Construction of a Collagen-Based, Split Thickness Cornea Substitute

Aylin Acun[3,4], Vasif Hasirci[1,2,3,4]
METU, Depts. [1]Biological Sciences, [2]Biomedical Engineering, [3]Biotechnology, Ankara 06800, Turkey
[4]BIOMATEN, Center of Excellence in Biomaterials and Tissue Engineering, Ankara 06800, Turkey
vhasirci@metu.edu.tr

Statement of Purpose: Keratoprosthesis and donor corneas that are used as replacements do not completely meet the requirements for proper recovery of vision. Tissue engineering can be an alternative method for preparing a biocompatible and stable cornea equivalent. In this study a novel, split thickness cornea replacement is proposed to substitute the two upper cellular layers (epithelium and stroma) of the native cornea.

Methods: The design includes a chondroitin sulfate impregnated collagen type I (isolated from rat tail) foam (CSXLF) produced by lyophilization carrying electrospun fibers of the same polymer collected directly on top of the foam, forming the bilayer structure (Fo-Fi). The fiber layer was intended to separate the epithelium and the stroma of the reconstructed cornea yet to allow material transfer in between. The foam layer (bottom) was crosslinked by N-ethyl-N-[3-dimethylaminopropyl] carbodiimide (EDC), and ***N***-hydroxy succinimide and after fiber deposition the bilayer was further stabilized with physical crosslinking (DHT method). The construct was characterized by SEM (and NIH-Image J program), microCT, fluorescence and confocal laser scanning microscopy,

Results: The physical characterization of the foam side showed that their pore sizes (10-200 μm) and porosities (around 70%) were well within the desired range for typical tissue engineering applications. The cell free wet thicknesses of both single and bilayer constructs were close to that of the native stroma and light transmittance through these scaffolds was quite high (around 82% in the 500-700 nm range). The scaffolds were also tested for their stability and shown to be suitable for *in vitro* testing. *In vitro* studies were performed using retinal pigment epithelial cells (RPE, D407 cell line) and isolated human corneal keratocytes (HK) to reconstruct the epithelium and the stroma, respectively. Three types of constructs were prepared; only HK seeded Fo-Fi constructs, RPE-HK seeded CSXLFs, and RPE-HK seeded Fo-Fi constructs. All were shown to support cell attachment and promoted cell proliferation (Figure 1) and the cells covered the bottom and the top of the scaffolds (Figure 2). The fiber layer prevented the mixing of the two cell types, without hindering material exchange between them. Moreover, when co-cultured for 14 days, the keratocytes started to deposit collagen type I, a specific marker of these cells. In contrast, ECM deposition could not be observed in the single type cell seeded samples. The co-cultured bilayer construct was tested for suturability at the end of 31 days of *in vitro* incubation and it was shown that it could be successfully sutured without any major tears. Under the light of these results it was concluded that both the single layer and the bilayer constructs are promising constructs for use as split thickness cornea replacements.

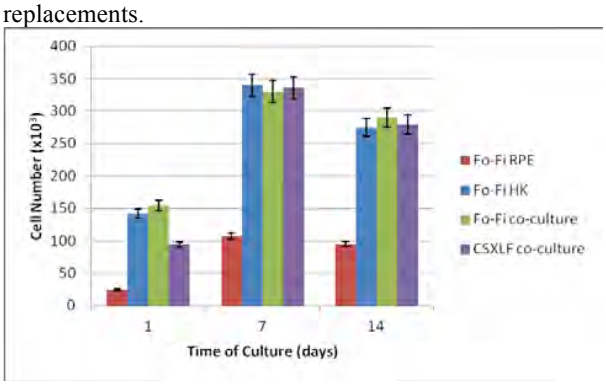

Figure 1. Proliferation of HK and RPE on bilayer (Fo-Fi) and single layer (CSXLF) scaffolds.

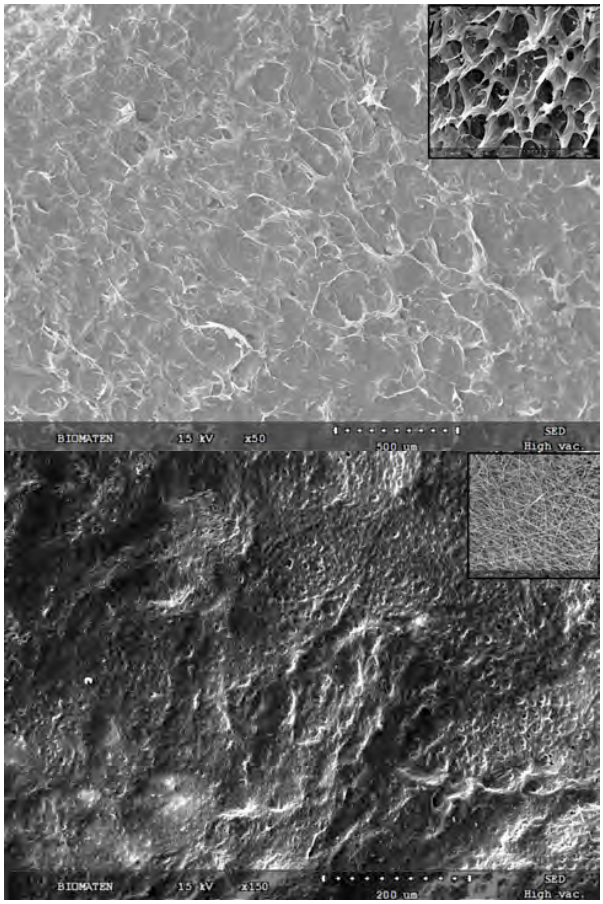

Figure 2. SEM of fiber-foam bilayer cell seeded construct. (Top) Microporous foam side with human keratocytes, (Bottom) Fibrous side with RPE. Day 14.

Conclusions: The fiber-foam bilayer construct appears to have a potential for use in split thickness cornea applications.