Assessment and Optimisation of Human Keratinocyte Attachment on Laminin Functionalised Titanium Alloy for use in ITAP

INTRODUCTION

Intraosseous transcutaneous amputation prostheses (ITAP) overcome the problems associated with the stump socket interface such as rubbing and development of sores, by directly loading the skeleton. For ITAP, the primary barrier to infection is the seal between the keratinized epithelium and the implant interface. Keratinocytes attach to the laminin component of the basement membrane via focal adhesions (FA’s) and hemidesmosomes (HD’s), however it is not known whether FA’s are prerequisite structures for the formation of HD’s or whether they are expressed independently. This study was performed to quantify and analyse keratinocyte expression patterns of FA’s and HD’s. We hypothesise that both FA and HD expression will be up-regulated by laminin functionalisation of titanium alloy.

METHODS

Human keratinocytes were cultured in DMEM supplemented with penicillin, streptomycin and FCS. To generate the semi-quantitative scoring system for HD expression, BP180 and plectin primary antibody labeling was performed at 1:500 and 1:112 respectively in a 70µl droplet of PBS with 5% triton x-100 for 2hrs. Primary labeled BP180 and plectin was then localised with TRITC and FITC labeled secondary antibodies for BP180 and plectin respectively for 1hr. Cells were cultured at 30,000 cells per disc on 10mm diameter polished titanium alloy discs for 24, 48, 72 and 96 hours. The vinculin component of FA’s was also immunolocalised using our previously published protocol. To assess the effects of laminin functionalisation on keratinocyte attachment, laminin was either adsorbed (AdLn) or silanized (SiLn) to polished titanium alloy discs. Silanized discs without laminin coating were also assessed as controls for SiLn. For adsorption, 1500 ng of laminin in a 50µl droplet of PBS was placed onto the disc surface and left for 4hrs at room temperature. The solution was then washed off and cells were seeded at 30,000 cells per disc for 24, 48, 72 and 96 hours. Vinculin, plectin and BP180 immunolocalisation protocols were used to immunolocalise FA’s and HD’s respectively. For silanized laminin coatings, the discs were passivated in 30% H2O2 for 2hrs, transferred to 2% gluteraldehyde solution for 2hrs and then thoroughly rinsed in sterile PBS prior to laminin addition. A scoring system was used to determine the effect of laminin functionalisation of titanium alloy substrates on keratinocyte attachment. Assessments were performed in triplicate and 5 images from each disc were analysed according to a semi-quantitative scoring system. The scoring system was based on the brightness of the stain observed and scores were allocated independently of time. A score of 1 to 4 was allocated with 1 being low.

RESULTS

The images of BP180, plectin and vinculin staining over 96 hours showed that the semi-quantitative score increased linearly such that grade 1 was observed at 24 hrs for vinculin, BP180 and plectin staining whilst at 96 hrs the score had increased to grade 4 for all immune stains.

CONCLUSION

Early keratinocyte attachment at the skin-implant interface is critical to the success of ITAP. We have developed a technique to immunolocalise the hemidesmosomal components, plectin and BP180, and have developed a time-independent semi-quantitative scoring system to assess the expression patterns of FA’s and HD’s in keratinocytes. This system gives a quantifiable method of assessing cell attachment. We have used the scoring system to demonstrate that the process of silanization alone is detrimental to keratinocyte adhesion, and speculate that this may be attributable to the increased surface roughness associated with the silanization technique. We have demonstrated that there is an up-regulation of FA and HD expression on laminin functionalized titanium alloy surfaces, with both adsorption and silanization. We have previously shown with fibronectin, that silanization creates a more durable coating4, so silanization of laminin may be beneficial in creating a strong seal at the skin-implant interface in ITAP. Further studies are warranted to determine whether functionalisation of ITAP with laminin could improve epidermal layer attachment in vivo.

REFERENCES