European Cells and Materials Vol. 2. Suppl. 1, 2001 (page 64-65) ISSN 1473-2262

FIBROBLAST MORPHOLOGY AND ADHESION ON CALCIUM PHOSPHATE SURFACES.

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INTRODUCTION: Studying the morphology and adhesion of cells on different metal surface coatings can give an indication as to the biocompatibility of the coating and its suitability for possible further applications on orthopaedic implants. The adhesion of either soft or hard tissue cells to implant materials is essential to the success or failure of an implant and is also important for the prevention of infection. Implant surface topography and chemistry are important factors in the adhesion of cells to surfaces.

The adhesion and morphology of fibroblast cells was studied on titanium discs coated with either: calcium phosphate, deposited using anodic plasma-chemical treatment (APC) at current densities of 200mA and 290mA; phosphoric acid (H_3PO_4) at temperatures of 25ºC and 75ºC; hydroxyapatite; anodisation at 57V. Thermanox culture plastic discs were also investigated.

METHODS: Balbc/3T3 fibroblasts were used throughout this study and were cultured in DMEM containing 10% foetal calf serum at 37ºC. Approximately 20,000 cells were seeded onto each sample 24 hours before fixation and immunolabelling. All the fixation and labelling protocols were conducted at room temperature (22ºC).

The cells used for morphological studies were fixed in 2.5% glutaraldehyde and were post-fixed in 0.5% osmium tetroxide. The cells were dehydrated through an acetone series, critical point dried, coated in chromium (10nm) and imaged with a Hitachi S-4700 Field Emission Scanning Electron Microscope.

Immunolabelling was used to identify adhesion areas. Cells were permeabilised with 0.1% Triton X-100 (1 minute) and fixed with 4% paraformaldehyde (5 minutes). Non-specific binding sites were blocked (30 minutes) and the vinculin (an integral focal adhesion protein) immunolabelled using an indirect labelling method with the secondary antibody attached to 5nm gold labels (primary antibody 1 hour and secondary antibody 2 hours). The cells were fixed with 1% glutaraldehyde (5 minutes). Gold enhancement was performed using a kit from Nanoprobes for 7 minutes. The cells were post-fixed with 1% osmium tetroxide (1 hour), dehydrated through an acetone series and critical point dried before coating and imaging. After imaging samples were embedded in LR White resin. The resin was polymerised and separated from the discs by rapid cooling, on a copper block immersed in liquid nitrogen, so that the cells remained in the resin. The undersurface of the cells was then directly imaged within the resin using backscattered electron imaging.

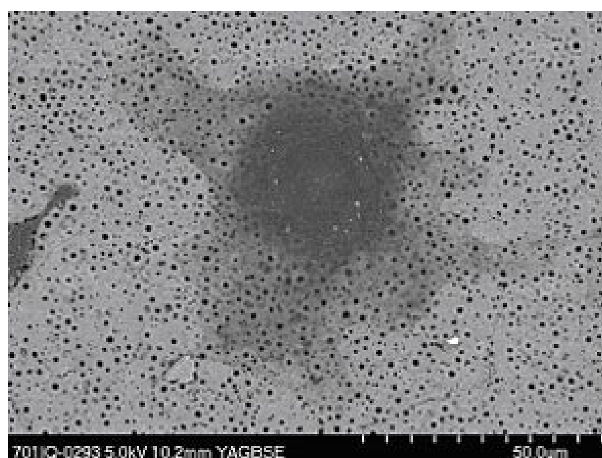

Fig 1. A well spread out fibroblast on the calcium phosphate (APC) surface (anodic plasma-chemical treatment at 75ºC).

Fig 2. Fibroblast attached to the phosphoric acid surface, which is not spread and is slightly raised compared to cells on the APC surface.

RESULTS: Studies of the cells on the different surfaces revealed variations in the morphology of the cells. Cells on the anodised titanium, Thermanox plastic and the calcium phosphate (APC) surfaces were very spread out, especially on the calcium phosphate (APC) surfaces On these APC surfaces the cells were extremely flat and extremely spread with few features on the surface of the cells. On the APC surface the majority of the cells tended to have very few or no filopodia.

The cells on the hydroxyapatite and the phosphoric acid surfaces were much less spread with numerous filopodia and many folds and blebs on their surface. They were closer to the expected size of fibroblasts but did not look as if they were comfortable i.e. their ventral surface was raised from the surface.

There were no observable differences between the two calcium phosphate (APC) surfaces. The cells on the phosphoric acid surfaces also did not appear to be noticeably different from each other.

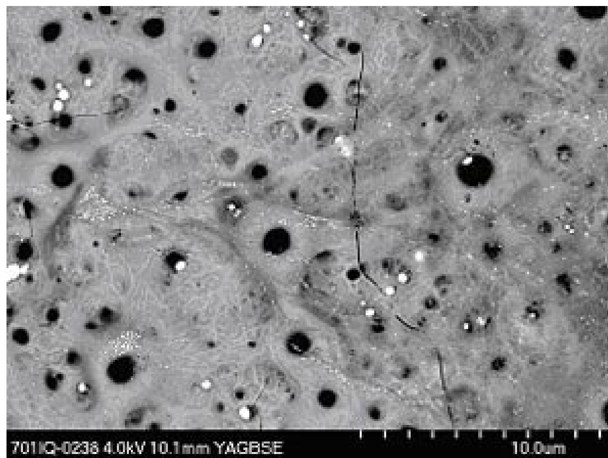

Fig 3. The periphery of an immunolabelled fibroblast on the calcium phosphate (APC) surface showing the gold label attached to the adhesion sites.

Cell adhesion sites were seen on all the surfaces at the cell periphery using immunolabelling. The adhesion sites for cells on the calcium phosphate (APC) surface appeared to be situated on and around the rough areas on the "volcanic like" structures. There were no particular areas of preference that could be seen for the other surfaces. Qualitatively "by eye" there were more adhesion sites on the APC surface than the other test surfaces (not including the Thermanox or anodised titanium).

DISCUSSION & CONCLUSIONS: The morphology and the adhesion of the cells indicate that the fibroblasts prefer the calcium phosphate (APC) surface to the other test surfaces (hydroxapertite and phosphoric acid surfaces) and show similar morphologies to the cells on the Thermanox plastic and the anodised titanium discs. However, the cells used for this study are fibroblastic, soft tissue cells, which are known to spread more on smoother surfaces[1]. For osteoblast proliferation and differentiation in an *in vivo* situation a three-dimensional matrix structure is essential[2, 3]. Therefore an *in vitro* osteoblast adhesion study should also be undertaken on these surfaces. *In vivo* studies are currently being undertaken to assess the adhesion and osteointegration of all the surfaces when applied to screws for orthopaedic use.

The cells do adhere to all the surfaces and show adhesion patterns on and around the periphery of the cells. The adhesion sites for the calcium phosphate (APC) surface appear to be located on the rougher surface texture indicating that the actual adhesion of the cells may be related to the micro-topography[4]. Quantitative analyses of the adhesion sites are currently being conducted.

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ACKNOWLEDGEMENTS: We would like to acknowledge Mathys Foundation and Stratec Medical KTI/Medtech 4729.1 for the implant materials and funding.