Isolation and immortalisation of tonsillar keratinocytes for three-dimensional tissue engineered tonsil mucosal models

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INTRODUCTION: The generation of tissue engineered epithelial models is often hampered by the limited proliferative capacity of primary epithelial cells. This study aimed to isolate primary keratinocytes from human tonsils removed during routine tonsillectomies by enzymatic digestion and then immortalise these cells using a Rho kinase inhibitor. Immortalised cells were used to create functional tissue-engineered tonsillar tissue that could be used to model tonsillitis.

METHODS: The efficiency of cell isolation using two different enzymes (trypsin and dispase) was compared. The growth of primary tonsil keratinocytes was measured and compared to cells cultured in the presence of the Rho kinase inhibitor Y27632. Immortalised keratinocytes were used to develop a tissue-engineered model of tonsil epithelium using primary tonsil fibroblasts and de-epithelialized dermis. These models were then incubated with *Streptococcus pyogenes* to model tonsillitis and the expression of pro-inflammatory cytokines measured by cytokine array and ELISA.

RESULTS: Enzymatic digestion of tonsillar tissue with trypsin resulted in the isolation of significantly more keratinocytes compared to dispase isolation. Keratinocytes cultured without the Rho kinase inhibitor Y27632 survived in culture for less than 10 population doublings whereas cells cultured in the presence of this inhibitor grew for over 30 population doublings without changing their phenotype. Tonsil keratinocytes and fibroblasts cultured in three dimensions produced a multi-layered differentiated epithelium that histologically resembled the surface epithelium of normal tonsils and responded to *S.pyogenes* by increasing expression of pro-inflammatory cytokines.

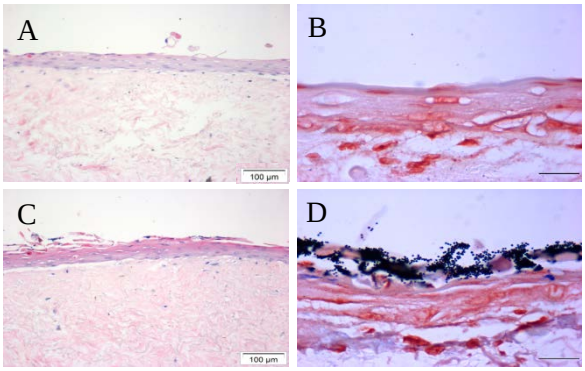

Fig. 1: H&E and Gram stained images of uninfected control (A-B) and S. pyogenes (C-D) infected tissue engineered tonsil mucosal models.

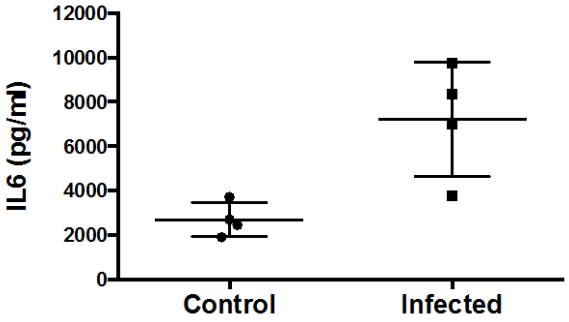

Fig. 2: IL-6 release from uninfected controls and S. pyogenes infected tissue engineered tonsil mucosal models.

DISCUSSION & CONCLUSIONS: Tonsil keratinocytes can be successfully isolated and cultured *in vitro*. Y27632 was able to markedly prolong the life-span of keratinocytes without any deleterious consequences to the cell phenotype making these cells useful for a number of applications which require longer term culture. A functional tissue engineered model of tonsil epithelium was generated which will provide a useful tool for studying cells in a more physiologically relevant way.