Controlling and online monitoring in a corneal stromal model [Abstract]

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NON-DESTRUCTIVE MONITORING OF THE EFFECT OF CONDITIONS ON CORNEAL STROMAL CELL DIFFERENTIATION IN HYDROGELS

Wilson SL, El Haj AJ, Yang Y.

Institute of Science and Technology in Medicine, School of Medicine,

Keele University, Stoke-on-Trent, UK

Introduction

Collagen hydrogels have been extensively used as scaffolds for corneal tissue engineering. However, corneal stromal cells differentiate into fibroblasts in the hydrogel in vitro culture, rather than keratocytes. The aim of this study is to optimize culture conditions via chemical cues (media supplements) in order to control keratocyte phenotype, which improves the current state of corneal stromal tissue engineering models. Novel, non-destructive monitoring protocols were established to reveal the stromal cells’ response under the different culture conditions in terms of the rate of contraction and mechanical properties.

Materials & Methods

A human corneal stromal model was constructed by seeding $5 \times 10^5$ stromal cells in 0.5 mL collagen gel. A non-destructive spherical indentation technique was used to examine the mechanical properties of the individual collagen hydrogel specimens under keratocyte or fibroblast media respectively every 3 days up to 28 days. The amount of gel contraction caused by the cells was measured by optical coherence tomography in parallel. The quantitative PCR with respect to the expression of keratocytic markers was conducted to cross-validate the observed physical properties.

Results & Discussion
It was confirmed that the culture conditions can induce the corneal fibroblasts to partially trans-differentiate into a keratocyte phenotype. Stromal cells cultured in hydrogels under keratocyte media with insulin and without serum exhibited constant elastic modulus and gel dimension, indicating that contraction was suppressed and that the quiescent characteristic of keratocytes was restored, which was cross-validated by the expression of keratocan and ALDH3; whilst stromal cell-gel cultured with serum demonstrated continuously increased modulus and reduction of thickness, typical of fibroblast phenotype.

Conclusions

The alteration of supplements in culture media can facilitate the differentiation of corneal stromal cells from fibroblasts towards a keratocytic lineage. 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.

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