Intraosseous Transcutaneous Amputation Prostheses vs Dental Implants: A Comparative Study of Keratinocyte and Gingival Epithelial Cell Adhesion In Vitro.

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Introduction

Infection is the primary failure modality for transcutaneous implants because the skin breach provides a route for pathogens to enter the body. Intraosseous transcutaneous amputation prostheses (ITAP) are being developed to overcome this problem by creating a seal at the skin-implant interface. Oral gingival epithelial cell adhesion creates an infection free seal around dental implants; however this has yet to be demonstrated outside the oral environment. Epithelial cells attach to metal substrates via hemidesmosomes (HD) and focal adhesions (FA) and their expression is an indicator of adhesion efficiency. The aim of this study was to compare epidermal keratinocyte with oral gingival epithelial cell adhesion on titanium alloy in vitro to determine whether these two cell types differ in their speed and strength of adhesion; it was hypothesised that oral gingival epithelial cells attach to titanium alloy earlier than epidermal keratinocytes; with greater expression of hemidesmosomes and focal adhesions.

Materials and Methods

Pool human oral gingival epithelial cell (HGEP) and primary human epidermal keratinocyte (HPEK) adhesion to titanium alloy, was assessed at 4, 24, 48 and 72 hrs. 30,000 cells were seeded onto 10 mm diameter discs. Six discs were used for each analysis and 15 randomly selected cells were assessed per disc. Adhesion was measured by the number of FAs per unit cell area (vinculin/area) and expression of HDs using a semi-quantitative scale from 1-5 (BP180 Grade). To determine whether the findings from the study held for matched cells, tissue samples were harvested from the gingiva and extra-oral tissue of 3 sheep. Gingival cells and extra-oral keratinocytes were culture expanded and the matched cells underwent identical analyses to those of the pooled human cell lines outlined above.

Results

For the pooled human cells, at 4 and 24hrs, there was a significant increase in vinculin marker expression per unit cell area of 4.3 and 4.7 times in HGEP compared with HPEK (p=0.000). At 48 and 72hrs there were no significant differences. HD expression was significantly greater in HGEP at 4 and 24hrs (p=0.002) compared with HPEK. Up-regulation of HD expression in HPEK lagged that of HGEP until 48hrs, after which no significant differences were observed.

For the matched ovine cells, there was significant increase in vinculin marker expression at all time points in the gingival cells compared with the extra-oral keratinocytes (all p values < 0.05) (Figure 1 & 2). HD expression was significantly greater in gingival cells compared with extra-oral keratinocytes at all time points (all p values < 0.05), with up-regulation of HD expression in extra-oral keratinocytes lagging that of gingival cells up to 72 hours (Figure 3 & 4).

Figure 2. Box and whiskers plot of vinculin/area with time for gingival cells (red) and extra-oral keratinocytes (blue). * Denotes significant differences at the 0.05 level.

Figure 3. 4 ((A) and E)), 24 ((B) and (F)), 48 ((C) and (G)) and 72 ((D) and (H)) hour images for BP180 staining of gingival (A-D) and extra-oral keratinocytes (E-H).

Figure 4. Box and whiskers plot of BP180 Grade over time for gingival cells (red) and extra-oral keratinocytes (blue). * Denotes significant differences at the 0.05 level.

CONCLUSION

This study has demonstrated that oral gingival cells up-regulate both focal adhesion and hemidesmoses expression at earlier time points compared with epidermal keratinocytes. Expression of hemidesmosomes lags that of focal adhesions, suggesting that focal adhesion formation may be a prerequisite for hemidesmosome assembly.

SIGNIFICANCE

We postulate that early attachment of oral gingival epithelial cells to dental implant biomaterials may be responsible for the formation of an infection-free seal.

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