Remotely-activated injectable materials for restoring thinning corneas

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BACKGROUND

Keratoconus is an example of a common corneal thinning disease that affects 21 per 1000 men and 18 per 1000 women.[1] While the etiology beyond both genetic and environmental origins is unclear, inflammation and oxidative stress have roles in disease development and progression. [2] The hallmarks of keratoconus are excessive degradation of the collagen fibers by matrix metalloproteases and loss of corneal stromal keratocytes.[3] Currently, the early stages of keratoconus are managed with therapeutic contact lenses, while later stages are treated by corneal crosslinking to stabilize remaining collagen and prevent further degradation. However, in severe cases, corneal transplantation (keratoplasty) is needed. [4] Although corneal transplantation for severe keratoconus where collagen crosslinking cannot be performed is successful, complications include severe postoperative astigmatism, delayed visual rehabilitation, and graft rejection. Furthermore, a lack of donor corneas, particularly in developing countries, results in less than 5% of individuals in need receiving a corneal transplant.[5] Even though biomaterial-based alternatives, such as our solid corneal implants made from recombinant human collagen which stimulate corneal tissue regeneration, offer an alternative to donor human corneal transplantation,[6,7] they require invasive surgery. Here, we expanded on the concept of using an injectable peptide-based material but as a bulking agent to rebuild corneal stromas with advanced thinning instead of only trying to stabilize them with UV crosslinking or replacing them with an invasive donor cornea transplantation. As these thinned corneas have an abnormal extracellular matrix, we will substitute not just the collagen but the corneal extracellular matrix which contains a significant proportion of water-retaining proteoglycans to ensure optimal hydration of the rebuilt corneal stroma.[8]

METHODOLOGY

Hydrogels with varying concentrations of biopolymers and peptides were synthesized and tested with the top formulations being selected for further characterization. The viscosity of the developed materials was then finetuned for intracorneal injections by initiating semi-controlled radical polymerization while mixing allowing for rapid thickening of the material as shown in Figure 1. After pre-activation of the peptide-based material in the lab, the material was injected into corneal stroma where the material was fully activated to thicken cornea tissue. The material was tested in *ex vivo* pig corneas and in vivo in a rat model as shown in Figure 2.

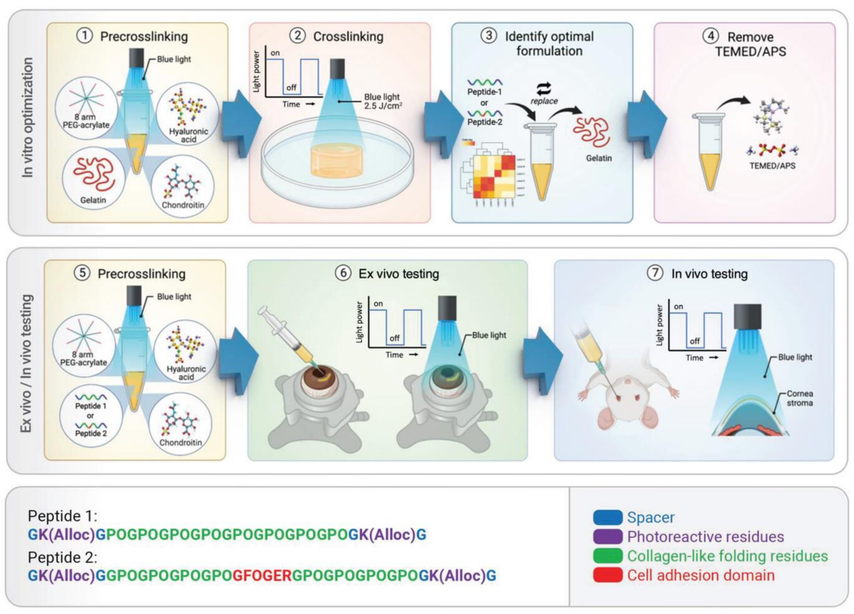

Figure 1: Schematic illustrating the process to create injectable materials.

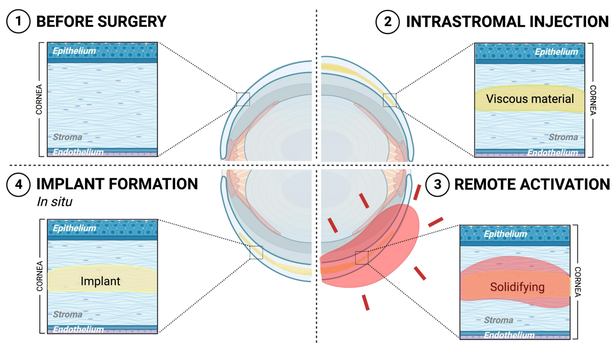

Figure 2: Schematic illustrating the protocol used to thicken rat corneas in vivo

RESULTS

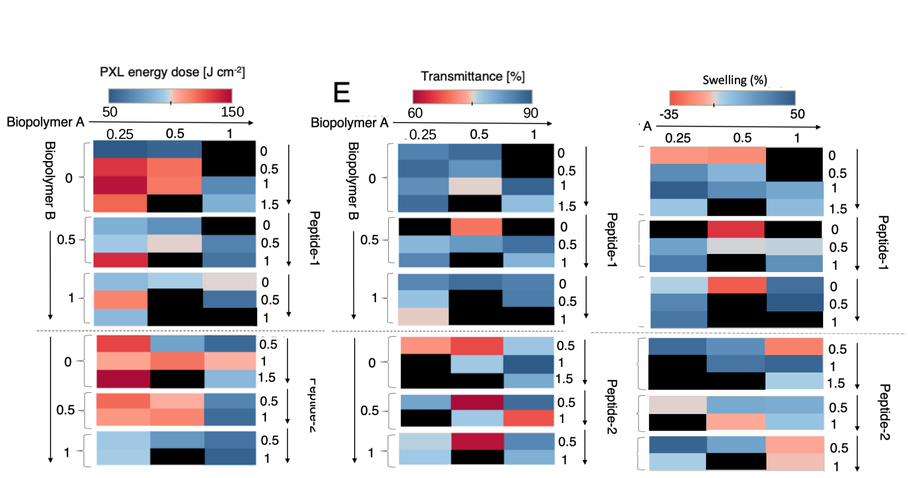

Figure 3: Physical characterization of hydrogels with varying concentrations of biopolymers and custom-made peptides.

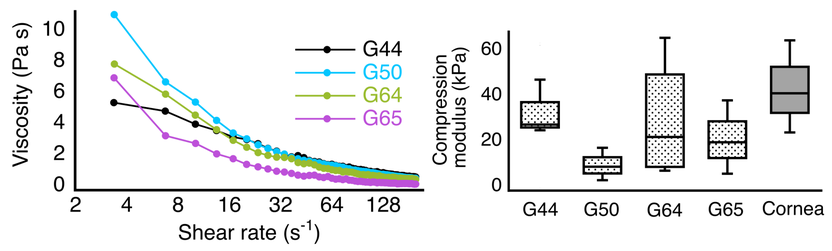

Figure 4: Shear thinning and mechanical properties of developed materials. Left: Viscosity as a function of shear rate (s^{-1}) measured for the four different peptide-based formulations. Right: Compression moduli for fully crosslinked materials (n≥3). Data showed the plot are represented as box plots where the box encloses 50% of the data, upper and lower quartile, with the median value of the variable displayed as a line inside the box.

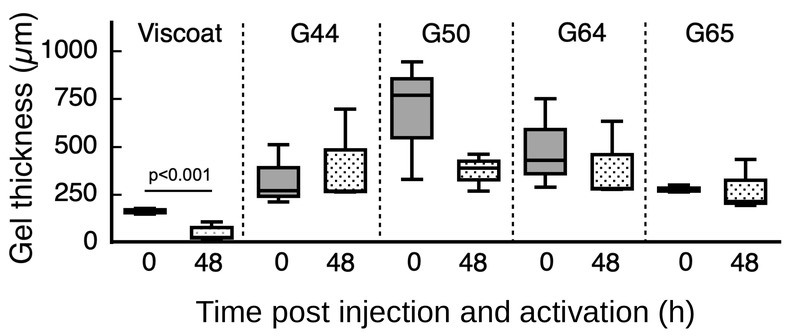

Figure 5: Stability of biomaterials post-injection into ex vivo porcine corneas after 48 h at physiological intraocular pressure. Viscoat is an ophthalmic viscosurgical device and was used as the control to show that our gels have greater retention inside cornea tissue.

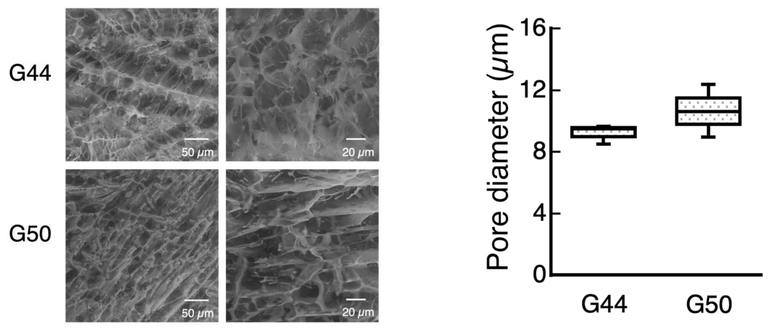

Figure 6: Cryo-scanning electron microscopy (Cryo-SEM) of G44 and G50 hydrogels. The pore size was determined using ImageJ software calculated from measuring +100 individual pores per sample from independent regions of the hydrogels.

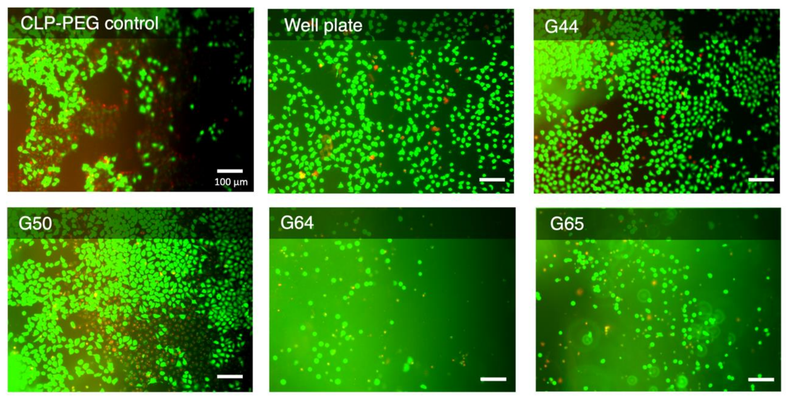

Figure 7: Representative images for LIVE/DEAD assay of corneal epithelial cells culture onto treated well plate and fully formed hydrogels.

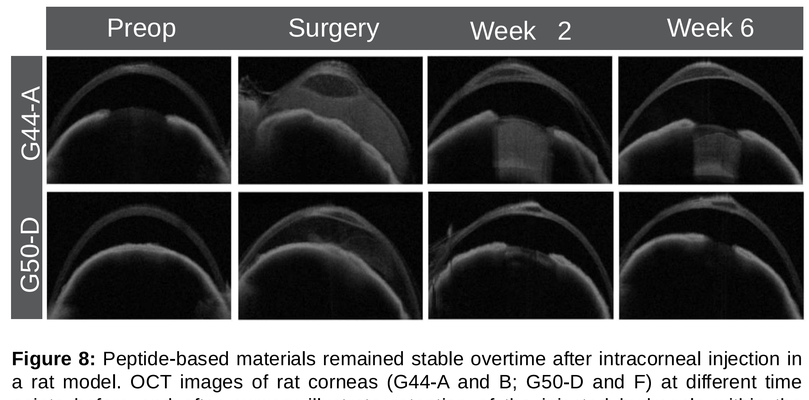

Figure 8: Peptide-based materials remained stable overtime after intracorneal injection in a rat model. OCT images of rat corneas (G44-A and B; G50-D and F) at different time points before and after surgery illustrate retention of the injected hydrogels within the corneal stroma 6 weeks post operation.

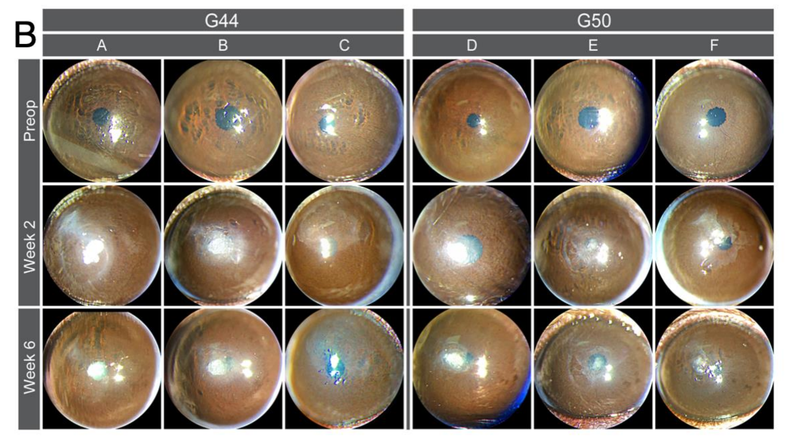

Figure 9: Peptide-based materials did not promote corneal vascularization and all corneas healed with minimal scarring. Cornea transparency was monitored after biomaterial intrastromal injection over 6-week period.

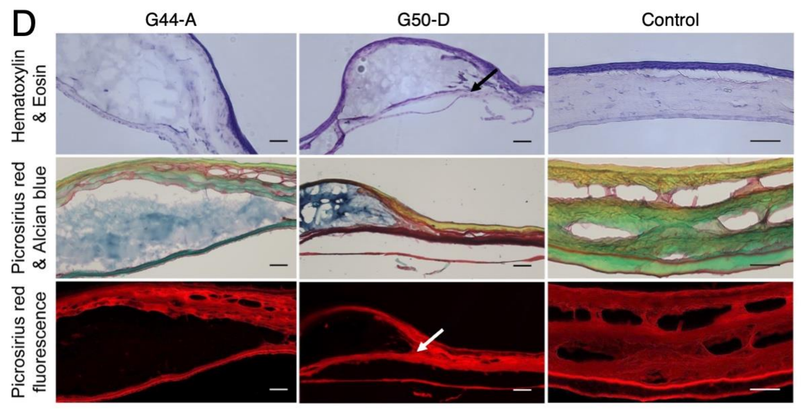

Figure 10: Histology of intracorneal injection in a rat model. Hematoxylin and eosin staining shows the retention of the changed shape of the cornea in two rats after injection with the bulking agents (G44-A and G50-D).

CONCLUSION

Corneal thinning is a significant problem for which there are no effective solutions. Corneal crosslinking only serves to stabilize already thinning or thinned corneas but does not replace the largely lost collagenous extracellular matrix. In this study, peptide-based injectable materials were developed to repair diseased or damaged corneal extracellular matrices. The best candidate formulations comprising of a mixture of biopolymers and custom-made peptides exhibited good biocompatibility, high transparency, and similar mechanical properties to cornea tissue. The injectable materials were able to change the cornea shape and thicken the corneas to varying degrees in an ex vivo pig cornea model. Intracorneal injection of these materials in rats showed that the top two materials, G44 and G50, caused no significant inflammation or neovascularization and remained stable in vivo for 6 weeks. The developed materials' ability to reshape and thicken corneas makes it a plausible alternative to corneal transplantation for future treatment of corneal thinning disorders.

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