Transparent, Thermoresponsive Hydrogels for Ophthalmic Drug Delivery

Mohammad. A. Jafar Mazumder[1], Scott Fitzpatrick[2], Ben Muirhead[2] and Heather Sheardown[1, 2]*

[1]McMaster University, Department of Chemical Engineering, 1280 Main Street West, Hamilton, ON, L8S 4L7, Canada.

[2]McMaster University, School of Biomedical Engineering, 1280 Main Street West, Hamilton, ON, L8S 4L7, Canada.

INTRODUCTION

Blood ocular barriers such as the blood retinal barrier (BRB) and the blood aqueous barrier (BAB) are effective at isolating the eye from systemic circulation, thereby preventing the infiltration of pathogens and immune cells, which could mount a host response and damage the sensitive ocular tissues. However, these restrictive barriers significantly limit the ability to deliver pharmaceuticals to the eye via the blood stream; only an estimated 1- 2% of systemically applied drugs reach the posterior segment of the eye, which houses the light-sensing retinal cells that are crucial for sight and are typically damaged or lost in retinal degenerative diseases such as age related macular degeneration (AMD) and diabetic retinopathy (DR)[1]. Furthermore, only 5% of topically applied drugs successfully enter the anterior segment of the eye, and negligible amounts enter the posterior segment[2]. The most effective modality of delivering into the posterior segment of the eye is through direct intravitreal injections. However, the eye contains efficient clearance mechanisms that effectively eliminate pharmaceuticals from the eye once they have successfully been introduced into the ocular environment. These clearance mechanisms necessitate the use of frequent injections to maintain therapeutic concentrations within the eye, which can result in a number of complications such as vitreous haemorrhage, cataract formation, retinal detachment and patient discomfort[3]. These obstacles make delivery of pharmaceuticals to the back of the eye one of the most significant unmet needs of visual health care. There is a clear need for new delivery modalities that are capable of safely providing sustained delivery of pharmaceuticals to the posterior segment of the eye in a minimally invasive fashion. We hypothesize that in situ forming hydrogels are ideal for the introduction of a solid drug-releasing scaffold to provide long-term release of pharmaceuticals directly within the vitreous body. Poly(N-isopropylacrylamide) (PNIPAAm), which undergoes a rapid phase transition from liquid to gel when heated above a lower critical solution temperature (LCST) of approximately 32^{o}C in aqueous conditions is an attractive candidate[4]. This thermoresponsive polymer would allow relatively non-invasive delivery of a room temperature suspension into the vitreous chamber via syringe and would gel quickly upon injection into the eye, entrapping the drug solution, creating a drug-infused polymer scaffold that slowly releases its therapeutic agent in a localized setting.

However, PNIPAAm suffers from two major drawbacks. Firstly, PNIPAAm is a non-degrading synthetic polymer[5]. Therefore, after all drug has been exhausted, the polymer will remain within the vitreous for the lifetime of the patient unless surgically removed. The second shortcoming of PNIPAAm is that as it transitions from a liquid to gel, it loses its optical transparency. This may not pose a significant limitation as, similar to other non-degrading opaque intravitreal drug releasing scaffolds, the copolymer is expected to reside on the floor of the vitreal chamber, outside the visual axis. However, an optically transparent scaffold would decrease the number of potential complications that may arise throughout the course of the release. It would not be suitable for a drug-releasing scaffold designed to combat retinal blindness to obstruct incoming light and become the cause of visual loss.

In this work, we discuss the development of several degradable, optically transparent copolymers based on NIPAAm for minimally invasive delivery of pharmaceuticals into the posterior segment of the eye to combat retinal degenerative diseases.

EXPERIMENTAL

Materials: Acrylic acid N-hydroxysuccinimide (NAS), Acryloyloxy dimethyl butyrolactone (95%) (DBA), dexamethasone (98%), and benzoyl peroxide (BPO, 97%) were purchased from Sigma- Aldrich (Oakville, ON, Canada), and used as received. N-isopropylacrylamide (NIPAAm) (97%) was purchased from Sigma, and was purified by recrystallization from a toluene/ hexane mixture. Acrylic Acid (AA) (99%) monomer and varying molecular weight (MW) PEG monomers (475, 526 and 1100 Da) were purchased from Sigma and purified. 1, 4-dioxane, toluene, hexane, THF, DMSO and ethyl ether from Caledon Laboratories (Caledon, ON) were used as received. Phosphate buffered saline solution (PBS, pH 7.4) was obtained from McMaster University Health Science Facilities and used as received.

Synthesis of poly(NIPAAm-NAS-AA-DBA): Several poly(NIPAAm-NAS-AA-DBA) copolymers with different compositions of AA and DBA were prepared by free radical polymerization. Briefly, NIPAAm (3.99 g, 35.30 mmol), NAS (0.298 g, 1.76 mmol), AA (0.382 g, 5.30 mmol), DBA (0.325 g, 1.76 mmol) and BPO (0.106 g, 0.441 mmol) were dissolved in 45 mL 1, 4- dioxane to form a 10 wt% monomer solution, and was heated to 70°C for 24hrs. The pNNAD-4 polymer was isolated by precipitation in ethyl ether, and purified by dialysis followed by freeze drying. Yield: 4.65 g (93%). pNNAD-8 and 12 were prepared and isolated in similar fashion, where the number refers to DBA content (mol %).

Synthesis of poly(NIPAAm-NAS-PEG$_x$-DBA): poly(NIPAAm-NAS-PEG$_x$-DBA) (pNNP$_x$D) copolymers with varying compositions and MW of PEG and DBA were prepared by free radical polymerization following the procedure described above.

Material Characterization: Copolymer structure and composition were determined using FT-IR (Nicolet 6700) and ^{1}H NMR (Bruker AV 600). Copolymer LCSTs were characterized by differential scanning calorimetry (DSC) and turbidity testing. Degradation studies were carried out in PBS at 37^{o}C. A Philips 515 scanning electron microscope (SEM) was used to assess changes in copolymer morphology as a function of degradation. Drug release kinetics were examined by dissolving 100 mg pNNAD copolymers in 0.5 mL PBS solutions containing 100 mg/mL dexamethasone and examining the release profile from the gelled pellets using a Waters high performance liquid chromatography (HPLC) system.

RESULTS AND DISCUSSION

To address the non-degradable nature of PNIPAAm, we have synthesized copolymers consisting of NIPAAm, NAS, AA and DBA. Copolymer compositions are shown in Table 1 where the number (i.e. pNNAD-4) refers to DBA content (mol %). AA was introduced into the copolymer to increase the hydrophilic content and NAS provided an amine reactive side group that could be utilized for facile conjugation of bioactives such as drugs or cell adhesive peptides. The hydrophobic DBA co-monomer contains a hydrolytically susceptible lactone ring that opens upon degradation, creating carboxylic acid and hydroxyl functional groups, which shift the co-monomer to a more hydrophilic state. The net impact of this ring-opening process is a dramatic increase in copolymer hydrophilicity, which leads to an increase in LCST. When the extent of hydrolytic ring-opening is sufficient to increase the LCST above body temperature, the copolymer re-hydrates, transitioning back into a liquid state and can be effectively cleared from the eye into the systemic circulation through the effective ocular clearance mechanisms. From the systemic circulation, the copolymer will be filtered from the body via the kidneys, provided the molecular weight is below the filtration threshold of approximately 30 KDa, without releasing any small molecular weight byproducts, which have been shown to induce inflammatory or cytotoxic responses in a number of degradable biomaterials[6].

DSC and ^{1}H-NMR analysis of partially degraded pNNAD reveal that hydrolytic opening of the DBA lactone ring is sufficient to elevate copolymer LCST above physiological temperature, Figure 1. These results indicate that copolymer degradation and clearance from the body should occur without the production of small molecular weight byproducts. Furthermore, degradation studies reveal that copolymer hydrolysis and subsequent re-hydration occur slowly as shown in mass loss over time in Figure 2, which should allow for long-term release of

low levels of drug within the posterior segment following injection into the eye. Sustained release would greatly decrease the number of injections required to maintain therapeutic concentrations of drug within the eye and copolymer degradability will promote clearance from the eye without the need for invasive surgical intervention.

Table 1. Polymer sample, final copolymer composition, LCST determined via DSC and scaffold appearance at 37°C.

Polymer	Composition	LCST	Gel appearance
pNNAD-4	76.0: 4.4: 15.1: 4.5	21.25	Stiff, opaque
pNNAD-8	74.8: 4.1: 12.9: 8.2	16.98	Stiff, opaque
pNNAD-12	75.2: 3.8: 8.6: 12.4	13.11	Stiff, opaque
$pNNP_{526}D$-4	75.8: 4.9: 14.1: 5.2	35.21	Soft, transparent
$pNNP_{526}D$-8	76.1: 4.8: 9.3: 9.8	27.71	Stiff, opaque
$pNNP_{526}D$-12	75.7: 4.5: 6.7: 13.1	18.67	Stiff, opaque
$pNNP_{475}D$-4	75.4: 4.7: 15.4: 4.5	39.12	No gel
$pNNP_{475}D$-8	75.9: 4.6: 10.2: 9.3	30.94	Stiff, opaque
$pNNP_{475}D$-12	75.3: 4.4: 7.3: 13.0	20.93	Stiff, opaque
$pNNP_{1100}D$-4	76.2: 4.7: 13.1: 6.0	-	No gel
$pNNP_{1100}D$-8	76.0: 5.4: 8.3: 10.7	-	No gel
$pNNP_{1100}D$-12	76.0: 5.3: 5.7: 13.0	23.59	Stiff, transparent

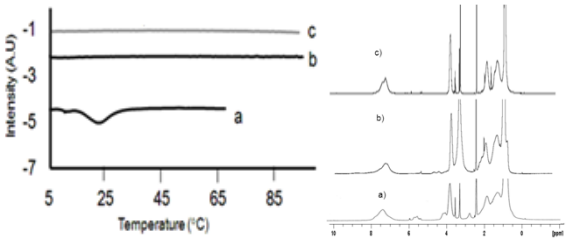

Figure 1. DSC (left) and NMR (right) analysis of a) pNNAD-4, b) partially degraded pNNAD-4, and c) completely degraded pNNAD-4 reveal elimination of LCST upon hydrolytic DBA ring opening.

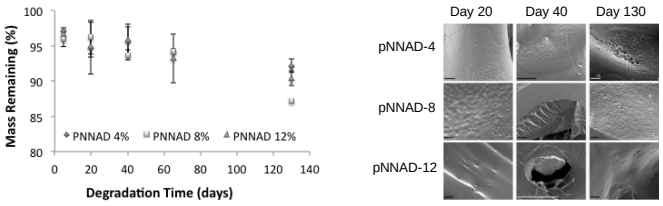

Figure 2: pNNAD mass loss (left) occurs slowly with over 80% mass remaining after 130 days. SEM micrographs reveal subtle changes in surface morphology as a function of degradation time. Bar = 100 μm.

Drug release studies reveal an initial burst release from the pNNAD copolymer scaffolds, which may be helpful in treating the initial state of the compromised ocular environment. A significantly lower rate of release is observed after the first 24 hours, which is expected to produce the desired low levels of corticosteroid within the posterior segment of the eye for sustained periods of time.

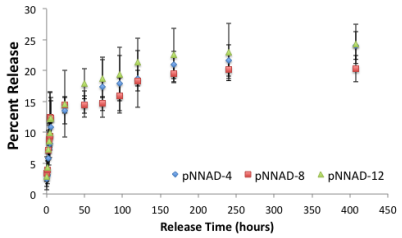

Figure 3. Dexamethasone release from pNNAD scaffolds reveals a small initial burst that plateaus to produce a slow-release scaffold.

To develop an optically transparent PNIPAAm-based thermo responsive hydrogel, we have synthesized copolymers consisting of NIPAAm, NAS, DBA and PEG of varying molecular weights. The motivation for the incorporation of PEG was the concept that large blocks of PEO within the NIPAAm-based copolymer would be able to create alternating micro-domains of hydrophilic and hydrophobic rich environments. Therefore, as the NIPAAm component undergoes a temperature-induced chain collapse resulting in the expulsion of its aqueous environment, the PEO domains would hold onto the water within the bulk of the hydrogel and prevent a macroscopic polymer collapse and precipitation from the aqueous environment. In theory, if the alternating hydrophilic and hydrophobic domains are smaller than the path-length of light (~500nm), then light travelling through the copolymer will not be scattered and gelled scaffold should remain transparent[7]. Therefore, it was crucial to find the correct co-monomer ratio and PEG chain length to obtain a copolymer that undergoes a rapid temperature-induced phase transition and results in the formation of micro-domains of appropriate size to yield an optically transparent thermoresponsive hydrogel. While the majority of copolymers synthesized generated opaque gels, two copolymers remained optically transparent following gel formation; $pNNP_{526}D$-4 (soft) and $pNNP_{1100}D$-12 (hard).

Figure 4. Incorporation of PEG into modified pNNAD copolymers produced optically transparent soft and hard copolymers $pNNP_{526}D$-4 (left) and $pNNP_{1100}D$-12 (right) respectively.

In addition to being transparent, it was found that $pNNP_{1100}D$-12 did not shrink upon gelation, which is favorable as scaffold contraction may be associated with an increased initial burst release. While the soft hydrogel would not serve as an adequate drug release scaffold, it may be a suitable as a vitreous replacement in vitreoretinal surgery.

CONCLUSIONS

A number of degradable thermoresponsive biomaterials have been synthesized for minimally invasive ophthalmic drug delivery. Degradation and drug release characteristics have been examined for the first generation pNNAD materials, and subsequent testing will elucidate these properties for the optically transparent, non-shrinking, degradable second generation hydrogels.

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