Fibronectin Behaviour and Cellular Response to Nanostructured Surfaces

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Introduction

Cells interact with a substrate via the attached protein layer, hence the concentration, composition and conformation of adsorbed proteins control the biological activity of the biomaterial surface. Fibronectin, an extracellular matrix (ECM) molecule, is recognised as playing a major role in cell-substrate interactions including cell attachment. Surface topography influences protein adsorption as well as adhesion, proliferation and orientation of mammalian cells. Substrates coated with colloidal silica have been investigated for their ability to influence ECM protein adsorption, such as fibronectin, as well as the biological response of a variety of primary and established cell lines, bacteria and fungi.

Materials and Methods

Tissue culture polystyrene (TCPS) well plates or gold quartz crystal microbalance (QCM) (Q Sense AB, Sweden) crystals were coated with a polycationic polymer (Zetag^{TM}) for 10 min, rinsed with deionised water and dried with nitrogen. Colloidal silica (7, 14 and 21 nm diameter) was applied to the Zetag^{TM} coated crystals for 5 min, rinsed with deionised water and dried with nitrogen. For cell culture experiments, well plates were sterilized with UV light for 30 min in a laminar flow hood. Retinal pigment epithelial cells (ARPE-19) were placed in direct contact with glass slides partially coated with silica. ARPE-19 cells were cultured in DMEM/Ham's F12 media supplemented with 10 % (v/v) FCS. The substrates were placed in incubation at 37 °C in 5 % CO_2/95 % air for 7 days.

Quantification of adsorbed fibronectin as well as conformation determination was carried out using a quartz crystal microbalance with dissipation monitoring (QCM-D) (Q-Sense) at 37 ± 0.1°C. Silica-coated QCM crystals (purchased from Q-Sense) and polystyrene (spin coated onto gold QCM-D crystals using a 0.5wt% solution in toluene and oxidized in a UVO chamber for 20s)-coated QCM crystals were used as controls. Coated QCM crystals were exposed to phosphate buffered saline (PBS), pH 7.4, incubated ovine fibronectin (300µg/ml) for 60 min and then rinsed twice with PBS, treated with 0.1wt% casein for 30 min, rinsed with PBS, incubated with A17 antibody (5µg/ml) for 30 min and rinsed twice with PBS. A17 is a monoclonal anti fibronectin (bovine) antibody which cross-reacts with ovine fibronectin (data not shown). Its epitope is located on the 120kDa cell binding fragment. Fibronectin conformation on surfaces was also examined by ELISA using A17.

Results and Discussion

The cellular response to silica-coated substrates is shown in Figure 1. ARPE-19 cells spread and proliferated in clusters forming a sheet of cells with typical 'cobblestone' morphology. On the silica coated portion of the surface distinct rounded clusters of cells could be seen that were poorly spread.

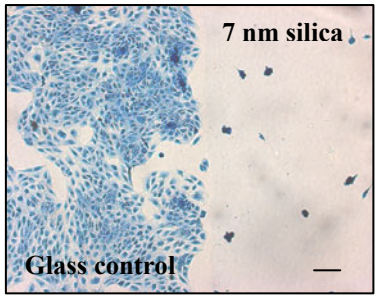

Figure 1. Optical image of ARPE-19 cells on glass partially coated with 7 nm silica after 7 days in cell culture [Scale bar = 30 µm].

Each of the substrates experienced comparable levels of fibronectin adsorption (Figure 2). A17 binding to the fibronectin on colloidal silica treated surfaces (7, 14 and 21nm) was significantly reduced in comparison with the fibronectin on control silica and oxidized polystyrene ($P<0.01$). Reduced A17 binding to fibronectin on colloidal silica treated surfaces in comparison with oxidized polystyrene was verified by ELISA (data not shown). This indicates that fewer cell-binding domains are available on the fibronectin pre-coated colloidal silica treated surfaces in comparison with the control silica and oxidized polystyrene.

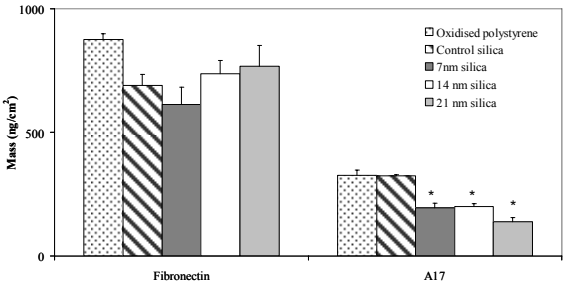

*Figure 2. Amount of fibronectin adsorbed to substrates and A17 adsorbed to fibronectin and casein pre-coated substrates. Error bars: SD (n=4). * = t test, P<0.01 relative to control silica and oxidized polystyrene.*

Conclusions

Conformation of attached proteins is critical for biological function. Creation of surface nanotopography using a range of colloidal silica sizes leads to a distinctive cellular response. The surface treatment is able to inhibit the spreading and proliferation of cells, which are round in morphology and weakly attached to the substrates, but remain viable. This is attributed to a change in the conformation of fibronectin leading to the cellular adhesion site being no longer available.