Functionalized fibronectin and RGD Titanium alloy surfaces used for Intraosseous Transcutaneous Amputation Prostheses *in vitro*

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Statement of Purpose: Intraosseous Transcutaneous Amputation Prostheses (ITAP) provide an alternative means of attaching artifical limbs to the body[1,2]. ITAP are bone anchored and penetrate the skin and their success relies upon a tight seal at the skin-implant interface to prevent infection[1,2]. Functionalising a titanium alloy surface with covalently tethered fibronectin (fn) has been shown to increase cell atttachment strength of human dermal fibroblasts (HDFs) *in vitro*. Fn contains the RGD (Arg-Gly-Asp) integrin-binding domain[3]. The use of specific RDG sequences may be justified compared with fn due to regulatory issues, sterilization protocols and the number of attachment sites available. Our aim was to assess cell attachment strength, metabolic activity and morpholgy of HDFs cultured on these functionalised surfaces of either fn or RGD. **We hypothesise that HDFs cultured on functionlaised fn and RGD coated titanium surfaces will produce larger, flatter cell morphology, higher levels of metabolic activity and be more strongly attached than on polished controls .**

Methods: Titanium alloy discs (10mm diameter) were prepared to orthopaedic implant manufacturing standards (roughness average = 0.03um). Fn coatings were applied at a concentration of 0.1mg/ml (most biologically active[4]) using adsorbed (ad) or silanization (si) methodologies[4]. RGD coatings were prepared in the same manner at a concentration of 7mM/disc. Polished titanium discs acted as a control surface. HDFs were seeded onto the substrates at 20,000 cells/disc, cultured for 1, 4, 24 or 96h[4]. Fluorescent immunolocatisation of vinculin was performed and focal adhesion (FA) density calculated and used as a measure of cell attachment. Cell metabolism was assessed using Alamar Blue assays and surfaces were characterised under SEM. An n of 6 was used and data were analysed in SPSS, results were considered significant at the p= 0.05 level.

Results: The cell attachment data presented in Fig 1 shows FA density was significantly greater on all coated surfaces (either ad or si) compared with polished controls and at 4hrs (p values < 0.05). Comparisons of cell attachment between sifn and siRGD showed no significant difference in FA Density at all time points (p>0.05) with the exception of the 4h assay, where sifn coated surfaces saw a significant increase compared with siRGD coated surfaces (p<0.001). (Fig1).

Metabolic activity of all surfaces significantly decreased between 1h and 4h time points (p =<0.05), followed by a significant increase between 4h and 24h, (all p values + <0.05). Polish surfaces continued to show a significant increase in metabolic activity at 96h (p =<0.001) but both si coated surfaces showed significant deceases in metabolic activity at the 96h time-point (p<0.001), these values were also significantly lower compared with those from polished surfaces (p<0.05). Si coated surfaces (both fn and RGD) significantly increased cell metabolism compared with uncoated controls at 1, 4 and 24h (all p values = <0.05, except the siRGD 4h assay (p=0.406). Comparisons between the coated surfaces showed no pattern. At 1h, there was no difference observed in cell metabolism between sifn and siRGD. At 4h siRGD coated surfaces significantly increased cell metabolism compared with sifn (p<0.001) but at 24h sifn significantly increasing cell metabolism compared with siRGD, p<0.001 (Fig 2).

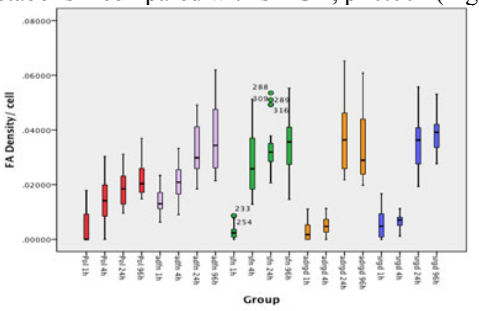

Fig 1. Box plot of FA density on adfn (purple), sifn (green) and adRGD (orange), siRGD (blue) compared with polished controls (red) over time.

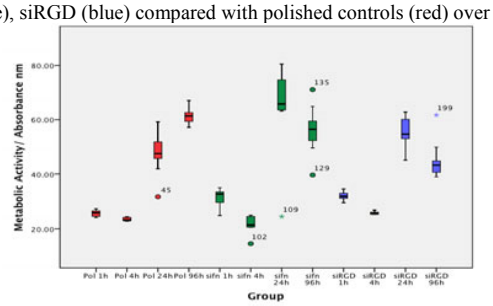

Fig 2. Box Plot demonstrating HDF Metabolic Activity on sifn (green), siRGD (blue) with polished surfaces as controls (red) over time.

SEM analysis allowed us to assess HDF morphology. Images showed larger, flatter cells on si coated surfaces compared with polished surfaces. More filapodia projections were visable on these surfaces interacting with the metallic surface and neighbouring cells.

Conclusions:

Dermal fibroblasts were attached to a greater degree on si coated surfaces, cells were larger, flatter and well spread. Metabolic activity of those attached cells was significantly higher than cells on polished surfaces. The use of RGD in a functionalised titanium alloys may be justified as this study indicates that attachement and proliferation is comparable to that of fn surfaces. It may be possible to use an RGD functionalized titanium alloy surface that will stabilize the skin seal at the transcutaneous portion of an implant, which will enhance the resistance to infection *in vivo*.

References:[1] Pendegrass CJ, *et al* J. Anat. 2006;209:59-67 [2] Pendegrass CJ, *et al* Biomaterials;27:4183-4191 [3] Verrier, S., *et al* Biomaterials 2002;23:585-596 [4] Middleton CA, *et al* JBMR 2007;83(A):1032-8.

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