Controlling corneal stromal cell phenotype via chemical cues [Abstract]

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Controlling Corneal Stromal Cell Phenotype via Chemical Cues

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INTRODUCTION: Collagen hydrogels have been extensively used as scaffolds for corneal tissue engineering¹. However, corneal keratocytes are notoriously difficult to culture and often differentiate into fibroblasts in the scaffolds in vitro². This causes the construct to contract and become opaque. The aim of this study is to encourage contractile corneal fibroblasts to transdifferentiate into non-contractile keratocytes via chemical cues supplied by the media and explore the phenomena occurring both macro- and microscopically with regards to the contraction of the collagen.

METHODS: Hydrogels with collagen concentrations of 3.5 mg/ml and 5x10⁵ cells per gel were manufactured. Accelar gels were also prepared to serve as a control. The hydrogels were cultured at 37°C, 5% CO₂ over a seven day period under fibroblast and keratocyte media respectively. Fibroblast media consisted of Dulbecco’s modified eagle medium (DMEM; Biowest, France), supplemented with 10% foetal calf serum (Biowest, France), 1% antibiotic and antimitotic solutions (Sigma, UK) and 1% 200mM L-glutamine (Sigma, UK). Keratocyte media consisted of DMEM-F12 (Biowest, France), supplemented with 1% 100mM ascorbic acid (Sigma-Aldrich, UK), 1% antibiotic and antimitotic solution (Sigma-Aldrich, UK) and 0.1% 10mg/mL insulin (Sigma-Aldrich, UK). Macroscopic contraction was monitored using digital imaging over a 7-day culture period and the percentage change in gel area calculated. Microscopic contraction was monitored using FESEM imaging.

RESULTS: Hydrogels cultured under keratocyte media contracted less than hydrogels cultured in fibroblast media and maintained gel area and volume (Fig 1). As the hydrogels contract the ratio of cells to total volume increases; the collagen concentration increases as the water is forced out of the gel; and the collagen lattice density increases (Fig 2).

DISCUSSION & CONCLUSION: It has been demonstrated that when cultured in serum-free, media containing insulin contractile fibroblasts can be encouraged to partially trans-differentiate into a non-contractile keratocyte phenotype.