Corneal Stromal Stem Cells Versus Corneal Fibroblasts in Generating Structurally Appropriate Corneal Stromal Tissue

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Statement of Purpose: The cornea is the transparent outermost layer of the eye. Trauma, bacterial and viral infections, and heritable conditions lead to loss of corneal function and visual impairment in over ten million individuals world-wide. The limited supply of healthy cornea donor tissue has stimulated efforts to develop biological equivalents of human cornea. In this study, we focused on tissue engineering corneal stroma, the central, structural region of cornea characterized by orthogonally oriented multi-layers of tightly-packed, highly aligned uniform collagen fibrils. Employing a strategy of surface contact guidance and growth factor supplementation, we compared the applicability of human corneal stromal stem cells (**hCSSCs**) versus human corneal fibroblasts (**hCFs**) in the generation of human corneal stromal tissue.

Methods: An aligned fibrous substrate was prepared by electrospinning biodegradable poly(ester urethane) urea (PEUU) onto a spinning mandrel. hCSSCs and hCFs were seeded on the substrate and cultured in a serum-free keratocyte differentiation media (KDM) supplemented with FGF-2 (10ng/mL) + TGFβ3 (0.1ng/mL). After 9 weeks of culture, extracellular matrix (ECM) deposited by hCSSCs and hCFs on the substrates were evaluated by transmission electron microscopy(TEM), immunohistochemistry, Western blotting and quantitative RT-PCR (qPCR).

Results: Immunohistochemistry demonstrated that hCSSCs deposited fibrous ECM abundant in type-I collagen (**Fig. 1a**) on aligned PEUU fibrous scaffold. Furthermore, the elaborated ECM was abundant in keratocan (**Fig. 1b**), a characteristic proteoglycan in native human corneal stromal tissue. TEM (**Fig. 1c**) revealed secreted ECM that was sandwiched between two monolayers of hCSSCs. The hCSSC-secreted collagen fibril construct was 60~70 μm thick and featured the uniform fiber diameter and inter-fiber spacing. The stratified multilayered collagen-fibril lamellae with orthogonal orientation were morphologically similar to those developed human corneal stromal tissue.

In contrast, hCFs secreted a type-I collagen-based fibrous ECM that was not as abundant as that from hCSSCs (**Fig. 1d**). In particular, characteristic keratocan expression was lacking (**Fig. 1e**). TEM (**Fig. 1f**) showed no preferred alignment or orderly organization in this ECM. Micro-structurally, hCF-secreted ECM was similar to scar tissue of human corneal stroma.

Gene expression profiles from hCSSCs seeded on the aligned fibrous substrate and cultured in serum-free KDM gradually lost generic markers present in many adult stem cells, and markedly upregulated several generic markers of keratocytes, including *KERA*, *ALDH*, *B3GnT7*, *CHST6* and others. Western blotting revealed that hCSSCs were able to secrete human cornea-specific ECM components, including keratan sulfate, lumican, and keratocan. In contrast, these keratocyte-specific gene markers were lower ($p<0.05$) for hCFs. The unique proteoglycans and proteoglycan/keratan sulfate present in native human cornea were hardly detectable in hCF-secreted ECM by Western blotting.

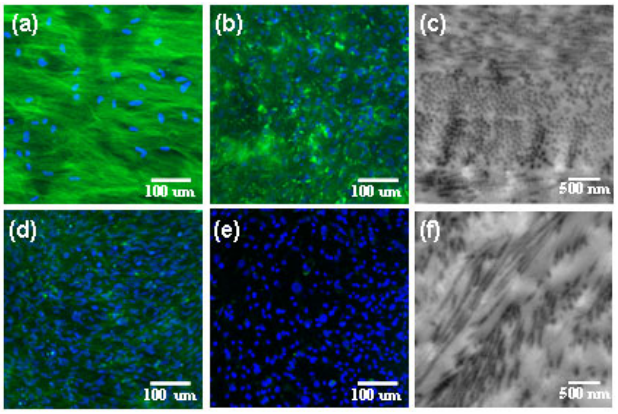

Fig. 1 Micrographs of ECM deposited by hCSSCs **(a-c)** and hCFs **(d-f)** on aligned fibrous substrates after 9 weeks culture. Immunofluorescent staining of Type-I collagen (**a,d**) and keratocan (**b,e**) is shown with DAPI nuclear staining (blue). TEM images **(c,f)** demonstrate biomimetic organized native structure and scar-like organization, respectively.

Conclusions: On aligned fibrous substrates made from biodegradable PEUU, hCSSCs could be induced to secrete and organize a type-I collagen-based ECM abundant in characteristic human corneal stromal ECM components. Spatial self-organization of the collagen-based ECM by hCSSCs featured stratified multilayered collagen-fibril lamellae with orthogonal orientation, and uniform fibril size and inter-fibril spacing in a pattern mimicking human corneal stromal tissue. In contrast, ECM secreted by hCF lacked cornea-specific ECM components, leading to less-organized microstructure in a pattern akin to corneal scar-tissue. These observations demonstrate the potential for hCSSCs, under proper substrate and growth factor guidance, to facilitate the generation of a biological human cornea equivalent. In contrast hCFs were not similar responsive to these environmental cues and generated an ECM that poorly mimicked that native, functional tissue structure and composition.

References:

[1] Wu J, et al. Biomaterials 2012; 33: 1343-1352.

[2] Du YQ. et al. Invest. Ophthalmol. Vis. Sci. 2007 ; 48 : 5038-5045.