

Development and Characterisation of a Full-thickness Engineered Human Oral Mucosal Model for *In Vitro* Studies

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INTRODUCTION: Restorative materials and oral health care products come into direct contact with oral mucosa and can cause adverse reactions. To obtain an accurate risk assessment, the *in vitro* test model must reflect the clinical situation as closely as possible. A three-dimensional full-thickness engineered human oral mucosal model for biological assessment of dental materials is reported.

METHODS: Oral keratinocytes were isolated from the biopsies from patients having canine exposures with their written informed consent and cultured in Greens medium. Fibroblasts were isolated from the connective tissue layer of the biopsy and cultured in serum containing DMEM. Fibroblasts were seeded onto a Collagen-Glycosaminoglycane-Chitosan scaffold and cultured for 7 days to produce an engineered lamina propria. Oral Keratinocytes were then seeded on the top of this connective tissue and grown at the air/liquid interface for 2 weeks to produce a full-thickness engineered human oral mucosa. The tissue was frozen sectioned and stained by haematoxylin and eosin and tiny pieces of engineered oral mucosa were cut, fixed, and processed for Transmission Electron Microscopy examination.

RESULTS: An engineered oral mucosa with a well-differentiated stratified epithelium and viable fibroblasts in the connective tissue layer was reconstructed (Fig. 1). Ultrastructural examination showed numerous desmosomes joining keratinocytes throughout the epithelium especially in the spinose layer and cytoplasmic keratin increasing towards the cornified layer. Newly synthesized ECM produced by fibroblasts present in sub-epithelial layer.

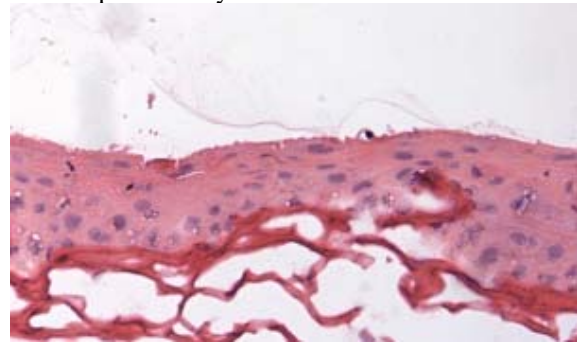


Fig. 1: Engineered oral mucosa on collagen-GAG-chitosan matrix

CONCLUSIONS: The optimised oral mucosa reconstruct resembled native human oral mucosa and it has the potential to be used as an accurate and highly reproducible test model in biocompatibility evaluation of dental materials