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SUMMARY

The work on this thesis provides new insights on the use of new hydrogelators functionalized with cell instructor peptides towards reduction of epithelial mesenchymal transition (EMT) in the eye lens. These hydrogelators are based in a self-assembled nanofibers core linked to peptides motifs with a gelation ration that can be used as an injectable gel.

Prior to the use of the self-assembled nanofibers as a gel, tests to identify the most suitable core of nanofibers were performed. In **chapter 2**, we demonstrate that the choice of building blocks in self-assembling nanofiber systems can be used to control cell behavior. The use of 2D-coated, self-assembled nanofibers in controlling lens epithelial cells, fibroblasts and mesenchymal stem cells was investigated, focusing on gene and protein expression related to the fibrotic response. Therefore, three nanofibers with different characteristics (morphology, topography and wettability) were compared to two standard materials frequently used in culturing cells, TCPS and a collagen *type I* coating. Cell metabolic activity, cell morphology and gene and protein expression were analyzed. The most hydrophilic nanofiber with more compact network consisting of small fibers proved to provide a beneficial 2D environment for cell proliferation and matrix formation while decreasing the fibrotic/stress behavior in all cell lines when compared with TCPS and the collagen *type I* coating. This nanofiber demonstrates the potential to be used as a biomimetic coating to study the development of fibrosis through epithelial-to-mesenchymal transition. This study also shows that nanofiber structures do not enhance cell function by definition, because the physico-chemical characteristics of the nanofibers also influence cell behavior and can be used to regulate cell behavior towards sub-optimal performance.

Based in the insights from **chapter 2** on the influence of chemistry and wettability properties on cell behavior, a low molecular weight gelator (LMWG) was created. The bioactivity of the LMWG was increased by adding peptide motifs to the hydrogelator. The choice of the peptides was based on the knowledge that cell polarity is lost during the EMT process and that detachment from the basement membrane enables cell migration, a characteristic behavior of EMT. In **chapter 3**, gels based on LMWG and functionalized with peptides derived from proteins from the basement membrane (laminin, collagen and fibronectin) were used to investigate the effects of matrix composition on their potential as modulators of EMT. LEC were seeded on top of or underneath the gels and were analyzed for metabolic activity, morphology, α-SMA expression and EMT/fibrotic gene expression. LEC seeded on LMWG mixed with peptides had a significant increase in metabolic activity compared with LMWG without peptides. Only LEC seeded on top of the basement membrane (BM) mixture and on matrigel were spreading and assembled into a monolayer resembling their natural morphology. The expression of α-SMA fibers at the protein level and the mRNA expression of other fibrotic genes however were higher on matrigel. In general, the BM mixture was able to maintain LEC in a lower fibrotic state than the other gels including matrigel. The data suggest that a cell instructing hydrogel with a proper peptide composition can attenuate EMT.

Once the LMWG BM mixture showed beneficial results, a new study model was developed as described in **chapter 4**. Although laminin is one of the major constituents of the basement membrane in the eye lens, the laminin combination of peptides (IKVAV+YIGSR) did not show advantage for LEC survival, as concluded in **chapter 3**. A new combination of fibronectin and collagen derived peptides was described in **chapter 4**. LMWG alone and in combination with laminin derivatives (IKVAV+YIGSR), fibronectin and collagen derivatives (RGD+DGEA), and with a basement membrane peptide mixture were studied in the presence of porcine LEC attached to the capsule bag. The use of LEC with the full capsule bag resembled more the natural environment. Cell alterations will be mostly due to the interaction with the hydrogelators, more so than to the stress induced by the sample preparation. In this chapter, a lens capsule model, to analyze the effects of lens epithelial cells exposure to bioactive hydrogels towards EMT, was investigated. Hydrogels incorporating adhesive peptide motifs present in fibronectin, laminin and collagens were in contact with porcine capsule bags and effects on EMT were analyzed. EMT cell alterations as, elongation of the cytoskeleton, increase of the nucleus size and consequently decrease of cell number were identified. Alteration on the protein level was also detected by the production of new proteins, as α-SMA. The laminin motifs created a large cellular apoptosis. Despite the large cell regeneration provided by the mixture of fibronectin and collagen motifs, EMT was enhanced. The hydrogel that most resembled the basement membrane have shown a large delay in EMT. In **chapter 4** an *ex vivo* model that allowed the interaction of lens epithelial cells with hydrogels in a similar postoperative environment was created. Besides the prevention on EMT by one of the bioactive hydrogels, the percentage of peptides should be further investigated toward a total inhibition of EMT.

In this thesis, new approaches to study and detect cornea epithelial damage were also investigated. In **chapter 5** the mechanical damage on the cornea and the effects of this damage by using lubricants were analyzed by a new tribometer associated with confocal techniques. Glycerine is a common lubricant added to the artificial tears. A friction measurement device with minimal intervention with the pig cornea tear film revealed a low friction coefficient of 0.011 in glycerin solution. Glycerine molecules presumably bind to water, mucins, and epithelial cells and therewith improve both squeeze film and boundary lubrication. Using confocal microscopy was visible that glycerine solution reduced damage to epithelial cells by 50% compared with the phosphate buffer saline.

In **chapter 6** a general discussion of the theory and practicability of the work performed in this thesis was discussed. Suggestions for further improvements of this work were also mentioned in this chapter.