Loading and Release Kinetics, and Fibroblast Attachment to Fibronectin Functionalised HA *In Vitro*.

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Introduction: Attachment of artificial limbs to the body is clinically challenging due to soft tissue complications that often render prostheses redundant. Osseointegrated transcutaneous implants are being developed so that the external prostheses can attach directly to the skeleton however; complications associated with the development of a microbiological seal at the soft tissue interface have not be solved.[1] Hydroxyapatite (HA) and fibronectin (Fn) have been shown to improve dermal fibroblast attachment to titanium alloy[2,3] and their use may enhance soft tissue attachment. This study determines the optimal loading and release kinetics of Fn on HA and the effect of the coatings on dermal fibroblast attachment.

Methods: Fn Loading, Release and Durability Kinetics: 10 mm sintered HA discs were used. Detection of gamma radiation in counts per minute (CPM) of ^{125}I-Fn was used to determine quantities of Fn on HA. A standard calibration curve up to 1000ng of Fn was produced to calculate the results of the loading and release kinetics. Fn was adsorbed onto the discs in 50µl droplets of sterile PBS and all assays run in triplicate. To investigate the optimal duration for loading Fn on HA, 500ng of ^{125}I-Fn was placed on disc surfaces for 0, 0.5, 1 and 2hrs prior to analysis. 100, 250, 500, 1000 and 1500ng of ^{125}I-Fn were assayed to determine the max coating concentration. To assess coating durability, ^{125}I-Fn was added to discs, and after washing in PBS, immediately analysed. Other discs after washing were immersed in foetal calf serum (FCS) and incubated at 37^{o}C prior to 1, 4, 8 and 24 hrs analyses.

Dermal Fibroblast Attachment Experiments: 10mm titanium alloy control discs (Pol) were compared with HA discs (HA). 1000ng of Fn was applied to the HA discs (HAFn) for 1hr, as established from the loading studies. 2500 Dermal fibroblasts (1BR.3) were seeded for 1, 4 and 24hrs on Pol, HA and HAFn discs prior to analysing the number of vinculin markers per unit cell area. 6 replicates and 15 randomly selected cells were analysed per disc.

Results: The optimal time for binding was 1hr, at which the maximum binding quantity of Fn was 1000ng (Fig 1). There were no significant differences in the amount of Fn remaining on the HA discs with initial loading quantities in excess of 1000ng ($p<0.05$) (Fig 1). With 1000ng loading concentration over 1hr, a significant decrease in Fn coupled to HA was observed within the first hour and between 8 and 24hrs only ($p<0.05$) (Fig 2) with the amount of Fn at 24hrs being to one fifth of its initial loading concentration. At all time points, the number of vinculin parkers per unit cell area was significantly greater on HAFn compared with HA and Pol ($P<0.05$) (Fig 3). Attachment was 3, 4 and 7.5 times greater on HAFn compared with HA alone at 1, 4 and 24hrs respectively. The number of vinculin markers per unit cell area was only significantly greater on HA compared with Pol at 1hr ($p<0.05$).

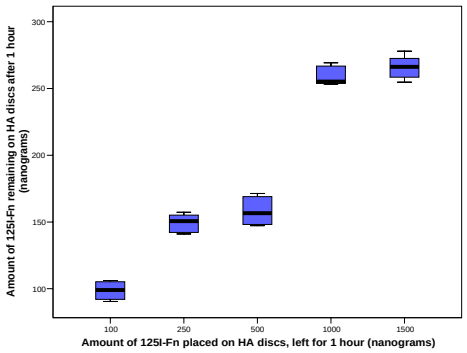

Figure 1: Box plot showing the amount of ^{125}I-Fn remaining on HA discs after 1 hr incubation with increasing initial loading quantities.

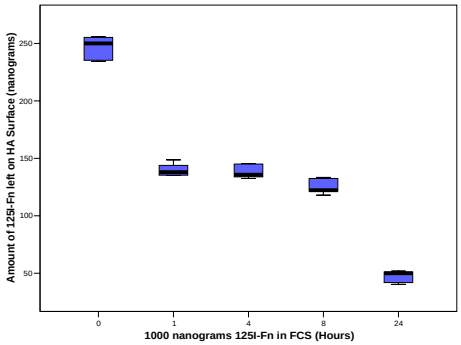

Figure 2: Box plot showing the amount of ^{125}I-Fn remaining on HA discs after increasing durations in FCS.

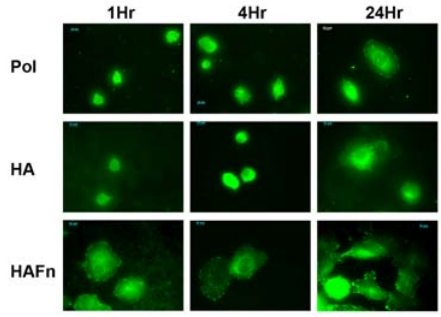

Figure 3: Immunofluorescent images of 1BR.3 on Pol, HA and HAFn at 1, 4 and 24hrs.

Conclusions: We have shown that, despite the reduction in Fn-HA bonding to 1/5 of its initial concentration after 24hrs, cell attachment strength remained 7.5 times greater compared with that on HA alone. Whether initial contact of cells on Fn-functionalised surfaces is sufficient to up-regulate attachment, or that smaller quantities of Fn could cause a similar phenomenon remains to be determined. Our results show that Fn functionalised HA could improve dermal fibroblast attachment and increase the efficacy of the skin-implant seal.

References: [1] Sullivan J, *et al* Prosthet Orthot Int 2003 [2] Middleton CA, *et al* JBMR 2007 [3] Pendegrass CJ, *et al* Adv Biomats 2010 Accepted and In Press.

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