Regulation and characterisation of corneal stromal cell contraction

[Abstract]

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Regulation and characterisation of corneal stromal cell contraction

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Objectives: Collagen hydrogels have been extensively used as scaffolds for corneal tissue engineering. However, corneal stromal cells differentiate into contractile fibroblasts in the hydrogel in vitro culture, rather than keratocytes. The aim of this study is to develop techniques to regulate the contraction by either chemical or topographical cues which mimic the native corneal environment, and characterize the cellular feedback in prolonged culture period via novel, non-destructive monitoring protocols.

Methods: 5x10^5 human corneal stromal cells were seeded in collagen hydrogels with and without the incorporation of poly-lactic acid aligned nanofibers. A non-destructive spherical indentation technique was used to examine the alteration of the mechanical properties of the individual collagen hydrogel specimens under different media respectively up to 28 days. The dimensional change of the specimens caused by the cells’ contraction was measured by optical coherence tomography in parallel. The quantitative PCR with respect to the expression of keratocytic and fibroblastic markers was conducted to cross-validate the observed physical properties.

Results and Discussion: It was revealed that stromal cells cultured under a media with insulin and without serum exhibited constant elastic modulus and gel dimension, indicating that contraction was suppressed, which was cross-validated by the expression of keratocan and ALDH3; whilst stromal cells cultured with serum demonstrated continuously increased modulus and reduction of thickness, typical of contraction process. The presence of the aligned nanofibers reduced the degree to which the cells were able to contract the hydrogel constructs in a vertical direction, thus encouraging the cells cultured in fibroblastic media to behave more like non-contractile keratocytes.

Conclusions: The alteration of culture conditions and the addition of topographical cues can regulate corneal stromal cell differentiation. This can potentially enhance the field of corneal tissue engineering using collagen hydrogel models. The non-destructive monitoring protocols provide convenient tools for observing biological phenomenon for prolonged culture periods in the same specimen.