Published in final edited form as:

Eur J Pharm Biopharm. 2015 September ; 95(0 0): 398–406. doi:10.1016/j.ejpb.2015.05.020.

Formulation to target delivery to the ciliary body and choroid via the suprachoroidal space of the eye using microneedles

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Abstract

In this work, we tested the hypothesis that particles injected into the suprachoroidal space can be localized at the site of injection or broadly distributed throughout the suprachoroidal space by controlling polymeric formulation properties. Single hollow microneedles were inserted into the sclera of New Zealand White rabbits and injected non-biodegradable fluorescently tagged nanoparticles and microparticles suspended in polymeric formulations into the suprachoroidal space of the eye. When formulated in saline, the particles were distributed over 29% - 42% of the suprachoroidal space immediately after injection. To spread particles over larger areas of the choroidal surface, addition of hyaluronic acid to make moderately non-Newtonian solutions increased particle spread to up to 100% of the suprachoroidal space. To localize particles at the site of injection adjacent to the ciliary body, strongly non-Newtonian polymer solutions localized particles to 8.3% – 20% of the suprachoroidal space, which exhibited a small increase in area over the course of two months. This study demonstrates targeted particle delivery within the suprachoroidal space using polymer formulations that spread particles over the whole choroidal surface or localized them adjacent to the ciliary body after injection.

Graphical abstract

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Mark Prausnitz, Henry Edelhauser and Yoo Chun Kim are inventors on patents that have been licensed, and Mark Prausnitz and Henry Edelhauser have significant financial interest in a company that is developing microneedle-based products for ocular delivery (Clearside Biomedical). This potential conflict of interest has been disclosed and is overseen by Georgia Tech and/or Emory University.

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Keywords

microneedles; eye; suprachoroidal space; particle formulation; ocular drug delivery

Introduction

Current ophthalmic drug delivery is constrained by the anatomical and physiological barriers of the eye, which prevent many drugs from effectively or selectively reaching their sites of pharmacological action [1-3]. As a result, pharmacotherapies for many vision-threatening diseases are not delivered efficiently and selectively to these sites of action in the eye, which limits treatment efficacy and can lead to side effects.

Ophthalmic drug formulations are typically administered as a bolus by topical eye drops on the corneal surface or intravitreal injection, and sustained-release formulations are typically delivered as implants placed in the vitreous humor. However, the sites of pharmacological action of most ophthalmic drugs are not located on the corneal surface or in the vitreous humor. The two most common causes of blindness in industrialized nations are glaucoma, for which drugs often act on the ciliary body or trabecular meshwork [4, 5], and age-related macular degeneration (AMD), for which most drugs act on the choroid and/or retina [6, 7].

The non-targeted nature of current delivery methods can also cause adverse side effects and toxicity in surrounding tissues. For example, the poorly targeted nature of eye drops leads to extensive systemic exposure, which causes as much as 8-53% of glaucoma patients taking topical anti-glaucoma drugs to experience side effects [8, 9]. In the case of intravitreal administration of steroids, unwanted contact with the lens promotes the formation of cataract in 7 – 14% of patients [10, 11]. In addition, administering sustained-release formulations often requires surgical procedures to insert the implants. A novel delivery method that can administer controlled release formulations to specific locations in the eye in a minimally invasive manner to maintain high therapeutic levels of a drug at the site of pharmacological action should provide more effective therapy for many vision-threatening eye diseases.

Injection into the suprachoroidal space (SCS) allows access to tissues in the eye located from right above the ciliary body anteriorly to immediately next to the choroid and adjacent retina posteriorly. The SCS is a potential space located between sclera and choroid that can expand to accommodate a fluid or drug formulation. The location of the SCS adjacent to the sites of pharmacological action for diseases like glaucoma (ciliary body) and wet AMD, diabetic retinopathy, and uveitis (choroid and/or retina) may provide a route of

administration that enables higher drug levels in these target tissues. For this reason, the SCS represents a promising new site of administration for treatment of many ocular diseases and has become a recent focus of drug delivery research [12-15].

Initial attempts to access the suprachoroidal space involved invasive techniques such as catheters or implants that were surgically placed in the eye [13, 16, 17]. More recently, we introduced the use of microneedles for minimally invasive, targeted SCS injection [18, 19]. In this case, a single, hollow microneedle measuring less than 1 mm in length (i.e., designed to match the scleral thickness) is pressed perpendicularly across the sclera to the sclera-choroid interface. A fluid formulation is then injected, and the hydrodynamic force of the injection opens the SCS so that the fluid flows circumferentially around the eye between the sclera and choroid (i.e., within the SCS). Previously, microneedles have been shown to deliver nanoparticles and microparticles into rabbit, pig, and human eyes ex vivo [18, 20]. An in vivo study in the rabbit further showed microneedles can deliver particles as large as 10 μm to the posterior segment through the SCS [19]. While these particles did not contain drug, their delivery provides information about how to deliver particles loaded with drug for localized controlled release.

While SCS injection enables improved drug targeting, in this study we seek still better targeting by controlling delivery within the SCS. Using conventional formulations, the particles injected into the SCS spread over a portion of the SCS [18, 19, 21, 22], but are not well targeted either to localize anteriorly adjacent to pharmacological sites of action in the ciliary body or to spread posteriorly across the whole choroidal surface adjacent to pharmacological sites of action in the choroid and/or retina.

To improve targeting within the SCS, we have developed formulations either to immobilize particles at the site of injection or to enhance the spreading of the particles throughout the SCS. To achieve this control, we used the properties of non-Newtonian fluids (made of non-toxic excipients) to modulate the movement of both polymers and embedded particles. Non-Newtonian fluids are characterized by viscosity that varies with shear rate, such that shear-thinning non-Newtonian fluids have viscosity that decreases as shear rate increases. To localize delivery at the site of injection, we used strongly shear-thinning non-Newtonian polymer solutions to enable low viscosity during injection (i.e., high shear) and then provide high viscosity after injection (i.e., low shear) to prevent further movement. To widely distribute particles throughout the SCS, we used moderately shear-thinning non-Newtonian polymer solutions to again have low viscosity during injection, but have only moderate viscosity after injection. We discovered that use of such polymer solutions facilitated spread of injected particles throughout the SCS over time after injection, whereas injection of polymer-free solutions did not, possibly by a mechanism involving keeping the SCS open for a longer time, thereby allowing particles to distribute throughout the SCS.

We first determined the distribution of particles after injection into the SCS as a function of particle size in polymer-free saline formulation. We then examined the extent to which polymeric formulation could affect the distribution of microparticles inside the SCS with the objectives of delivering particles localized immediately above the ciliary body or distributed throughout the SCS. To image and quantify movement of particles, non-biodegradable

fluorescent particles were used throughout the study. For the first time, this study presents methods to deliver particles up to 10 μm in size targeted to the ciliary body or throughout the choroid using non-Newtonian formulations of polymers having different viscosity, molecular weight and hydrophobicity.

Materials and Methods

Microneedle Fabrication

Microneedles were fabricated from 33-gauge needle cannulas (TSK Laboratories, Tochigi, Japan). The cannulas were shortened to approximately 750 μm in length and the bevel at the orifice was shaped using a laser (Resonetics Maestro, Nashua, NH). The microneedles were electropolished using an E399 electropolisher (ESMA, South Holland, IL) and cleaned with deionized water, as described previously [19]. Microneedles were attached to gas-tight, 100 – 250 ml glass syringes (Thermo Scientific Gas-Tight GC Syringes, Waltham, MA) containing the formulation to be injected.

Formulations

Solutions for injection were prepared by mixing 2 wt% FluoSpheres in water (Invitrogen, Grand Island, NY), 0.2 wt% Sky Blue particles in water (Spherotech, Lake Forest, IL) and Hank's balanced salt solution (HBSS, Manassas, VA) containing polymer formulations described below at a volumetric ratio of 1:1:2. Particles with carboxylate-modified surfaces were used to minimize aggregation. When carboxymethyl cellulose or methyl cellulose were used, they were dissolved in deionized water rather than HBSS. Fluospheres were labeled with red-fluorescent dye and Sky Blue particles were labeled with infrared-fluorescent dye. Particles having diameters of 20 nm, 200 nm, 2 μm or 10 μm were used, but in a given formulation, only one diameter particle was used, and the FluoSpheres and Sky Blue particles both had the same diameter. The polymeric formulations were made using carboxymethyl cellulose (Sigma Aldrich, St. Louis, MO), hyaluronic acid (R&D Systems, Minneapolis, MN), methylcellulose (Alfa Aesar, Ward Hill, MA) or DiscoVisc[®] (Alcon, Fort Worth, TX).

Viscosity Measurements

The viscosity (η) measurements were carried out on an MCR300 controlled-stress rheometer (Anton Paar, Ashland, VA) equipped with Peltier elements for temperature control and an evaporation blocker that enables measurements of polymer solutions at elevated temperature in a cone-plate geometry. The viscosities of samples were measured at shear rates from 0.01 s^{-1} to 100 s^{-1} at a temperature of 20 and 40 $^{\circ}\text{C}$. The viscosity reported for each sample in this study was matched at a shear rate of 0.1 s^{-1} . Multiple measurements were performed ($n = 3$), and the mean value is reported.

Ex Vivo Injection Procedure

Whole rabbit eyes were obtained with the optic nerve attached (Pel-Freez Biologicals, Rogers, AR). Eyes were shipped on ice and stored wet at 4 $^{\circ}\text{C}$ for up to 2 days prior to use. Before use, eyes were allowed to come to room temperature ($\sim 21\text{--}24^{\circ}\text{C}$), and any fat and conjunctiva were removed to expose the sclera. A catheter was inserted through the optic

nerve into the vitreous and connected to a bottle of HBSS raised to a height that generated an internal eye pressure of 10 mm Hg, which mimics the lowered intraocular pressure in the rabbit eye under general anesthesia. The microneedle was then inserted perpendicular to the sclera surface 3 mm posterior from the limbus. A volume of 50 μ L or 100 μ L was injected within 15 s, followed by a 30 s delay before removing the microneedle from the eye to prevent excessive reflux.

In Vivo Injection Procedure

Microneedle injections were carried out in New Zealand White rabbits (Charles River Breeding Laboratories, Wilmington, MA). All injections were done under systemic anesthesia by subcutaneous injection of a mixture of ketamine/xylazine/acepromazine at a concentration of 17.5/8.5/0.5 mg/kg. A drop of 0.5% proparacaine was given 2 - 3 min before injection as a topical anesthetic. To perform an SCS injection, the eyelid of the rabbit eye was pushed back and the microneedle was inserted into the sclera 3 mm posterior to the limbus in the superior temporal quadrant of the eye. A volume of 50 μ L or 100 μ L was injected within 15 s, followed by a 30 s delay before removing the microneedle from the eye to prevent excessive reflux. At terminal study endpoints, rabbits were euthanized with an injection of pentobarbital through the ear vein. The eyes were enucleated after death and processed for further analysis. All animal studies were carried out with approval from the Georgia Institute of Technology Institutional Animal Care and Use Committee (IACUC, protocol number A14030).

Tissue Processing and Measurement of Fluorescence Intensity

Immediately after SCS injection into rabbit eyes ex vivo and immediately after enucleation of rabbit eyes in vivo, eyes were snap frozen in an isopropyl alcohol (2-isopropanol, Sigma Aldrich, St. Louis, MO) bath, which was cooled in dry ice. After the eyes are completely frozen, they were removed and eight radial cuts were made from the optic nerve on the posterior side to the limbus on the anterior side of each eye. Each of the eight pieces of cut tissue was then peeled away outward exposing the chorioretinal surface inside the eye. This made the eyes into a flat mount-like configuration, exposing the injected dyes for imaging. Brightfield and fluorescence images were taken using a digital camera (Cannon Rebel T1i, Melville, NY). A green light bulb (Feit Electric, Pico Rivera, CA) was used to excite the fluorescent particles and a red camera filter (Tiffen red filter, Hauppauge, NY) was mounted on the digital camera to image the distribution of particles inside the SCS.

Obtained images were used to quantify the SCS area containing injected particles using Adobe Photoshop (Adobe, San Jose, CA). Each of the eight tissue pieces was then divided into additional two pieces. The cuts were made 6 mm antero-posteriorly from the ciliary body, which is approximately at the mid-point of the SCS. In this study, we refer to ocular tissue between 0 mm and 6 mm from the ciliary body as “anterior SCS” and ocular tissue more than 6 mm away from the ciliary body as “posterior SCS”.

This method produces a total of 16 tissue pieces from each eye. Each of the 16 pieces was then put into separate vials containing HBSS and homogenized (Fisher Scientific PowerGen, Pittsburgh, PA) to extract injected fluorescent particles. Because particles were injected in a

potential space between the scleral and choroidal tissues (i.e., in the suprachoroidal space), as opposed to being entrapped within a tissue, we expected the extraction of particles from the eye to be efficient, although we did not quantify particles possibly left in the tissue. The liquid part of the homogenate was pipetted into 96-well plates to measure fluorescent signal intensity (Synergy Microplate Reader, Winooski, VT). To quantify radial distribution of particles, data were designated into two categories radially: ocular tissue between -90° and 90° from the injection sites (referred to as “superior SCS”) and ocular tissue between 90° and 270° from the injection site (referred to as “inferior SCS”).

Statistical Analysis

Replicate experiments were done for each treatment group, from which the mean and standard deviation were calculated. Experimental data were analyzed using both one- and two-way analysis of variance (ANOVA) to examine differences between treatments. In all cases, a value of $p < 0.05$ was considered statistically significant.

Results and Discussion

Distribution of Nanoparticles and Microparticles in the SCS

Fluorescently tagged, polystyrene particles with various diameters (20 nm, 200 nm, 2 μ m, 10 μ m) were suspended in 50 μ L of HBSS and injected into the SCS of New Zealand White rabbit eyes using a hollow microneedle inserted 3 mm posterior to the limbus. The distribution and number of particles in the SCS was determined immediately after injection into rabbit cadaver eyes ex vivo and was determined 14 or 112 days after injection into living rabbit eyes in vivo.

Fig. 1 displays images of a representative eye cut open in a flat-mount presentation showing the distribution of 10- μ m fluorescent particles in the SCS. Fig 1a shows a brightfield image, where the lightly colored interior region is the cornea and the tips of the “petals” all were formally joined at the optic nerve before dissection and mounting. Figs. 1b and 1c show the distribution of red-fluorescent and infrared-fluorescent particles, respectively, which exhibit similar distributions after co-injection. The sharp circular line where fluorescent signal abruptly ends toward the center of the tissue is interpreted as the anterior end of the SCS near the limbus. Quantitative analysis of images like these was used to generate the SCS surface area coverage data described immediately below.

Immediately after injection, particles covered 29% - 42% of the SCS surface area (Fig. 2a). There was no significant effect of particle size on SCS surface area coverage (one-way ANOVA, $p > 0.10$). Fourteen days after injection, the SCS coverage area did not significantly change for any of the particle sizes studied. Two-way ANOVA analysis showed no significant effect of particle size ($p = 0.11$) or time ($p = 0.75$) on SCS surface coverage area at 0 and 14 days after injection. Likewise, there was no significant interaction between particle size and time ($p = 0.16$). It is worth noting that the day 0 measurements were made ex vivo, whereas the day 14 measurements were made in vivo, yet the results are similar.

Between days 14 and 112, there was a significant decrease in the SCS coverage area to 24% -32% of the SCS. This represents a reduction of 9% - 35% of SCS coverage area relative to the day 14 value. Two-way ANOVA analysis showed a significant difference in SCS coverage area between day 14 and 112 ($p < 0.001$), but there was no significant effect of particle size ($p = 0.17$). There was also no significant interaction between time and particle size ($p = 0.21$).

In addition to measuring SCS coverage area, fluorescence signal intensity of the particles was measured. The fluorescence signal intensity of particles in the SCS between days 0 and 14 showed no significant difference (two-way ANOVA) as a function of time ($p = 0.13$) and particle size ($p = 0.05$). There was also no significant interaction between time and particle size ($p = 0.1$). This suggests that there was no significant clearance of particles during the first 14 days after injection.

However, the fluorescence intensity from particles decreased between days 14 and 112, as shown by fluorescence intensities falling to 31% - 61% of original values (at day 0). This suggests a 39% - 69% reduction in the number of particles remaining in the SCS. Two-way ANOVA analysis showed a significant difference in particle fluorescence between days 14 and 112 ($p < 0.001$), but not as a function of particle size ($p = 0.17$). There was also no significant interaction between time and particle size ($p = 0.21$).

Histological analysis of tissue three months after injection showed that the particles as small as 20 nm were largely located in the SCS and had not penetrated significantly into the vascular region of the choroid (data not shown). Furthermore, the majority of the particles were still present even after three months based on fluorescence signal intensity (Fig. 1), which is inconsistent with the rapid (< 1 day) clearance of small molecules and macromolecules from the SCS by the vasculature [19]. Therefore, we expect that loss of fluorescence from particles is either due to removal of the particles (e.g., by macrophages) or a reduction of the fluorescence signal intensity over time (i.e., artifact). To assess the relative roles of these two possible mechanisms, we measured the decrease in fluorescence intensity of 20 nm, 200 nm, 20 μ m, and 10 μ m particles in HBSS after storage for 112 days in the dark at 39°C to mimic conditions in the SCS of the rabbit eye. These particles lost $25 \pm 6.5\%$ of their fluorescence signal intensity. This suggests that particle clearance from the eye may not be as extensive as reported, because loss of fluorescence signal may at least partially explain the loss.

Overall, these data show that the injected particles spread over a coverage area of about one-third of the SCS. Within 14 days, there was little movement or loss of particles in the SCS, but after 112 days, there was a reduction in coverage area to about one-quarter of the SCS and there was an apparent reduction in the number of particles in the SCS of up to about half of the particles originally injected.

Polymer characterization

The main objective of this study is to develop formulations that target delivery within the SCS. For treatment of macular degeneration, uveitis and other chorioretinal diseases, we seek to spread injected formulations throughout the SCS. For treatment of glaucoma, we

seek to target the ciliary body by immobilizing injected formulations at the injection site. In particular, we want to deliver polymeric particles that simulate controlled-release formulations and to use materials expected to be safe based on prior use in parenteral formulations.

When designing formulations to achieve this objective, we considered two time scales for particle transport. One is during the injection itself and the other is after the injection is over. Our data above (Fig. 2) indicate that a simple HBSS formulation enabled spread at the time of injection over about one-third of the SCS and that no significant further spreading occurred afterwards. This amount of spreading is too little for complete SCS coverage and too much for localized delivery at the site of injection.

Our prior study indicated that the SCS closes within minutes after saline injection, which then appears to trap particles in place [20], which is consistent with the data in Fig. 2. We therefore hypothesized that addition of polymer to the injected formulation could slow down clearance of the formulation from the SCS, thereby allowing it to keep the SCS open for longer due to smaller polymer diffusivity and increased solution viscosity. This would allow particles to distribute further within the SCS after injection through the expanded SCS. Because we want to inject as easily as possible (i.e., low injection pressure) and distribute the particles as much as possible during the injection (i.e., throughout the SCS), we want low viscosity at high shear during injection. We estimate the shear rate during injection through our microneedles to be on the order of $10,000 \text{ s}^{-1}$ (see Supplemental Information). But, because we want slow clearance of the polymer after injection, we want a high polymer molecular weight and concentration and a high solution viscosity at low shear after injection. We expect the shear rate after injection to be close to zero.

We selected hyaluronic acid (HA) as a material that meets these criteria. HA is extensively used in the eye with an excellent safety record [23-26]. It also exhibits shear-thinning non-Newtonian behavior [27, 28], so that it has low viscosity during injection and high viscosity afterwards. It is also available at high molecular weight (i.e., we selected 950 kDa). In addition to a pure HA solution, we also studied the use of a commercial product, DisCoVisc (DCV), which is a dispersive and cohesive viscoelastic material used in ophthalmic surgery [28]. DCV contains 17% (w/v) HA (1.7 MDa), as well as sodium chondroitin sulfate (22 kDa).

Fig. 3 shows a rheological characterization of a pure HA formulation, as well as the DCV formulation. Both formulations exhibited similar rheological behavior. At high shear rate (100 s^{-1}), the viscosity was low ($\sim 4 \text{ Pa}\cdot\text{s}$), but at low shear rate (0.01 s^{-1}) it was about 17 fold higher ($\sim 70 \text{ Pa}\cdot\text{s}$). While we did not also measure the rheological behavior of these solutions with polymer particles added to the formulation, we believe that the general shear-thinning behavior shown in Fig. 3 will remain even after the addition of the relatively low particle concentration used in this study (0.5 wt%).

For immobilizing particles at the site of injection, we need a different formulation that also has low viscosity at high shear during injection, but has extremely high viscosity at low shear after injection in order to hold the particles in place. Increased viscosity after injection

can be achieving using in situ gelling polymers, for example triggered by solvent removal, temperature change, pH change, or light exposure [29-32]. However, we preferred a shear-mediated trigger of increased viscosity of a shear-thinning fluid, because of its simplicity (e.g., no need for organic solvents, refrigerated storage or special equipment).

For this application, we selected 700 kDa carboxymethylcellulose (CMC) and 90 kDa methylcellulose (MC), which are polysaccharides with excellent biocompatibility [33] and strong shear-thinning properties (see Supplemental Figure S1 and S2). Solutions made of these materials had even stronger non-Newtonian behavior than HA, exhibiting low-shear viscosity many fold higher (Fig. 4), suggesting the ability to retain injected materials at the site of injection.

Formulations to enhance spreading of particles throughout the suprachoroidal space

To test the hypothesis that high molecular weight and moderately non-Newtonian polymeric solutions enhance the spreading of particles inside SCS, we injected HA-based formulations containing pure HA and DCV into the SCS and measured the area over which particles spread. As a negative control, we first injected particles formulated in 50 μ l of HBSS and found that the particles spread over 42% of the SCS immediately after injection and 35% after 14 days (Fig. 5a,b), in general agreement with previous results shown in Fig. 2a. Increasing the injection volume to 100 μ l of HBSS had a significant effect to increase the particle spreading area ($p = 0.004$, Fig. 5a,b).

Addition of HA to the formulation led to particles spreading over 9% of the SCS immediately after injection and 61% after 14 days (Fig. 5a,b). This represents a 7-fold increase in spreading area from day 0 to day 14. The small initial spreading area can probably be explained by the higher viscosity of the HA formulation compared to pure HBSS. However, what is notable is that 14 days after injection, the particles spread over a significantly larger area than the negative control of injection with 50 μ l of HBSS ($p = 0.018$). Formulation with DCV at 1 $\times$, 2 $\times$ and 4 $\times$ concentrations led to similar effect, where the particles were highly localized immediately after injection, but spread over large areas of the SCS afterwards (Fig. 5a,b), which were again significantly large than after injection with HBSS ($p < 0.01$). Finally, injection of 100 μ l of DCV at 4 $\times$ concentration led to spreading over 99% of the SCS (Fig. 5a,b), which achieved our goal of delivering particle throughout the SCS. This result is shown through imaging of a representative flat-mounted eye, where the red-colored particles are seen completely covering the SCS (Fig. 5c).

While these results demonstrate delivery of particles throughout the SCS, we wanted to further assess the uniformity of delivery in the SCS. When measuring the fraction of particles in the anterior versus posterior portion of the SCS (delineated by the equator of the eye), injection of particles in 50 μ L of HBSS led to 71 $\pm$ 15% of particles in the anterior SCS and 29 $\pm$ 15% in the posterior SCS (Fig. 5a,b), which corresponds to an anterior-to-posterior ratio of 4.2 $\pm$ 4.5. In contrast, injection with 50 μ L of HA and DCV formulations led to anterior-to-posterior ratios of 1.1 to 2.6 and injection with 100 μ L of 4 $\times$ DCV led to an anterior-to-posterior ratio of 2.0 $\pm$ 0.8 (Fig 5a,b). These ratios are not significantly different than after injection of 50 and 100 μ L HBSS ($p < 0.31$ and 0.1, respectively).

We also considered the uniformity of delivery in the superior (upper) and inferior (lower) halves of the SCS and determined superior-to-inferior ratios of 17 ± 14 and 7.8 ± 4.6 after injection in 50 and 100 μL of HBSS, respectively. These ratios were 2.4 – 4.6 after injection in 50 μL of HA and DCV formulations and 3.0 ± 0.94 after injection in 100 μL of 4 $\times$ DCV (Fig. 6a,b). Although these ratios were much lower for the HA-based formulations, they were not significantly different compared to injection with HBSS ($p = 0.08 - 0.18$).

We also hypothesized that increasing injection flow rate into the SCS could increase spreading, but over the range of parameters tested (i.e., injection of 50 μL within ~ 2 s to 30 s), we did not see a correlation between flow rate and particle spreading in the rabbit eye ex vivo during injection of particles in unformulated HBSS (data not shown).

Formulations to immobilize particles at the site of injection in the suprachoroidal space

We next tested the hypothesis that strongly non-Newtonian solutions immobilize particles at the injection site not only at the time of injection, but afterwards too. Three different polymer formulations were used – 750 kDa CMC, 90 kDa CMC and 90 kDa MC – and their concentrations were adjusted to have approximately the same viscosity (~ 50 Pa-s at a shear rate of 0.1 s^{-1} at 20°C). This value was chosen because as one that could be high enough to localize at the site of injection, but still low enough to permit controlled injection through microneedles.

Immediately after injection, all three polymeric formulations covered 7 – 10 % of the SCS. These areas are significantly smaller than the SCS spreading area after injection using the pure HBSS formulation, which was 42% of the SCS ($p < 0.00065$, Fig. 7). Fourteen days after injection, SCS spreading after injection using the 700 kDa CMC and 90 kDa MC formulations did not significantly change compared to day 0 ($p > 0.16$ and 0.32 , respectively, Fig. 7). In contrast, the 90 kDa CMC formulation led to a 5-fold increase in SCS spreading area between day 0 and day 14 ($p < 0.00066$, Fig. 7). Forty days after injection, SCS spreading still did not significantly increase for the 700 kDa CMC formulation, but 60 days after injection, there was a significant increase in spreading to 20% of the SCS ($p < 0.031$, Fig. 7).

Overall, these data show that strongly non-Newtonian solutions of 700 kDa CMC and 90 kDa MC were able to localize particles at the site of injection immediately after injection and for up to 2 months after that. To localize particles even more effectively, we propose that increasing the concentration of 700 kDa CMC will further reduce particle spreading due to higher viscosity at low shear rate, because increasing the CMC concentration from 1.7 wt % (as used in this study) to 2.5 wt% increased viscosity by 3 fold at a low shear rate of 0.01 s^{-1} (Supplemental Fig. S2). We tested solutions containing up to 3 wt% 700 kDa CMC and found that we were able to reliably inject such solutions through microneedles (data not shown). Additional studies are needed to further optimize formulations that immobilize particles at the site of injection long term.

Discussion

While conventional ocular delivery via eye drops and intraocular injections targets drug delivery to the eye as opposed to the rest of the body, injection into the SCS enables even more specific targeting to sites within the eye. Of particular interest, and the subject of human clinical trials [34, 35], is targeting the choroid and adjacent retina, for example, with steroids to treat inflammation, as well as other drugs. We have also recently proposed targeting drug delivery to the ciliary body via the anterior SCS for treatment of glaucoma [36]. The use of a microneedle simplifies delivery to the SCS by designing the microneedle to penetrate just to the base of the SCS but into deeper tissues. Previous research in this area showed that microneedles could be used to inject particles as large as 10 μm into the SCS [19]. This study builds on previous results to deliver particles into the SCS by further targeting delivery within the SCS by controlling the movement of the particles using non-Newtonian polymeric formulations.

Our long-term vision for this project is to combine targeted delivery either spread across the SCS adjacent to the whole surface of the choroid or localized to the SCS above the ciliary body with microparticles that release drugs of interest at sustained rates over extended periods of time. As compared to, for example, implants that deliver steroids from the intravitreal space [37, 38] or anti-glaucoma drugs from the intracameral space [39], a microneedle-based injection of microparticles is less invasive than surgical placement of an implant and allows better intraocular targeting than drug delivery from the intravitreal space. In this way, a microneedle injection into the SCS of slow-release microparticles containing drug could place the microparticles at the site of the drug's therapeutic action for extended treatment for months. This study demonstrates targeted delivery; future studies will need to combine this technology with slow-releasing particles.

Microneedles are expected to be a simple, safe and reliable way to deliver polymeric controlled release formulations into the SCS in a minimally invasive way. Currently, retina specialists give millions of intravitreal injections per year at the same site of injection as used for microneedles located 2-5 mm from the limbus [40, 41]. This similarity should make the injection procedure straightforward for an ophthalmologist, which is supported by initial clinical study results [35]. SCS injection using a microneedle is also expected to carry fewer safety concerns, because the microneedle only penetrates less than 1 mm into the eye. In contrast, intravitreal injection requires a hypodermic needle to penetrate completely across the ocular sheath into the center of the eye.

This study used the shear-dependent viscosity of non-Newtonian fluids as a means to target delivery of particles to specific regions within the SCS. All of the polymeric formulations had low viscosity at high shear rate during injection, which facilitated controlled injections through microneedles. All of the formulations also had high viscosity at low shear rate after injection, which caused the particles to remain localized at the site of injection. Over time, the highly non-Newtonian fluids (with the very high viscosity at low shear rate) continued to immobilize the particles largely at the site of injection, thereby keeping the particles in the anterior SCS adjacent to the ciliary body. The moderately non-Newtonian fluids (with more moderate viscosity at low shear rate) facilitated spreading of particles throughout the SCS.

We hypothesize that this latter effect is due to spreading of the polymers within the SCS that keeps the space open for movement of particles within the SCS before the polymers are eventually cleared and the SCS closes. This mechanistic hypothesis needs further testing.

Conclusion

This study demonstrates that injection of particles as large as 10 μm formulated in pure HBSS lead to spreading of the particles over a portion of the SCS at the time of injection and that little additional spreading occurs for up to 112 days after. Formulation instead with HA-based formulations led to initial localization of the particles at the site of injection followed by spreading of the particles throughout the SCS. This complete coverage of the choroidal surface via the SCS may enable future drug delivery efficiently targeted to the choroid and adjacent retina. Formulation with high molecular weight CMC and MC formulations immobilized particles at the site of injection and for up to 60 days after. This localized delivery in the anterior SCS adjacent to the ciliary body may enable future drug delivery efficiently targeted to the ciliary body for glaucoma treatment. The use of non-Newtonian properties of polymeric formulations is appealing because it takes advantage of the inherently highly shear-rate environment during injection and the inherently low shear-rate environment after injection. This contrasts with more complex methods that rely on triggered gelation or on surgical placement of an implant. Additional studies will need to optimize formulations more fully to target specific locations in the eye using microneedles and thereby pave the way for future clinical applications of this drug delivery method in ophthalmology

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

We would like to thank Donna Bondy for administrative support. This work was carried out at the Center for Drug Design, Development and Delivery and the Institute for Bioengineering and Bioscience at the Georgia Institute of Technology. This work was supported in part by the National Eye Institute (R24-EY017045 and R01-EY022097).

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Figure 1.

Ocular imaging after delivery of particles to the suprachoroidal space of a rabbit eye ex vivo. (a) Brightfield image of flat mounted eye. (b) Fluorescence image of the red-fluorescent particles in the SCS. (c) Fluorescence image of near-infrared particles. Images of the same eye are shown in all three parts of this figure. These results are representative of 3 different similar experiments that generated similar results.

Figure 2. Distribution of 20-nm, 200-nm, 2-μm, and 10-μm particles in the SCS after injection using a microneedle in the rabbit eye *ex vivo* (time = 0 days) and *in vivo* (time = 14 and 112 days).

(a) SCS coverage area as function of time and particle size. * Significant difference (oneway ANOVA, $p < 0.054$, 0.0175, 0.075, and 0.13, respectively) between day 14 and 112. (b) Total fluorescence of particles in the SCS as a function of time and particle size. * Significant difference (one-way ANOVA, $p < 0.029$, 0.0000034, 0.0018, and 0.052, respectively) between day 14 and 112. Data represent the average of 3 replications $\pm$ standard deviation.

Figure 3. Rheological behavior of hyaluronic acid-based solutions. Viscosity is shown as a function of shear rate for solutions of 3 wt% hyaluronic acid in HBSS and the commercial preparation of DisCovisc measured at 40°C. Data represent the average of 3 replications $\pm$ standard deviation.

Figure 4.

Rheological behavior of polysaccharide solutions. Viscosity is shown as a function of shear rate for solutions of 1.7 wt% CMC (700 kDa and 90 kDa), 3 wt% MC (90 kDa), and 2.2 wt % HA (950 kDa). All measurements were made at 40°C except MC, which was measured at 20°C. Data represent the average of 3 replications $\pm$ standard deviation.

Figure 5.

Formulations to enhance spreading of particles throughout the suprachoroidal space. Distribution of 10- μ m particles in the SCS is shown after injection with a microneedle in the rabbit eye in vivo. (a) SCS coverage area as function of time and particle size for different formulations after injection of 50 μ l of solution (unless otherwise noted as 100 μ l). (b) Percent coverage of SCS as function of time and particle size for different formulations after injection of 50 μ l of solution (unless otherwise noted as 100 μ l). (c) Brightfield image of a flat-mounted eye 14 days after injection of particles in 100 μ l of a 4 $\times$ DisCoVisc formulation. Data represent the average of 3 replications $\pm$ standard deviation.

Figure 6.

Formulations to enhance spreading of particles throughout the suprachoroidal space. Distribution of 10- μ m particles in the SCS is shown among hemispheres of the eye after injection with a microneedle in the rabbit eye in vivo. (a) Distribution of particles in the anterior versus posterior SCS for different formulations. The anterior and posterior regions were delineated by the equator of the eye. (b) Distribution of particles in the superior versus inferior SCS for different formulations. The superior and inferior regions were delineated by the site of the injection. Injections were performed using 50 μ l of solution (unless otherwise noted as 100 μ l). Data represent the average of 3 replications $\pm$ standard deviation.

Figure 7.

Formulations to immobilize particles at the site of injection in the suprachoroidal space. Distribution of 10- μ m particles in the SCS is shown after injection with a microneedle in the rabbit eye in vivo. SCS coverage area is shown as a function of time and particle size for different formulations after injection of 50 μ l of solution. Data represent the average of 3 replications $\pm$ standard deviation.