Intraosseous Transcutaneous Amputation Prostheses vs Dental Implants: A comparison between keratinocyte and gingival epithelial cell adhesion in vitro.

+1Pendegrass, C J; 1Fontaine, C; 1Bunn, G W
+1University College London, The Institute or Orthopaedics & Musculoskeletal Science, Centre for Biomedical Engineering, The RNOH, Brockley Hill, Stanmore, Middlesex, HA7 4LP.
c.pendegrass@ucl.ac.uk

INTRODUCTION
In the 1960’s Brånemark successfully pioneered the use of transcutaneous implants in the dental field, however subsequent attempts to translate these findings into a solution for amputees have been beset with complications. Infection is the primary failure modality 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 to prevent bacterial invasion and provide secure artificial limb attachments for amputees. For ITAP to be successful a tight seal between cells in the epidermis and the implant is essential. Oral gingival epithelial cell adhesion creates an infection free seal around dental implants; however this has yet to be demonstrated outside the oral environment. All epithelial cells attach via hemidesmosomes (HD) and focal adhesions (FA) and their expression is an indicator of the efficiency of cell adhesion. The aim of this study was to compare epidermal keratinocyte and oral gingival epithelial cell adhesion to titanium alloy in vitro to determine whether these two cell types differ in their speed and strength of adhesion to titanium alloy. It was hypothesised that oral gingival epithelial cells attach to titanium alloy earlier than epidermal keratinocytes; with earlier and greater expression of hemidesmosomes and focal adhesions.

METHODS
10mm titanium alloys discs (TiAlV) were prepared to clinical orthopaedic implant manufacturing standards. 30,000, passage 1-3 primary human oral gingival epithelial cells (HGEP) and primary human epidermal keratinocytes (HPEK) were seeded per disc and incubated for 4, 24, 48 and 72 hrs. HDs and FAs were immuno-labelled with anti-BP180 and anti-vinculin antibodies 1:200 in PBS with 0.025% Triton X-100 for 1.5 hrs respectively. Primary antibodies were localised with Alexa Fluor labelled secondary IgGs for 1 hr and HDs and FAs were visualised and quantified using a Carl Zeiss photomicroscope and Axiovision Image Analysis Software. Assays at 4, 24, 48 and 72 hours were performed in triplicate for each cell type, adhesion complex and time point, with 15 randomly selected cells analysed per disc. Adhesion was measured by counting the number of HDs and measuring the cell area for 15 randomly selected cells. The results were expressed as the number of FAs per unit cell area. The expression of HDs was assessed using a semi-quantitative scale of 1 to 5; with 1 being poor fluorescence and 5 high.

RESULTS
The results for FAs analyses are shown in Figure 1. At 4 and 24hrs, significant increases of 4.3 and 4.7 times greater vinculin marker expression per unit cell area were observed in HGEP compared with HPEK (p=0.000). At 48 and 72hrs there were no significant differences.

Table 1: Median and 95% confidence intervals (CI) for BP180 score over time for HPEK and HGEP.

<table>
<thead>
<tr>
<th>Time (hr)</th>
<th>Cell type</th>
<th>Median</th>
<th>95% CI</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>HPEK</td>
<td>1.79 – 2.88</td>
<td>2.00 – 3.29</td>
<td></td>
</tr>
<tr>
<td></td>
<td>HGEP</td>
<td>3.00 – 3.85</td>
<td>4.00 – 4.96</td>
<td></td>
</tr>
</tbody>
</table>

CONCLUSION
This is the first study to have directly compared oral gingival epithelial cell and epidermal keratinocyte adhesion to titanium alloy in vitro. Oral gingival cells up-regulate both focal adhesion and hemidesmosome expression at earlier time points compared with epidermal keratinocytes. The expression of hemidesmosomes has been shown to lag that of focal adhesions, suggesting that the formation of focal adhesions is a prerequisite for hemidesmosome assembly. This study has shown that epidermal keratinocytes lag oral gingival epithelial cells in expression of both focal adhesions and hemidesmosomes and 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 which has yet to be observed in extra-oral transcutaneous devices. Advances in biomaterial development and augmentation techniques to facilitate earlier FA and HD expression in epidermal keratinocytes may reduce infection rates and lead to an infection-free seal around transcutaneous implants including ITAP.

REFERENCES

ACKNOWLEDGEMENTS
This study was supported by Stanmore Implants and the National Institute of Health.