Fibronectin Functionalized Hydroxyapatite Coatings: Improving soft tissue adhesion to a transcutaneous osseointegrated implant in Vitro and in Vivo

INTRODUCTION

Most amputees encounter complications with artificial limb attachment, ranging from mild skin irritation to complete limb disuse. Bone anchored prostheses overcome these problems by attaching directly to the skeleton, giving amputees increased osseoperception and proprioception. However, infection due to a lack of soft tissue attachment at the transcutaneous interface results in a failure rate of approximately 25%. Intraosseous transcutaneous amputation prostheses (ITAP) reduce infection rates by creating an infection resistant seal at the skin-implant interface. Fibronectin (Fn) is known to enhance fibroblast adhesion, whilst hydroxyapatite (HA) coating of transcutaneous devices has been shown to enhance dermal fibroblastic attachment in vivo. This study assesses the effects of Fn functionalized HA coating on dermal fibroblastic cell and tissue adhesion in vitro and in vivo respectively. We hypothesise that Fn adsorbed onto an HA coated surface will increase cell adhesion and that soft tissue ingrowth into porous HA will be effected not only by the pore size but also by adsorption with fibronectin.

METHODS

In Vitro Study: Human dermal fibroblasts were cultured on polished titanium alloy (Pol), fibronectin coated Pol (PolFn), HA and Fn functionalized HA (HAFn) 10mm diameter discs, at a seeding density of 2500 cells per disc for 1, 4 and 24 hrs. 1000ng of human plasma Fn was adsorbed onto the Pol and HA discs in 50µl droplets of PBS for 1hr prior to cell seeding. Cell attachment was measured by immunolocalisation of the vinculin component of focal adhesions (FAs) and image analysis was used to quantify the number of FAs per unit cell area for 30 cells/disc (n = 6 for each experimental group).

In Vivo Study: Six skeletally mature female ewes were used. 10 titanium alloy rectangular flat plates, 24x8x1mm (with rounded corners and a 2.8mm diameter hole at either end) were surgically implanted bilaterally (5 per tibia) into the medial aspect of the tibia. Coatings and pores were positioned in a 10x5mm region over the centre of each plate. The implants were raised from the underlying bone surface with 2mm high washers to enable full soft tissue ingrowth. Soft tissue attachment was assessed by measuring soft tissue ingrowth (% contact) and cell orientation at the tissue-implant interface. 70-100µm thick sections were prepared using hard grade resin histology and were analysed using image analysis. For cell orientation, a semi-quantitative score from 1-5 was used based on the shape of the cell nucleus (5 indicating nuclei parallel, and 0 perpendicular to the implant surface). Plates were divided into 10 regions and 3 cell nuclei within each region were scored. Qualitative histological analysis was also performed.

RESULTS

In Vitro Study: At all time points, HAFn significantly increased cell attachment compared with HA and Pol controls (p < 0.05) respectively. At 24hrs, no differences between Pol, HA and PolFn were observed. Based on the number of adhesion plaques per cell, at 4hrs, HAFn supported a 4.9, 4.6 and 3.8 fold increase in cell attachment compared with Pol, PolFn and HA respectively. At 24hrs, 8.2, 6.8 and 7.4 fold increases were observed. Figure 1 shows the appearance of the cells on the surfaces at each time point.

In Vivo Study: Encapsulation of solid controls was observed (Fig 2A). Clear gaps between the tissue and the internal pore margins were observed for all the 0.5mm pores irrespective of coatings (Fig 2B), with HA and HAFn having no significant effect on % contact or cell alignment (Fig 3). 0.7mm pores significantly increased dermal attachment (Fig 3), with bands of tissue fully traversing the pores in all sections (Fig 2C-E). 0.7mm HA significantly increased % contact and nuclei orientation scores; indicating dermal integration as opposed to encapsulation. 0.7mm HAFn significantly increased dermal integration further (Fig 2C-E & 3). Pegs of dermis not completely traversing the pores with one sided dermal attachment was observed around 1mm pores irrespective of the coating regime (Fig 2F). 0.7mm HAFn significantly increased dermal ingrowth and % contact compared with all other implants.

CONCLUSION

In vitro, Fn functionalisation of HA results in a significant increase in dermal fibroblastic attachment, with a maximum 8 fold increase compared with polished titanium alloy controls. In vivo, 0.7mm pores were shown to be optimal for dermal tissue ingrowth and attachment compared with 0.5mm and 1mm pores. Fn functionalized HA coating of implants with 0.7mm pores significantly further increased dermal attachment and ingrowth.

HAFn coating 0.7mm pores could be applied to transcutaneous osseointegrated implants to create an infection resistant seal at the dermal tissue-implant interface.

REFERENCES