Dual Coating Proteins Silanized to Titanium Alloy: A Durable Substance enhancing Fibroblast Growth and Adhesion

INTRODUCTION

Intraosseous transfemoral amputation prostheses (ITAP) provide an alternative means of attaching artificial limbs for amputees [1]. Conventional stump-socket devices are associated with soft tissue complications including; pressure sores and tissue necrosis [2]. ITAP resolves these problems by attaching the exo-prosthesis transfemorally to the skeleton. Other transfemoral amputation prosthetics are limited by infection [3,4], however ITAP aims to overcome this by creating an infection-resistant transfemoral seal. Previous work has demonstrated that early dermal attachment prevents epithelial downgrowth and infection [5], hence the aim of this study is to increase the attachment of dermal fibroblasts to titanium alloy in vitro. Fibronectin (Fn) and laminin 332 (Ln) enhance early cell growth and adhesion [6,7]. Covalent bonding of those proteins to titanium alloy (Ti) through silanization has shown to improve cell attachment [7]. We hypothesize that silanized dual coatings of fibronectin and laminin (SiFnLn) will be more durable when compared with adsorbed dual coating (AdFnLn), and will enhance fibroblast growth and adhesion compared to single coatings (AdFn, AdLn, SiFn, SiLn).

METHODS

The kinetics of dual single and dual protein coating attachment onto titanium alloy (Al 6%, V 4%) was quantified on silanized 10mm diameter discs using radiolabelled Fn (125I-Fn) and Ln (125I-Ln). 60 discs were polished, sterilized and silanized with 636.62 ng/cm² of 125I-Fn, 125I-Ln, 125I-Fn+Ln or 125I-Ln+Fn (n=3). Coating durability was assessed when soaked in fetal calf serum (FCS) for 0, 1, 24, 48 and 72hrs. Data was compared to un-silanized Ti discs with the same coatings. Five thousand human dermal fibroblasts were seeded on discs (n=6) of Ti polished alone (Pol), Ti with adsorbed fibronectin (AdFn), Ti with adsorbed laminin (AdLn), Ti adsorbed dual coating (AdFnLn), Ti silanized (Si), Ti silanized with fibronectin (SiFn), Ti silanized with laminin (SiLn), Ti silanized with a dual coating (SiFnLn) for 24hrs. Cells were fixed, vinculin stained using mouse monoclonal antibody (1:200) for 2hrs and alexa fluor (1:100) for 1hr. Axiovision Image Analysis software was used to measure cell area, vinculin markers per cell and per unit cell area. Data was analysed in SPSS and significance was assumed at the 0.05 level. The data presented are median values with 95% confidence intervals.

RESULTS

Silanized dual coatings bonded to Ti alloy in significantly larger quantities compared with adsorbed coatings at all time points (Table 1) (all p values < 0.05).

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<th>Silanized discs</th>
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<tr>
<td>125I-Fn</td>
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Table 1. Median amount of protein attached to Ti (ng/cm²) over time

* Dual coating proteins in which Fn was radioactively labelled and measured. + Dual coating proteins in which Ln was radioactively labelled and measured.

CONCLUSION

This study has demonstrated that covalently bonding both fibronectin and laminin to Ti alloy provides a durable dual coating that enhances early fibroblast growth and attachment compared with both protein coating alone in vitro. Our study showed that there is non-competitive binding of laminin on Ti surfaces in the presence of fibronectin. Dual coatings may be applied to the skin-penetrating region of transfemoral devices to improve the skin seal and this may have positive implications for the development of ITAP.

REFERENCES


ACKNOWLEDGEMENTS

This study was supported by Stanmore Implants Worldwide and the National Institute of Health.