

Dual Coating Proteins Silanized to Titanium Alloy: A Bioinductive Surface for Fibroblasts

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Introduction: Intraosseous transcutaneous amputation prostheses could solve stump-socket complications for amputees [1]. Early dermal attachment is essential for a seal that prevents epithelial downgrowth and infection [2], hence the aim of this study is to increase attachment of dermal fibroblasts to titanium alloy (Ti) *in vitro*. Fibronectin (Fn) and laminin 332 (Ln), enhance early cell growth and adhesion [3,4]. Covalently bonding each to titanium alloy (Ti) by silanization has shown to improve cell attachment [2]. We hypothesize that silanized dual coatings of Fn and Ln (SiFnLn) will provide more durable bioinductive surfaces for fibroblast adhesion compared with silanized Ti controls and single coating proteins.

Methods: Ten millimetres diameter Ti discs were polished, sterilized and silanized by immersing in 10% aminopropyltriethoxysilane for 2 hrs followed by 1% glutaraldehyde for 2 more hrs. Five hundred nanograms of dual coating proteins FnLn and single coating protein were added to the surface. Five thousand human dermal fibroblasts were seeded on the discs (n=6): silanized (Si), silanized fibronectin (SiFn), silanized laminin (SiLn), silanized dual coating (SiFnLn) for 1, 4 and 24hrs. Cells were fixed, vinculin stained using mouse vinculin 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: Fibroblasts cultured on silanized dual coatings were significantly larger, produced more vinculin markers per cell, and per unit cell area compared with single coatings at all time points (Table 1 and Figures 1 & 2).

		Si	SiFn	SiLn	SiFnLn
Cell area	1 hour	593.9	1384.1	1185.3	1769.8
	4 hours	735.5	1705.6	1566.3	2049.8
	24 hours	955.2	1999.9	1824.0	2477.9
Vinc/ cell unit	1 hour	6	77	57	131
	4 hours	11	102	79	164
	24 hours	15	136	104	220

Table 1. Median cell area and vinculin markers/cell unit at 1, 4 and 24 hours

Conclusion: This study has demonstrated that silanized dual coating proteins on Ti alloy enhance early fibroblast growth and attachment compared with single coatings *in vitro*. This may lead to improved dermal attachment to ITAP which will prevent epithelial downgrowth and subsequent infection.

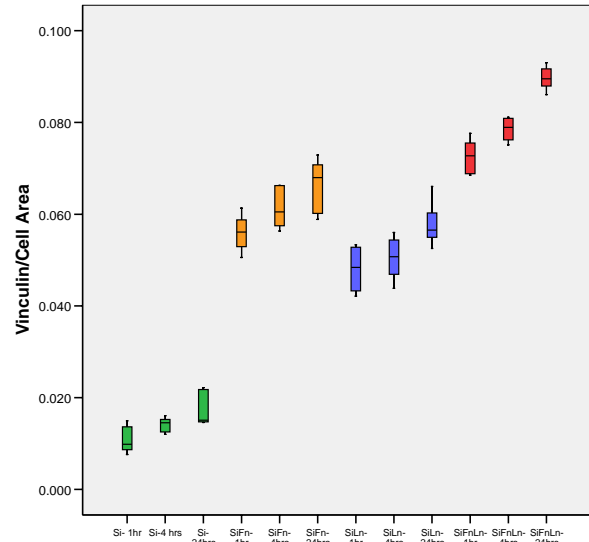


Figure 1. Box Plots showing vinculin markers/ cell area at 1, 4 and 24 hours

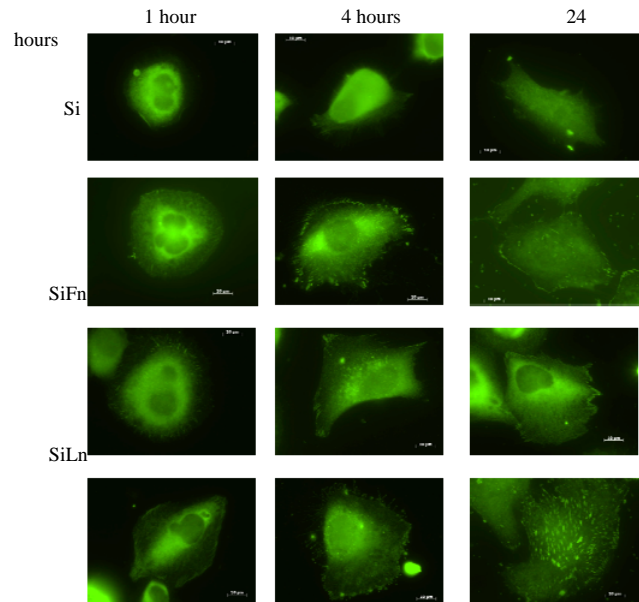


Figure 2. Fibroblasts cultured at 1,4 and 24 hrs on single and dual coating protein surfaces

References: [1] Pendegrass CJ. Biomaterials 2006. [2] Pendegrass CJ. J Biomed Mater Res A 2010. [3] Cooke CA. Invest Ophthalmol Vis Sci 2006. [4] Gordon DJ. J Biomed Mater Res Part A: 2010. (Article in Press)

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