Intraosseous Transcutaneous Amputation Prostheses: An *In Vitro* investigation of the effect of fibroblast pre-seeding on keratinocyte attachment to titanium alloy.

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**INTRODUCTION**

Transcutaneous implants have been successfully employed in dentistry since the 1960’s, however subsequent attempts to translate this into extra-oral solutions for amputees have been beset with complications, with infection being the primary failure modality. Intraosseous transcutaneous amputation prostheses (ITAP) are being developed to overcome this problem by creating a seal at the skin-implant interface to prevent bacterial invasion and provide secure attachment of artificial limbs for amputees. In normal skin, dermal fibroblasts and epidermal keratinocytes are known to effect one another’s differentiation and cellular activities, for example; production and constitution of a basement membrane. Cells do not adhere directly to biomaterial substrate, but attach to the surface via a layer of ECM extruded from cells. In the case of keratinocytes this layer is principally laminin-5 (Ln5). It is possible that pre-seeding ITAP biomaterials with fibroblasts could up-regulate Ln5 expression and result in improved adherence of keratinocytes, and this could improve the infection-resistance of the skin-ITAP seal *in vivo*. We hypothesise that adhesion of keratinocytes, measured by the number of focal adhesions per unit cell area, and the order and intensity of Ln5 production will be significantly greater on titanium alloy substrates pre-seeded with fibroblasts compared with controls.

**METHODS**

10mm diameter Ti<sub>6</sub>Al<sub>4</sub>V discs were ground, polished and sterilised to orthopaedic implant manufacturing standards. Discs were divided into 2 groups, keratinocytes only (PNT-2) and co-culture keratinocytes + pre-seeded fibroblasts (PNT-2 + 1BR3). In the PNT-2 group, 25,000 cells were seeded in 70µl of media and incubated for 4 and 24hrs. In the PNT-2 + 1BR3 group, 30,000 1BR3 cells were pre-seeded for 24hrs prior to secondary seeding with 25,000 PNT-2 cells as before for 4 and 24hrs. Ln5 deposition analysis was performed at 24hrs with a semi-quantitative score for order and intensity. A score of patchy/discontinuous (0) to linear/continuous (5) was assigned for Order, and low (0) to high (5) for Intensity (Fig 1). Vinculin was immunolocalised to assess the number of focal adhesions per cell, and cell area was measured using Image Analysis. Cell adhesion was expressed as the number of vinculin markers per unit cell area at 4 and 24hrs. All assays performed in triplicate and for assessment of cell adhesion, 15 randomly selected cells per disc were analysed.

**RESULTS**

Median order of Ln5 deposition for PNT-2 and PNT-2 + 1BR3 were 1.50 (1.43 to 1.81) and 4.00 (3.94 to 4.32) respectively. Corresponding Intensity scores were 2.00 (2.07 to 2.57) and 3.50 (3.52 to 3.80). Order and Intensity of Ln5 deposition were significantly greater in co-culture (PNT-2 + 1BR3) compared with PNT-2 alone (p < 0.05) (Fig 2).

The median vinculin count per unit cell area at both 4 and 24hrs was significantly greater in co-culture (PNT-2 + 1BR3) compared with PNT-2 only (p < 0.05) (Fig 3).

**CONCLUSION**

This study has shown that the presence of fibroblasts in co-culture significantly increases keratinocyte adhesion *in vitro*. We have also demonstrated that in fibroblast co-culture, keratinocytes not only secrete more laminin 5, but also show an increased order of deposition compared with those in mono-culture. Factors responsible for these observations may be direct, with integrin mediated attachment of fibroblasts leading to up-regulation of vinculin and talin recruitment in keratinocytes, or indirect; via fibroblast ECM or via exogenous factors, however irrespective of the mechanism; this phenomenon may prove beneficial for tissue engineering approaches at improving the skin-implant seal around ITAP.

**REFERENCES**


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