The Intraosseous Transcutaneous Amputation Prosthesis (ITAP): Optimization of fibroblast adhesion to fibronectin silanized titanium alloy

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INTRODUCTION

Osseointegrated transcutaneous amputation prostheses avoid soft tissue complications commonly associated with traditional socket prostheses and allow improved function due to increased sensory feedback (osseoperception). However, approximately 50% of implants become infected due to the lack of a successful skin-implant interface. Intraosseous Transcutaneous amputation prostheses (ITAP) are designed to create a tight dermal attachment at the skin-implant interface which is necessary to prevent epithelial downgrowth and subsequent infection.

Cells attach to fibronectin via its Arg-Gly-Asp-Ser (RGD) sequence which binds integrins. In vitro studies have shown that fibronectin adsorption enhances fibroblast adhesion; however, in vivo, fibronectin becomes desorbed from the implant surface due to protein competition. Covalent attachment of fibronectin by silanization has been shown to be durable in vitro. The silanization process for fibronectin is preceded by a stage of passivation with sulphuric acid and hydrogen peroxide which alters surface characteristics. Radiolabelling studies have shown that passivation reduces the amount of fibronectin that may be silanized to titanium alloy.

The aim of this study was to determine if in vitro fibroblast adhesion to silanized fibronectin (SiFn) titanium alloy could be improved by omitting or reducing the length of the passivation stage. Additionally, the study aimed to assess the effect of silanized and adsorbed fibronectin substrates on dermal tissue attachment in vivo, compared with uncoated controls.

METHODS

The surfaces tested in vitro were polished (Pol), silanized only (Si), passivation for five minutes (5Pass), passivation for two hours (120Pass), silanization following passivation for five minutes (5PassSi), silanization following passivation for two hours (120PassSi), adsorbed fibronectin (AdFn), silanized fibronectin (SiFn), silanized fibroenctin following five minutes passivation (5PassSiFn), silanized fibronectin following two hours passivation (120PassSiFn).

Scanning electron microscopy and Ra profilometry (n=6) were used for topographical characterization of surfaces. Contact angle measurement (n=6) was performed to study the wettability of surfaces. Anti-vinculin antibodies were used to immunolocalize fibroblast adhesion sites and to assess cell areas on modified SiFn surfaces and controls after 24 hours. The morphology of fibroblasts on each surface was evaluated using scanning electron microscopy.

An in vivo ovine model was used to evaluate the performance of the SiFn surface without passivation compared to simple adsorption of fibronectin. The study was performed in accordance with UK Home Office Animals Scientific Procedures Act 1986. 2cm x 1cm titanium alloy plates were implanted bilaterally onto the tibiae of an ovine model (n=3). The surfaces tested in vivo were Pol, AdFn, Si, SiFn, HA (hydroxyapatite) and HAFn (hydroxyapatite with fibronectin). The plates were attached to the medial aspect of the tibial bone using cortical screws positioned subcutaneously using spacers to raise the plate off the underlying bone surface. HA and HAFn were also tested because HA coatings are currently applied to the dermal section of ITAP in clinical trials. After four weeks, percentage soft-tissue attachment and cell alignment relative to the implant were evaluated using high-grade resin histology. Data was analyzed using SPSS (version 14.0 for windows, Chicago, IL). Data were considered significantly different if p<0.05.

RESULTS

Passivation produced rougher, more hydrophobic surfaces with numerous microcracks and was associated with poorer fibroblast adhesion and spreading than un-passivated controls in vitro. Figure 1 demonstrates the appearance of cells on a silanized substrate with and without passivation.

The addition of fibronectin to surfaces, irrespective of coating regime, resulted in significantly better cell adhesion and larger cell areas (p<0.05). However, it did not completely overcome the detrimental effect of passivation on fibroblast adhesion. SiFn with passivation resulted in poorer cell adhesion than SiFn without passivation. Reducing the time period for passivation did not remove the detrimental effects of passivation. Cell adhesion data is presented in Figure 2. Both contact angles and surface roughness showed a negative linear correlation with fibroblast adhesion (p<0.000 and p=0.002 respectively). The number of vinculin markers increased with increasing cell area. (p=0.000)

Figure 1: Scanning electron micrographs of fibroblasts on a silanized surface (Si) and a silanized surface following passivation (120PassSi). Microcracks are seen on the passivated surface and there is reduced cell spreading.

Figure 2: Box plot showing the numbers of vinculin markers per cell for the surfaces tested in vitro.

In vivo, HAFn and SiFn resulted in higher median values for soft-tissue attachment than simple adsorption of fibronectin; however, the differences were not statistically significant. Cell alignment was significantly better for HAFn and SiFn than for controls (p<0.05).

DISCUSSION

Omission of the passivation stage improves fibroblast adhesion to SiFn surfaces in vitro. Reducing the time period for passivation does not significantly reduce the detrimental effects that passivation has on fibroblasts. A time course may be of value to determine if fibronectin coatings (silanized or otherwise) are lost over time in vivo.

REFERENCES