FIBRONECTIN SILANIZED TO TITANIUM ALLOY : A BIOINDUCTIVE SURFACE FOR DERMAL FIBROBLASTS

INTRODUCTION: Bone-anchored transcutaneous implants have been shown to fail due to epithelial downgrowth, infection and avulsion (1,2). A stable skin-implant interface is promoted by increasing dermal fibroblast attachment(3). Fibronecin (Fn), an extracellular matrix glycoprotein, has been shown to enhance fibroblast cell growth and proliferation when adsorbed to titanium(4,5) and this may improve dermal attachment and reduce infection in bone-anchored transcutaneous implants. Adsorbed fibronectin is subject to desorption as described by Vroman(6). Silanisation covalently bonds proteins to titanium surfaces. Our hypothesis is that Fn silanized to titanium alloy (Ti) will provide a more durable, bioinductive surface for fibroblast adhesion and spread when compared with polished Ti control and polished Ti with adsorbed Fn. We postulate these surfaces will potentially improve the skin-implant interface for Intranasal Transcutaneous Amputation Prothesis (ITAP).

MATERIALS AND METHODS: 10mm Ti discs were polished and cleaned to orthopaedic implant standard. For silanization, discs were treated at 25°C for 2hrs with a 1:1 solution of H2SO4: H2O2(30%), placed in acetone containing 10% (3 aminopropyl) triethoxysilane (APS) for 2hrs at room temperature and dried overnight at 37°C. Fn was placed on the disc surface. The amount of Fn silanized was optimized by using 125I labelled Fn. To establish the response of the protein. Fn was placed on the disc surface. The amount of Fn silanized was optimized by using 125I labelled Fn. To establish the response of the silanized surface to body fluid passivation, silanized Fn was compared to adsorbed Fn when soaked in foetal calf serum (FCS) for 5mins, 3hrs, 20hrs and 144hrs.

In vitro studies used immortalized dermal fibroblasts (Fb) at a seeding density of 2500 cells/disc. Silanized Fn Ti discs were compared with polished Ti discs, +/− adsorbed Fn. Fb were cultured for 1,4,24 and 96hrs, fixed in formal saline and vinculin stained (2hrs with 1:100 mouse vinculin antibody; 1hr with 1:100 FITC mouse antibody). Images were taken using a Zeiss microscope linked to image analysis software. Cell area and the number of vinculin markers were measured per cell. SEM was performed for cell morphology. N=6 was used for all experiments.

RESULTS: All results are expressed as median (95% confidence intervals). The optimum method for covalent coupling was addition of Fn within 20hrs of silanization for a duration of 4hrs or more. 4000ng of Fn were needed to gain the optimum amount silanizable -1616ng Fn (AdFn) in FCS.

Adsorbed Fn: Cell area 1693µm² (1422-1697) and vinculin/cell area 0.0639 (0.0538-0.0738). Similar patterns were observed at 4, 24 and 96hrs.

In vitro at 1hr cells on polished discs were significantly smaller (392µm² (299-502)) with fewer vinculin markers/cell area (0.0083 (0.0057-0.0099)) than all other groups (all p values < 0.05). There was no significant difference in cell area (p=0.24) or vinculin markers/cell area (p=0.18) between the discs with adsorbed Fn and silanized Fn. Adsorbed Fn: Cell area 1693µm² (1422-1879) and vinculin/cell area 0.0582 (0.0527-0.0625. Silanized Fn: Cell area 1386µm² (1204-1697) and vinculin/cell area 0.0639 (0.0538-0.0738). Similar patterns were observed at 4, 24 and 96hrs.

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ACKNOWLEDGEMENTS: This study was supported by Stanmore Implants Worldwide Ltd.