Keratinocyte growth and expression of adhesion complex molecules is influenced by titanium alloy surface topography

Pendegrass CJ, Gordon D, Ng Man Sun S, Middleton C, Blunn G.
The Centre for Biomedical Engineering, Institute of Orthopaedics & Musculoskeletal Science, Brockley Hill, Stanmore, Middlesex, HA7 4LP, UK.

Introduction: Current prostheses for amputees reply on the stump-socket interface for attachment, where rubbing often causes pressure sores, pain and infection. Intraosseous transcutaneous amputation prostheses (ITAP) could overcome this by attaching the external implant to the skeleton; however the transcutaneous abutment is a potential route for infection. For ITAP to be successful, a biological seal at the skin-implant interface is a prerequisite. Epithelial cells attach to dental implants via hemidesmosomes (HDs)[1] and focal contacts (FCs)[2], however these interfaces have not been observed between transcutaneous implants and extra-oral skin cells (keratinocytes). This study was performed to determine which titanium alloy surface finish would provide an optimal topography for keratinocyte growth and adhesion via FCs and HDs *in vitro*. This study tests the hypothesis that substrate topography will influence keratinocyte morphology, proliferation and HD/FC expression *in vitro*.

Methods: 4 titanium alloy (Ti_6Al_4V) surface topographies were analyzed; smooth polished (SP), machine finished (MF), sandblasted (SB) and hydrofluoric acid (HF) etched. 10mm diameter 4mm thick substrates were prepared and image profiled to determine surface roughness (Ra), average max profile height (Rz) and mean spacing of profile irregularities (Sm) (Fig. 1). Human keratinocytes were seeded onto the substrates at 30,000 cells per disc for 1, 2, 3 and 4 days. HDs and FCs were quantified with immunolocalization of plectin and BP180, and vinculin respectively, and visualized with confocal and light microscopy. Proliferation was assessed using Alamar Blue, and morphology with scanning (Fig. 1) and transmission electron microscopy.

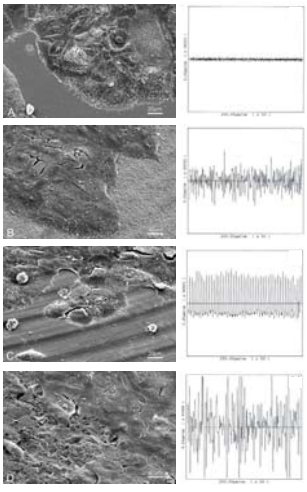

Figure 1
Surface profile images (right) and SEM (left) taken at x550 of keratinocytes for 24 hours *in vitro*, on SP (A), MF (B), SB (C) and HF (D) substrates. Ra's: SP = 0.030 (0.012 to 0.048), MF = 0.205 (0.175 to 0.230), SB = 0.935 (0.821 to 1.034), HF = 0.205 (0.175 to 0.230).

Results/Discussion: FC and HD numbers increased with time on all surfaces. Substrates with lower Ra's supported cells with significantly more HDs and FCs at all times ($p < 0.05$) (Fig. 2 & 3).

Cells on smoother substrates demonstrated increased spreading and filopodia, whilst no significant differences in proliferation were observed at any time ($p > 0.05$).

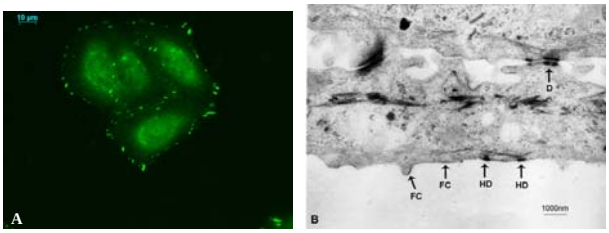

Figure 2 A) Immunolocalization of vinculin in keratinocytes cultured on smooth polished substrates @ 24hrs. B) TEM image showing HDs and FCs in keratinocytes on machined substrates @ 24hrs.

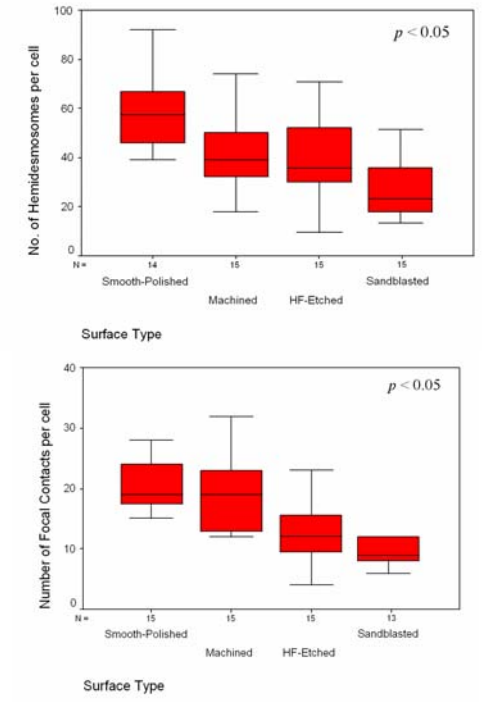

Figure 3 Box plots of HD and FC data @ 48hrs.

Conclusions: The aim of this study was to identify which surface topography would optimize keratinocyte attachment *in vitro*. We have demonstrated that human HaCaTs are significantly influenced by the topography of the titanium alloy substrates used to support their growth and attachment. We have shown that smooth polished substrates increase HaCaT expression of HDs and FCs; increasing adhesion at the cellular level. Our results suggest that a smooth polished surface may enhance epithelial cell attachment to ITAP *in vivo*. Further work is required to assess the effects of changing surface topography on epithelial attachment to ITAP on a tissue level *in vivo*.

References:

1) Gould TR *et al*. J Prosthet Dent 1984;52:418-420.
2) Raisanen L *et al*. J Biomed Mater Res 2000;49:79-87.