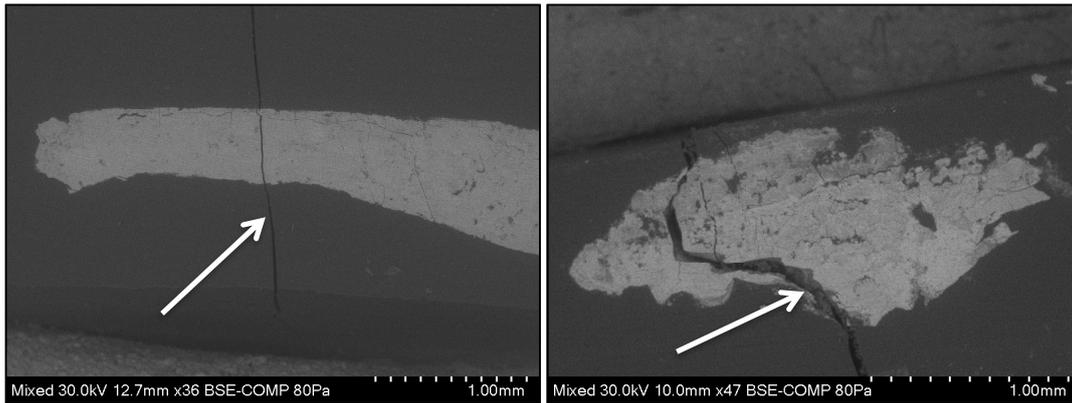


Figure 6.6.9 Scanning electron microscopy images showing the cracks that extended through the specimen and into the resin, indicated by the white arrow (scale included for each image).

Overall, the presence of large cracks being seen in specimens after the embedding process can be attributed to resin clamp. However, the origin of the small cracks within specimens cannot be determined post-removal. The possible presence of them in the n-CT data indicates that they were not because of embedding, however they may not have been present when the sample was in situ on the tooth. The cracking could have occurred during the sampling procedure or during transportation and handling. To test this, further, n-CT would need to be conducted on specimens that were still adhered to a tooth (see section 9.3).

6.7 Specimen Sectioning

In this study, microtome sectioning of dental calculus was employed to achieve a smooth cross-sectional surface, which was primarily required for micro-beam X-ray diffraction analysis to be performed. The development of the embedding and sectioning of the specimens for this study was carried out in collaboration with Debbie Martin and Andrew Griffiths at the Histology Services Unit, University of Bristol.

6.7.1 Wax Embedding Testing

It was initially intended to embed the specimens in wax and section these into multiple thin slices to have sequential cross-sections that could be analysed. Testing was carried

out using dental calculus specimens from Cranfield University that had been collected for a previous study.

The test specimens were transferred through an ascending concentration of ethanol up to 100% ethanol over several hours. The length of time that this was carried out was dependant on size of sample. Following this, the specimens were immersed in Histo-clear, a histological clearing agent, and left overnight.

The specimens were then infiltrated in Fibrowax in an oven at 56 °C and during this process, the wax was replaced three times. Following this, each infiltrated specimen was placed in a mould containing molten Fibrowax and covered with a processing cassette. This cassette was then topped up with more Fibrowax. Once a skin had formed over the wax surface, the mould was then lowered into a vessel of cooled water and submerged. Once the block had solidified and cooled it was removed from the water and placed on a cold plate, then the wax block was removed from the mould.

This specimen preparation was tested and sections were achieved of between 5 and 20 microns. However, it was found that the embedding wax did not properly infiltrate the dental calculus and the resulting sections were too fragile to successful analyse.

6.7.2 London Resin Embedding

Following the above wax embedding tests, the feasibility for such thin sections to be cut was discussed. Difficulties with the brittle nature of the dental calculus meant that thinner sections had more chance of fracturing, losing the desired cross-sectional surface. In addition, the micro-beam X-ray diffraction required a specimen thickness of at least 50 microns and the more sections cut the more time-consuming the process. Considering the timescale of the project and the availability of the Histology Services Unit, it was decided that one cut through each specimen would be best, to provide cross-sections through the widest part of the specimen.

The specimen widths were determined using the 2-D slices from the nano-computed tomography data. Using this data and ImageJ software, the width of the specimens from the superficial to the deep surfaces was determined at ten points throughout the specimen. The approximate position of the widest point and therefore the largest cross-section, was

marked on a 3-D image of each specimen for the histology technicians to be guided as to where the specimen should be cut. To fix the specimens, resin embedding was employed.

Initially, the dental calculus specimens were dehydrated using ascending ethanol dilutions to 100%, the time taken was dependant on the size of the deposit. The specimens were then transferred to 50:50 solution of LR White acrylic resin and pure ethanol for 2 hours. This solution was replaced with LR White twice for an additional hour per refill. Following this, the specimens were placed in an embedding mould of resin and covered with a sheet of parafilm to minimise air contact. The mould was then placed into a staining dish with the lid sealed overnight.

The resulting resin blocks were removed from the mould and placed under a dissecting microscope to identify the required plane of cut, from the diagrams provided. The desired cut line was then marked onto the surface of the resin with permanent marker. The resin block was then clamped into a specimen head and cut part way through using a Cambridge Microslice 2, which uses a revolving diamond tipped circular blade cooled with running water. As it was not possible to cut all the way through the blocks because of the specimen clamp, the second cut was done on a Dremel Moto-Saw.

A test was carried out on whether the resulting cross-sections should undergo polishing to increase the smoothness of the surface. The two halves of a specimen, one half having been polished and other left as cut, were analysed by micro-beam X-ray diffraction. The resulting data showed no increased benefit for the polished surface and therefore due to time efficiency, it was decided that polishing would not be carried out.

6.8 Mineralogical Analysis

6.8.1 Powder X-ray Diffraction

Specimen Preparation

Powder X-ray diffraction was performed on powdered specimens that were placed on silicon discs. These discs were used because of their low background scatter properties. For each dental calculus deposit, the specimen was powdered using an agate micro pestle and mortar and transferred to a silicon disc. For each specimen, the edge of a clean glass

microscope slide was used to carefully push the powder to the centre of the disc. The gathered powder was then gently flattened with the face of the glass slide to create an even layer of powder. The silicon disc was then secured in a back-loaded sample holder and placed in the batch holder. Three specimens were prepared for each batch.

Between preparations of each specimen, the pestle and mortar was cleaned with ultra-pure water and acetone and left to air dry. Nitrile gloves were changed between handling of each specimen and a clean microscope slide was used for each preparation. Following data collection, specimens were transferred to clean dry glass vials and sealed in their original specimen bags. The silicon discs were then cleaned with ultra-pure water and a drop of acetone and left to dry in air. In addition, to minimise any contamination between sample groups, the silicon discs were sonicated for 30 minutes at 40 °C after data acquisition of the specimens from each population.

Data Collection

Powder X-ray diffraction analysis of all powdered dental calculus specimens was carried out using a PANalytical X'Pert Pro diffractometer with Cu K α radiation (approximately 15 Å) (Figure 6.8.1). Each specimen was subjected to two programs of data collection, 'Full' and 'OCP'. For the 'Full' program, the X-ray conditions were set at 40 kV and 40 mA and the detector collected data as stepped scans across an angular range of 10 – 80 °/2 θ (4.43 – 0.78 Å d-spacing). The count time at each step was 700 s, with a 0.0131 °/2 θ step size. To determine the presence of octacalcium phosphate, the 'OCP' program was performed under the same conditions but across an angular range of 4.2 – 5.5 °/2 θ (10.51 – 8.50 Å d-spacing).



Figure 6.8.1 Photograph of the PANalytical X'PertPro diffractometer situated in the Cranfield Forensic Institute X-ray Diffraction Laboratory. [Image taken by author (September 2015)].

Each silicon disc was assigned a letter (A-C) and blank acquisitions were run in both the 'Full' and 'OCP' programs for each disc. The letter of each disc was included in the data file name of specimen data collections. There were no peaks identifiable in the blank runs for both the full angular range (10-80°) and the OCP range (4.2-5.5°). As these were performed before each group of specimens it was concluded that there had been no contamination between populations.

Data Analysis

Characterisation of peaks was performed using Crystallographica Search-Match v.2.1.1.1. The following mineral phase database cards from the International Centre for Diffraction Data (ICDD) were used: Hydroxylapatite (9-432); Whitlockite (9-169); Quartz (46-1045); Calcite (5-586).

Occasional noise peaks were visually identified in the data. These were characterised by a sharp line peak. Noise peaks were removed prior to data analysis by taking the average of the adjacent peaks on either side. For each diffractogram, 'whole pattern' fitting was carried out using Topas (Bruker-AXS) v.4.1 and diffractogram stacks were compiled using EVA (Bruker-AXS v.14.0).

There are associated errors related to this pattern fitting analysis and as such mineral phases that were detected below 0.5% were not positively identified. In addition, although the lattice parameters were reported, due to the poorly crystalline nature of the material being analysed, these should also be treated with caution as they can have an error of between 0.001-0.018 Å (see Beckett 2009).

6.8.2 Micro-beam X-ray Diffraction

Specimen Preparation

Two dental calculus specimens that had undergone the embedding and sectioning process outlined in section 6.7 were analysed by micro-beam X-ray diffraction (μ -XRD). Embedded specimens were positioned on the mounting block with the exposed cross section facing up (Figure 6.8.2). A small foam block was placed beneath each specimen to ensure the exposed surface was at the correct height and to ensure there was no slippage during data acquisition. Specimens were orientated to ensure the X-ray beam would collect data from the deep surface of the dental calculus to the superficial surface.

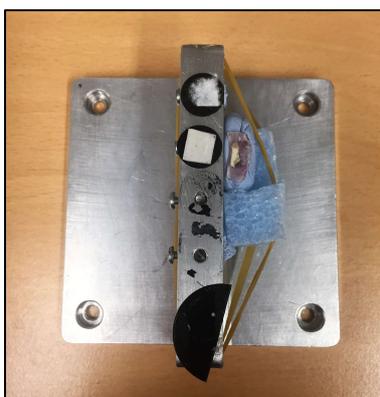


Figure 6.8.2 Photograph of the mounting block with a secured specimen for the micro-beam X-ray diffraction analysis.

Data Collection

Micro-beam X-ray diffraction analysis was carried out using a Bruker D8 Discover micro-beam X-ray diffractometer with Cu K α radiation (1.54 Å) (Figure 6.8.3). For each specimen, a linear path from the deep surface to the superficial surface of the dental calculus specimen was chosen to collect data from (Figure 6.8.4). Along this path, 100

μm steps were taken and at each step, data was collected for 1 hour in reflectance mode and the beam condition were set at 40 kV and 40 mA. Data was collected using a General Area Detector Diffraction System (GADDS) and a 50 μm monochromator collimator was used to collect data over an angular range of approximately $20\text{-}55^\circ/2\theta$ (2.25-0.94 \AA d-spacing). Prior to data collection, beam calibration was performed using an aluminium oxide standard (NIST CAS Registry Number 1344-28-1).



Figure 6.8.3 Photograph of the Bruker D8 Discover micro-beam X-ray diffractometer situated in the Cranfield Forensic Institute X-ray Diffraction Laboratory [Image taken by author (September 2015)].

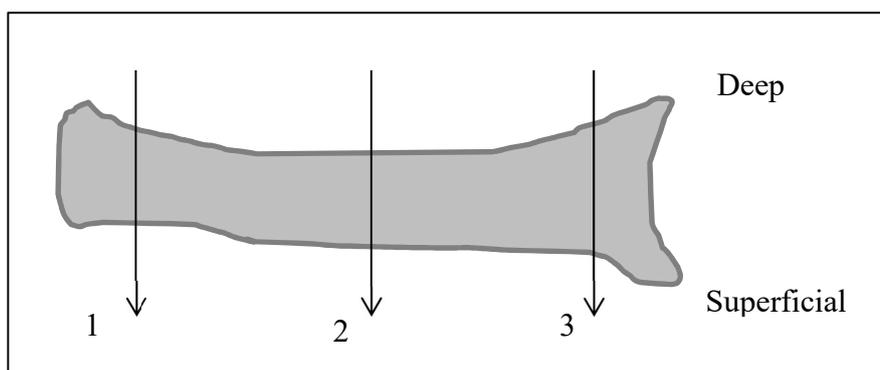


Figure 6.8.4 Diagram showing the linear paths along which micro-beam X-ray diffraction data was collected for a specimen of dental calculus; the arrow direction indicates the direction of the beam during data collection.

Data Analysis

The data for each line collection was exported into separate RAW files. For each diffractogram within each line file, 'whole pattern' fitting was carried out using Topas (Bruker-AXS) v.4.1. Diffractogram stacks were compiled using EVA (Bruker-AXS v.14.0).

6.9 Elemental Analysis

6.9.1 Inductively-Coupled Plasma Mass Spectrometry (Solution)

A sub-sample of ten dental calculus specimens from each population were analysed by quantitative elemental analysis using solution inductively-coupled plasma mass spectrometry (ICP-MS(Sol)).

Specimen Preparation

To prepare the solutions required, the powdered specimens in Eppendorf tubes were dissolved in 1 ml of hydrochloric acid (0.5 M), and left overnight. The following day, the HCl supernatant was pipetted out into clean Eppendorf tubes³³. From the extractions, 0.5 ml of the HCl supernatant was auto-pipetted, using a Thermo Scientific Finnpiquette (10 - 1000 μ l), into a clean 50 ml specimen tube. The supernatant was then diluted with 49.5 ml Ultra-Pure Water (from a Millipore Direct-Q 3 filtration system), which was auto-pipetted, using a Thermo Scientific Finnpiquette (1 - 10 ml). This resulted in a 50 ml stock solution, consisting of diluted supernatant solution (dilution factor (D_f) = 100).

Subsequently, for each specimen an aliquot of the stock solution was further diluted for analysis. Into a clean 10 ml specimen tube, 5 ml of stock solution was auto-pipetted, using a Thermo Scientific Finnpiquette (50-500 μ l) and added to this was 4.9 ml of nitric acid (1%). Additionally, an internal standard was added consisting of 0.1 ml of gallium (1

³³ This process left behind starch and microfossil inclusions from within the dental calculus specimens. These were washed twice with ultra-pure water, left to dry and retained for future work, due to the timescale of this project (see section 5.4.1).

ppm) in nitric acid (1%). This resulted in 10 ml of diluted supernatant solution (dilution factor (D_f) = 200). The tubes were lidded and inverted twice to mix the solution.

A set of standard solutions were prepared for each of the three acquisition runs, a calcium phosphate standard; iron standard; and a multielement standard. Standards of calcium phosphate solution were prepared consisting of 0.25, 0.5 and 1 ppm. In addition, multielement standard solutions were prepared at 0.15, 0.25 and 0.5 ppm and iron at 0.5, 0.75 and 1 ppm. The standards were also matrix-matched to the prepared specimen solutions by the addition of 0.5 ml hydrochloric acid (0.5 M).

Data Collection

The prepared solutions were analysed using a Thermo X Series II ICP-MS with a Cetac ASX-520 auto-sampler. The detection limit for this equipment varies for each element from 4×10^{-5} to 2 ppb (See Thermo Fisher Document AN_40854). The ICP-MS ran in standard mode with Argon carrier at (>80 psi). Each solution was analysed in triplicate, with each analysis consisting of 100 main sweeps with 20 ms dwell time each for element, with a total acquisition time for each run of 8 secs.

Following the first calcium phosphate run, it was found that some solutions were too concentrated for the prepared standards. Consequently, solutions were further diluted down to the relevant concentration. A list of the specimens and their final dilution factors can be found in *Supplementary Material: Appendix E.4.1*.

Data Analysis

The resulting data was adjusted for each solutions dilution factor and the concentration was calculated from the counts per second using Thermo Scientific PlasmaLab software by Dr Fiona Brock (Cranfield University). Further analysis of the results was carried out using Microsoft Excel 2016.

Unfortunately, the second standard in the multi-element run was incorrect, even after a second preparation. It was therefore excluded from the standard calibration plot and only standard one and three were used to calculate the concentrations of the multi-element acquisition. In addition, for this standard, the nickel and the zinc within the solution did not produce counts within an acceptable range, which may be due to the age of the

standard solution. Unfortunately, these elements therefore had to be excluded from the results.

Additionally, in the iron run, the third standard did not produce the correct concentration and was excluded from the standard calibration plots. The iron concentrations were still able to be calculated from the remaining two standard solutions. The standard calibration plots for all acquisition runs are included in *Supplementary Material: Appendix E.4.1*.

6.9.2 Energy-Dispersive X-ray Analysis

Specimens that were imaged using scanning electron microscopy (see section 6.6.3) were also analysed by energy dispersive X-ray analysis during SEM imaging. The analysis was performed using an Ametek energy dispersive X-ray spectrometer (Figure 6.9.1), which has elemental detection limits of between 1000 and 5000 ppm (EDAX 2015).



Figure 6.9.1 Photograph of the EDX spectrometer configuration (circled) on the Hitachi SU3500 scanning electron microscope in Cranfield Forensic Institute Microscopy Suite [Image taken by author (November 2016)].

Three areas on each specimen were marked out for point analysis and data was collected for 300 secs at 30 kV. The areas analysed were the deep, central and superficial thirds, as indicated on Figure 6.9.2. The peaks of the resulting spectra were identified using the in-built TEAM software package and the resulting reports were exported to Microsoft Word (2016).

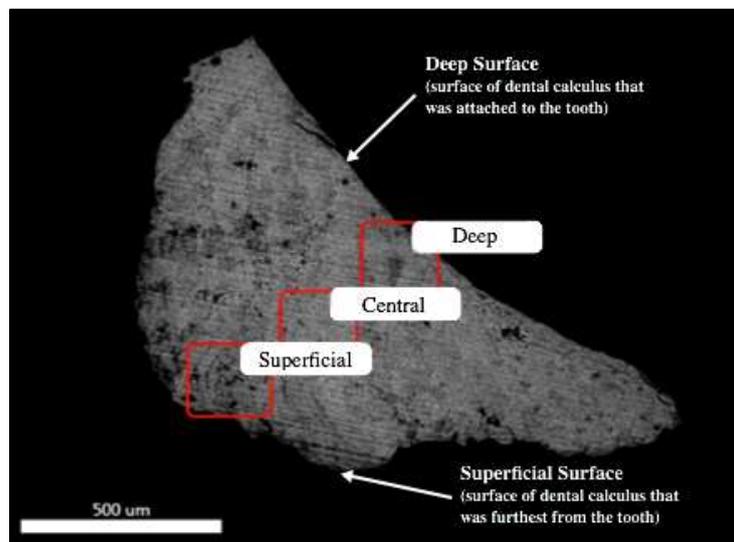


Figure 6.9.2 Labelled scanning electron microscope image indicating an example of the three areas that were analysed for semi-quantitative elemental composition for each specimen.

6.10 Chapter Summary

It is intended that this chapter has provided the reader with a clear understanding of the materials and methods that have been used in this research. This information is the specific detail on how experimental work was carried out and the data was collected. It is intended that the sequence of data collection has been clear and this is summarised below in Figure 6.10.1. In the next chapter, the results of the dental recording and the dental calculus analysis are compiled.

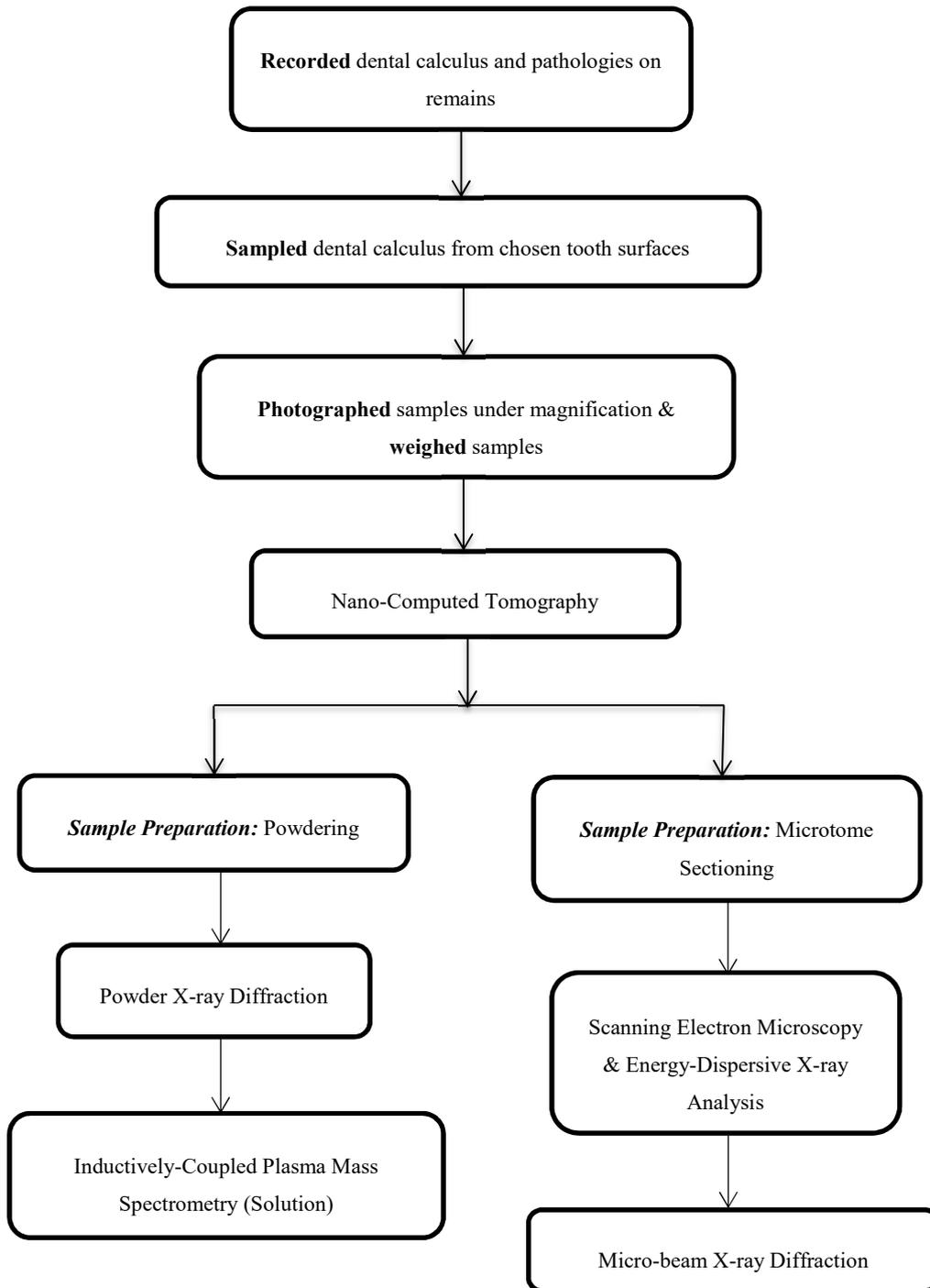


Figure 6.10.1 Diagram showing the workflow of the stages of recording, sampling and analysis performed in this study on specimens of archaeological dental calculus.

CHAPTER 7: RESULTS

A substance which accumulates on the surface of the teeth, and which becomes when left there, a stony crust of more or less considerable volume

- Pierre Fauchard 1728

7.1 Overview

This chapter presents the results of the archaeological dental calculus analysis performed for this thesis (all additional tables and graphs can be found in *Supplementary Material: Appendix E*). The results detail the analysis of archaeological dental calculus from three populations; the Capuchin Catacombs; Cementeri Vell; and San Agustín (see Chapter 3).

The chapter begins with the results of the in-situ recording that was performed on the remains prior to removal of any dental calculus specimens (section 7.2). Where relevant, results have been reported for all individuals in each population and presented separately for just the calculus-exhibitors of the populations. Following on, the imaging results are presented in section 7.3. Subsequently, the mineralogical and elemental analyses are presented separately (section 7.4 and 7.5 respectively). For each of these sections, the results are reported separately for bulk and cross-sectional analysis.

Overall, these results present a comprehensive analysis of the physical characteristics and the mineral and elemental composition of archaeological dental calculus from three populations.

7.2 In-Situ Dental Recording

7.2.1 Tooth Presence

The values for MNI, T_{EXP}, S_{EXP}, T_{OBS}, S_{POT}, S_{OBS}, were calculated for the whole sample of each population (Table 7.2.1). Also, reported in Table 7.2.1 are these values for only the calculus-exhibitors in each population. These values were used to calculate T% and S%, the methods for these calculations are explained in section 6.4.2.

When considering each population sample, the percentages of teeth present (T%) were similar for all populations (Table 7.2.1). Even so, T% for the Capuchin Catacombs (28%) and Cementeri Vell (29%) were similar and lower than T% for San Agustín (35%). In both the Capuchin Catacombs (CC) and Cementeri Vell (CV), there was a mix of male and female individuals; additionally, CV contained individuals of unknown sex. Within CC, there was a difference in T%, between male (25%) and female individuals (40%). Within CV, T% for males (20%) was similar to females (21%) and both male and female percentages were lower than individuals of unknown sex (34%). There were only male individuals in San Agustín (SA).

For all individuals in each population, the percentage of surfaces that were observable (S%) was similar for CV (94%) and SA (91%), however much lower for CC (65%). In CC, S% for male individuals (62%) was lower than for females (73%), however, in CV the opposite was true (female: 74%; male: 91%). In addition, CV individuals of unknown sex (97%) had the highest S%. As stated above, there were no individuals of female or unknown sex in SA, therefore S% for the whole population represents male individuals (91%).

In terms of T% for calculus-exhibitors, the percentages between populations were similar for the Capuchin Catacombs (35%) and Cementeri Vell (28%) but much higher for San Agustín (61%). Additionally, SA calculus-exhibitors (61%) had a far greater percentage of present teeth than the overall population (35%). The T% for CC calculus-exhibitors (34%) was also higher than for the whole population (28%), however for CV, the percentage for calculus-exhibitors (28%) was similar to all individuals (29%).

For each population, S% for the dentition of calculus-exhibitors was similar to S% for the dentition of all individuals. Cementeri Vell (94%) and San Agustín (92%) had high percentages of observable surfaces. In comparison, calculus-exhibitors from Capuchin Catacombs (61%) had a much lower percentage of observable surfaces.

		Capuchin Catacombs, Sicily				Cementerí Vell, Formentera				San Agustín, La Rioja			
		M	F	?	T	M	F	?	T	M	F	?	T
		<i>All Individuals</i>											
MNI _(TOT)		102	24	0	126	26	5	51	82	31	0	0	31
<i>Teeth</i>	T _{EXP}	3264	768	0	4032	832	160	1632	2624	992	0	0	992
	T _{OBS}	812	303	-	1115	171	34	559	764	350	-	-	350
	T%	25	40	-	28	20	21	34	29	35	-	-	35
<i>Surfaces</i>	S _{EXP}	10608	2496	-	13104	2704	520	5304	8528	3224	-	-	3224
	S _{POT}	2978	1050	-	4028	638	122	2006	2766	1110	-	-	1110
	S _{OBS}	1834	769	-	2603	580	90	1936	2606	1011	-	-	1011
	S%	62	73	-	65	91	74	97	94	91	-	-	91
		<i>Calculus-Exhibiting Individuals</i>											
MNI _(CALC)		65	13	0	78	11	0	17	28	19	0	0	19
<i>Teeth</i>	T _{EXP}	2080	416	0	2496	352	0	544	896	608	0	0	608
	T _{OBS}	654	191	-	845	85	-	164	249	327	-	-	327
	T%	31	46	-	34	24	-	30	28	61	-	-	61
<i>Surfaces</i>	S _{EXP}	6760	1352	-	8112	1144	-	1768	2912	1976	-	-	1976
	S _{POT}	2378	662	-	3040	316	-	582	898	1038	-	-	1038
	S _{OBS}	1429	437	-	1866	281	-	560	841	954	-	-	954
	S%	60	66	-	61	89	-	96	94	92	-	-	92

Table 7.2.1 Table showing the teeth and surfaces that were observable in relation to the expected teeth and surfaces for the minimum number of individuals in each population. Results are presented for all individuals and separately for individuals who exhibited calculus in each population sample.

In terms of the distribution of present teeth by tooth type, there were no major differences between the pattern of present teeth found for all calculus-exhibitors compared to all individuals (Figure 7.2.1).

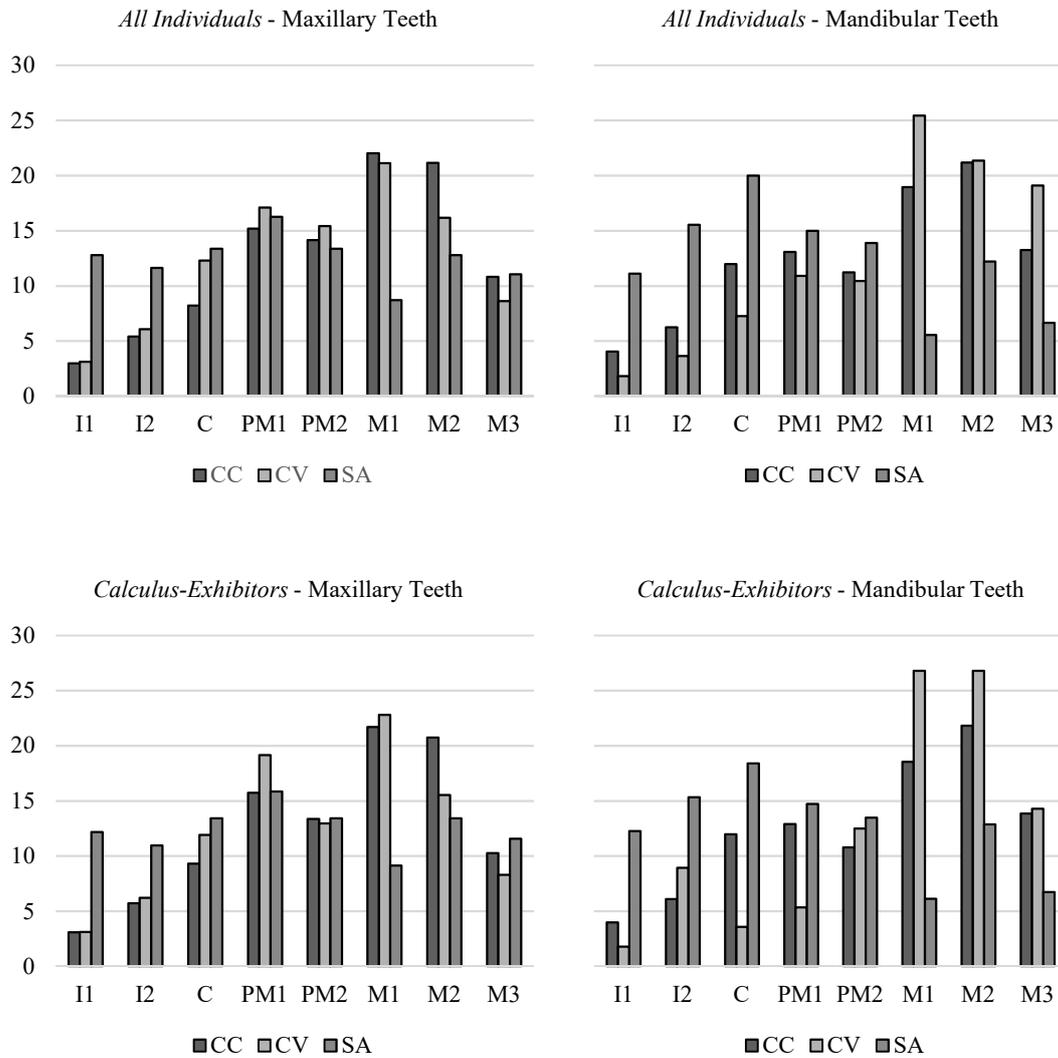


Figure 7.2.1 Graphs showing the distribution of teeth present by tooth position in the maxillary (left) and mandibular (right) jaws. Results are presented according to each population for all individuals (top) and separately for calculus-exhibitors (bottom).

In general, the percentages of anterior teeth increased from first incisors to canines for both maxillary and mandibular teeth. This trend was seen in the maxillary teeth for all

populations and mandibular teeth for the Capuchin Catacombs and Cementeri Vell. For San Agustín, mandibular teeth, a higher percentage of second incisors were seen in comparison to other anterior teeth (Figure 7.2.1). For premolar teeth in all populations there were more first than second premolars in the maxilla, however for the mandible the opposite was observed.

In terms of molar teeth, there were more first and second molars present in both jaws compared to all other tooth types in the Capuchin Catacombs. In Cementeri Vell, this was also the case for the mandibular teeth but for maxillary teeth, the percentage of second molars was lower than first premolars. In San Agustín, the percentages of present molar teeth were lower than anterior teeth, with canines in both jaws being the most abundant tooth type (Figure 7.2.1).

7.2.1.1 Tooth-Loss (*post-mortem and ante-mortem*)

When all individuals were considered (Table 7.2.2), the Capuchin Catacombs (CC) (37%) and Cementeri Vell (CV) (38%) had similar amounts of post-mortem tooth loss (PMTL). In contrast, San Agustín (6%) had a far lower percentage of teeth lost post-mortem. For CC, there was a difference in PMTL between male (41%) and female (25%) individuals, however for CV all groups showed similar percentages for male (41%), female (41%) and slightly lower for unknown (36%) individuals.

When comparing ante-mortem tooth loss (AMTL) between populations, for all individuals, San Agustín (SA) (53%) had the highest percentage. Cementeri Vell (36%) had a much lower percentage than SA, with the Capuchin Catacombs (26%) being lower still. There were minimal differences between the percentages of AMTL between male and female individuals in both CC (25%, 31% respectively) and CV (26%, 24% respectively), although individuals of unknown sex (43%) in CV had a higher percentage than other individuals.

For calculus-exhibitors in each population, the percentages of PMTL were not dissimilar to values for the whole population (Table 7.2.2). However, percentages of AMTL for calculus-exhibitors were much lower than the total population for all populations, Cementeri Vell (9%), Capuchin Catacombs (17%) and San Agustín (31%). Again, there was little difference between male (16%) and female (18%) AMTL, in the Capuchin

Catacombs calculus-exhibitors and in Cementeri Vell, unknown sex (19%) individuals showed greater AMTL than male (7%) calculus-exhibitors.

		Capuchin Catacombs, Sicily				Cementeri Vell, Formentera				San Agustín, La Rioja			
		M	F	?	T	M	F	?	T	M	F	?	T
		<i>All Individuals</i>											
P _{OBS}		2531	760	0	3291	561	83	803	1447	866	0	0	866
PMTL	P _{PMTL}	1038	190	-	1228	229	34	288	551	51	-	-	51
	P _P (%)	41	25	-	37	41	41	36	38	6	-	-	6
AMTL	P _{AMTL}	625	239	-	864	145	20	343	508	456	-	-	456
	P _A (%)	25	31	-	26	26	24	43	36	53	-	-	53
		<i>Calculus-Exhibitors</i>											
P _{OBS}		1765	411	0	2176	192	0	43	235	533	0	0	533
PMTL	P _{PMTL}	773	128	-	901	80	-	12	92	36	-	-	36
	P _{PMTL} /P _{OBS} (%)	44	31	-	41	42	-	28	39	7	-	-	7
AMTL	P _{AMTL}	285	76	-	361	13	-	8	21	163	-	-	163
	T _{AMTL} /P _{OBS} (%)	16	18	-	17	7	-	19	9	31	-	-	31

Table 7.2.2 Table showing the number of observable tooth positions (P_{OBS}) for each population with the number of positions exhibiting post-mortem tooth loss (P_{PMTL}) and the number of positions exhibiting ante-mortem tooth loss (P_{AMTL}). Results are presented for all individuals in each population sample and individuals who exhibited calculus in each population sample.

In terms of the general distribution of PMTL by tooth type, the pattern of teeth lost for all calculus-exhibitors was similar compared to all individuals (Figure 7.2.2). However, in the Cementeri Vell and San Agustín calculus-exhibitors, there were some mandibular tooth positions that were not represented by alveolar positions that exhibited post-mortem

tooth loss (second incisors and canines for San Agustín and posterior tooth positions for Cementeri Vell see Figure 7.2.2-bottom right). For all populations, the general trend was that PMTL decreased from anterior teeth to posterior teeth in both jaws (Figure 7.2.2)

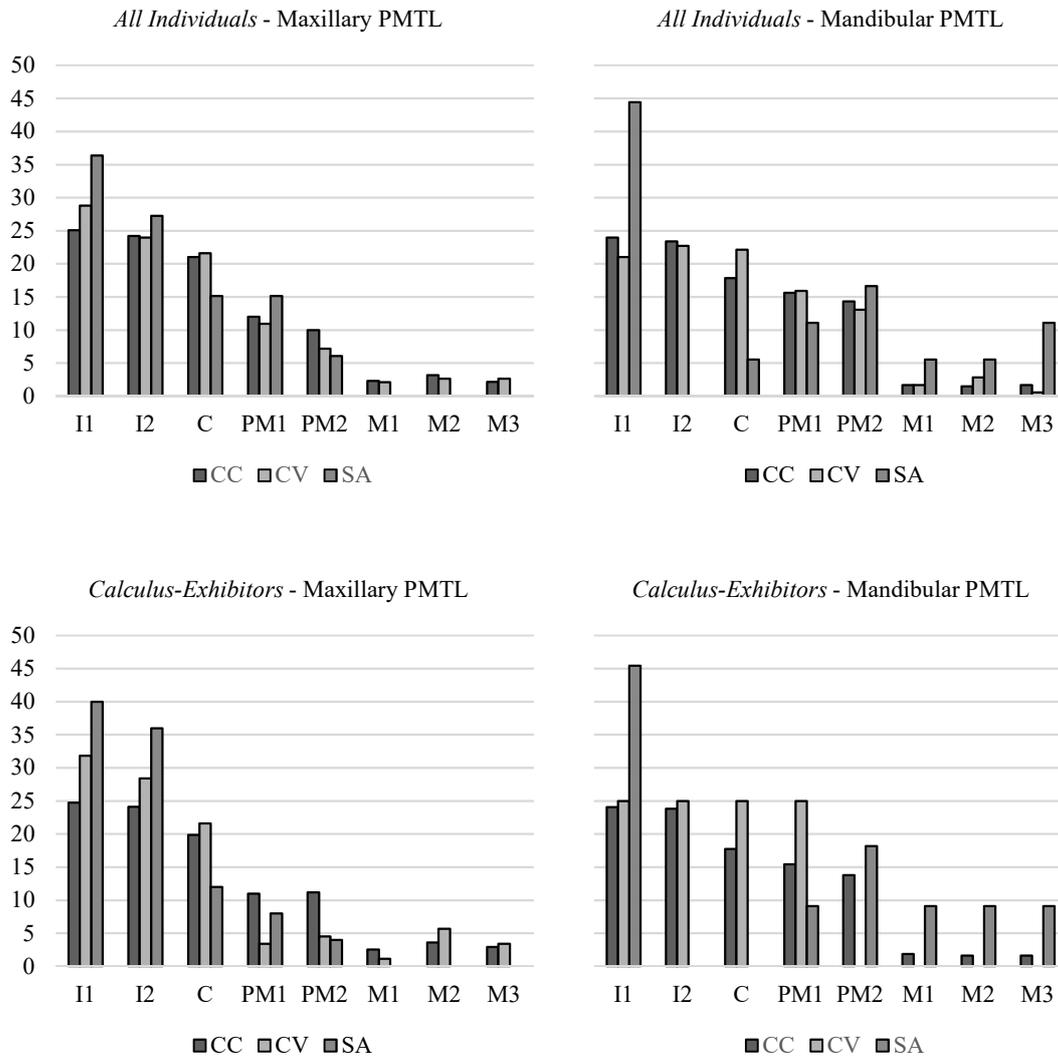


Figure 7.2.2 Graphs showing the distribution of post-mortem tooth loss by tooth position in the maxillary (left) and mandibular (right) jaws. Results are presented according to each population for all individuals (top) and separately for calculus-exhibitors (bottom).

For the general distribution of AMTL by tooth type, the general trends were more obvious in the calculus-exhibitor sub-groups of each population. When compared to all individuals (Figure 7.2.3).

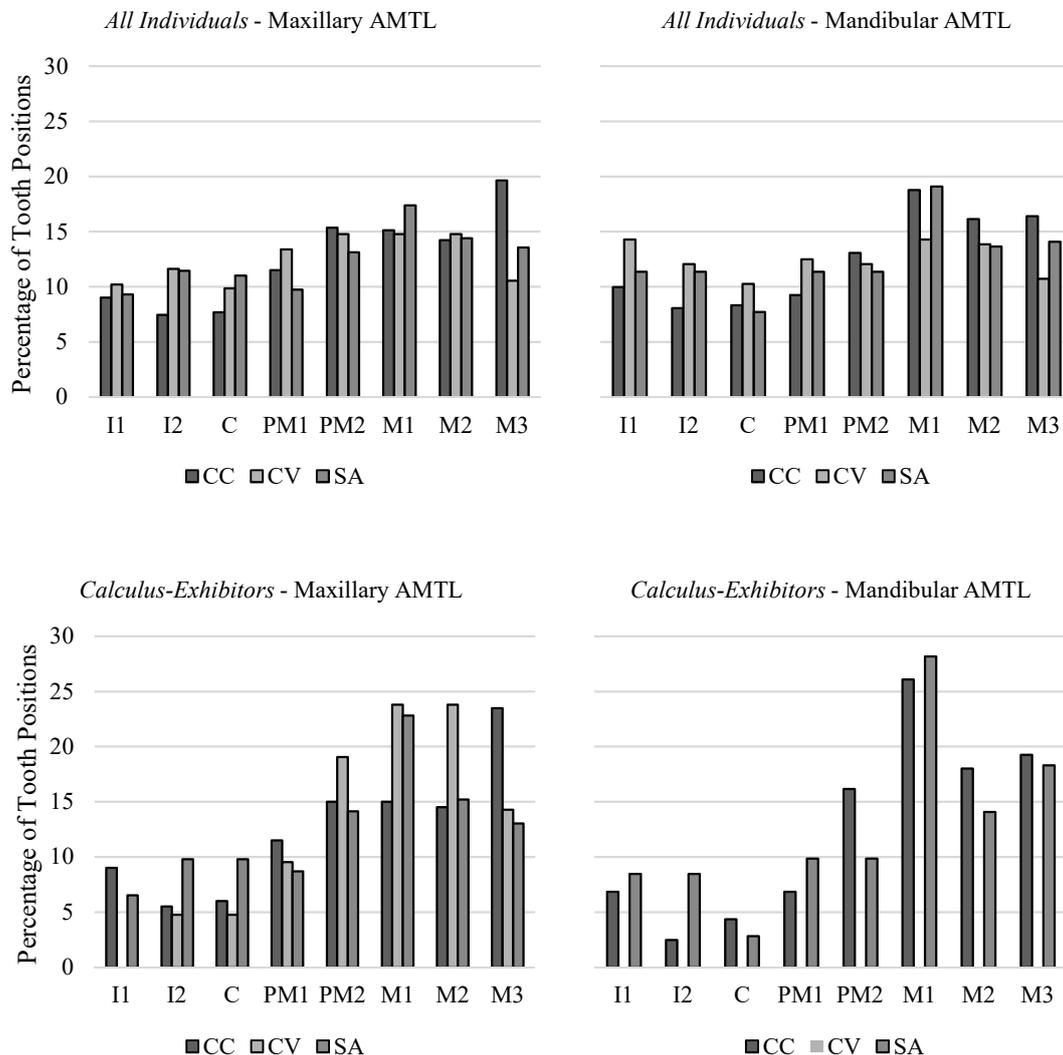


Figure 7.2.3 Graphs showing the distribution of ante-mortem tooth loss by tooth position in the maxillary (left) and mandibular (right) jaws. Results are presented according to each population for all individuals (top) and separately for calculus-exhibitors (bottom).

For all individuals, there was a slight trend of increasing percentage of maxillary tooth positions that showed AMTL from anterior to first molar positions (Figure 7.2.3). This trend dropped off for the second and third molars in Cementeri Vell and San Agustín. For calculus-exhibitors, this trend was more evident in all populations. The distribution of AMTL in mandibular teeth for all individuals was similar to maxillary teeth. However, the percentage of lower incisors did not fit this trend. Again, the trends seen were more

obvious for the calculus-exhibitors sub-group for the Capuchin Catacombs and San Agustín. There were no mandibular tooth positions that exhibited AMTL in Cementeri Vell in the calculus-exhibiting individuals (Figure 7.2.3).

7.2.2 Dental Calculus

Across the three populations it was found that the percentage of minimum number of individuals with dental calculus for the Capuchin Catacombs (62%) was similar to that of San Agustín (61%). In contrast, a lower percentage of individuals were affected by dental calculus in Cementeri Vell (34%). There was a small difference in the percentages of male (64%) and female (54%) individuals affected in the Capuchin Catacombs and male (42%) and individuals of unknown sex (33%) in Cementeri Vell. There were only male individuals (61%) in San Agustín (See Table 7.2.3).

For all individuals and in terms of the percentage of teeth affected, there was a similar pattern to individuals affected per population. Affected teeth in the Capuchin Catacombs (35%) were of a similar percentage to San Agustín (34%). In contrast to the percentage of individuals affected, the percentage of teeth affected for Cementeri Vell (28%) was not vastly lower than the other two populations, but still had the least affected teeth of the three populations (See Table 7.2.3 - *All Individuals*).

Within the total population of the Capuchin Catacombs there was a small difference in the percentage of teeth affected between male (37%) and female (29%) individuals, and the female percentage was lower. Similarly, for all individuals in Cementeri Vell, there was little difference between the percentage of male (32%) and unknown sex (29%) teeth affected. In San Agustín, where there were only male individuals present, the percentage of teeth affected for all individuals (34%) was in between the values for the Capuchin Catacombs and Cementeri Vell males (See Table 7.2.3 - *All Individuals*).

When the percentages of surfaces affected were considered for all individuals Cementeri Vell (10%) had the lowest percentage, followed by San Agustín (16%) and the Capuchin Catacombs (21%) had the highest. Within the Capuchin Catacombs, the same pattern was seen between male (22%) and female (17%) individuals, with male surfaces being more affected. Additionally, male individuals (18%) in Cementeri Vell had a higher percentage of surfaces affected than unknown sex individuals (8%). However, unlike the percentage

of teeth affected, the percentage of surfaces affected for male individuals in San Agustín (16%) was the lowest compared to males in the Capuchin Catacombs (22%) and Cementeri Vell (18%) (See Table 7.2.3 - *All Individuals*).

When the teeth of only calculus-exhibitors were considered for the percentage affected by dental calculus, the values were somewhat different to the whole population. As expected, the percentages of teeth affected were higher as there were fewer teeth in total for individuals with calculus compared to all individuals.

For the calculus-exhibitors in the Capuchin Catacombs (46%), nearly half of observed teeth had dental calculus accumulations and there was little difference between male (46%) and female (47%) individuals. The percentage of teeth affected in calculus-exhibitors in San Agustín (36%) was the lowest of the three populations. In contrast, Cementeri Vell (61%) had the highest percentage of affected teeth in calculus-exhibitors. However, this percentage for Cementeri Vell only represents the observed dentition from male individuals and does not include the percentage of loose teeth that had calculus deposits (see below Table 7.2.3 for explanation).

For the surfaces affected in calculus-exhibitors, the same pattern was seen between populations. Cementeri Vell male exhibitors (37%) had the highest percentages of surfaces affected (see below Table 7.2.3 for the exclusion of unknown individuals from this percentage) and San Agustín exhibitors (17%) had the lowest percentage. The male (28%) and female (30%) calculus-exhibiting individuals of the Capuchin Catacombs had similar percentages of surfaces affected by dental calculus.

	Capuchin Catacombs, Sicily				Cementerí Vell, Formentera				San Agustín, La Rioja			
	M	F	?	T	M	F	?	T	M	F	?	T
<i>Individuals Affected</i>												
MNI _{TOT}	102	24	0	126	26	5	51	82	31	0	0	31
MNI _{CALC}	65	13	0	78	11	0	17	28	19	0	0	19
ICALC%	64	54	0	62	42	0	33	34	61	0	0	61

<i>All Individuals -Teeth & Surfaces Affected</i>												
T _{CALC}	300	89	-	389	52	0	161	213	118	-	-	118
T_{CALC}%	37	29	-	35	32	0	29	28	34	-	-	34
S _{CALC}	404	132	-	536	104	0	(158)*	262	158	-	-	158
S_{CALC}%	22	17	-	21	18	0	(8)*	18	16	-	-	16

<i>Calculus-Exhibitors -Teeth & Surfaces Affected</i>												
T _{CALC}	300	89	-	389	52	0	161	213	118	-	-	118
T_{CALC}%	46	47	-	46	61	-	(98)^	61	36	-	-	36
S _{CALC}	404	132	-	536	104	-	158*	262	158	-	-	158
S_{CALC}%	28	30	-	29	37	-	(28)*	37	17	-	-	17

^ T_{CALC}% (*Calculus-Exhibitors*) of unknown sex individuals in Cementerí Vell is based on alveolar fragments and loose teeth and most teeth that had dental calculus were singular. Consequently, this value does not truly reflect the teeth that belonged to calculus-exhibitors (as loose teeth without calculus could not be attributed to be the same individual as a tooth with calculus).

* S_{CALC} (*All Individuals*); S_{CALC} (*Calculus-Exhibitors*); S_{CALC}% (*Calculus-Exhibitors*) for unknown sex individuals in Cementerí Vell does not include all loose teeth. Teeth recorded by Dr Nicholas Márquez-Grant did not include a dental calculus score per surface, only per tooth. The value for the percentage of surfaces affected was not included in calculating the percentage for the total population.

Table 7.2.3 Table showing the number of teeth with dental calculus (T_{CALC}) and the percentage of teeth present that had dental calculus (T_{CALC}%), where T_{OBS} is the value included in Table 7.2.1 (all individuals).

To further analyse the teeth that had dental calculus accumulations, the percentage of each type of tooth that had calculus was calculated, for the maxillary (upper) and mandibular (lower) (Figure 7.2.4) teeth. These percentages are also divided by the Brothwell score assigned to the calculus deposit observed.

Regarding the maxillary teeth in all populations, there was a general increase in teeth affected from the posterior to the anterior teeth, except for the third molars. The only exception to this was in Cementeri Vell, there was a low percentage of second premolars that were observed with calculus deposits (Figure 7.2.4 - top). For the Capuchin Catacombs, the deposits that were assigned a Brothwell score of three, were mainly found on second premolars and first and second molars. In Cementeri Vell, the highest scored deposits were found on canines, first premolars and third molars. Whereas for San Agustín, the three upper molar teeth had deposits with a Brothwell score of three (Figure 7.2.4 - top).

The distribution of mandibular teeth with calculus increased from anterior to posterior for the Capuchin Catacombs and Cementeri Vell, however the opposite was true for San Agustín (Figure 7.2.4 - bottom). This may have been due to oral hygiene by the individuals being carried out on the larger and more accessible teeth. However, it is more likely that dental calculus deposits on these teeth have suffered from post-mortem damage to the remains during excavation, storage and handling, as there was obvious evidence of dental calculus damage in this population (see section 2.5.3). If dental calculus deposits on the teeth exhibited features that indicated the whole deposit was not present, they were not included in the recording statistics (see section 6.4.1).

For mandibular teeth, in all populations there were higher percentages of teeth that were assigned a score of three. For the Capuchin Catacombs and Cementeri Vell, these were mainly found on anterior teeth, whereas for San Agustín the highest Brothwell scores were found on nearly all tooth types (Figure 7.2.4 - bottom).

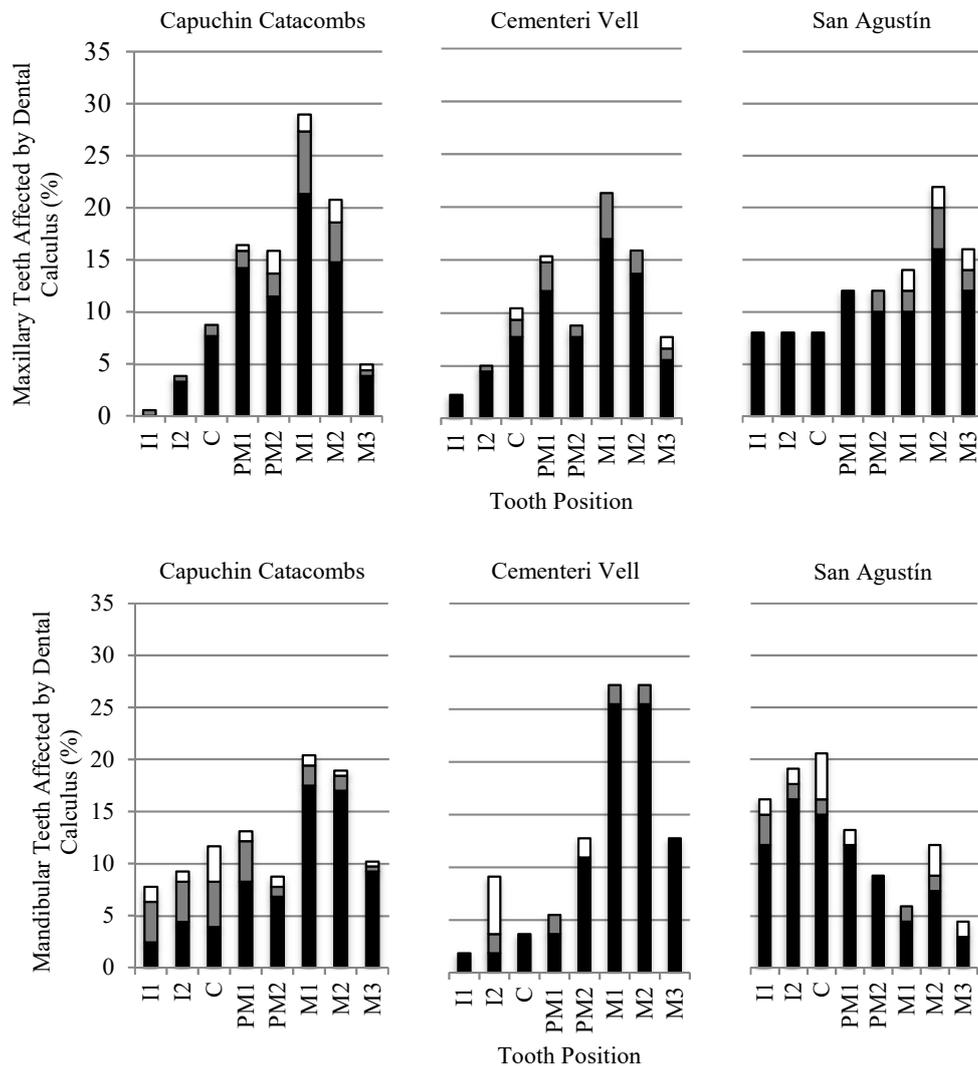


Figure 7.2.4 Graphs for the calculus-exhibitors in each population showing the percentages of maxillary teeth (top) mandibular teeth (bottom) scored for dental calculus according to tooth position. Each bar is divided according to the Brothwell Score assigned (Brothwell = 1 – black; 2 – grey; 3 – white).

In terms of the distribution of dental calculus on different tooth surfaces, the Capuchin Catacombs and San Agustín populations were similar. Both had buccal or labial surfaces affected the most, with much lower percentages of mesial and distal surfaces affected. Cementeri Vell had the same pattern of distribution, however there was a much smaller difference between the two highest affected and two lowest affect surfaces. These trends were seen in both the maxillary and mandibular surfaces (Figure 7.2.5).

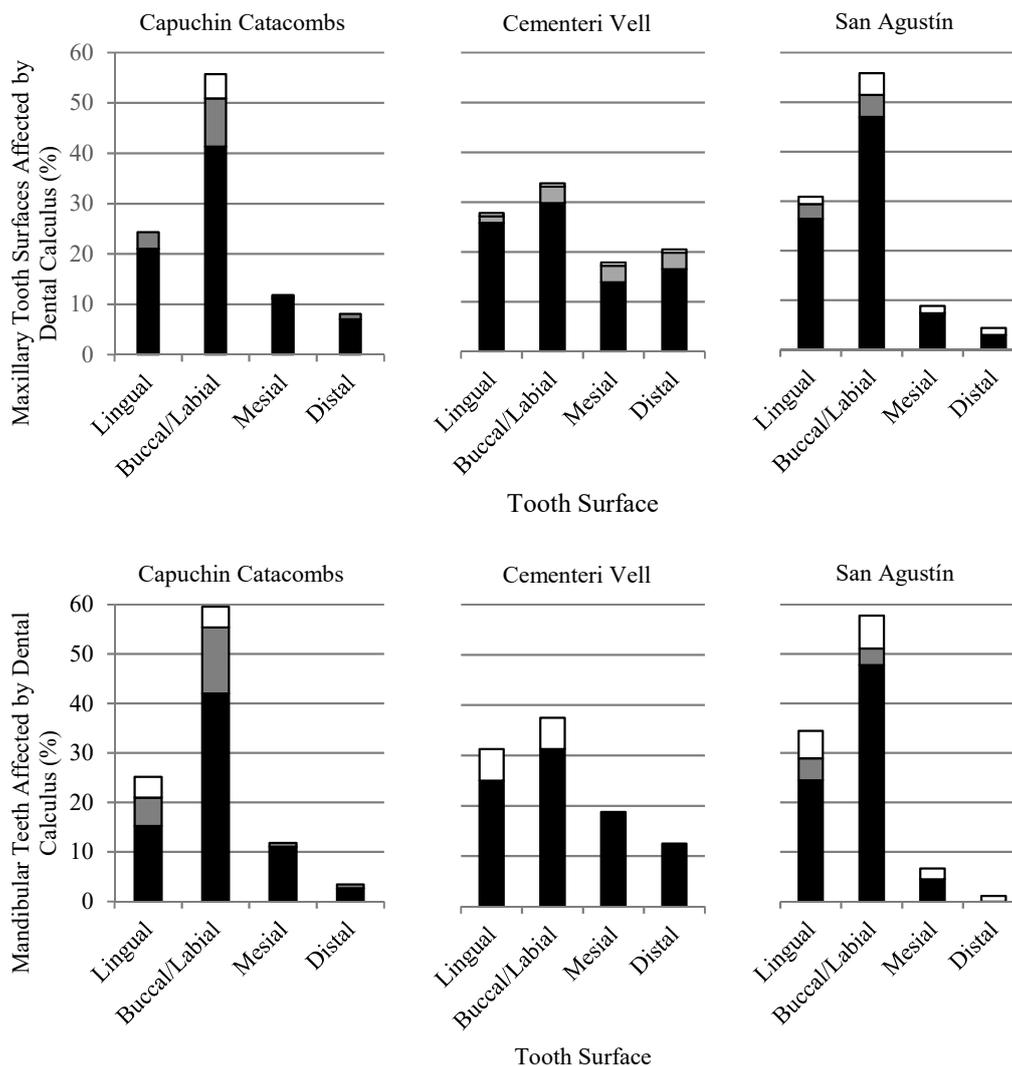


Figure 7.2.5 Graphs for the calculus-exhibitors in each population showing the percentages of maxillary teeth (top) and mandibular teeth (bottom) scored for dental calculus according to tooth surface. Each bar is divided according to the Brothwell Score assigned (Brothwell = 1 – black; 2 – grey; 3 – white).

In the Capuchin Catacombs teeth, the maxillary deposits that were assigned higher Brothwell scores were observed on buccal/labial and mesial surfaces. In Cementeri Vell and San Agustín, the percentages of deposits scores with a three were evenly spread over all surfaces (Figure 7.2.5 – top). On mandibular teeth, lingual and buccal/labial surfaces had deposits with Brothwell scores of three for all populations. In San Agustín, deposits of Brothwell score three were also seen on mesial and distal surfaces (Figure 7.2.5).

7.2.3 Dental Pathologies

The following results regarding the dentition from each population are specific to individuals in the populations that exhibited dental calculus. For the individuals, pathological conditions (carious lesions, periapical cavities and periodontal disease) are presented in Table 7.2.4. The results of pathological conditions for all individuals (i.e. not only calculus-exhibitors) in each population are presented in *Supplementary Material: Appendix E.1.1*.

For carious lesions, Cementeri Vell (15%) and San Agustín (13%) exhibited similar percentages of teeth affected and the Capuchin Catacombs (5%) was lower. Caries correction factors were not calculated for any of the populations because the primary focus of the dental recording in this study was the dental calculus present (Lukacs 1995). Additional pathologies were recorded in relation to their presence in dental calculus-exhibitors, rather than for the population although population-wide dental pathology percentages can be found in *Supplementary Material: Appendix E.1*.

The percentage of periapical cavities observed on alveolar bone positions was under 5% for calculus-exhibitors in all three populations. The population with the most periapical cavities observed on calculus-exhibiting individuals was San Agustín (5%), followed by Cementeri Vell (3%) and then the Capuchin Catacombs (1%).

In all three populations, the percentages of teeth in alveolar sockets that exhibited periodontal disease were high. For San Agustín (100%) all observable teeth in sockets exhibited periodontal disease.

		Capuchin Catacombs, Sicily				Cementerí Vell, Formentera				San Agustín, La Rioja			
		M	F	?	T	M	F	?	T	M	F	?	T
<i>Carious Lesions</i>	TCL	27	13	-	40	12	-	4	16	41	-	-	41
	TCL%	4	7	-	5	14	-	19	15	13	-	-	13
<i>Periapical Cavities</i>	P _{PAC}	25	5	-	30	6	-	2	8	26	-	-	26
	P_{PAC}%	1	1	-	1	3	-	5	3	5	-	-	5
<i>Periodontal Disease</i>	PPD	596	170	-	766	85	-	16	101	325	-	-	325
	PPD%	91	89	-	91	100	-	76	95	100	-	-	100

Table 7.2.4 Table showing the number and percentage of teeth or tooth positions affected by each dental pathology for calculus-exhibitors in each population.

7.3 Physical Characterisation

Throughout this section external surfaces of calculus deposits are described in terms of the ‘superficial’ (i.e. surfaces that were furthest from the attached tooth) and ‘deep’ (i.e. the surfaces attached to the tooth) surfaces of a deposit, for diagrammatical explanation of these terms see section 6.9.2.

7.3.1 External Morphology

The optical microscopy of the dental calculus deposits revealed visible differences between the specimens from different populations. The specific observations for each population sample are detailed below. Five deposit shapes were identified and the assigned names and diagrams of these can be found in *Supplementary Material: Appendix E.2.1*.

In general, the superficial surfaces of the Capuchin Catacombs specimens were varying shades of dark brown or reddish-brown in colour with speckles of black and white (Figure 7.3.1). These surfaces were textured and ‘gritty’ in appearance.

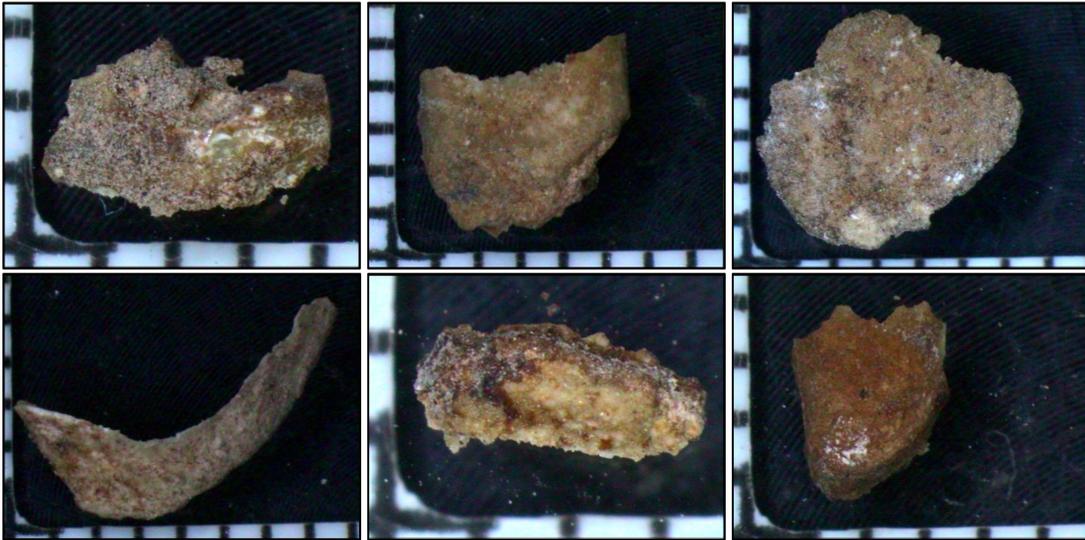


Figure 7.3.1 Example photographs showing the colouring and texture of the superficial surfaces of dental calculus specimens from the Capuchin Catacombs (each image includes a separate scale in millimetres).

In contrast, the deep surfaces of the Capuchin Catacombs specimens were lighter in colour, exhibiting shades of white, yellow, yellow-brown, pale green and dark green. In most deposits, the colours appeared in graduating bands that followed the shape of the deposit (Figure 7.3.2). In contrast to the gritty, textured superficial surfaces, the deep surfaces appeared to be smooth and glossy (Figure 7.3.2).

Many specimens were removed as an entire piece of calculus. Where specimens from CC were fragmented, they were easily orientated to resemble the whole specimen (Figure 7.3.3).

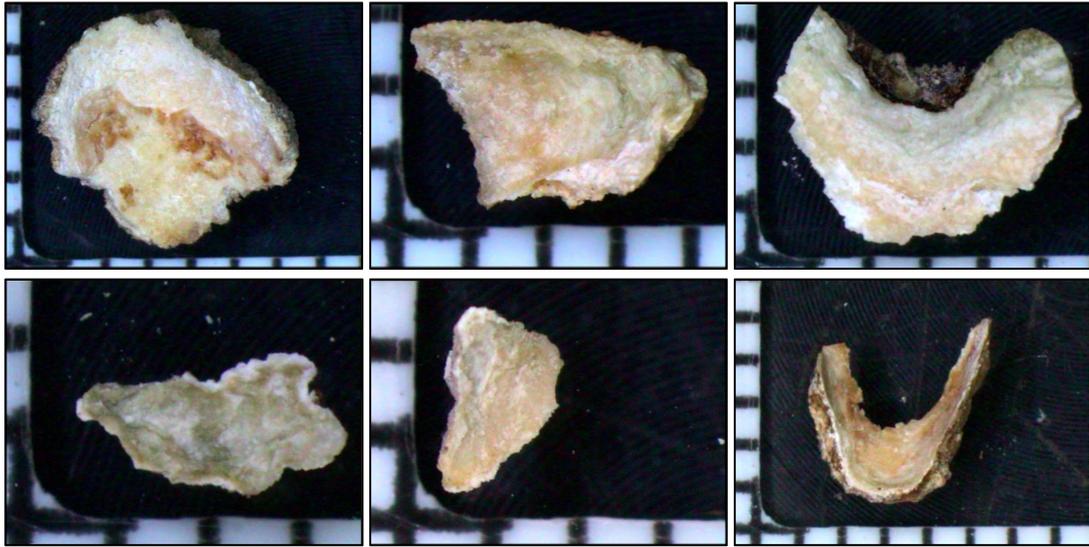


Figure 7.3.2 Example photographs showing the colouring and texture of the deep surfaces of dental calculus specimens from the Capuchin Catacombs (each image includes a separate scale in millimetres).



Figure 7.3.3 Example photographs showing dental calculus specimens from the Capuchin Catacombs that have broken. The specimens have fractured in a line from the bottom of the specimen to the top (each image includes a separate scale in millimetres).

In the Cementeri Vell sample, the superficial surfaces of dental calculus were consistently light sandy brown and white in colour with some speckles of reddish brown in places (Figure 7.3.4). These surfaces were textured and ‘gritty’ in appearance. For many of these deposits, powdery residue was shed from the specimens upon handling and during storage in their specimen bags.

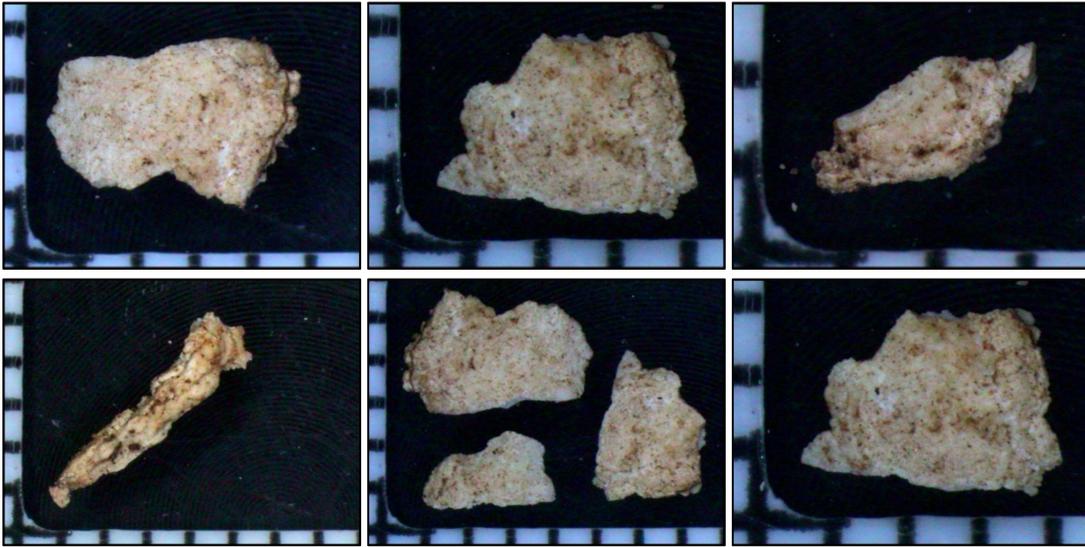


Figure 7.3.4 Example photographs showing the colouring and texture of the superficial surfaces of dental calculus specimens from Cementeri Vell (each image includes a separate scale in millimetres).

The deep surfaces of these specimens were similar in appearance to the superficial surfaces in both colour and texture. In general, they were white and pale yellow in colour with no obvious striations to the colours or speckling. These surfaces were smooth and glossy (Figure 7.3.5).

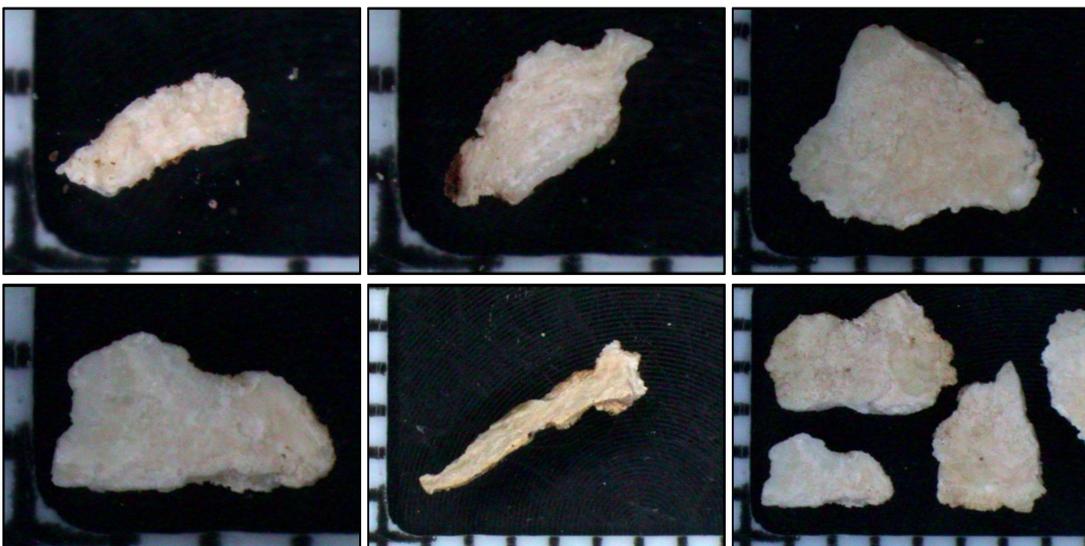


Figure 7.3.5 Example photographs showing the colouring and texture of the deep surfaces of dental calculus specimens from Cementeri Vell (each image includes a separate scale in millimetres).

In general, these specimens were thin and fragile, and most specimens were fragmented . Only a small number of specimens were removed as an entire piece of calculus. The breakage of specimens did not occur in a predictable way, which caused fragments of different shapes and size, often with multiple fracture lines per specimen.

The specimens from San Agustín had superficial surfaces that were predominantly pale-brown in colour with few speckles of dark brown/black (Figure 7.3.6). The surface colouring was consistent across specimens taken from all tooth locations.

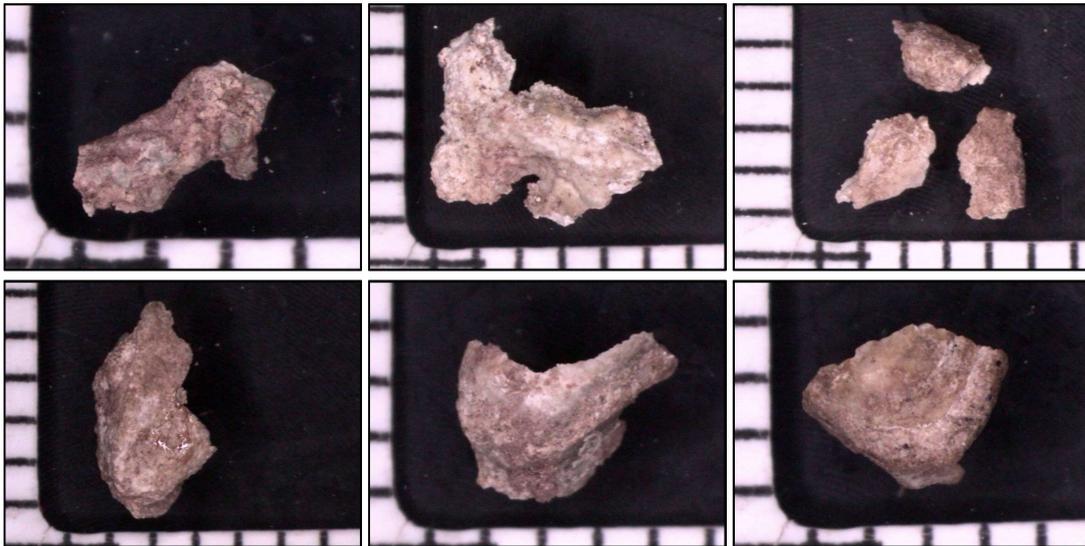


Figure 7.3.6 Example photographs showing the colouring and texture of the superficial surfaces of dental calculus specimens from San Agustín (each image includes a separate scale in millimetres).

The deep surfaces of the San Agustín specimens were shades of white, yellow, yellow-brown; yellow-green and dark green. In many deposits, the colours appeared in graduating bands that followed the shape of the deposit (Figure 7.3.7). These surfaces appeared to be smooth and glossy (Figure 7.3.7).

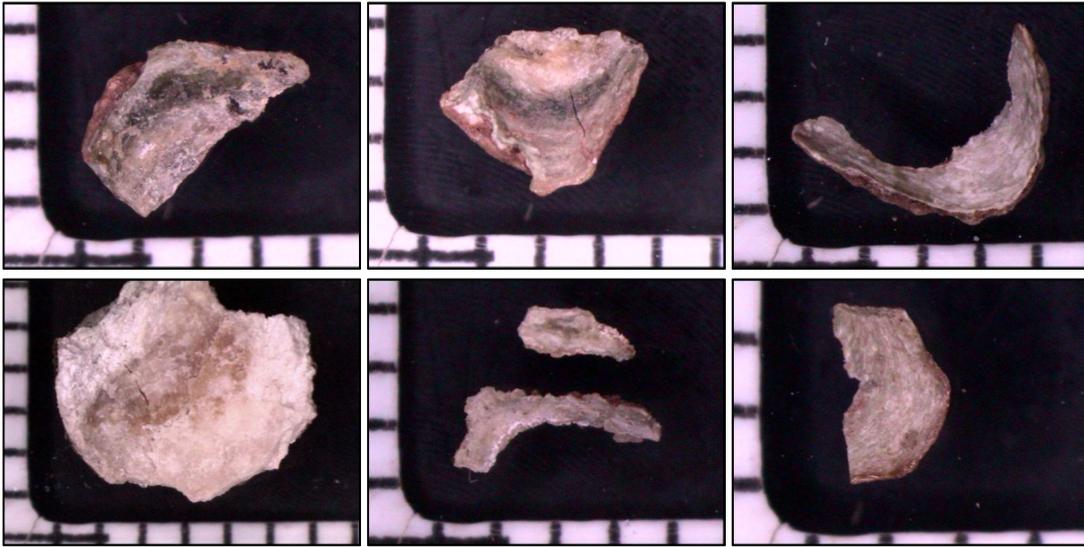


Figure 7.3.7 Example photographs showing the colouring and texture of the deep surfaces of dental calculus specimens from San Agustín (each image includes a separate scale in millimetres).

These specimens were of varying thickness but were brittle in nature and most specimens were fragmented and produced dust on contact. Many specimens were removed as an entire piece of calculus, however some of these broke after removal, even during careful handling. As with the Cementeri Vell specimens, the breakage of specimens did not occur in a predictable way, which caused fragments of different shapes and size, often with multiple fracture lines per specimen.

7.3.1.1 Population Comparison of External Morphology

The optical microscopy observations of the external features of the specimens of dental calculus described above for each population, are summarised in Table 7.3.1. The entries in the table are further compared with reference to the additional imaging techniques of nano-computed tomography and scanning electron microscopy in sections 7.3.1.1.1 to 7.3.1.1.3.

		Capuchin Catacombs, Sicily	Cementerí Vell, Formentera	San Agustín, La Rioja
No. of Specimens		62	40	33
Surface Texture	Superficial	mottled colouring; gritty	mottled colouring; gritty	mottled colouring; gritty
	Deep	glossy, smooth	glossy, smooth	glossy, smooth
Robustness		reasonably robust/ fractures generally occurred in one direction	brittle with dust produced on contact/ fractures occurred in any direction	brittle with dust produced on contact/ fractures occurred in any direction
Surface Colour	Superficial	tan; reddish brown; white (flecks); black (speckles)	light sandy brown; white; reddish-brown (speckles)	pale brown; dark brown/black (speckles)
	Deep	white; yellow; yellow-brown; pale green; dark green	white; pale-yellow; very pale green	white; yellow; yellow-brown; yellow-green; dark green
Shape (%)	Crescent	6	5	6
	Semi-Circular	24	5	15
	Ledge/Linear	50	38	39
	Planar	15	28	21
	Bead	5	8	6
	Uncategorised*	0	18	12

*Specimens that were too small to be categorised

Table 7.3.1 Table showing a summary of the visual observations made from the optical microscopy analysis regarding the archaeological dental calculus specimens from each population, including the percentage of specimens observed as each morphological shape.

7.3.1.1.1 Texture and Robustness

For specimens in all populations, the observations regarding the texture of the superficial and deep surfaces were similar (Table 7.3.1). These observations were supported in both the nano-computed tomography imaging and the scanning electron microscopy (Figure 7.3.8 and 7.3.9 respectively). In terms of the robustness of the specimens, the Cementerí Vell were the most fragile, followed by San Agustín specimens. The specimens in these populations also produced dust on contact with surfaces and easily fractured.

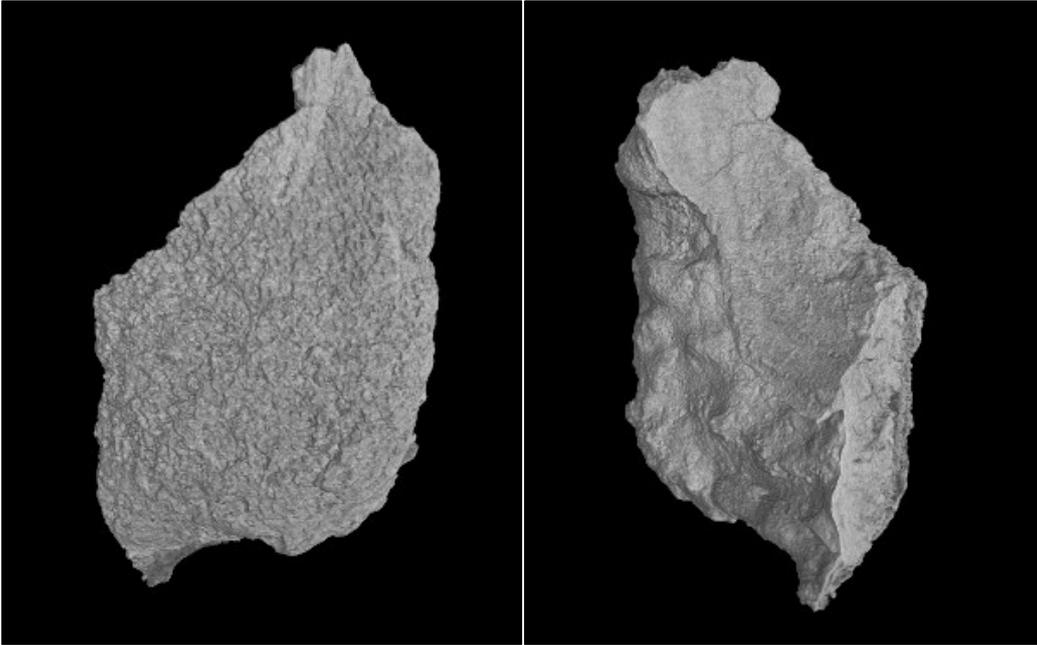


Figure 7.3.8 3-D CT image of a specimen of archaeological dental calculus, showing the gritty superficial surface (left) and the smooth deep surface (right) of the fragment (specimen shown, CCFMR63).

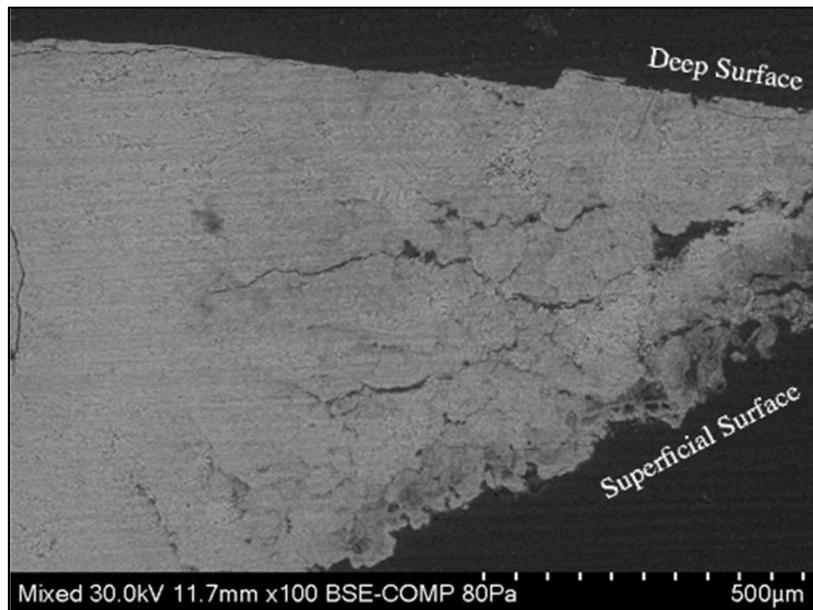


Figure 7.3.9 Scanning electron microscopy image of a specimen that shows the textural differences between the edges at the deep and superficial surfaces, observed in the dental calculus cross-sections (scale included) (specimen shown, CCSMR257).

7.3.1.1.2 Colour

The general observations regarding colour were that for all populations the superficial surfaces of specimens were darker than the deep surfaces. Unfortunately, colourimetry could not be applied for this study, due to the small size of the specimens and the colour variation across the surfaces, therefore colour-based observations were subjective.

All specimens exhibited superficial surfaces which were brown in colour however differences in the shades of brown were observed. The Capuchin Catacombs had the darkest superficial surfaces and had flecks of white, which were not observed in the other populations. For the deep surfaces, the general colour observations were shades of yellow, which were darker in both the Capuchin Catacombs and San Agustín. Additionally, these specimens had shades of green in their deep surfaces, which were not observed in the Cementeri Vell specimens.

The n-CT data is displayed in grey scale in terms of the density changes that occur within a material. Therefore, if the observed differences in colour correspond to differences in the composition and these materials have different densities, these colour changes would be observed as density changes in the n-CT images.

Figure 7.3.10 (A) shows an optical microscope image of the deep surface of a dental calculus specimen where definite colour bands can be seen. In the comparative nano-computed tomography 2-D slices through the specimen, these colour bands can be identified as regions of different densities. Of note is the dark green band that can be observed as a region of more dense material (Figure 7.3.10). This, high density band can be observed through the specimen and not just on the surface, as shown by Figure 7.3.10 (B-D). Also, shown in Figure 7.3.10 (C-D) is a low-density band of material that corresponds to the white coloured material in the optical microscopy image (indicated by white arrows).

This correlation between the colour on the surface and the density differences seen in the computed tomography data was found in specimens that had contrasting bands of darker or lighter material. Additional density differences were seen in the cross-sections of specimens and these are discussed in section 7.3.2.

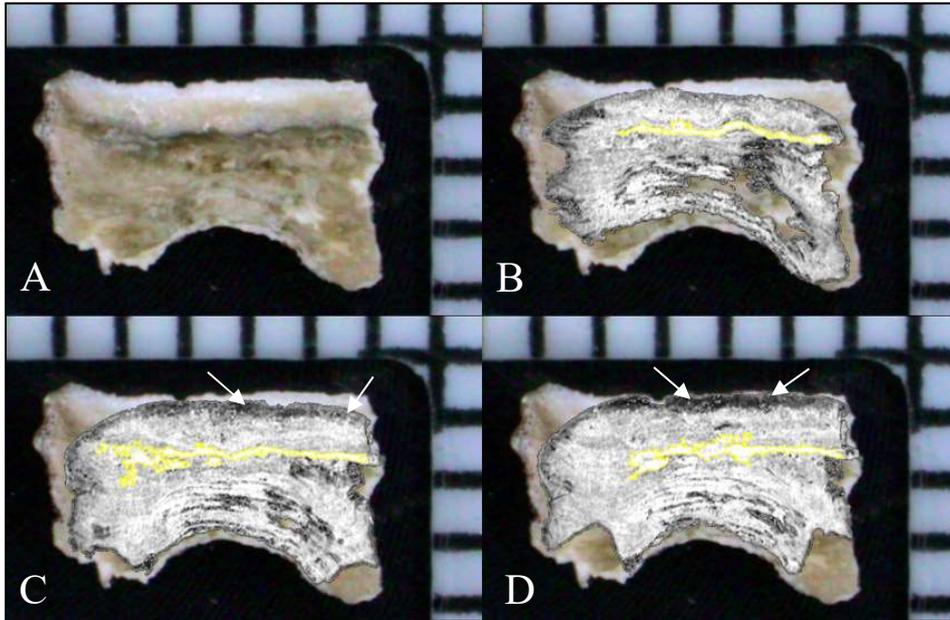


Figure 7.3.10 Images showing the colour differences observed using optical microscopy and corresponding density differences in the computed tomography data. A: optical microscopy image; B – D: nano-computed tomography internal slice overlays showing the higher density band that corresponds to the dark green band in the optical microscopy image (yellow outline). Also, shown in C-D are white arrows indicate a low-density band that corresponds to the white band in the optical microscopy image (specimen shown, CCNMR1142).

Scanning electron microscopy was performed for cross-sectional specimens however, colour changes could not be observed due to the nature of the technique. As no elemental changes were observed across a specimen using SEM, this indicated that the colour and density differences observed, could be contributed to differences in materials that have similar elemental compositions.

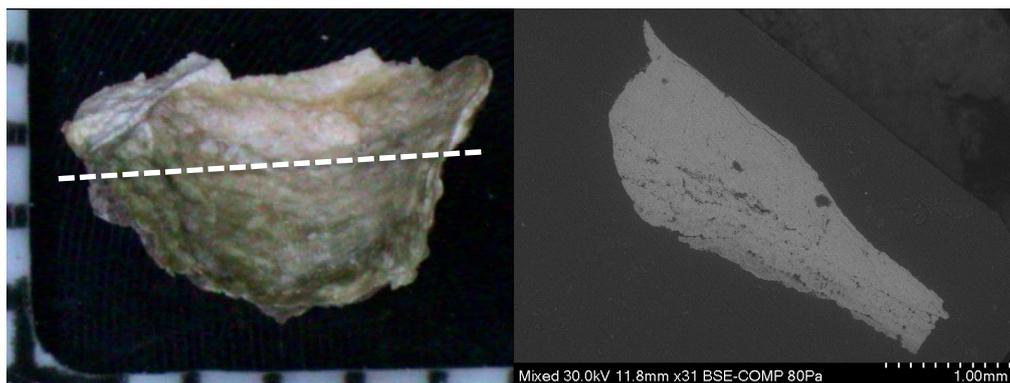


Figure 7.3.11 Example SEM image (right) showing no elemental composition changes or bands that may have corresponded to the colour changes seen on the external surface of the specimen (left), or the density changes observed through the 2-D slices of the n-CT data (specimen shown, CCMMR203).

7.3.1.1.3 Shape

The results of the shape categorisation showed that the most common deposit shape in all populations, was ledge (CC: 50%; CV: 38%; SA: 39%) (Table 7.3.1). In CC, the second most common shape was semi-circular (24%), whereas for CV and SA, this was planar (28% and 21% respectively). In all populations, there were less than 10% of specimens that could be assigned to bead and crescent shaped deposits (Table 7.3.1). There were also specimens in CV and SA that could not be categorised due to the amount of fracturing that had occurred (18%; 12% respectively).

On further analysis, it was found that the shape of the deposits does not appear to be dependent on where the deposit was found in the mouth with regard to dental quadrant, tooth type or surface (see *Supplementary Material: Appendix E.2.1*).

7.3.2 Cross-Sectional Morphology

Cross-sectional observations were made from both the nano-computed tomography data of whole specimens of dental calculus and the scanning electron microscopy of sectioned specimens. The changes in composition of the cross-sections are reported for the mineral and elemental composition in section 7.4 and 7.5 respectively. This section reports the physical morphology of the specimens in terms of their cross-section through the specimen, in the direction of deposit build up on the tooth.

7.3.2.1 Density Changes

Although a density standard was not analysed for the specimens during the n-CT and SEM analysis, qualitative density changes could be observed. As mentioned in section 7.3.1.1.2, the cross-sectional data from scanning electron microscopy showed a homogenous elemental composition through a deposit. Despite this, the cross-sectional analysis of the deposits using n-CT showed differences in material density through the specimens (Figure 7.3.12).

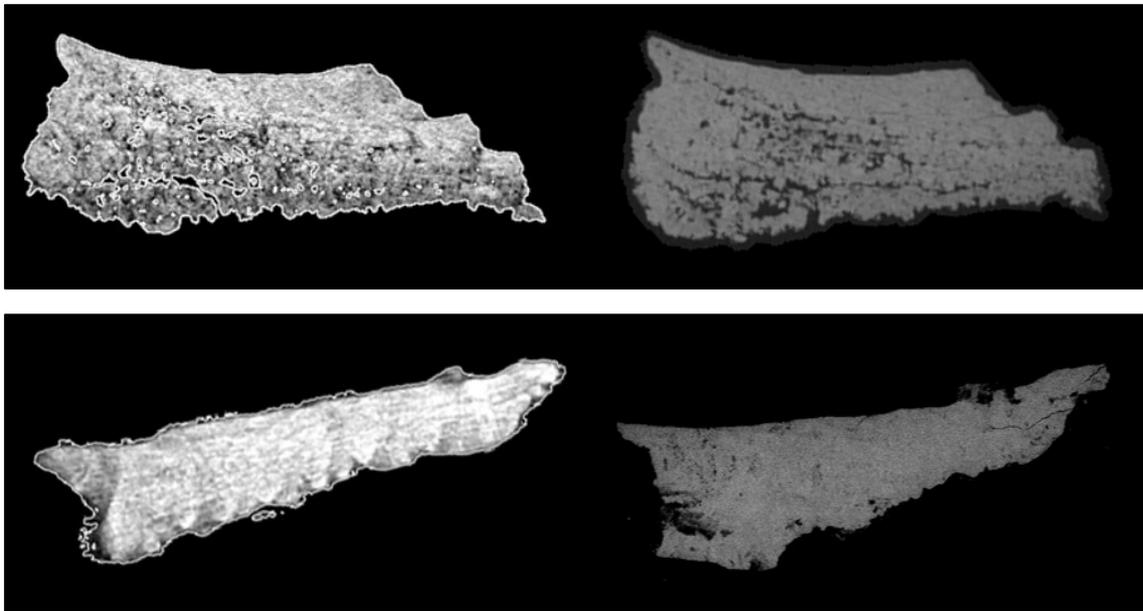


Figure 7.3.12 Example images showing the observable density differences across a specimen in the n-CT data (left) compared with the homogenous elemental composition across a specimen observable from the SEM data (right) (specimen shown, top: CCUS08; bottom: CCM200).

As well as the surface texture differences identified in section 7.3.1.1.1., within some specimens, there were differences in density from the deep surface to the superficial surface (Figure 7.3.13). However, the observed regions of different densities were not observable in all specimens and some specimens exhibited multiple density differences internally, which formed layers within the material (CC: 10%; CV: 16%; SA: 14%). In some specimens, these layers were more distinctive than others.

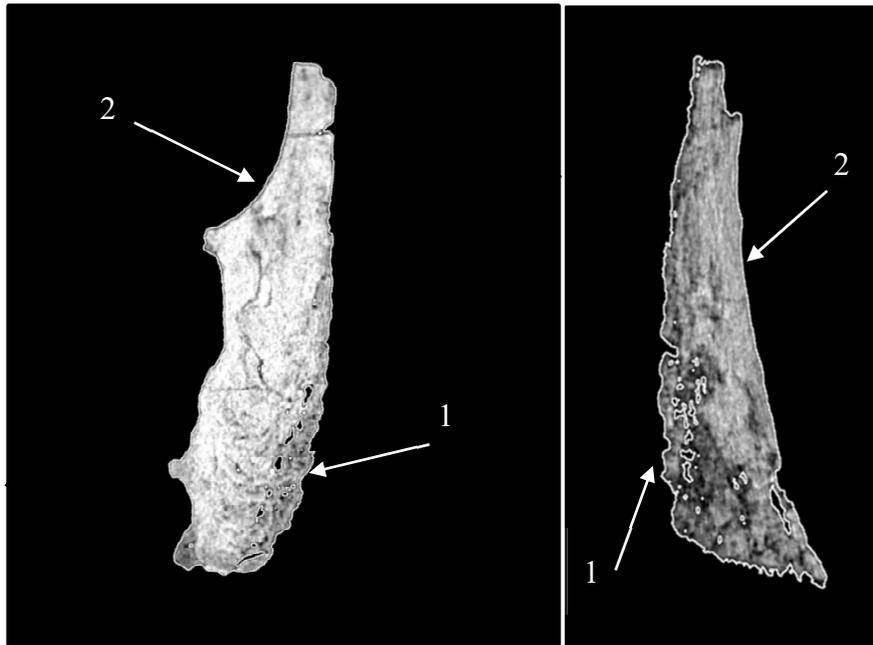


Figure 7.3.13 Example 2-D CT image slices of archaeological dental calculus, showing the density and texture differences between the superficial surface (1) and the smooth deep surface (2) (specimen shown, left: CCFMR63; right: CCNMR1142).

From the observed qualitative density differences, the specimens were grouped into three different types (Type I, II and III). These types were objectively determined for each specimen based on the number of density changes across each specimen. The specimens that showed no discrete density differences within the specimen were classed as ‘Type I’ (Figure 7.3.14). Type II specimens exhibited two discrete density bands within the dental calculus of lower density at the superficial surface and higher density in the remaining material (Figure 7.3.15). Finally, specimens with multiple alternating density differences were classed as Type III (Figure 7.3.16).

Type I

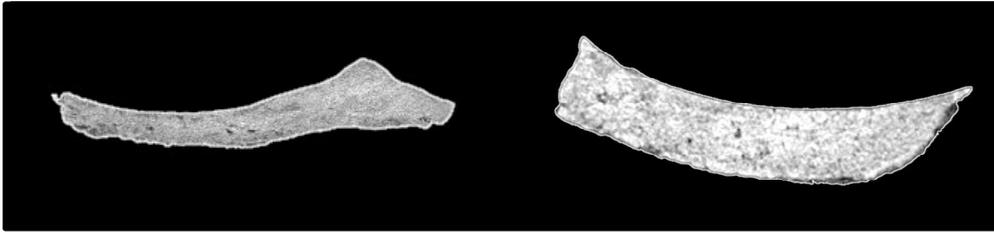


Figure 7.3.14 Example 2-D CT image slices of Type I specimens as classified by the density changes from the superficial to deep surfaces with no discrete changes in density across the deposits.

Type II

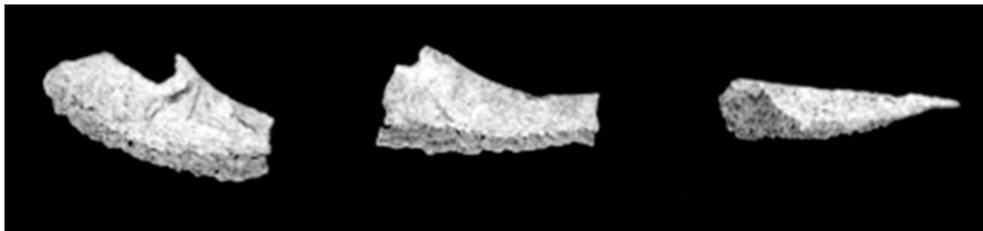


Figure 7.3.15 Example 2-D CT image slices of Type II specimens as classified by the density changes from the superficial to deep surfaces with two discrete bands of different density across the deposit.

Type III

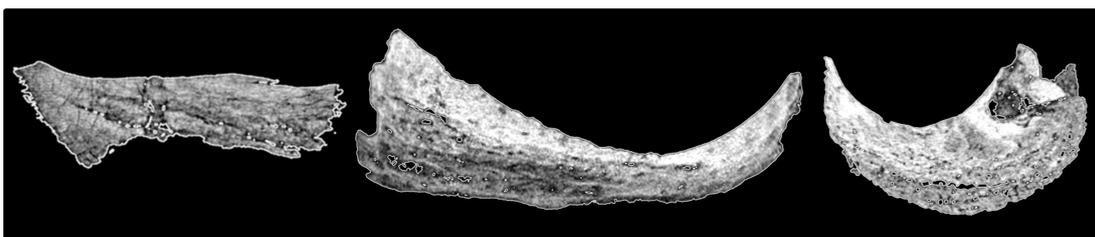


Figure 7.3.16 Example 2-D CT image slices showing Type III specimens as classified by the density changes across a specimen from the superficial to deep surfaces. Type III specimens exhibit multiple changes in density across the deposit.

In each population, the highest proportion of specimens were assigned to Type I (CC: 53%; CV: 68%; SA: 79%) (Table 7.3.2). In the Capuchin Catacombs, this percentage was the lowest as more specimens were assigned to the other two categories. In both Cementeri Vell and San Agustín, only a small percentage of specimens were assigned to Type II (CV: 3/19, 16%, SA: 1/14; 7%) and Type III (CV: 3/19, 16%, SA: 2/14; 14%).

		Capuchin Catacombs, Sicily	Cementeri Vell, Formentera	San Agustín, La Rioja
No. of specimens		40	19	14
Category	Type I	53%	68%	79%
	Type II	38%	16%	7%
	Type III	10%	16%	14%

Table 7.3.2 Table showing the percentage of specimens for each population that were identified as belonging to each density category (Type I, II, III).

The category that was assigned to each specimen, did not appear to relate to the dental location or the shape of the deposit (see *Supplementary Material: Appendix E.2.1*). However, there was a relationship between the mass of the dental calculus deposit and the type that they were categorised into.

In all three populations, the mean mass of the specimens increased for each category, I to III (Figure 7.3.17). In San Agustín, the difference in mass between the categories was more pronounced with no overlap between categories. In the Capuchin Catacombs and Cementeri Vell, the range of masses for Type III was large considering the small percentage of specimens in this category (10% and 16% respectively). Additionally, in the Capuchin Catacombs, an outlier specimen in the Type I category had a mass more in line with the upper quartile of Type II. However, all other specimens were with the mass standard deviations of their respective groups (Figure 7.3.17).

ANOVA results indicate that in all three populations, there was a statistically significant difference between the deposits masses in each category ($p < 0.05$). Additional ANOVA results of each category compared between populations indicate that there was no significant statistical difference between the deposit masses in Type I and Type II specimens across populations. However, Type III specimens did exhibit a significant

difference and further analysis using 2-tailed t-tests indicate that this difference was between the Capuchin Catacombs and San Agustín Type III specimens.

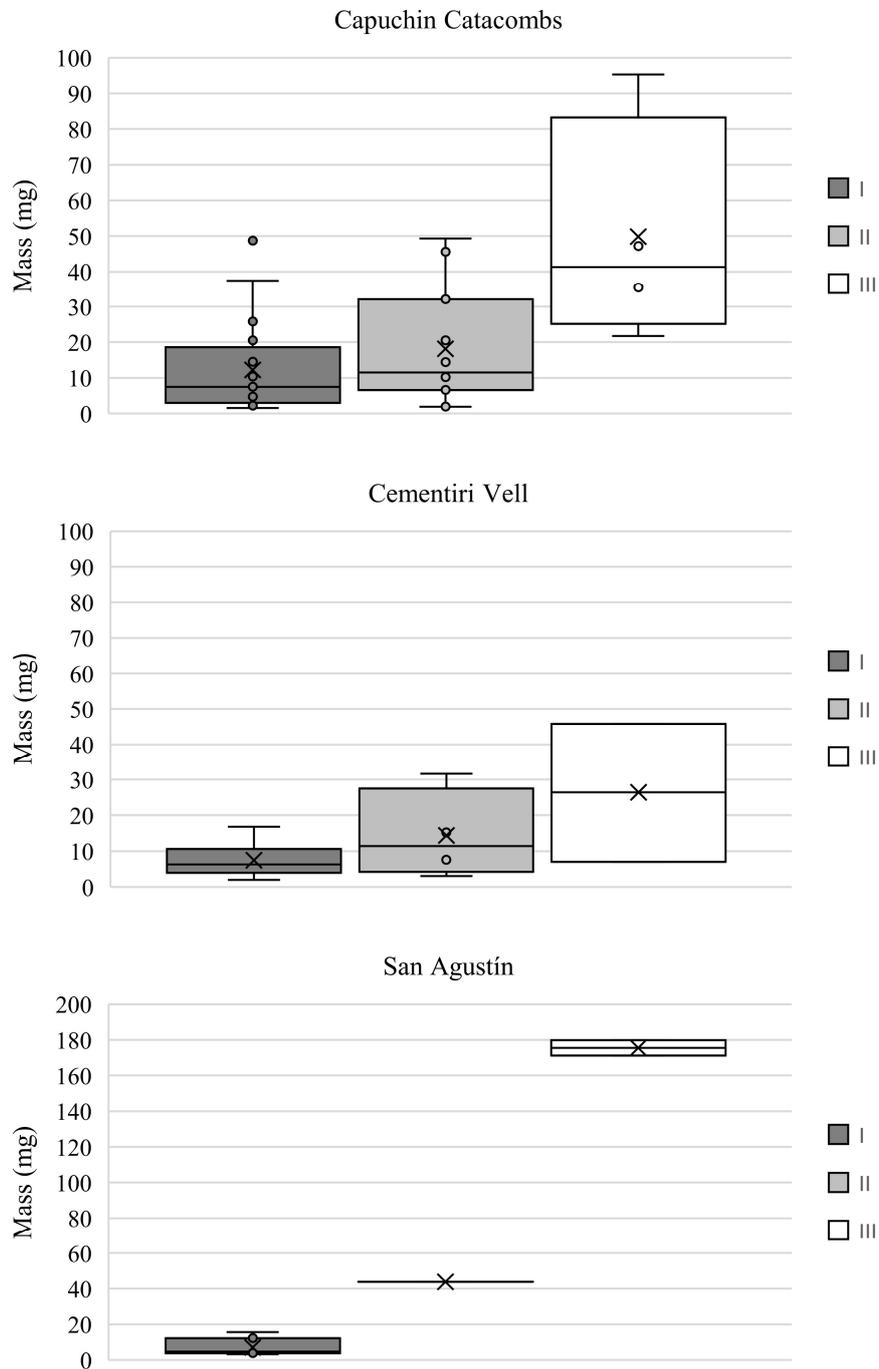


Figure 7.3.17 Box plots for each population showing the deposit masses of specimens in each category as assigned according to density changes observed in the n-CT data.

7.3.2.2 Porosity

The scanning electron microscopy and nano-computed tomography also showed differences in the porosity of the calculus material. Some specimens displayed predominately uniform, compact cross-sections, with few voids whereas other specimens had numerous voids, of differing size (Figure 7.3.18).

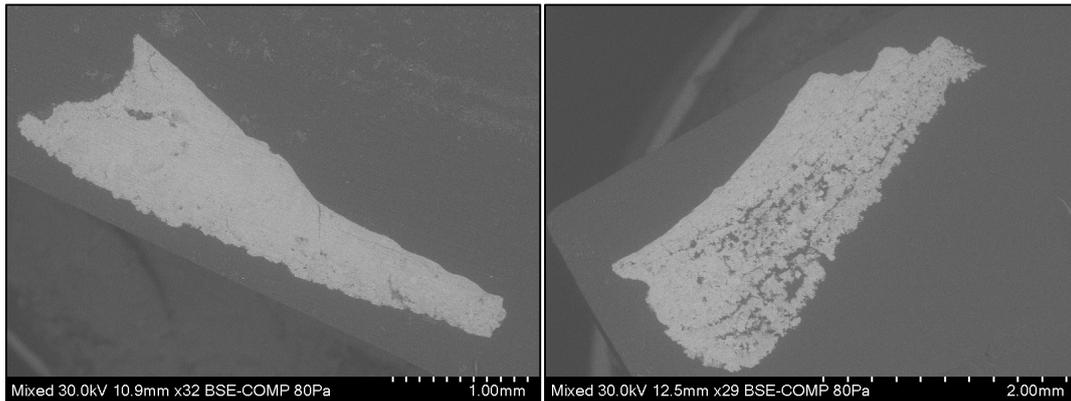


Figure 7.3.18 Example scanning electron microscopy images showing the differences between specimens in terms of the voids present across the cross-sectional surfaces of the specimens, as viewed using scanning electron microscopy; top: uniform cross-section with few voids; bottom: numerous voids of differing sizes, (scale included for each image) (specimens shown, left: CCSMR64; right: CCUS08).

The differences in specimen porosity were also observable in the defect detection analysis performed on the nano-computed tomography data. This determined the 3-D void sizes within a deposit and showed their location on the specimen images. Some specimens had discrete areas of voids that were larger sizes, although in most specimens, the void size was consistent across a specimen. In addition, two specimens from molar teeth are shown to have larger voids in the calculus that accumulated in the grooves of the cusp on the buccal surface (Figure 7.3.19).

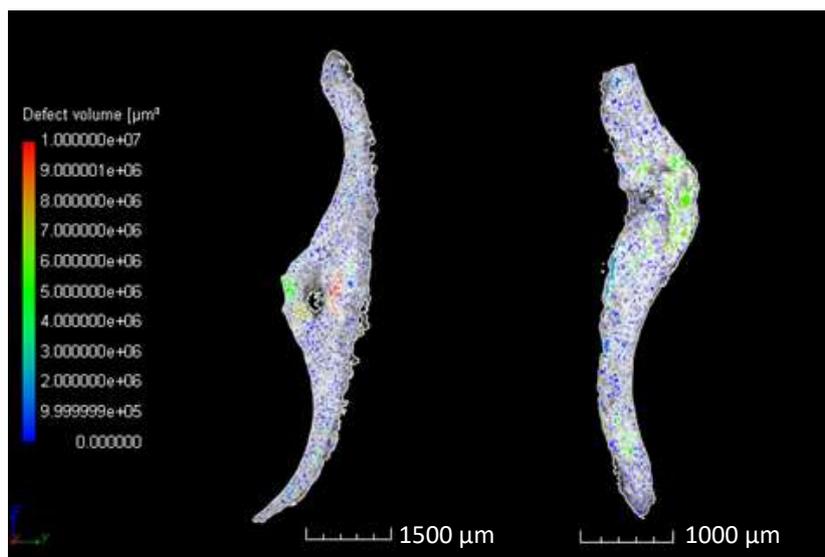


Figure 7.3.19 Cross-sectional images from the nano-computed tomography data that show specimens of dental calculus from the buccal surface of molar teeth. In the void analysis shown on these images, larger voids can be seen in the calculus that is associated with the cusp grooves of the tooth, as indicated by the different colours (specimen shown, left: CCFUM04; right: CCMR200).

7.4 Mineralogical Analysis

The following sections present mineralogical results from the archaeological specimens of dental calculus from the three populations sampled. Within these results, two approaches to X-ray diffraction analysis were utilised, powder X-ray diffraction and micro-beam X-ray diffraction. The rationale for using these techniques is presented in section 5.2 and the methods are explained in section 6.8.

7.4.1 Bulk Mineral Composition

The diffractograms from all specimens analysed by powder X-ray diffraction (pXRD) exhibited broad overlapping peaks. In all specimens, both mineral phases of hydroxyapatite (HAp) and whitlockite (WHT) were identified. The ratio of these minerals varied between specimens and this is further detailed in the following results sections.

Additional mineral phases of quartz and calcite were identified in varying amounts in some specimens (Calcite – CC: 35%; CV: 57%; SA: 34%; Quartz – CC: 15%; CV: 43%; SA: 14%). The percentage of specimens which exhibited the identified phases for

specimens from the three populations are detailed in Table 7.4.1. There were no specimens that contained brushite (BRU) or octacalcium phosphate (OCP). An example plot of the OCP range is shown in Figure 7.4.1, the diffractograms for the OCP range for all specimens in each population are included in *Supplementary Material: Appendix E.3*.

		Capuchin Catacombs, Sicily	Cementeri Vell, Formentera	San Agustín, La Rioja
No. of Specimens		20	28	29
Specimens containing each identified mineral phase (%)	Hydroxyapatite (HAp)	100	100	100
	Whitlockite (WHT)	100	100	100
	Calcite	35	57	34
	Quartz	15	43	14

Table 7.4.1 Table showing the mineral phases identified in specimens of archaeological dental calculus from the populations of the Capuchin Catacombs, Cementeri Vell and San Agustín and the percentage of specimens per population that were observed as containing each mineral phase.

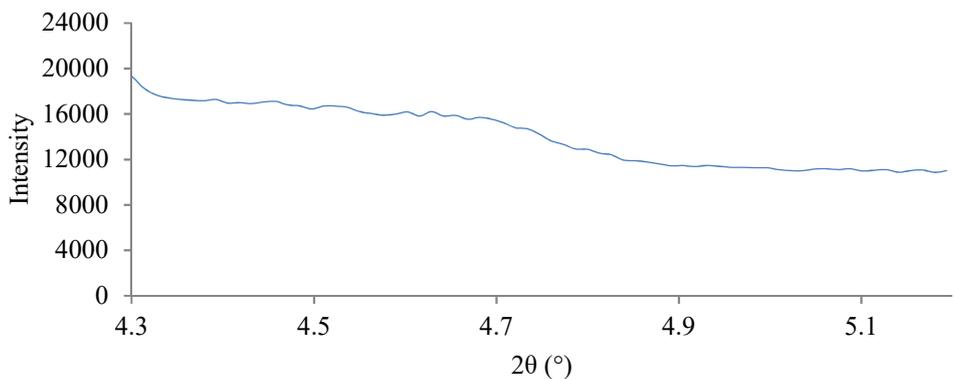


Figure 7.4.1 An example plot of the 2θ range (4.2-5.5°) scanned for octacalcium phosphate showing no peaks present.

7.4.1.1 Major Phases

For the major mineral phases of hydroxyapatite and whitlockite, the descriptive statistics for the proportion of each phase in specimens from each population are detailed in Table 7.4.2 and Table 7.4.3 respectively.

The specimens from Cementeri Vell (CV) had the lowest mean hydroxyapatite (HAp) percentage, followed by the Capuchin Catacombs (CC) and then San Agustín (SA) (72%, 76%, 83% respectively) (Table 7.4.2). As expected, the percentage of whitlockite (WHT) in these population displayed the inverse trend (Table 7.4.3). Despite the difference in mean for CV and SA, the range of percentages for these populations was similar and larger than the range in CC (CC: HAp – 39%; WHT – 35%; CV: HAp - 54%; WHT - 52%; SA: HAp - 58%; WHT – 58%). This was due to both CV and SA having one specimen which contained a hydroxyapatite percentage below 50%, which brought down the mean percentages for this phase, and consequently raised the mean percentage of whitlockite. Both these outliers were from upper molar lingual surfaces, however there were other specimens from this location which were within the standard deviation range of the mean. Without these outliers, the mean percentages of both CV and SA were far more like CC.

In terms of the lattice parameters for hydroxyapatite, the values obtained for ‘a’ in each population were higher than the value of 9.41 Å for stoichiometric hydroxyapatite (Table 7.4.2). Whereas the value for ‘c’ was in line with the expected value of 6.88 Å. Kruskal-Wallis results of the lattice parameter values between populations for hydroxyapatite indicated that there was no significant difference between the ‘a’ parameters but there was between the ‘c’ parameter values (Table 7.4.2). The CC specimens exhibited the largest expansion of the hydroxyapatite ‘a’ lattice and SA exhibited the largest expansion of the hydroxyapatite ‘c’ lattice.

For whitlockite, the mean values obtained for ‘a’ and ‘c’ in each population were slightly higher than the values for stoichiometric whitlockite (10.33 Å and 37.10 Å respectively), indicating lattice expansion in both directions. The lattice parameters were approximately the same between populations for the ‘a’ lattice and showed no significant statistical difference (Table 7.4.3). However, the values for the ‘c’ lattice parameters did indicate a

significant difference between populations, with SA having the highest mean parameter, followed by CV and then CC (Table 7.4.3).

		Capuchin Catacombs, Sicily	Cementeri Vell, Formentera	San Agustín, La Rioja
No. of Specimens		20	28	29
HAp (%)	Mean (%)	76	72	83
	Std Dev.	11	12	12
	Min (%)	51	38	38
	Max (%)	90	92	96
HAp 'a' (Å)	Mean (Å)	9.446	9.444	9.441
	Std Dev.	0.009	0.010	0.008
	Min (Å)	9.428	9.425	9.425
	Max (Å)	9.463	9.468	9.458
	H	3.01		
	<i>p</i>	0.22		
HAp 'c' (Å)	Mean (Å)	6.878	6.879	6.884
	Std Dev.	0.005	0.005	0.004
	Min (Å)	6.868	6.866	6.874
	Max (Å)	6.885	6.888	6.892
	H	17.73		
	<i>p</i>	0.00014*		

* Significant result ($p \leq 0.05$)

Table 7.4.2 Table showing descriptive statistics of hydroxyapatite present in the specimens analysed by powder X-ray diffraction. The values for the lattice parameters of 'a' and 'c' in angstroms (Å) for hydroxyapatite in specimens from each population are also included.

		Capuchin Catacombs, Sicily	Cementeri Vell, Formentera	San Agustín, La Rioja
No. of Specimens		20	28	29
WHT (%)	Mean (%)	23	26	17
	Std Dev.	10	12	12
	Min (%)	10	8	4
	Max (%)	45	60	62
WHT 'a' (Å)	Mean (%)	10.355	10.356	10.354
	Std Dev.	0.010	0.0010	0.010
	Min (%)	10.335	10.335	10.335
	Max (%)	10.374	10.377	10.373
	H	1.98		
	<i>p</i>	0.37		
WHT 'c' (Å)	Mean (%)	37.220	37.211	37.247
	Std Dev.	0.049	0.042	0.038
	Min (%)	37.112	37.161	37.188
	Max (%)	37.293	37.338	37.336
	H	11.99		
	<i>p</i>	0.0025*		

* Significant result ($p \leq 0.05$)

Table 7.4.3 Table showing the descriptive statistics of whitlockite present in the specimens analysed by powder X-ray diffraction. The values for the lattice parameters of 'a' and 'c' in angstroms for whitlockite in specimens from each population are also included.

The following results are displayed for the hydroxyapatite percentage, the corresponding results for whitlockite percentage are included in *Supplementary Material: Appendix E.3*.

Table 7.4.4 shows the descriptive statistics of the percentage of hydroxyapatite (HAp) in specimens, where the specimens are separated by their jaw location in the mouth. In all populations, there was a small difference between the percentages of HAp in specimens from the upper and lower jaws. Additionally, the mean percentage of lower jaw specimens was slightly higher than upper jaw specimens for all populations. These differences were found to not be significant following Kruskal-Wallis tests of the upper and lower percentages in each population (Table 7.4.4).

Further division of the specimens from each jaw into the side of the jaw that they were sampled from, is also shown in Table 7.4.4. There were no significant differences between the left and right sides of each jaw for any of the populations.

		Capuchin Catacombs, Sicily		Cementeri Vell, Formentera				San Agustín, La Rioja					
		<i>By Jaw</i>											
		Upper		Lower		Upper		Lower		Upper		Lower	
No. of Specimens		10		10		22		6		14		15	
HAp (%)	Mean (%)	75		77		71		73		82		83	
	St. Dev.	11		11		12		13		14		9	
	Min (%)	51		60		38		55		38		66	
	Max (%)	90		88		92		89		94		96	
H		0.09				0.08				0.07			
p		0.76				0.78				0.79			
		<i>By Dental Quadrant (per Jaw)</i>											
		UR	UL	LL	LR	UR	UL	LL	LR	UR	UL	LL	LR
No. of Specimens		6	4	4	6	12	10	4	2	10	4	6	9
HAp (%)	Mean (%)	78	71	74	79	70	73	79	63	87	70	86	81
	St. Dev.	13	2	12	10	12	11	11	8	6	20	8	10
	Min (%)	51	68	60	60	38	54	61	55	72	38	70	66
	Max (%)	90	75	88	87	84	92	89	70	94	89	96	91
H		2.23		0.41		0.11		1.93		2.88		0.89	
p		0.14		0.52		0.74		0.16		0.09		0.35	

(UR = Upper Right; UL = Upper Left; LL = Lower Left; LR = Lower Right)

* Significant result ($p \leq 0.05$)

Table 7.4.4 Table comparing the descriptive statistics of hydroxyapatite in each jaw and the dental quadrant of each jaw for specimens analysed by powder X-ray diffraction for each population. Also shown are the results of the Kruskal-Wallis tests for significance ($p \leq 0.05$).

In addition to statistically testing for differences in each jaw, upper and low, the overall differences in the side from which the specimens were sampled are detailed in Table 7.4.5. In all populations, there was a higher percentage of hydroxyapatite found in right-hand specimens, however the difference between left and right was not found to be statistically significant (Table 7.4.5). There were also no statistically significant differences between the upper and lower jaws for the left and right sides of the mouth, in any population (Table 7.4.5).

		Capuchin Catacombs, Sicily				Cementerí Vell, Formentera				San Agustín, La Rioja			
		<i>By Side</i>											
		Left		Right		Left		Right		Left		Right	
No. of Specimens		8		12		14		14		10		19	
HAp (%)	Mean (%)	73		78		69		74		80		84	
	St. Dev.	9		12		12		11		16		8	
	Min (%)	60		51		38		54		38		66	
	Max (%)	88		90		84		92		96		94	
H		1.72				1.02				0.17			
<i>p</i>		0.19				0.31				0.68			
		<i>By Dental Quadrant (per Side)</i>											
		UL	LL	LR	UR	UL	LL	LR	UR	UL	LL	LR	UR
No. of Specimens		4	4	6	6	10	4	2	12	4	6	9	10
HAp (%)	Mean (%)	71	74	79	78	73	79	63	70	70	86	81	87
	St. Dev.	2	12	10	13	11	11	8	12	20	8	9	6
	Min (%)	68	60	60	51	54	61	55	38	38	70	66	72
	Max (%)	75	88	87	90	92	89	70	84	89	96	91	94
H		0.00		0.03		0.98		1.20		1.14		2.16	
<i>p</i>		1.00		0.87		0.32		0.27		0.29		0.14	

(UR = Upper Right; UL = Upper Left; LL = Lower Left; LR = Lower Right)

* Significant result ($p \leq 0.05$)

Table 7.4.5 Table comparing the descriptive statistics of hydroxyapatite in each side and the dental quadrant of each side for specimens analysed by powder X-ray diffraction for each population. Also shown are the results of the Kruskal-Wallis tests for significance ($p \leq 0.05$).

The results of comparing the type of tooth from which a specimen was removed yielded a statistically significant result. In all populations, there was a higher percentage of hydroxyapatite found in anterior teeth compared to posterior teeth. Additionally, when statistically tested with Kruskal-Wallis tests, this difference was found to be significant (with a confidence level of 95%) in the San Agustín specimens (Table 7.4.6). Within both the posterior and anterior categories of teeth, no significant difference was found either between incisor and canine teeth or premolar and molar teeth (Table 7.4.6).

		Capuchin Catacombs, Sicily				Cementeri Vell, Formentera				San Agustín, La Rioja			
		<i>By Tooth Positions</i>											
		Anterior		Posterior		Anterior		Posterior		Anterior		Posterior	
No. of Specimens		5		15		4		24		12		17	
HAp (%)	Mean (%)	83		74		73		71		89		78	
	St. Dev.	4		12		10		12		5		13	
	Min (%)	75		51		61		38		76		38	
	Max (%)	87		90		89		92		96		91	
H		1.83				0.00				9.61			
<i>p</i>		0.18				0.95				0.0019*			
		<i>By Tooth Type</i>											
		I	C	P	M	I	C	P	M	I	C	P	M
No. of Specimens		2	3	5	10	2	2	10	14	7	5	8	9
HAp (%)	Mean (%)	86	80	78	72	75	72	74	69	89	89	84	73
	St. Dev.	1	4	8	12	14	4	7	15	3	7	6	15
	Min (%)	85	75	70	51	61	68	63	38	85	76	70	38
	Max (%)	87	85	90	88	89	76	84	92	93	96	91	88
H		3.00		1.81		0.00		0.28		0.53		3.34	
<i>p</i>		0.08		0.18		1.00		0.60		0.46		0.07	

(I = Incisors; C = Canines; P = Premolars; M = Molars)

* Significant result ($p \leq 0.05$)

Table 7.4.6 Table comparing the descriptive statistics of hydroxyapatite between tooth positions and tooth types for specimens analysed by powder X-ray diffraction for each population. Also shown are the results of the Kruskal-Wallis tests for significance ($p \leq 0.05$).

In addition to the dental location, the pathological environment that the calculus specimens were recorded as being in, was also compared in terms of the hydroxyapatite percentage content. The specimens were grouped into whether the individual, from which the specimen was taken, was recorded as having one or more carious lesion or periapical cavities, or neither of these recordable pathologies (Table 7.4.7).

In the Capuchin Catacombs and Cementeri Vell, the groups containing specimens with and without pathologies was reasonably balanced. San Agustín, however, had a disproportionate number of specimens that came from individuals with either of the pathologies (Table 7.4.7). From Kruskal-Wallis statistical testing there were no statistical differences in hydroxyapatite percentage for the Capuchin Catacombs and Cementeri Vell populations. However, the statistical testing demonstrated a significant

difference in the San Agustín population, although this is questionable due to the extremely unbalanced sample groups (Table 7.4.7).

		Capuchin Catacombs, Sicily	Cementerí Vell, Formentera	San Agustín, La Rioja			
		<i>By Pathology Presence</i>					
		Pathologies absent	Pathologies present	Pathologies absent	Pathologies present	Pathologies absent	Pathologies present
No. of Specimens		9	11	14	14	2	26
HAP (%)	Mean (%)	79	74	74	69	74	83
	St. Dev.	12	10	13	10	3	12
	Min (%)	51	60	38	54	71	38
	Max (%)	91	88	92	84	76	96
H		1.57		1.90		15.15	
<i>p</i>		0.21		0.17		0.0001*	

(Pathologies present/absent = carious lesions and periapical cavities)

* Significant result ($p \leq 0.05$)

Table 7.4.7 Table comparing the descriptive statistics of hydroxyapatite between specimens from individuals recorded with/without carious lesions and periapical cavities on their dentition. Also shown are the results of the Kruskal-Wallis tests for significance ($p \leq 0.05$).

The percentage of hydroxyapatite in specimens was not analysed in terms of the sex of the individual from which it was from. This is because only two specimens from the Capuchin Catacombs were from female individuals. Neither of these specimens had a percentage of hydroxyapatite that was noticeably different to the rest of the Capuchin Catacombs specimens.

7.4.1.2 Minor Phases

In addition to the major mineral phases of hydroxyapatite and whitlockite, which were found in all analysed specimens, several specimens in each population contained the phases of calcite and quartz (Table 7.4.8).

In the Capuchin Catacombs and San Agustín samples, similar percentages of specimens were identified as having a calcite mineral phase (35% and 34% respectively). For Cementerí Vell the percentage of specimens was much higher (57%). In all populations, the mean percentage of calcite identified was under 5%. However, the maximum calcite percentage found in Cementerí Vell was far higher than the other populations (18%) (Table 7.4.8).

For specimens with quartz present, the mean percentage present was around 1% for all populations. Again, similar percentages of specimens contained this phase in the Capuchin Catacombs and San Agustín samples, the percentage of specimens was higher in Cementeri Vell (15%, 14%, 57% respectively). The range of percentages for this phase was lowest in the Capuchin Catacombs specimens, with a maximum content of 1.6%. For the other two populations, the maximum percentage was around 2.5% (Table 7.4.8).

These additional phases were not commonly present together in the same specimen for the Capuchin Catacombs and San Agustín. However, in the Cementeri Vell, specimens found to contain these minor phases, generally had both quartz and calcite present together (61% of specimens). For tables containing the percentages found for each phase in all specimens see *Supplementary Material: Appendix E.3*.

		Capuchin Catacombs, Sicily	Cementeri Vell, Formentera	San Agustín, La Rioja
Total no. of Specimens		20	28	29
Calcite %	No. of Specimens	7 (35%)	16 (57%)	10 (34%)
	Mean (%)	2.1	3.7	1.4
	Std Dev.	1.4	4.7	0.5
	Min (%)	0.7	0.6	0.7
	Max (%)	4.5	17.7	2.2
Quartz %	No. of Specimens	3 (15%)	12 (43%)	4 (14%)
	Mean (%)	0.9	1.0	1.1
	Std Dev.	0.5	0.5	0.9
	Min (%)	0.5	0.6	0.5
	Max (%)	1.6	2.5	2.6

Table 7.4.8 Table showing the descriptive statistics for the additional mineral phases of calcite and quartz for each population, also included are the number and percentage of specimens identified as containing these phases.

Kruskal-Wallis analysis of the hydroxyapatite percentages for specimens grouped according to the minor phases absent or present, showed no significant difference (Table 7.4.9). The descriptive statistics for these groups show that the CV and SA outliers described in section 7.4.1.1, did not both contain the minor phases. Indeed, the CV outlier

specimen did contain both calcite and quartz, however the SA specimen contained neither phase.

		Capuchin Catacombs, Sicily				Cementerí Vell, Formentera				San Agustín, La Rioja			
		<i>By Minor Phase Present</i>											
		No minor phases	Calcite Present	Quartz Present	Both minor phases	No minor phases	Calcite Present	Quartz Present	Both minor phases	No minor phases	Calcite Present	Quartz Present	Both minor phases
No. of Specimens		12	5	1	2	10	6	2	10	17	8	2	2
HAp (%)	Mean (%)	79	75	84	57	73	71	80	69	84	82	76	79
	St. Dev.	9	10	-	51	12	10	4	13	14	6	5	6
	Min (%)	60	60	-	64	54	55	76	38	38	70	71	72
	Max (%)	90	88	-	6	92	88	84	83	96	89	81	85
H		4.48				2.00				5.90			
<i>p</i>		0.21				0.57				0.12			

* Significant result ($p \leq 0.05$)

Table 7.4.9 Table showing the descriptive statistics and Kruskal-Wallis results for the specimens of dental calculus as grouped by present additional mineral phases of calcite and quartz for each population.

7.4.2 Cross-Sectional Mineral Composition

The results of the micro-beam X-ray diffraction demonstrated that the mineral composition of the dental calculus specimens analysed is not consistent throughout the deposit. As shown in the stacked diffractograms in Figure 7.4.2, the shoulder of the hydroxyapatite peak that is associated with whitlockite (at about $32^\circ 2\theta$) increases in size from the deep surface towards the centre of the deposit and then decreases towards the superficial surface. This is also shown in the 3-D plot of the diffractograms in Figure 7.4.3. This mineral distribution was found in both specimens analysed.

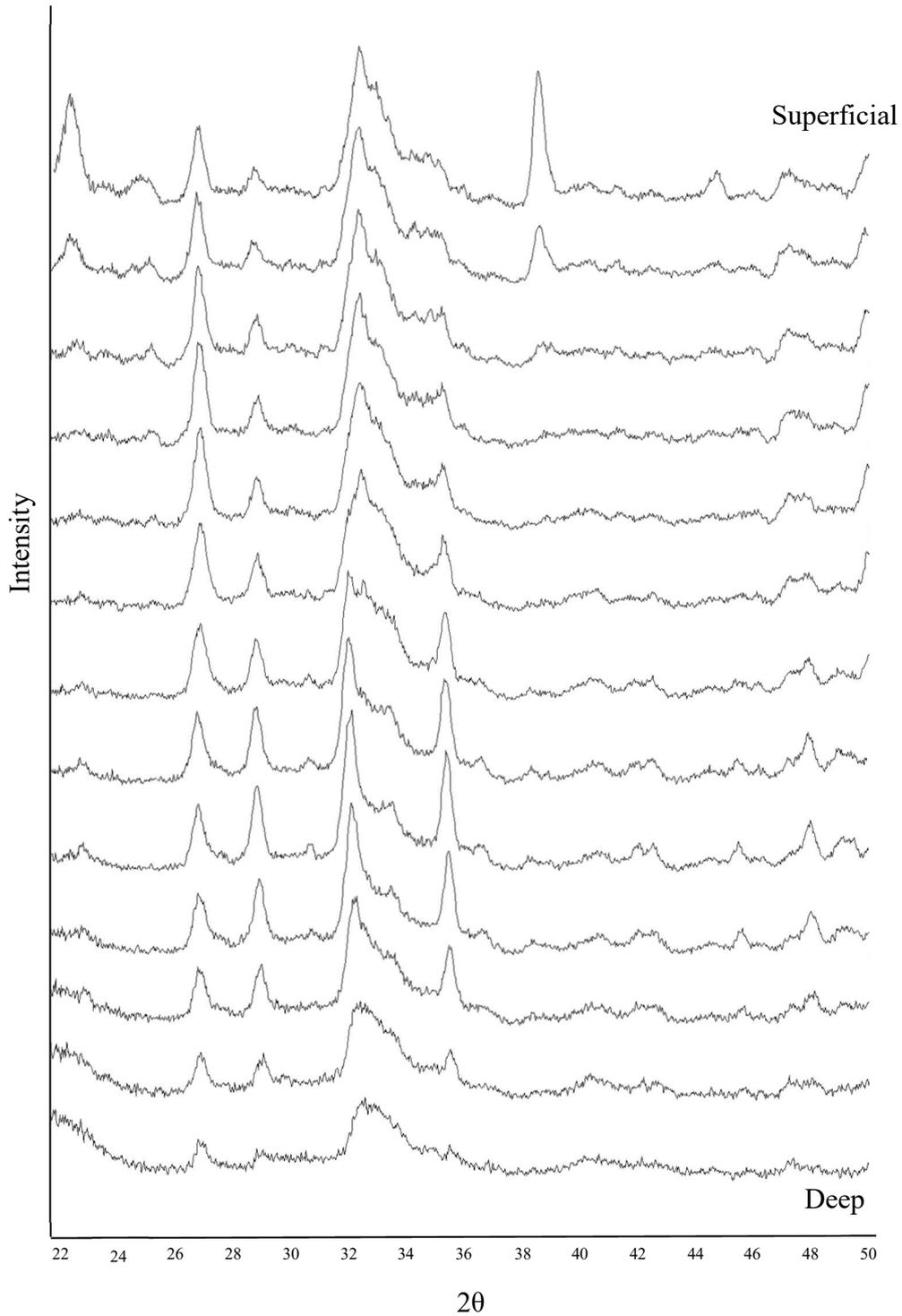


Figure 7.4.2 Stacked micro-beam X-ray diffractograms showing the change in mineral composition in the cross-section of specimen CCNMR1144 of dental calculus from the Capuchin Catacombs. The peak shoulder at around 32° 2θ indicates the presence of whitlockite (the peak at around 40° 2θ is related to the resin the specimen was mounted in).

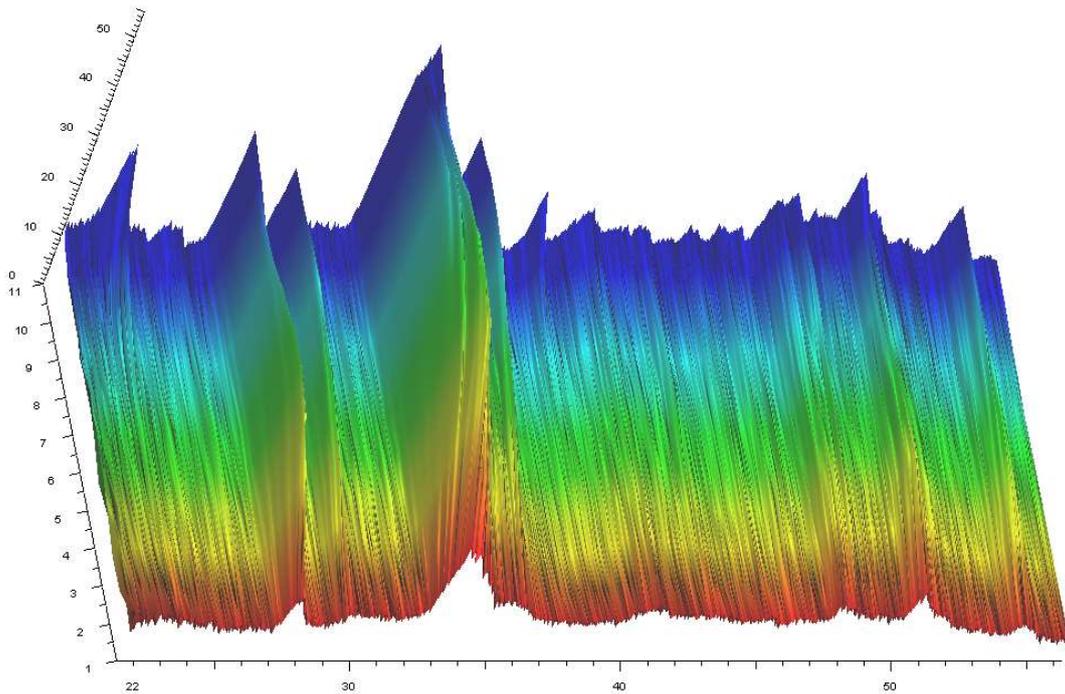


Figure 7.4.3 3-D plot of the sequential diffractograms along a line across the cross-section of dental calculus as analysed by micro-beam X-ray diffraction (data shown for specimen CCNMR1144).

The ratio of hydroxyapatite to whitlockite for this specimen also demonstrates the increase in whitlockite towards the middle of the deposit and subsequent decrease towards the superficial surface (Figure 7.4.4). This was the case in both lines analysed (line 2 and 3) for this deposit, unfortunately line 1, did not produce sufficient results to analyse the percentage content. The peak percentage of whitlockite for line 2 is closer to the deep surface (around 0.4 mm from the deep surface), whereas for line 3, the peak whitlockite percentage is more central (at around 0.7 mm) (Figure 7.4.4).

The corresponding nano-computed tomography image for the cross-section analysed in these results is shown in Figure 7.4.5. The changes in mineral ratio do not seem to be detectable in the n-CT of this specimen; or the other specimen analysed. However, this may be due to the low-quality data of the micro-beam XRD because of the material being poorly crystalline and having interference from the surrounding resin.

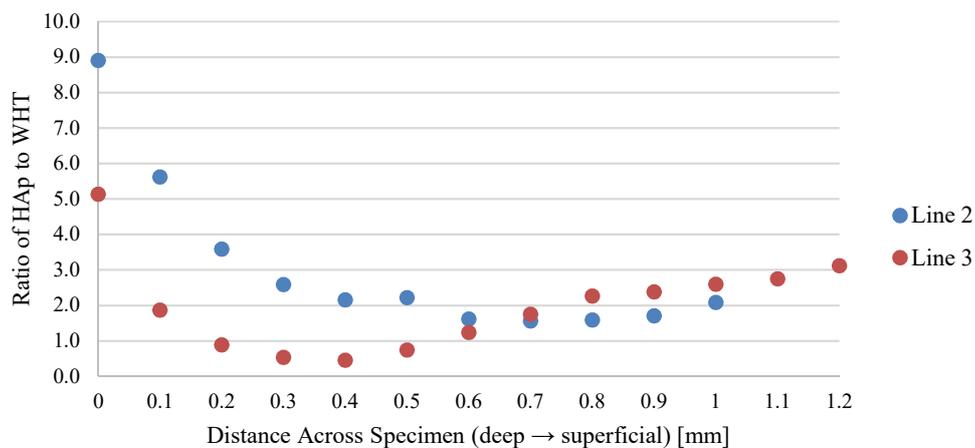


Figure 7.4.4 Graph showing the decrease in HAp:WHT from the deep surface of the deposit towards the centre and the subsequent ratio increase from the centre to the superficial surface for specimen CCNMR1144 (see Appendix E, Figure E.3.4 for graph with error bars).

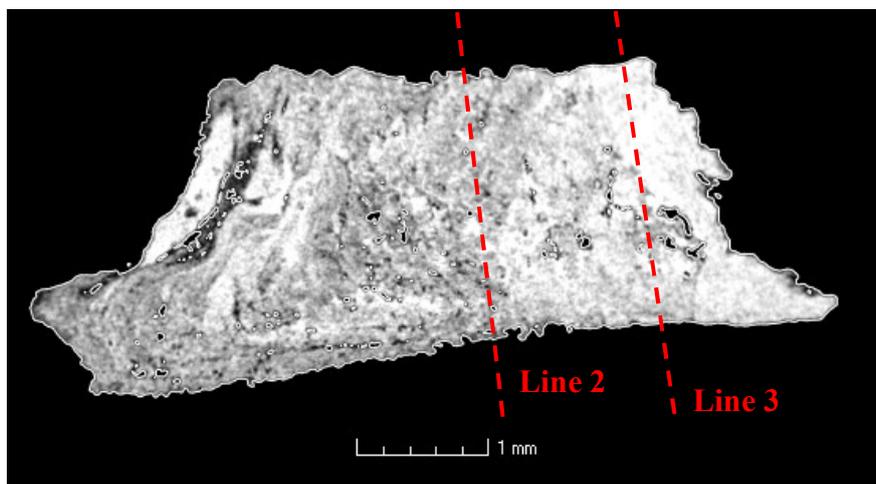


Figure 7.4.5 Nano-computed tomography image of the cross-section relating to the above micro-beam X-ray diffraction data (specimen shown, CCNMR1144).

7.5 Elemental Analysis

The following sections present elemental results from the archaeological specimens of dental calculus from the three populations sampled. Within these results, two methods of

inductively-coupled plasma mass spectrometry in solution mode and energy dispersive X-ray analysis were utilised. The rationale for using these techniques is presented in section 5.4 and the methods are explained in section 6.9. The results are presented in terms of the bulk and cross-sectional composition of the dental calculus specimens analysed.

7.5.1 Bulk Elemental Composition

7.5.1.1 Calcium-Phosphorus Ratios

The major elements of calcium and phosphorus showed consistent ratios between each population for all specimens, although this was higher than the stoichiometric values in both hydroxyapatite and whitlockite (1.67 and 1.51 respectively). The Ca/P ratio for the Capuchin Catacombs specimens was slightly lower than the other two populations and the Cementeri Vell specimens had the largest range of Ca/P ratios (Table 7.5.1). However, ANOVA results show that there is no significant difference between the population regarding Ca/P ratio (Table 7.5.1).

		Capuchin Catacombs, Sicily	Cementeri Vell, Formentera	San Agustín, La Rioja
No. of Specimens		9	9	9
Ca/P Ratio	Mean (%)	2.08	2.20	2.17
	Std Dev.	0.20	0.28	0.17
	Min (%)	1.74	1.97	1.85
	Max (%)	2.31	2.92	2.49
ANOVA	F test	0.66		
	F-Critical	3.40		
	p value	0.52		

Table 7.5.1 Table showing descriptive statistics of the Ca/P ratios for all specimens analysed in each population.

7.5.1.2 Iron Content vs. Periodontal Health

The concentrations of iron found in the analysed specimens from each population showed that Cementeri Vell had the highest mean concentration and the largest range of values (Table 7.5.2). However, this included one outlier that increased the maximum

concentration from 23.5 mg kg⁻¹ to 43.0 mg kg⁻¹. If this outlier is excluded, the mean iron concentration is more like the Capuchin Catacombs result and both these sample group contained only specimens from posterior teeth.

In contrast, the San Agustín sample group contained an equal amount of anterior and posterior specimens and had a lower mean and range than the other two sample groups (Table 7.5.2). Although a 2-tailed t-test found that there was no significant difference between the anterior and posterior iron concentration in San Agustín ($p = 0.20$; $p < 0.05$). There were three specimens from San Agustín that were from the lower anterior teeth of the same individual, these specimens showed iron concentrations from 2 to 5 mg kg⁻¹. Of these specimens, the deposit from the lingual surface was lower than the two specimens from labial surfaces (1.9; 4.8 and 5.1 mg kg⁻¹ respectively). However, a two-tailed t-test of the buccal/labial surface specimens and the lingual specimens for all populations indicated that the iron concentrations were not statistically significant ($p = 0.08$; $p < 0.05$).

		Capuchin Catacombs, Sicily	Cementeri Vell, Formentera	San Agustín, La Rioja
No. of Specimens		10	10	10
Mean Iron Concentration (mg kg ⁻¹)	Mean (%)	9.1	12.3	5.1
	Std Dev.	8.6	12.5	3.1
	Min (%)	1.0	0.5	0.8
	Max (%)	28.5	43.0	10.3
ANOVA	F test	1.47		
	F-Critical	3.35		
	<i>p</i>	0.25		

Table 7.5.2 Table showing descriptive statistics of the iron concentrations for all specimens analysed in each population.

The iron concentration did not appear to be related to the dental pathologies recorded on the dentition of the individuals analysed (see *Supplementary Material*: Appendix E.4.1). However, in the Capuchin Catacombs, there was a positive trend between the iron concentration and the percentage of teeth affected by dental calculus and this trend was negative in the other two populations (Figure 7.5.2).

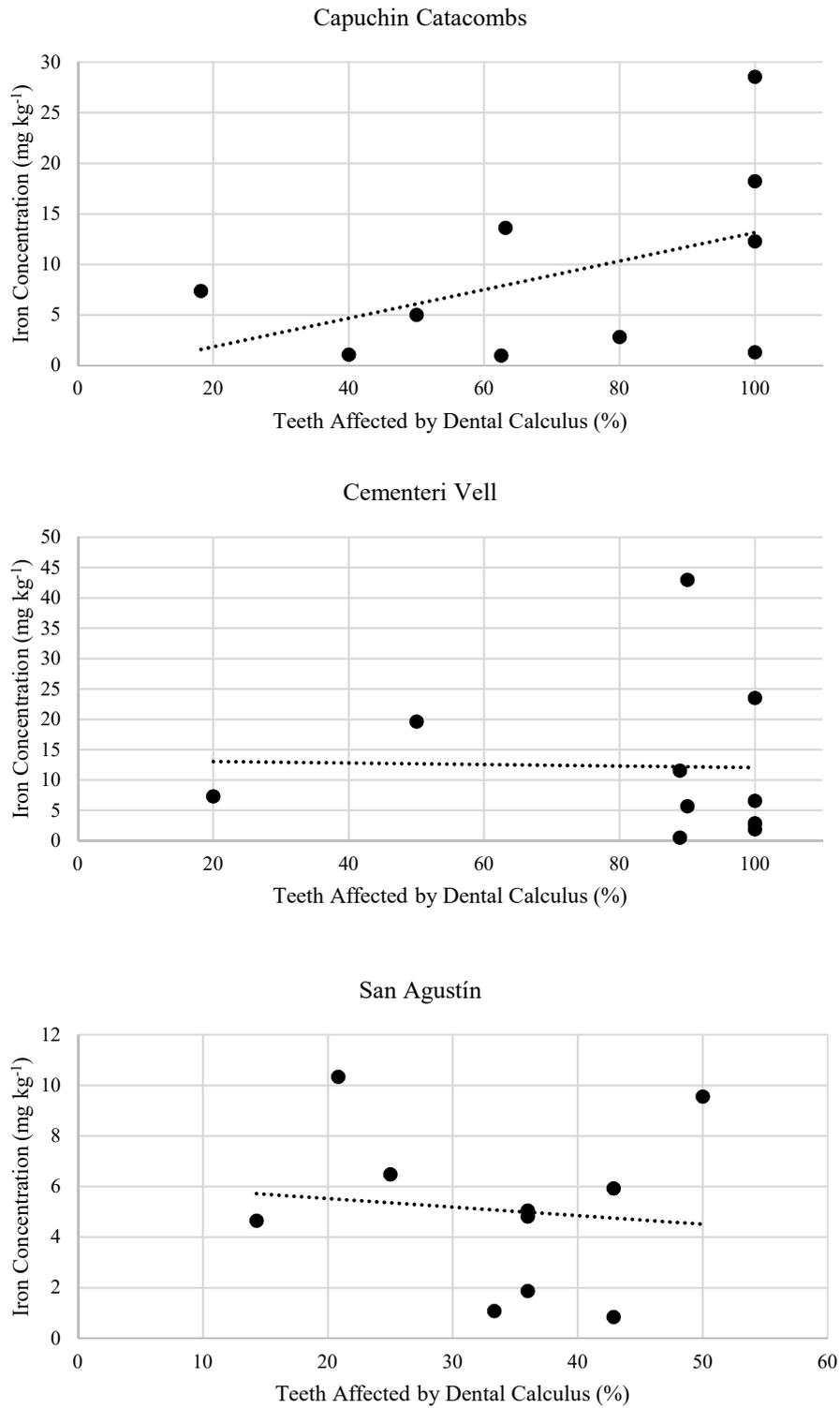


Figure 7.5.1 Graphs to show the relationship between the iron concentration (mg kg⁻¹) as determined by ICP-MS(Sol) against the percentage of teeth affected by dental calculus in the individual from which the specimen was taken.

There were six specimens from Cementeri Vell that had associated data regarding periodontal recession (see section 6.4.1.3). The amount of recession did not seem to correspond to an increase in iron concentration in the specimens (Figure 7.5.3). The specimen with the outlying iron concentration, was not from the tooth with the largest recession measurement. Additionally, the specimen from the tooth with the largest recession measurement had the lowest iron concentration (Figure 7.5.3).

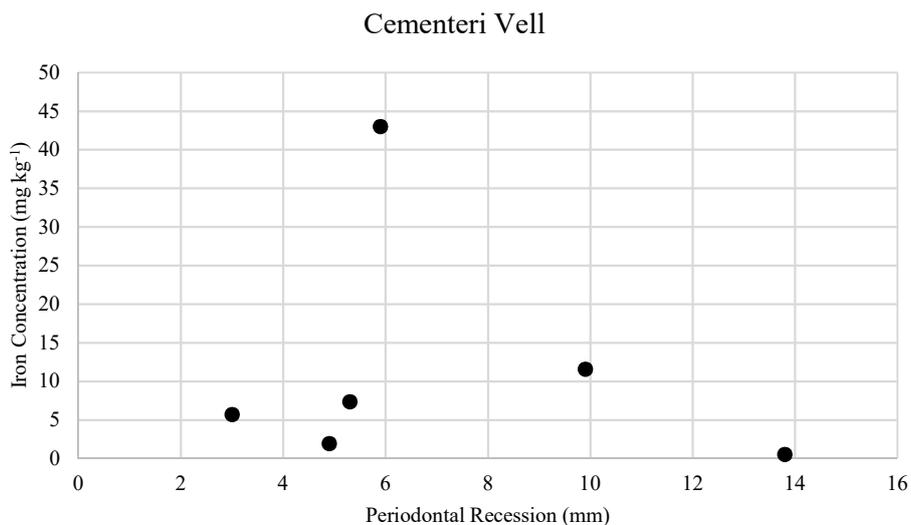


Figure 7.5.2 Graph showing the dental calculus concentration of iron (mg kg⁻¹), as determined by ICP-MS(Sol) against the amount of periodontal recession (mm) measured on the tooth face from which each specimen was taken.

7.5.1.3 Multi-element Analysis

The multi-element analysis of the specimen solutions enabled the comparison of the trace elements in specimens from each population (Table 7.5.3). The Capuchin Catacombs and San Agustín specimens had the most similar elemental concentrations although the CC concentrations were predominantly lower than SA. In contrast, Cementeri Vell had markedly higher concentrations than the other two populations, with aluminium, vanadium, chromium, copper and strontium all being the most different elements compared to the other two populations.

		Capuchin Catacombs, Sicily	Cementeri Vell, Formentera	San Agustín, La Rioja
No. of Specimens		10	10	10
Mean Element Concentration (mg kg ⁻¹)	Al	0.19	0.53	0.25
	V	0.12	0.52	0.37
	Cr	0.55	2.90	0.57
	Mn	0.16	0.44	0.52
	Cu	1.20	2.19	0.73
	As	0.02	0.12	0.10
	Sr	0.35	3.23	2.41
	Ba	0.19	0.53	0.25

Table 7.5.3 Table showing the mean concentrations of trace elements present in specimens analysed by ICP-MS(Sol).

7.5.2 Cross-Sectional Elemental Composition

During the scanning electron microscopy analysis (results described in section 7.3), energy dispersive X-ray analysis was performed on cross-sectional specimens from the Capuchin Catacombs. The elemental results gained from this analysis were semi-quantitative, as described in the methods (section 6.9.2).

7.5.2.1 Major Elements

All specimens contained the major elements identified, which were calcium, phosphorus, carbon and oxygen. The oxide percentages of these elements were compared between locations (deep/central/superficial) in each cross-section (Table 7.5.4 and 7.5.5). In Table 7.5.4, the descriptive statistics for each element are displayed per their location in the cross-section. Also included are the results of the analysis of variance (ANOVA) between the locations for each element.

For calcium, the central third exhibited the highest average percentage (29%) as well as the highest minimum percentage (18%) of calcium. The range of percentages was marginally lower for the central compared to the deep and superficial locations (8%; 10%; 10% respectively). This relationship was also present for the phosphorus percentages in each location (see Table 7.5.4).

For the oxygen percentages, the central location was only marginally higher than the deep and superficial locations, which were themselves the same (36%; 34%; 34% respectively). However, the percentage range for the deep location was slightly larger than the central and superficial locations (13%; 10%; 10%).

The carbon content, showed an inverse relationship between the central and deep and superficial locations, when compared to the other three major elements. The mean carbon percentage of the central location was lower than the deep and superficial locations (19%; 27%; 30% respectively). The ranges of carbon percentages found in each location larger than the ranges of the other three major elements (deep: 37%; central: 31%; superficial 28%).

The results of the ANOVA statistical test for variance between the percentages of each elements per location, indicated that the null hypotheses should be rejected in all cases. Therefore, it was concluded that there were location differences between the percentages of each element. Further statistical tests were conducted on the possible pairings of locations to ascertain where the differences were statistically significant (Table 7.5.4).

		Deep	Central	Superficial
<i>Calcium</i>	Mean (%)	25	29	23
	St Dev.	4	4	5
	Min (%)	15	18	15
	Max (%)	35	36	35
	<i>ANOVA</i>	F	11.577	
	F-Critical	3.098		
	H ₀	Rejected		
<i>Phosphorus</i>	Mean (%)	12	14	11
	St Dev.	2	2	2
	Min (%)	7	9	7
	Max (%)	16	16	15
	<i>ANOVA</i>	F	15.868	
	F-Critical	3.098		
	H ₀	Rejected		
<i>Oxygen</i>	Mean (%)	34	36	34
	St Dev.	3	3	3
	Min (%)	27	29	29
	Max (%)	40	41	41
	<i>ANOVA</i>	F	7.612	
	F-Critical	3.098		
	H ₀	Rejected		
<i>Carbon</i>	Mean (%)	27	19	30
	St Dev.	9	7	9
	Min (%)	14	11	14
	Max (%)	51	42	48
	<i>ANOVA</i>	F	16.452	
	F-Critical	3.098		
	H ₀	Rejected		

H₀ = null hypothesis; there is no difference between each location

Table 7.5.4 Table showing the descriptive statistics for each element, displayed per their location in the cross-section. Also shown are the ANOVA results for each element to determine if there were statistical differences in the percentage of major element between locations.

To determine the significant differences within the data, the possible pairs of variables (deep/central; deep/superficial; central/superficial) were tested using 2-tailed t-tests. Prior to this, F-tests for variance were conducted to determine if the t-tests needed for each pair were of equal or unequal variance. All pairings exhibited equal variance. For all four

major elements, there were significant differences between the percentages of the deep/central and central/superficial locations within the cross-sections (using a 95% confidence limit) (Table 7.5.5).

		D	C	S			D	C	S
		<i>Calcium</i>							
Calcium	C	1.149			Phosphorus	1.799			
	S	1.841	1.366			1.841	1.691		
	D	0.002*	1.841	1.189		0.001*	1.841	1.064	
			0.000*	1.841			0.053		
		<i>Oxygen</i>							
Oxygen	C	1.433			Carbon	1.768			
	S	1.841	1.005			1.841	1.742		
	D	0.004*	0.841	1.440		0.000*	1.841	1.015	
			0.000*	1.841			0.139		
		<i>Carbon</i>							

Key of Values	
F	F-Critical
t-test	

(D = deep third; C = central third; S = superficial third, see Figure 6.9.2)

* Significant result ($p \leq 0.05$)

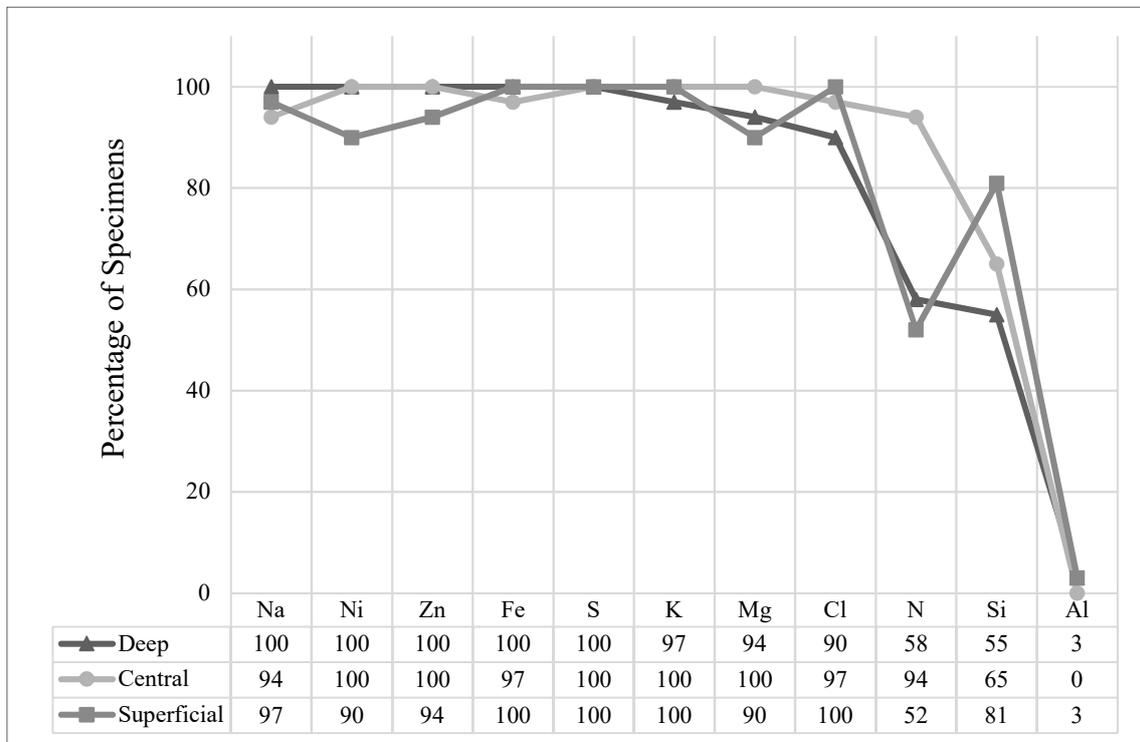
Table 7.5.5 Table showing the F-test for variance (F values and F-critical values) and the 2-tailed t-test results for each element. Results displayed show the significance testing of location differences for each element.

7.5.2.2 Minor Elements

The minor elements identified in the cross-sectional specimens were not all found in all locations of all specimens, except for sulphur (Figure 7.5.4). The percentage of specimens that contained Na, Ni, Zn, Fe, K, Mg and Cl varied from 90-100% depending on which third of the cross-section was analysed (see Figure 7.5.4).

The percentage of specimens with nitrogen identified was highest in more central locations than deep and superficial locations (94%; 58% and 52% respectively). The percentage of specimens identified as having silicon present decreased from superficial (81%) to central (65%) locations and further decreased for the deep surfaces (55%). Only one specimens had aluminium present and this was found to be in the deep and superficial locations (Figure 7.5.4).

Unfortunately, the sensitivity of the EDAX analysis was not enough to accurately quantify the minor elements. The results tables detailing the elements present for each specimen analysed are included in *Supplementary Material: Appendix E.4.2*.



(Na= Sodium; Ni=Nickel; Zn = Zinc; Fe=Iron; S=Sulphur; K=Potassium; Mg=Magnesium; Cl=Chlorine; N=Nitrogen; Si=Silicon; Al=Aluminium)

Figure 7.5.3 Line graph showing the percentage of specimens having each minor elements identified in their EDX spectra for each location analysed.

7.6 Chapter Summary

This chapter has demonstrated a wide range of results from the recording of the dentition, the multi-technique imaging of dental calculus and the mineralogical and elemental analyses.

This research has several key findings that are a significant contribution to the field of dental calculus analysis. The novel use of imaging for these specimens, has provided valuable information, regarding the colour, texture and shapes of archaeological dental calculus, observations which have previously been under-reported. Furthermore, the multiple complementary analytical techniques have provided compositional information for both the mineral and elemental components of the archaeological specimens, both in terms of the bulk material and cross-sectional changes.

In addition, these results demonstrate the novel application and successful imaging of dental calculus specimens using nano-computed tomography, as well as the identification of different internal densities using this technique. Furthermore, the mineralogical and elemental analysis of many specimens from multiple populations has provided more comprehensive data on the composition of archaeological dental calculus, that has previously been derived from clinical research. As well, the different burial environments that the dental calculus has been subjected to, has provided a comparison between characteristics and composition, that has not been explored previously.

The subsequent chapter will explore these results to discuss the potential implication of these findings for the wider research context and the specific populations studied, with reference to the research hypotheses of this thesis.

CHAPTER 8: DISCUSSION

*Scrape the teeth that have the scales
or the sand like substance
until nothing remains of them.*

- Albucasis 1000 C.E.

8.1 Overview

The work in this thesis was focused on providing insight about the variation in physical characteristics and the mineral and elemental composition of archaeological dental calculus from three Mediterranean populations. As such, four research aims were outlined which investigated features of archaeological dental calculus that had not been previously explored. These were, (1) to investigate if layering can be detected in archaeological dental calculus and whether the mineral and elemental composition is consistent through the accumulation plane (cross-section) of a deposit; (2) investigate whether dental calculus composition varies within and between archaeological populations; (3) investigate if there is a compositional influence on dental calculus from the presence of oral diseases and pathologies and (4) demonstrate whether modern dental calculus research accurately reflects the archaeological findings presented in this research.

The results presented in this thesis represent a study that includes a large sample size of archaeological dental calculus from three Mediterranean populations. This research includes the first known analysis of dental calculus by non-destructive nano-computed tomography. As well, it is believed to be the largest compositional study of archaeological dental calculus to date. In addition, the study includes cross-sectional compositional analysis, which has not been investigated for past population specimens.

The results were presented in Chapter 7 with additional data included in *Supplementary Material: Appendix E*. The results obtained are discussed in this chapter and where relevant are discussed regarding the published literature, from both archaeology and dentistry.

8.2 Cross-Sectional Variation

The results of the cross-sectional morphology analysis demonstrated that nano-computed tomography (n-CT) was an ideal method to detect density differences and pores within a

specimen. These differences were not observable in scanning electron microscopy analysis due to the similarity in elemental composition between the regions of different density. Consequently, the results of the n-CT in this research identified some interesting cross-sectional variation between archaeological dental calculus specimens.

In the literature, there is little difference in the density of the major phases of pure mineral hydroxyapatite and whitlockite (3.16 and 3.11 g cm⁻³ respectively) (Calvo and Gopal 1975; Fleet 2004). However, the nano-computed tomography (n-CT) demonstrated that there are density differences that can be detected within archaeological calculus and these relate to colour differences observed on the attachment (deep) surface.

The specimens with least variation in external colour had homogenous densities (Type I) but did not have a different mineral composition to other types. This suggests that the observed differences in density may be due to the compaction rather than the composition of the mineral. Alternatively, density differences may be related to apatitic substitutions in the mineral phases, however confirmation of this would require further analysis. Whether the density differences are due to compaction of the mineral or ionic substitution, this implies that the density differences may be related to the manner of formation or the oral environment that the dental calculus accumulated in.

The three different types (I, II, III) of specimen identified show that dental calculus density can be homogenous (Type I), have two discrete regions of homogeneity (Type II) or can be heterogenous (Type III). These types were not found to relate to the dental location from which the deposit was taken, but did relate to the mass of the deposit. The most common type of density distribution in all populations was Type I, however these were also the least massive deposits. Across all populations, smaller deposits (Brothwell Score = 1 'slight') were more common and as such there was a higher percentage of Type I specimens.

The homogeneity of density in Type I specimens indicates that in these deposits there was an accumulation of calculus material without disruption. The homogenous density exhibited few pores or defects in the cross-section of the calculus. With these deposits being the smallest in terms of mass, this may indicate that they began to accumulate not long before the individual died, rather than having increased in size over a longer time. As Type I specimens were found to be less massive, as well as having a dense and

homogenous structure, they may have formed over a short period when continuous and possibly quick accumulation occurred. This rapid accumulation may have occurred in the weeks before death due to a change in lifestyle that promoted the formation of dental calculus.

Alternatively, rather than accumulating before death, these Type I deposits may have decreased in size, due to a change in conditions adverse to calculus formation. The density of these Type I specimens was similar to the density and structure of the inner layer of material in the Type II specimens. Therefore, it is feasible to suggest that a Type I specimen may have previously been Type II, but prior to death had experienced some reversal phenomenon, possibly due to a change in oral conditions. Additionally, rather than by dissolution, these specimens may have been physically decreased in size by scraping or picking at the deposit to remove it from the tooth.

However, it is more plausible that the former explanation is applicable and dental calculus has accumulated prior to death rather than eroded. For the deposits to have been eroded, the oral environment would have had to increase in acidity to dissolve the calcified dental plaque. If this were to occur, the enamel of the teeth would also have been highly susceptible to erosion, causing lesions. As such this was not observed in any of the populations as the percentages of caries were low for calculus-exhibitors. These observations are contrary to the conditions that would be required for considerable calculus dissolution.

Regarding the possible physical alteration of the specimens through picking or scraping, this cannot be ruled out and could explain the small size of the deposits. If this had occurred close to the time of death, a more jagged, superficial surface would be expected, however this was not observed. If the size of the deposits had been physically reduced in size a greater amount of time prior to death, a jagged superficial edge may have smoothed. However, following this, it is likely that further calculus deposition would occur, resulting in an observable layer that indicated the disrupted accumulation, potentially like the Type II specimens, that are discussed below. Consequently, considering the lack of observable features that would be expected for both chemical erosion or physical alteration, it is more likely that these specimens of calculus accumulated because of a change of health, diet or mobility.

For calculus accumulation to occur rapidly, prior to death, it is possible that the host individual had become afflicted with illness or injury. This implies that the 'normal' diet and health of the individual did not promote the formation of deposits or that the teeth were cleaned sufficiently to deter plaque accumulation and calcification. However, close to the time of death, these conditions changed. It is possible that a change in diet or the consumption of herbal treatments promoted calculus formation. Alternatively, the oral hygiene in an individual may have decreased, possibly due to mobility or illness which may have led to plaque accumulation and consequently, resulted in calculus formation.

In Sicily, the advice of doctors was not trusted during the period in question, therefore it would be family members or close friends that would decide on the treatment for the sick or injured (Pitrè 1870). This is likely to have been similar across Europe, especially in rural areas where access to surgeons and physicians was limited (Pitrè 1870). Consequently, the treatment of the sick and injured would most likely have been done through the modification of diet, herbal treatment or through prayer (Pitrè 1870; Piombino-Mascali et al. 2013; Byrne 2013). Therefore, a change in diet close to death, due to ill health is feasible and may have influenced calculus-formation.

In the group of Type I specimens, an outlier was observed in the Capuchin Catacombs specimen that had a higher mass than the upper quartile. By examining the dental recording of this individual, there did not appear to be any outstanding differences in this individual's dental health compared to the rest of the population. The larger mass of this deposit compared to other Type I specimens, may indicate a prolonged episode of illness prior to death. A longer period of eating a modified diet that induced calculus formation may have caused this specimen to be larger. However, this was a specimen sampled from a lower incisor lingual surface, which was uncommon due to the high amount of anterior tooth loss in the population. As this surface is known to be a common site for calculus formation due to the proximity to the sublingual salivary ducts, the presence of a larger specimen here is not unexpected. However, this combined with the homogenous density of the specimen indicates that the individual may have had a disturbed metabolism, possibly due to kidney disease, that caused increased calculus formation (Davidovich et al. 2009).

In Type II specimens, a distinction was seen between two regions of differing density of dental calculus. At first this was thought to be attributed to a taphonomic layer, that may have accumulated on the specimens, while the remains were buried. However, with the presence of Type II specimens in the mummified remains of the Capuchin Catacomb sample, combined with the thickness of the outer layer in most cases, this was discounted. In addition, the bulk mineral analysis of these specimens did not prove remarkably different to the other two types of specimens.

As mentioned above, another cause for observing two distinct layers of dental calculus density may relate to the physical debridement of a specimen by picking or scraping. This would cause a disruption in the accumulation of calculus and cause an observable marker for where the original deposit had been reduced. Following, this further calculus accumulation would cover this original layer. The problem with this theory is the difference in density between the inner and outer layers of calculus. A disruption followed by further accumulation in an individual that had not changed their diet, health or oral hygiene, would therefore be expected to exhibit as a line rather than a separation of bands. As such this was not seen and the density of the layers was found to differ, with the outer layer being more porous and less dense.

The presence of two discrete densities of dental calculus suggest a change in formation conditions. This may be related to an episode of illness or injury, that was overcome. As in Type I specimens, the first set of conditions caused a dense inner layer which may have been due to a change in diet, the consumption of herbal medication or due to a change in mobility. However, following this, the conditions altered so as calculus formation was not as compact.

The presence of a second layer of dental calculus may indicate that the initial episode that caused formation was overcome. Consequently, it may be that the diet was reverted to pre-illness; the medication was halted; or mobility was regained. Even if the individual regained their health and mobility, the calculus had still accumulated and would be difficult to remove. Additionally, as dental calculus provides a rough surface for dental plaque to accumulate on, this would enhance calculus formation, even if prior to illness or injury, the teeth had been kept clean. Furthermore, if a change in diet back to pre-illness was less favourable for calculus formation, this may explain the porous nature of

the secondary layer. Although promoted by the increased plaque accumulation on the rough calculus surface, the alkaline conditions required may have been less consistent than during the bout of illness, causing a less compact layer.

Finally, the Type III specimens indicate that dental calculus can be heterogenous with respect to density. The results show that larger specimens of dental calculus exhibit multiple regions of differing density. This implies that calculus accumulation was prolonged and underwent fluctuations in accumulation. These fluctuations may have occurred because of periods of ill health or seasonal variation in diet. The lines of disruption and changes in density could also be attributed to the removal of calculus material by the individual as it became uncomfortable.

The green colouration of dental calculus that potentially relate to a carbonate-containing hydroxyapatite (see section 8.3) were found to be high density layers in Type III specimens. This supports the evidence that density can indicate fluctuations in oral conditions during dental calculus formation. The hydroxyapatite in this layer was formed containing carbonate, rather than phosphate, or the phosphate was replaced by carbonate. In either case, this implies that for a discrete period during calculus formation, there was an increase in carbonate ions in the oral environment.

In addition to observing density changes in the specimens, there were also defect features that were observable in the computed tomography. The results of the void analysis algorithms indicated that void size can vary across a deposit. Larger voids can be associated with cusp crevices, which may relate to food entrapment being incorporated into the calculus matrix. Further work is required to understand void distribution in archaeological dental calculus and how it may relate to microfossil or starch inclusions. However, this study has highlighted that there is variation in the spatial distribution of voids within archaeological calculus.

The cross-sectional micro-beam X-ray diffraction results demonstrated that changes in hydroxyapatite and whitlockite percentages are detectable across a deposit of archaeological dental calculus. This means that the mineral composition is not homogenous and the two phases are formed in varying proportions as the deposit accumulates. The results demonstrated that there was a detectable increase in whitlockite from the attachment surface to the middle of the deposit, followed by a decrease towards

the superficial surface. This indicates a change in calculus formation conditions, which may be related to seasonal dietary changes or the health of the individual. However, analysis of more specimens would be required to understand if these changes are typical of calculus aetiology or because of external factors that influence calculus deposition, such as diet and health.

The results of the micro-beam X-ray Diffraction combined with the nano-computed tomography data did not seem to suggest that the observed density changes are due to different mineral phases. This is supported by the similarity in density of the minerals found to be present, hydroxyapatite and whitlockite. Instead the density changes seem to relate to the compaction of the mineral content in the dental calculus, possibly due to rapid accumulation.

The cross-sectional elemental analysis of the sectioned specimens of dental calculus from the Capuchin Catacombs qualitatively determined the elemental composition of the deposits in three locations, close to the attachment (deep) surface, centrally and close to the superficial surface. The major elements present were found to be calcium, phosphorus, oxygen and carbon. The first three of these major elements are consistent with the EDX analysis of archaeological dental calculus by Charlier et al. (2010) and are expected considering the calcium phosphate mineral phases that were found in the mineralogic results.

The presence of carbon as a major element in the specimens is possibly related the substitution of phosphate for carbonate in the hydroxyapatite mineral phases. However, the results also indicated that the percentage of carbon was higher in the superficial and deep thirds of the specimens. As the specimens were embedded in resin, which is an organic polymer, the increased carbon content at the resin/calculus boundaries can be attributed to the embedding medium penetrating the deposit. The statistical results supported this by showing that there was no statistical significant difference between the superficial and deep surface regarding carbon content. However, there were statistical differences between both external thirds and the central third. The presence of carbon in the central third may be related to the presence of starch granules in the deposits, which are around 40% carbon (see Power et al. 2014). Unfortunately, due to the surface damage

by the micro-tome blade this was not able to be corroborated by visualisation of any inclusions (section 6.6.3).

The same statistical differences were also seen between the thirds for calcium, phosphorus and oxygen. This suggests that the central third of the deposits is different regarding all major elements, not just due to the resin contamination for carbon. The differences between the central and superficial surfaces may be due to the porosity of the specimen seen at the superficial surface resulting in lower percentages of elements and possible inclusions, in the less compact material.

For the lower percentages of major elements detected in the deep compared to the central third, this may suggest a greater proportion of elemental substitution. The calculus at the deep third is the oldest material in the deposit and as such has been in the oral environment the longest. Therefore, the mineral phases in this portion of the accumulation may have undergone substitution during the time that it has been present on the tooth. This may also be related to reversal phenomenon or dental intervention to remove the deposits. If this region of the calculus had been exposed on numerous occasions because of picking or scraping at the deposit, the mineral phases may have been more susceptible to ionic exchange, depending on the oral environment.

Overall, the major elements determined were consistent with what was expected for the mineral phases identified and the previous EDX elemental analysis of archaeological dental calculus (Charlier et al. 2010; Salazar-García et al. 2014). Despite these results not being fully-quantitative, they have shown that there are significant differences in the distribution of major elements across a deposit of dental calculus. These differences could be further investigated using LA-ICP-MS (see section 9.2).

The EDX results of the minor elements that were present in the different portions of the cross-sections of dental calculus could not be reported semi-quantitatively. Due to the sensitivity of the technique, the percentages of the minor elements being below 5% could only be reliable enough to confirm the presence of the element. Consequently, the results were displayed according to whether the element was found to be present, rather than by percentage. This was done for each third of the cross-section analysed (see Figure 6.9.2).

These results indicated that the trace elements detected were not limited to the external (superficial) surface of the deposit. Although, the specimens analysed were from the Capuchin Catacombs population and as such had not been buried, this demonstrates that the minor elements can be identified throughout the deposit, rather than solely on the external surface. In a further study, the analysis of the cross-sections of specimens that had been buried would need to be conducted to determine how much elemental difference there is between these thirds, when a specimen has been exposed to soil.

Unfortunately, the EDX results were insufficient to determine the changes in concentration of trace elements across the specimen. In addition, the sensitivity of the technique may have caused some low percentages of minor elements to be undetected. To solve this and gain better in-situ elemental data, further analysis using LA-ICP-MS would be beneficial.

This study has shown that colour and density differences in the dental calculus that appear in bands or layers can be detected in archaeological dental calculus. This supports the literature that dental calculus accumulation occurs in sequential layers (LeGeros 1981; Hayashi 1993; White 1997; Roberts-Harry and Clerehugh 2000). Consequently, hypothesis 1(a) was confirmed to be true. In addition, the colour observations that indicated layering, demonstrate that as well as accumulating out from the tooth's surface a dental calculus deposit also builds up in layers across the tooth's surface.

However, not all specimens exhibited layering. The Type I specimens had minimal colour differences in the attachment surfaces and no density changes in their cross-section. It is possible that the layering in these specimens was not detected, rather than not being present. Although, the colour observations support the homogeneity of these deposit. While hypothesis 1(a) is accepted, this study demonstrates that dental calculus may not always contain layers.

Regarding hypotheses 1(b) and (c), the composition of archaeological dental calculus was found to vary across a deposit. The micro-beam XRD results demonstrated that the ratio of major mineral phases, hydroxyapatite and whitlockite, varied from the attachment surface to the superficial surface. Similarly, the EDX elemental analysis of sectioned specimens suggested that the trace element composition was different between the deep, central and superficial thirds of the cross-sections. However, this study was not able to

definitively show that the compositional variation corresponded to the layers that were observed in the optical microscopy and nano-computed tomography results. There are further analyses and techniques that could be employed to test these hypotheses and these are discussed in section 9.3.

Overall cross-sectional variation in archaeological dental calculus was detected. The density differences observed in the nano-computed tomography may provide important information regarding the nature of accumulation. The differences in density changes through a deposit indicate changes in the oral environment during accumulation. This may enable dental calculus accumulation to be an indicator of life events, such as changes in health, diet or oral hygiene habits, in the weeks or months prior to death. In addition, these changes could be connected to an individual's regular oral hygiene habits, providing an indication of when a deposit was manually reduced in size and then reaccumulated.

8.3 Inter/Intra-Population Variation

The recording of tooth presence in each population indicated that approximately a third of the expected teeth were present in all populations. This was important because it gave an indication of how under-reported the prevalence of dental calculus and other pathologies may be. For each population, there were different potential reasons as to why the percentage of teeth was low compared to the expected number of teeth for the minimum number of individuals.

Firstly, from the results of the positions affected by ante-mortem tooth loss (AMTL), it is possible to determine that this contributed to the proportion of missing teeth. This was particularly high in San Agustín, where the individuals had just over half alveolar positions, recorded as AMTL. The causes of tooth loss during life cannot be conclusively determined but if occurring naturally, the main causes are periodontal disease and dental decay (Cucina and Tiesler 2003; Hillson 2005; Roberts and Manchester 2010). While periodontal disease is promoted by calculus accumulation and dental plaque presence, it is not possible to say that the AMTL observed is due to the populations forming calculus rather than suffering from dental decay (Roberts and Manchester 2010).

Some studies have suggested that there is an inverse relationship between calculus accumulation and carious lesion formation (Yardeni 1948; Dawes and MacPherson 1993;

Hillson 2001; Bonsall and Pickard 2015). This might suggest that individuals exhibiting dental calculus and AMTL, have a higher likelihood of having lost their teeth by periodontal disease, than decay. However, other clinical studies have noted the presence of dental calculus and carious lesions in the mouth of the same individual (Little et al. 1960; de Sousa et al. 2013). Consequently, the AMTL percentages can be used to suggest overall dental health, rather than definitively confirm that teeth were lost due to lesions or periodontal disease (Roberts and Manchester 2010). As such, the high percentages of AMTL in San Agustín suggest that this population suffered from prolific dental disease.

The proportion of post-mortem tooth loss (PMTL) indicated how much missing data there was, that could have potentially been available to determine calculus presence. In the Capuchin Catacombs, the post-mortem tooth loss was above 30% and nearly as high as the commingled remains from Cementeri Vell. This was unexpected because the mummified remains had been interred on site and had not been subjected to burial and excavation. In addition, the San Agustín population, only had 6% PMTL, far lower than the other two populations. The potential reasons for these unexpected proportions of missing teeth and consequently, missing data, differ between populations.

Firstly, in the Capuchin Catacombs, the results indicated a difference between male and female individuals in terms of tooth presence, however this may not be due to oral health. The female individuals recorded were all in a supine position in coffins and in an enclosed part of the Catacombs, where visitors cannot access. In contrast, the male individuals were predominantly upright in wall niches, many within arm's reach of the visitor walkways. Consequently, the lower percentage of male teeth present may be influenced by two factors, the upright position of the remains and potential vandalism (D. Piombino-Mascali per comms. May 2014).

The results of the distribution of present teeth in the Capuchin Catacombs sample, indicates that more posterior teeth were found to be present, compared to anterior teeth. This supports the potential loss of the anterior teeth either through falling loss from the upright positioning of the remains or vandalism. As the anterior teeth are only held in by one root, they generally become loose in the alveolar sockets and are easy to remove. If only the upright position of the remains was the cause of this tooth loss, it would be expected that mandibular anterior teeth should still be present as they would not fall from

the socket. However, this was not the case and equally low percentages of anterior teeth were found to be present in both jaws, indicating that vandalism or souvenir-taking is potentially the cause.

In Cementeri Vell, tooth presence was potentially influenced by the survivability of dentition fragments. The excavated remains in this population were subjected to initial burial and then secondary deposition in the ossuary. In addition, the remains had been exposed to changes in weather and had suffered high fragmentation. Most fragments present were maxillary, whole or partial. Within these fragments, the anterior dentition was less likely to be found in sockets, potentially due to the decreased security by which they are fixed into the alveolar bone, with only one root.

In contrast to the other two populations, San Agustín had a very low proportion of teeth lost post-mortem. This may be due to the burial nature of the individuals, as most were found in separate burial tombs and had not suffered from disturbance prior to excavation. In addition, the high proportion of AMTL in this population, means that there were less teeth present overall. With over half of the teeth lost before death, there would have been less to lose post-mortem.

Despite the various reasons for missing teeth in each population, the resulting percentages of observable teeth were similar. However, the percentage of tooth surfaces, excluding the occlusal surface, was not. Almost all surfaces were observable for both CV and SA, with unobservable surfaces being due to post-mortem damage or large carious lesions. However, the percentage of surfaces observable in CC was much lower. This was both due to the height of some of the remains causing difficulty in observing the posterior teeth surfaces and the presence of soft tissue on the mummified remains. Consequently, the amount of dental calculus reported on the CC sample is likely to be under-reported due to this lack of observability.

For individuals who were recorded as having calculus deposits on at least one surface, the percentages of teeth observable were similar or higher than the total population. In addition, the percentage of AMTL in these individuals was lower than the total population and this was likely due to older individuals who were edentulous, increasing the AMTL percentages in the total population. There was little difference in the percentage of alveolar positions affected by PMTL between the total population and the calculus-

exhibitors, indicating that missing data for each population did not affect calculus-exhibitors more than other individuals.

Overall, for all three populations it is likely that the dental calculus prevalence is under-reported due to post-mortem tooth loss. However, the amount of post-mortem tooth loss affected individuals with dental calculus in similar percentages to the total population. This means that the individuals with calculus did not have a disproportionate amount of missing tooth data. Consequently, the differences between all individuals and just the calculus-exhibitors was limited to ante-mortem tooth loss. This is understandable considering the total population included AMTL edentulous individuals that could not have had calculus deposits.

The result of recording the dental calculus deposits on the dentition indicated that the individuals in Cementeri Vell were least affected by the condition. However, the remains in this population had been subjected to burial, exhumation and re-deposition in the ossuary, followed by archaeological excavation. This disturbance may have resulted in post-mortem damage occurring to the dental calculus deposits that were present at death. Consequently, the number of individuals affected by dental calculus for this population may be under-estimated. This is supported by the result that there was a lower percentage of unknown sex individuals to exhibit dental calculus. These individuals were highly fragmented and did not have enough sex estimation landmarks present to indicate whether they were male or female.

The results indicated that the non-commingled populations had similar percentages of overall individuals affected by dental calculus. Furthermore, the percentages of male individuals affected were similar in both the Capuchin Catacombs and San Agustín. The percentage of Capuchin Catacombs females affected were lower indicating a clear difference between the sexes, in terms of dental calculus accumulation, which does match clinical studies of modern individuals and other archaeological assemblages (Christersson et al. 1992; Greene et al. 2005; Carneiro and Kabulwa 2012). This was also the case in Cementeri Vell, where the five crania with dentition that were estimated as female, exhibited no dental calculus deposits.

In all populations, dental calculus was recorded on all teeth types and all surface types. However, these results were greatly affected by the presence of teeth in each population

and the observability of surfaces in the Capuchin Catacombs. Despite this, in the Capuchin Catacombs and San Agustín, the largest deposits, according to Brothwell (1981) scores, were observed on the buccal surfaces of the posterior teeth and the lingual surfaces of the mandibular anterior teeth. This corresponds well with the literature that surface proximity to the parotid salivary gland and the submandibular gland increases the accumulation of dental calculus (Corbett and Dawes 1998; Dawes 2007).

An additional result in San Agustín was the presence of larger deposits on mesial and distal surfaces of the posterior teeth, which is understandable considering the high ante-mortem tooth loss in these individuals. The absence of an adjacent tooth consequently allows for a dental calculus accumulation to continue unhindered by the neighbouring surface. In Cementeri Vell, there was an overall lower percentage of deposits scored as being larger in size (Brothwell score = 3 ‘considerable’). This may have been due to individuals attempting to remove their deposits, however is more likely to be because of the potential post-mortem damage mentioned earlier.

To summarise, the dental calculus recording of the three populations indicates several points. Firstly, individuals in all three populations were observed as having deposits of dental calculus although the condition did not affect all individuals in each population. In addition, the size of the deposits recorded were not unusually large, compared to the recording literature; in fact, most deposits were recorded as slight (Brothwell 1981). Similarly, the site specificity of deposits corresponded with the literature, with mainly posterior buccal and anterior lingual surfaces affected (Corbett and Dawes 1998; Dawes 2007; Hayashizaki et al. 2008).

It is clear from the preceding section that the calculus recorded across each population is likely to be underestimated due to post-mortem damage and missing teeth. However, the observations of the dental calculus deposits that were recordable, indicate that all three populations were not extraordinary sample groups for dental calculus analysis, nor did the calculus-exhibitors represent a few select individuals in the population.

The results of the non-destructive imaging techniques highlighted intra- and inter-population differences in the physical characteristics of the archaeological dental calculus deposits. However, a feature that was consistent between all specimens was the textural nature of the deep and superficial surfaces. The deep surface being observable as smooth

corresponds with the intimate attachment that calculus has with the enamel surface of the tooth, as found in clinical calculus (Rohanizadeh and LeGeros 2005). Similarly, clinical statements that the external surface of dental calculus is rough which promotes plaque adhesion was supported in these results (Rohanizadeh and LeGeros 2005; Hayashizaki et al. 2008) (see section 8.5).

Because of the textural features being consistent within these studied populations, this characteristic has the potential to be used for future studies to orientate loose fragments of archaeological dental calculus. This may be valuable when sampling is not permitted unless accidental calculus removal occurs, such as through handling of the remains or during cleaning. Indeed, the surface texture differences were extremely useful in this project, when orientating a specimen for cross sectioning, especially because in-situ photographs had not been permitted for the specimens that were sectioned.

The orientation of dental calculus once off the tooth is important when considering the external factors that may affect analysis. The superficial surface, found to be rough and granular, represents the last calculus to have been deposited prior to death. Consequently, this surface has been exposed to the processes of taphonomy and the burial environment. As such, the colour observations of this surface potentially reflect the environment that the calculus has been buried in, rather than the colour it was in a living individual, resulting in post-mortem inter-population variation.

Alternatively, the deep surface, which corresponds to the attachment surface represents the oldest material in the deposit. While deposits can be subject to reversal phenomenon (see section 2.3.2.1) it is unlikely that this would remove an entire deposit without external intervention, as in modern dental scaling. Therefore, in past populations, this surface of dental calculus may have formed several months or years prior to death. It is also the calculus which has been shielded from the burial environment and therefore the colours observed on this surface are indicative of the material as formed in an individual, prior to post-mortem changes.

The regions of green dental calculus likely relate to the presence of hydroxyapatite, which is reported to exhibit a range of colours including white, yellow and green in naturally occurring deposits of this mineral (Ben-Nissan 2014). During the analysis of these deposits, X-ray diffraction confirmed the presence of hydroxyapatite in all the specimens

analysed. Furthermore, the green colouration can be linked to a carbonate-rich form of calcium hydroxyapatite (Britannica 2009). This is expected because biological forms of hydroxyapatite, in bones and teeth, have been found to contain carbonate due to the physiological conditions of the body (Crowley 2007). The substitution of carbonate into the mineral structure of hydroxyapatite, can occur at the hydroxide ion, phosphate ion or at both sites (LeGeros 1981; Fleet 2014). If substituted at the hydroxide group, the lattice parameters are changed by an expansion of the 'a'-axis and contraction of the 'c'-axis, whereas substitution of the phosphate causes the opposite parameter changes. (LeGeros 1981).

In the mineralogical results, the Capuchin Catacombs specimens had a mean 'a' lattice parameter that was higher and 'c' lattice parameter that was lower, than stoichiometric values. This indicates that substituted carbonate in the hydroxyapatite of these specimens may have occurred at the hydroxide group. Conversely, the San Agustín specimens had a mean 'a'-axis that was lower and 'c'-axis that was higher than stoichiometric hydroxyapatite, indicating phosphate substitution. However, Fourier-transform infrared spectroscopy (FTIR) would need to be employed to confirm the cause of these lattice parameter changes (LeGeros 1981; Fleet 2014).

Most of the Cementeri Vell specimens did not exhibit this green colouration, implying that there was no carbonate-containing hydroxyapatite present. This was supported by the mean lattice parameters for this population, which were values expected for stoichiometric hydroxyapatite, with no expansion or contraction of either axis by carbonate-substitution. However, for all the populations, carbonate-substitution cannot be the only consideration for lattice parameter changes and these are further discussed later in this section.

A reason for why most Cementeri Vell specimens did not contain any green, carbonate-substituted hydroxyapatite may be related to taphonomy. The incorporation of carbonate into the hydroxyapatite structure decreases its crystallinity and increases its solubility (LeGeros 1981; Fleet 2014). The dissolution of this portion of hydroxyapatite in the specimens may explain the fragility of these deposits compared to the other two populations. Indeed, the percentage of hydroxyapatite present in these specimens, as

determined from the powder X-ray diffraction analysis, was the lowest mean percentage of the three populations.

However, in the San Agustín sample, the outlier with a very low percentage of hydroxyapatite (38%), still exhibited a green colouration on the deep surface. Indicating that despite the difference in burial conditions between the two populations, a low hydroxyapatite percentage cannot necessarily be attributed to carbonate-containing hydroxyapatite loss. An alternative explanation for the lack of green carbonate-containing hydroxyapatite, may relate to the conditions that calculus formation occurred in. Consequently, implying that the general oral environment of individuals from Cementeri Vell was different to the individuals from the other two populations.

As explained in section 2.2.1.1, there are two main buffer systems in saliva, which influence the oral environment, the bicarbonate buffer and the phosphate buffer. The precipitation of calcium phosphates from saliva to form dental calculus occurs when there is a highly alkaline pH (9.0 or over). This can occur when proteins are digested and the urea concentration in saliva increases. In contrast, the bicarbonate buffer is the predominant regulator of oral pH and as such, works to balance out acidic products that come from the digestion of sugars.

The presence of carbonate-containing hydroxyapatite in dental calculus is potentially an indicator that the bicarbonate buffer has played a major role in the composition of the deposits. This also indicates that the oral environment has experienced high alkalinity for the dental calculus to accumulate on the teeth, as well as high acidity, for the bicarbonate buffer to regulate the low pH levels. This change from alkaline to acidic conditions suggests that a varied diet containing proteins and fermentable sugars has been consumed.

The lack of carbonate-containing hydroxyapatite in Cementeri Vell specimens may therefore be because this dental calculus has formed in individuals, where the oral environment has not required consistent regulation from the bicarbonate buffer. This implies fermentable sugars were not readily consumed, but proteins were, for the dental calculus to have formed. The observation of carbonate-containing hydroxyapatite in the dental calculus specimens from CC and SA, may indicate that dietary sugars, combined with proteins were being alternatively consumed by these populations. Consequently,

there may have been a continuously high concentration of carbonate ions in the oral environment as well as the alkaline conditions necessary for calculus formation.

Despite this theory, without confirmation by FTIR analysis, it cannot be confirmed that the lack of green colouration in the CV specimens means that there was no carbonate substitution in the hydroxyapatite (Leventouri et al. 2009). Indeed, a couple of specimens did have green bands in their superficial structure, but this was not the general trend. It may be that the amount of substitution or the position where the substitution occurs, influences the colour of the hydroxyapatite, rather than just the presence of carbonate in the mineral structure.

When there was green-coloured material observed in all specimens, this was found to correspond to regions of higher density in the n-CT data, as shown in Figure 7.3.10. In the literature, there is little difference in the density of the major phases of pure mineral hydroxyapatite and whitlockite (3.16 and 3.11 g cm⁻³ respectively) (Calvo and Gopal 1975; Fleet 2004). However, the visual observation of the green material that is likely hydroxyapatite rather than whitlockite, suggests that this phase is of higher density or that it accumulates in a more compact manner than whitlockite.

Returning to the colours observed on the deep surfaces, the Capuchin Catacombs and San Agustín specimens featured observable bands of colour that followed the shape of the deposits. This supports the observation in clinical literature that dental calculus builds up in layers (LeGeros 1974). In addition, the orientation of the observed bands demonstrates that dental calculus accumulates in layers across the tooth's surface, not just outwards into the oral cavity. These bands of colour were generally not observed in Cementeri Vell specimens and this may be due to the lack of carbonate-containing hydroxyapatite as previously discussed, or the post-mortem conditions which are discussed below.

All specimens exhibited areas of white colouration. This white dental calculus could be attributed to either the hydroxyapatite or whitlockite phase (Ben-Nissan 2014; Barthelmy 2014). The minor phases of quartz and calcite are also usually white in colour, however the coverage of white observed did not seem to relate to the percentages identified from the mineralogical results. In addition, there were specimens that had this white colouration, but had no minor phases of quartz or calcite present.

This colouration is also not thought to be attributed to enamel ‘peel-off’ that may have occurred during specimen removal from the dentition. In the publication, Henry and Piperno (2008), enamel damage following calculus removal using a dental pick was investigated using scanning electron microscopy. It was found that the method did not produce any new scratches onto the enamel surface and it was this method that was utilised in this research (Henry and Piperno 2008). In addition, the nano-computed tomography data of the specimens did not indicate that there was discrete layer on this surface of the calculus that could be attributed to enamel. In fact, the whiter regions of dental calculus were found to be less dense than the regions of dark colour (Figure 7.3.10). Even though tooth enamel has a similar material density to the major phases in dental calculus, it is much more crystalline and therefore would be expected to be more dense than dental calculus in computed tomography imaging.

Another difference between the populations was that Cementeri Vell specimens had superficial surfaces that were also much lighter in colour than the other two populations. This may, as with the absence of green carbonate-containing hydroxyapatite, be an indicator of compositional differences between the populations. Even though the mineral composition was determined to be the same, albeit with moderately different mean percentages of phases, the elemental composition for this population was found to be quite different.

However, due to the superficial surface being exposed to the burial soil and environmental conditions it seems more likely that this surface has been affected by diagenesis. This is supported by the taphonomic changes observed in the bone of these individuals, which included erosion, flaking and staining (Wilson 2014). Therefore, because of the deposition conditions of the remains in Cementeri Vell, the lighter colouration is potentially due to the environmental exposure that these specimens were subjected to in the open-air ossuary.

This exposure diagenesis is also likely the reason why these specimens were less robust than the specimens from the other two populations. These results indicate that dental calculus can suffer from taphonomic weathering, like bone (Pate et al. 1989; Nicholson 1996; Tütken and Vennemann 2011). This fragility may have also affected the recording of the dental calculus.

Indeed, due to this fragility the Cementeri Vell sample had the most specimens that could not be categorised by shape, due to the fracturing of the deposits. San Agustín also had specimens that could not be categorised by shape. This indicates that even though the visual observations of SA specimens did not indicate degradation by exposure, alternative degradation processes are likely to have occurred, increasing the fragility of the deposits.

The results of the mineralogical analysis demonstrate that even though all specimens contained hydroxyapatite and whitlockite, they were present in different percentages. The analysis of where the specimen had been taken from in terms of tooth location indicated that there were no significant differences in mineral composition between specimens sampled from left or right teeth. Clinical studies have found that most individuals favour the right-hand side of the mouth for mastication, and that this is not linked to handedness (Christensen and Radue 1985; Pond et al. 1986; Martinez-Gomis et al. 2009). However, a lack of significant difference in these results suggests that dental calculus composition is not affected by this preference for the right-hand side of the mouth.

It might be expected that if a preferred side of the mouth were used for mastication, there would be a difference in calculus composition between left and right teeth. The breakdown and holding of food on one side of the mouth may cause local oral environment differences that influence the mineral formation of each phase. For example, the presence of different dental plaque bacteria, or a local increase in alkalinity during protein consumption. In terms of the alkalinity, this would only be true if direct food consumption was the influencing factor on the calcification of dental plaque.

Although it is known that protein intake influences dental calculus accumulation by creating an alkaline environment, it is not the direct mastication of protein-based foods that causes local pH rises (Lieverse 1999). As explained in section 2.3.1, it is the secondary effect of protein consumption causing urea concentrations in the body to rise, increases salivary alkalinity (Lieverse 1999). As such, an increase in the urea concentration of saliva should not be side specific to the mouth. These results support this by showing no difference in the composition from either side.

Similarly, when the upper and lower quadrants of each side were statistically compared, there was no significant difference in mineral composition. This, as described above for

the left and right sides, suggests that the conditions for mineral precipitation are not locally different between the upper and lower jaws.

The results did however indicate that in the San Agustín specimens, there was a significant difference between the mineral percentages in the anterior and posterior teeth. There was a higher proportion of whitlockite found in specimens from the back of the mouth compared to the front. As stable whitlockite forms in high alkaline conditions with the presence of magnesium, there may be a few reasons for there being more of this mineral in posterior specimens (Knuuttila et al. 1980; Kani et al. 1983; Lagier and Baud 2003; Hayashizaki et al. 2008).

Firstly, it may be due to the consumption of foods that contain magnesium, requiring more chewing from the grinding, posterior occlusal surfaces. Magnesium-rich foods include dark leafy greens, seeds, bran, whole grains, dairy, meat and some species of fish (Spiegel 2010; Hruby and McKeown 2013). However, most magnesium absorbed by the body, is done so in the digestive track, indicating, that magnesium from these food sources may not be fully released from chewing alone (Spiegel 2010). Consequently, the consumption of these foods may not be enough to explain why posterior teeth would form more whitlockite.

An alternative explanation may be related to gingival health and the recession of soft tissue around the teeth. It has been found that magnesium concentrations are higher in the gingival crevice, than in saliva, especially when the gingivae are inflamed (Kani et al. 1983; Embery and Waddington 1994; Roberts-Harry and Clerehugh 2000). This suggests that the posterior teeth may have been more affected by gingival inflammation, causing magnesium in the local environment to promote whitlockite over hydroxyapatite in the dental calculus.

As dental plaque is a causative factor for gingival inflammation, this corresponds with oral hygiene being lower at the back of the mouth than the front (Bernimoulin 2003; Demir 2008; Arabacı and Demir 2013). Even if oral hygiene is regularly practised, the posterior teeth can be difficult to successfully clean, due to their numerous cusps and grooves. As such, in individuals where oral hygiene was low or insufficient, the posterior teeth would suffer greatly from dental plaque accumulation and consequently gingival inflammation.

In populations where oral hygiene for health was limited or unpractised, it may have been the case that tooth cleaning was carried out for aesthetic purposes. This may have been limited to the anterior teeth which were seen by others. Although there is no written evidence of this occurring in the populations studied, this does support the anterior and posterior differences seen in the mineral composition of dental calculus.

All the elements detected in the ICP-MS(Sol) results were also detected in the archaeological dental calculus analysed by Lazzati et al. (2015) using the same technique. Within these results, the highest concentrations of trace elements were found in the Cementeri Vell specimens and these specimens also showed the least lattice expansion in the powder XRD data. This suggests that the trace elements detected in the specimens were not chemically incorporated into the mineral lattices, but instead were ‘trapped’ in the dental calculus material (Lazzati et al. 2015). This also suggests that the trace elements detected may not have become part of the dental calculus composition because of diet, but rather during post-mortem processes. However, the lack of lattice expansion does not necessarily mean that substitution did not occur. The incorporation of a species at one position in the unit cell may have expanded the lattice, while another species contracted the lattice at a different position, resulting in a negligible net change.

The nature of apatitic mineral phases means they readily undergo substitution at multiple positions in their structure. Additionally, the values obtained for the lattice parameters were based on the Rietveld fitting analysis, which has associated errors (see section 5.2.1). Therefore, it is difficult to say with certainty where the elemental species detected, were present in the mineral phases.

The elemental species identified in this analysis are found naturally in the human body in varying concentrations (Emsley 2011). Even though Lazzati et al. (2015) suggest that the concentrations of trace elements in archaeological dental calculus can indicate diet, this may be optimistic. Clinical studies have found a large amount of variation between individuals in terms of trace element composition of dental calculus and within the material of one calculus deposit (Sánchez et al. 2000; Pérez et al. 2004; Abraham et al. 2007). In addition, the evidence by Huang et al. (1997) that fluoride from drinking water can be incorporated into dental calculus also complicates any dietary analysis. Therefore,

the results of this study are more relevant to discussing the inter-population differences, in terms of post-mortem environment.

Except for copper, the Capuchin Catacombs had the lowest concentrations of trace elements, compared to the two buried populations. This suggests that a proportion of trace element composition for Cementeri Vell and San Agustín, may be attributed to contact with the burial environment. Additionally, apart from manganese, all elements were found in higher concentrations in Cementeri Vell than San Agustín. This supports the idea that the burial environment influences the trace element composition of dental calculus. As Cementeri Vell was subjected to two types of burial environment, firstly a grave and then an ossuary, this may have increased the amount of ionic exchange in the calculus (Dudgeon et al. 2016). Further analysis of the soil around the remains may have helped to assess this, however soil samples were not included in the permissions granted for this study.

The much higher concentrations of strontium in Cementeri Vell and San Agustín, compared with the Capuchin Catacombs may also be indicative of soil contamination. Strontium has been detected in clinical researchers such as Söremark and Samsahl (1962) and Abraham et al. (2007). However, the comparison between the populations in this study, indicates that like in archaeological bone, strontium may be an element that can readily exchange with calcium during burial (Lambert et al. 1985; Pate and Hutton 1988).

The presence of aluminium and chromium in the Cementeri Vell specimens was higher than the other two populations. This may be an indicator that instead of carbonate-containing hydroxyapatite as observed in the Capuchin Catacombs and San Agustín specimens, the Cementeri Vell specimens were substituted at the phosphate group with CrO_4^{3-} or AlO_4^{3-} (LeGeros 1981). However, this would only be confirmed with the application of an additional technique such as FTIR.

For the specimens that could be categorised by shape, the most common was ledge across all populations. This deposit shape may be common because it provides the least discomfort to the individuals that exhibit it. In addition, this shape indicates that the dental plaque on the upper portion of the crown, above the ledge-shaped deposit, was regularly disturbed and therefore unable to mature (Marsh 2004). The process of maturation in a bacterial colony is required to develop the bacterial species that calcify to form dental

calculus (Mislowsky and Mazzella 1974). This disturbance may have come from the actions of eating and drinking or indeed from oral hygiene processes. As discussed in section 2.3.1, modern individuals who undertake regular tooth-brushing, can still form dental calculus in locations that are missed during cleaning. Unfortunately, this is not able to be confirmed as any oral hygiene undertaken by these populations was most likely on an individual basis rather than professional and therefore has gone undocumented.

The second most common shapes were planar and semi-circular which unlike teeth with ledge-shaped deposits, indicate that the plaque clearance on these teeth was not as thorough. These two shapes extend across a larger area of the crown and therefore dental plaque colonization must have been allowed to mature for the calculus to form in this manner. Consequently, the presence of this shape of deposit may indicate that oral hygiene was less practised in these individuals or that it was not as meticulous and these surfaces were missed. Finally, the crescent and bead-shaped deposits are potentially immature versions of the semi-circular and ledge deposits respectively.

There did not seem to be a correspondence between specimen shape and the dental location that it had accumulated. This is particularly surprising regarding the tooth type. It was expected that the coronal shapes of the different tooth types would influence the shape of the calculus formed. However, this does not appear to be the case. This strengthens the above theory that the shape is connected to plaque clearance and consequently oral hygiene habits.

To summarise, the results of physically characterising of specimens by their external features has provided both similarities and differences between the specimens from each population. The surface morphology of the superficial and deep surfaces was found to be consistent in all specimens of archaeological calculus and this corresponded with clinical literature. However, various colour differences were seen between populations, which likely relates to both the burial or interment environments and possibly, the formation conditions. Finally, the shapes of the deposits have provided potential clues towards individual oral hygiene habits that may have influenced the way the dental calculus has accumulated on the dentition.

Overall, the compositional results indicate that the present mineral phases are consistent between populations, although there are intra-population variations in mineral phases

ratios. Additionally, the presence of minor mineral phases is variable between populations, however this is likely due to the burial environment that the dental calculus has contact with or is exposed to. Finally, the values for the trace element concentrations in this study indicate that post-mortem environment may also have an influence on archaeological dental calculus composition. While this cannot be confirmed, the differences between the non-buried and buried specimens support calcium-phosphate ionic exchange from soil contact (Dudgeon et al. 2016).

In terms of the second aim of this study, the results have shown that the general external morphology is consistent within and between dental calculus specimens from different archaeological populations. All deposits had the same external textures and the colours were largely consistent. However, subtle differences were seen in the fragility and superficial surface colour of the deposits. These were attributed to the burial or interment environment that the remains had been subjected to (see below regarding hypothesis 2(d)). Consequently, hypothesis 2(a) can be accepted with the point being emphasised that within and between populations the deposits are consistent but not identical.

Hypothesis 2(b) and (c) can also be accepted. The same major mineral phases were found in all specimens in the three populations. Equally the precursor phases of brushite and octacalcium phosphate were absent in all archaeological deposits. Even though the presence of minor phases was variable within each population, these minerals can be attributed to post-mortem contamination. As such, they are not truly dental calculus phases and hypothesis 2(b) still stands. Similarly, despite variation in the ratios of major mineral phases within each population, all specimens did have both minerals present. The presence of the same mineral phases dictated that the major elements in all specimens were similar. Indeed, the same major and trace elements were found in all specimens, even though in differing concentrations and distributions. Regarding the distribution of mineral phases, the results from San Agustín also allow the proposition of an additional hypothesis, that the ratio of Hap and WHT is significantly different between the anterior and posterior teeth. The analysis of further archaeological as well as modern populations would be beneficial to test this hypothesis.

Despite the acceptance of hypotheses 2(a)-(c), the post-mortem conditions of dental calculus do influence the physical, and elemental composition. Comparison of the

deposits from the mummified and buried remains demonstrates that the physical characteristics, mineral and elemental compositions are consistent. However, the burial environment increases the fragility, influences the superficial colouration and potentially changes the trace element composition of dental calculus. Further investigation into the post-mortem changes that effect dental calculus and the effect of soil diagenesis is required. However, based on the observations in this study, hypothesis 2(d) must be rejected.

8.4 Evidence of Periodontal Health

The results of recording additional pathologies for the calculus-exhibitors, indicated that the percentage of teeth affected by carious lesions was low. This corresponds with the expected inverse relationship between dental calculus and caries, as dental calculus formation requires an alkaline environment and lesions form in an acidic environment (Hillson 2001). The percentage of teeth affected by dental caries in the Capuchin Catacombs was much lower than the other two populations however, this may have been due to the percentage of surfaces that were unobservable, causing carious lesions to be under-recorded.

The low percentages of carious lesions may indicate that sugary food or fermentable carbohydrates were not readily consumed in any of the populations (Bowen 1994; Hillson 2005; Bonsall and Pickard 2015). Alternatively, it may have been the case that these foods were consumed and caries were formed, however the lesions progressed quickly due to a lack of oral hygiene, causing ante-mortem tooth loss (Roberts and Manchester 2010). The pattern of ante-mortem tooth loss (AMTL) occurring more in the posterior teeth for all populations supports this as these teeth are generally more susceptible to caries due to the fissures and grooves in the tooth surfaces and their role in chewing (Hillson 2001). However, the loss of teeth due to lesions would likely be supported by the presence of carious lesions on the teeth that were remaining, as well as the lack of dental calculus in these individuals and this was not the case.

Ante-mortem tooth loss is not always caused by carious lesions but can occur from periodontal disease, continued eruption or deliberate extraction (Lavelle 1988; Mays 2010; Roberts and Manchester 2010; Masotti et al. 2013). Even if all the positions recorded as indicating AMTL are assumed to be due to carious lesions, this still amounts

to low caries percentages in calculus-exhibitors in the island populations. However, using this assumption about AMTL, the mainland population of San Agustín are more greatly affected by caries, which may be due to differences in availability of carbohydrate-based produce, such as wheat, from the surrounding countryside, compared to the potentially limited supplies available for the island populations.

Results of the percentages of alveolar positions affected by periapical cavities indicate that for all populations this was very low. San Agustín had the highest percentage and again this may have been linked to the potentially higher rates of carious lesions. A periapical cavity is usually linked to carious lesion development and can lead to subsequent tooth loss (Hillson 2001; Roberts and Manchester 2010; Masotti et al. 2013). Therefore, observations of periapical cavities, when the tooth has been lost ante-mortem, support the presence of carious lesions.

In terms of periodontal disease, this was high in all populations for calculus-exhibitors. This was expected because the presence of dental calculus means that plaque could colonise on the deposits. The persistent presence of dental plaque bacteria on and around the gingivae causes inflammation progressing to gingivitis and periodontitis (White et al. 2011(2)).

The high prevalence of periodontal disease in these populations is not unexpected as it is a condition that has affected humans for centuries (Fujita 2011). However, some archaeological studies have found a higher prevalence of periodontal disease in men than women (Albandar 2002; Delgado-Darias et al. 2006; Bonsall 2014). In this study, for calculus-exhibitors, slightly higher percentages of periodontal disease were indeed recorded in male individuals from the Capuchin Catacombs. This difference was minimal and may suggest that if calculus is observed, there is less disparity between male and female periodontal disease prevalence. Unfortunately, there were no female individuals with calculus in the Cementeri Vell populations to compare periodontal disease prevalence and the San Agustín sample was male only.

Overall, the results demonstrated that in all three populations there were individuals with dental calculus who also exhibited other oral pathologies. The most common of these was periodontal disease, which affected nearly all the remains. The presence of dental caries

and periapical cavities in conjunction with dental calculus indicates that the conditions in the oral environment varied between acidic and alkaline.

One of the research aims in this study was concerned with investigating whether the composition of archaeological dental calculus was influenced by pathological conditions present in the mouth. To do this, the bulk mineralogical results were statistically compared according to whether carious lesions and/or periapical cavities were present or absent in the individual that the specimen was sampled from. The San Agustín population was found to have a statistically significant difference between specimens from individuals with and without pathologies present however, there were some issues with the statistical testing due to sample sizes. Consequently, this result is questionable to should not be heavily invested in for discussion purposes.

The lack of statistical significance in the populations that did have suitable sample sizes indicates that the acidic conditions required causing lesions on the teeth and the production of acidic pus from periapical lesions, do not have a significant influence on the minerals that are formed in dental calculus (Bowen 1994; Nekoofar et al. 2009; Costolonga and Herzberg 2014). This may be due to the localised bacterial species involved, rather than the conditions of the oral environment; therefore this is could be a worthwhile topic for a future bacteriological study.

The percentages of hydroxyapatite and whitlockite in archaeological dental calculus were found to be statistically significant between specimens from anterior and posterior specimens. These percentages were not however found to differ between sides of the mouth of in terms of which jaw the sample was taken from. Despite this, many of the specimens analysed in this study were taken different individuals due to ethical procedure regarding the human remains involved. As such, it may be possible that the results would show greater statistical variation in calculus composition between specimens if taken from multiple locations around the mouth of the same individual. In addition, the hypothesis that oral health influences dental calculus mineral composition was rejected as no statistical difference was found between specimens that were recorded in dentition that also exhibited periapical cavities or carious lesions.

The results of the iron concentration analysis by ICP-MS(Sol) indicated that for these populations iron was present in all the specimens analysed. Unfortunately, this could not

be compared to the ICP-MS analysis by Lazzati et al. (2015) as iron was not analysed in their study. In the clinical XRF studies by Sánchez et al. (2000) and Abraham et al. (2007), the presence of iron in the deposits was not discussed despite it being detected. However, this does demonstrate that the presence of iron in the results of this study is not unique to only archaeological specimens of dental calculus (Sánchez et al. 2000, Abraham et al. 2007).

In this study, iron was of interest to determine whether its contribution could be connected to periodontal health, particularly from gingival bleeding in the oral cavity. The results indicated that there were no significant statistical differences between the dental location of the specimens and the iron concentrations measured. However, most individuals from all populations exhibited indicators of periodontal disease at all tooth positions. The nature of archaeological remains means that the amount of bleeding that might have been present is unknown. Therefore, the lack of any difference in iron concentration around the mouth could be related to gingival bleeding occurring at multiple tooth positions and not being localised to one tooth or quadrant.

If the iron content of the specimens is not present due to gingival bleeding, it may relate to dietary sources such as red meat or plant products, including dark leafy greens (Berdanier 2007). As discussed above with magnesium, dietary iron is absorbed by the intestinal tract after it has been digested by the stomach acids (O'Bryant and Thompson 2013). Therefore, for it to be introduced to dental calculus by the salivary fluid, it would need to be there after iron metabolism, not during direct consumption of iron-containing foods. There does not appear to be any literature regarding how iron concentrations in saliva change with the consumption of different amounts of iron-containing foods or supplements. Consequently, it cannot be said whether the differences in iron concentration in these populations was due to dietary variation or not.

Of note though, is that it seems unlikely for the iron that is present, to be there solely due to soil contamination. In fact, there was a higher concentration of iron in the unburied, mummified Capuchin Catacombs specimens compared to buried San Agustín specimens. This is surprising considering the La Rioja region of Spain where the San Agustín population is from, is known for its red ferric clay (Rabe 2015; Rojo-Smith 2016). However, the iron-rich clays are generally found in the lowlands of the region and the

monastery where the graves were situated is in the mountainous village of San Millán de Cogolla. Despite this, the higher concentration of iron in the Capuchin Catacombs individuals does demonstrate that iron content in archaeological dental calculus should not be ruled out as a soil contaminant.

Additionally, the results comparing the amount of calculus per individual and the iron concentration in the mummified remains, indicated there may be a positive correlation. Dental calculus provides a rough surface for plaque accumulation, which can cause gingival inflammation and plaque-induced gingivitis (Nield-Gehrig and Willmann 2007). A symptom of gingivitis can be gingival bleeding, particularly on probing of the gums (Nield-Gehrig and Willmann 2007). If an individual has more calculus present, they in turn must have more dental plaque present thus increasing the potential for gingivitis and gingival bleeding. As gingivitis is not observable in archaeological remains, due to the soft tissue changes that have occurred, these results indicate that iron concentration may be an indicator of this disease.

The lower mean concentrations of iron in the San Agustín population may be due to the high percentages of AMTL seen in these individuals. If tooth loss has occurred and the alveolar socket can resorb, the symptoms of plaque-induced gingivitis are no longer exacerbated by the presence of dental calculus or plaque (Nield-Gehrig and Willmann 2007). Consequently, these individuals may not have suffered from as much gingival bleeding close to death, as the Capuchin Catacombs individuals did.

In Cementeri Vell, there did not seem to be any relationship between the amount of dental calculus in an individual and the iron concentration. However, four of the specimens in this analysis were taken from loose teeth. This resulted in skewed results showing 100% of teeth affected, when for that individual only one tooth was available. Additionally, in this population the periodontal recession measurements did not seem to relate to the concentration of iron. However, these results were only based on six specimens, due to the inclusion of the four loose tooth specimens.

Further work is required to assess the concentration of iron in dental calculus from archaeological populations to determine its connection to gingival bleeding. However, these results do show that iron should not be discounted as purely a taphonomic contribution to the trace element composition of archaeological dental calculus.

An additional aim of this study was to analyse whether concomitant dental pathologies might affect the composition of archaeological dental calculus. Firstly, hypothesis 3(a) was accepted, as some dental calculus-exhibitors in all populations did exhibit additional oral pathologies. However, the statistical analysis of the powder XRD did not show that mineral composition was related to the presence of these oral pathologies. This may be due to missing data in the archaeological material which unfortunately cannot be overcome. As such, the results of this study mean that hypothesis 3(b) must be rejected. However, it would be worth performing mineralogical analysis on specimens from clinical patients, where post-mortem tooth loss is not an issue and a full dental history is known.

The analysis of clinical specimens for iron presence in relation to gingival bleeding would also be beneficial. There is the potential that the iron concentration in dental calculus is related to periodontal health. The specimens from the mummified remains demonstrated that iron is present in archaeological dental calculus and that it should not only be attributed to sediment contact. However, it cannot be confirmed that trace iron in dental calculus is present due to blood in the oral environment, rather than physiological iron in the saliva. Unfortunately, it is clinical research that would be best placed to continue this line of research before it can benefit archaeology. Consequently, for the time being, hypothesis 3(c) must be rejected.

8.5 Clinical vs. Archaeological Dental Calculus

The physical characteristics of the dental calculus deposits, have demonstrated features of archaeological dental calculus that are consistent with clinical literature and others that have so far been unexplored. The recording of where the deposits were found on the teeth of the archaeological populations was consistent with clinical literature of site-specificity (see section 8.3). Additionally, the results demonstrated that physically, the layering and textures observed in the archaeological dental calculus specimens was as expected from clinical data (see section 8.2). However, the external colouration of the specimens seemed to be heavily influenced by the burial environment and did not match published clinical colour descriptions (see section 8.2). Despite this, the deep surface colours did correspond to the literature regarding clinical supragingival dental calculus colour (Roberts-Harry and Clerehugh 2000; Hayashizaki et al. 2008). However, in the Capuchin Catacombs and

San Agustín specimens, shades of green were also observed on most specimens, which were not reported in Roberts-Harry and Clerehugh (2000) for either supragingival or subgingival calculus (see section 8.2).

The results obtained from the mineralogical analysis of archaeological dental calculus demonstrate that the major mineral phases for all specimens were calcium hydroxyapatite (HAp) and whitlockite (WHT). Also, identified in some specimens were the mineral phases of calcite and quartz. There were no specimens that contained the mineral phases of brushite or octacalcium phosphate.

The presence of HAp and WHT as the major phases is consistent with the previous research that has analysed archaeological dental calculus for mineral composition (Klepinger et al. 1977; Greenwood 2009; Wood 2012). In addition, the presence of the minor phases of calcite and quartz as well as the lack of brushite and octacalcium phosphate, are also consistent (Klepinger et al. 1977; Greenwood 2010; Wood 2012). These findings demonstrate that the composition of dental calculus from archaeological remains is also consistent with reported compositions of clinical dental calculus. Both brushite and octacalcium have only been reported as precursor phases in newly formed calculus deposits, and as such they were not expected for these accumulations (Barone and Nancollas 1978; Kani et al. 1983; Driessens and Verbeeck 1989; Mandel 1987; Hayashizaki et al. 2008; Rau et al. 2010). However, it is possible that these less stable phases were present at death and they have been affected by taphonomic influences over time.

The presence of calcite and quartz as minor phases is most likely due to the burial environment of the archaeological dental calculus, thus explaining why clinical specimens do not contain these phases (Klepinger et al. 1977; Jin and Yip 2002; Greenwood 2009; Wood 2012). It is likely that soil contamination has introduced these minerals to the dental calculus matrix, as both calcite and quartz are readily found in sediments (Klepinger et al. 1977; Monge et al. 2014).

Although expected on the exterior of the calculus deposits (superficial surface), unfortunately, it was not possible to confirm this. The location of these minor phases in relation to the cross-section of the calculus using micro-beam X-ray diffraction was

inhibited by the low percentage content of the phases and the presence of the highly crystalline resin at the edges of the cross-section (see section 5.2.2).

An unexpected result was the identification of these minor phases in the specimens from the Capuchin Catacombs, which had not been buried. However, this may be explained by two potential occurrences. Firstly, the Catacombs have been open to visitors for several decades, and the consistent foot traffic past the remains, may have had the potential to kick-up particles of soil and debris. In addition, maintenance work and the installation of lighting, walkways etc. have been carried out around the remains, without any protective shielding. Consequently, drilling into the tuff walls, may have promoted calcareous dust within the catacomb environment, which could have settled on the remains.

Another explanation for the presence of calcite and quartz is the porous nature of dental calculus combined with water saturation. This may have occurred post-mortem as the deposits were displayed in the Catacombs. The underground environment is subjected to bouts of humidity when Palermo suffers from heavy rains. Potentially, if water in the air, repeatedly infiltrates the pores of the calculus and subsequently evaporates in drier weather, dissolved geological minerals will be left behind in the dental calculus matrix. The influence of environmental water on the dental calculus composition may also have affected the buried remains through water percolation, rather than just soil contact. This may explain the higher mean percentage of calcite in the Cementeri Vell specimens, which were exposed to environmental factors due to their presence in an open-air ossuary.

Alternatively, these minor phases may have been introduced to the oral environment during life and been incorporated into the dental calculus through the intake of water. As evidenced by the study by Huang et al. (1997), fluoride levels in dental calculus can be related to drinking water. Therefore, if dental calculus composition is affected by drinking water in the living individual, this may be how calcite and quartz have been introduced into the oral environment.

However, it is widely believed that past populations were more inclined to drink wine rather than water, potentially because of contamination from human or animal waste (Mitchell 2016). This potentially is true for these populations, particularly in Sicily and La Rioja, which are prominent grape-growing regions (Musshoff et al. 2013; Rojo-Smith 2016). In addition, the individuals in the Capuchin Catacombs and San Agustín samples

were religious personnel or upper classes, who would be more likely to drink wine than the lower social classes (Vess 2004). It has also been suggested that individuals from Formentera, despite its small size, would have drunk wine, due to Ibiza having a good production economy of grapes (Fuller et al. 2010). Consequently, while water intake is a valid suggestion for the presence of calcite and quartz in dental calculus, it may not be more likely that the contribution from environmental water post-mortem.

Unfortunately, these minor phases were not identifiable in the nano-computed tomography data as a separate material density. The standard densities of calcite and quartz are lower than the major mineral phases of hydroxyapatite and whitlockite (2.71, 2.65, 3.16 and 3.11 g cm⁻³ respectively) (Calvo and Gopal 1975; Fleet 2004; Přikryl 2004; Osman 2013). However, they could not be observed as discrete regions in the data. Although lower density regions of material were seen in the specimen cross-sections, the amount of material did not correlate with the low percentages of minor phases. Consequently, these regions could not be attributed to calcite or quartz. It may be that these minor phases were distributed through the deposits, rather than collected in a layer or region within the matrix.

The results of the specimens analysed by ICP-MS(Sol) demonstrated that specimens from all three populations had a mean Ca/P ratio that was higher than the stoichiometric ratios of the major phases. This indicates that the specimens were more calcium-rich than reported clinical data (Little et al. 1963; Hoyer et al. 1984; Hayashizaki et al. 2008).

The calcium-rich ratios could be assumed to be attributed to the presence of the minor phase of calcite (CaCO₃). This would explain why there would be a difference between clinical and archaeological Ca/P ratios, as calcite is not reported as a mineral phase of modern calculus. However, on inspection of the mineral phase percentages for the specimens that were analysed by ICP-MS(Sol), there was no correlation between the amount of calcite detected in the specimen and the Ca/P ratio calculated (see *Supplementary Material: Appendix E.4.1*). It is therefore feasible that instead of the calcium-rich Ca/P ratios being caused by the minor phases in the specimens, they can instead be attributed to the major phases.

A high Ca/P ratio indicates that regarding the stoichiometric ratio of calcium the phosphorus, there was a higher contribution of calcium, not that there was more calcium.

As explained in previous sections, hydroxyapatite and whitlockite are apatitic and can undergo substitutions at multiple locations within their structure (see Table 2.4.2). Consequently, the high Ca/P ratios do not necessarily mean that calcium was not substituted, but that the phosphate groups were more substituted. This corresponds well with the external colour observations of the specimens. The green coloured material observed indicated carbonate-containing hydroxyapatite was present and carbonate can substitute the phosphate group.

However, the specimens with the highest mean Ca/P ratio were in Cementeri Vell and these were the specimens that did not exhibit green colouration, indicating no carbonate-containing hydroxyapatite. This suggests that the phosphate groups in these specimens were not substituted by carbonate, but another ionic group.

The results have demonstrated that the site specificity, external texture and the presence of layers correspond with observations made about clinical dental calculus. The colours observed on the attachment surfaces of the specimens largely correspond with the colours outlined in Roberts-Harry and Clerehugh (2000) regarding clinical deposits, except for the regions of green. Further work is required to confirm whether these regions correspond with carbonate-containing hydroxyapatite (section 9.3). The colours of the superficial surfaces of the deposits were markedly different to the attachment surface but this is understandable considering this surface has been exposed to the interment environment. As this visual difference in colour between clinical and archaeological deposits was observed, consequently, hypothesis 4(a) can only partially be accepted. In addition, the fragility of some of the archaeological deposits is contrary to dental calculus in patients, which does not break off easily and requires physical or chemical intervention (White 1997).

The results of the mineralogical analysis in this study demonstrated that archaeological dental calculus consists mainly of two poorly crystalline phases, calcium hydroxyapatite and whitlockite. These phases have both been reported as major phases in clinical dental calculus (LeGeros 1974; Jin and Yip 2002; Hayashizaki et al. 2008). However, clinical literature also reports brushite and octacalcium phosphate as major phases and neither of these minerals were found in any specimen analysed (LeGeros 1974; Jin and Yip 2002; Hayashizaki et al. 2008). This is likely to be because these phases are thought to be

transient precursors to HAp and WHT and would not persist through the post-mortem environment, if they had been there at the time of death (LeGeros 1981; Abraham 2007). In addition, these phases have been found in immature accumulations of dental calculus in *in vivo* studies (Abraham 2007). Considering the unavailability of a full dental scale and polish in past populations, the deposits of dental calculus in archaeological remains are unlikely to have been freshly accumulated prior to death. Indeed, this is evident in the recording of the dental calculus deposits. Newly mineralised dental plaque that would contain these precursor species would potentially not be present as a formed deposit of calculus, which all the archaeological specimens were.

The presence of calcite and quartz as minor mineral phases also provided a difference between archaeological and clinical dental calculus. As these phases are associated with the burial environment, their absence in clinical deposits is understandable. Further work to determine their location within a deposit may be able to confirm the post-mortem deposition of these minerals. Consequently, regarding the mineral composition, hypothesis 4(b) can largely be accepted. The major phases that were identified were expected and the ones that were absent were not. The minor phases were deemed to not be associated with the composition of dental calculus and even though present, can be attributed to the environment rather than through biological calcification, so do not conflict with the clinical literature (LeGeros 1974; Klepinger 1977; Greenwood 2009; Wood 2012).

Regarding the elemental composition, this was the most difficult feature of dental calculus to compare. Clinical studies have found considerable trace elemental variation between deposits from different individuals and even across the material of one deposit (Sánchez et al. 2000; Pérez et al. 2004; Abraham 2007). This study has confirmed that calcium, phosphorus and oxygen as major elements is consistent between past and modern populations, although this is not surprising considering the consistencies between mineral composition. It is possible that archaeological dental calculus contains more carbonate than clinical dental calculus based on the green regions observed and the high concentration of carbon observed in the EDX data. However, confirmation to determine if this carbon is in a carbonate form is required.

The variability in trace element composition is complicated further in archaeological dental calculus due to the post-mortem ionic exchange that can potentially occur. However, hypothesis 4(c) can tentatively be accepted at this stage. Both clinical and archaeological dental calculi are composed of calcium phosphates and have variable trace element compositions. Further clinical data on the trace elemental composition, as well as taphonomic studies for ionic exchange in archaeological calculus would be very beneficial to both fields.

8.6 Chapter Summary

The results obtained from this study have demonstrated that there are physical and compositional differences and similarities between clinical and archaeological dental calculus. In addition, intra- and inter-population differences can be observed within these archaeological samples. The cross-sectional analyses have demonstrated that dental calculus composition can vary through a deposit, although further work using alternative techniques would be beneficial. The analysis of compositional differences in relation to concomitant dental pathologies has proved inconclusive. The mineral composition does not seem to change with the presence of carious lesions or periapical cavities, however the presence of iron in the trace element composition may indicate gingival bleeding.

CHAPTER 9: CONCLUSIONS

*Daher ist die Aufgabe nicht sowohl zu
sehen was noch keiner gesehen hat,
als bei Dem was Jeder sieht,
zu denken was noch Keiner gedacht hat.*

- Arthur Schopenhauer 1851

9.1 Research Conclusions

This research has explored areas of archaeological dental calculus that have previously been assumed based on clinical literature. As such, the results of this work have contributed to the knowledge of archaeological dental calculus and the potential differences between assumed clinical data and the direct observation and analysis of archaeological data. The hypotheses that this research explored were developed based on gaps in the literature and following this work, there are further areas that would greatly benefit from further research (see section 9.3).

Overall, this research has concluded several key points. Firstly that depositional layering can be detected in archaeological dental calculus and also that not all specimens exhibit layering and those that do have different patterns of density across their accumulation pane. This is potentially key consequence for the manner of accumulation that has occurred in an individual. It is likely that the type of layering is closely linked to the speed of plaque calcification, the oral hygiene practices of an individual and their mobility and health. The layering observed may also help to inform the theories of calcification that have been suggested in clinical studies, although more work is required. What can be concluded in terms of these theories is that there may not be a single calcification path for all individuals. The presence of different layering patterns may in fact be linked to the chemistry of the oral environment, thus changing the calcification method that proceeds. The results of the cross-sectional mineral and elemental analysis were unfortunately not as successful as hoped. However, future work with a greater focus on just this aspect of dental calculus analysis would provide valuable information about changes in chemistry during accumulation (see section 9.3).

Additionally, this research has concluded that burial environment has an observable effect on the physical integrity and composition of archaeological dental calculus. In particular,

the trace elemental composition of archaeological specimens may be heavily contaminated by diagenesis and this can be indicated by a change in external colouring and fragility. This is a key finding, not only in terms of the potential dietary information that may be analysed using composition, but also in terms of sampling and archaeological ethics of specimen removal.

Additionally, this research has found that despite the potential chemical effect of additional oral pathologies being present in the mouth, this does not seem to affect dental calculus composition. However, this finding may be affected by the nature of the material and the unavoidable consequence of having incomplete dentition to examine. A future study of clinical patients where the oral health can be documented in full and the calculus has not been affected by post-mortem conditions would be key to progressing this research path.

Finally, this project has been able to conclude that dental calculus from archaeological sources does share commonality with clinical literature. However, there are definite differences between archaeological and clinical calculus in terms of the present mineral phases, elemental species and physical characteristics such as superficial colour.

9.2 Contribution to Knowledge

Despite there being an abundance of archaeological dental calculus research, this is the first large scale compositional study of specimens from three separate past populations. By applying a non-destructive to destructive methodology, complementary techniques have determined the physical characteristics, mineral and elemental composition of specimens. This approach has maximised the physical and compositional information from each specimen.

The novel application of nano-computed tomography has enabled the detection of layering in archaeological specimens. As such, this study has provided valuable insight into the way dental calculus accumulates. In addition, this opens the possibility of dental calculus layering being able to provide insight into health, diet and mobility changes, as well as oral hygiene habits. This feature of archaeological dental calculus will hopefully be able to be further understood in future studies.

This study has also contributed to the field of archaeological dental calculus analysis by demonstrating the differences that post-mortem interment conditions can have. This will assist in future studies when dental calculus analysis is being considered as well as the conservation of remains. The results that exposure and environmental conditions increases the fragility of deposits informs the curators of remains that special care should be taken to protect dental calculus deposits. As well, for studies that may wish to analysis whole specimens, like the nano-CT in this study, sample integrity would potentially be a key feature in choosing a sample to analyse.

The mineralogical analysis performed on the three populations in this study has provided corroborating evidence that the mineral phases of archaeological dental calculus are hydroxyapatite and whitlockite. This means that in future, the mineral component of archaeological dental calculus should not be described as containing four major phases, as clinical dental calculus is. In addition, it should be noted that mineral phases of the burial environment should be expected.

Overall this research has filled some gaps that were missing from archaeological calculus knowledge, that had previously relied on clinical data. This research has also explored new ideas concerning the influence of oral pathology on dental calculus composition and periodontal health. While further research is needed, this study has provided an opening for research that might be able to increase the identification of dental pathologies in archaeological remains through dental calculus analysis. This may prove to be valuable in confirming the distinction between the markers of periodontal disease and natural periodontal recession, or when remains are incomplete.

9.3 Future Work

Dental calculus has been the subject of many studies in both archaeology and dentistry. Researchers that have extracted microfossils and bacterial aDNA in archaeological dental calculus have demonstrated its value for past population studies. This study pursued a different research focus, however it has hopefully promoted the value in the analysis of the composition of the calculus, as well the plant and bacterial species trapped within it.

With hindsight, there are a couple of aspects of the project that would have been carried out differently. Firstly, the cross-sectional elemental analysis would have greatly benefited from the use of La-ICP-MS rather than EDX as this would have produced more valuable data of the present trace elements. In addition, the microtome sectioning process caused irreversible damage to the specimens. While I am grateful to Bristol University for their collaboration, it would have been preferential to consult an expert in archaeological sectioning rather than biological. Despite this, the development of knowledge of dental calculus physical structure and composition from archaeological populations should continue in future work.

This is particularly key in terms of the cross-sectional analysis of dental calculus. Dental calculus layering has been demonstrated using nano-computed tomography on whole specimens, however this also means that dental calculus still attached to the tooth may provide valuable information for archaeological remains that do not get permission for sampling. In addition, the development of techniques that can be combined with computed tomography may allow in-situ compositional analysis to be performed without removal of deposits.

The cross-sectional elemental analysis would also benefit from the application of LA-ICP-MS. As shown in this study, EDX analysis is a convenient technique when scanning electron microscopy is being performed, however the trace elemental analysis is not sensitive enough for dental calculus. Alternatively, the use of synchrotron-based analyses that have been used in clinical studies, could easily be applied to archaeological specimens so that the elemental profiles could be compared.

This study also identified variation in void distribution and further research to understand this should be considered. These voids may relate to inclusions in the dental calculus and this could provide spatial analysis of microfossils. The ability to determine which inclusions are from the deeper layers of the calculus, would increase the validity of them being introduced to the oral cavity rather than having been deposited post-mortem.

The results in this thesis have shown that post-mortem conditions have an influence on dental calculus, physically and compositionally. However, more work in the determination of post-mortem changes in dental calculus would provide valuable insight into how to interpret compositional results. As this has already been carried out for human

bone and teeth, dental calculus should also be the focus of taphonomic studies, especially if it is to be used as a palaeodietary source of information.

Finally, the strength of inter-population comparisons, lies with analysing as many populations as possible. Therefore, the results presented here would benefit from comparison to other populations from different areas of the world.

Additional techniques should be considered for future analyses, such as FTIR which could identify possible substitution groups in the mineral phases. However, future researchers should also consider the methodological approach to dental calculus taken in this work. The process used, has demonstrated that multiple techniques can be performed on the same specimens to maximise the information gained. This is particularly important considering the ethical approval required for human remains sampling.

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SUPPLEMENTARY MATERIAL

CRANFIELD DEFENCE AND SECURITY
POSTGRADUATE RESEARCH DEGREE

MARCH 2017

CRANFIELD UNIVERSITY

CRANFIELD FORENSIC INSTITUTE
CRANFIELD DEFENCE AND SECURITY

PhD Thesis

KAYLEIGH ANNE COOPER

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SUPPLEMENTARY MATERIAL

Supervisors: Dr Sophie Beckett and Dr Nicholas Márquez-Grant

March 2017

This thesis is submitted in partial fulfilment of the requirements for
the degree of PhD

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APPENDIX A: ORAL NOMENCLATURE AND NOTATION

This thesis includes a large amount of dental terminology; therefore, this section explains the relevant terminology for the reader that is unfamiliar with dental anthropology and the skeletal system. In this terminology, deciduous (primary) dentition (i.e. the teeth of young children) is not covered because all specimens in this research were removed from adult dentition.

A.1 Osteological Anatomy of the Mouth

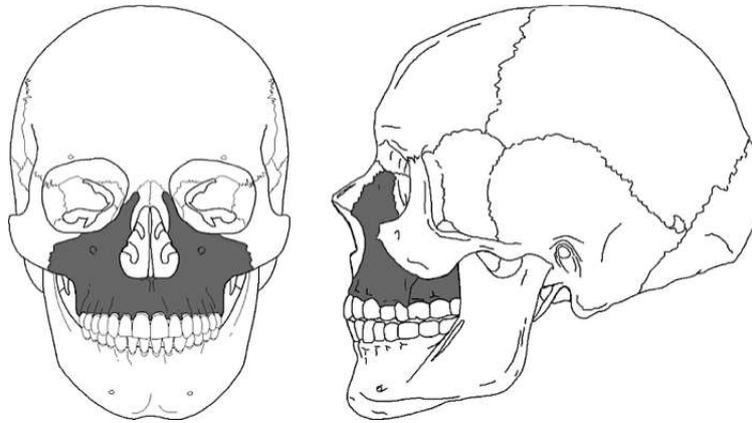
The bones of the human mouth consist of the upper jaw (maxilla), lower jaw (mandible) and the palatine bones³⁴ (Appx_Figure A.1.1 and A.1.2 respectively). The maxilla is two symmetrical bones (left maxillae and right maxillae) and has the function of holding the upper teeth as well as being the palate (roof of the mouth) and a portion of the nasal aperture and eye orbits (White et al. 2011(1)). The maxilla articulates with several other facial and cranial bones to form the superior portion of the mouth and central portion of the face (White et al. 2011(1)). The maxillae are fragile due to the hollow maxillary sinuses in each side and the inter-maxillary suture, which connects the left and right maxillae³⁵.

The mandible consists of one bone, which is articulated to the cranium with the left and right condyles via the temporo-mandibular joint (TMJ) (White et al. 2011(1)). This jaw holds the lower teeth and with the condylar movement provides mastication (White et al.

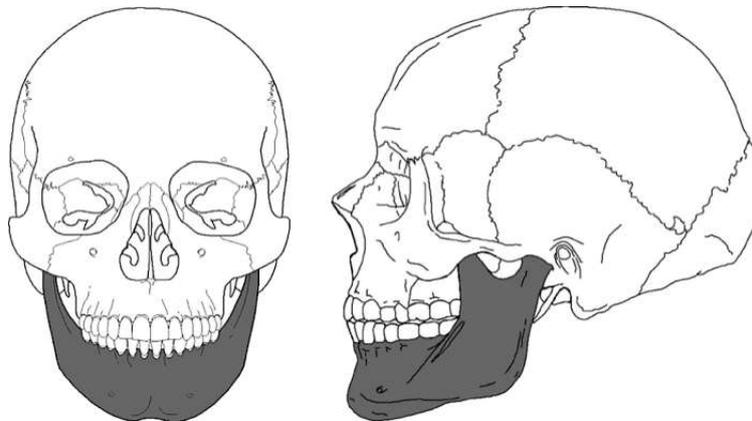
³⁴ The palatine bones (left and right) form the roof of the mouth and separate the mouth from the nasal cavity. They articulate with the maxillae, sphenoid, vomer, nasal conchae, and ethmoid (White and Folkens 2005). The palatine bones have no alveolar bone (i.e. they do not feature the dentition).

³⁵ From personal experience with excavated archaeological remains (All Saints, Oxford, UK (2011); Via Punic, Ibiza, Spain (2012); Av España, Ibiza, Spain (2012), MACE, Ibiza, Spain (2012), SATO, Ibiza, Spain (2012); SHARP, Norfolk, UK (2013); Capuchin Catacombs, Sicily, Italy (2014); Cementeri Vell, Formentera, Spain (2014)) the maxillae often break at the maxillary suture into their respective left and right sides and damage occurs to frontal process and zygomatic process, leaving the alveolar process containing the teeth relatively intact.

2011(1)). The mandible is a thick compact bone and while sometimes fragmented³⁶, is more durable than the maxilla.



Appx_Figure A.1.1 Diagram of human skull (adult), indicating the maxilla (shown in grey) in the anterior view (left) and lateral view (right) [Adapted by author from <http://phs.psd3.org> (Retrieved 15th July 2015)].



Appx_Figure A.1.2 Diagram of human skull (adult), indicating the mandible (shown in grey) in the anterior view (left) and lateral view (right) [Adapted by author from <http://phs.psd3.org> (Retrieved 15th July 2015)].

³⁶ From personal experience, with excavated archaeological remains (All Saints, Oxford, UK (2011); Via Punica, Ibiza, Spain (2012); Av España, Ibiza, Spain (2012), MACE, Ibiza, Spain (2012), SATO, Ibiza, Spain (2012); SHARP, Norfolk, UK (2013); Capuchin Catacombs, Sicily, Italy (2014); Cementeri Vell, Formentera, Spain (2014)), the mandible is often found fragmented in three pieces (the body, the left ramus and the right ramus). These fragments can often be reconstructed to provide inspection of the mandible.

Compared to the rest of the skeleton, the maxilla and the mandible are unique in that they feature alveolar bone. This type of bone provides the alveoli (sockets) that hold the roots of the teeth in the mouth (White et al. 2011(1)). This alveolar bone is present along the dental arches of both the maxilla and the mandible, except in places where the tooth has been lost ante-mortem (Appx_Figure A.1.3). When a tooth is lost ante-mortem the alveoli that had surrounded the tooth (i.e. the socket) is resorbed and new bone forms to fill the empty socket.

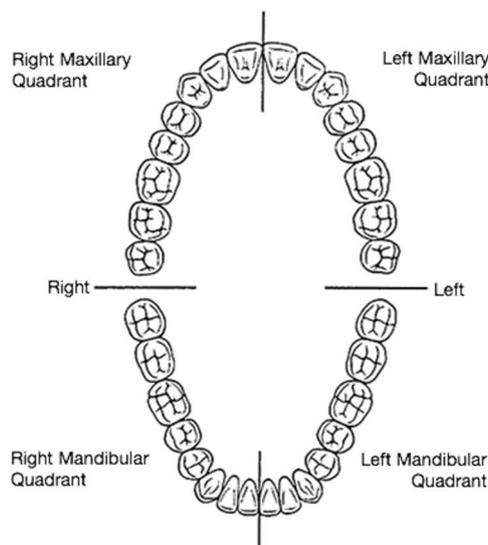
The high physiological and metabolic activity of alveolar bone separates it from many bones found in mammals (Hall 2015). These characteristics enable the bone to adapt as the dentition grows and changes (Brand and Isselhard 2014). This activity also causes fast resorption (Brand and Isselhard 2014; Hall 2015). Alveolar bone is closely linked to the development of the dentition and remodelling causes the formation of bundle bone in the socket to strengthen the attachment of the tooth to the periodontal ligament (Brand and Isselhard 2014).



Appx_Figure A.1.3 Photograph of a skull from Cementeri Vell, Formentera showing the maxillary arch and indicating the alveoli (tooth sockets) as well as partial resorption of the alveolar bone [Photograph taken by author (June 2014)].

A.2 Identification of the Teeth and their Surfaces

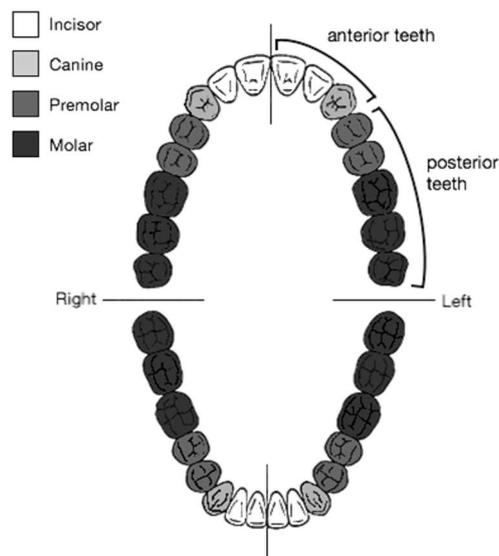
In the average adult, human mouth there are 32 permanent (adult) teeth³⁷ that are divided equally between the maxilla and mandible. The maxilla contains the upper dental arcade and the mandible contains the lower dental arcade, with each arcade containing 16 teeth. When studying the teeth, common practice is to use the natural midline of the dental arcades to divide the mouth into left and right. This results in four quadrants of eight teeth as shown in Appx_Figure A.2.1. The quadrants are named regarding the side (left or right) and the jaw (maxilla or mandible).



Appx_Figure A.2.1 Diagram to illustrate the permanent upper and lower dental arcades that are further divided into quadrants by the natural midline of the mouth with the names of the quadrants included [Adapted by author from Finkbeiner & Johnson (1997) (July 2015)].

³⁷ The number of teeth described regarding usual dental development however, in some individuals the third molars do not develop (Scheid and Weiss 2012). Other conditions can also change the total number of teeth present such as agenesis (the lack of tooth development) and supernumerary teeth (development of extra teeth).

The teeth can be categorised into four types, incisors, canines, premolars and molars, each with a different function during mastication³⁸. For each quadrant of eight teeth, there are two incisors, one canine, two premolars and three molars, as shown in Appx_Figure A.2.2. The incisors and canines are termed anterior teeth, because they are at the front of the mouth and the premolars and molars are posterior teeth due to their location at the back of the mouth.

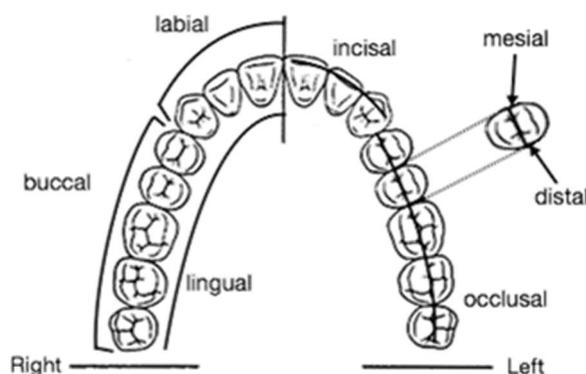


Appx_Figure A.2.2 Diagram to illustrate the categories of teeth found in the upper and lower dental quadrants and the distinction between anterior and posterior teeth (as shown in the left maxillary quadrant) [Adapted by author from Finkbeiner & Johnson (1997) (July 2015)].

As well as the category of tooth, the terminology for the surfaces of the teeth is important. Often pathological conditions of the dentition are described regarding the surface that they are present on which provides greater location specificity. The surfaces are generally described in relation to the crown of the tooth as these are the surfaces often examined by dentists but the same surfaces can also be applied to the root of the tooth.

³⁸ The mastication functions of the teeth are reflected in their coronal shape. The incisors are for cutting; canines are for cutting and tearing; premolars are for shearing and grinding and molars are for grinding (Scheid and Weiss 2012).

The names given to tooth surfaces are in relation to the direction that they face. For example, the lingual surface faces the tongue. All surfaces and their names are shown in Appx_Figure A.2.3.

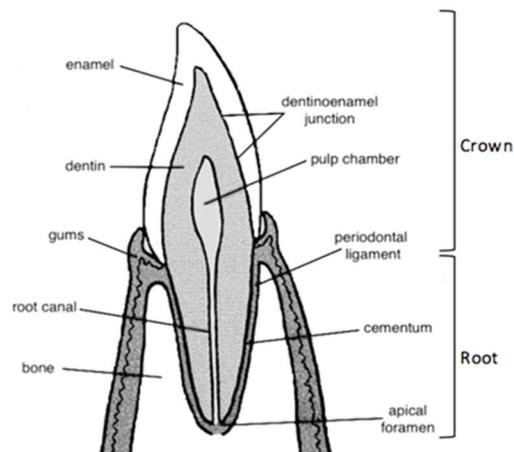


Appx_Figure A.2.3 Diagram of the upper dental arch with tooth surfaces labelled [Adapted by author from Finkbeiner & Johnson (1997) (July 2015)].

A.3 Tooth Anatomy

Despite the different types of tooth, all categories of teeth comprise of a crown and a root. There is variety in crown and root shape for differing categories of tooth but the anatomy is consistent throughout the dental arch (Appx_Figure A.3.1). The crown of a tooth is covered in enamel, a hard tissue that is composed of 97% mineral, with the most abundant mineral being calcium hydroxyapatite (Nanci 2013). The root of a tooth is covered with cementum, which is also composed of hydroxyapatite (around 40-50%) as well as proteins and water (Nanci 2013).

The differing compositions of the crown and root reflect their function. The crown is the surface that interacts with the natural environment and requires hardness to endure contact with different surfaces. In contrast, the root is the means of anchoring the tooth into the alveolar bone via the periodontal ligament and therefore has connective tissue attachments requiring organic material and water (Ho et al. 2009; Ren et al. 2010; Nanci 2013). The enamel of the crown and the cementum of the root surround the internal dentin and pulp of the tooth.



Appx_Figure A.3.1 Cross-sectional diagram of the anatomy of a human tooth showing the main features of the tooth crown and root [Adapted by author from White et al. 2011(1) (July 2015)].

A.4 Dental Notation

There are several different notation systems that can be used to record human dentition. Within this thesis, the FDI numbering system (the 2-digit system), the forensic notation system and shorthand tooth codes are used, all of which are explained below.

A.4.1 The Fédération Dentaire Internationale Tooth Numbering System

In this thesis, the Fédération Dentaire Internationale³⁹ (FDI) Tooth Numbering System (known by some as the 2-digit system) is primarily used in tables and charts within the recording forms. This system is useful when dental data is being recorded in a chart or used in a digital format because of the simplicity and uniformity of the 2-digit code rather than the tooth code system explained below. This system does not include the surface of the tooth.

This notational system assigns a unique 2-digit number to each tooth and the siding of charts is displayed in terms of the individual being examined (i.e. left is the patient

³⁹ The Fédération Dentaire Internationale are the world's leading professional dental organisation. In 1997 (revised in 2009) the FDI tooth numbering system was registered as an International Standard (ISO 2016).

/individual's left, not the examiners). The first digit of the code relates to the relative tooth quadrant (Appx_Table A.4.1) and the second digit of the code relates to the category of tooth (Appx_Table A.4.2).

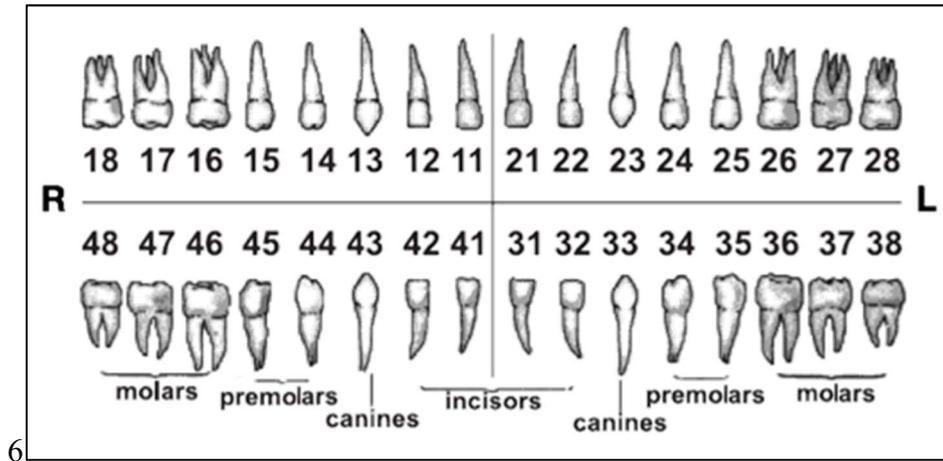
UR	UL
1	2
4	3
LR	LL

Appx_Table A.4.1 The first digit assignment of the FDI Tooth Numbering System. Each quadrant is given a number, which forms the first half of the 2-digit tooth number.

First Incisor	1
Second Incisor	2
Canine	3
First Premolar	4
Second Premolar	5
First Molar	6
Second Molar	7
Third Molar	8

Appx_Table A.4.2 The second digit assignment of the FDI Tooth Numbering System. Each tooth category is given a number, which forms the second half of the 2-digit tooth number.

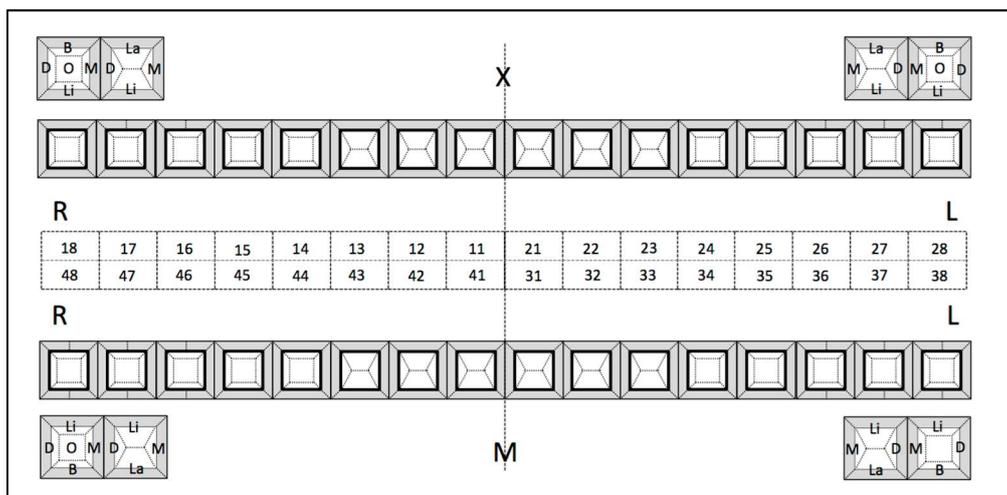
The usual format of a dental chart using the FDI system is shown below with the codes for each tooth in the position of the tooth they represent (Appx_Figure A.4.1).



6 **Appx_Figure A.4.1** A dental chart showing the FDI Number system and corresponding dentition [Adapted by author from <http://www.ictph.org.in/gip-2009/> (Retrieved 15th July 2015)].

A.4.2 Forensic-Style Notation System

In forensic odontology, the FDI Tooth Numbering System is commonly used in conjunction with drawings or diagrams of the teeth. However, in computer based software (WinID and Interpol DVI), these drawings are more difficult to implement therefore, dental charting is compiled in simplistic-looking charts with codes to identify pathologies, traits, dental work or restoration etc. This digital code system enables the software to automatically search for matches between the data entered from dental records and from post-mortem examinations, which is timesaving for cases of disaster victim identification. This charting used a square box to represent a tooth, with divisions for each tooth and root surface (Appx_Figure A.4.2).



Appx_Figure A.4.2 Example of the forensic notation system of dental recording, which is used in this thesis for the representation of dental calculus presence across the dentition of individuals. The FDI Tooth numbers are also included [Diagram compiled by author (February 2015)].

A.4.3 Tooth Codes

Codes given to the teeth are shorthand abbreviations that are primarily used for recording dentition and labelling samples. The codes are an easier way to identifying a tooth (or teeth) of interest for readers or examiners who are not familiar with the FDI numbering system. They are a quick method of including all relevant information without constantly writing the entire descriptive text for a given tooth. For example, upper left first premolar can be shortened to ‘UL PM1’.

For greater specificity, the tooth code can also be followed by a surface code. For example, the distal face of the upper left first premolar can be shortened to ‘UL PM1 (D)’. The codes take the general format of jaw:side:tooth:surface. Appx_Table A.4.3 details all codes and the words they represent, these are then combined to give the full tooth descriptor. When a component of the code is unknown, it is replaced with ‘?’⁴⁰ for

⁴⁰ For example, loose teeth with a large amount of wear are sometimes difficult to assign. It may be possible to determine the jaw and type of tooth but not which side it was from or the position of the tooth. A loose upper premolar of unknown side may then be denoted as U? PM? The code is as specific as the examiner can achieve within reason and based on the present data.

missing data. In this thesis, tooth codes are used to label sample bags and within analysis files to avoid complication when additional numbering is used (i.e. dates and acquisition numbers).

<i>Jaw</i>	
Maxilla	U
Mandible	L
Missing Data (if the jaw location cannot be determined)	?

<i>Side</i>	
Left	L
Right	R
Missing Data (if the side cannot be determined)	?

<i>Tooth</i>	
First Incisor; Second Incisor	I1; I2
Canine	C
First Premolar; Second Premolar	PM1; PM2
First Molar; Second Molar; Third Molar	M1; M2; M3
Missing Data (if the tooth cannot be identified)	?

<i>Surface</i>	
Buccal	B
Distal	D
Labial	La
Lingual	Li
Mesial	M
Occlusal	O

Appx_Table A.4.3 Terminology used for describing the teeth and the related shorthand code used to formulate a tooth code. The general format of a code is jaw:side:tooth:surface although surface is usually only applied if describing the location of a condition or feature on the tooth.

APPENDIX B: RECORDING METHODS

B.1 Recording Dental Calculus

On examination of archaeological remains, the first task is to record the remains, as they are post-excavation. This initial recording is an important stage in the analysis of past populations because it compiles data, which can be utilised to determine demography, stature, health and disease and trauma for an individual and subsequently a population. Depending on the preservation of the remains, the handling of skeletal elements may cause unintentional damage or breakage. Similarly, intentional destructive sampling for research will alter the overall context of the remains, even if done responsibly and ethically. Therefore, prior recording of the remains ensures the maximum amount of data available is collected before such damage or sampling occurs.

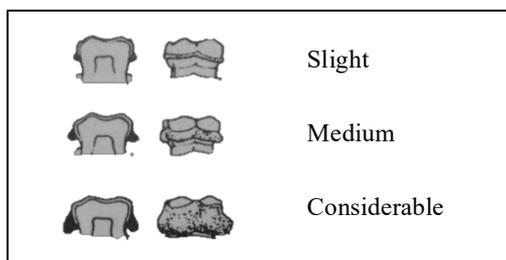
Despite the importance of recording human remains in archaeology, there are vast amounts of information to record in often a limited amount of time. Depending on the amount of time the skeletal collection can be studied and the reason for recording the information for each individual it is not always possible or relevant to detail every feature in a comprehensive manner.

Usually when dental pathology of a population is recorded, each disease or condition is recorded per the most widely adopted method. This can allow an overview of the general dental health of each individual and consequently the overall dental health of the population. However, using these quick, simple methods can make it difficult to return to the data after the skeletal material is no longer available, especially if more detailed information on a condition (such as dental calculus) is required.

This appendix is specifically focused on dental calculus recording, as a precursor to dental calculus analysis. The purpose of examining dental calculus recording is to analyse the current methods employed in archaeology. Furthermore, it is important to determine if the current methods are suitable considering the advances that have been made in understanding the importance of dental calculus as an archaeological source of evidence.

B.1.1 Archaeological Recording Methods

The most widely adopted method for recording calculus deposits on archaeological remains is Brothwell (1981), which gives a numerical value between 1-3 for the quantity of calculus present (Fox et al. 1996; Valentin et al. 2006).



Appx_Figure B.1.1 Diagram of amounts of calculus for scoring by Brothwell (1981), scores are given as follows; 0: none; 1: slight calculus; 2: medium calculus; 3: considerable calculus.

Similarly, the calculus scoring method in Buikstra and Ubelaker (1994) from the Standards of Data Collection from Human Skeletal Remains, uses Brothwell (1981) with an additional score of 9 for 'unobservable' (such as when the tooth is missing or in crypt). The Buikstra and Ubelaker (1994) method also indicates that the face on which the score is given should be identified.

There have been attempts to create more quantitative and comprehensive scoring systems for an archaeological application. One publication that addresses this is Greene et al. (2005), who suggested a combined method of scoring via the Buikstra and Ubelaker (1994) index and an adapted Simplified Calculus Index (explained fully in section 5.1.2). This process of recording, aims to create a more representative value for calculus for quadrants of the mouth by scoring 3 surfaces per tooth and creating an average score depending on the number of surfaces able to be scored. The frequencies of calculus presence gained from this process as opposed to an overall dental calculus score for the mouth is more useful for when teeth are missing or there is an above average deposition in an individual on specific teeth.

An alternative index based on experimentation of dental calculus amount on human and animal teeth adds to Brothwell (1981) with a score for the quantification of thickness of

the deposit (Dobney and Brothwell 1987). This method was tested on a range of samples to work out the values that should be assigned for different levels of thickness (i.e. what constitutes a thin, moderate or thick deposition).

Another, simpler, novel method was used by Klepinger et al. (1977) which involved 5 scores (extreme, very heavy, moderately heavy, slight and none) for a population, which was described as having above average calculus deposits. However, this method had no diagram or description of how the quantities were determined so it can be assumed it was based on visual assignment by the author. Unfortunately, methods like this cannot be used in studies of calculus formation between populations because the scale cannot be translated into a comparable score to effectively compare quantity of calculus.

Prior to Brothwell (1981), Evans (1973) used an index of 0-4 based on a suggested index by Stewart and Burnett (1960) and a diagram of the scale is featured in the paper. This study involved the comparison of calculus scores across five time periods of Mayan population and originally a mean was calculated of the total calculus score divided by the number of teeth that could be scored. However, Evans found that this method biased the overall mean towards individuals, which had more teeth present. To overcome this data skew, a mean index per individual was calculated with the number of teeth present for that burial and then a mean was calculated for the group of burials for that time.

The indices that are available for dental calculus recording are often sufficient for the broad contribution to knowledge that calculus scoring achieves. However, when analysing dental calculus for mineralogical, elemental, microfossil and biological content, quantitative analysis could be related to the size and location of the sample. With an increasing comprehension of the value that dental calculus can hold for past population studies, the position that this research takes is that more detail would be a benefit.

B.1.2 Problems with Archaeological Methods

Widely accepted methods for recording dental calculus found on archaeological remains involve subjective visual assignment of scores to document the amount of calculus (Brothwell 1981; Dobney and Brothwell 1987). This process of visual assignment is highly dependent on the observer as well as the collection to be scored. An issue that can affect the scores given for a deposit can include the observer's experience of using the

scoring system, which is particularly detrimental when comparing scores from multiple populations when different observers have scored them. The consistency of the scores given cannot be confirmed without one observer re-scoring all the dentition that is to be compared for dental calculus. Additionally, a scale of one to three (Brothwell 1981) or one to four (Dobney and Brothwell 1987) is not always sufficient to differentiate between the varying amounts of calculus present.

The recording methods described are a mixture of qualitative and quantitative with much of quantitative methods taking the form of scoring indices. A scoring index is defined as 'a number giving the magnitude of a physical property or other measured phenomenon in terms of a standard' (Oxford English Dictionary). Therefore, a scoring index is classed as a method of quantification because of the assignment of a number in relation to magnitude. However, due to an index being a standard range of values that can be assigned, there is limited variation and therefore discrete levels of magnitude.

This method is highly subjective even with diagrams of each score as it relies on the visual assignment of a value by the recorder. Unless the individual recording has seen many examples of dental calculus, it can be difficult to assign scores in a consistent manner as the collection is worked through. It also varies; again, on time available, whether the separate surfaces of each tooth are scored individually or whether one score is given per tooth for the most affected face.

When a scoring index is the only method of measurement assigned, the amount of information is being limited from the start. For example, a group of individuals could be recorded in terms of their hair length by a scoring index (Appx_Table B.1.1). In this table, there is limited information about the hair length of the individuals and assuming the data could only be collected once, more detailed information is not available. In comparison, Appx_Table B.1.2 shows the measured hair length of the individuals in question, which could be simplified if required. In addition to the limited information collected in the scoring index table, the categories of 'short', 'medium' and 'long' are vague and subjective.

Index	No. of People
1 (short)	3
2 (medium)	5
3 (long)	3

Appx_Table B.1.1 Table showing an example of data collection using an index scoring method; the index relates to hair length for individuals.

Individual	1	2	3	4	5	6	7	8	9	10
Hair Length (mm)	55	78	115	165	187	202	210	287	309	325

Appx_Table B.1.2 Table showing an example of data collection for recording the hair length with measurements rather than indices.

The example shown in these tables demonstrates that while the index scores are an effective way of quickly grouping individuals into categories, it relies on the scorer to subjectively decide on which category each individual best aligns. This is adequate for cases, which fit into those, predefined standards, however an individual that lies between two categories is harder to assign an index to. This is often the case in dental calculus recording due to the difficulty in assigning a standard measurement for the observable shapes, thicknesses and quantities of calculus deposits.

The recording of dental calculus has been completed in a qualitative way by a few studies to document the presence of calculus. For example, Arnay-del-la-Rosa et al. (2009) and Delgado-Darias et al. (2006) recorded the number of teeth affected by calculus without any scale for the amount of calculus deposit per tooth. These studies were not solely focused on dental calculus composition or development in a population. Instead the researchers used dental calculus in combination with other pathologies to create an overall summary of population health or diet.

Greene et al. (2005) have produced a method for archaeological application using Buikstra and Ubelaker (1994) (See section 5.1.1) and elements of the Simplified Calculus Index (Greene and Vermillion 1964) (See section 5.2.2). The method relies on a well-

known scoring system already employed in archaeology (Buikstra and Ubelaker 1994) with the addition of dentition segments to increase the reliability of data gained from calculus score averages in an individual and consequently a population.

B.1.3 Dentistry Recording Methods

There are by far the most methods used for calculus scoring, within periodontal health assessments in the dentistry field. The aim of reducing the number and severity of periodontal disease cases means there is an abundance of literature on recording dental health from clinical and epidemiological studies. The differences in the two investigations are that clinical studies are concerned with the treatment and removal of pathologies (such as calculus deposits) in individuals. In contrast, epidemiological studies are concerned with the wider population health. Specifically, how calculus is formed and the effects that calculus has in relation to other symptoms or conditions. The methods that are used in dentistry have been summarised in Appx_Table B.1.3.

The earliest published quantitative method is Ramfjord (1959), which was developed after the World Health Organisation consulted Ramfjord for a study of Indian Periodontal Disease in 1957. During his consultation, Ramfjord found that there was no published method to record his data and therefore draw conclusions about the state of the periodontal health of India. Consequently, he designed his Periodontal Index, which includes scoring gingival inflammation, calculus depositions, gingival pockets and plaque depositions. The calculus component of this Index was the first scoring method that enabled scale to be assigned to mineralised depositions on the teeth. Despite the development of subsequent methods since Ramfjord (1959), this index is still used in some fields (Bathla 2011).

An interesting review of scoring methods published by Volpe in 1974 aimed to compare the method available at the time. Volpe assessed the method based on reproducibility, reliability and objectivity, specifically in the field of oral hygiene and treatment of periodontal disease. The methods included for assessment included Volpe's own Probe Method (1965), the Calculus Surface Index by Ennever et al. (1961), the Marginalised Line Calculus Index by Mühlemann and Villa (1967) and the Standardised Foil

Technique by Marthaler et al. (1962). All these methods and the conclusions of Volpe's comparison are detailed in section 5.1.2.

Method	Publisher	Year	Scoring System
Periodontal Disease Index (Calculus Component)	Ramfjord	1959	C0 C1 C2 C3
Oral Hygiene Index	Greene and Vermillion	1960	Debris (DI) and Calculus (CI) Scored Separately: 0 1 OHI = Average DI 2 + Average CI 3 One tooth per segment examined and two faces of tooth scored (buccal/labial and lingual)
Calculus Surface Index	Ennever et al	1961	Four incisors scored for presence of calculus on all four surfaces 0 1
Simplified Oral Hygiene Index	Greene and Vermillion	1964	Debris (DI) and Calculus (CI) Scored Separately: 0 1 OHI = Average DI 2 + Average CI 3 One tooth per segment examined and one face of tooth scored (buccal/labial for upper teeth and lingual for lower teeth)
Volpe-Manhold Probe Method	Volpe and Manhold	1965	Periodontal probe used to measure calculus deposits in millimetres (usually of the six lower anterior teeth)
Marginal Line Calculus Index	Mühlemann and Villa	1967	Percentage of tooth covered - determined by the average of coverage over three sections of tooth
CSSI	Conroy and Sturzenberger	1968	Four incisors scored for presence of calculus on all four surfaces 0 1 2 3
Sign Grading System	Agrawal 2011	2011	- + ++ +++

Appx_Table B.1.3 Summary of the dental calculus recording methods published for use in dentistry (full descriptions of scoring values are included in Appendix B.2).

B.1.4 Veterinary and Zoological Recording Methods

Many veterinary practitioners record calculus according to the method by Ramfjord (1959) for cats and dogs (Bellows 2004). This is a simple and quick visual index for the qualification of calculus deposits to give an overall summary of the animals' dental health. The latter is also a method approved for use by The Royal Veterinary College, University of London and the Veterinary Oral Health Council in the United States, who also state that any novel method used must be justified in a statement to the council (Royal Veterinary College 2002).

While calculus recording has not been widespread in Zooarchaeology, some researchers have employed a range of methods. Some publications have utilised accepted methods from dentistry as well as novel methods (Kyllar and Witter 2005; Wenker et al 1999; Willis et al. 1999). In Wenker et al. (1999), a study of bears included a simple method of scoring a yes or no result for each tooth surface (mesial, distal, facial, lingual). This yielded a percentage of total surfaces that had been affected by calculus but with no scale of the extent of calculus present.

A more complex system of scoring was employed for the study of lemurs and baboons in Willis et al. (1999). This involved a Ramfjord style index of 0-4 combined with a 1-3 score of thickness (light (<0.5mm); moderate (0.5-1.0mm); heavy (>1.0mm)). The teeth scored were different for each species of lemur and were chosen according to sites of highest calculus accumulation in each species from previous studies of the same animals (Willis et al. 1999).

B.2 Published Dentistry Recording Methods

Periodontal Disease Index (Calculus Component) (Ramfjord 1959)

- C0: Absence of calculus
- C1: Supragingival calculus extending only slightly below the free gingival margin (not more than 1 mm)
- C2: Moderate amount of supra and subgingival calculus, or subgingival calculus only
- C3: An abundance of supra and subgingival calculus

Oral Hygiene Index (OHI) (Greene and Vermillion 1960)

The mouth is divided into six segments and each one is given a OHI score based on the teeth present for the buccal/labial and lingual faces. One tooth is scored per segment and both buccal/labial and lingual faces are scored for each tooth.

Debris Index

- 0 No debris or stain present.
- 1 Soft debris covering not more than one third of the tooth surface, or presence of extrinsic stains without other debris regardless of surface area covered.
- 2 Soft debris covering more than one third, but not more than two thirds, of the exposed tooth surface.
- 3 Soft debris covering more than two thirds of the exposed tooth surface.

Calculus Index

- 0 No calculus present.
- 1 Supragingival calculus covering not more than third of the exposed tooth surface.
- 2 Supragingival calculus covering more than one third but not more than two thirds of the exposed tooth surface or the presence of individual flecks of subgingival calculus around the cervical portion of the tooth or both.
- 3 Supragingival calculus covering more than two third of the exposed tooth surface or a continuous heavy band of subgingival calculus around the cervical portion of the tooth or both.

Oral Hygiene Index = Average Debris Index + Average Calculus Index
--

Calculus Surface Index (Ennever et al. 1961)

Four mandibular incisors scored on facial, mesial, lingual and distal surfaces

- 0 No calculus present
- 1 Calculus Present

Volpe-Manhold Probe Method (Volpe and Manhold 1965)

Generally used on the lingual surface of the six anterior teeth. Three planes are taken and measured in millimetres using a periodontal probe. Scores are averaged per tooth depending on the number of planes measured with calculus. Measurements can be averaged for a measurement score (total scores divided by number of measurements); tooth score (total scores divided by the number of teeth scored); or a subject score (total scores divided by the number of scores for individual).

Simplified Oral Hygiene Index Greene and Vermillion 1964)

The mouth is divided into six segments and each one is given a OHI score based on the teeth present for the buccal/labial and lingual faces.

Calculus Index:

- 4 No calculus present.
- 5 Supragingival calculus covering not more than third of the exposed tooth surface.
- 6 Supragingival calculus covering more than one third but not more than two thirds of the exposed tooth surface or the presence of individual flecks of subgingival calculus around the cervical portion of the tooth or both.
- 7 Supragingival calculus covering more than two third of the exposed tooth surface or a continuous heavy band of subgingival calculus around the cervical portion of the tooth or both.

Oral Hygiene Index = Average Debris Index + Average Calculus Index
--

Marginal Line Calculus Index (Mühlemann and Villa 1967)

Generally used on the lingual surface of the lower incisors. The tooth is sectioned into three sections and each one is scored for percentage covered. Percentages are averaged per tooth and total score is percentage scored divided by the number of teeth scored.

Calculus Surface Severity Index (Conroy and Sturzenberger 1968)

Four mandibular incisors scored on facial, mesial, lingual and distal surfaces

- | | |
|---|---|
| 0 | No calculus present |
| 1 | Calculus observable, but less than 0.5 mm in width and/or thickness |
| 2 | Calculus not exceeding 1.0 mm in width and/or thickness |
| 3 | Calculus exceeding 1.0 mm in width and/or thickness |

Sign Grading System (SGS) (Agrawal 2011)

Grade	Examination
-	Absence of supra or subgingival calculus
+	Isolated flakes or continuous band of calculus present supragingivally not covering more than one-third of the crown and without presence of subgingival calculus or supragingival/subgingival calculus present only on the proximal surface
++	Isolated flakes or continuous band of subgingival calculus present on labial/lingual surface of crown without presence of supragingival calculus or Supragingival calculus in cervical one-third of labial or lingual surface of crown along with presence of subgingival calculus or Supragingival calculus extending more than one-third but less than two-thirds of labial or lingual surface or crown, with or without the presence of subgingival calculus
+++	Supragingival calculus extending more than two-thirds of the crown, with or without the presence of subgingival calculus

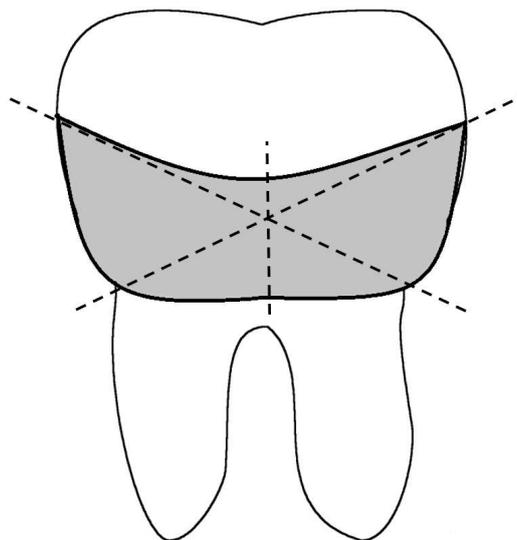
B.3 Recording Protocol

B.3.1 Proposed Quantitative Recording Method

This new method for scoring dental calculus deposits is adapted from a clinical method of quantification used by the dentistry profession. The Volpe-Manhold Probe Method (1965) has been validated as a reproducible method between scorers and individuals (Volpe-Manhold et al. 1965; Baumhammers et al. 1973; Barnett et al. 1989). It aims to comprehensively detail the amount of calculus present per surface of each tooth to allow accurate representation of calculus prevalence in individuals, across populations and between collections. By using graduated dental probes, this novel method allows the amount of calculus to be quantified to allow more accurate documentation of this dental pathology, prior to subsequent destructive analysis. The method is as follows:

Cooper Method (2014):

1. Ensure tooth has been cleaned carefully to remove any dirt or debris from the enamel, cementum and calculus. Before scoring the tooth for calculus deposits, make sure the surface is air dried.
2. Using a UNC15 periodontal probe, measure the length of calculus along each plane shown in diagram A. Do this for every lingual and labial face of the anterior teeth present and the mesial and distal faces of the molar teeth present.



Appx_Figure B.3.1 Diagram showing the planes to be measured in the Cooper (2014) method for quantifying dental calculus.

B.4 Recording Protocol Results

B.4.1 Recording Forms

Overall the designed recording forms (included in Appendix D) performed well with all relevant information being recorded. The completion of a form for an individual took differing lengths of time depending on the number of teeth present to record. The time taken was severely increased by drawing the calculus deposits on the tooth diagrams (Probe Scoring Record) so this section was discarded early in the recording of the Capuchin Catacombs. Overall, there was enough space to fill in each section and plenty of extra lines for photographs and samples, should more be required.

B.4.2 Scoring Methods

The results for comparing the Brothwell scores and Probe scores were compiled to determine the validity of using the Probe method for calculus recording. Currently, methods such as Brothwell (1981) are based on the subjective decision of the recorder to assign a discrete score for calculus deposits, which vary in size, location and shape. The deposits seen do not always fit neatly into one of the indices described and are therefore given an overstated or understated score. By implementing a quantitative method based on measuring the deposit it was intended that the deposits could be more objectively compared.

For both collections, a considerable disadvantage to using the Probe method was no measurement being possible. For some teeth, there were problems with accessibility to deposits and others where there were flecks of calculus on the tooth surface. This type of deposit was not measurable because the flecks of calculus were in some cases smaller than 0.5 mm and any plane measurement would not accurately represent the actual amount of calculus present. For the teeth where the Probe could not be inserted to measure the calculus, the Brothwell score could still be given by inspecting the interproximal space by eye. Similarly, the flecks of calculus were given a score of 1 for Brothwell because there was calculus present, however this is a case where the score over-estimates the amount of calculus.

From the comparing the results of the total measurements against the Brothwell score given for a surface, a very loose correlation can be seen, which is expected. However,

there was a large amount of overlap between measurements taken and Brothwell score given. This means that for deposits, which produced the same total measurement score, it was possible that different Brothwell scores were given.

The qualitative Probe method has shown that for the same amount of calculus, the subjective nature of scoring with indices produces inconsistent results. However, the biggest limitation to recording with only this method would be the loss of information where measurements are not possible. These results are presented from scores taken by the same recorder and further analysis will include the Probe measurements compared to Brothwell scores taken by Emily Wilson for the Cementeri Vell Collection.

The recording and sampling components of the protocol have been designed and implemented during the sample collection fieldwork. From this implementation, a few elements have been found to work well and some have required changing to improve the final protocol. The work carried out so far has also tested the use of a quantitative method of calculus recording and compared it against a commonly used index. This method has proved to eliminate the subjectivity of assigning indices. The major downfall of this method is however the problems with measuring deposits that are situated in interproximal spaces and that are flecks or speckles of calculus.

APPENDIX C: MATERIALS

C.1 Accessibility Log

Friar's Corridor

CCFMR_01	Access due to soft tissue / No teeth visible for exposed alveolar
CCFMR_02	Layman placed in Friars corridor (record for Male population)
CCFMR_03	Layman placed in Friars corridor (record for Male population)
CCFMR_04	Layman placed in Friars corridor / Access due to soft tissue
CCFMR_05	Record
CCFMR_06	Record - High wear, no calculus (?)
CCFMR_07	Access due to soft tissue and remains position (head on chest)
CCFMR_08	Access due to soft tissue / Edentulous
CCFMR_09	Edentulous
CCFMR_10	Access due to soft tissue / Coffin
CCFMR_11	Degrading remains possibly due to microbial activity and exposed internal soft tissue
CCFMR_12	Access to posterior teeth due to soft tissue / No calculus on accessible anterior teeth
CCFMR_13	Remains removed from alcove (likely due to failure of hanging method)
CCFMR_14	No calculus present
CCFMR_15	Record
CCFMR_16	Record
CCFMR_17	Record
CCFMR_18	Access due to soft tissue and position of remains (head on chest)
CCFMR_19	Soft tissue
CCFMR_20	Physical Access due to metal fencing
CCFMR_21	Soft tissue
CCFMR_22	No maxilla / Mandible Edentulous
CCFMR_23	Record
CCFMR_24	Edentulous
CCFMR_25	Record (lots of soft tissue around posterior teeth)
CCFMR_26	Soft tissue around posterior teeth and debris
CCFMR_27	Record
CCFMR_28	Record
CCFMR_29	Soft tissue
CCFMR_30	Soft tissue
CCFMR_31	Edentulous
CCFMR_32	No mandible / Maxilla Edentulous
CCFMR_33	4 LR teeth present and accessible but too high to reach (stacked coffins in front)
CCFMR_34	Soft tissue
CCFMR_35	Soft tissue
CCFMR_36	Soft tissue / height of remains (stacked coffins)
CCFMR_37	Soft tissue
CCFMR_38	Edentulous
CCFMR_39	Record
CCFMR_40	Soft tissue
CCFMR_41	Soft tissue
CCFMR_42	Soft tissue
CCFMR_43	No calculus present on visible teeth surfaces
CCFMR_44	No calculus present on visible teeth surfaces
CCFMR_45	Soft tissue / Height of remains
CCFMR_46	Soft tissue / No calculus present on visible/accessible teeth surfaces

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CCFMR_47	Soft tissue / Edentulous (note: 47 positioned between 48 and 49)
CCFMR_48	Soft tissue / Edentulous (note: 48 positioned between 46 and 47)
CCFMR_49	Edentulous (one LR anterior root remaining)
CCFMR_50	Record (no sample - only one tooth (calculus present) visible due to soft tissue (left))
CCFMR_51	Soft tissue
CCFMR_52	Soft tissue / Height of remains
CCFMR_53	Soft tissue
CCFMR_54	Record (no sample - only one tooth surface with calculus present accessible)
CCFMR_55	Soft tissue / No mandible
CCFMR_56	Record
CCFMR_57	Access due to height of remains
CCFMR_58	Edentulous (expect one LL anterior - post mortem crown damage)
CCFMR_59	Soft tissue / height of remains
CCFMR_60	Soft tissue (left side) / Access to height of remains (right side)
CCFMR_61	No mandible / Maxilla Edentulous
CCFMR_62	Access due to position of remains (head on chest)
CCFMR_63	Record
CCFMR_64	No mandible / 1 UL posterior tooth - no access
CCFMR_65	Edentulous
CCFMR_66	Soft tissue
CCFMR_67	LR M2 and LL C present - no calculus on visible surfaces
CCFMR_68	Edentulous
CCFMR_69	Soft tissue
CCFMR_70	Soft tissue
CCFMR_71	Soft tissue
CCFMR_72	Soft tissue
CCFMR_73	Edentulous
CCFMR_74	Soft tissue
CCFMR_75	Mandible Edentulous / Maxilla not accessible due to positioning
CCFMR_76	Soft tissue
CCFMR_77	Inter-oral Soft tissue / LR PM1 and LR M1 Visible but high wear and no calculus
CCFMR_78	Record (no sample)
CCFMR_79	Record

Priest's Corridor

CCSMR_252	Record
CCSMR_253	No mandible / Access due to position of skull (resting on sternum)
CCSMR_254	Record
CCSMR_255	Soft tissue
CCSMR_256	Mandible Edentulous / Maxilla not observable due to position)
CCSMR_257	Record
CCSMR_258	Record (no sample - 1 tooth observable)
CCSMR_259	No mandible / Soft tissue / Position of skull (head on chest)
CCSMR_260	Access due to height of remains
CCSMR_261	Record
CCSMR_262	Record (no sample - 1 tooth present (UL posterior))
CCSMR_263	Soft tissue
CCSMR_264	Record
CCSMR_265	No mandible / 1 tooth damaged PM
CCSMR_266	No mandible / Access (stacked coffins)
CCSMR_267	No mandible / Edentulous
CCSMR_268	No mandible / Access due to position of skull (head on chest)
CCSMR_269	Soft tissue
CCSMR_270	Record
CCSMR_271	Soft tissue
CCSMR_272	Record
CCSMR_273	Record (?)
CCSMR_274	Access due to position of skull (head on chest) / Soft tissue

CCSMR_275	No calculus visible on observable teeth
CCSMR_276	Access due to height / Debris on teeth
CCSMR_277	Soft tissue (individual with no skull between 277 and 278)
CCSMR_278	Record (individual with no skull between 277 and 278)
CCSMR_279	No mandible / Access due to position of skull
CCSMR_280	Soft tissue
CCSMR_281	Soft tissue (not priest)
CCSMR_282	Child
CCSMR_283	Record
CCSMR_284	Debris
CCSMR_285	Edentulous
CCSMR_286	No mandible / No calculus visible on observable teeth
CCSMR_287	Access due to position of skull (head on chest)
CCSMR_288	Soft tissue
CCSMR_289	Soft tissue
CCSMR_290	Access due to position
CCSMR_291	Record
CCSMR_292	Record
CCSMR_293	Soft tissue

Professionals' Corridor

CCPMR_314	Soft tissue / Only upper anterior labial observable (no calculus)
CCPMR_315	Soft tissue / Only upper anterior labial observable (no calculus)
CCPMR_316	No mandible / Edentulous
CCPMR_317	Remains too high
CCPMR_318	Record (Maxilla only)
CCPMR_319	Soft tissue / position of head (turned to left)
CCPMR_320	No mandible / Edentulous
CCPMR_321	Record
CCPMR_322	No calculus
CCPMR_323	Record
CCPMR_324	Mandible Edentulous; Maxilla not observable (positional)
CCPMR_325	No mandible / Posterior teeth present but not accessible
CCPMR_326	No mandible / Edentulous
CCPMR_327	No mandible / 1 tooth no calculus visible on observable surfaces
CCPMR_328	Record
CCPMR_329	No calculus visible on observable surfaces
CCPMR_330	Record
CCPMR_331	Edentulous
CCPMR_332	Record
CCPMR_333	Access due to position of skull (skull on chest and turned slightly left)

Men's Corridor

CCMMR_180	Access due to hood
CCMMR_181	Record (no sample - only 1 tooth with visible calculus)
CCMMR_182	Record
CCMMR_183	Record
CCMMR_184	Soft tissue
CCMMR_185	Record (no sample - only 1 tooth with visible calculus)
CCMMR_186	Soft tissue
CCMMR_187	Soft tissue
CCMMR_188	Access due to position of skull (head on chest)
CCMMR_189	Record
CCMMR_190	Soft tissue
CCMMR_191	No calculus visible on observable surfaces (charring to enamel)
CCMMR_192	Record (no sample - only 1 tooth with visible calculus)
CCMMR_193	Record

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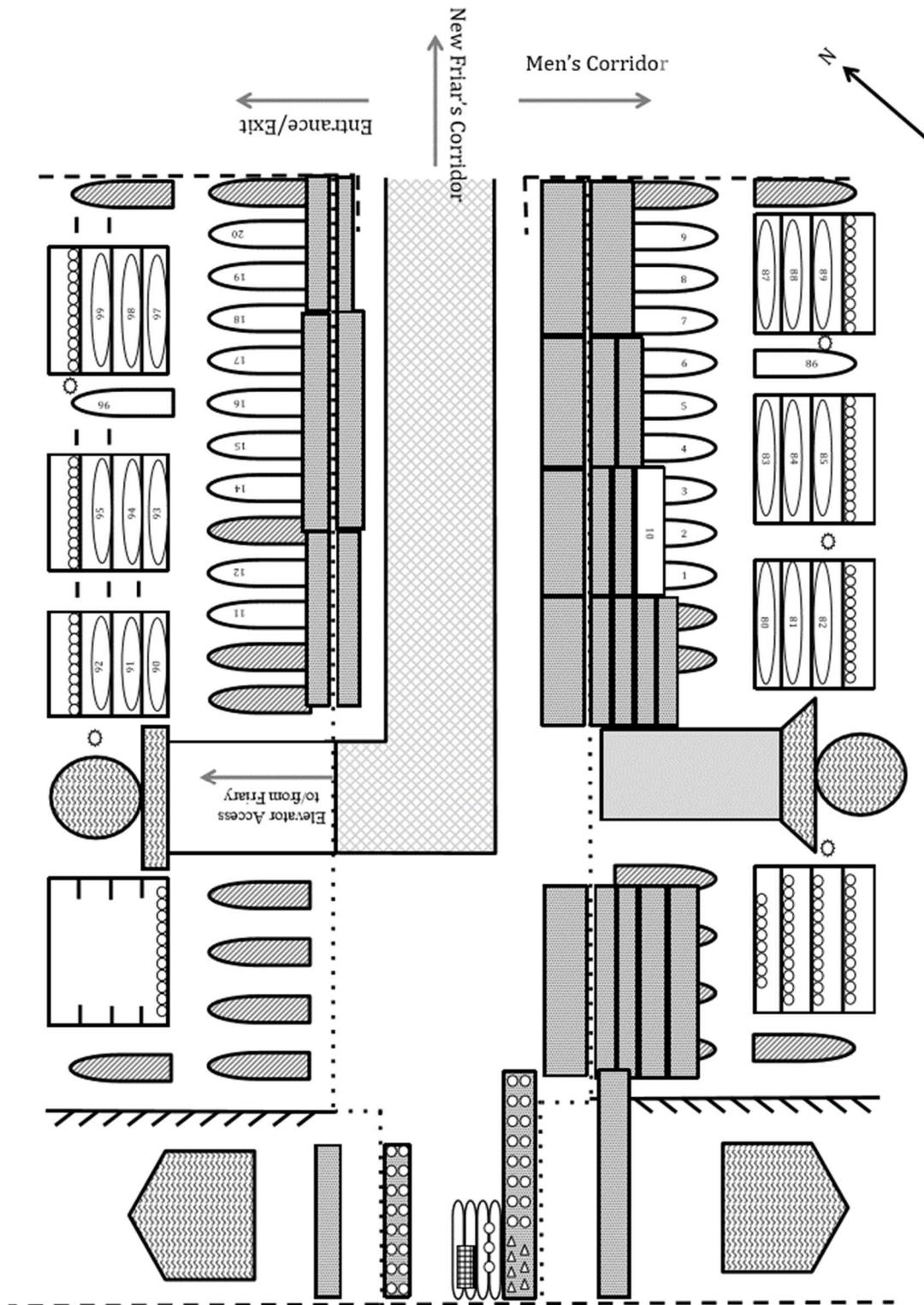
CCMMR_194	Soft tissue
CCMMR_195	Record (no sample - only 1 tooth with visible calculus)
CCMMR_196	Record
CCMMR_197	Record
CCMMR_198	Access due to metal fencing
CCMMR_199	Soft tissue
CCMMR_200	Record
CCMMR_201	No mandible access due to position of skull head on chest
CCMMR_202	Soft tissue
CCMMR_203	Record (lots of soft tissue)
CCMMR_204	Only 1 tooth present (pm damaged only root remaining)
CCMMR_205	No mandible / Edentulous
CCMMR_206	Mandible Edentulous / Maxilla access due to position of skull (head on chest)
CCMMR_207	Record
CCMMR_208	Record
CCMMR_209	Record
CCMMR_210	Record
CCMMR_211	Edentulous
CCMMR_212	No calculus visible on observable surfaces
CCMMR_213	Record
CCMMR_214	Edentulous
CCMMR_215	Edentulous

Women's Corridor

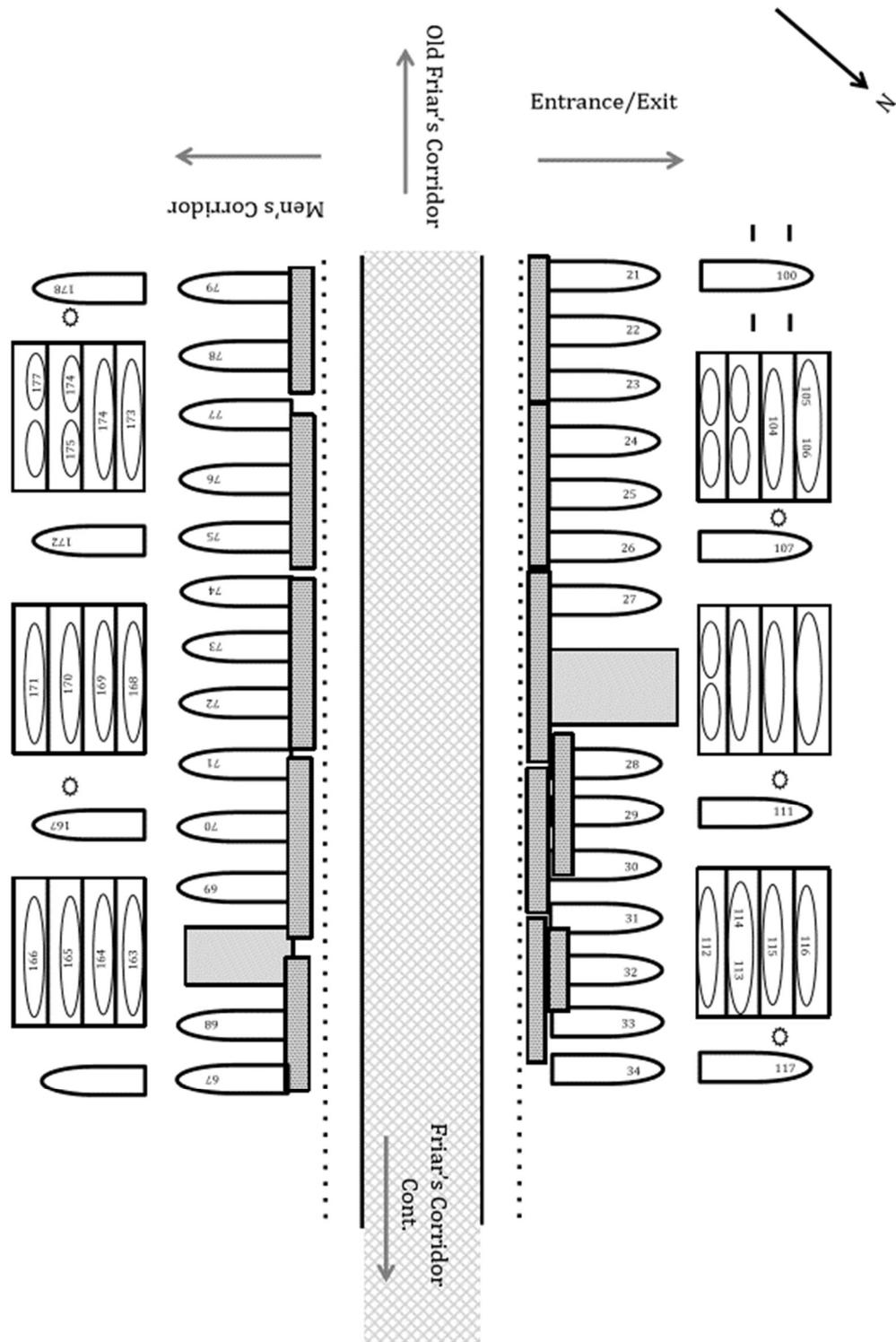
CCNMR_1119	Access due to height (positioned on wall above stacked coffins)
CCNMR_1120	Access due to height (positioned on wall above stacked coffins)
CCNMR_1121	Access due to height (positioned on wall above stacked coffins)
CCNMR_1122	Access due to height (positioned on wall above stacked coffins)
CCNMR_1123	Access due to height (positioned on wall above stacked coffins)
CCNMR_1124	Access due to height (positioned on wall above stacked coffins)
CCNMR_1125	Edentulous
CCNMR_NN	Access due to wire mesh covering coffin opening (no reference code)
CCNMR_1126	Record
CCNMR_1127	No Calculus Observable on Accessible teeth
CCNMR_1128	No Calculus Observable on Accessible teeth
CCNMR_1129	Access due to height (stacked coffin)
CCNMR_1130	Edentulous
CCNMR_1131	Record (lots of soft tissue)
CCNMR_1132	Soft tissue
CCNMR_1133	Record
CCNMR_1134	Access due to height (stacked coffin)/ Safety due to unstable stacking
CCNMR_1135	Edentulous
CCNMR_1136	One tooth observable, no calculus visible
CCNMR_1137	Record (?)
CCNMR_1138	Record
CCNMR_1139	Record
CCNMR_1140	Record
CCNMR_1141	Edentulous
CCNMR_1142	Record
CCNMR_1143	Edentulous
CCNMR_1144	Record
CCNMR_1145	Debris
CCNMR_1146	Record
CCNMR_1147	No Calculus visible
CCNMR_1148	One tooth, Record but no Sample
CCNMR_1149	No Calculus
CCNMR_1150	Edentulous
CCNMR_1151	Access due to height (stacked coffin)

CCNMR_1152 Male in coffin next to Rosalia Lombardo mummy case
CCNMR_1153 Soft tissue / male?

C.2 Corridor Plans

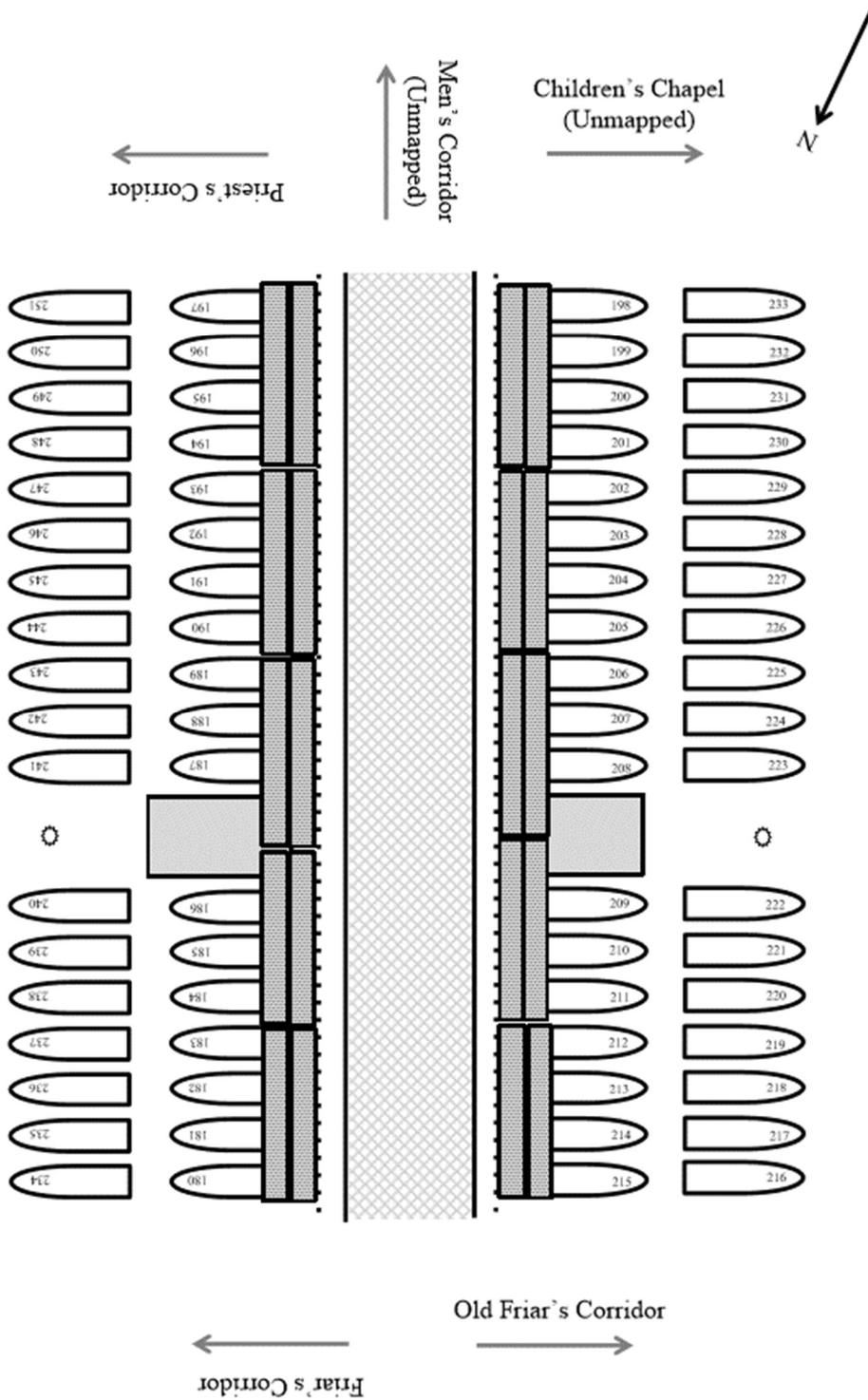


Appx_Figure C.2.1 Plan of the Old Friar's Corridor (no scale).

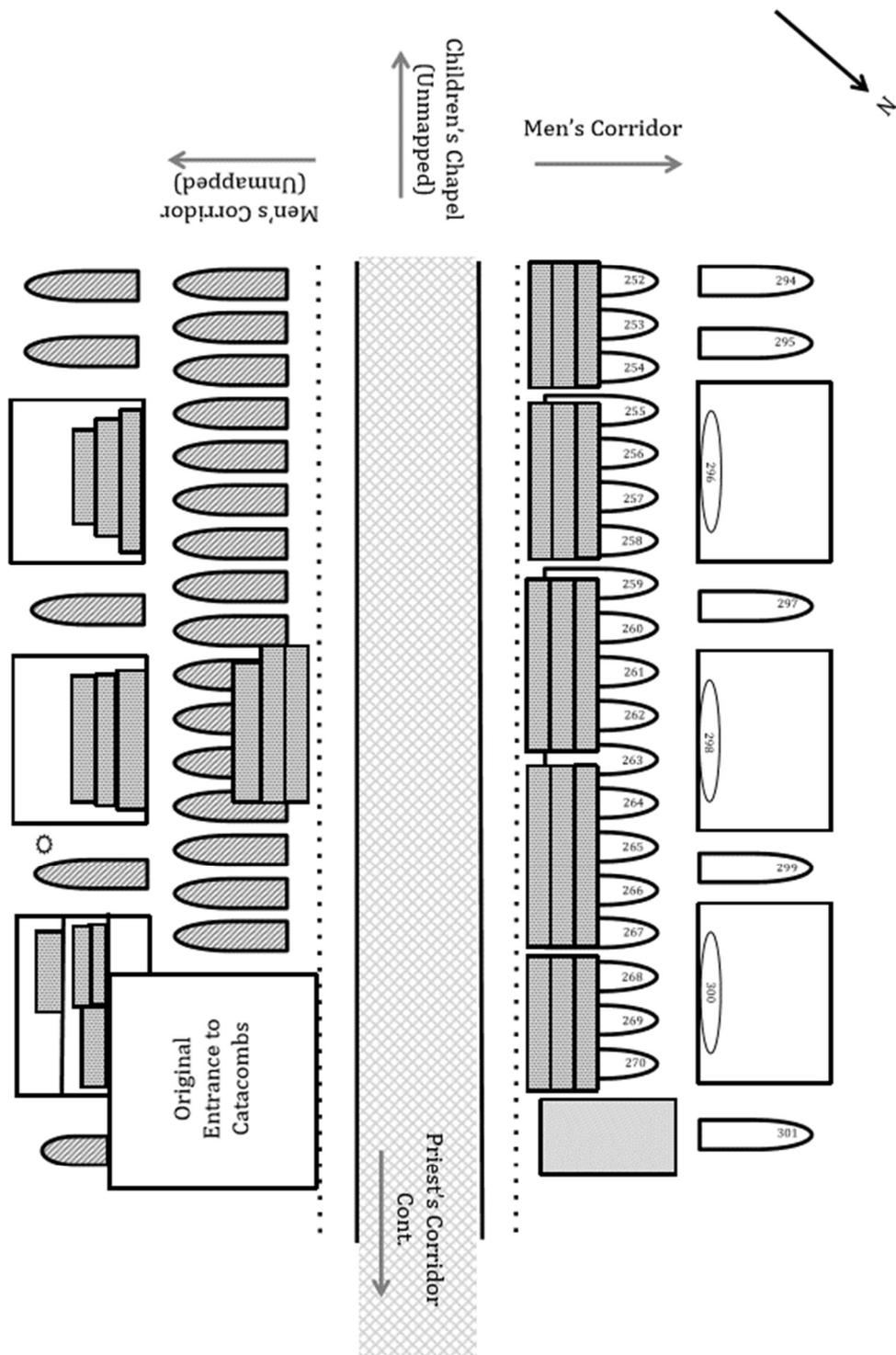


Appx_Figure C.2.2

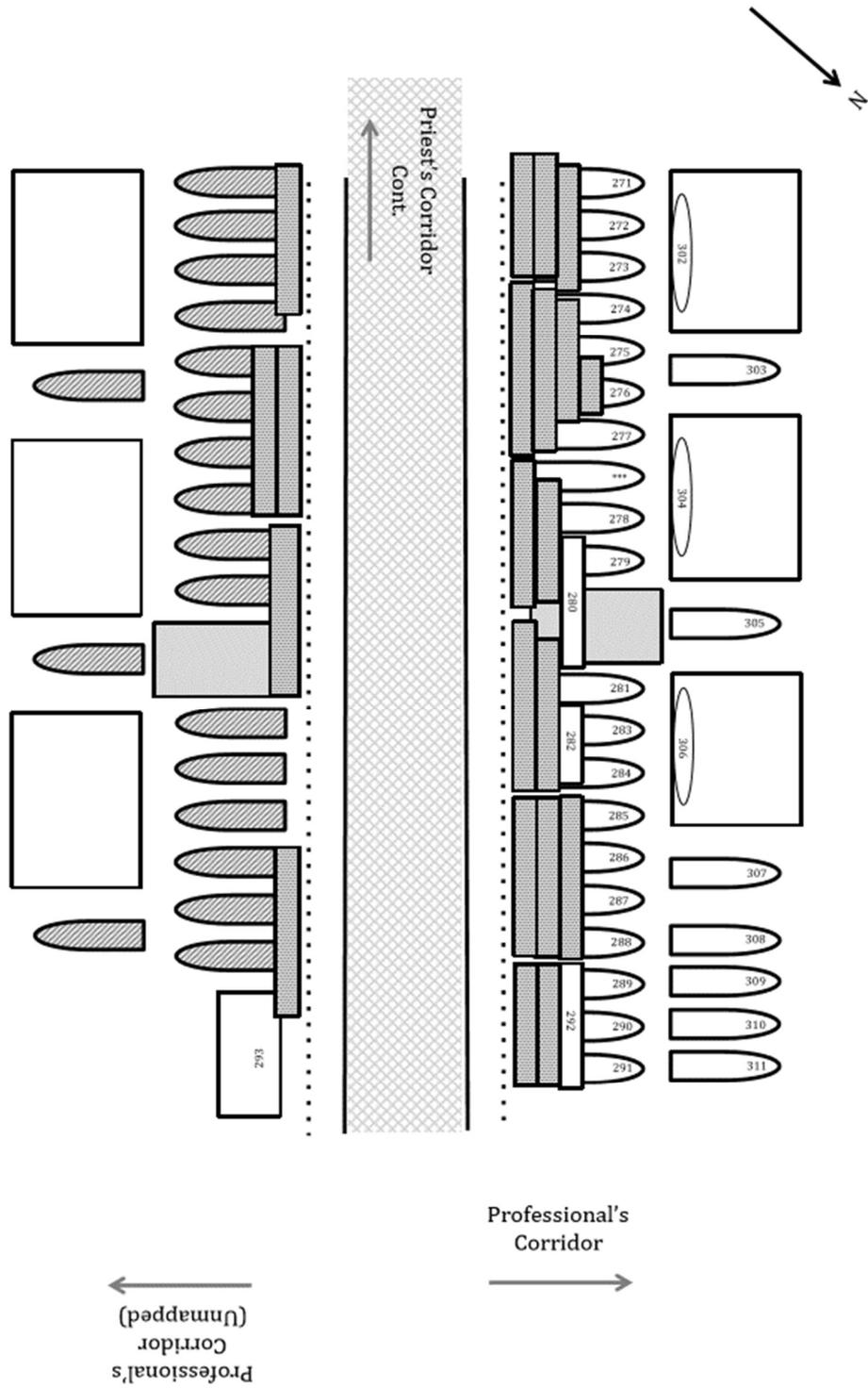
Plan of the Friar's Corridor (West) (no scale).



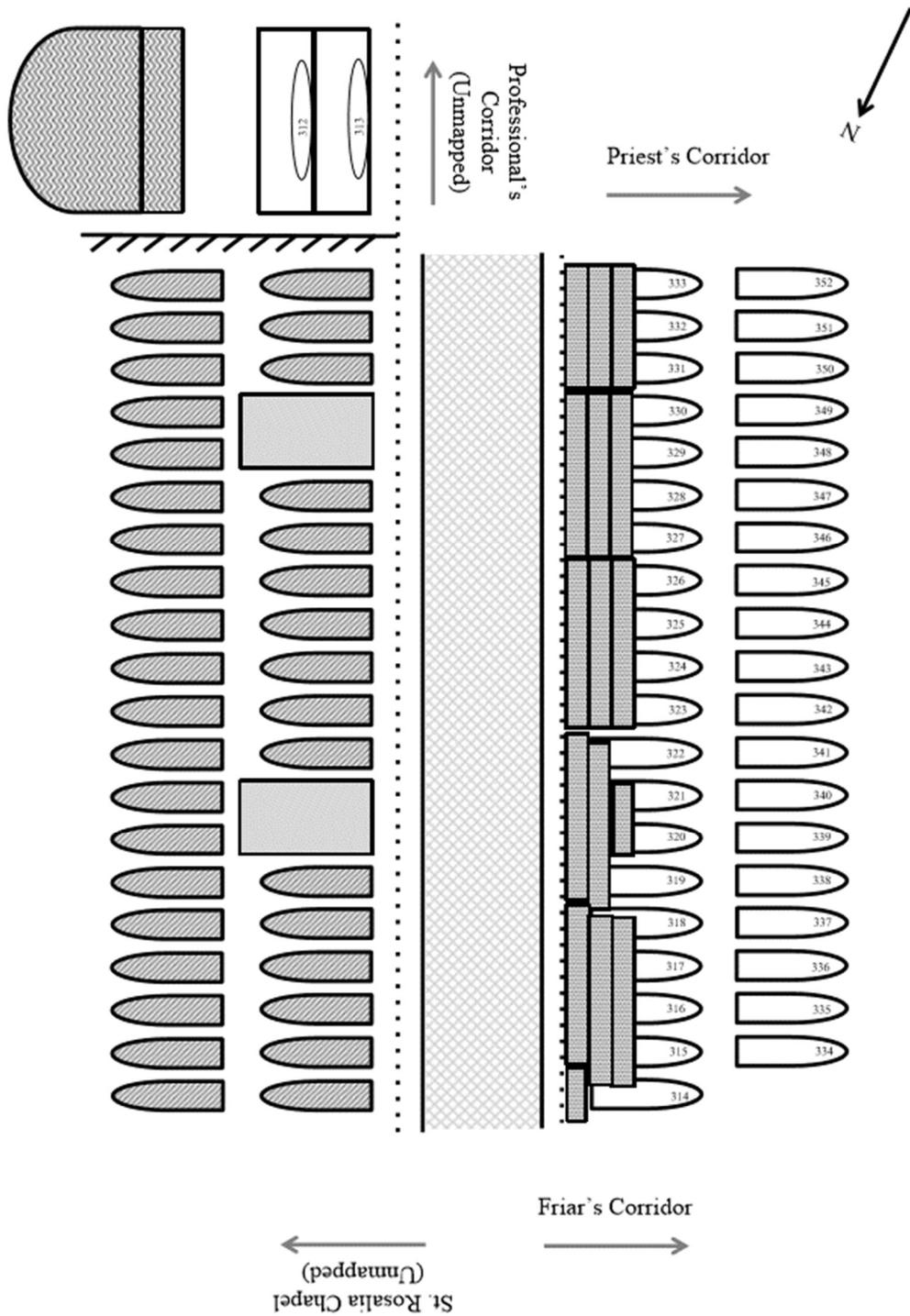
Appx_Figure C.2.4 Plan of the Men's Corridor (no scale).



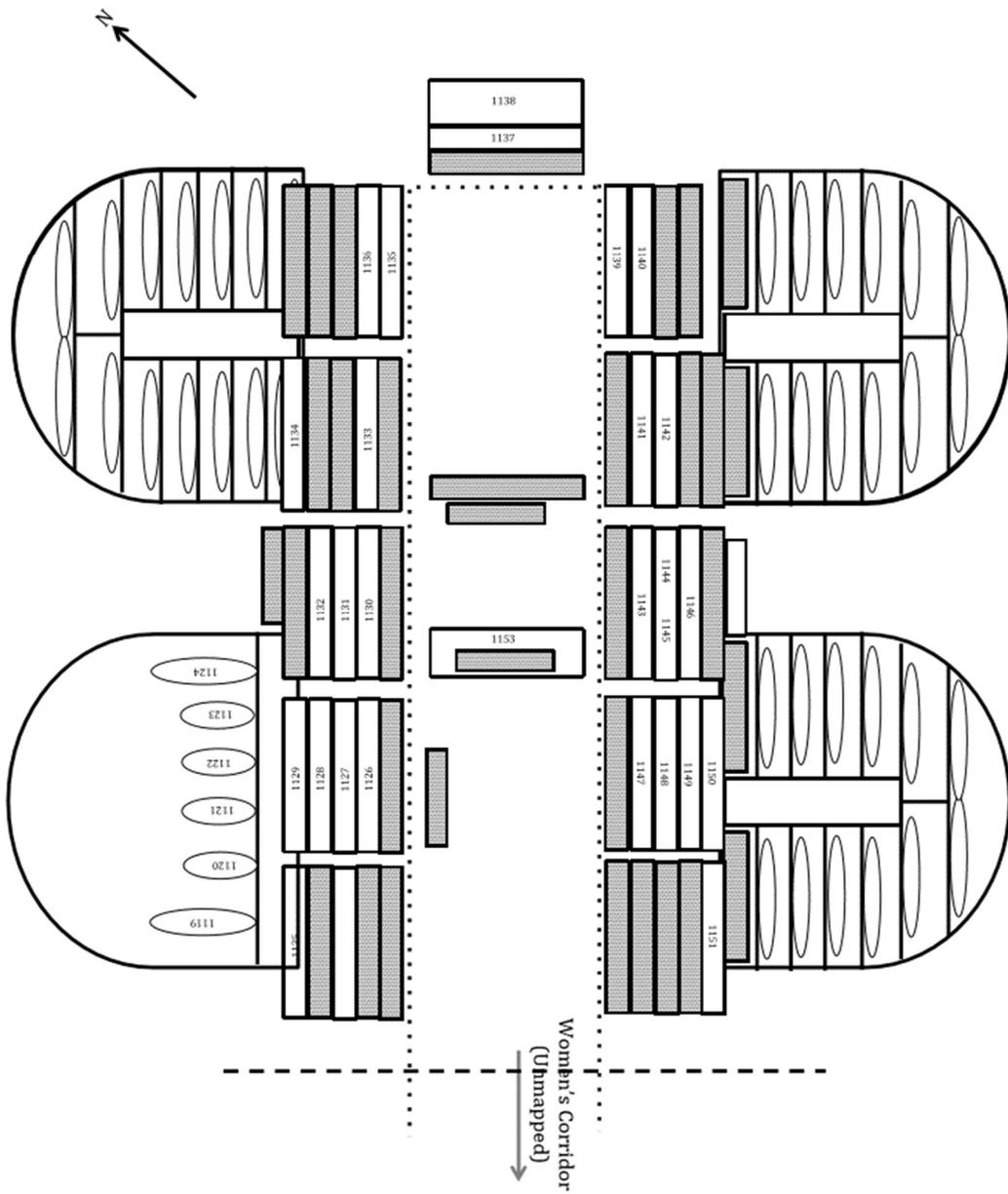
Appx_Figure C.2.5 Plan of the Priest's Corridor (West) (no scale).



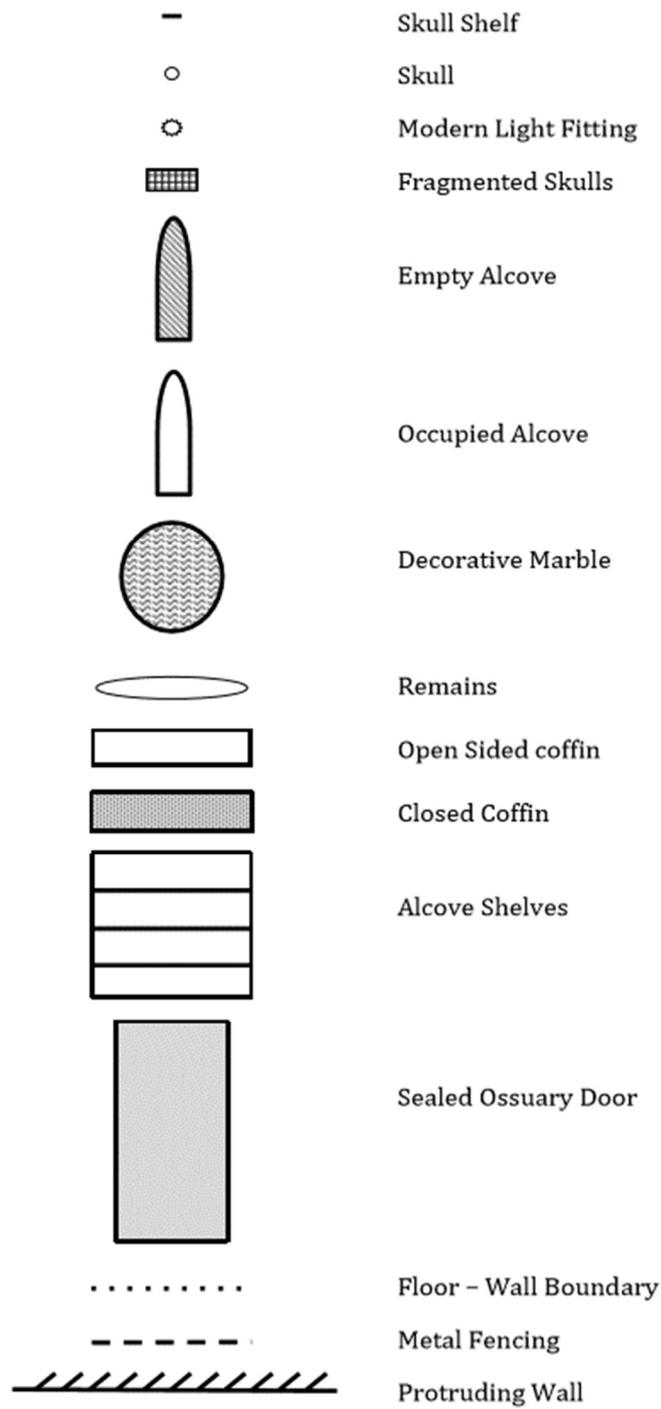
Appx_Figure C.2.6 Plan of the Priest's Corridor (East) (no scale).



Appx_Figure C.2.7 Plan of the Professionals' Corridor (no scale).



Appx_Figure C.2.8 Plan of the Women's Corridor (no scale).



Appx_Figure C.2.9 Key to plans for the Capuchin Catacombs (no scale).

APPENDIX D: RECORDING FORMS

Collection Name: _____
 Skeleton Reference: _____



Cover Form

<u>Form</u>	<u>No. of Pages</u>	<u>Completed?</u>	<u>Date of Completion</u>
Initial Dentition Record Recorder.....	(1/2)	<input type="radio"/>	Date
Initial Dentition Record Recorder.....	(2/2)	<input type="radio"/>	Date
Visual Record Recorder.....	(1/1)	<input type="radio"/>	Date
Calculus Removal Record Recorder.....	(...)	<input type="radio"/>	Date
Brothwell Scoring Record Recorder.....	(1/1)	<input type="radio"/>	Date
Probe Scoring Record Recorder.....	(1/1)	<input type="radio"/>	Date
Analysis Record Recorder.....	(...)	<input type="radio"/>	Date
Photographic Record Recorder.....	(...)	<input type="radio"/>	Date

Collection Name: _____

Skeleton Reference: _____

Initial Dentition Record (1/2)

General

Date _____

Collection Name: _____

Location of Remain: _____

Context Number: _____

Excavation Date: _____

Remains Reference: _____

Sex: M F U

Method of Sex Estimation: _____

Age: Deciduous Adult Both

Method of Age Estimation: _____

State of remains: _____

General - Dentition

Debris Present on Dentition? Y N

Brief Description: _____

Debris Sample Collected? Y N

If 'Y': Sample Type: _____

Sample Reference: _____

Standard Cleaning Method Followed? Y N

Photographs taken? Y N (If Y, complete Photographic Recording Form)

Collection Name: _____

Skeleton Reference: _____

Initial Dentition Record (2/2)

Initial Dentition Record

Date

Teeth Present	UL	UR	LL	LR	
	I1	I1	I1	I1	<input type="checkbox"/> Present in occlusion
	I2	I2	I2	I2	<input type="checkbox"/> Present not in occlusion (no associated alveolar bone)
	C	C	C	C	<input type="checkbox"/> Present not in occlusion (associated alveolar bone)
	PM1	PM1	PM1	PM1	<input type="checkbox"/> Present but unobservable
	PM2	PM2	PM2	PM2	<input type="checkbox"/> Ante-Mortem Tooth Loss
	M1	M1	M1	M1	
	M2	M2	M2	M2	
	M3	M3	M3	M3	

Notes on Presence:

.....

Comments on Wear:

Pathologies Present:

Caries:

- Enamel Cementum Dentine
- Pinprick Small Medium Large

Lesions:

- Periapical Granuloma Radicular Cyst Abscess

Enamel Hypoplasia

Periodontal Disease

Notes on Pathologies:

.....

.....

Collection Name: _____

Skeleton Reference: _____



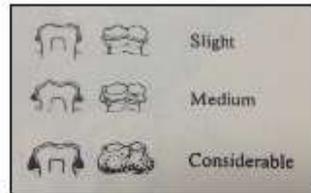
Brothwell Scoring Record (1/1)

Date: _____

Tooth	Lingual	Labial/Buccal	Mesial	Distal
11			-	-
12			-	-
13			-	-
14			-	-
15			-	-
16				
17				
18				
21			-	-
22			-	-
23			-	-
24			-	-
25			-	-
26				
27				
28				
31			-	-
32			-	-
33			-	-
34			-	-
35			-	-
36				
37				
38				
41			-	-
42			-	-
43			-	-
44			-	-
45			-	-
46				
47				
48				

Brothwell Scoring Index

- 0 None
- 1 Slight
- 2 Medium
- 3 Considerable



Notes: _____

Collection Name: _____

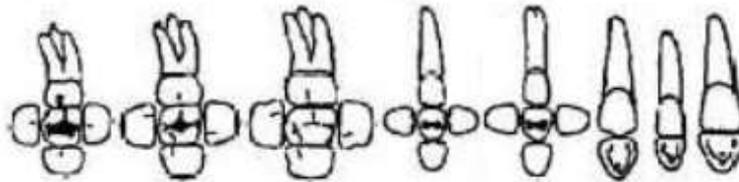
Skeleton Reference: _____



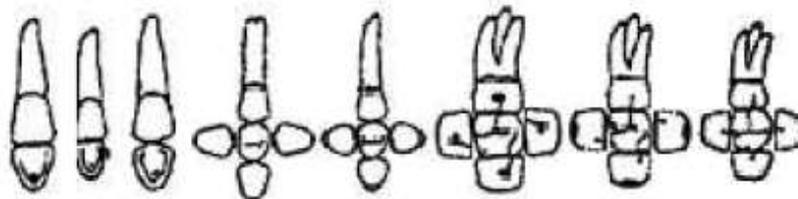
Probe Scoring Record (1/2)

Date: _____

UR	Lingual					Labial														
	A	B	C	Total	Index	A	B	C	Total	Index										
11																				
12																				
13											Mesial			Index		Distal			Index	
14											A	B	C	Total	Index	A	B	C	Total	Index
15																				
16																				
17																				
18																				



UL	Lingual					Labial														
	A	B	C	Total	Index	A	B	C	Total	Index										
21																				
22																				
23											Mesial			Index		Distal			Index	
24											A	B	C	Total	Index	A	B	C	Total	Index
25																				
26																				
27																				
28																				



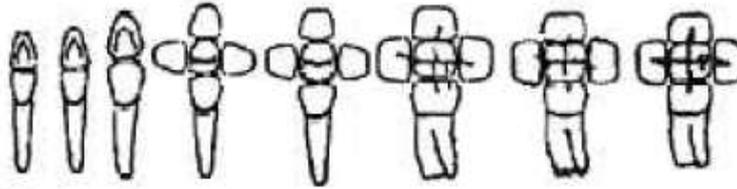
Collection Name: _____

Skeleton Reference: _____

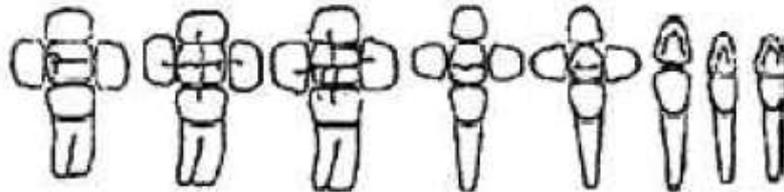


Probe Scoring Record (2/2)

LL	Lingual					Labial														
	A	B	C	Total	Index	A	B	C	Total	Index										
31																				
32																				
33											Mesial			Index		Distal			Index	
34											A	B	C	Total	Index	A	B	C	Total	Index
35																				
36																				
37																				
38																				



LR	Lingual					Labial														
	A	B	C	Total	Index	A	B	C	Total	Index										
41																				
42																				
43											Mesial			Index		Distal			Index	
44											A	B	C	Total	Index	A	B	C	Total	Index
45																				
46																				
47																				
48																				



APPENDIX E: RESULTS

E.1 In-Situ Dental Recording

E.1.1 Dental Pathologies (All Individuals)

		Capuchin Catacombs, Sicily				Cementeri Vell, Formentera				San Agustín, La Rioja			
		M	F	?	T	M	F	?	T	M	F	?	T
<i>Carious Lesions</i>	T _{CL}	42	14	-	56	31	9	77	117	44	-	-	44
	T _{CL} %	5	6	-	5	20	26	14	16	14	-	-	14
<i>Periapical Cavities</i>	P _{PAC}	48	8	-	56	7	1	4	12	26	-	-	26
	P _{PAC} %	2	1	-	2	1	1	*	1	5	-	-	5
<i>Periodontal Disease</i>	P _{PD}	711	282	-	993	170	24	144	338	350	-	-	350
	P _{PD} %	88	93	-	89	99	71	86	91	100	-	-	100

(* percentage < 1)

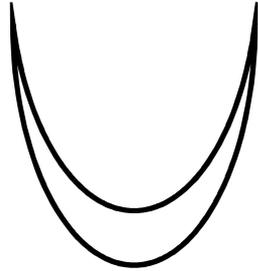
Appx_Table E.1.1 Table showing the number and percentage of teeth or tooth positions affected by each dental pathology for all individuals in each population.

E.2 Physical Characterisation

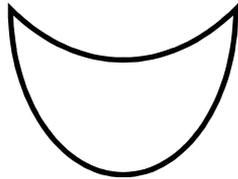
E.2.1 Optical Microscopy

Generalised Deposit Shapes

Crescent:



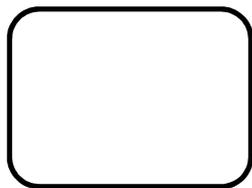
Semi-circular:



Ledge:



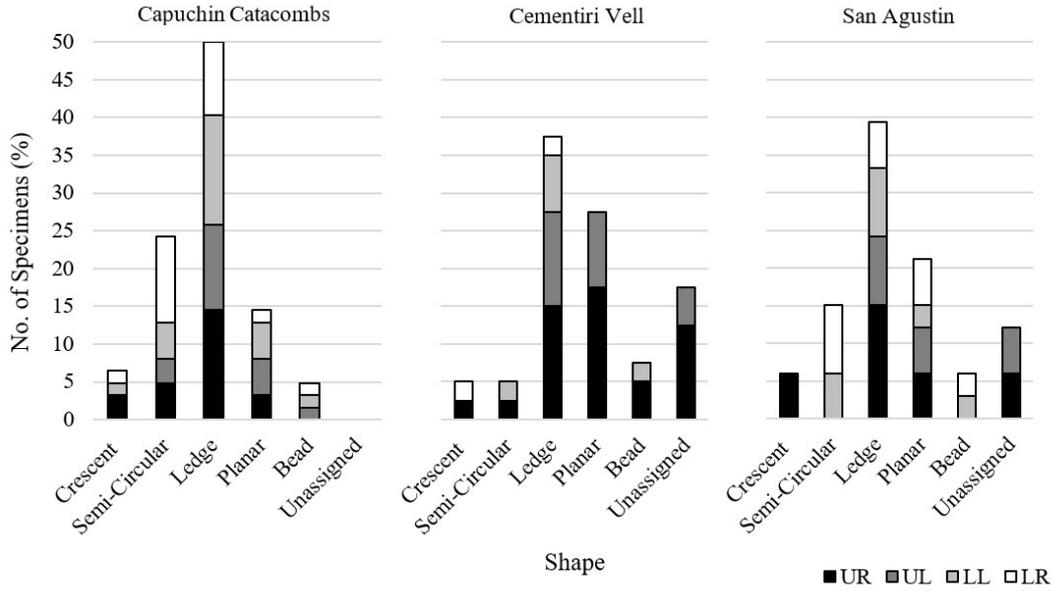
Planar:



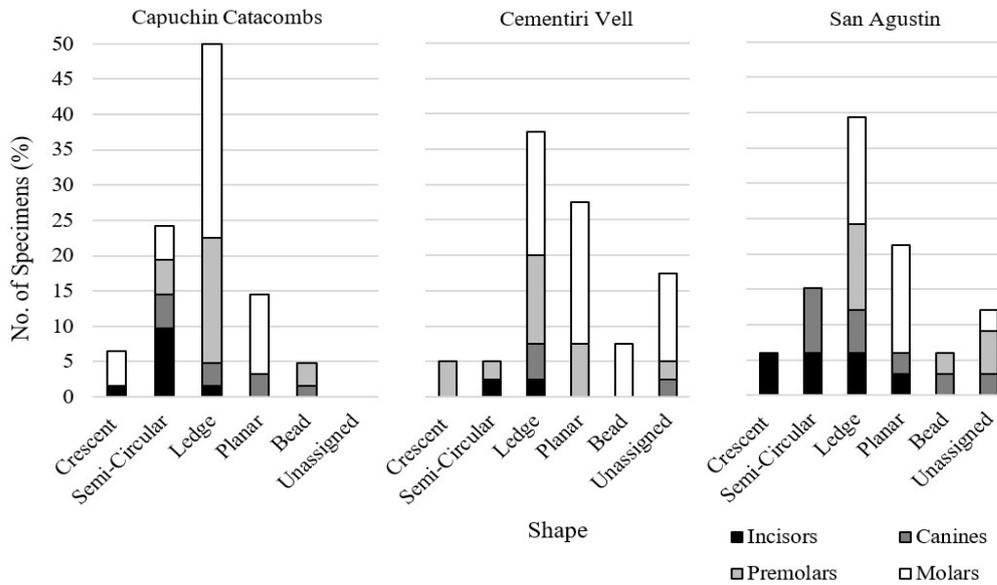
Bead:



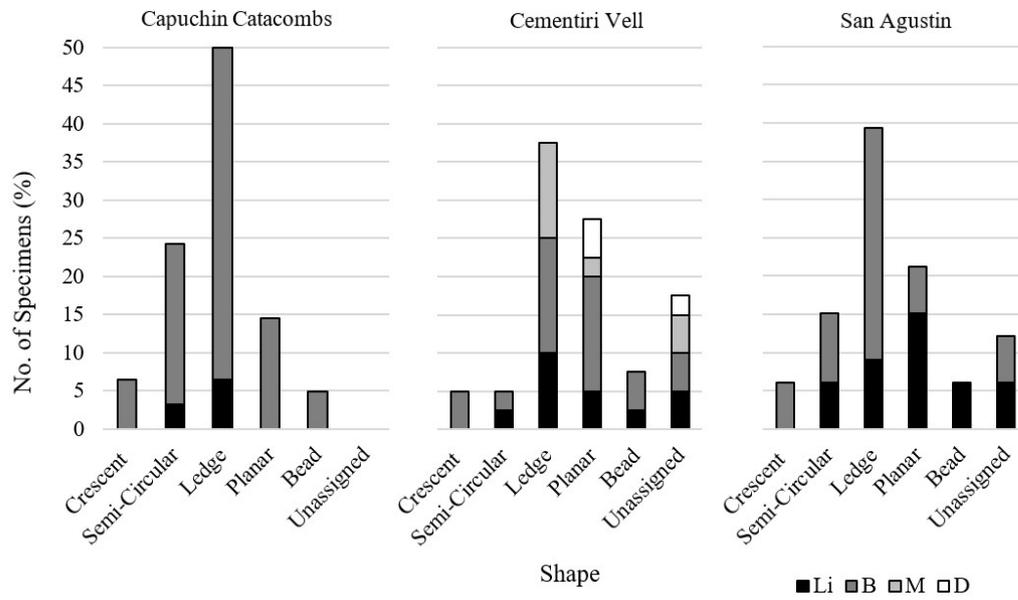
Deposit Shape Distribution



Appx_Figure E.2.1 Graphs showing the percentage distribution of dental calculus shapes per the dental quadrant that the specimen had been sampled from.



Appx_Figure E.2.2 Graphs showing the percentage distribution of dental calculus shapes per the tooth type that the specimen had been sampled from.



Appx_Figure E.2.3 **Graphs showing the percentage distribution of dental calculus shapes per the tooth surface that the specimen had been sampled from.**

E.2.2 Computed Tomography

Voxel Size vs. Type of Density Change

		Capuchin Catacombs, Sicily	Cementiri Vell, Formentera	San Agustín, La Rioja
Overall	<i>N</i>	40	19	14
	Mean	9.2	7.9	8.2
	S.D.	1.9	1.7	1.7
	Min	4.4	5.4	7.0
	Max	14.0	13.2	14.2
Type I	<i>n</i>	21	13	11
	Mean	9.3	7.6	7.6
	S.D.	1.5	1.2	0.4
	Min	5.5	6.1	7.0
	Max	11.5	9.4	8.5
Type II	<i>n</i>	15	3	1
	Mean	8.9	9.2	8.7
	S.D.	2.1	2.9	0.0
	Min	4.4	6.8	8.7
	Max	12.9	13.2	8.7
Type III	<i>n</i>	4	3	2
	Mean	9.7	7.6	11.4
	S.D.	2.6	1.6	2.9
	Min	6.8	5.4	8.5
	Max	14.0	8.8	14.2

Appx_Table E.2.1 Table detailing the descriptive statistics for the voxel size (μm) per specimen in the n-CT data.

ANOVA						
<i>Source of Variation</i>	<i>SS</i>	<i>df</i>	<i>MS</i>	<i>F</i>	<i>P-value</i>	<i>F crit</i>
Between Groups	9.33458	2	4.66729	1.23615	0.29676	3.12768
Within Groups	264.29550	70	3.77565			
Total	273.63007	72				

Appx_Table E.2.2 Table detailing the ANOVA results of comparing the voxel size (μm) for each type of specimen from all populations, where the type of specimen relates to the density changes seen.

Capuchin Catacombs

	Type I	Type II	Type III
Voxel Size for each Specimen (µm)	11.5	10.7	9.4
	10.7	10.6	8.8
	10.7	12.9	6.8
	10.7	5.8	14.0
	10.7	8.1	
	9.9	8.1	
	9.9	8.5	
	9.8	7.3	
	5.5	7.3	
	8.5	4.4	
	9.7	10.4	
	7.3	10.4	
	11.2	9.2	
	9.1	10.9	
	7.6	8.5	
	7.6		
	9.1		
	9.5		
	7.6		
	10.9		
7.7			

Appx_Table E.2.3 Table showing the n-CT data voxel size (µm) for each specimen in the Capuchin Catacombs sample, as per the type of density changes.

ANOVA						
Source of Variation	SS	df	MS	F	P-value	F crit
Between Groups	2.96474	2	1.48237	0.37690	0.68859	3.25192
Within Groups	145.52357	37	3.93307			
Total	148.48832	39				

Appx_Table E.2.4 Table detailing the ANOVA results of comparing the voxel size (µm) for each type of specimen from the Capuchin Catacomb sample, where the type of specimen relates to the density changes seen.

Cementerri Vell

	Type I	Type II	Type III
Voxel Size for each Specimen (µm)	6.8	6.8	5.4
	6.1	13.2	8.7
	7.3	7.6	8.8
	7.3		
	8.3		
	9.4		
	9.4		
	9.4		
	8.1		
	6.9		
	6.9		
	6.7		
	6.2		

Appx_Table E.2.5 Table showing the n-CT data voxel size (µm) for each specimen in the Cementerri Vell sample, as per the type of density changes.

ANOVA						
<i>Source of Variation</i>	<i>SS</i>	<i>df</i>	<i>MS</i>	<i>F</i>	<i>P-value</i>	<i>F crit</i>
Between Groups	6.37042	2	3.18521	1.02551	0.38102	3.63372
Within Groups	49.69575	16	3.10598			
Total	56.06617	18				

Appx_Table E.2.6 Table detailing the ANOVA results of comparing the voxel size (µm) for each type of specimen from the Cementerri Vell sample, where the type of specimen relates to the density changes seen.

San Agustín

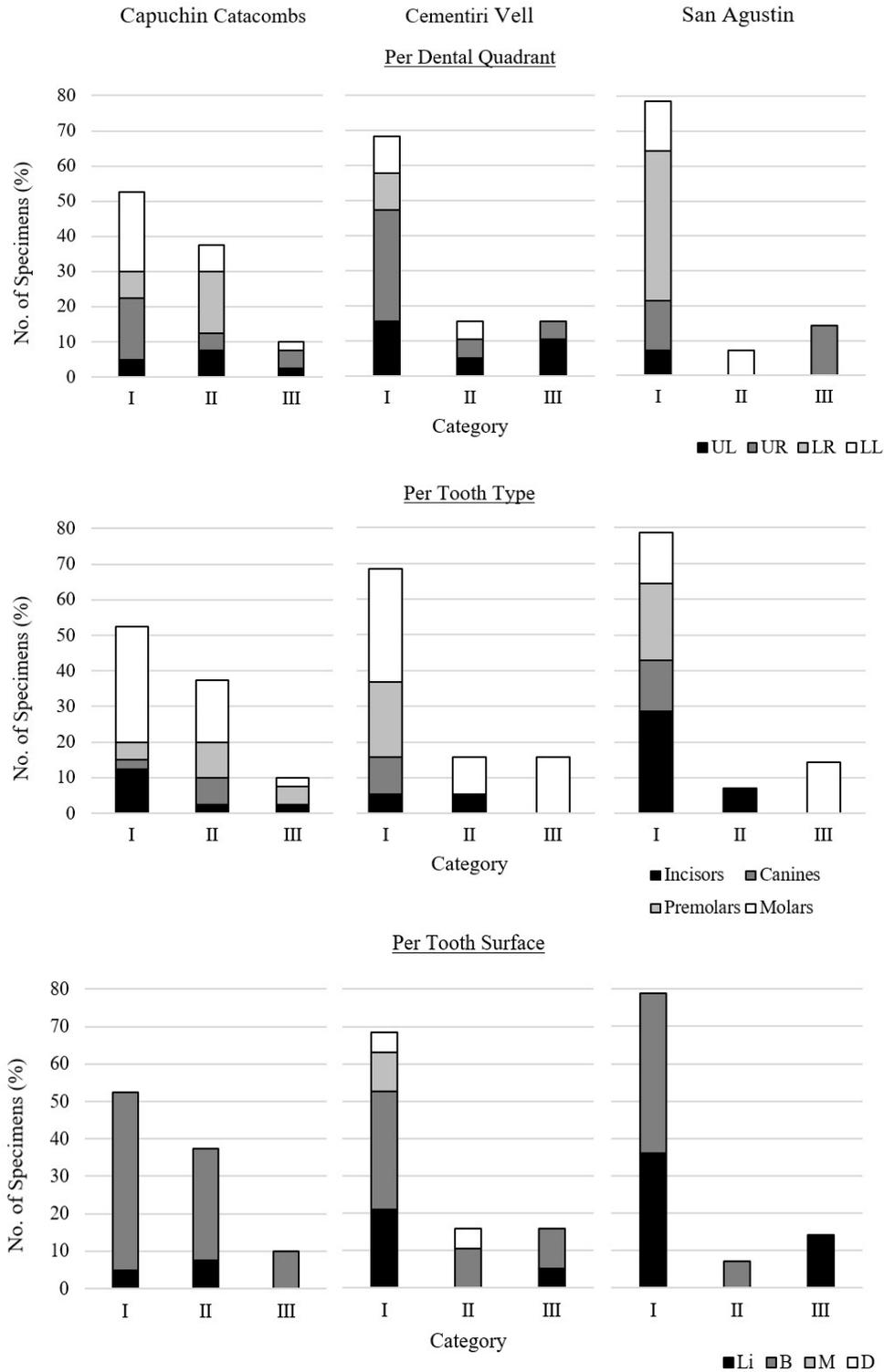
	Type I	Type II	Type III
Voxel Size for each Specimen (µm)	8.5	8.7	8.5
	7.4		14.2
	7.4		
	7.4		
	7.6		
	7.6		
	7.0		
	7.9		
	7.9		
	7.2		
	7.2		

Appx_Table E.2.7 Table showing the n-CT data voxel size (µm) for each specimen in the San Agustín sample, as per the type of density changes.

ANOVA						
Source of Variation	SS	df	MS	F	P-value	F crit
Between Groups	24.72975	2	12.36487	7.55211	0.00862	3.98230
Within Groups	18.01001	11	1.63727			
Total	42.73976	13				

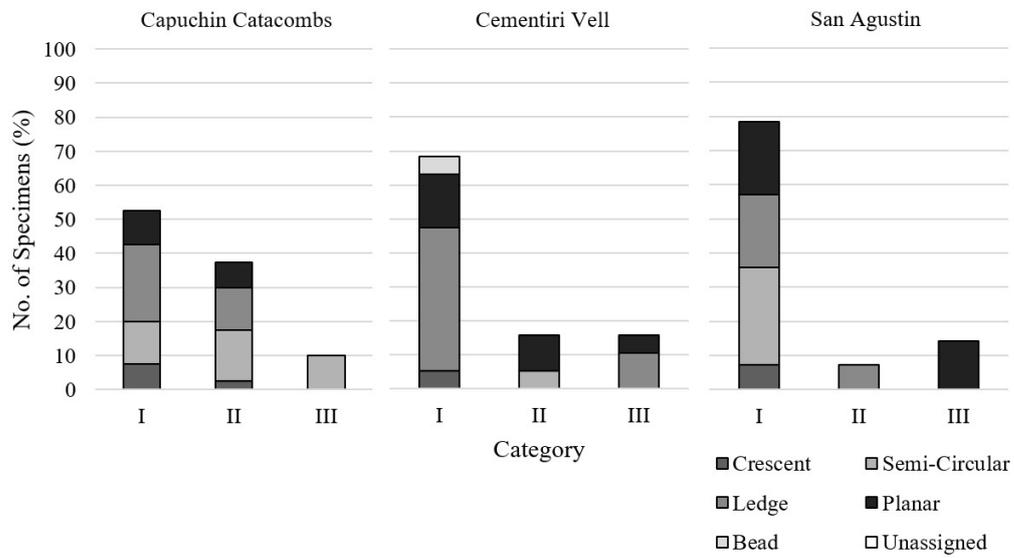
Appx_Table E.2.8 Table detailing the ANOVA results of comparing the voxel size (µm) for each type of specimen from the San Agustín sample, where the type of specimen relates to the density changes seen. N.B. a significant statistical difference was seen for this population, however it was deemed unreliable due to the extraordinarily large sample that was analysed and determined as a type III specimen).

Type of Density Change vs. Dental Location



Appx_Figure E.2.4 Graphs showing the type of specimen assigned to each density change category, according to dental quadrant (top), tooth type (middle), tooth surface (bottom).

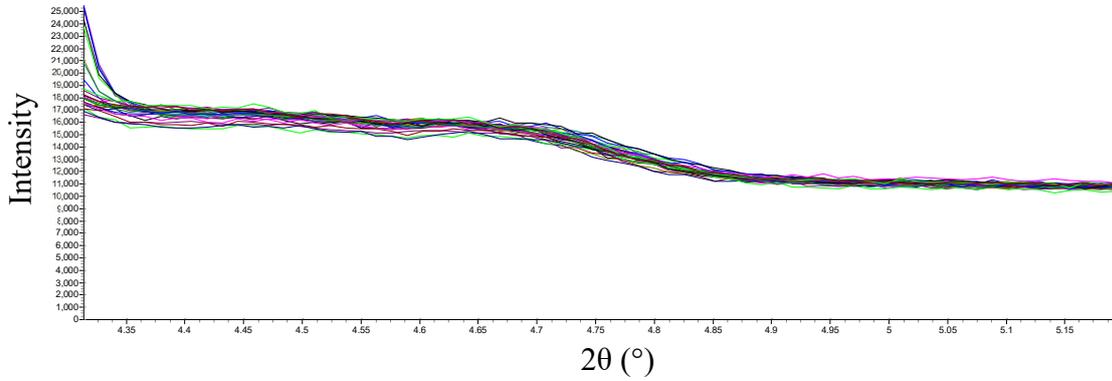
Type of Density Change vs. Deposit Shape



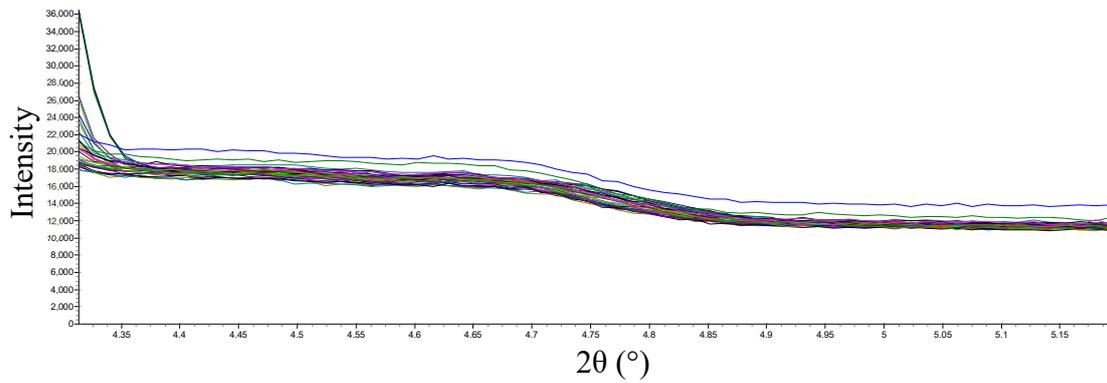
Appx_Figure E.2.5 Graph showing the type of specimen assigned to each density change category, according to deposit shape as determined in the optical microscopy analysis.

E.3 Mineralogical Analysis

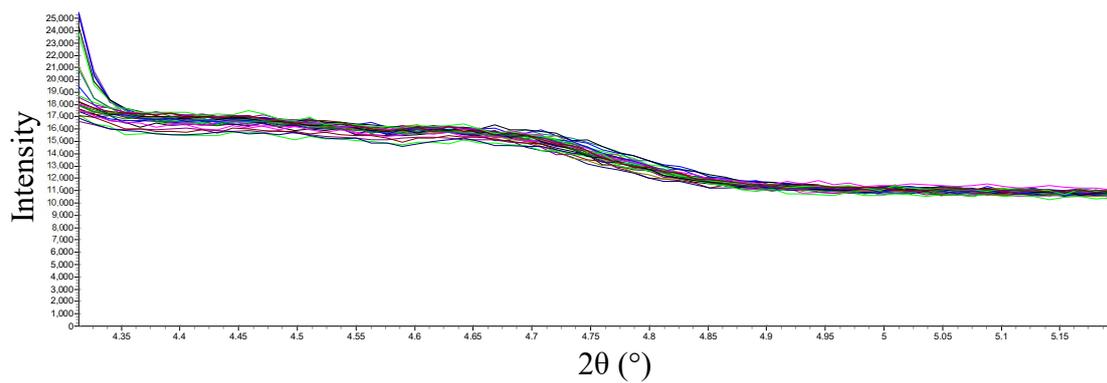
E.3.1 OCP Diffractograms



Appx_Figure E.3.1 The stacked diffractograms for the range (4.2–5.5°) of analysis for Capuchin Catacombs specimens showing no OCP peaks present.



Appx_Figure E.3.2 The stacked diffractograms for the range (4.2–5.5°) of analysis for the Cementeri Vell specimens showing no OCP peaks present.



Appx_Figure E.3.3 The stacked diffractograms for the range (4.2–5.5°) of analysis for the San Agustín specimens showing no OCP peaks present.

E.3.2 Mineral Percentages (All Phases)

Capuchin Catacombs

Individual	Jaw	Tooth	Surface	Hydroxyapatite (%)	Whitlockite (%)	Calcite (%)	Quartz (%)
F15	LL	M1	B	88.4	10.7	0.9	/
F16	LL	M3	B	59.7	39.7	0.7	/
F25	LR	I1	B	85.3	14.7	/	/
F27	UR	PM2	B	74.2	25.8	/	/
F54	LL	M2	B	63.8	30.0	4.5	1.6
N1126	LL	PM1	B	86.9	13.1	/	/
N1138	LR	PM1	La	70.3	29.7	/	/
M193	UL	C	La	74.5	25.5	/	/
M203	UR	PM1	B	85.8	14.1	/	/
M207	UL	PM1	B	71.7	27.0	1.3	/
M213	UR	M2	B	51.0	44.6	3.9	0.5
P318	UL	M1	B	71.6	25.9	2.5	/
P321	LR	M1	B	60.2	39.8	/	/
P323	UR	PM2	B	90.5	9.5	/	/
P328	LR	C	La	85.0	14.1	0.9	/
S291	UR	C	La	81.4	18.6	/	/
US01	UR	I2	La	84.0	15.3	/	0.7
US31	UL	M1	B	68.0	32.0	/	/
UM02	LL	M1	B	83.4	16.6	/	/
UM04	UR	M1	B	85.6	14.4	/	/

(/ = phase not present; * = phase present below (0.5%))

Appx_Table E.3.1 Table detailing the mineral percentages of each specimen analysed from the Capuchin Catacombs sample using pXRD (the specimens from the two female specimens are indicated by the Nxxx code).

Cementeri Vell

Individual	Jaw	Tooth	Surface	Hydroxyapatite (%)	Whitlockite (%)	Calcite (%)	Quartz (%)
BB02	UL	M3	B	91.9	8.1	/	*
BB02	UL	M2	B	76.2	23.0	/	0.7
BB03	UR	C	Li	76.0	24.0	/	/
BB04	LL	I2	La	61.4	35.4	2.3	0.9
BB04	LL	I2	Li	88.6	11.4	/	/
4D101	UR	PM2	B	84.0	15.3	/	0.8
4D101	UR	M1	B	62.5	37.5	/	/
4D102	UR	PM1	B	62.6	18.6	17.7	1.1
4D102	UL	PM1	M	73.3	26.7	/	*
4D02	UL	M1	M	72.4	26.7	0.9	/
4B01	UR	PM1	B	80.9	15.0	3.4	0.8
4B02	LR	PM2	B	70.4	29.2	/	*
4B02	LR	M1	B	54.6	43.2	1.8	*
4B03	LL	M1	B	76.4	23.6	/	*
4B04	LL	M1	B	87.8	9.4	2.3	*
2C01	UL	M1	D	56.8	43.2	/	/
2C02	UR	M1	Li	38.4	60.5	0.6	0.6
2C02	UR	M1	B	73.9	20.9	4.1	1.1
2B02	UL	M1	Li	54.0	46.0	/	/
US01	UL	C	M	68.0	30.6	1.4	*
US02	UR	PM1	M	66.5	18.8	13.7	1.0
US02	UL	PM1	M	68.0	31.3	0.6	/
US02	UR	PM2	B	73.0	24.9	1.8	*
US05	UL	M1	Li	82.2	17.8	/	*
US05	UL	M3	Li	83.0	11.7	2.8	2.5
US06	UR	PM1	Li	79.1	18.6	1.6	0.7
US10	UR	PM2	B	78.4	18.9	1.5	1.2
US12	UR	M1	M	62.7	34.1	2.1	1.1

(/ = phase not present; * = phase present below (0.5%))

Appx_Table E.3.2 Table detailing the mineral percentages of each specimen analysed from the Cementeri Vell sample using pXRD.

The Physical Characterisation and Composition of Archaeological Dental Calculus

San Agustín

Individual	Jaw	Tooth	Surface	Hydroxyapatite (%)	Whitlockite (%)	Calcite (%)	Quartz (%)
01	UR	M2	Li	86.6	13.4	/	/
04	UR	M3	Li	72.1	23.6	1.7	2.6
08	UR	M1	B	86.2	11.8	1.9	/
10	UL	M3	Li	37.5	62.5	/	/
12	LR	M2	Li	66.7	33.3	/	/
16	UR	C	La	76.2	22.4	1.4	/
20	UR	M2	B	80.1	19.2	0.7	/
20	UR	PM2	B	87.7	12.3	/	/
39	LR	C	Li	90.6	9.4	/	/
39	UL	PM1	Li	81.4	17.9	/	0.6
39	UL	C	Li	88.5	11.5	/	/
39	LL	C	Li	95.8	4.2	/	/
42	UL	M2	Li	70.8	28.6	/	0.6
43	LL	M1	B	88.4	11.6	/	/
46	UL	PM1	B	87.0	11.0	2.0	/
46	UR	I2	La	84.9	13.7	0.9	0.5
46	LR	M1	B	66.3	33.7	/	/
46	LL	PM2	Li	87.3	11.7	0.9	/
46	LL	PM2	B	70.2	28.8	1.0	/
46	LL	PM1	B	86.8	13.2	/	/
46	LR	PM1	Li	80.0	19.0	1.0	/
50	UR	I1	La	93.4	6.6	/	/
50	UR	C	La	94.0	6.0	/	/
50	UR	I2	La	91.7	7.8	/	*
50	LR	PM1	B	91.0	9.0	/	/
56	LL	I2	La	88.9	8.9	2.2	/
56	LL	I1	Li	88.4	11.6	/	/
56	LR	I1	La	85.7	14.3	/	/
56	LR	I2	La	89.3	10.7	/	/

(/ = phase not present; * = phase present below (0.5%))

Appx_Table E.3.3 Table detailing the mineral percentages of each specimen analysed from the San Agustín sample using pXRD.

E.3.3 Mineral Percentages (Major Phases).

Capuchin Catacombs

Individual	Jaw	Tooth	Surface	Calculus Score (Brothwell (1981))	%T_Calculus	%T_Caries	%P_PAC	Periodontal Disease	Mass (g)	Hydroxyapatite (%)	Whitlockite (%)	HAp:WHT
F15	LL	M1	B	1	100	0	4	100	0.0015	88	11	8.3
F16	LL	M3	B	1	63	13	3	100	0.0027	60	40	1.5
F25	LR	I1	B	2	30	0	0	100	0.0010	85	15	5.8
F27	UR	PM2	B	1	63	13	5	100	0.0005	74	26	2.9
F54	LL	M2	B	1	40	40	0	100	0.0014	64	30	2.1
N1126	LR	I1	La	2	7	0	0	100	0.0010	87	13	6.6
N1138	LR	PM1	Li	2	58	0	0	100	0.0019	70	30	2.4
M193	UL	C	La	1	60	7	0	100	0.0021	75	25	2.9
M203	UR	PM1	B	3	33	0	0	100	0.0023	86	14	6.1
M207	UL	PM1	B	1	75	25	6	100	0.0018	72	27	2.7
M213	UR	M2	B	1	18	0	0	100	0.0033	51	45	1.1
P318	UL	M1	B	3	80	0	0	100	0.0029	72	26	2.8
P321	LR	M1	B	1	63	26	3	100	0.0012	60	40	1.5
P323	UR	PM2	B	1	71	0	0	100	0.0019	90	10	9.5
P328	LR	C	La	1	19	5	0	100	0.0014	85	14	6.0
S291	UR	C	La	1	50	0	0	100	0.0013	81	19	4.4
US01	UR	M2	B	2	100	6	6	100	0.0035	84	15	5.5
US31	UL	M1	B	2	50	0	6	0	0.0010	68	32	2.1
UM02	LL	M1	B	1	100	0	6	0	0.0024	83	17	5.0
UM04	LR	M2	B	2	100	0	0	0	0.0238	86	14	5.9

(%T_Calculus = percentage of teeth with calculus in individual; %T_Caries = percentage of teeth with calculus in individual; %P_PAC = percentage of alveolar positions with periapical cavities)

Appx_Table E.3.4 Table detailing the major mineral percentages of each specimen analysed from the Capuchin Catacombs sample. Also included are the pathologies recorded for the individual from which calculus was removed (the specimens from the two female specimens are indicated by the Nxxx code).

The Physical Characterisation and Composition of Archaeological Dental Calculus

Cementeri Vell

Individual	Jaw	Tooth	Surface	Calculus Score (Brothwell (1981))	%T_Calculus	%T_Caries	%P_PAC	Periodontal Disease	Weight	Hydroxyapatite (%)	Whitlockite (%)	HAp:WHT
BB02	UL	M3	B	2	100	0	/	/	0.007	92	8	11.4
BB02	UL	M2	B	2	100	0	/	/	0.0458	76	23	3.3
BB03	UR	C	Li	2	100	0	/	/	0.0122	76	24	3.2
BB04	LL	I2	La	3	100	0	/	/	0.0152	61	35	1.7
BB04	LL	I2	Li	3	100	0	/	/	0.003	89	11	7.8
4D101	UR	PM2	B	1	90	10	0	100	0.0114	84	15	5.5
4D101	UR	M1	B	2	90	10	0	100	0.0191	62	38	1.7
4D102	UR	PM1	B	1	64	0	0	100	0.001	63	19	3.4
4D102	UL	PM1	M	1	64	0	0	100	0.0013	73	27	2.7
4D02	UL	M1	M	1	100	0	/	/	0.0011	72	27	2.7
4B01	UR	PM1	B	2	20	25	17	100	0.0076	81	15	5.4
4B02	LR	PM2	B	1	75	25	0	100	0.002	70	29	2.4
4B02	LR	M1	B	1	75	25	0	100	0.0168	55	43	1.3
4B03	LL	M1	B	1	100	0	/	/	0.001	76	24	3.2
4B04	LL	M1	B	1	100	0	/	/	0.003	88	9	9.3
2C01	UL	M1	D	2	50	0	6	100	0.0316	57	43	1.3
2C02	UR	M1	Li	1	100	0	0	100	0.0027	38	60	0.6
2C02	UR	M1	B	1	100	0	0	100	0.054	74	21	3.5
2B02	UL	M1	Li	1	100	100	/	/	0.0076	54	46	1.2
US01	UL	C	M	1	89	11	0	100	0.003	68	31	2.2
US02	UR	PM1	M	2	88	25	0	100	0.0029	66	19	3.5
US02	UL	PM1	M	2	88	25	0	100	0.005	68	31	2.2
US02	UR	PM2	B	1	88	25	0	100	0.0011	73	25	2.9
US05	UL	M1	Li	1	89	11	13	100	0.0029	82	18	4.6
US05	UL	M3	Li	1	89	11	13	100	0.002	83	12	7.1
US06	UR	PM1	Li	1	100	0	0	100	0.0022	79	19	4.3
US10	UR	PM2	B	1	100	0	/	/	0.0018	78	19	4.1
US12	UR	M1	M	1	100	100	/	/	0.0013	63	34	1.8

(%T_Calculus = percentage of teeth with calculus in individual; %T_Caries = percentage of teeth with calculus in individual; %P_PAC = percentage of alveolar positions with periapical cavities)

Appx_Table E.3.5 Table detailing the major mineral percentages of each specimen analysed from the Cementeri Vell sample. Also included are the pathologies recorded for the individual from which calculus was removed (the specimens with a strike-through in their pathological information were loose teeth and as such had no associated alveolar bone to observe periapical cavities or periodontal disease).

San Agustín

Individual	Jaw	Tooth	Surface	Calculus Score (Brothwell (1981))	%T_Calculus	%T_Caries	%P_PAC	Periodontal Disease	Weight	Hydroxyapatite (%)	Whitlockite (%)	HAp:WHT
01	UR	M2	Li	3	21	25	9	100	0.1799	87	13	6.5
04	UR	M3	Li	1	25	33	0	100	0.0013	72	24	3.1
08	UR	M1	Li	1	14	14	0	100	0.002	86	12	7.3
10	UL	M3	Li	1	33	33	13	100	0.0019	38	62	0.6
12	LR	M2	Li	2	44	11	0	100	0.0075	67	33	2.0
16	LR	C	Li	3	80	0	0	100	0.0022	76	22	3.4
20	UR	M2	B	2	50	25	0	100	0.0052	80	19	4.2
20	UR	PM2	B	2	50	25	0	100	0.0029	88	12	7.1
39	LR	C	Li	1	43	2	7	100	0.0039	91	9	9.7
39	UL	PM1	Li	1	43	2	7	100	0.0039	81	18	4.5
39	UL	C	Li	1	43	2	7	100	0.0009	89	11	7.7
39	LL	C	Li	1	43	2	7	100	0.0095	96	4	22.6
42	UL	M2	Li	2	42	0	0	100	0.0022	71	29	2.5
43	LL	M1	B	2	50	1	0	100	0.006	88	12	7.6
46	UR	PM1	B	1	72	2	0	100	0.0034	87	11	7.9
46	LR	I2	La	1	72	2	0	100	0.004	85	14	6.2
46	LR	M1	B	1	72	2	0	100	0.0038	66	34	2.0
46	LL	PM2	Li	1	72	2	0	100	0.0054	87	12	7.4
46	LL	PM2	B	1	72	2	0	100	0.0054	70	29	2.4
46	LL	PM1	B	1	72	2	0	100	0.0078	87	13	6.6
46	LR	PM1	La	1	72	2	0	100	0.123	80	19	4.2
50	UR	I1	La	1	32	1	3	100	0.0026	93	7	14.3
50	UR	C	La	1	32	1	3	100	0.0039	94	6	15.6
50	UR	I2	La	1	32	1	3	100	0.0032	92	8	11.7
50	LR	PM1	B	1	32	1	3	100	0.0042	91	9	10.1
56	UR	I2	Li	3	36	0	3	100	0.0032	89	9	10.0
56	LL	I1	Li	3	36	0	3	100	0.0038	88	12	7.6
56	LR	I1	La	3	36	0	3	100	0.0156	86	14	6.0
56	LR	I2	La	3	36	0	3	100	0.007	89	11	8.4

(%T_Calculus = percentage of teeth with calculus in individual; %T_Caries = percentage of teeth with calculus in individual; %P_PAC = percentage of alveolar positions with periapical cavities)

Appx_Table E.3.6 Table detailing the major mineral percentages of each specimen analysed from the San Agustín sample. Also included are the pathologies recorded for the individual from which calculus was removed.

E.3.4 Whitlockite Analysis

In all populations, there was a small difference between the percentages of WHT in specimens from the upper and lower jaws. Additionally, the mean percentage of lower jaw specimens was slightly lower than upper jaw specimens for all populations. These differences were found to not be significant followed 2-tailed t-tests of the upper and lower percentages in each population (Appx_Table E.3.7). Further division of the specimens from each jaw into the side of the jaw that they were sampled from showed there no significant differences between right and left specimens (Appx_Table E.3.7).

		Capuchin Catacombs, Sicily		Cementerí Vell, Formentera		San Agustín, La Rioja							
		<i>By Jaw</i>											
Jaw		Upper	Lower	Upper	Lower	Upper	Lower						
No. of Specimens		10	10	22	6	14	15						
WHT (%)	Mean (%)	24	22	26	25	17	16						
	St. Dev.	10	11	12	12	14	9						
	Min (%)	10	11	8	9	6	4						
	Max (%)	45	40	60	43	62	34						
F-test		1.26		1.16		2.57							
F Critical		3.18		2.68		2.51							
t-test		0.75		0.91		0.87							
		<i>By Dental Quadrant</i>											
Dental Quadrant		UR	UL	LL	LR	UR	UL	LL	LR	UR	UL	LL	LR
No. of Specimens		6	4	4	6	12	10	4	2	10	4	6	9
WHT (%)	Mean (%)	21	28	24	21	26	27	20	36	12	30	14	18
	St. Dev.	12	3	11	10	12	12	10	7	5	20	7	10
	Min (%)	10	25	11	13	15	8	9	29	6	11	4	9
	Max (%)	45	32	40	40	60	46	35	43	24	62	29	34
F-test		17.89		1.37		1.13		1.49		16.50		1.40	
F Critical		9.01		5.41		3.10		215.71		3.86		4.82	
t-test		0.29		0.68		0.87		0.18		0.21		0.33	

(UR = Upper Right; UL = Upper Left; LL = Lower Left; LR = Lower Right)

* Significant result ($p \leq 0.05$)

Appx_Table E.3.7 Table comparing the descriptive statistics of whitlockite in each jaw and the dental quadrant of each jaw for specimens analysed by powder X-ray diffraction for each population. Also shown are the results of the F-tests, indicating the type of variance for each pairing and the 2-tailed t-test for significance ($p \leq 0.05$).

In addition to statistically testing for whitlockite differences in each jaw, upper and low, the overall differences in the side from which the specimens were sampled are detailed in Appx_Table E.3.8. Unlike for hydroxyapatite, not all populations, had a higher percentage of whitlockite found in specimens of the same side. In the Capuchin Catacombs and San Agustín, the left-hand specimens had higher percentages of whitlockite than the right hand-side. Despite this difference, none of the populations were found to have statistically significant differences between left and right (Appx_Table E.3.8). There were also no statistically significant differences between the upper and lower jaws for the left and right sides of the mouth, in any population (Appx_Table E.3.8).

		Capuchin Catacombs, Sicily		Cementerí Vell, Formentera				San Agustín, La Rioja					
		<i>By Side</i>											
		Left		Right		Left		Right		Left		Right	
No. of Specimens		8		12		14		14		10		19	
WHT (%)	Mean (%)	26		21		25		27		20		15	
	St. Dev.	8		11		12		12		16		8	
	Min (%)	11		10		8		15		4		6	
	Max (%)	40		45		46		60		62		34	
F-test		1.61				1.13				4.19			
F Critical		3.60				2.58				2.46			
t-test		0.33				0.61				0.38			
		<i>By Dental Quadrant (per Side)</i>											
		UL	LL	LR	UR	UL	LL	LR	UR	UL	LL	LR	UR
No. of Specimens		4	4	6	6	10	4	2	12	4	6	9	10
WHT (%)	Mean (%)	28	24	21	21	27	20	36	26	30	14	18	12
	St. Dev.	3	11	10	12	12	10	7	12	20	7	9	5
	Min (%)	25	11	13	10	8	9	29	15	11	4	9	6
	Max (%)	32	40	40	45	46	35	43	60	62	29	34	24
F-test		19.18		1.28		1.02		1.73		7.79		2.97	
F Critical		9.28		5.05		8.81		242.98		5.41		3.23	
t-test		0.65		0.33		0.38		0.30		0.23		0.09	

(UR = Upper Right; UL = Upper Left; LL = Lower Left; LR = Lower Right)

* Significant result ($p \leq 0.05$)

Appx_Table E.3.8 Table comparing the descriptive statistics of whitlockite in each jaw and the dental quadrant of each side for specimens analysed by powder X-ray diffraction for each population. Also shown are the results of the F-tests, indicating the type of variance for each pairing and the 2-tailed t-test for significance ($p \leq 0.05$).

In all populations, there was a higher percentage of whitlockite found in posterior teeth compared to anterior teeth, however in Cementeri Vell this difference was only slight

The Physical Characterisation and Composition of Archaeological Dental Calculus

(Appx_Table E.3.9). When statistically tested with 2-tailed t-tests, this difference was found to be significant (with a confidence level of 95%) only for and the San Agustín specimens (Appx_Table E.3.9). Within both the posterior and anterior categories of teeth, no significant difference was found either between incisor and canine teeth or premolar and molar teeth (Appx_Table E.3.9).

		Capuchin Catacombs, Sicily				Cementerí Vell, Formentera				San Agustín, La Rioja			
		<i>By Tooth</i>											
		Anterior		Posterior		Anterior		Posterior		Anterior		Posterior	
No. of Specimens		5		15		4		24		12		17	
WHT (%)	Mean (%)	17		25		25		26		11		21	
	St. Dev.	5		11		9		13		5		13	
	Min (%)	13		10		11		8		4		9	
	Max (%)	25		45		35		60		22		62	
F-test		4.85				1.51				7.78			
F Critical		5.87				8.64				2.70			
t-test		0.15				0.93				0.01*			
		<i>By Tooth Type</i>											
		I	C	P	M	I	C	P	M	I	C	P	M
No. of Specimens		2	3	5	10	2	2	10	14	7	5	8	9
WHT (%)	Mean (%)	14	19	21	27	23	27	22	29	11	11	15	26
	St. Dev.	1	5	8	12	12	3	6	15	3	6	6	15
	Min (%)	13	14	10	11	11	24	15	8	7	4	9	12
	Max (%)	15	25	30	45	35	31	31	60	14	22	29	62
F-test		26.06		1.89		13.28		7.18		5.96		6.31	
F Critical		199.50		6.00		161.45		3.05		4.53		3.73	
t-test		0.29		0.38		0.78		0.13		0.96		0.08	

(I = Incisors; C = Canines; P = Premolars; M = Molars)

* Significant result ($p \leq 0.05$)

Appx_Table E.3.9 Table comparing the descriptive statistics of whitlockite between tooth positions and tooth types of specimens analysed by powder X-ray diffraction for each population. Also shown are the results of the F-tests, indicating the type of variance for each pairing and the 2-tailed t-test for significance ($p \leq 0.05$).

In addition to the dental location, the pathological environment that the calculus specimens were recorded as being in, was also compared in terms of the whitlockite percentage content. The specimens were grouped into whether the individual, from which the specimen was taken, was recorded as having one or more carious lesion or periapical cavities, both pathologies or neither (Appx_Table E.3.10). From ANOVA, statistical testing of the four possible groups described above, there were no differences in whitlockite percentage. This was the case in all populations (Appx_Table E.3.10)

As with the hydroxyapatite percentages, the whitlockite in specimens was not analysed in terms of the sex of the individual from which it was from. This is because only two specimens from the Capuchin Catacombs were from female individuals. Neither of these specimens have a percentage of whitlockite that is noticeably different to the rest of the Capuchin Catacombs specimens (see Appendix E.3.2 and E.3.3).

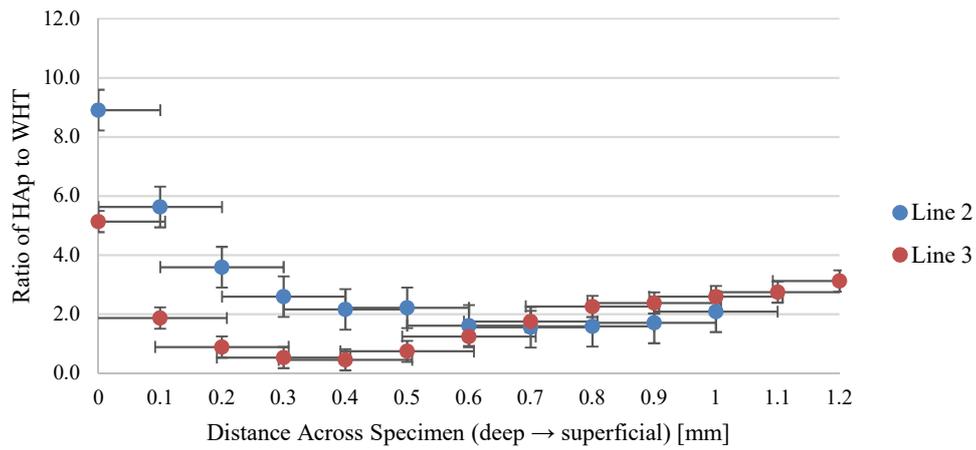
		Capuchin Catacombs, Sicily				Cementerí Vell, Formentera				San Agustín, La Rioja			
		<i>By Pathology Present</i>											
		No CL+ No P _{AC}	No CL+ P _{AC}	CL+ No P _{AC}	CL+ P _{AC}	No CL+ No P _{AC}	No CL+ P _{AC}	CL+ No P _{AC}	CL+ P _{AC}	No CL+ No P _{AC}	No CL+ P _{AC}	CL+ No P _{AC}	CL+ P _{AC}
No. of Specimens		9	3	3	5	14	1	10	3	2	4	13	10
WHT (%)	Mean (%)	21	20	23	30	23	43	31	15	25	11	19	15
	St. Dev.	10	9	7	9	12	0	9	2	3	2	8	16
	Min (%)	10	11	14	15	8	43	15	12	22	9	11	4
	Max (%)	45	32	30	40	60	43	46	18	29	14	34	62
F-test		0.89				2.69				0.83			
F Critical		3.24				3.01				2.99			
t-test		0.47				0.07				0.49			

(CL = carious lesion; P_{AC} = periapical cavity)

* Significant result ($p \leq 0.05$)

Appx_Table E.3.10 Table comparing the descriptive statistics of whitlockite between specimens from individuals recorded with/without carious lesions and/or periapical cavities on their dentition. Also shown are the results of the F-tests, indicating the type of variance for each pairing and the 2-tailed t-test for significance ($p \leq 0.05$).

E.3.5 Cross-sectional Mineral Analysis



Appx_Figure E.3.4 Graph showing the decrease in HAp:WHT from the deep surface of the deposit towards the centre and the subsequent ratio increase from the centre to the superficial surface with error bars included.

E.4 Elemental Analysis

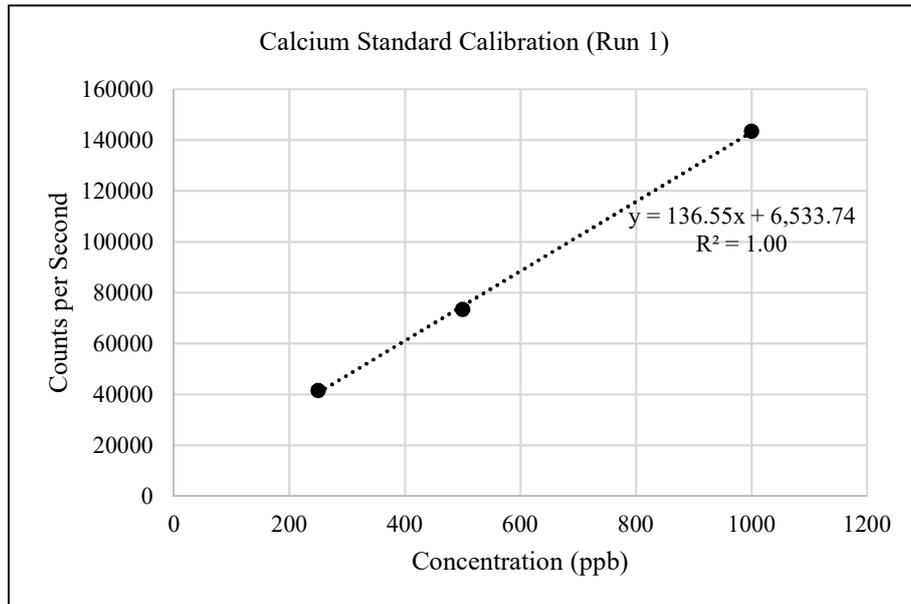
E.4.1 Inductively-Coupled Plasma Mass Spectrometry (Solution) Analysis

Dilution Factors

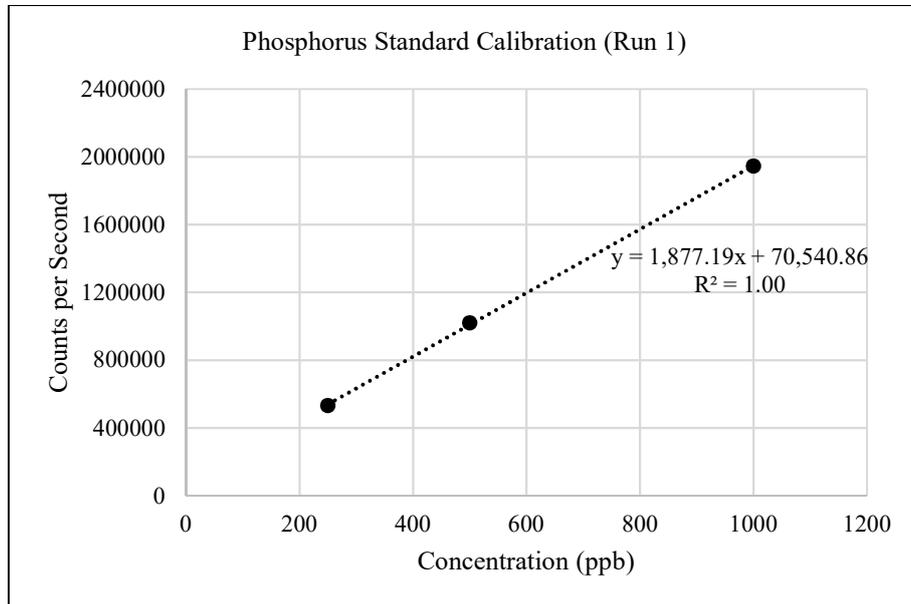
	<i>Specimen</i>	<i>Stock Volume (ml)</i>	<i>Nitric Acid (1%) (ml)</i>	<i>Ga (1 ppm) in nitric acid (1%) (ml)</i>	<i>Dilution Factor (D_f)</i>
<i>Capuchin Catacombs, Sicily</i>	F15	5.000	4.900	0.1	200
	F54	5.000	4.900	0.1	200
	P318	2.500	7.400	0.1	400
	P321	5.000	4.900	0.1	200
	M213	2.500	7.400	0.1	400
	US01	1.000	8.900	0.1	1000
	US31	5.000	4.900	0.1	200
	US32	5.000	4.900	0.1	200
	UM02	1.250	8.650	0.1	800
	UM04	0.385	9.515	0.1	2600
<i>Cementerí Vell, Formentera</i>	BB02	0.125	9.775	0.1	8000
	2C01	0.125	9.775	0.1	8000
	2C02	2.500	7.400	0.1	400
	4B01	0.500	9.400	0.1	2000
	4D01	1.000	8.900	0.1	1000
	4D101_1	1.000	8.900	0.1	1000
	4D101_2	0.166	9.734	0.1	6000
	US05_1	5.000	4.900	0.1	200
	US05_2	2.500	7.400	0.1	400
	US12	1.600	8.300	0.1	600
<i>San Agustín, La Rioja</i>	SA01	0.250	9.650	0.1	4000
	SA04	0.625	9.275	0.1	1600
	SA08	2.500	7.400	0.1	400
	SA10	5.000	4.900	0.1	200
	SA20	0.625	9.275	0.1	1600
	SA39_1	2.500	7.400	0.1	400
	SA39_2	1.000	8.900	0.1	1000
	SA56_1	2.500	7.400	0.1	400
	SA56_2	0.250	9.650	0.1	4000
	SA56_3	1.250	8.650	0.1	800

Appx_Table E.4.1 Table showing the dilution factors of the final specimen solutions analysed by ICP-MS(Sol).

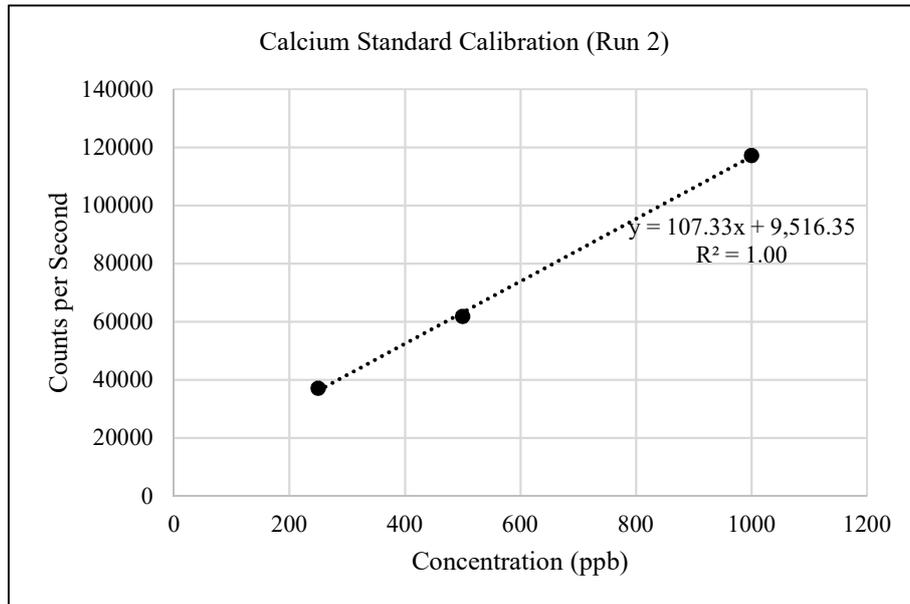
Calcium/Phosphorus Standard Calibration



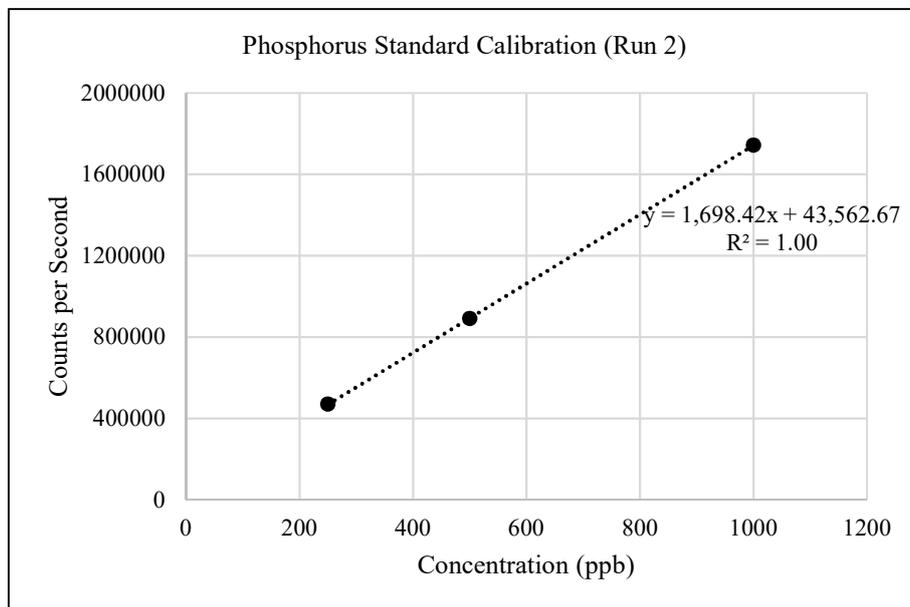
Appx_Figure E.4.1 Standard Calibration plot for calcium (0.25, 0.5 and 1.0 ppm) for the first run of Ca/P ratios.



Appx_Figure E.4.2 Standard Calibration plot for phosphorus (0.25, 0.5 and 1.0 ppm) for the first run of Ca/P ratios.

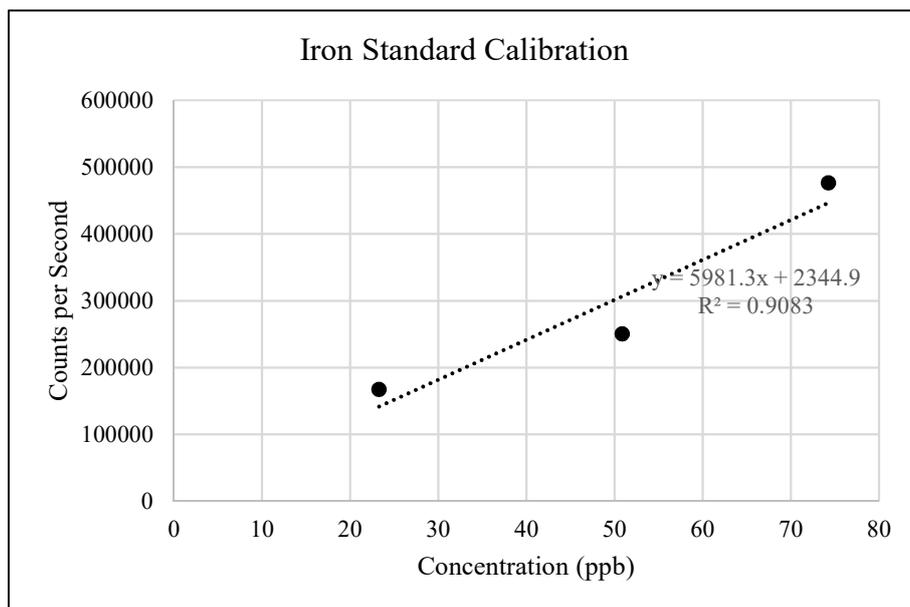


Appx_Figure E.4.3 Standard Calibration plot for calcium (0.25, 0.5 and 1.0 ppm) for the second run of Ca/P ratios.



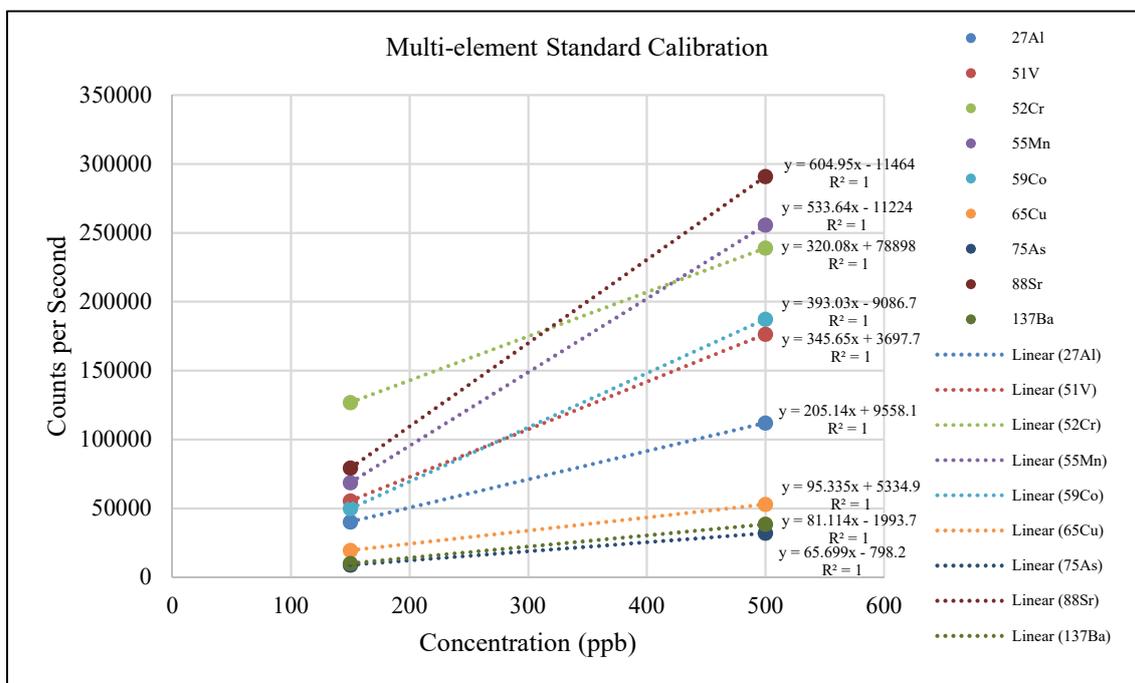
Appx_Figure E.4.4 Standard Calibration plot for phosphorus (0.25, 0.5 and 1.0 ppm) for the second run of Ca/P ratios.

Iron Standard Calibration



Appx_Figure E.4.5 Standard Calibration plot for iron standard (0.02, 0.05, and 0.075 ppm).

Multi-element Standard Calibration



Appx_Figure E.4.6 Standard Calibration plot for multi-element standard (0.15 and 0.5 ppm).

Ca/P vs. Calcite Percentage

	Individual	Jaw	Tooth	Surface	HAp	WHT	HAp:WHT	⁴⁸ Ca: ³¹ P	Calcite Present?
CC	US31	UL	M1	B	68	32	2	1.90	N
	F54	LL	M2	B	64	30	2	1.83	Y
	P321	LR	M1	B	60	40	2	1.74	N
	F15	LL	M1	B	88	11	8	2.02	Y
	M213	UR	M2	B	51	45	1	2.13	Y
	UM02	LL	M1	B	83	17	5	2.31	N
	US01	UR	M2	B	84	15	5	2.28	N
	P318	UL	M1	B	72	26	3	2.20	Y
	UM04	LR	M2	B	86	14	6	2.28	N
CV	US05	UL	M1	Li	82	18	5	2.09	Y
	US05	UL	M3	Li	83	12	7	2.92	Y
	US12	UR	M1	M	63	34	2	1.99	Y
	4D101	UR	PM2	B	84	15	5	2.42	Y
	4B01	UR	PM1	B	81	15	5	2.15	Y
	2C02	UR	M1	Li	38	60	1	1.97	Y
	2C01	UL	M1	D	57	43	1	2.14	
	4D101	UR	M1	B	62	38	2	2.03	Y
	BB02	UL	M2	B	76	23	3	2.07	N
SA	SA39	LL	C	Li	96	4	23	2.09	N
	SA10	UL	M3	Li	38	62	1	1.85	
	SA08	UR	M1	Li	86	12	7	2.49	Y
	SA39	LR	C	Li	91	9	10	2.19	N
	SA20	UR	M2	B	80	19	4	2.09	Y
	SA01	UR	M2	Li	87	13	6	2.15	N
	SA56	LL	I1	Li	88	12	8	2.19	
	SA56	LR	I2	La	89	11	8	2.40	
	SA04	UR	M3	Li	72	24	3	2.11	

Appx_Table E.4.2 Table showing the Ca/P ratio determined from ICP-MS(Sol) analysis and whether the specimen exhibited calcite in the pXRD data (see Appendix E.3.2 for mineral percentage tables).

The Physical Characterisation and Composition of Archaeological Dental Calculus

Fe Concentration vs. Pathologies Present

	Individual	Jaw	Tooth	Surface	Calculus Score (Brothwell (1981))	%T_Calculus	%T_Caries	%P_P _A C	Periodontal Disease	Fe (ppb)
CC	US31	UL	M1	B	2	50	0	6	0	4996
	F54	LL	M2	B	1	40	40	0	100	1065
	P321	LR	M1	B	1	63	26	3	100	13590
	F15	LL	M1	B	1	100	0	4	100	1303
	M213	UR	M2	B	1	63	3	0	100	984
	UM02	LL	M1	B	1	18	0	0	100	7367
	US01	UR	M2	B	1	100	0	6	0	12260
	P318	UL	M1	B	2	100	6	6	100	18210
	UM04	LR	M2	B	3	80	0	0	100	2800
CV	US05	UL	M1	Li	2	100	0	0	0	28530
	US05	UL	M3	Li	1	89	11	13	100	531
	US12	UR	M1	M	1	89	11	13	100	11560
	4D101	UR	PM2	B	1	100	100	-	-	2905
	4B01	UR	PM1	B	1	90	10	0	100	5701
	2C02	UR	M1	Li	2	20	25	17	100	7357
	2C01	UL	M1	D	1	100	0	0	100	1902
	4D101	UR	M1	B	3	100	0	-	-	6585
	BB02	UL	M2	B	2	50	0	6	100	19660
SA	SA39	LL	C	Li	2	90	10	0	100	42990
	SA10	UL	M3	Li	2	100	0	-	-	23530
	SA08	UR	M1	Li	1	43	2	7	100	848
	SA39	LR	C	Li	1	33	33	13	100	1087
	SA20	UR	M2	B	1	14	14	0	100	4650
	SA01	UR	M2	Li	1	43	2	7	100	5927
	SA56	LL	I1	Li	2	50	25	0	100	9560
	SA56	LR	I2	La	3	21	25	9	100	10340
	SA04	UR	M3	Li	3	36	0	3.13	100	5060

Appx_Table E.4.3 Table showing the Fe concentration (ppb) determined from ICP-MS(Sol) analysis and the recorded pathologies in each individual.

E.4.2 Energy Dispersive X-ray Analysis

	Ca	P	O	C	Na	Ni	Zn	Fe	S	K	Mg	Cl	N	Si	Al
F17	15	7	27	51	*	*	*	*	*	*	*	*		*	
M189	26	12	34	27	*	*	*	*	*	*	*	*	*		
M200_1_01	35	15	32	17	*	*	*	*	*	*	*		*	*	
M200_1_02	29	15	36	16	*	*	*	*	*	*	*	*	*	*	
M203	24	11	32	33	*	*	*	*	*	*	*	*	*	*	
M208	26	13	37	22	*	*	*	*	*	*	*	*	*	*	
M209_1_01	22	11	35	31	*	*	*	*	*	*	*	*		*	
M209_1_01	29	13	36	20	*	*	*	*	*	*	*	*	*	*	
M210	29	14	38	18	*	*	*	*	*	*	*	*	*		
P318_01_03	25	10	32	31	*	*	*	*	*	*	*	*			
P318_01_04	32	14	35	16	*	*	*	*	*	*	*	*	*	*	
P318_01_05	28	12	34	23	*	*	*	*	*	*	*	*	*		
P330	31	14	36	17	*	*	*	*	*	*	*	*	*		
S254	24	12	37	25	*	*	*	*	*	*	*	*		*	
S257	26	14	34	24	*	*	*	*	*	*	*	*	*		
S264	26	14	38	21	*	*	*	*	*	*	*	*	*		
S270	30	14	34	21	*	*	*	*	*	*	*	*	*	*	
N1131	26	12	34	28	*	*	*	*	*	*	*	*		*	
N1133	23	11	35	31	*	*	*	*	*						
N1142_1	28	12	32	28	*	*	*	*	*	*		*			
N1142_2	22	10	29	37	*	*	*	*	*	*	*			*	
N1144	28	15	40	15	*	*	*	*	*	*	*	*	*		
US01	24	11	31	34	*	*	*	*	*	*	*	*			
US08	20	10	38	30	*	*	*	*	*	*	*	*	*	*	
US11_01_01	28	13	36	22	*	*	*	*	*	*	*	*	*		
US11_01_02	23	11	32	34	*	*	*	*	*	*	*	*			
US15	21	11	35	32	*	*	*	*	*	*	*	*	*	*	
US17	28	16	40	14	*	*	*	*	*	*	*	*	*		
US26	16	7	29	45	*	*	*	*	*	*	*	*		*	*
UM01	21	9	32	38	*	*	*	*	*	*	*	*		*	
UM04	20	11	32	35	*	*	*	*	*	*	*	*		*	

* indicates that element was identified as present in the spectra in quantities below 5%; blank entries indicate that the element was not present in the spectra

Appx_Table E.4.4 Semi-quantitative elemental results pertaining to the deep third (see Figure 5.9.1) of the cross-sectional surfaces of sectioned dental calculus specimens. Results are presented as the oxide percentage for each element.

	Ca	P	O	C	Na	Ni	Zn	Fe	S	K	Mg	Cl	N	Si	Al
F17	26	13	35	24	*	*	*	*	*	*	*	*	*	*	*
M189	31	14	36	16	*	*	*	*	*	*	*	*	*	*	*
M200_1_01	36	15	31	17	*	*	*	*	*	*	*	*	*	*	*
M200_1_02	31	16	36	14	*	*	*	*	*	*	*	*	*	*	*
M203	30	14	38	14	*	*	*	*	*	*	*	*	*	*	*
M208	23	12	34	29	*	*	*		*	*	*	*	*	*	*
M209_1_01	26	13	37	24	*	*	*	*	*	*	*	*		*	*
M209_1_01	28	15	36	19	*	*	*	*	*	*	*	*	*	*	*
M210	27	14	39	18	*	*	*	*	*	*	*	*	*		*
P318_01_03	33	14	34	16	*	*	*	*	*	*	*	*	*	*	*
P318_01_04	19	9	29	42	*	*	*	*	*	*	*	*	*	*	*
P318_01_05	34	15	35	13	*	*	*	*	*	*	*	*	*		*
P330	32	15	37	14	*	*	*	*	*	*	*	*	*		*
S254	28	14	38	18	*	*	*	*	*	*	*	*	*	*	*
S257	28	14	37	19	*	*	*	*	*	*	*	*	*	*	*
S264	31	15	39	12	*	*	*	*	*	*	*	*	*		*
S270	32	14	36	15	*	*	*	*	*	*	*	*	*	*	*
N1131	32	15	36	14	*	*	*	*	*	*	*	*	*	*	*
N1133	25	13	40	21		*	*	*	*	*	*	*	*		*
N1142_1	33	15	35	14	*	*	*	*	*	*	*	*	*		*
N1142_2	33	16	34	16	*	*	*	*	*	*	*	*	*	*	*
N1144	28	15	41	14		*	*	*	*	*	*	*	*	*	*
US01	32	15	35	16	*	*	*	*	*	*	*	*	*	*	*
US08	18	9	36	35	*	*	*	*	*	*	*	*	*	*	*
US11_01_01	32	15	39	11	*	*	*	*	*	*	*	*	*	*	*
US11_01_02	31	14	37	15	*	*	*	*	*	*	*	*	*	*	*
US15	26	13	37	22	*	*	*	*	*	*	*	*	*	*	*
US17	28	15	41	14	*	*	*	*	*	*	*	*	*		*
US26	28	13	35	21	*	*	*	*	*	*	*	*	*	*	*
UM01	30	13	38	16	*	*	*	*	*	*	*	*	*		*
UM04	24	12	39	24	*	*	*	*	*	*	*				*

* indicates that element was identified as present in the spectra in quantities below 5%; blank entries indicate that the element was not present in the spectra

Appx_Table E.4.5 Semi-quantitative elemental results pertaining to the central third (see Figure 5.9.1) of the cross-sectional surfaces of sectioned dental calculus specimens. Results are presented as the oxide percentage for each element.

	Ca	P	O	C	Na	Ni	Zn	Fe	S	K	Mg	Cl	N	Si	Al
F17	15	7	29	48	*			*	*	*		*		*	
M189	28	13	35	22	*	*	*	*	*	*		*	*	*	
M200_1_01	35	14	31	19	*	*	*	*	*	*	*	*	*	*	
M200_1_02	32	14	36	16	*	*	*	*	*	*	*	*	*	*	
M203	20	10	32	37	*	*	*	*	*	*	*	*		*	
M208	22	11	35	31	*	*	*	*	*	*	*	*	*	*	
M209_1_01	16	8	31	43	*	*	*	*	*	*	*	*		*	
M209_1_01	26	12	35	24	*	*	*	*	*	*	*	*	*		
M210	25	13	36	25	*	*	*	*	*	*	*	*	*	*	
P318_01_03	25	10	32	31	*	*	*	*	*	*	*	*		*	
P318_01_04	25	11	34	30	*	*	*	*	*	*	*	*	*	*	
P318_01_05	22	9	31	37	*	*	*	*	*	*	*	*		*	
P330	26	12	35	26	*	*	*	*	*	*	*	*			
S254	27	14	39	18	*	*	*	*	*	*	*	*	*	*	*
S257	28	14	36	20	*	*	*	*	*	*	*	*	*	*	*
S264	21	10	34	34	*	*	*	*	*	*	*	*	*	*	*
S270	25	11	33	29	*	*	*	*	*	*	*	*		*	
N1131	29	13	35	21	*	*	*	*	*	*	*	*	*	*	*
N1133	16	8	33	43	*	*	*	*	*	*	*	*			
N1142_1	20	9	30	42	*	*	*	*	*	*		*		*	
N1142_2	26	12	32	29	*	*	*	*	*	*	*	*		*	
N1144	28	15	41	14	*	*	*	*	*	*	*	*	*		
US01	24	11	32	33	*			*	*	*	*	*			
US08	18	9	36	37		*	*	*	*	*	*	*	*	*	*
US11_01_01	28	13	37	20	*	*	*	*	*	*	*	*	*		
US11_01_02	21	10	35	33	*	*	*	*	*	*	*	*	*	*	*
US15	22	11	34	32	*	*	*	*	*	*	*	*		*	
US17	18	9	35	37	*	*	*	*	*	*	*	*		*	
US26	22	10	33	32	*		*	*	*	*	*	*		*	*
UM01	21	9	33	35	*	*	*	*	*	*	*	*	*	*	*
UM04	16	8	31	44	*	*	*	*	*	*	*	*		*	

* indicates that element was identified as present in the spectra in quantities below 5%; blank entries indicate that the element was not present in the spectra

Appx_Table E.4.6 Semi-quantitative elemental results pertaining to the superficial third (see Figure 5.9.1) of the cross-sectional surfaces of sectioned dental calculus specimens. Results are presented as the oxide percentage for each element.

APPENDIX F: PUBLICATIONS

- Cooper, K.; Beckett, S.; Marquez-Grant, N. (2014). Is there Forensic Potential in Dental Calculus Analysis? *EMFA Conference Poster, Cranfield University*.
- Cooper, K.; Beckett, S.; Marquez-Grant, N.; Piombino-Mascali D. (2014). A Quantitative Method for Dental Calculus Analysis? Method, Testing and Conclusion. *BABAO Conference Poster, Durham University*.
- Willis, C.; Osborn, C.; Cooper, K.; Marquez-Grant, N.; Piombino-Mascali, D. (2015). Paleopathology, Non-metric Traits and Dental Calculus Analysis of Individuals from the Capuchin Catacomb Collection (Palermo, Sicily). *Journal of Paleopathology* 25(S), p. 44, XIII Congreso Nacional de Paleopatología (Seville, Spain).
- Cooper, K.; Beckett, S.; Marquez-Grant, N.; Piombino-Mascali D. (2015). A Quantitative Method for Dental Calculus Analysis? Method, Testing and Conclusion. *Journal of Paleopathology* 25(S), p. 2, XIII Congreso Nacional de Paleopatología (Seville, Spain).
- Cooper, K.; Greenwood, C.; Beckett, S.; Rogers, K. D. Shortland, A. A Combined Mineral and Microfossil Approach to Dental Calculus Analysis (*In preparation for submission to the Journal of Archaeological Science*)
- Cooper, K.; Beckett, S.; Marquez-Grant, N. Archaeological Dental Calculus: Considerations and Proposed Guidelines for Sampling and Analysis (*In preparation for submission to the Journal of Paleopathology*).
- Cooper, K.; Piombino-Mascali D.; Beckett, S.; Marquez-Grant N. A Preliminary Study of Dental Caries, Dental Calculus and Ante-Mortem Tooth-Loss in Mummified Remains From the Capuchin Catacombs, Palermo, Sicily: A Report Of The Sicilian Mummy Project (*In preparation for submission to the International Journal of Osteoarchaeology*).

Is There Forensic Potential In Dental Calculus Analysis?

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What is Dental Calculus?

When we don't brush our teeth, the saliva in our mouth coats them with a biofilm of ions, minerals and bacteria, which is dental plaque. When we eat, food particles also become trapped in this biofilm and the deposits become noticeable. If we don't brush plaque away it hardens into calcified deposits called dental tartar or calculus. This can cause inflammation of the gums and can be quite uncomfortable as the deposits get larger.



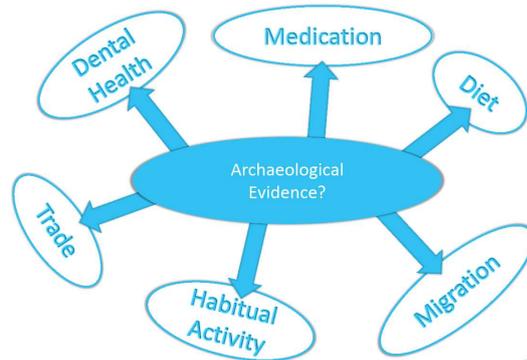
What Information Do We Gain From Dental Calculus Analysis In Archaeology?

Firstly, by recording of the presence and degree of dental calculus we can discover the dental health and hygiene habits for a population. Trapped microfossils can also provide information of whether the mouth was used as a 'third-hand' for activities such as basket-weaving or rope-making. Additionally, plant microfossils extracted from the deposits can indicate plant species consumed for their nutritional, medicinal or cultural value.

The dental calculus analysis, in conjunction with local literature and geographical profiles, can also indicate whether the food consumed was representative of the resources available or whether different materials were imported or exported through trade routes.

By analysing dental calculus deposits from archaeological remains we are able to gain primary information from the skeleton rather than secondary information from the soil, artefacts or food and animal remains within a burial. The changing nature of surface soil and naturally growing plants in an area from time of burial to excavation can produce a markedly different picture of life for past populations compared modern populations living in the same area.

Therefore, dental calculus can provide evidence regarding the resources that were available when it was formed. This information has persisted in death and burial we can extract that evidence to give us knowledge of the population from the time they were alive.

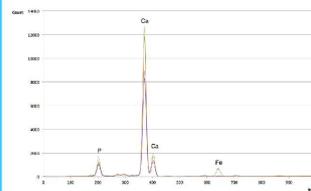


What Is The Aim Of Researching Dental Calculus At Cranfield?

The aim of researching dental calculus is to create standard protocols that can be applied to dental calculus analysis. The standardisation of the methodology will enable comparison between results from different collections and different research groups.

The research undertaken will also evaluate the evidence gained from analysing dental calculus. This will allow more clarity for future researchers to be able to choose which techniques to use for limited samples. During this evaluation, the potential for the analysis of dental calculus in forensic situations will also be considered.

Analysis of dental calculus by X-Ray Fluorescence produces the elemental composition of the sample. Samples from the HASLAR Naval Hospital were analysed and the above graph shows the presence of calcium and phosphorus which are the main elements in the mineral component of dental calculus.



One sample (green) showed the presence of iron which can be attributed to gingival bleeding causing iron to become trapped in the calculus matrix.

What Additional Information Could Be Gained For Forensic Use?

It may be possible that dental calculus could help with determining provenance for unidentified individuals based on the diatoms, pollen or fibres identified from microfossil analysis. Another type of case may be in chronic poisonings (Charlier et al. 2010) by identifying poison components in elemental analysis of the calculus that could have been absorbed during calcification. In previous research, Huang et al. 1997 investigated fluoride profiles of dental calculus to compare with fluoride levels of water supplies in different countries.



My name is Kayleigh Cooper and I am a current PhD Researcher at Cranfield Forensic Institute, Cranfield University. I have achieved an undergraduate Masters in Chemistry with Forensic Science from University of Leicester and a subsequent Masters of Science in Archaeology and Anthropology from Cranfield University.

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Charlier et al. (2010). 'The microscopic (optical and SEM) examination of dental calculus deposits (DCD). Potential interest in forensic anthropology of a bio-archaeological method'. *Legal Medicine* 12, pp. 153-171.
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Acknowledgements

The authors would like to thank Cranfield University for enabling this research to be carried out. Access to the Capuchin Catacombs was granted by the Order of the Capuchin Friars, Palermo, Sicily and the Superintendent to the Cultural and Environment Heritage of Palermo. Access to Cementini Vell was granted by the Consell Insular de Formentera and the Church of St. Francesc de Xavier.

A Quantitative Method For Dental Calculus Analysis? Method, Testing And Conclusion



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Introduction

Dental calculus (Figure 1) has been successfully analysed for past population studies and has been shown to produce valuable evidence of diet, health and habitual activity¹⁻³. While the removal of dental calculus is not considered destructive to human remains, it is an irreversible process. With this in mind and prior to removal, it is vital to comprehensively record the amount of calculus present on the individual and for future research. The importance of not only recording but quantifying dental calculus is increased when further laboratory analysis is carried out. Many indices for recording archaeological dental calculus^{4-6,8} are highly subjective and require visual assignment of a score by the recorder. Therefore, it is the ongoing aim of this research to develop a recording method that is objective rather than subjective. Therefore, the Volpe-Manhold Probe method⁷ used in dentistry to quantify dental calculus was adapted for use in this study. This adapted Probe method was tested during the recording of two collections to determine its feasibility as an anthropometric method for quantifying dental calculus. Additionally, all calculus deposits were recorded using the widely accepted Brothwell⁹ method of calculus scoring and to determine if the subjective assignment of scores corresponded to the objective method of measurement.



Figure 1. - A tooth with calculus from the Cemenitri Vell Collection

Materials & Methods

In total, 92 individuals were recorded for dental calculus from the Capuchin Catacombs, Sicily and 32 individuals were recorded from Cemenitri Vell, Formentera (Figure 3 & 4). For the results presented, the two collections were merged because all measurements and scores were recorded by the same individual.

The Probe Method utilized a UNC-15 periodontal probe, graduated in millimeters. Three measurements were taken to assess the coverage of calculus on the tooth⁷. The probe was held adjacent to the tooth and the calculus band was measured to within 0.5 mm. The Total Probe Measurement is the sum of the three measurements.

The dental calculus was also recorded using Brothwell⁹ to give each deposit a score between 0-3. On each tooth, the surfaces, lingual, labial/buccal, mesial and distal, were recorded using both methods to score the calculus present.

Results

From the results shown, a limitation of the suggested Probe method was the ability to record dental calculus in certain positions around the mouth. When adjacent teeth were present in the alveoli, for mesial and distal surfaces, the probe was not able to be inserted sufficiently enough to accurately record the three planes. In contrast, the Brothwell method could be used in all cases by viewing by eye the calculus in the interproximal spaces (Table 1).

By comparing the calculus deposits that were recorded using both methods, it is possible to see the distribution of Probe scores for each Brothwell score (Figure 2). Only labial/buccal surfaces had more than one calculus deposit that was scored as a Brothwell '3'. However, it can be seen from all surfaces that the median of Probe measurements did increase with Brothwell score.

The measurements for mesial surfaces had a small range which corresponded well with the Brothwell scores, however all other surfaces had significant overlap. This indicates that two calculus deposits with same measurement via the Probe method, may have been assigned different Brothwell scores.

Tooth Surface	No. of Observable Teeth Scored by Brothwell	No. of Observable Teeth Scored by Probe	Total Number of Observable Teeth	Percentage of Teeth Scored by Brothwell	Percentage of Teeth Scored by Probe
Lingual	151	73	151	100	48
Labial/Buccal	337	267	337	100	79
Mesial	93	45	93	100	48
Distal	62	30	62	100	48

Table 1. - Table showing the number of teeth with calculus that were observable and recordable via each method. Also shown are the percentages of teeth that were scored with each method.

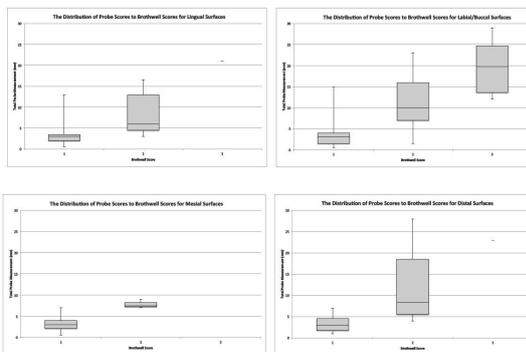


Figure 2. - Graphs showing the distribution of Probe measurements against Brothwell scores for the lingual (top left); labial/buccal surfaces (top right); mesial (bottom left) and distal (bottom right).

Discussion

For both collections, a considerable disadvantage to using the Probe method was no measurement being possible. For some teeth there were problems with accessibility to deposits and others where there were flecks of calculus on the tooth surface. This type of deposit was not measurable because the flecks of calculus were in some cases smaller than 0.5 mm and any plane measurement would not accurately represent the actual amount of calculus present. For the teeth where the Probe could not be inserted to measure the calculus, the Brothwell score could still be given by inspecting the interproximal space by eye. Similarly, the flecks of calculus were given a score of 1 for Brothwell because there was calculus present, however this is a case where the score can over-estimate the amount of calculus.

The qualitative Probe method has shown that for the same amount of calculus, the subjective nature of scoring with indices produces inconsistent results. However, the biggest limitation to recording with only this method would be the loss of information where measurements are not possible.



Figure 3 (above) & 4 (below). - Dentition from Cemenitri Vell showing varying degrees of calculus present.



Conclusion

The results for comparing the Brothwell scores and Probe scores were compiled in order to determine the validity of using the Probe method for calculus recording. Currently, methods such as Brothwell (1981) are based on the subjective decision of the recorder to assign a discrete score for calculus deposits, which vary in size, location and shape. The deposits seen do not always fit neatly into one of the indices described and are therefore given an overstated or understated score. By implementing a quantitative method based on measuring the deposit it was intended that the deposits could be more objectively compared.

From this initial testing of the Probe method, it has been found that alterations are required to reduce the amount of missing data. Additionally, further testing to determine intra-observer error of the Probe measurements will be performed. Alongside, the intra-observer error for the Brothwell method will also be investigated.

Further work will enable clarification of whether the Probe method is a valid quantitative method for dental calculus recording.



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Acknowledgements

The authors would like to thank Cranfield University for enabling this research to be carried out. Access to the Capuchin Catacombs was granted by the Order of the Capuchin Friars, Palermo, Sicily and the Superintendent to the Cultural and Environment Heritage of Palermo. Access to Cemenitri Vell was granted by the Consell Insular de Formentera and the Church of St. Francesc de Xavier.



3D Nano-Computed Tomography of Archaeological Dental Calculus

Introduction

Computed Tomography (CT) is a powerful imaging technique that is increasingly being applied to archaeological material (Brough et al. 2016; Miles et al. 2016; Sanger 2016). The technique uses non-destructive X-Rays to take numerous 2D radiographs that are then reconstructed to produce a 3D visualisation of the object. The resulting data shows differences in densities within a material or object on a gradient of grey-scale voxels.

Traditionally, medical CT has been applied to archaeological finds and human remains. However, medical imaging suffers from low resolution when looking at small objects. The commercialisation of micro-Computed Tomography (μ -CT) negates this resolution issue. The research presented here uses a Nikon Metrology XT H225 scanner with a 180kV Transmission target with a 1-10 μ m focal spot size.

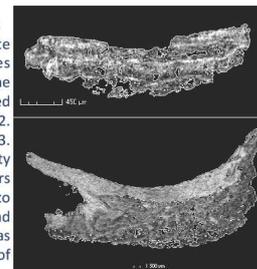


Background

Dental calculus begins to form in the oldest dental plaque on a tooth's surface and builds up in layers (LeGeros 1974; Hayashi 1993; White 1997; Roberts-Harry and Clerehugh 2000). The presence of these layers could be indicative of different phases of dental calculus formation. This research has applied Nano-Computed Tomography to specimens of archaeological dental calculus in order to determine the possibility of visualizing dental calculus layering without destructive preparation techniques (such as histological sectioning). In addition, void analysis and inclusion analysis of the specimens has been applied to the resulting images, in order to determine the possibility of identifying microfossils in situ as well as vacancies in the mineral matrix.

Layering

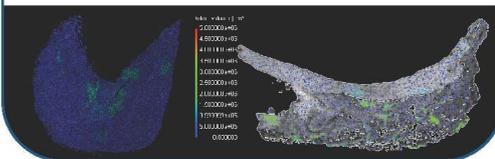
In a number of specimens it was possible to observe discrete layers of different densities within the dental calculus. The specimens could be separated into three types; 1. no layers, 2. multiple alternating bands or 3. two layers of distinct density difference. In all cases, layers were observed perpendicular to the calculus surface that had been attached to the tooth, as expected for the accumulation of the deposit.



The images shown are examples of a category 2 (top) and category 3 (bottom).

Void/Inclusion Analysis

The void and inclusion analysis of the images produced interesting results. Firstly, there were category 3 specimens that had inclusions of different size in each distinct layer (below right). Secondly, there were specimens that had groups of larger sized voids and inclusions towards the centre of the deposits. In cases where deposits had been removed from molars, there was a distinct collection of larger sized voids, in the calculus that had formed in the buccal development groove (BDG).



Conclusions

This novel application of micro-Computed Tomography has demonstrated the potential wealth of knowledge that can be gained from specimen imaging of dental calculus deposits from archaeological remains. This technique has provided a non-destructive means of observing the layers and porosity, that is most commonly lost during sample preparation techniques.

Further to this analysis, the specimens in question have been analysed by Scanning Electron Microscopy and micro-beam X-Ray Diffraction in order to determine whether the observed layers can be related to changes in mineral or elemental composition.

The determination of layer presence and depth may be beneficial in assessing a potential time-line of fluctuating dietary or health-related changes in an individual prior to death.

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This work has been funded by Cranfield Forensic Institute and the authors would like to acknowledge Dr Dario Piombino-Mascali and The Department of Sicilian Cultural Heritage: Sonia Cardona Ferrer, Jaume Castello Guash, Miguel Angel Riera Planells and the Formentera Council for Heritage and Culture and the Roman Catholic Diocese of Ibiza; and Almudena Garcia-Rubio for access for the collections.

