Corneal Wound Healing, Diseases and Ocular Surface

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Goals and Projects:

The Wilson lab goals are to (I) identify and characterize the growth factor-receptor systems through which the functions of corneal, immune, and other cells of the anterior segment of the eye are controlled during development, homeostasis, and wound healing; (II) understand at the molecular and cellular level, the factors that lead to corneal opacity, and its resolution, after injury, surgery or infection; (III) explore the mechanism of epithelial basement membrane regeneration after injury and the importance of the corneal epithelial basement membrane in modulating epithelial-stromal interactions in the cornea, including the development of myofibroblasts associated with corneal stromal opacity.

Research and Innovations:

Recently our laboratory has characterized myofibroblasts (Fig. 1) responsible for corneal scarring or opacity that are generated after injury, infection or some surgical procedures and shown that the progenitor cells that develop into myofibroblasts can be derived either from bone marrow-derived cells or the fibroblastic cells in the cornea called keratocytes. We have also demonstrated that the development of haze is directly related to corneal surface irregularity which can interfere with normal regeneration of the epithelial basement membrane after surgery.

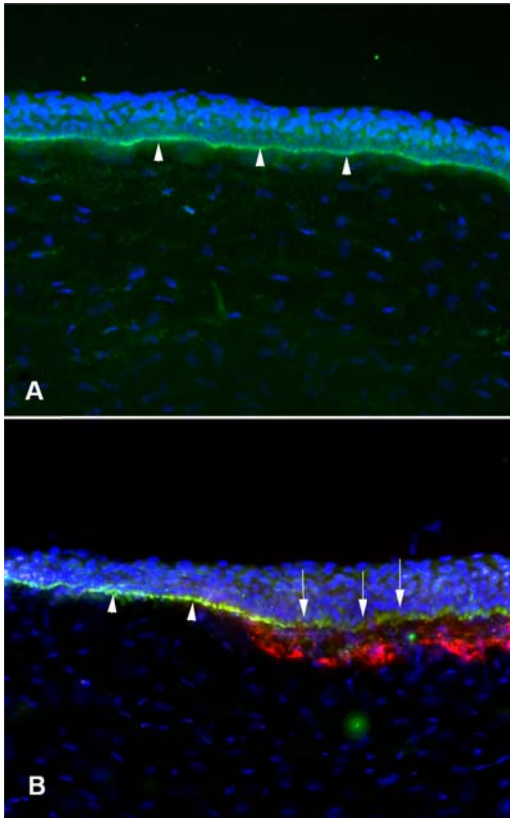

Fig. 1. Basement membrane (BM) defects and myofibroblasts in corneas with irregular surfaces after PRK. Triple staining of the central cornea for SMA-expressing myofibroblasts (red) and integrin beta-4 (green), along with DAPI (blue). (A) Note the homogeneous BM regeneration (arrowheads) in a cornea without haze after -4.5 D PRK. (B) BM with obvious disruptions (arrows) adjacent to an area with more uniform basement membrane (arrowheads) after -4.5 D PRK with 50% screening of the excimer laser pulses over a fine mesh screen. Similar disruptions were noted in corneas that had -9 D PRK without screening of pulses. Note red SMA+ cells below the defects in the BM. 400X.

Myofibroblasts are generated in the anterior subepithelial stroma after many forms of corneal injury (Fig. 2). Our work has demonstrated that these cells develop and persist there because they are dependent on TGFß and PDGF cytokines produced by the overlying corneal epithelium.

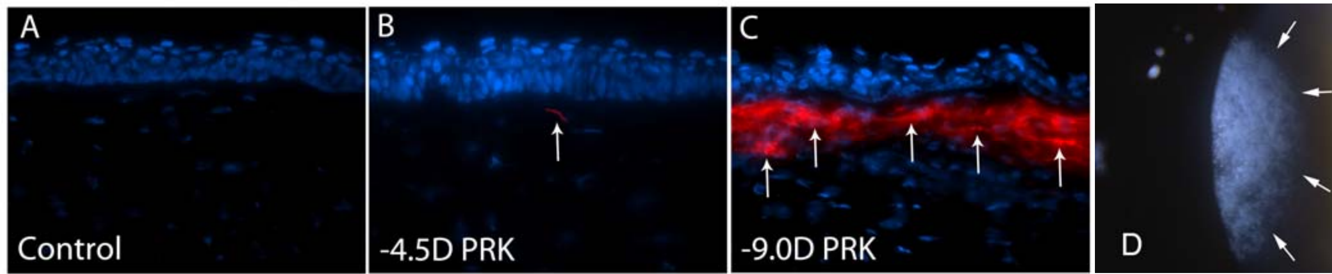

Fig. 2. IHC for myofibroblast marker SMA (red, arrows) on 7 μm thick central corneal sections from an unwounded control cornea (A), a cornea that had -4.5D PRK (B) and a cornea that had –9D PRK (C). Blue is DAPI for cell nuclei and reveals the epithelial cells (top blue band in each panel) and presumed keratocytes, and possibly some inflammatory cells, scattered in the stroma of A and B, and beneath the layer of myofibroblasts in C. Note a dense layer of subepithelial SMA+ myofibroblasts (arrows in C) noted in all -9D PRK corneas (C) (40 to 62 SMA+ cells/400X column) compared to rare (<1 SMA+ cells/400X column) myofibroblasts (arrow in B) in all -4.5D PRK corneas (B) and no detectible SMA+ cells in the unwounded control (A). 400X. D. Stromal haze after PRK. 10X

An important discovery recently made in the lab is that corneas that have stromal opacity, also called haze, after high correction photorefractive keratectomy (PRK) to correct myopia have an abnormality of the epithelial basement membrane when it regenerates after surgery (Fig. 3). Our working hypothesis is that this abnormality occurs because the myofibroblasts that develop in the subepithelial stroma do not produce one or more of the epithelial basement membrane components normally produced by the keratocyte cells in the stroma. These normal stromal keratocytes are blocked from approaching the subepithelial zone where the epithelial basement membrane is regenerated by the presence of the myofibroblasts in these corneas after high PRK correction.

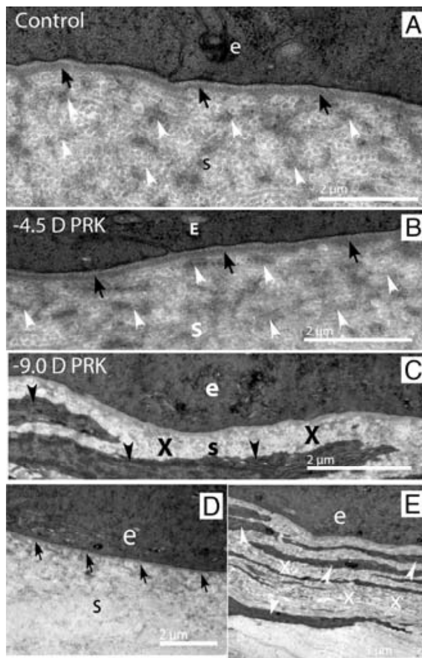

Fig. 3. TEM at 30,000X of unwounded control (A), -4.5D PRK (B), and -9.0 D PRK (C) central rabbit corneas at one month after surgery. White E or e indicates epithelium and s indicates stroma in all panels. Black arrows in panels A and B indicate the lamina densa. The lamina lucida is the less dense band between the lamina densa and the basal epithelium. Also note the normal regular arrangement of stromal collagen fibrils seen in cross-section in some areas of A and B, with scattered keratocytes (white arrowheads) in the anterior stroma. In all -9.0D PRK corneas (C) no normal BM morphology was detected and the subepithelial stroma is packed with abnormal extracellular matrix (X) and cells with large amounts of rough endoplasmic reticulum (arrowheads) that are the SMA+ myofibroblasts detected by IHC in Fig 2C. Lower mag TEM images from a -4.5D PRK cornea at 13,000X (D) and a -9.0D PRK cornea at 68000X show deeper stroma. Arrows in D indicate the epithelial BM. White arrowheads in E are several stacked myofibroblasts corresponding to SMA+ cells in Fig 2C and X indicates areas of disorganized extracellular matrix anterior and posterior to myofibroblasts extending from the basal epithelial cells to the deepest myofibroblasts.

We have developed animal models in rabbits and mice that can be used to test the efficacy of potential drugs that could be used to block myofibroblast development and haze generation in the cornea.

Lab Staff Members:

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