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Micro-patterned biological interfaces manufactured by diamond turning with CVD diamond micro-tools

S. Marson[1], R. Villa[2], I.Cardenas-Durazo[3], R.W. Evans[1], A. Storti[1], A. Heaume[1], F. Marinello[4], S. Carmignato[5] and D.M.Allen[1]
[1]*Precision Engineering Centre, Cranfield University, UK*
[2]*Centre for Energy and Resource Technologies, Cranfield University, UK*
[3]*Materials Department, Cranfield University, UK*
[4]*DIMEG, Dip. di Innovazione Meccanica e Gestionale, University of Padova, Italy*
[5]*DTG, Dip. di Tecnica e Gestione dei Sistemi Industriali, University of Padova, Italy*
s.marson@cranfield.ac.uk

Abstract

The generation of microstructured interfaces which enhance cell adhesion and proliferation is of great interest in bioremediation, i.e. in all those applications where biological reactions result in the destruction of contaminants. Diamond turning has been implemented for the manufacture of microstructures, taking advantage of bespoke CVD diamond micro-tools in which the edge profile was successfully modified using a combined laser/FIB machining strategy. The CVD micro-tools show good cutting performance in terms of the achievable cutting volume and repeatability of the fabricated microstructure.

1 Introduction

Bioremediation is one of several methods used for the removal of fats, oils and grease (FOGs) from waste-water. Destruction of contaminants is carried out by biological reactions, including those of microorganisms such as yeast, fungi or, more typically, bacteria. The method is said to rely on the formation of biofilms (three-dimensional communities encapsulated by a self-generated polysaccharide matrix) on sewer surfaces which act as a catalyst for the in-situ degradation of the greasy material. In particular, adhesion of bacteria cells to material interfaces is the first step in bacterial colonization (i.e. the proliferation of bacteria into multicelled communities) as well as bacterial biofilm maturation. Of particular interest, as reported by literature [1], is the fact that properly designed microstructured interfaces enhance the tendency of bacteria cells to adhere and proliferate. To be commercially viable, such microstructures are needed on relatively large surfaces.A possibility would be to manufacture polymer films by replication technologies using microstructured drums as the metal master, as per ref [2]. The resultant polymer microstructured films would be compatible with the current fabrication technologies of sewer pipes and could potentially be lined into new pipes or retrofitted into existing pipework to modify their surface properties.

The structured surface subject of this work was designed to enhance the biofilm formation of *Bacillus sp.*, one of the most commonly-used, commercially available microbial bioadditions. The design strategy was to create features of dimensions comparable to the *Bacillus sp.* i.e. 1-10µm in length. An additional criterion was to provide a sinusoidal surface to better accommodate the bacteria and to improve cell-substrate adhesion thus cell attachment and proliferation. Diamond turning was implemented for micro-patterning of aluminium plates, taking advantage of bespoke diamond micro-tools.

2 Specially designed tools

For this work, innovative CVD diamond micro-tools were used in combination with diamond turning [3]. Chemical vapour deposited (CVD) polycrystalline diamond was selected as the tool material with the aim of achieving higher fracture toughness than single crystal. Additional benefits of CVD diamonds compared to single crystal natural diamonds are that they are lower cost, harder, have better wear properties and are chemically and thermally more stable [4]. Polycrystalline blanks were mounted on kite-shaped shanks (Figure 1a) and then laser processed to produce a rough shape in the tool (Figure 1b). Micrometric features and sharp edges were eventually generated on the tool by means of a focused ion beam source (FIB). The tool had a sinusoidal profile with a pitch of 9.1 µm and a height of the prongs of 5.9 µm (Figure 1c).

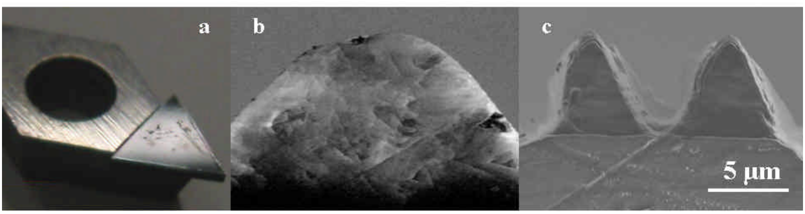

Figure 1: CVD tool blank mounted on shank (a) after laser cut (b) and after final FIB machining (c).

3 Microstructured surfaces

The proposed novel process effected the generation of very high performance diamond tools, in terms of stability, wear resistance and machining accuracy. Machining tests were performed on Al 6061-T6 plates using the bespoke CVD diamond tool shown in Figure 1c. A periodical sinusoidal geometry was produced by diamond turning three plates on 50 concentric circular trajectories, with diameters ranging between 24.5 and 25.5 mm for a total path of over 12 m. The total amount of removed material was estimated to be around 80mm³. Machining was performed on the diamond turning machine Nanotech 350 UPL - Moore Nanotechnology System using the following processing conditions: cutting speed: 1200 rpm (corresponding to a speed of about 3m/s); feed rate (depth of cut per rotation): 5 nm/rev; coolant: white spirit. The target groove depth was 5 µm.

The machining strategy was defined so that the first prong of the sinusoidal profile performed the roughing cut (thus removing the majority of the material) while the second prong only performed the finishing cut. In this way, most of the wear was concentrated on the first prong, while the second one (showing negligible wear following the machining) ensured the high quality and repeatability of the machining operation. The quality of the machined component was evaluated by analyses on scanning electron, scanning probe and confocal microscopes (Figure 2). Measurements performed at the beginning (after 100 mm), middle (after 2000 mm) and end (after 3900 mm) of the machined path evidenced low standard deviations on average machined pitch (distance between two consecutive machined channels: $p=9.1$ µm, $\sigma=0.1$ µm) and height (depth of cut: $h=5.6$ µm, $\sigma=0.15$ µm).

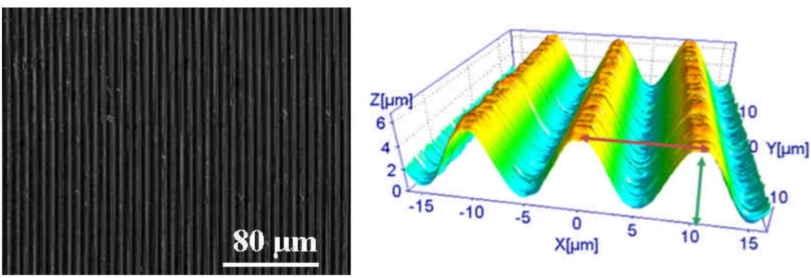

Figure 2: Periodic sinusoidal geometry machined on Al 6061-T6 using a sinusoidal profile CVD diamond tool (SEM and optical profilometer maps).

4 Biofilm proliferation

Biofilm growth was observed on the machined sinusoidal microstructures. Commercially available microbial additions were grown on Nutrient Broth for 12 hours at 37°C. The structured surfaces were aseptically added to the microbial cultures and left at 37°C under gentle agitation (150 rpm) for one week and three weeks. Following incubation, the samples were analysed using SEM in order to evaluate biofilm formation on the surface. After one week's incubation, tests evidenced the preference of the biofilm for the microstructured surface compared to the original smooth surface of the aluminium samples. This initial observation was confirmed from the analysis of the structured surfaces after 3 weeks' incubation, with 100% of the sinusoidal profile covered by bacteria against only partial coverage in the case of the smooth surface [5].

5 Conclusions

The proposed processing route shows great potential in the fabrication of large areas of complex microstructured surfaces for bioremediation applications. In particular: (1) the edge profile of CVD polycrystalline diamond tools was successfully modified using a combined laser/FIB machining strategy; (2) the CVD micro-tools show good cutting performance in terms of the achievable cutting length and microstructure repeatability; (3) bacterial growth is enhanced by the machined microstructure, with 100% coverage after three weeks' incubation.

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Durazo-Cardenas, Isidro

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