Engineering bioresponsive hydrogels for corneal stromal cell guidance

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Corneal stroma is a highly organised collagen-rich tissue (Fig. 1) responsible for corneal transparency[1]. Stromal keratocytes undergo TGF-β-mediated transformation and activate into fibroblasts (that migrate to the wound area) and subsequently myofibroblasts (providing high contractile force needed to close wounds) to repair stromal injuries. **Uncontrolled stromal remodelling** and overactivation of the myofibroblast phenotype can lead to progressive **loss of corneal transparency** (Fig. 2)[2].

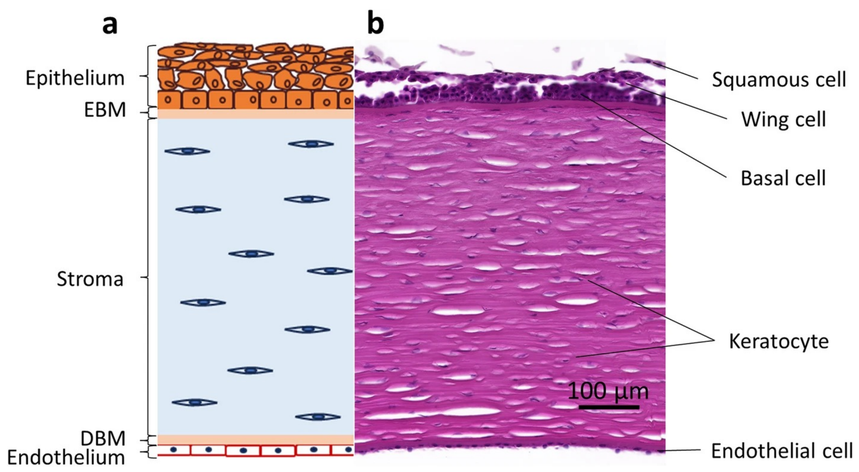


Fig. 1. A schematic diagram of the **(a)** human cornea and **(b)** its cell types. EBM, epithelial basement membrane; DBM, Descemet's basement membrane[1]

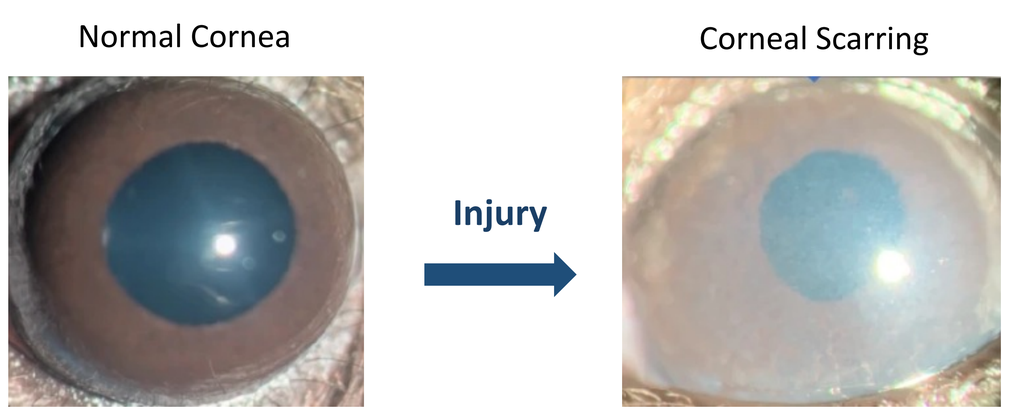


Fig. 2. Corneal scarring is a frequent complication of corneal injury[2]

HYPOTHESIS: Effective regeneration strategies would employ **biomaterials that respond to cell activity** to enable dynamic cell–material interaction and controlled corneal remodelling.

RESULTS

Enzyme-responsive peptides enable stromal cell attachment on bioinert hydrogels

MMP-responsive RGDS-functionalised alginate hydrogels promoted cell survival and spreading of immortalised keratocytes (Fig. 3)

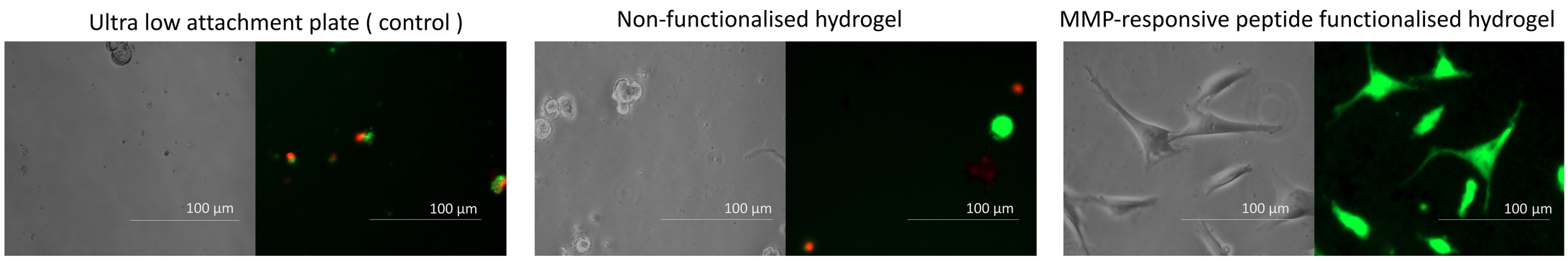


Fig. 3. Brightfield and Live (green)/Dead (red) imaging of keratocytes grown on ultra low attachment plate, alginate and MMP-responsive RGDS functionalised hydrogel for 72h.

Cell priming differentially regulates ECM remodelling and fibrotic activation

FGF2 (10 ng/mL) priming for quiescent keratocytes maintained *ALDH3A1* expression (keratocyte marker) and led to an increase in *MMP1* expression, suggesting extracellular matrix (ECM) remodelling. TGF-β1 (5 ng/mL) stimulation increased *ACTA2* expression suggesting fibrosis. Interestingly, cells primed with FGF2 were protected from this effect (Fig. 4 A).

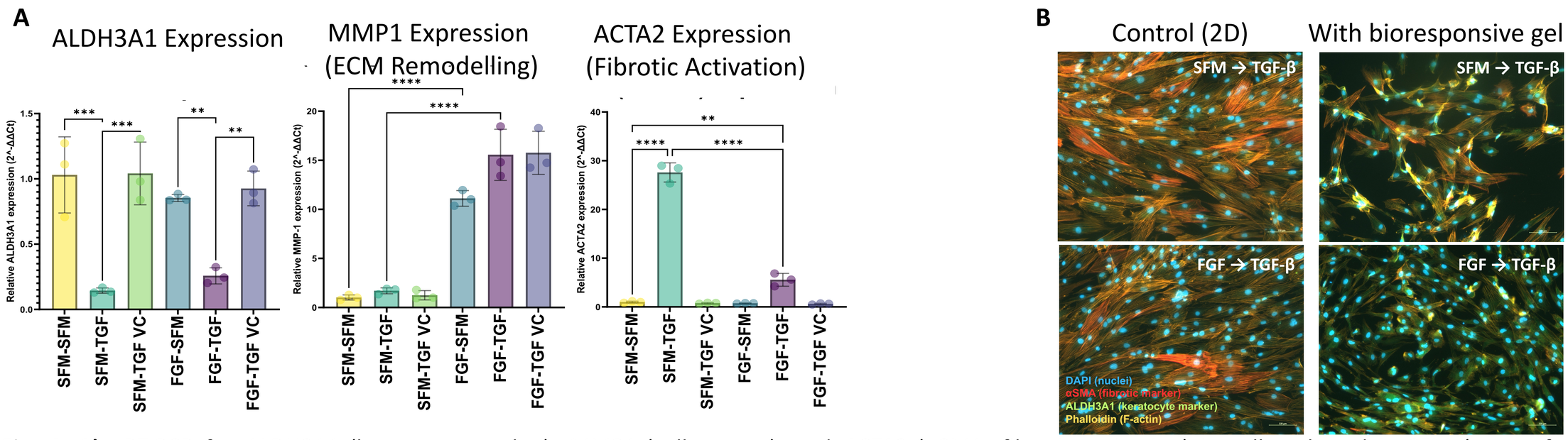


Fig. 4. A) qRT-PCR for *ALDH3A1* (keratocyte marker), *MMP1* (collagenase), and *ACTA2* (αSMA, fibrotic activation) in cells cultured in SFM (serum free media) or SFM supplemented with FGF2 for 72h and followed by TGF-β1, TGF-β1 VC – vehicle control (4 mM HCl + 0.1% BSA) or SFM for 72h, One Way ANOVA * P<0.05, ** P<0.01, *** P<0.001, *** P<0.0001. **B)** immunocytochemistry for αSMA (alpha smooth muscle actin) (red), ALDH3A1 (green), F-actin (yellow) and nuclei (blue) in cells cultured on tissue culture plastic or on functionalised hydrogel with and without FGF2 priming. Scale bar: 100 µm

Cells in bioresponsive gels exhibit reduced αSMA expression

Hydrogel microenvironment altered stromal cell differentiation and resulted in decreased αSMA expression in TGF-β1-stimulated cells (Fig. 4B).

CONCLUSION: Elevated MMP1 expression in the continuum of keratocyte transformation combined with decreased fibrotic activation in hydrogel supported cultures highlights the potential of bioresponsive biomaterials to regulate stromal cell behaviour and support regenerative corneal healing.

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[1] Jin, L. et al. *Eye and Vis,*2024
[2] Torricelli AAM et al., *Exp Eye Res*, 2016

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