Effect of Keratocyte Presence on the Mechanical Properties of Micropatterned Collagen Films

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Introduction:

Corneal defects are among the most common causes of blindness. Although allograft transplantation has been quite successful, donor shortage necessitates the development of alternative methods to replace corneal tissue. Cornea is composed of 5 different layers, where the stroma is the thickest layer. It has a lamellar structure, and in each lamella the collagen fibrils are parallel to each other but perpendicular to the subsequent lamellae. This oriented structure is important for both transparency and mechanical integrity of the cornea.

Effects of surface features at microscale have been extensively studied. Surface topography has been shown to affect cell alignment, ECM secretion and differentiation. Thus, utilization of a surface patterned carrier for corneal stroma should be beneficial in terms of triggering oriented ECM secretion by the seeded cells. Our previous studies have shown that, corneal stroma cells, keratocytes, responded to the surface topography of micropatterned collagen films and aligned in the direction of the grooves and also secreted ECM molecules within the confines of the groove. However, since natural cornea is inhabited by three different cell types, differences in the response of these cells to collagen based membranes remains as a question. Present study deals with the mechanical properties of keratocyte seeded and unseeded, patterned collagen films in relation to their suitability as artificial cornea cell carriers. Changes in the mechanical strength due to the keratocytes were also compared to the films seeded with D407 epithelium cells, which served as a model cell line for corneal epithelium.

Methods:

Micropatterned collagen films were prepared by solvent casting. Collagen solutions were poured onto a patterned elastomeric template and air-dried overnight. Films were crosslinked with EDC/NHS. Human corneal keratocytes isolated at Eduard Herriot Hospital (Lyon, France) and D407 epithelial cells were seeded onto collagen membranes at a density of 50 $x10^3$ cell/cm^2. Tensile strength tests were performed on unseeded, keratocyte seeded and D407 seeded films on a weekly basis for a three week period. Resistance of the films to collagenase was also tested. Morphology of keratocytes and D407 cells were determined by staining with DAPI. Proliferation of keratocytes on films during three week period was determined with MTS assay. Actin filament staining was done with FITC-labelled phalloidin and Collagen type I secretion was monitored with indirect immunolabelling.

Results/Discussion:

Crosslinking increased the resistance of the films to collagenase; films lost only 47.8% of their weight after 3 h of incubation in a collagenase solution whereas uncrosslinked films disintegrate in 10 min. In the absence of the enzyme, the weight loss was 52.2% after one month for the crosslinked carrier while the uncrosslinked eroded within a day. Microscopical observations showed that keratocytes respond better to the micropatterns than the epithelial cells. There was collagen type I secretion in the direction of the grooves and also cellular cytoskeleton was aligned in groove direction too; whereas for D407 cells secretion was minimal. Keratocytes reached confluency by day 7 of culture period, but for D407 only a small portion of the surface was covered at that time point. MTS assay supported this observation, cell number reached 2.2 x 10^5 by day 7 and increased to 2.6 x 10^5 by day 21. D407 cells covered the film surface by day 14.

These differences in behavior reflected on the mechanical properties. Keratocytes seeded films gained strength during 21 days period. Degree of increase was in close correlation with the cell number. Films seeded with D407 cells, on the other hand, lost their mechanical integrity significantly after 7 days. This was followed by a slight improvement in the following culture periods. Both of the specimens had higher tensile strength with respect to the unseeded films.

This difference in mechanical strength can be attributed to the difference in cell growth rate, ECM secretion and also collagenase activity. Improvement in the keratocyte seeded films demonstrated that the keratocyte activity compensated for the loss due to degradation of the scaffold, whereas such an effect was attained later with D407 cells. Also, micropatterning affected the tensile strength of the system by aligning the cells and restricting their secretion in the direction of grooves. Thus, this may increase the strength of the structure in that particular direction. So such a system can be exploited to increase the mechanical strength of a three dimensional artificial cornea substitute.

Conclusions:

Keratocyte presence improves the mechanical properties of a micropatterned collagenous scaffold. This improvement is important in the design of safe, biodegradable cell carriers for cornea engineering. For a full thickness cornea, the scaffold should have the same integrity throughout its structure. The difference between the behavior of the epithelial cells and the keratocytes demonstrate the need for a co-culture system involving all the cell types for a better understanding of the in vitro behavior..

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