Tissue engineered human corneal stromal tissue

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Statement of Purpose: As the transparent outmost layer of the eye, the cornea provides three fundamental functions including chemical and mechanical protection, transparency for transmission of light and refraction of light. With ~10 million people worldwide suffering vision loss due to corneal disease or injury, the availability of healthy donor tissue for corneal grafting lags behind demand in most regions, stimulating efforts to produce biological human corneal equivalents. In this study, we focused on bioengineering the corneal stroma, which is characterized by layers of tightly-packed, highly aligned uniform collagen fibrils. We utilized a strategy of surface contact guidance to initiate and guide organization of a human corneal stroma-like matrix generated by human corneal stromal stem cells (hCSSCs) and examined the effect of varying scaffold topography.

Methods: Both solution-cast films and highly aligned electrospun fibrous scaffolds were prepared from biodegradable polycaprolactone-based poly(ester urethane) urea (PEUU).[1] Two types of PEUU-based scaffolds were seeded with hCSSCs and cultured under serum-free keratocyte differentiation media (KDM).[2] After 6 wks, the influence of scaffold topography on hCSSC-secreted extracellular matrix (ECM) was evaluated by immunohistochemistry, scanning electron microscopy (SEM), transmission electron microscopy (TEM), and gene expression as examined by quantitative reverse transcriptase polymerase chain reaction (qPCR).

Results: While solution-cast PEUU films were smooth and flat, electrospun fibrous scaffolds consisted of highly oriented nano-scale fibers (165±55nm in diameter). Immunohistochemistry demonstrated that hCSSCs deposited fibrous ECM abundant in type-I collagen on both scaffolds. However, only highly-aligned nano-fibrous scaffolds guided the expressed ECM to orient in one preferred direction, as shown in **Fig. 1a**. This observation was confirmed by SEM (**Fig. 1b**). Moreover, hCSSCs were substantially elongated and followed the same orientation direction as aligned collagen fibrils. TEM (**Fig. 1c**) revealed that hCSSC-secreted ECM deposited on aligned PEUU scaffold was sandwiched by two mono-layers of hCSSCs. All of the secreted collagen fibrils possessed uniform size and were aligned in one preferred direction. Furthermore, the fibrils were packed into a so-called "*pseudo-hexagonal*" lattice with fiber diameter and fiber spacing at 40.2±2.7nm and 60.9±6.5nm, respectively. Uniform periodic banding was also seen along each fiber with a 67nm periodicity approximating the *D*-spacing of native Type-I collagen.

In contrast, **Fig. 1d-f** show the fibrous ECM deposited on cast film to be random in nature. The cells exhibited a dendritic morphology and the collagen fibrils grew out of the cells in a radial manner. Microstructurally, there was no preferred alignment or orderly organization found in this collagen construct.

Gene expression profiles from hCSSCs seeded on both scaffold types and cultured in serum-free KDM gradually lost generic markers present in many adult stem cells, and markedly upregulated several generic markers of keratocytes. The relevant collagens and proteoglycans typifying human corneal stromal tissue were also expressed in immunofluorescent staining of hCSSC-secreted ECM (not shown here).

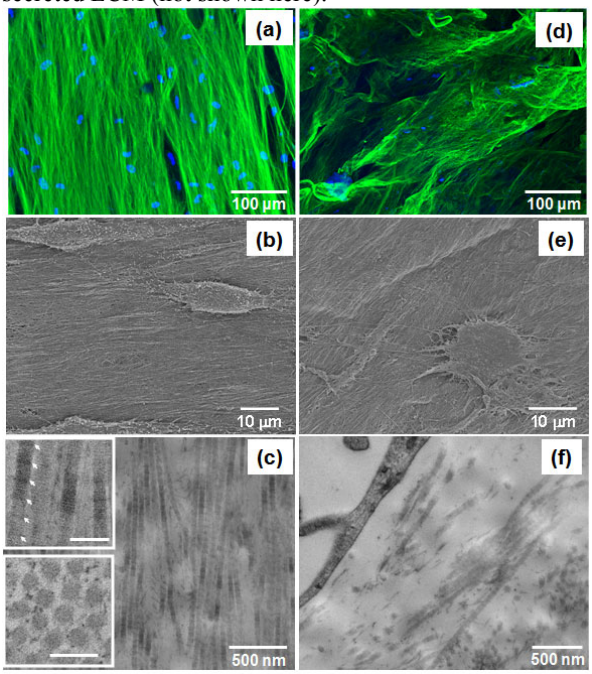

Fig. 1. Micrographs of hCSSC-secreted ECM deposited on **(a-c)** aligned nano-fibrous sheets and **(d-f)** cast films after 6-wk culture. (**a,d**) Immunofluorescent staining of Type-I collagen, cell nuclei (blue; DAPI), **(b,e)** scanning electron microscopy, and **(c,f)** transmission electron microscopy. **(c inset)** top, collagen banding; bottom, hexagonal collagen lattice. Scale bar = 100nm

Conclusions: Scaffold topography is critical to initiate and guide the organized expression of a human corneal stroma-like matrix by human corneal stromal stem cells (hCSSCs). Only the aligned nano-fibrous scaffold resulted in a collagen type-I-based ECM characterized by lamellae with oriented fibers as well as small and uniform fiber diameters and spacing. The protein expression profiles typifying human corneal stromal tissue suggest that the generated ECM mimicked human stroma-like tissue. These striking results provide evidence of the feasibility of generating corneal stromal tissue by differentiated hCSSCs under the guidance of an oriented fibrous scaffold, and represent an important first step in a bottom-up strategy to bioengineer a complete bioequivalent human cornea.

References:

[1] Stankus JJ, et al. *J Biomed Mater Res*. 70A, 603, 2004.

[2] Du YQ. et al. *Invest. Ophthalmol. Vis. Sci*., 48, 5038, 2007.