Minimally Invasive Cell and Drug Delivery Biomaterials for Posterior Segment Ocular Therapy

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Statement of Purpose: Sustained delivery of pharmaceuticals to the back of the eye is one of the most significant unmet needs in vision health care. The current standard of care for pharmaceutical treatment of patients with posterior segment complications, such as age related macular degeneration (AMD), is frequent injections (every 4 – 6 weeks) directly into the vitreous chamber. These frequent injections are associated with increased risk of infection, cataract, hemorrhages, increased intraocular pressure, retinal detachment in addition to patient discomfort. There is a large market demand for an ophthalmic drug delivery device that is minimally invasive, easily administered, and provides sustained, long-term delivery of pharmaceuticals locally to the retina. We hypothesize that PNIPAAm, a thermo-responsive polymer that undergoes a rapid phase transition from liquid to gel when heated above a lower critical solution temperature (LCST), roughly 32°C, may serve as an ideal vehicle for delivery of pharmaceuticals into the back of the eye. The thermopeutic polymer would allow a drug-infused liquid suspension to be injected into the vitreous chamber and the subsequent temperature-induced gelation would entrap the delivered drug thereby providing a means to introduce a solid drug delivery reservoir into the eye for long-term sustained release. However, one of the major drawbacks of PNIPAAm is that it is non-degradable. Ideally, an injected drug delivery scaffold will degrade and be cleared from the body upon exhaustion of its drug. Therefore, to introduce degradability into the PNIPAAm-based material, we have copolymerized NIPAAm with dimethyl butyrolactone acrylate (DBA), which undergoes a hydrolytic ring opening process that increases the LCST above physiological temperature. The ring opening process triggers the gelled material to transition back to a liquid state, which should promote clearance from the eyes into the systemic circulation where it can be filtered from the body via the kidneys.

Methods: Copolymers of NIPAAm, N-acryloxysuccinimide (NAS), acrylic acid (AA) and DBA (Sigma), denoted as PNNAD, were synthesized via free radical polymerization using benzoyl peroxide (BPO) as an initiator. Copolymer composition was verified by ^{1}H-NMR. Phase transition properties were examined using DSC and turbidity testing. Drug release kinetics were observed by loading copolymers with bovine serum albumin (BSA) as a model protein and incubating in PBS (pH 7.2) at 37°C. Degradation rates of the copolymers were examined by observing change in mass as a function of time when samples were incubated in PBS at 37°C. SEM images were used to observe the change in copolymer morphology as a function of degradation. Copolymers were incubated with pre-adhered retinal pigment epithelial (RPE) cells to examine cellular compatibility. Finally, cell adhesive RGDS peptides (American Peptides) were grafted along the PNNAD backbone to examine potential application of these polymers as temporary cell scaffolds for retinal therapy.

Results: Three degradable PNIPAAm-based copolymers were synthesized and all displayed sub-physiological LCSTs that were strongly influenced by DBA content. Copolymer composition was determined by ^{1}H-NMR.

Table 1: Final composition and LCST of the copolymers.

Feed Ratio NIPAAm-NAS-AA-DBA	Yield (%)	Composition (^{1}H NMR)	LCST (°C)
PNNAD 1 (80 - 4 - 12 - 4)	93	76.0 - 3.4 - 14.7 - 3.9	21.3
PNNAD 2 (80 - 4 - 8 - 8)	90	74.8 - 4.1 - 12.9 - 8.2	17
PNNAD 3 (80 - 4 - 4 - 12)	87	75.2 - 3.8 - 8.6 - 12.4	13.1

Slow copolymer degradation rates were observed, which is crucial for sustained drug release. Slower degrading scaffolds can delivery drugs for longer periods of time, thus decreasing the frequency of treatment.

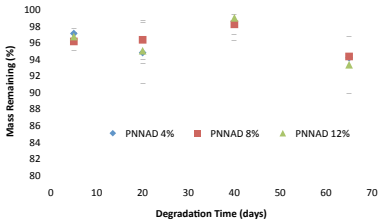

Figure 1: Degradation profile of PNNAD copolymers

Although very little change in mass was observed following 65 days of incubation in PBS, surface morphology appeared to have undergone structural changes, suggesting that degradation may have commenced.

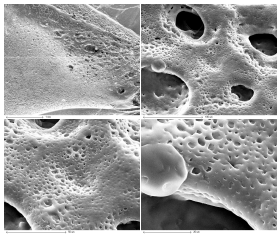

Figure 2: SEM micrograph of PNNAD 2 at day 65. Scale bar: top left 1 mm, top right 100 μm, bottom left 50 μm, bottom right 20 μm.

Following complete degradation, which was achieved through harsh basic conditions, there was no observable phase transition between 0 – 100 °C. All scaffolds displayed excellent compatibility with RPE cells and NMR revealed successful incorporation of RGDS. Delivery studies are ongoing.

Conclusions: Degradable PNIPAAm-based copolymers were developed in attempts to facilitate sustained delivery of pharmaceuticals to the back of the eye. PNIPAAm-based polymers offer a means to introduce solid drug reservoirs into the vitreous using minimally invasive techniques and slow degradation kinetics should allow long-term delivery, reducing the frequency of treatment.