**Title**

Nanotopography of substrates directs the deposition of organised fibrillar collagen by corneal stromal cells

**Authors**

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**Introduction**

The corneal stroma constitutes 90% of the cornea. It consists of around 200 collagen lamellae, oriented roughly orthogonally throughout. Within each of these are highly aligned collagen fibrils interspersed with keratocytes which are responsible for maintaining transparency. Corneal scarring is a leading cause of blindness globally. A shortage of donors, with only 1 cornea available for every 70 corneas needed, has led to a tissue engineering solution being required. Furthermore, current *in vitro* models of the stroma focus on using a collagen gel or layers of fibroblasts that fail to replicate the microstructure. By being able to more faithfully recapitulate the stroma we aim to develop an *in vitro* model to better explore the mechanisms of collagen alignment.

**Materials and Methods**

Cells isolated from human corneo-scleral rims were cultured on coverslips with polytetrafluoroethylene (PTFE) nanofibres. Cell Tracker and a collagen probe (CNA35) were added to culture media and imaged for up to 3 days to observe the live cell deposition of collagen. Separate samples were cultured for several weeks to form a cell layer and the alignment of the extracellular matrix (ECM) was analysed using OrientationJ, an ImageJ plugin.

**Results**

Collagen fibres could be visualised using a FITC-labelled collagen probe (CNA35) whilst cells were highlighted using Cell Tracker. OrientationJ analysis of collagen fibres showed that when the cells were cultured on PTFE nanofibres around 73% of the fibrils were aligned within ± 10° of the dominant direction, compared to just 44% when cultured on a control substrate.

**Discussion**

It has been well documented that cells respond to topographical cues and this has been exploited in a variety of applications. The PTFE nanofibres provided the topographical cues for cells, and thus collagen, to align along. Interestingly, we observed that as stromal cells stratified they maintained their alignment but rotated by 37°. Literature suggests that cell layers that maintain organisation have improved tissue functionality. By manipulating cell sheets, we hypothesise that we’ll be able to produce multiple cell layers that can be organised to recapitulate the native cornea for use as either a graft alternative or an *in vitro* model.