THERMORESPONSIVE HYDROGELS AS A NEW OCULAR DRUG DELIVERY PLATFORM TO THE POSTERIOR SEGMENT OF THE EYE

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ABSTRACT

Purpose: To characterize thermoresponsive hydrogels (liquids at room temperature, gels at body temperature) as a novel drug delivery platform to the posterior segment.

Methods: Thermoresponsive hydrogels were synthesized using poly(*N*-isopropylacrylamide) (PNIPAAm), cross-linked with poly(ethylene glycol) diacrylate (PEG-DA). Proteins were then encapsulated into the hydrogels, including bovine serum albumin (BSA), immunoglobulin G (IgG), and, finally, bevacizumab and ranibiumab. By varying the degree of cross-linker density, the rate of protein release could be adjusted. The rate of release was assessed at various time points with Bradford assay, and the bioactivity of the released anti–vascular endothelial growth factor agents was studied in an in vitro cell culture assay.

Results: Cross-linked PNIPAAm hydrogel exhibited a fast and reversible phase change with alteration in temperature. The rate of protein release was examined as a function of cross-link density. Release profiles of the proteins showed that there was an initial burst of release within 48 hours, and then a steady state was reached, which was sustained for approximately 3 weeks. Hydrogels with less cross-linking showed faster release and yielded a more pliable gel for intravitreal injection via small-gauge needles. Examination of the gels after the release experiment revealed significant residual entrapped protein.

Conclusion: Thermoresponsive hydrogels were successfully synthesized and exhibited fast and reversible phase changes. The gel was able to encapsulate and release various proteins. Current formulation of the gel will be modified to extend the release time and to be made fully biodegradable. Thermoresponsive hydrogels appear to be a promising, minimally invasive platform for extended drug delivery to the posterior segment.

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INTRODUCTION

Vascular endothelial growth factor (VEGF) has been identified as a key regulator of angiogenesis. It can act as an endothelial cell mitogen and increase vascular permeability along with angiogenesis.[1-3] Elevated VEGF level has been correlated with several ocular diseases, such as age-related macular degeneration and diabetic retinopathy.[4,5] On the basis of these findings, in the past several years, considerable progress has been made in the treatment of the wet form of age-related macular degeneration and diabetic retinopathy by using anti-VEGF therapy. Several clinical trials employing ranibizumab, including ANCHOR and MARINA, have demonstrated the success of anti-VEGF therapy.

Although intravitreal anti-VEGF therapy is a very promising treatment, the major drawback is that the treatment must be repeated every 4 to 6 weeks. This is not a desirable method of delivery for several reasons: patient discomfort; the need for repetitive injections with inherent complications, including endophthalmitis, retinal tear and detachment, intraocular hemorrhage, and cataract formation; and bolus administration of the agent. Currently, there is no alternative method for delivery of the anti-VEGF agent into the eye; hence, there is a great need and desire to develop a relatively noninvasive delivery method that is more effective and longer lasting than the current clinical regimen.

Since the development of hydrogels in 1960, they have been of great interest to biomaterial scientists and tissue engineers. Hydrogels are polymers that have the ability to swell in water or aqueous solvent system, and they hold the solvents in a swollen cross-linked gel system for delivery. Through manipulation of permeation and diffusion characteristics, they can retain hydrophobic and hydrophilic agents, small molecules, and macromolecules. Depending on the specific structure, they can be nondegradable or degradable in their application.[6,7] Numerous advantages make hydrogels an attractive platform.[6,8] The aqueous environment of hydrogels can protect cells and fragile drugs (such as peptides, proteins, oligonucleotides, and DNA). They serve as a good means of transport of nutrients to cells and products from cells. They can be modified with cell adhesion ligands and can change physical state (liquid to solid) in response to pH or temperature changes. Most important, they are highly biocompatible.

Among all the hydrogel systems investigated over the years, temperature- and pH-responsive hydrogels have demonstrated great promise in drug delivery owing to their novel ability to change physical state. Poly(*N*-isopropylacrylamide) (PNIPAAm) hydrogel is one of the well-known thermosensitive materials that has a lower critical solution temperature (LCST) or transition temperature at ~32ºC.[9,10] Below the LCST the hydrogel is swollen, and above the LCST the hydrogel will collapse (shrink). The change in physical state is rapid and reversible, which makes the thermoresponsive hydrogel an attractive means of drug delivery. However, a potential drawback of PNIPAAm hydrogel is the limited amount of drug released in response to a change in temperature. With a fast response to temperature stimuli, the drug can be released from the hydrogel quickly and act as an on-off switching release system.[11]

One of the key design constraints of the drug delivery system is that it should be able to release in a sustained manner. To address this limitation, recent studies have proposed the use of poly(ethylene glycol) (PEG) as a pore-forming agent to obtain macroporous

PNIPAAm hydrogels.[12-15] By incorporating PEG in the synthesis, Zhang and colleagues[12] have shown that cross-linked PNIPAAm hydrogels have a homogeneous structure. Zhang and Chu[16] demonstrated that by introducing poly(ethylene glycol) diacrylate (PEG-DA) to PNIPAAm, the hydrogel retained better thermoresponsive characteristics and had homogeneous pores. The pore size of the hydrogels can be modified by the amount of PEG-DA added to the system. PNIPAAm–PEG-DA hydrogels showed a significant improvement in mechanical properties. The main objective of our work is to demonstrate that PNIPAAm–PEG-DA hydrogels can be utilized to encapsulate and release protein for ocular delivery to the posterior segment.

MATERIALS AND METHODS

POLYMERIZATION AND PROTEIN ENCAPSULATION

In a 20-mL sterile plastic vial, 5 mL 0.01M phosphate-buffered saline (PBS; Sigma, pH 7.4) buffer was sonicated to purge any excess oxygen and nitrogen. Either fluorescently labeled bovine serum albumin (BSA; Invitrogen) (25 g/L) or fluorescently labeled immunoglobulin G (Invitrogen) was dissolved in the PBS, followed by the addition of PEG-DA (Aldrich). *N*-isopropylacrylamide (NIPAAm; Aldrich) (0.35 mM) was dissolved in the mixture, followed by the addition of 13.2 mg (0.07 mM) of ammonium persulfate (Sigma). Finally, 126 µL of *N,N,N',N'*-tetramethylethylenediamine (Sigma) was gradually pipetted into the mixture to initiate free radical polymerization at 7°C for 24 hours. After the 24-hour polymerization, the PNIPAAm–PEG-DA hydrogel was washed with 50 mL of PBS 5 times to remove any unpolymerized substances. Each wash (supernatant) was saved for in vitro toxicity testing. All hydrogels were stored at 7°C until the testing. The weight of wet and dry (at 40°C for 72 hours) hydrogels was measured to calculate the percent mass loss at various time points to confirm the thermoresponsiveness of the hydrogels.

LOWER CRITICAL SOLUTION TEMPERATURE MEASUREMENT

The thermoresponsive hydrogel has a LCST, or transition temperature, of ~32ºC.[9,10] Below the LCST the hydrogel is swollen, and above the LCST the hydrogel will collapse (shrink). It has been shown that introducing cross-linker has a small change on the LCST owing to change in hydrophilicity.[23] To confirm that the LCST was not modified by addition of PEG-DA as cross-linker, the LCST was measured with and without PEG-DA. LCST was determined by measuring the average absorbance of the hydrogels (SpectraMax Plus plate reader at wavelengths of 450, 500, 550, and 600 nm; Molecular Devices Corp). Temperature was increased at 0.5°C increments from 25°C to 43°C. Temperature was increased incrementally every 15 minutes to ensure that the hydrogel reached the intended experimental temperature.

PROTEIN RELEASE

Two types of protein release study were conducted. First, the effect of cross-link density was examined with BSA and IgG. The release profiles of BSA and IgG were obtained from 7 different cross-linked hydrogels. The hydrogels were placed in PBS at 37°C, which was defined as time zero. At various time points, a sample was measured and percent release was calculated based on the amount of encapsulated protein. Second, experiments examined a longer release time period with BSA for up to 3 weeks (Bradford assay, Coomassie-binding colorimetric method; Sigma-Aldrich).

IN VITRO TOXICITY EXAMINATION

The PNIPAAm-PEG-DA hydrogels were examined by in vitro cell culture testing to determine the possibility of toxic effect. Each wash of the hydrogels was added to human umbilical vein endothelial cells (HUVECs) to determine how many washes were needed to prevent toxic effects.

Cells were maintained in growth-factor supplements medium under standard tissue culture conditions for 3 days to approximately 80% confluence. Cells were then cultured in 0% serum for 24 hours before adding hydrogel washes and incubating the cells for 48 hours. Growth response to samples was determined by incubating cells in Hanks balanced salt solution with (3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2*H*-tetrazolium, inner salt) (MTS assay). The MTS assay produces a tetrazolium salt that is reduced by only living cells to form an insoluble formazan product. The plate was then incubated for 4 hours before reading at 570 nm in plate reader. Cells were counted and compared to the positive control.

IN VIVO INTRAVITREAL INJECTION

To determine if the thermoresponsive hydrogel can be injected into the vitreous cavity, a rodent model was used. To image the hydrogel in the eye, the hydrogel was labeled with fluorescein isothiocyanate (FITC) so that the retina and hydrogel could be observed using the scanning laser ophthalmoscope. The protocol was approved by the Institutional Animal Care and Use Committee at the Illinois Institute of Technology. Long-Evans pigmented adult rats were anesthetized with ketamine and xylazine, and the pupils were dilated. Control images of infrared reflectance and fluorescein angiographic images were obtained. Approximately 3 to 5 µL of the FITC-hydrogel was injected via a 30-gauge needle. The images were then obtained immediately after the injection and weekly for 2 months. At 2 months the animals were sacrificed, and the retinas were harvested for histologic examination.

RESULTS

CHARACTERIZATION OF THERMORESPONSIVE HYDROGELS

The PNIPAAm-PEG-DA hydrogel is an ideal polymer because it has unique biocompatibility and polymerization characteristics. It is soluble in water and is readily cleared by the body.[17] It can be immobilized either chemically or physically, it is highly resistant to protein adsorption and cell adhesion,[18,19] and it is not readily recognized by the immune system.[20,21] Acrylates are used as end groups because they undergo very rapid photopolymerization.[22] By incorporating PEG-DA with PNIPAAm in the polymerization process, a nondegradable formulation was achieved. Sample images of the hydrogel at room temperature and at body temperature (37ºC) are seen in Figure 1. At room temperature, the hydrogel exists in a liquid gel–like phase; however, once the temperature was raised to 37ºC, a solid gel rapidly formed (within 1 minute). Bright-field images of the gel surface and edge show a relatively uniform pore surface created by the cross-linking process (Figure 1, bottom).
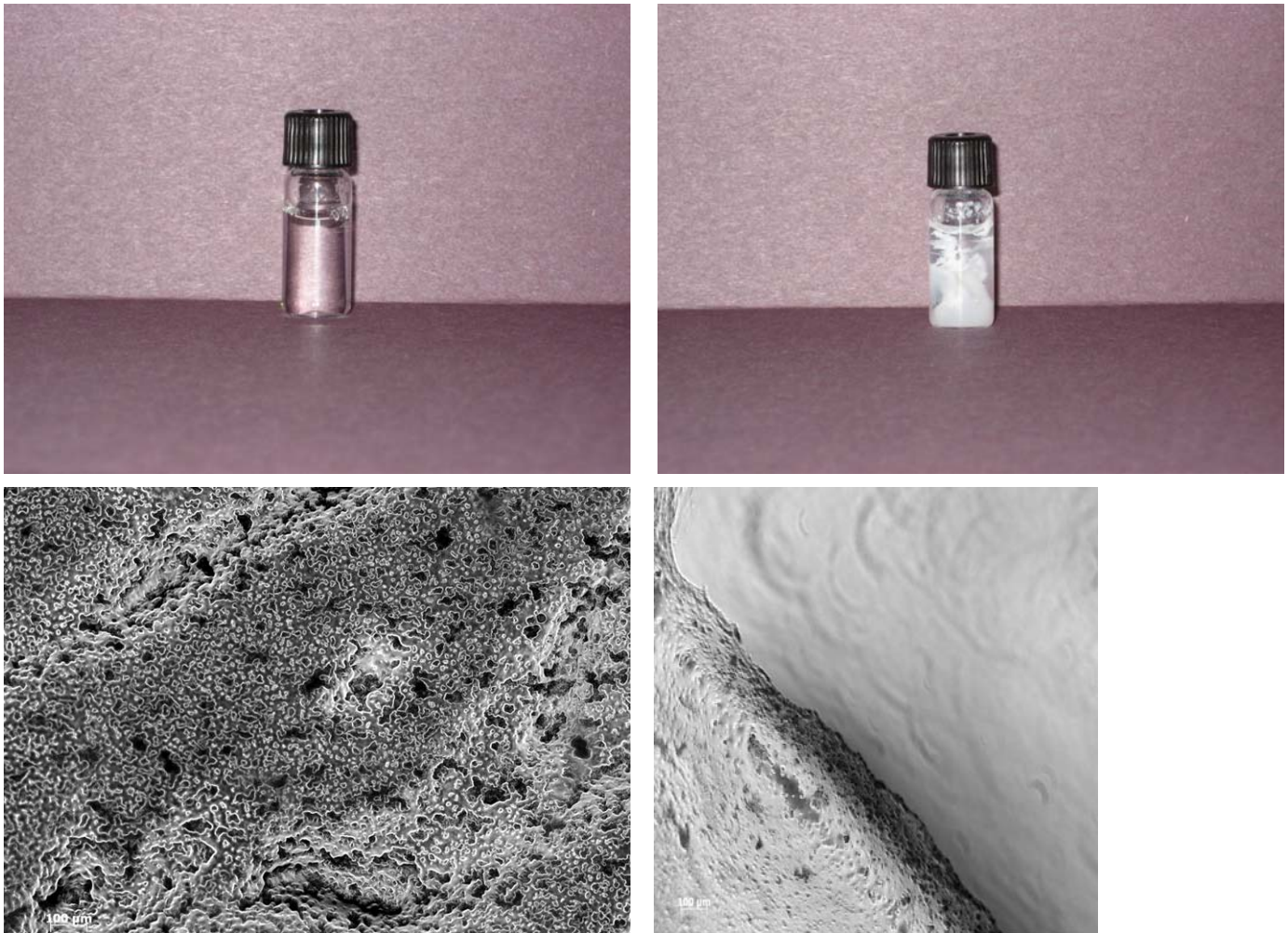

FIGURE 1

Images of cross-linked thermoresponsive gels: vial containing the hydrogel at room temperature (top left) and at 37ºC (top right), and bright-field images of gel surface (bottom left) and gel edge (bottom right).

The LCST of PNIPAAm alone and cross-linked PNIPAAm–PEG-DA, obtained by measuring the average absorbance of the hydrogel as a function of temperature, is shown in Figure 2. PNIPAAm alone hydrogel changed its phase (LCST) at ~31ºC, which correlated with previous studies.[9,10] PNIPAAm–PEG-DA hydrogel changed its phase at ~32ºC. By cross-linking with PEG-DA, LCST was shifted by ~1ºC owing to the increased hydrophilicity[23]; however, the change is still within an optimal range of injection.

Figure 3 shows the equilibrium swelling measurement above and below the LCST. Three different PNIPAAm–PEG-DA hydrogels with varying amount of cross-linker were used to examine the equilibrium swelling. The hydrogels were weighed before and after

swelling to calculate the percent mass ratio. Data suggest that the cross-linked hydrogels exhibit temperature sensitivity as well as the ability to swell and de-swell in response to temperature. This characteristic is important because de-swelling (collapsing) of the hydrogel will provide an initial burst release of protein.
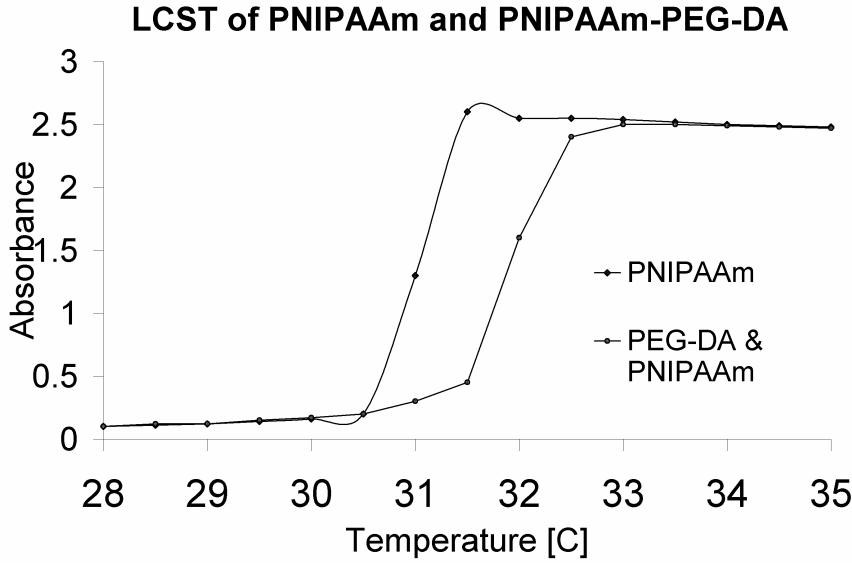

FIGURE 2
Lower critical solution temperature of PNIPAAm alone and cross-linked PNIPAAm–PEG-DA hydrogels.
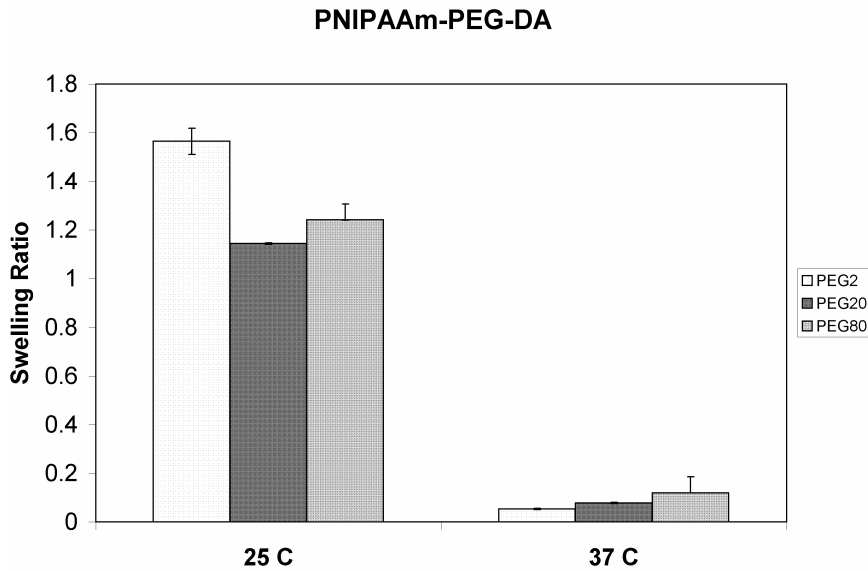

FIGURE 3
Equilibrium swelling ratios for 3 different PNIPAAm–PEG-DA cross-linked gels. A ratio of PNIPAAm and PEG-DA was varied and represented by different shaded bars.

PROTEIN RELEASE STUDIES

To examine the thermoresponsive hydrogel's ability to encapsulate and release protein, the effect of cross-link density on the protein release rate was investigated. The protein release rate is governed by 2 factors: the initial release due to compression of the hydrogel in response to temperature, and the pore size of the hydrogel. The initial compression due to change in temperature is difficult to control, though it is partially related to pore size. The pore size or cross-link density is easily controlled by varying the ratio of PNIPAAm and PEG-DA in the polymerization process. A higher PEG-DA concentration in the system yields a higher cross-link density and smaller pore size. Two different fluorescently labeled proteins, BSA (mol wt = 66 kDa) and IgG (mol wt = 150 kDa), were used for the study. The rationale for using BSA and IgG is that their sizes are similar to ranibizumab and bevacizumab (Avastin), respectively, which is the key anticipated application of this drug delivery system. The release profiles of BSA and IgG are seen in Figure 4. Seven different cross-link densities were examined. Immediately after temperature change, there was a burst of initial release of proteins, and then the rate of release reached a steady state.

Lower cross-link density hydrogels released protein faster compared to the higher cross-link density hydrogels. In contrast, the more highly cross-linked hydrogels yielded smaller pore size and longer release times. However, when the pore size was smaller, the hydrogel became stiffer in composition, making it more difficult to inject through small-gauge (eg, 27- to 30-gauge) needles. The

inability to inject through small-gauge needles is an important design constraint, as the goal is to develop a minimally invasive delivery system to the target sites, such as the vitreous cavity or juxtascleral region. Through multiple trials of cross-link density and the ability to inject through small-gauge needles, it was identified that ~8 μM PEG-DA is an optimal ratio. Figure 5 shows the BSA release profiles for ~20 days using this PEG-DA ratio in the hydrogel. The encapsulation efficiency was ~80%. There was a rapid BSA release in the first 48 hours, followed by a steady-state release of 40% within 1 week. However, a significant amount of protein was still entrapped within the hydrogel when examined under a fluorescent microscope after 3 weeks. Based on this study, it seems that a significant amount of protein can be further released, if the pore size could be adjusted in a controlled manner. To achieve this, a current plan is to add additional copolymer, which will slowly degrade and further extend the delivery time frame. This work is currently in progress.

Additionally, bevacizumab (1.25 mg/mL) and ranibizumab (1.0 mg/mL) were successfully encapsulated into and released from the thermoresponsive hydrogels. The bioactivity of the released anti-VEGF agents is currently being studied via in vitro cell culture assay.
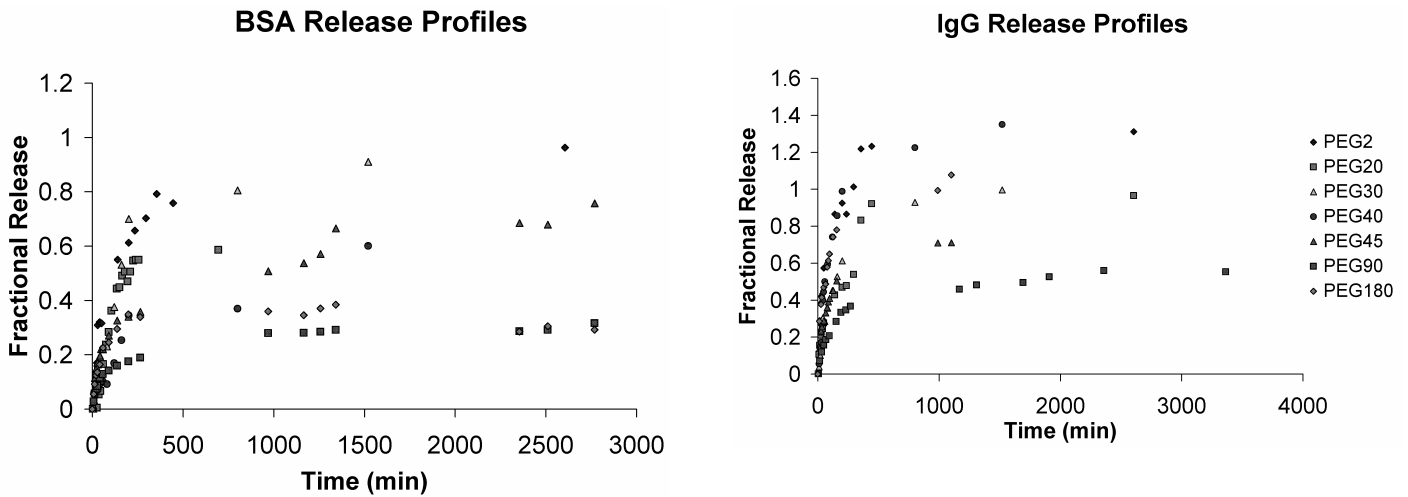

FIGURE 4

Effects of cross-link density on the protein release. Left, Bovine serum albumin release profiles. Right, IgG release profiles. Different symbols represent different cross-link density, where PEG2 has the lowest density of cross-linker and PEG180 has the highest density of cross-linker.
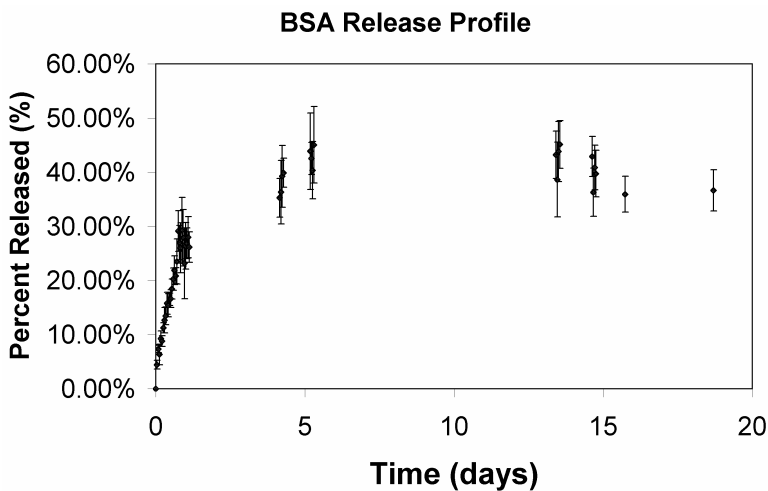

FIGURE 5

Bovine serum albumin (BSA) release profiles over 3 weeks. The encapsulation efficiency rate was ~80%.

IN VITRO TOXICITY STUDY

The pure NIPAAM (unpolymerized form), especially acrylamide, has been shown to be toxic in the nervous system.[24] However, a number of studies have shown that PNIPAAm (polymerized form) is not toxic.[25,26] Since there were no studies describing the potential toxicity of cross-linked PNIPAAm–PEG-DA hydrogels, we examined the washes of the hydrogels for evidence of possible toxicity. The rationale was that the washes may contain unpolymerized PNIPAAm. We therefore needed to assess the number of washes that would be necessary to prevent any possible toxic effects. Human umbilical vein endothelial cells and MTS assay were used to test for toxicity. Cells were counted and compared to the positive control (Figure 6). Compared to the control (20% fetal bovine serum), there

was a significant decrease in cell count in response to the first wash of the hydrogel, suggesting that it may contain unpolymerized PNIPAAm, which was toxic to the HUVECs. However, after the first wash, there was no significant difference between the second wash and the fifth wash compared to the control. To ensure that unpolymerized PNIPAAm is eliminated from the delivery system, a standard protocol of 5 washes was adapted.
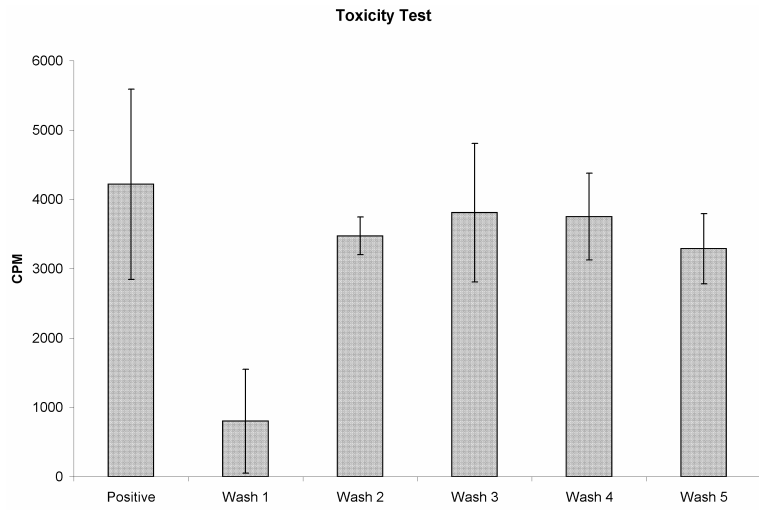

FIGURE 6

Toxicity test of the washes. After the overnight polymerization, the hydrogels were washed 5 times with buffer. Each wash was then added to the cell culture system to test for toxicity. The cell counts from each wash are compared to the positive control.

IN VIVO INTRAVITREAL INJECTION OF THE HYDROGEL

Approximately 3 to 5 µL of the FITC-labeled hydrogel was injected via a 30-gauge needle into the vitreous cavity of the adult rat to test if the thermoresponsive hydrogel could be readily and safely placed there. The scanning laser ophthalmoscope images of infrared reflectance and fluorescein angiography were then obtained before and immediately after the injection (Figure 7) and weekly for 2 months. Since the hydrogel was labeled with FITC, the hydrogel can be readily seen in the fluorescein angiography image. There was no other fluorescence emitted from any other location of retina, suggesting that the injection was localized. Furthermore, after 2 months, there was no movement of hydrogel location. Once the animals were sacrificed, the retinas were harvested for histologic examination (which is currently in progress). At the initial assessment, there seems to be no adverse impact on the retina. Two animals showed no retinal inflammatory response when the hydrogel was injected into the vitreous cavity. The third animal had an inadvertent injection of the hydrogel into the subretinal space. In this animal, a slight localized inflammatory response was noted immediately adjacent to the hydrogel. Further in-depth histologic assessment is pending.

FIGURE 7

Injection of fluorescein isothiocyanate (FITC)–labeled thermoresponsive hydrogel in a rodent model. Left, Infrared reflectance image before the injection. Middle, Fluorescein angiography image before the injection. Right, Fluorescein angiography image after the injection. A bright white spot indicates the FITC-labeled thermoresponsive hydrogel.

DISCUSSION

Pharmacologic treatment of ocular vascular disease has rapidly evolved over the past several years. The majority of treatment is with anti-VEGF agents, requiring multiple repetitive intravitreal injections, every 4 to 6 weeks, often for a period of time ranging up to a year or two. There are numerous other existing methods and routes for drug delivery to the posterior segment, though achieving adequate delivery of drug continues to pose significant challenges.

An ideal prolonged drug delivery system would strive to achieve the following criteria: relatively noninvasive delivery method, high encapsulation efficiency of the drug, sustained release, and high safety profile; also, it should be easy to manufacture, use, and store. Currently, the most efficient delivery system to the posterior segment is via intravitreal injection. However, injections are invasive with numerous potentially serious complications, such as endophthalmitis, retinal tears and detachment, cataract formation, and bolus drug delivery.

A controlled-release system is needed to overcome the complications associated with intravitreal injections. There are numerous advantages of an extended controlled-release system.[27,28] It will significantly decrease the frequency of treatments and provide sustained and controlled release of drug over an extended period of time. It will also provide protection of the drug from inactivation and degradation, plus provide spatial treatment to localized tissues in the body.

To design a localized, sustained-release drug delivery platform that can encapsulate and release anti-VEGF agents, a thermoresponsive hydrogel delivery system was developed. Because of its thermoresponsive characteristic, the hydrogel can be injected in a liquid form to the juxtascleral region or the vitreous cavity via a small-gauge needle. Once exposed to body temperature, the solution rapidly becomes a solid gel that releases the encapsulated protein (anti-VEGF agent). This system is designed to optimize the antiangiogenic effects and minimize the potential ectopic effects of a large bolus delivery. Our current data also showed that the thermoresponsive hydrogel can encapsulate protein at a high rate, is nontoxic, and is able to release protein for a period of at least 3 weeks (duration of initial study). The goal is to extend the release time, and currently a biodegradable cross-linker copolymer is being investigated, with the intent of achieving release for up to 3 to 6 months. In summary, thermosensitized hydrogels are capable of delivering drug to the posterior segment in a prolonged, controlled fashion. Further work assessing this technique is ongoing.

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PEER DISCUSSION

DR. BARRETT G. HAIK: The authors have devised an intraocular sustained drug delivery system with the potential to significantly prolong drug resident time and improve drug bioavailability to the retina. This innovation in drug delivery could dramatically improve our ability to care for patients with complex retinal diseases by improving the medications pharmacokinetics and reducing the frequency of intraocular injections.

The concept of using thermo-sensitive hydrogel is innovative. The hydrogel carrier is liquid at room temperature and can be injected into the posterior vitreous through a small gauge needle. As the hydrogel warms to body temperature at (37º C) it collapses into a more solid form and modulates the rate of drug release. Depending on the hydrogel carrier formulation the active ingredients can be released in a sustained fashion. The carrier's characteristics can be manipulated to facilitate delivery of chemical or biologic materials over varying time periods.

The authors show that non-polymerized thermo-responsive hydrogels are toxic so the exacting pharmacologic preparation is critical to utilizing this delivery platform. Further studies are anticipated to define the ocular immunologic response to the most promising therapeutic formulations.

I would like to ask the authors if the location of drug placement is critical. Does the status of the vitreous affect the placement, location of the therapeutic gel, its adherence, or the rate of drug delivery? Does the hyaloid anatomy matter in situations such as partial or total vitreous detachment or in post vitrectomy patients? Do vitreous fluid dynamics, convection currents, and small variations in intraocular temperature matter in choosing the optimal location for placement of the thermo-responsive hydrogel drugs?

The authors are to be commended for bringing this promising work to our attention. Hopefully this therapeutic strategy will advance rapidly for the benefit of our patients.

ACKNOWLEDGMENTS

DR. JOHN D. BULLOCK: No conflicts. I was fascinated by your beautiful slide showing the appearance of polymers at room temperature versus 37º C. In view of the paper that I gave yesterday and the fact that there are several polymers in multipurpose contact lens solutions, one wonders if this may be a mechanism for the increasing entrapment of antimicrobial agents in polymers as a function of elevated temperature. Thank you.

DR. ALLAN J. FLACH: I have no conflict of interest, but I am very interested in what was presented. Dr. Derwent and Dr. Mieler, this is a fascinating approach to drug delivery; however, I am a little confused. Maybe you can help me understand this more thoroughly. Historically, we are familiar with osmotic delivery systems, which depend upon water entering a device that results in a drug leaving the device. These systems, like the Ocusert pilocarpine, result in a fixed rate of diffusion. During your talk you mentioned diffusion and during the discussion by Dr. Haik the term "diffusion" was not used. He spoke in terms of collapse of the hydrogel and the subsequent drug release. To be honest, I do not understand the pharmacodynamic properties of your drug delivery

system. It is certainly not an osmotic system and I do not believe it is a true diffusion system. It seems that the hydrogel physically changes and that this results in a sequential compartmental release. An initial burst effect of 40% is not an unusual with a diffusion system, such as the Ocusert pilocarpine system. That delivery method has a measurable initial burst effect after which zero order time-release kinetics are observed. In your system it seems there is a large burst effect that is related to release from a large compartment and then prolonged release occurs. I hope you can further clarify this issue. You may have also problems with these systems if they require removal from the vitreous cavity, which will require surgical excision. With the osmotic system, such as the Ocusert, you simply remove it from the fornix. Your abstract suggests that having a biodegradable material is essential because the hydrogels will linger within the eye for prolonged periods of time and this would be particularly important if they are be repetitively injected. This is a key component. I wonder if you assembled sequential layers and if each layer different had a different release and you might achieve a sequential compartmental release. If you could think about those confused thoughts, then I would appreciate it. Thank you.

DR. DENNIS HAN: I have no conflicts of interest in this regard. I would like to congratulate Bill and Jennifer for this tremendously interesting work that you are doing. It has a lot of potential to make a big difference in patient care. I just have a few questions. Are you aware of the actual absolute temperature within the vitreous cavity? We all assume 37ºC is body temperature, but that is actually core body temperature. I am wondering what it is in the vitreous itself on an absolute basis. You have injected these hydrogels into some rats and I wonder if you saw any inflammatory effects or any signs of clinical toxicity related to the material. What do you believe would be the amount of material required to inject in the human eye to allow release of a therapeutic dose? Do you believe that the opacity of the material would have any visual significance? Thank you.

JENNIFER J. KANG DERWENT, PHD: I would like to thank the members of the American Ophthalmological Society (AOS) for asking me these questions regarding our work. I am going to answer in order. First, Dr. Haik, thank you for your opening discussion. The location of the injection does matter. We considered delivery of the medication via eye drops, or via injection into the vitreous cavity or into the juxtascleral space. In this study, our goal was to deliver medication to the vitreous cavity as we have experience in this delivery method in our animal models. The question is whether the location of injection plays a role in how effective the medication is taken up. The diffusion characteristics will be different in different sites, such as vitreous cavity versus juxtascleral; however, within the vitreous cavity, we assumed that the diffusion was quite uniform. This is based on a simple computation model as well as previous pharmacological studies performed in my laboratory. When we inject the hydrogel into the vitreous cavity of adult rats, the hydrogel stayed in place for at least two months, the maximum duration of our current studies. We are currently investigating longer timeframes, however.

The concern that Dr. Haik raised regarding the difference of vitreous consistency for older patients versus younger patients is a potential concern. We do not believe that this will pose a problem, however. In fact, in older patients, the vitreous is more liquefied and it should actually facilitate the diffusion characteristics of the medication released from the hydrogel.

In response to Dr. Bullock's question on whether thermo-responsive polymer may be involved in entrapment of antimicrobial agents in contact lens solutions-we are not aware that this particular thermo-responsive polymer is used in contact lens solutions. However, it is possible to encapsulate antimicrobial agents via thermo-responsive hydrogel. At the present time, we do not have experience in antimicrobial agents in thermo-responsive hydrogels or its possible role in multipurpose contact lens solutions.

Regarding the question on the actual temperature of the vitreous cavity raised by Dr. Han, that is an interesting question. There are several animal studies that have tried to measure the actual temperature of the vitreous and found that it may be slightly lower that the body temperature of 37^0 C. However, this should not pose a problem in our system as our least critical solution (LCS) temperature is approximately 32^0 C. That is, our hydrogel will change from liquid-gel into solid-gel as soon as the temperature reaches 32^0 C. The transformation is rapid, occurring within minutes. We also do not believe that even if there is a small variation in temperature between the front and the back of the eye, that it would pose a problem based on the reasons as outlined above.

Regarding a possible inflammatory response to the hydrogel in the animal model, we are currently investigating this issue. Our initial assessment is that there was no inflammation around the injection site based on visual inspection. Also, there was no obvious inflammation in the vitreous cavity based on the scanning laser ophthalmoscope examination. However, we are currently investigating this question via histological assessment of several rat eyes, and we should have this information within the next month or two.

A question arose regarding the apparent opaqueness of the hydrogel in the gel phase. The reason for the opaqueness is due to high amounts of PNIPAAm used to illustrate the hydrogel in my presentation. The PNIPAAm concentration used in our delivery system is substantially lower, and the hydrogel is transparent. We do not believe that the transparent hydrogel will interfere with visual function; however, this issue will require further investigation.