Biomimetic Injectable Hydrogels for Novel Myopia Treatment

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Statement of Purpose: This project targets myopia (near-sightedness), which has become the foremost public health problem in some parts of the world due to its very high prevalence (e.g. >80% in Singapore & Taiwan) and sight-threatening complications (especially in high myopia). There are currently no effective treatments for myopia. We explored the application of injectable, thermoresponsive, and enzymatically degradable semi-interpenetrating polymer network (edsIPN) hydrogels for stabilizing the disorder in the established myopic chick eye model[1]. Our purpose was to test the hypothesis that edsIPN, injected intraorbitally, adjacent to the sclera at the posterior pole of the eye, will promote scleral fibroblast migration and proliferation, effectively increasing the thickness and strength of the native sclera, and thus inhibit myopic growth. Apart from the principal goal of developing new anti-myopia therapies, this project has also provided new insight into the mechanisms underlying normal and myopic eye enlargement, which remain poorly understood.

Methods: The thermoresponsive hydrogel, p(NIPAAm-*co*-AAc), was comprised of NIPAAm, acrylic acid (AAc), and acrylated peptide crosslinker Gln-Pro-Gln-Gly-Leu-Ala-Lys-NH_2 (QPQGLAK-NH_2; American Peptide Co.; Sunnyvale, CA)[2,3]. The crosslinker was proteolytically degradable by matrix metalloproteinases (MMPs) and other collagenases. The p(NIPAAm-*co*-AAc) was interpenetrated by polyacrylic acid-graft-Ac-CGGNGEP**RGD**TYRAY-NH_2 [p(AAc)-*g*-RGD] linear polymer chains to promote cellular adhesion. The RGD motif was known to bind readily to several integrin receptors reported to be actively involved during normal and myopic eye growth in the mammalian sclera[4]. The

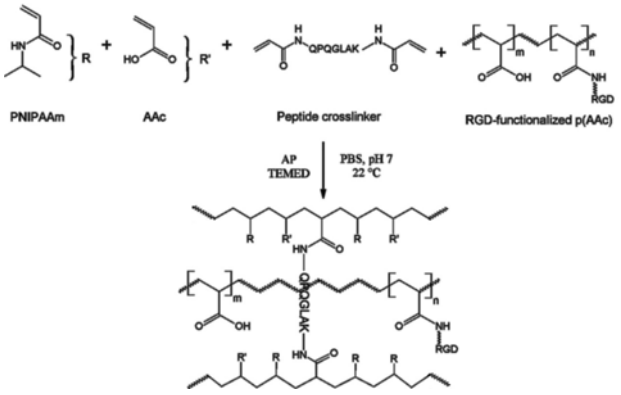

Figure 1. Polymerization scheme of edsIPN in the study.

edsIPN was synthesized by redox free radical polymerization (Fig. 1) at room temperature with molar ratios of 95:5:0.3 (NIPAAm: AAc: crosslinker). The viscoelastic properties of the edsIPN were characterized by dynamic oscillatory shear measurements, using a parallel plate rheometer. The complex shear modulus (G^*) was characterized as a function of frequency and temperature at 5% strain. Primary chick scleral fibroblasts were cultured *in vitro* on edsIPN and tissue-culture polystyrene (TCPS) surfaces. Cell proliferation was characterized daily over 11 days with an initial seeding density of 2.0×10^3 cells/mL using 48-well tissue-culture plates. The edsIPN was introduced into the chick sclera through retrobulbar injection at the posterior pole of the eye. Two groups of 9 chicks (*Gallus gallus domesticus*) each were used for *in vivo* study: 1) edsIPN injection alone, and 2) edsIPN injection and -10 D lens treatment to induce myopia progression. All treatments were monocular, with left or right eye assigned randomly. The fellow eyes served as non-treated controls. Scleral thickness and axial length of the eye was monitored *in vivo* over 28 days using a custom high frequency (30MHz) A-scan ultrasonography set-up.

Results: The mean G^* at 1 Hz and 22 ºC was 14.13 ± 6.13 Pa, and at 1 Hz and 37 ºC was 63.18 ± 12.24 Pa, representing a 4.5 fold increase in stiffness due to temperature increase. All edsIPNs synthesized for this study were transparent and easily injectable through a 19G needle at room temperature, as required for *in vivo* scleral application. Scleral fibroblasts cultured on edsIPN showed more favorable morphology than when cultured on TCPS (i.e. more rounded), but proliferated more slowly. Cell counts for the edsIPN surface also plateaued at a lower value, i.e. $3.22 \pm 0.39 \times 10^4$ cells/mL compare to $7.98 \pm 0.48 \times 10^4$ cells/mL for the TCPS surface plateaued. Normalized scleral thickness significantly increased by 6 % by day 28 for eyes treated with -10 D lenses + edsIPN injection (176 ± 4 vs.166 ± 4 µm for fellow eyes); changes in edsIPN injected eyes left without lenses and their fellow untreated eyes were 142 ± 6 µm and 148 ± 4 µm respectively. Over the same time interval, axial lengths increased to 13.27 ± 0.12 mm for edsIPN treated eyes with -10 D lenses, and 12.74 ± 0.17 mm for their fellow eyes. EdsIPN treated eyes without lenses and their fellow eye showed similar increases in axial length (12.46 ± 0.05 mm).

Conclusions: A mechanically tunable edsIPN allowing cell attachment and degradation by MMPs was synthesized and utilized in this study. Favorable *in vitro* scleral cell morphology was observed with slowed cellular proliferation. Most importantly, scleral thickness was increased significantly in myopic eyes injected with edsIPN, although it did not lead to inhibition of myopic growth. These findings represent the first steps in the development of a novel approach to treat high myopia using biomimetic edsIPNs. Future studies will use the guinea pigs as mammalian eye models and include the use of these biomimetic edsIPNs as drug delivery vehicles for anti-myopia pharmacological agents.

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