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**LS-2-P-1930 Keratocyte interaction with stromal microfibril bundles in murine cornea**

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Corneal transparency is made possible by uniform size and spacing of stromal collagen fibrils which are organized into sheets of parallel fibrils laid down in criss-crossed fashion. Keratocytes, interspersed between collagen lamellae, make extensive lateral connections and form layers parallel with the corneal surface. Understanding the architecture of ECM is limited when using conventional 2D ultrastructural information from thin sections. Serial block-face imaging with a scanning electron microscope (SB-SEM) provides a z-stack of images that can be reconstructed to show 3D details of spatial distribution and organization of ECM ultrastructural components.

Our initial TEM observations of the mouse cornea revealed what appeared to be small random electron dense patches within the corneal stroma. Computer-aided 3D reconstructions of images generated from SB-SEM showed these patches were in fact fibers, distinctly different than stromal collagen lamellae. In this study, we used SB-SEM imaging and 3D reconstruction to characterize these stromal fibers and their relationship to the ECM and keratocytes in the mouse cornea.

Corneas from adult mice were fixed and embedded in resin blocks for histological sectioning using a custom protocol for enhanced contrast. SB-SEM images (100 nm intervals between images) were obtained using a Gatan 3 View system mounted in Quanta 200FEG SEM. Z-stacks were obtained from the stroma in the limbus, paralimbus, and central cornea. Keratocytes and stromal fibers were segmented and reconstructed in 3D using Amira 5.2 software.

Reconstruction of segmented z-stacks provided evidence the corneal stroma contains a layered network of ~100nm diameter fibers running parallel with the corneal surface and lying between and within the collagen lamellae. High magnification TEM images of the fibers show that each fiber consists of numerous microfibrils ~10nm in diameter (Fig 1). Some fibers appear to attach posteriorly to Descemet’s membrane and anteriorly to peripheral lymphatic vessels (Fig. 2). The fibers were frequently observed in juxtaposition with keratocytes and the surface of keratocytes contained complementary invaginations or “grooves” which encompassed these fibers.

3D reconstruction of SB-SEM images revealed a network of fibers composed of microfibrils within the murine cornea (Figs. 3 and 4). The association of these fibers with Descemet’s membrane and lymphatics suggests they may serve as mechanical force transducers capable of opening lymphatic vessels for drainage when an injured cornea becomes edematous. The physical association of fibers with stromal keratocytes suggests the fibers are secreted by and/or assembled by keratocytes. Additionally, the fibers may serve as a scaffold for the keratocyte network.

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Fig. 1: Electronmicrograph of corneal stroma showing a fibril bundle made of numerous microfibrils that are 10 nm in diameter (arrows).

Fig. 2: TEM image showing the cable (arrows) associated with lymphatic endothelial cell.

Fig. 3: Serial Block-face image reconstruction of cables from the stack of electronmicrographs from central cornea. The reconstructed cables form an intricate network in layers parallel with the corneal surface.

Fig. 4: Stromal elements segmented and reconstructed from the stack of electronmicrographs from paralimbus area. Keratocytes are in yellow, orange, light and dark blue, light and dark green, and purple. Microfibril bundles are in red.