Fibronectin Silanized Titanium Enhances Fibroblast Attachment.

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Introduction: Intraosseous transcutaneous amputation prostheses (ITAP) could provide a solution to stump-socket complications for amputees[1]. Early dermal cell attachment is vital to produce a barrier to infection and prevent epithelial downgrowth leading to marsupilization and avulsion[2]. Fibronectin (Fn) is known to enhance fibroblast attachment to titanium *in vitro*[3], however it is postulated that the Fn is desorbed by competition from serum proteins *in vivo*[4]. Silanization covalently bonds Fn to titanium alloy (Ti). **We hypothesize that silanized Fn (SiFn) will be more durable when soaked in protein rich fluid compared with adsorbed Fn (AdFn), and will enhance fibroblast attachment compared with uncoated Ti.**

Methods: A two-stage silanization protocol was used to covalently couple Fn to polished discs (Pol). Fn was added to the silanized (SiFn) or untreated discs (AdFn) in a 70µl droplet. Quantification with ^{125}I-labelled Fn was used to optimize the silanization of Fn and assess the durability of the Fn coating when soaked in FCS for 5mins, 3, 20 and 144hrs. Data were compared with AdFn. Human dermal fibroblasts (Fb) were seeded on Pol, AdFn and SiFn for 1, 4, 24 and 96 hours before fixing with formal saline and vinculin staining (mouse vinculin antibody (1:200) 2 hrs; FITC mouse antibody (1:100) 1 hr). Images were analyzed with Zeiss microscope linked to image analysis software and the number of vinculin markers were counted per cell area. Cell morphology was analyzed with SEM. 6 replicate samples were performed for all analyses.

Results/Discussion: Results are expressed as median values. Addition of 4000ng Fn prior to 20hrs, and left for 4hrs on silanized substrates achieved optimal Fn binding. A maximum of 15.5ng of Fn per mm^2 was achieved. Silanized discs attached significantly more Fn ($p<0.005$) than untreated Ti (SiFn=32.23ng, AdFn=13.67ng). On soaking in FCS, there was no significant loss of Fn from SiFn discs ($p=0.58$), but a 44% loss of Fn from AdFn ($p=0.002$)(Fig 1).

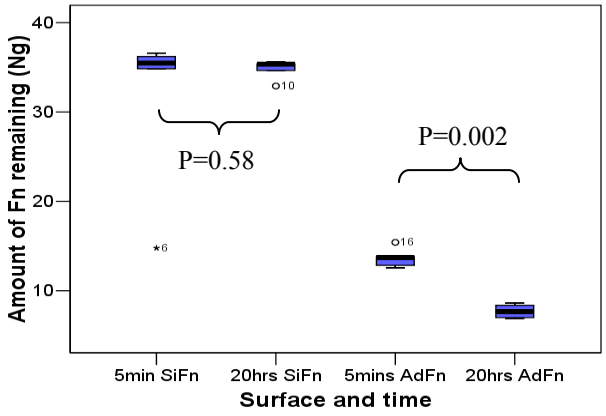

Figure 1. Amount of Fn remaining after soaking 100ng of silanized Fn (SiFn) or adsorbed Fn (AdFn) in FCS for 5mins or 20hrs.

Fb cultured on Pol were significantly smaller and produced fewer vinculin markers at all time points compared with SiFn ($p<0.05$). SiFn and AdFn both produced large flattened cells with numerous vinculin markers at 1hr and continued to increase in size and number of vinculin markers across all time points (Fig. 2 & 3). At 1hr, cell area was 392.28µm^2, 1386.39µm^2, and 1692.54µm^2 on Pol, SiFn and AdFn respectively.

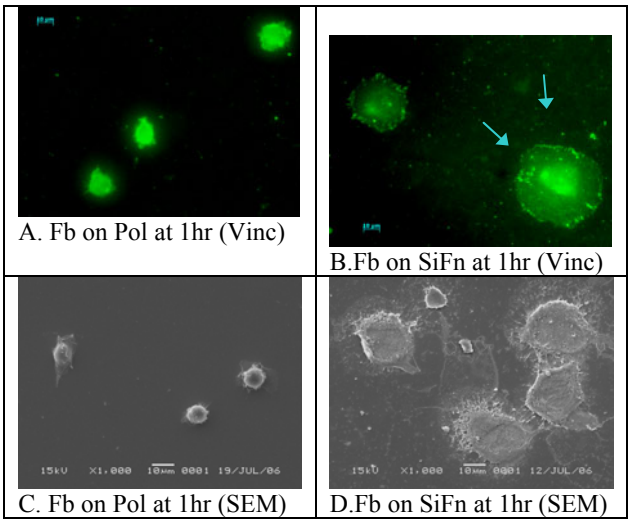

Figure 2. Fb cultured for 1hr on Pol (A) and SiFn (B) and vinculin stained (Vinc)(Arrows show vinculin staining of focal contacts). SEM of Fb cultured for 4 hrs on Pol (C) and SiFn (D).

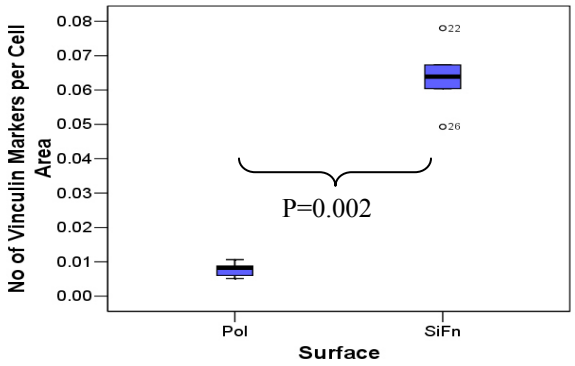

Figure 3. Vinculin markers/cell area at 1hr on Pol and SiFn.

Conclusions: This study has demonstrated a method to attach fibronectin to titanium alloy which is both durable in protein rich fluid and induces earlier and greater fibroblast attachment. It is postulated that this may lead to improved dermal attachment to ITAP *in vivo*.

References:

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