Nanofibrous Scaffolds for Orderly Corneal Wound Healing

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Statement of Purpose: Corneal blindness deprives 10 million people worldwide of their sight. Current treatments for corneal blindness mainly rely on implants that replace the injured tissue and have been clinically unsuccessful thus far. The goal of this project is to *prevent* corneal blindness by developing clinically-viable scaffolds that promote orderly corneal wound healing using biochemical and topological cues. Specifically, we have created a composite scaffold, comprised of oriented protein nanofibers embedded within a soft, degradable hydrogel matrix, reminiscent of the native cornea. We have studied our composite scaffold extensively *in vitro* using rabbit corneal fibroblasts, *ex vivo* using intact rabbit eyes and *in vivo* using a mouse and rabbit model.

Methods: *In vitro* experiments: corneal fibroblasts, isolated from young rabbit eyes, were seeded on electronspun collagen nanofibers. Rate of cell migration was observed using a mock wound healing assay. Cellular gene and protein expression levels were analyzed using quantitative PCR (qPCR), immunoblotting and immunocytochemistry. *Ex vivo* experiments: Lamellar keratectomies (6mm diameter; 150µm deep) were performed on fresh rabbit eyeballs to create corneal wounds, which were then treated with our scaffold. The corneas were excised from the eyeballs and cultured in a humidified incubator (37°C, 5% CO_2) on an agar support in DMEM/F-12 media. *In vivo* experiments: Mice experiments were approved by the Institutional Animal Care and Use Committee of the California Institute of Technology. Following anesthesia administration, the epithelium of the cornea was debrided and the wound was treated with our scaffold. Wound closure was visualized using fluorescein staining. Harvested corneal sections were subjected to H&E staining and immunolabeling.

Results: The success of an effective treatment for corneal blindness hinges on the ability to modulate myofibroblasts present in a corneal wound. These cells are normally quiescent in a healthy cornea but become hyperactive following corneal injury, during which they deposit disorganized extracellular matrix proteins that cause corneal scarring and express α-smooth muscle actin (α-SMA) stress fibers that distort the corneas' smooth refracting surface. We hypothesized that a composite scaffold reminiscent of the highly-ordered structure of the native cornea will regulate the behavior of myofibroblasts and promote a calmer wound healing response.

The collagen nanofibers used in our scaffold were fabricated by electrospinning and are transparent and non-cytotoxic. We have shown that corneal fibroblasts seeded on oriented nanofibers have a faster rate of migration compared to those on planar surfaces. In addition, we confirmed that the presence of nanofibers modulates the behavior of myofibroblasts by analyzing the relative gene and protein expression levels of α-SMA of seeded cells.

The *in situ* forming hydrogel used in our scaffold was developed in collaboration with Giyoong Tae and cures under visible-light using eosin Y as a photoinitiator with triethanolamine as an electron donor to initiate reaction of thiolated-heparin and acrylate-ended poly(ethylene glycol). We have shown that the hydrogel forms quickly (< 5minutes) under irradiation conditions compatible with safety standards for corneal and retinal exposure, and is easily tunable in terms of both the mechanical properties and gelation kinetics. The incorporation of heparin in the hydrogel allows for the binding and release of growth factors that modulate the myofibroblast phenotype.

To test the efficacy of our scaffold in a more clinically relevant setting, we developed an *ex vivo* full tissue model that allows us to keep excised corneas alive for 21 days during which the corneas retain their ability to heal injuries. Using this model, a wound was created on the cornea and then treated with our scaffold. Results from *ex vivo* studies helped screen for promising scaffold candidates prior to *in vivo* testing.

To test the *in-situ* toxicity of our scaffold and photocrosslinking procedure, *in vivo* experiments were carried out in mice: the epithelium of the cornea was debrided and the wound was treated with our scaffold. When observed the next day, the mice showed no signs of distress and no inflammation was observed in their eyes. Our treatment supported re-epithelialization at rates comparable to control eyes and histological analysis of harvested corneas showed normal corneal morphology following wound closure. To gain further insight in the efficacy of our scaffold in treating corneal wounds, a rabbit model was used where the creation of deeper wounds (150µm deep) was possible. Preliminary results show that our scaffold reduces the number of myofibroblasts present in the wound (**Figure 1**), calms down the eye's immune response and minimizes the formation of fibrosis.

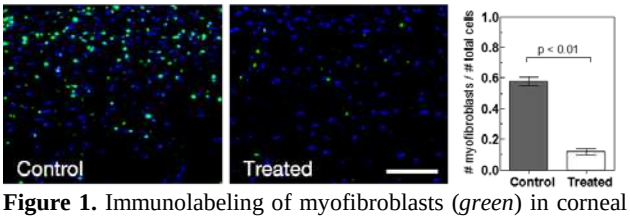

Figure 1. Immunolabeling of myofibroblasts (*green*) in corneal tissue sections. Blue dots: cell nuclei. Scale bar is 50 microns.

Conclusions: We have demonstrated that our scaffold is well-suited for corneal wound healing applications due to its transparency and ability to recruit healthy corneal cells into the wound area and modulate the behavior of hyperactive repair cells *in vitro* and *in vivo*.