Attachment and Spreading of Human Embryonal Carcinoma Stem Cells on Nanosurfaces Monitored by Optical Waveguides

A. Aref, R. Horvath, F. Ansari, and J.J. Ramsden

Nanotechnology Centre, Cranfield University, Bedfordshire, MK43 0AL, UK

E-mail: a.aref@cranfield.ac.uk

ABSTRACT

Cell adhesion is an active process, carried out in vivo via receptor ligand-like interactions between cell surface adhesion molecules and the extracellular matrix. Initial cell surface reactions following contact may trigger multiple responses, which in turn result in either spreading or detachment of the cell. The set of adhesion and attachment molecules mediating the adhesive behaviour of stem cells and the kinetics of their interactions are as yet largely unknown. In this paper we have investigated the attachment and spreading kinetics of human embryonal carcinoma stem cells (TERA2.sp12) onto the planar Si(Ti)O_2 waveguides, and covered with poly-L-lysine (PLL) or mucin, acting as substrata for the cells.

Keywords: *stem cells; optical waveguide; kinetics; attachment; spreading*

1. INTRODUCTION

Substratum-stem cell investigations are important for therapeutic (such as 3D tissue growth) and non therapeutic applications, e.g. cell development for disease modelling and protein production. Tissue engineering applications such as injury repair and tissue growth attempt to mimic the assimilatory role of natural substrata whilst designers of biomedical coatings (such as those of certain implants and surgical instruments) may seek to assimilate or inhibit protein and cell attachment [1]. Initial cell surface reactions following contact may trigger multiple responses, which in turn result in either spreading or detachment of the cell. However the set of adhesion and attachment molecules the adhesive behaviour of stem cells and the kinetics of their interactions are as yet largely unknown.

Optical waveguide lightmode spectroscopy (OWLS) is a powerful technique (fig.1) for the precise quantification of thin film material deposition on a solid surface [2] (within the sensing depth ~150 nm), and has also been used to measure the kinetics of cell adhesion and spreading on predefined surfaces [3], and the attachment of bacteria [4]. This technique is also very well suited for cell-based experiments to investigate cell binding and cell spreading parameters [5]. Hug *et al.* [6] have also explored the possibility of OWLS as a whole cell sensor by looking at cell adhesion and morphology changes of fibroblasts.

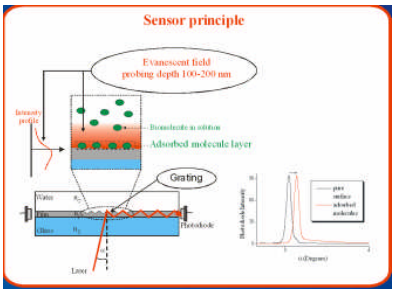


Fig1: Schematic illustration of the basic principle of optical waveguide lightmode spectroscopy for sensing.

Optical waveguide lightmode spectroscopy technique confines the light within a thin, high refractive index material whereby the upper and lower interfaces confine the light which propagation via total internal reflection. A grating coupler couples the light into the high refractive index material [7].

The waveguide is roated on a goniometer between -6 to +6 degrees. At a critical angle (α) light is coupled into the waveguide and propagates to the photodiode. As it propagates it travels via total internal reflection within the Si(Ti)O_2 layer and the electromagnetic evanescent field (penetrates 100-200 nm above the surface) is the sensing zone. Marerial adsorbed at the surface of the waveguide will interfere with the evanescent field causing a shift in the measured propagation constant [8].

1.1 Stem cells

Embryonal carcinoma (EC) stem cells derived from germ cell tumors are valuable tools for the study of embryogenesis and closely resemble embryonic stem cells [5, 6]. During embryonic development cells communicate with their local surroundings and receive information from their neighbors via secreted factors and membrane-bound molecules. Such interactions play a pivotal role in the modulation of cell differentiation and the determination of cell fate [9, 10, 11, 13].

2. MATERIALS & METHODS

2.1 Cell culture & biochemicals

Human EC cell lines, TERA2, were generously provided by S. Przyborski, Durham University, UK. All cells were maintained at 37 °C, under 5% CO_2 in DMEMFG medium, consisting of 90% Dulbecco's Modified Eagle's medium (DMEM; high glucose (4500 mg/L); pyridoxine HCl; $NaHCO_3$; without L-glutamine) (Sigma); supplemented with 10% fetal bovine serum (FBS; GIBCO), batch tested, heat inactivated at 56 °C for 30 min in a shaking water bath; 2 mM L-glutamine (Sigma); and penicillin (2.5 U/ml) (Invitrogen).

Pharmaceutical grade porcine gastric mucin (PGM) was purchased from A/S Orthana Kemisk Fabrik, Kastrup, Denmark. The commercial mucin preparation with a mean molecular weight estimated as 565 kDa was dialysed to remove all salts and low molecular weight additives and lyophilized for storage. All solutions were made by dissolving weighed PGM in Elga ultrapure water (resistance 18.2 Mohm cm, filtered through 200 nm pores) previously dialysed to remove all salts and low molecular weight additives and lyophilized for storage.

2.2 Substratum modification

Waveguides were cleaned with chromic acid (Fisher Scientific, U.K.) or under sonication in Roche cleaning solution or SDS/Roche treatment for 10 minutes, rinsed using ultrapure water and O_2-plasma treated (20 mW for 2 min). The PLL (0.01% solution; Sigma) and mucin (0.1% w/w) stock solution were made up and pre-equilibrated overnight. Solutions were applied to the substrata for 20 minutes, washed twice with ultrapure water and incubated for at least 40 minutes in cell culture media (DMEM; high glucose (4500 mg/L); pyridoxine HCl; $NaHCO_3$; without L-glutamine) (Sigma) at 37 °C, 5% CO_2 environment.

2.3 Cell attachment

Cells were detached from the culture flask using 0.05% trypsin/EDTA and collected using centrifugation (1500 rpm for 3 minutes). Cells were counted by eye using a hemocytometer, with the number of cells and surface coverage (60-70%) confirmed using phase contrast microscopy inspection of the waveguide after the experiments.

2.4 Optical waveguide lightmode spectroscopy

Waveguides were made from amorphous silica:titania at a ratio of approximately 2:1 and incorporated a shallow (5-10 nm) grating coupler (type 2400, grating constant equal to 416.667 nm). The incoupling resonance peaks for the TM_0 mode of the waveguides were measured every 40 seconds and saved for subsequent analysis [12, 14].

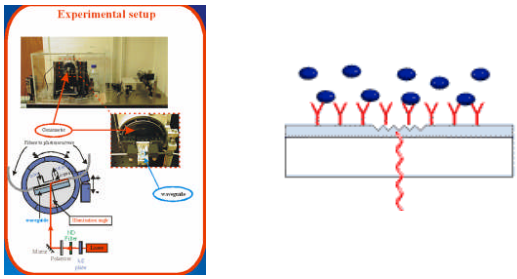


Fig2: Setups for the optical waveguide lightmode spectroscopy.

3. RESULTS & DISCUSSION

Behavior is dramatically different for bare silica-titania and mucin. We can see that mucin sharply inhibits spreading compared with bare waveguide (silica-titania), but the PLL layer is practically as good as an early spread-promoting substrate as the bar silica-titania waveguide (Fig. 3).

Microscopy images of cells living on identical substra show the distribution of the cell (fig 4). Microscopy data support the conclusion that spreading is inhibited by mucin and the inhibition is initially abolished for the PLL layer. Confirming the OWLS data mucin clearly inhibits spreading compared with uncoated silica-titania, but the inhibition is abolished by the PLL layer.

Synthetic scaffolds that support tissue growth by serving as the extracellular matrix for the cells do not represent the natural ECM associated with each cell type and tissue [15]. Embryonic stem cells and their progeny before and during development reside in a dynamic environment; thus synthetic or natural substrata that aim to mimic the developing embryo must present similar dynamics of signaling and structural element [16].

A number of approaches are currently under development, which may prove useful for stem cells. Materials that release particular factors or control the temporal expression of various molecules could be used to induce attachment and spreading of stem cells. If we need to inhibit the attachments and spreading of the cells we could use mucin to inhibit cell attachment within scaffolds.

Note that upon contacting substratum stem cells (and many other cell types) will modify it by synthesizing and releasing onto the surface protein required for attachment and spreading (the extracellular matrix) [5].

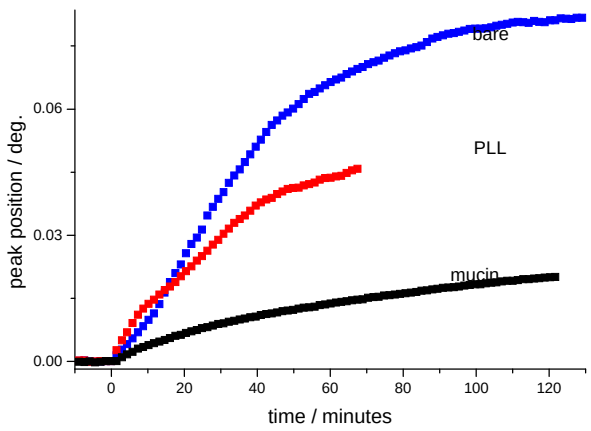
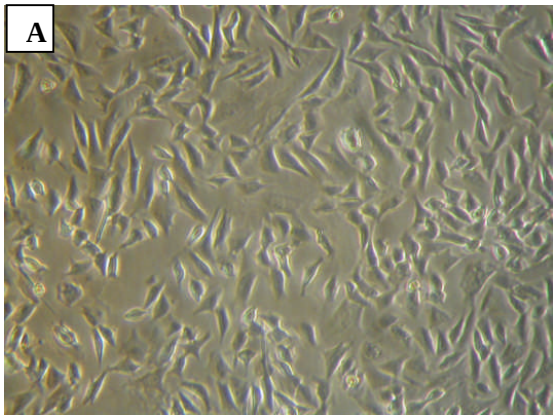
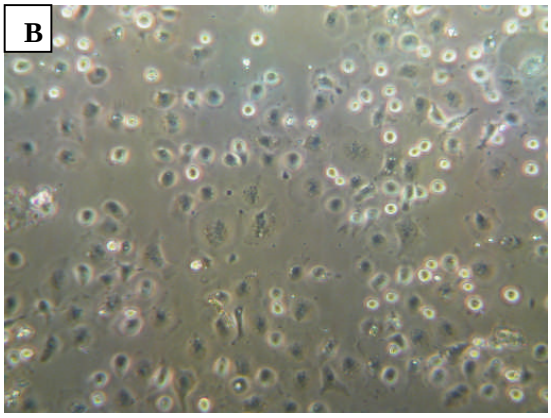


Fig 3: Cell-substrata interactions monitored by OWLS (monitored as mean incoupling peak position shift with time). Stem cells spread on silica-titania (top curve), PLL (middle curve), mucin (lower curve).





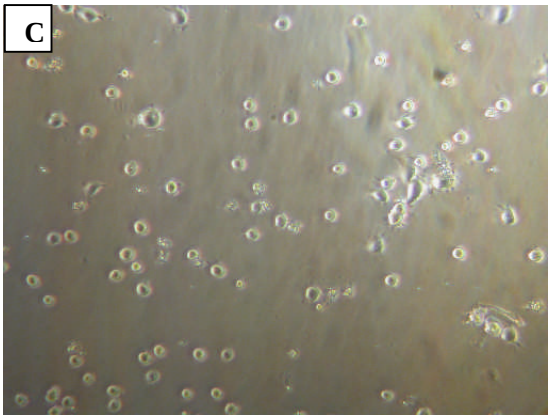


Fig 4. Microscopy images of human embryonal carcinoma stem cells on (A) bare waveguide $Si(Ti)O_2$, (B) PLL, (C) mucin after 120 minutes. The width of the images are 850 microns.

4. CONCLUSIONS

Stem cell attachment and spreading is greatly reduced on mucin coated substrates when compared with uncoated silica-titania but can be countered by incorporating PLL into the coating. We have also demonstrated the utility of OWLS for the quantitatives label-free investigation of stem cell attachment and spreading. OWLS can be used to monitor the kinetics of these processes in real time.

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