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Intrascleral Drug Delivery to the Eye Using Hollow Microneedles

Jason Jiang[1], **Jason S. Moore**[1], **Henry F. Edelhauser**[2], and **Mark R. Prausnitz**[1,3]

[1]School of Chemical and Biomolecular Engineering, Georgia Institute of Technology, Atlanta, GA 30332 USA

[2]Emory Eye Center, Emory University, Atlanta, GA 30322 USA

Abstract

Purpose—This study tested the hypothesis that hollow microneedles can infuse solutions containing soluble molecules, nanoparticles, and microparticles into sclera in a minimally invasive manner.

Methods—Individual hollow microneedles were inserted into, but not across, human cadaver sclera and aqueous solutions containing sulforhodamine or fluorescently-tagged nanoparticles or microparticles were infused into sclera at constant pressure. The infused volume of fluid was measured and imaged histologically as a function of scleral thickness, infusion pressure, needle retraction depth and the presence of spreading enzymes (hyaluronidase and collagenase).

Results—Individual hollow microneedles were able to insert into sclera. Fluid infusion was extremely slow after microneedle insertion into the sclera without retraction, but partial retraction of the microneedle over a distance of 200–300 μm enabled infusion of 10–35 μl of fluid into the tissue. Scleral thickness and infusion pressure had insignificant effects on fluid delivery. Nanoparticle suspensions were also delivered into sclera, but microparticles were delivered only in the presence of hyaluronidase and collagenase spreading enzymes, which suggested the role of scleral glycosaminoglycans and collagen fibers as rate-limiting barriers.

Conclusion—This study shows that hollow microneedles can infuse solutions into the sclera for minimally invasive delivery of soluble molecules, nanoparticles and microparticles.

INTRODUCTION

One of the greatest challenges in ocular drug delivery is effectively administering drugs to the back of the eye to treat diseases of the retina, choroid and vitreous body (1,2). Such posterior segment diseases include macular degeneration and diabetic retinopathy, which are two of the leading cause of blindness. While conventional delivery using topical eye drops can transport drug to the anterior segment, this method generally cannot deliver useful amounts of drug to the posterior segment, due to rapid elimination via precorneal tears and conjunctival blood flow, low corneal permeability coupled with counter-directional intraocular flow of aqueous humor, and long diffusional distances from the ocular surface to the back of the eye (3). Posterior segment delivery is often achieved via systemic delivery, which generally does not target the eye and thereby can cause side effects, or intravitreal injection, which is unpleasant for patients, requires expert clinical administration, and carries the risk of complications, such as retinal detachment, cataract, endophthalmitis and pain (4).

[3]To whom correspondence should be addressed. Address editorial correspondence to: Mark R. Prausnitz School of Chemical and Biomolecular Engineering Georgia Institute of Technology 311 Ferst Drive Atlanta, GA 30332-0100 Phone: 404-894-5135 Fax: 404-894-2291 Prausnitz@gatech.edu.

There has been growing attention to another route of administration to the back of the eye, i.e., across the sclera (5,6). The sclera is the white, structural sheath around the circumference of the eye and is a largely acellular tissue composed of interweaving collagen fibers embedded in an aqueous, glycosaminoglycan matrix (7). Because the sclera has a relatively large surface area and is highly permeable to many drugs, including macromolecules, it has been used as a site for delivery from extraocular depots made using periocular injection (8,9) and ocular implants of gel formulations (10) and biodegradable polymers (11), and across the conjunctiva using iontophoresis (12). While avoiding the complications of intraocular injection, these transscleral methods nonetheless involve hypodermic injection or implantation or the use of sophisticated electronic devices applied to the ocular surface.

In this study, we propose a novel approach to drug delivery via the sclera involving microneedles. Originally developed for drug delivery to the skin, microneedles are solid or hollow needles of microns dimension that penetrate into tissue for targeted delivery to that tissue in a minimally invasive manner (13,14). We hypothesize that hollow microneedles can infuse solutions containing soluble molecules, nanoparticles, and microparticles into the sclera in a minimally invasive manner. This would enable delivery of free drug or drug encapsulated within nanoparticles or microparticles for controlled release over time within the sclera. Drug could then diffuse from the sclera to neighboring choroidal and retinal tissues to treated diseases in the posterior of the eye.

Hollow microneedles can be made for prototype experiments using conventional laboratory techniques and for eventual mass production using low-cost microfabrication methods adapted from the microelectronics and other industries (15). Hollow microneedles have been used in animals for delivery of insulin and vaccines and, more recently, in human trials for delivery of lidocaine, insulin and seasonal influenza vaccine (13,14). Additional studies have shown that infusion rate into the skin can be increased by partially retracting microneedles after inserting them into the skin, which relieves skin compaction under the needle tip and thereby increases local flow conductivity (16,17).

Solid microneedles have also been used for drug delivery, either by creating micron-scale holes in the skin to increase permeability or by associating drug with the needle by coating or encapsulation for rapid or controlled release by dissolution within the skin. A number of different drugs have been delivered to animals and human subjects in this way (13). Microneedles have been reported as painless by human subjects (18). Coated microneedles have also been used for delivery of pilocarpine and other model compounds into the cornea in vitro and in rabbits (19).

MATERIALS AND METHODS

Tissue preparation

Human cadaver eyes were obtained from the Georgia Eye Bank (Atlanta, GA) with approval from the Georgia Tech Institutional Review Board and stored in moist containers at 4 °C for 2-7 days before use. A piece of sclera tissue (1 × 1 cm) was cut from the globe using surgical scissors. Adherent tissues associated with the retina, choroid, episclera and conjunctiva were gently removed with cotton swabs. The sclera tissue was soaked in water for 5 min prior to each infusion experiment to approximate its physiological hydration level. The scleral piece was placed onto a hemi-spherical surface (0.6 cm in radius) that was obtained by cutting off the end of a polystyrene round-bottom flow tube (BD Falcon, Bedford, MA), which mimicked the ocular curvature.

Microneedle fabrication

Individual, hollow, glass microneedles were prepared as previously described (17). Briefly, fire-polished borosilicate glass pipettes (outer diameter of 1.5 mm, inner diameter of 0.86 mm, B150-86-15, Sutter Instrument, Novato, CA) were pulled using a micropipette puller (P-97, Sutter Instrument) and detached into two microneedles at desired settings. These blunt-tip microneedles were then beveled at a setting of 20° tip angle using a glass grinder (BV-10, Sutter Instrument). For cleaning, each needle was connected to a 3 ml syringe by a small piece of tubing and then cleaned by dipping into a chromic acid bath (Mallinckrodt, Hazelwood, MO) while blowing air through the needle shaft for 15 s, which was followed by the same dipping procedure using DI water and then acetone (J.T. Baker, Phillipsburg, NJ) for 15 s each. The resulting microneedles were imaged using brightfield microscopy (Leica DC 300; Leica Microsystems, Bannockburn, IL) and image analysis (Image Pro Plus, Media Cybernetics, Silver Spring, MD).

As shown in Fig. 1, a representative microneedle has an approximately elliptical tip opening with a long axis of ~100 μm and a short axis of ~40 μm. Although these microneedles were long (i.e., 3 – 4 cm), they were held in an insertion device that allowed only a fraction of that length to protrude, effectively making them shorter (see below). In this way, we could easily make microneedles suitable for initial studies, that can later be reproduced in metal using microfabrication techniques suitable for inexpensive mass production.

Experimental apparatus

A 1-ml glass syringe (Gastight Syringe, Hamilton Company, Reno, NV) was used as the drug solution reservoir. One end of the syringe was connected using rubber tubing (1/4 inch inner diameter, Fisher Scientific, Hampton, NH) to a high-pressure CO_2 tank (Airgas, Radnor, PA) fitted with a pressure regulator (Two-Stage Regulator, Fisher Scientific, Hampton, NH) and the other end was connected to a 2.1 mm inner diameter metal tubing line, which was connected to the end of a glass microneedle by a flexible tube linker (500 μm inner diameter, Cole-Parmer, Vernon Hills, IL). Using a custom-made device (20), microneedles could be inserted into and retracted out of the scleral tissue in a controllable fashion by rotation (i.e., 1 full rotation equaled 1440 μm in microneedle displacement). The entire assembly was held by a stainless-steel adapter mounted to a Z-stage (Graduated Knob Unislide, Velmex, Bloomfield, NY) to control the vertical motion of the microneedle holder.

Infusion of sulforhodamine solution

Sulforhodamine (558 Da; Molecular Probes, Eugene, OR), which served as a model drug and a visual marker of fluid flow, was added to phosphate buffered saline (PBS) to make a $1 \chi 10^{-3}$ M sulforhodamine solution to be delivered into the human sclera tissue. The solution was loaded into the glass syringe of the apparatus using a 5-ml syringe. Individual, bevel-tipped, microneedles were then inserted into human cadaver sclera and used to deliver sulforhodamine solution at a constant pressure of 5, 10, 15, 20 or 25 psi.

To examine the effect of scleral thickness on intrascleral delivery, the sclera was divided into three different regions around the globe known to have different thicknesses: anterior (near the limbus), medial (at the equator), and posterior (near the optic nerve). To assess the impact of scleral tissue deformation during microneedle insertion, microneedles were initially extended 700 – 1080 μm out of the holder assembly, which only inserted the microneedles to a fraction of that length into the sclera due to tissue deformation, and then retracted away from the tissue in increments of 60 μm every 3 min while continuously applying pressure to infuse the solution. Throughout each experiment, the volumetric delivery of sulforhodamine solution was monitored by the movement of the gas-fluid meniscus in the glass syringe, and the amount

delivered was recorded between each retraction. The experiment was stopped when a retraction caused leakage of sulforhodamine solution onto the surface of the tissue.

Delivery of nanoparticles and microparticles

Nanospheres (provided courtesy of Dr. Uday Kompella, University of Colorado) were dispersed with vortexing in 1 ml PBS at solids contents of 0.5, 1, 5 and 10 wt %. These nanospheres were made out of poly-lactic acid (PLA), had an effective diameter of 278 ± 13 nm and encapsulated Nile Red as a fluorescent marker within the particle (21). Latex, fluorescein-labeled microspheres (1.0 micron in diameter, Polysciences, Warrington, PA) were added to 1 ml PBS solutions at solids contents of 0.3, 1.3 and 2.6 wt% for infusion into sclera. A drop of 5 μl polyoxyethylenesorbitan monooleate (Tween 80, Sigma Chemical, St. Louis, MO) was also added to each milliliter of solution, which was then vortexed and ultrasonicated to prevent microsphere aggregation. Microneedles were loaded with 20 μl nanosphere or microsphere solution, which was delivered into each region of the sclera at 15 psi using the same insertion and retraction procedures described above.

Effect of collagenase and hyaluronidase

In one scenario, scleral tissue was soaked in a 200 U/ml hyaluronidase solution (Vitrase, ISTA Pharmaceuticals, Irvine, CA) or 200 U/ml collagenase type I solution (Sigma Aldrich, St. Louis, MO) for 1 h, after which microneedle infusion experiments were performed. In another scenario, there was no pretreatment, but 200 U/ml hyaluronidase or 200 U/ml collagenase was added to the microneedle infusion solution and delivered into the sclera as a mixture with the sulforhodamine or microsphere solution.

Histological and microscopic image analysis

After each microneedle infusion experiment, the scleral tissue was examined by bright-field microscopy (Leica DC 300) to ensure that no solution leakage occurred onto the backside of the tissue. For histological analysis to visualize microneedle penetration pathways and the distribution of infused solution within the sclera, scleral tissues were rinsed with water immediately after an infusion experiment to wash off residual solution on the tissue surface, individually placed into sample blocks containing freezing agent (OCT; Sakura Finetechnical, Tokyo, Japan), and snap frozen with liquid nitrogen. Each tissue sample was sectioned into 10-μm thick pieces using a cryostat microtome (Richard Allan Scientific, Kalmazoo, MI), which were collected as consecutive sections onto glass slides. Each histological section was then examined using both bright-field (Leica DC 300) and fluorescence microscopy (Eclipse E600W, Nikon, Melville, NY).

Statistical Analysis

Replicate microneedle infusion experiments ($n \geq 3$) were performed at each condition, from which the mean and standard deviation were calculated. A one-way analysis of variance (ANOVA, $\alpha = 0.05$) was used to examine the impact of scleral position and pressure on infusion volume. In all cases, a value of $p < 0.05$ was considered statistically significant.

RESULTS AND DISCUSSION

Microneedle insertion and infusion into sclera

Individual microneedles were inserted into human cadaver sclera *in vitro*, after which sulforhodamine solution was infused into the tissue. Fig. 2a shows a top view of the sclera after microinfusion of sulforhodamine. In a typical experiment, the distribution of sulforhodamine in the sclera covered an approximately circular area with a diameter of 1 – 10 mm, depending on infusion conditions. These observations demonstrated that microneedles were strong

enough to insert into sclera and were capable of infusing sulforhodamine solution into the tissue. Because the scleral tissue used in this study did not have associated conjunctiva, future studies will need to address the effect of conjunctiva on microneedle insertion and infusion. Given that sclera is mechanically much stronger than conjunctiva (7), we expect that microneedles will be able to penetrate conjunctiva too.

To better examine the microneedle insertion site, Fig. 2b shows a representative histological cross-section of scleral tissue after infusion. In this case, the microneedle was initially fully retracted within the insertion device and placed on the surface of the sclera. The microneedle was then driven 720 μm out of the insertion device using a rotating motion and inserted into the sclera. A pressure of 15 psi was applied for 3 min, but very little sulforhodamine solution was infused (i.e., < 1 μl). The microneedle was then partially retracted (i.e., rotating backwards) by 240 μm, after which approximately 15 μl sulforhodamine solution was infused into the tissue within 3 min. The microneedle was then removed and the tissue was prepared for histological analysis.

Inspection of Fig. 2b, shows that the hole left in the sclera by the microneedle went only to a depth of approximately 300 μm, even though the microneedle was inserted 720 μm out of the insertion device. This result is similar to findings during microneedle insertion into skin, where microneedle translation initially deforms the tissue surface until sufficient force is applied to pierce into the tissue, after which further microneedle translation penetrates deeper into the tissue (16). In Fig. 2b, it appears that less than half of the microneedle length penetrated into the tissue and the rest could be accounted for by deformation of the scleral tissue surface.

Further inspection of Fig. 2b shows only mild sulforhodamine staining at the bottom half of the microneedle track in the tissue and intense and distributed staining in the upper half. This result is consistent with the observation that infusion was difficult upon full insertion of the microneedle into the sclera tissue, but infusion was much easier upon partial retraction of the microneedle. This effect has been observed previously in skin and was explained by compression of tissue at the needle tip during insertion, which created a dense tissue plug with poor flow conductivity (16). In sclera it is known that the fibers in the lower part of sclera are more elastic than those in the upper part of sclera (7,22). This suggests that the lower sclera might be compressed more easily, thereby blocking fluid flow, whereas the upper sclera keeps its shape, thereby maintaining fluid flow pathways.

Effect of retraction depth and scleral thickness

Having observed that microneedle retraction increased infusion into sclera, we next sought to quantify the amount of retraction needed as a function of sclera tissue thickness. We studied infusion into sclera excised from three regions around the globe, because sclera is moderately thick anteriorly near the limbus (0.5 mm), thins medially at the equator (0.4 mm) and becomes substantially thicker posteriorly near the optic nerve (1.0 mm) in the human eye (23).

Using tissue from each of these regions, we inserted microneedles to a controlled depth (720 – 1080 μm), applied an infusion pressure of 15 psi and monitored infusion volume over time. Every 3 min, the microneedle was retracted 60 μm. We consistently observed that infusion flow rate low upon initial insertion (Fig. 3, gray bars) and remained low until a critical retraction distance was achieved. At that point, flow rate dramatically increased and 10 – 15 μl of fluid was infused within 3 min (Fig. 3, black bars). The critical retraction distances were 287 ± 182 μm in the anterior, 209 ± 92 μm in the medial and 262 ± 145 μm in the posterior regions of the sclera, which were not significantly different from each other (ANOVA, $p = 0.07$). Further retraction did not appreciably further increase infusion volume until finally the microneedle was fully retracted from the sclera and began to leak onto the tissue surface. Attempting

infusion for a longer time (i.e., at the same retraction depth) also did not appreciably increase infusion volume (data not shown).

Effect of infusion pressure

We also studied the effect of infusion pressure on volumetric delivery to tissue in the three regions of the sclera. In this study, microneedles were inserted to a depth of 720 μm and then retracted in 60 μm increments every 3 min until rapid infusion began, which occurred at 140 – 300 μm. A constant infusion pressure of 5 – 25 psi was applied throughout. Fig. 4 shows that infusion into scleral tissue was not significantly affected by pressure over the range of pressures studied (ANOVA, $p = 0.83$). This contrasts with previous findings in human skin, in which pressure increased flow rate (17). Scleral tissue region also did not significantly affect infusion volume (ANOVA, $p = 0.07$). Overall, infusion volumes were generally between 10 – 35 μl, with an average of 22 ± 7 μl. The fact that increased pressure, increased infusion time (after 3 min) and further retraction (beyond the critical retraction distance) did not increase infusion volume suggests that there is a specific volume of potential space available for infusion into the sclera and once this volume is filled, it is difficult to infuse more fluid.

Delivery of nanoparticles and microparticles

Infusion of particles into the sclera for controlled drug release over time could facilitate extended therapies in the back of the eye. To assess this possibility, we infused suspensions of fluorescently tagged nanoparticles measuring 280 μm in diameter using the insertion-retraction protocol described above. As shown Fig. 5, microneedles were able to infuse nanoparticle suspensions into the sclera. Essentially all 20 μl of solution was infused into the tissue and very little was seen to leak onto the scleral surface.

For additional insight, Fig. 6 shows that increasing nanoparticle concentration in the infusion solution increased nanoparticle delivery into the sclera and, at higher concentration, the nanoparticles spread over a larger volume within the sclera tissue. In addition, nanoparticles were infused into all three regions of the sclera. It appears that nanoparticles distributed over a larger tissue volume in the posterior region of the sclera, which may reflect differences in regional tissue microstructure.

We carried out a parallel study to assess infusion of microparticles measuring 1 μm in diameter. However, we failed to deliver any significant number of microparticles into scleral tissue with any reproducibility (data not shown). Visual inspection suggested that the microparticles had difficulty flowing out of the microneedles and appeared to clog the needle tip. A control experiment showed that these microparticles flowed readily out of microneedles when infused into air. This indicates that the microparticles could flow through the tip orifice, but could not flow into the sclera, perhaps due to blockage by the fibrous microstructure of sclera tissue. Indeed, the edge-to-edge spacing between collagen fibrils in human sclera varies considerably, but is on the order of 100 nm (24). Therefore, we hypothesize that our nanoparticles were able to flow between scleral collagen fibrils, but microparticles are too big to pass through.

Effect of collagenase and hyaluronidase

If scleral collagen fibrils were blocking microparticle delivery, we hypothesized that the use of collagenase to break down collagen structure (25) should enable microparticle delivery into sclera. Consistent with this hypothesis, microparticle delivery without collagenase (Fig. 7a, control) showed very little microparticle delivery, whereas either incubating the tissue in collagenase solution for 1 h prior to microparticle infusion (Fig. 7a, pretreatment) or simply mixing collagenase with the microparticle suspension and infusing them mixed together (Fig. 7a, co-injection) enable microparticle infusion into the sclera.

Because administration of collagenase is of uncertain safety if used clinically, we also tested the use of another enzyme, hyaluronidase, to open up the tissue for microparticle infusion. Hyaluronidase has been shown to increase flow conductivity of dermis during injection by a mechanism involving hydrolysis of hyaluronic acid found in extracellular ground substance (25). Hyaluronidase has also been approved by the FDA to increase dispersion of injected drugs (26), is found as a naturally occurring enzyme in human corneoscleral tissue (27) and was well tolerated in patients when used in the eye for management of vitreous hemorrhage (28).

As shown in Fig. 7b, both pretreatment and co-injection with hyaluronidase enable microparticle delivery into the scleral tissue. This suggests that the extracellular matrix is a rate-limiting barrier to microparticle delivery, or possibly that partially degrading the extracellular matrix loosened collagen fibril structure and thereby permitted passage of microparticles. It also suggests that co-injection with hyaluronidase may provide a useful method to deliver controlled-release microparticles into the sclera.

Finally, we wondered if hyaluronidase might also facilitate infusion of sulforhodamine solution into sclera. However, when we repeated the sulforhodamine solution infusion experiments with the addition of hyaluronidase, we did not observe any significant changes in volumetric delivery (data not shown). This suggests that extracellular matrix structure did not significantly constrain sulforhodamine solution infusion into the sclera.

Implications for ocular drug delivery

This study assessed the effects of microneedles on intrascleral delivery and found that fluid could be infused into the sclera using individual microneedles. In this way, a small molecule model drug, as well as nanoparticles and microparticles were delivered into the sclera. Intrascleral delivery using microneedles may provide a minimally invasive method for localized delivery to the eye. After forming a drug depot within the sclera, drug could diffuse to neighboring tissues, such as choroid, retina or ciliary body, to treat diseases such as macular degeneration or glaucoma.

This approach contrasts with intravitreal injection, which uses a larger hypodermic needle that fully penetrates across the sclera, choroid, retina and into the vitreous body (4). Because microneedles penetrate just hundreds of microns into the sclera without fully crossing the tissue, they are expected to reduce the risk of complications, such as retinal detachment, cataract, and infection. Microneedles also contrast with topical eye drops, periocular injections and systemic administration, all of which are very inefficient at localizing delivery into the eye, which results in very low bioavailability and possible side effects (1,2). Additional safety studies will be needed to assess the mechanism and timescale of recovery of the sclera to repair the hole made by a microneedle. However, this level of tissue trauma is much smaller than holes made in sclera during intravitreal injection or ocular surgical procedures, which generally repair safely.

Individual microneedles were able to deliver 10 – 35 μl of fluid into the sclera. Although it was not studied here, we expect that multiple microneedles should be able to deliver more fluid. For example, we propose that a ten-needle array might be able to deliver 100 μl of fluid or more into the sclera. Given the small doses needed in the eye, delivery of 100 μl should be sufficient for many ophthalmic drugs. For example, the conventional intravitreal dose of ranibizumab (Lucentis®) given once per month is 50 μl (i.e., a dose of 0.5 mg from a 10 mg/ml solution) (29). As another example, the volume of a typical eye drop is approximately 40 μl (30).

Intrascleral delivery of nanoparticles and microparticles presents the opportunity for controlled release drug delivery over extended periods of time. In this scenario, drug-loaded particles

could be injected into the sclera, where they form a slow-release depot for delivery into the eye. Based again on the estimate that 100 μl of fluid can be injected, we predict that 10 mg of particles could be injected (i.e., based on 10% solids content (Fig. 6) and a specific gravity of one) and that they could encapsulate 1 mg of drug (i.e., based on 10% encapsulation within the particles). One milligram of drug can be enough for weeks or months of delivery for many ophthalmic drugs. For example, the monthly dose of ranibizumab is 0.5 mg. Thus, delivery of 1 mg encapsulated within microspheres corresponds to two months of controlled release delivery. Just increasing the amount of drug a few fold by increasing the fluid delivery, the solids content or the encapsulation content could enable ranibizumab delivery for many months and possibly for a full year.

Although the fluid volume delivered into the sclera appears sufficient for many applications, it is notable that this volume was limited to 10 – 35 μl per microneedle. Increasing pressure, increasing time (beyond a few minutes) and increasing retraction depth (beyond the minimum for infusion) did not appreciably increase this volume. Even breaking down tissue microstructure through the addition of hyaluronidase did not increase infusion of sulforhodamine solution. This indicates that there is a limited potential space accessible for fluid infusion into the sclera. It is currently not clear what controls its volume or how to increase it.

Although fluid and nanospheres could be infused into intact sclera, delivery of microparticles required the use of a spreading enzyme, such as hyaluronidase or collagenase. This observation is consistent with the known spacing between collagen fibrils in the sclera on the order of 100 nm (24), which would be expected to block microparticle movement. The possible safety of using hyaluronidase with microneedles for intrascleral delivery needs to be studied. It is worth noting that the approved indications for hyaluranidase (Vitrase®) use are not tissue specific, although there is a warning against direct application to the cornea (26) and possible degradation of the vitreous body should be avoided (28).

Finally, this study used individual glass microneedles fabricated using a low-throughput laboratory device. Clinical applications would benefit from stronger materials, possible multi-needle arrays, and high-throughput fabrication methods. To address this, a number of studies motivated by transdermal delivery applications have developed ways to make metal microneedles, both as individual needles and as multi-needle arrays, using low-cost fabrication methods suitable for mass production (13,15). Future studies should investigate the use of the commercializable microneedles for intrascleral delivery.

CONCLUSIONS

This study showed for the first time that hollow microneedles can infuse solutions containing soluble molecules, nanoparticles, and microparticles into the sclera in a minimally invasive manner. Infusion volumes of 10 – 35 μl were delivered into sclera using individual microneedles; multiple microneedles may be able to deliver more. Soluble molecules and nanoparticles were delivered using an insertion-retraction protocol, whereas microparticles required the addition of a spreading enzyme, i.e., hyaluronidase or collagenase, to disrupt scleral tissue microstructure. Infusion pressure and scleral thickness did not affect volumetric delivery into sclera. Altogether, this study suggests that hollow microneedles can be used to deliver drugs and particles into the sclera in a minimally invasive manner for rapid or possible controlled release drug delivery to the eye.

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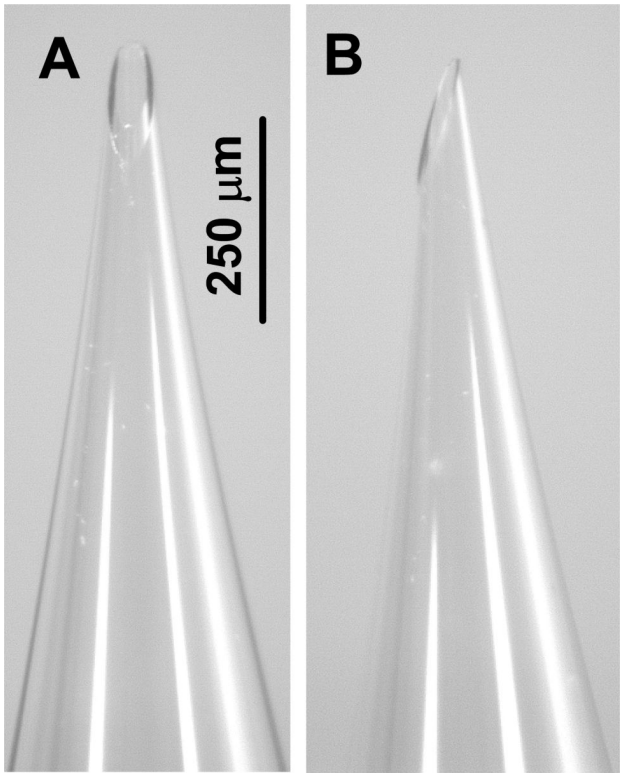

Figure 1.
Representative hollow microneedle: (A) front view and (B) side view. The microneedle shown has an approximately elliptical tip opening with a long axis of ~100 μm and a short axis of ~40 μm with a bevel tip angle of 25°.

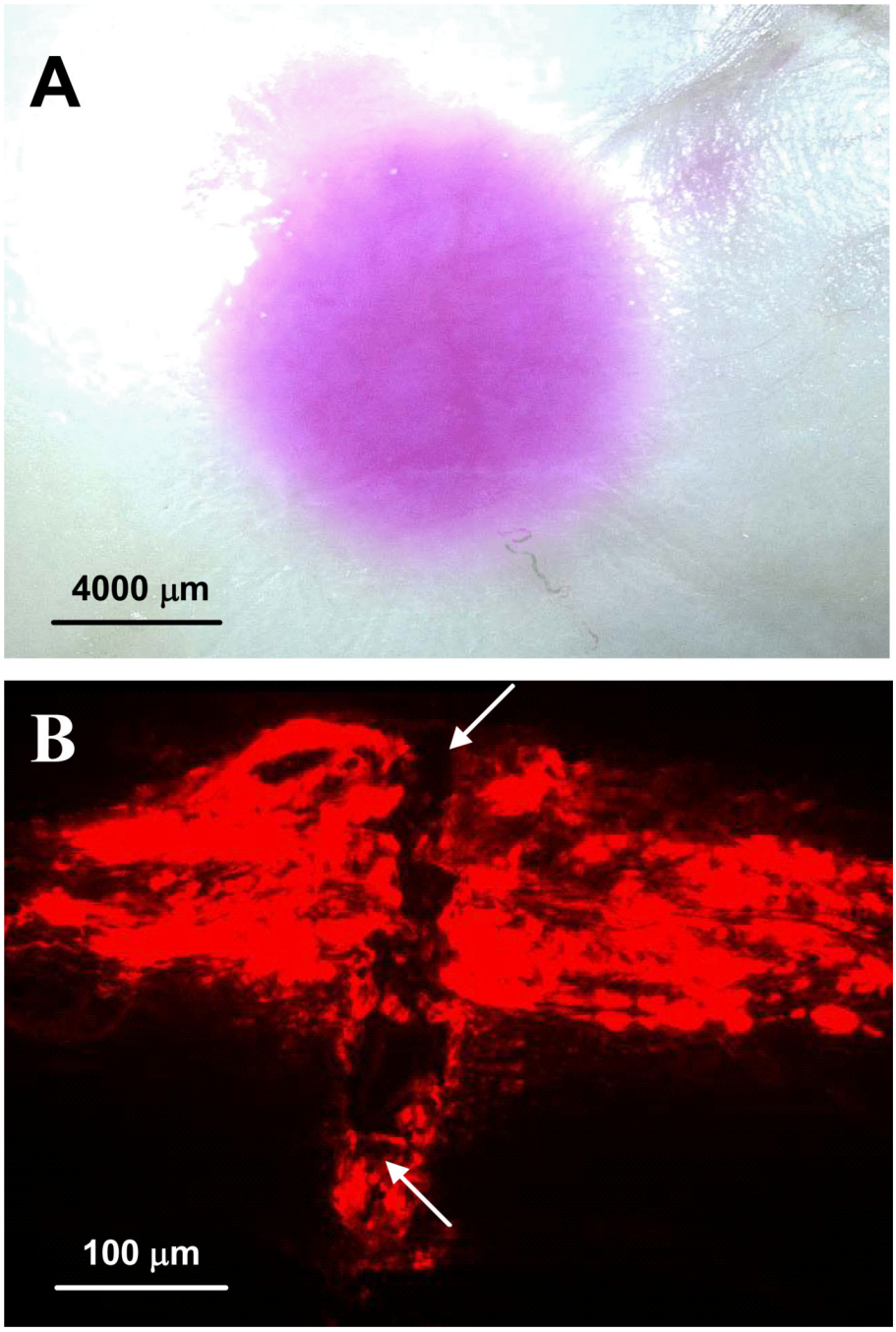

Figure 2.
Representative human cadaver sclera after microneedle infusion of sulforhodamine solution. (A) Top view image of the scleral surface using bright-field microscopy shows infusion of sulforhodamine over an area of many square millimeters. (B) Histological section using fluorescence microscopy shows the site of microneedle insertion (indicated by arrows) and the distribution of injected sulforhodamine preferentially localized to the upper portion of the tissue. A microneedle was inserted 720 μm into the sclera and then retracted 60 μm every 3 min to a maximum retraction of 240 μm. Sulforhodamine solution was infused into the tissue at a pressure of 15 psi.

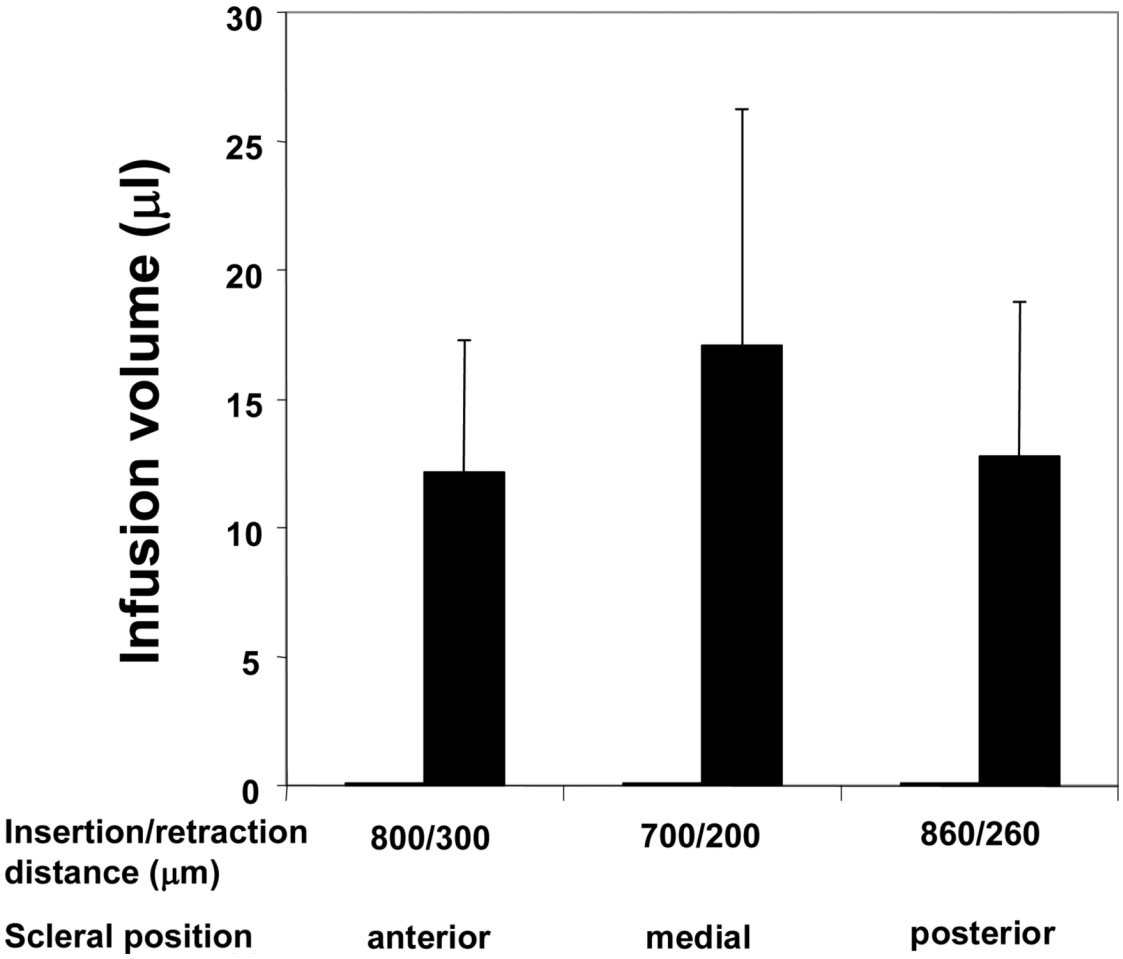

Figure 3.

Effect of microneedle retraction on fluid delivery into anterior, medial and posterior regions of human cadaver sclera. In each experiment, a microneedle was inserted into the sclera at a distance of 720 – 1080 μm and sulforhodamine solution was infused at a pressure of 15 psi. Every 3 min, the microneedle was retracted 60 μm. The bar on the left of each pair of bars shows the infusion volume after insertion without retraction. The bar on the right of each pair shows the infusion volume after insertion and retraction. Along the x axis, the reported insertion distance is the average insertion distance of that experimental data set. The reported retraction distance is the average amount of microneedle retraction needed before appreciable fluid flow began. Data are expressed as mean values ($n \geq 17$) with standard deviation bars.

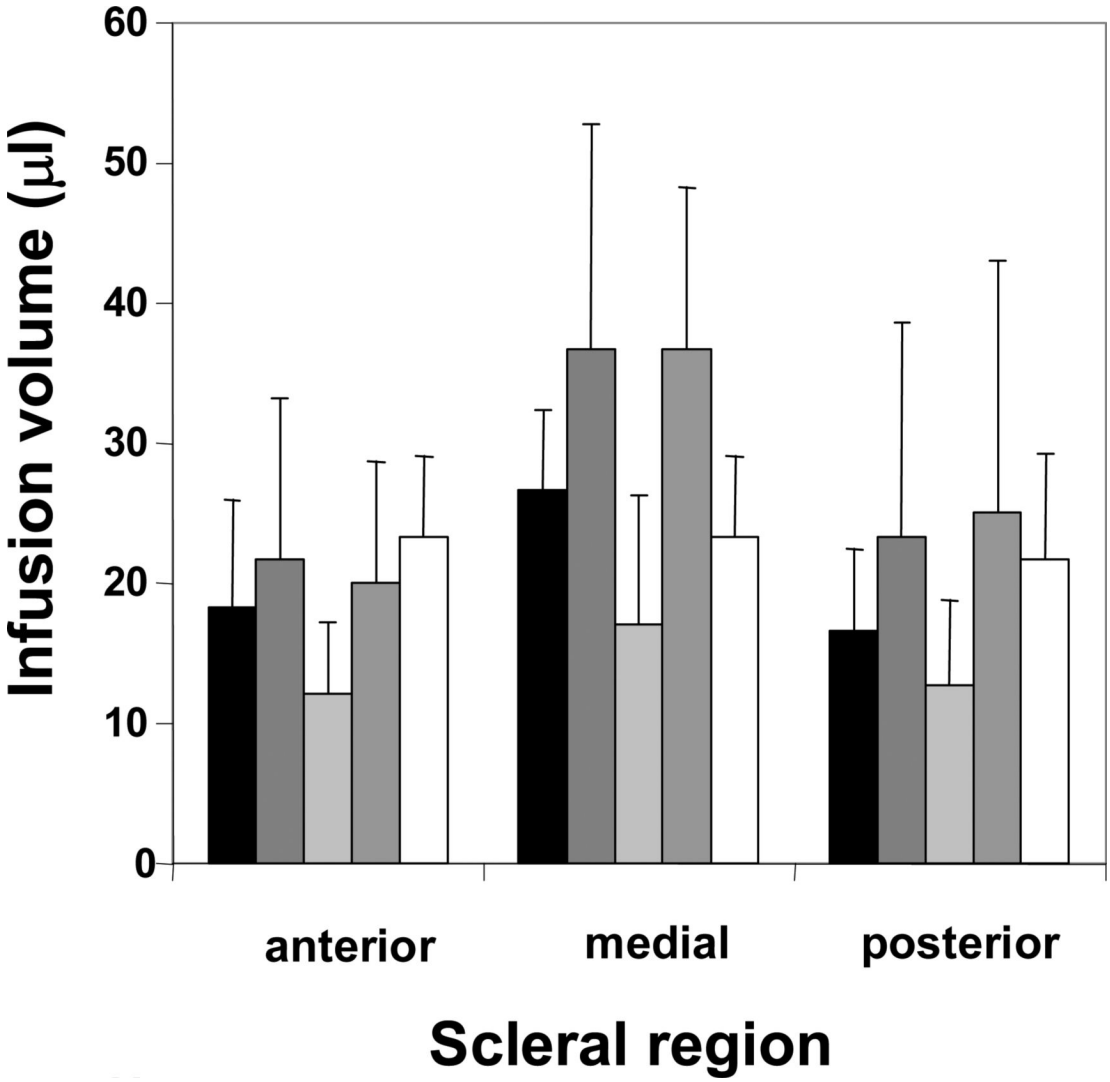

Figure 4.

Effect of pressure on fluid delivery into anterior, medial and posterior regions of human cadaver sclera. Individual microneedles were inserted 720 μm into the sclera and then retracted 60 μm every 3 min to a maximum retraction of 140 – 300 μm with an infusion pressure of 5 (■), 10 (■), 15 (■), 20 (■) and 25 (□) psi. Data are expressed as mean values ($n \geq 3$) with standard deviation bars.

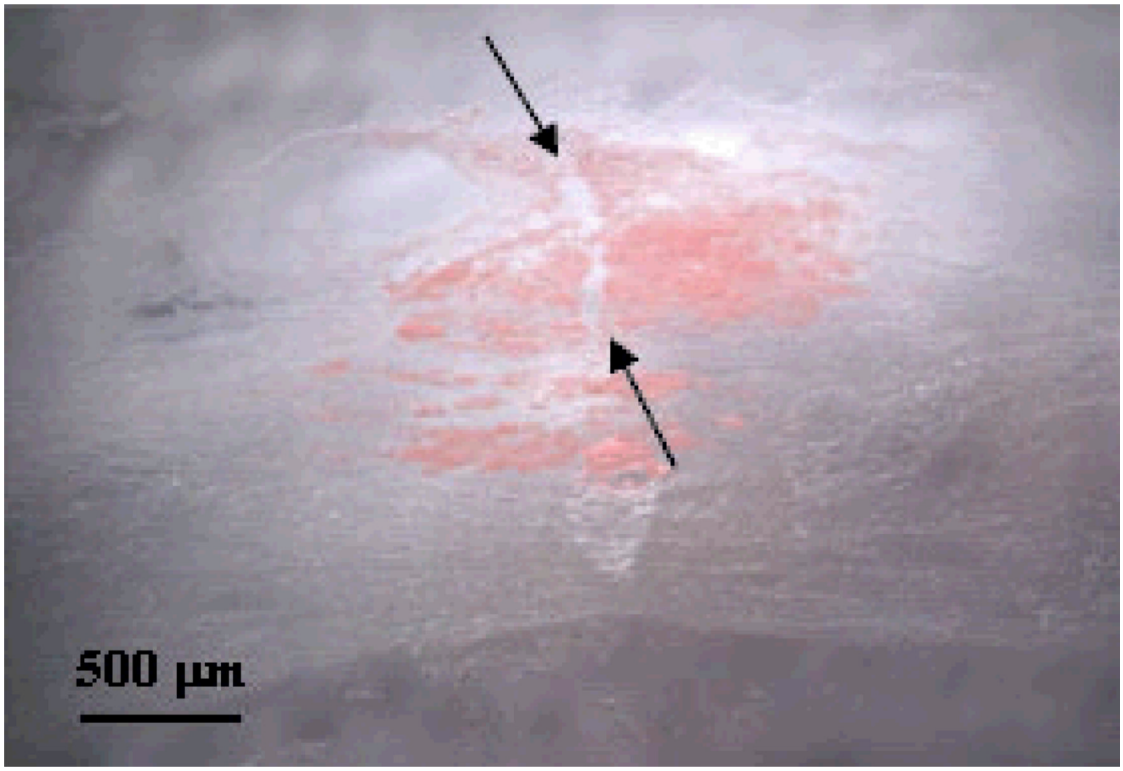

Figure 5.
Representative histological image of human cadaver sclera after infusion of fluorescent nanoparticles (280 µm diameter). The microneedle was inserted 720 µm into the anterior region of sclera and then retracted 60 µm every 3 min to a maximum retraction of 240 µm. A 1.0 wt % nanoparticles suspension was infused into the tissue at a pressure of 15 psi over 15 min. The site of microneedle insertion is indicated by arrows.

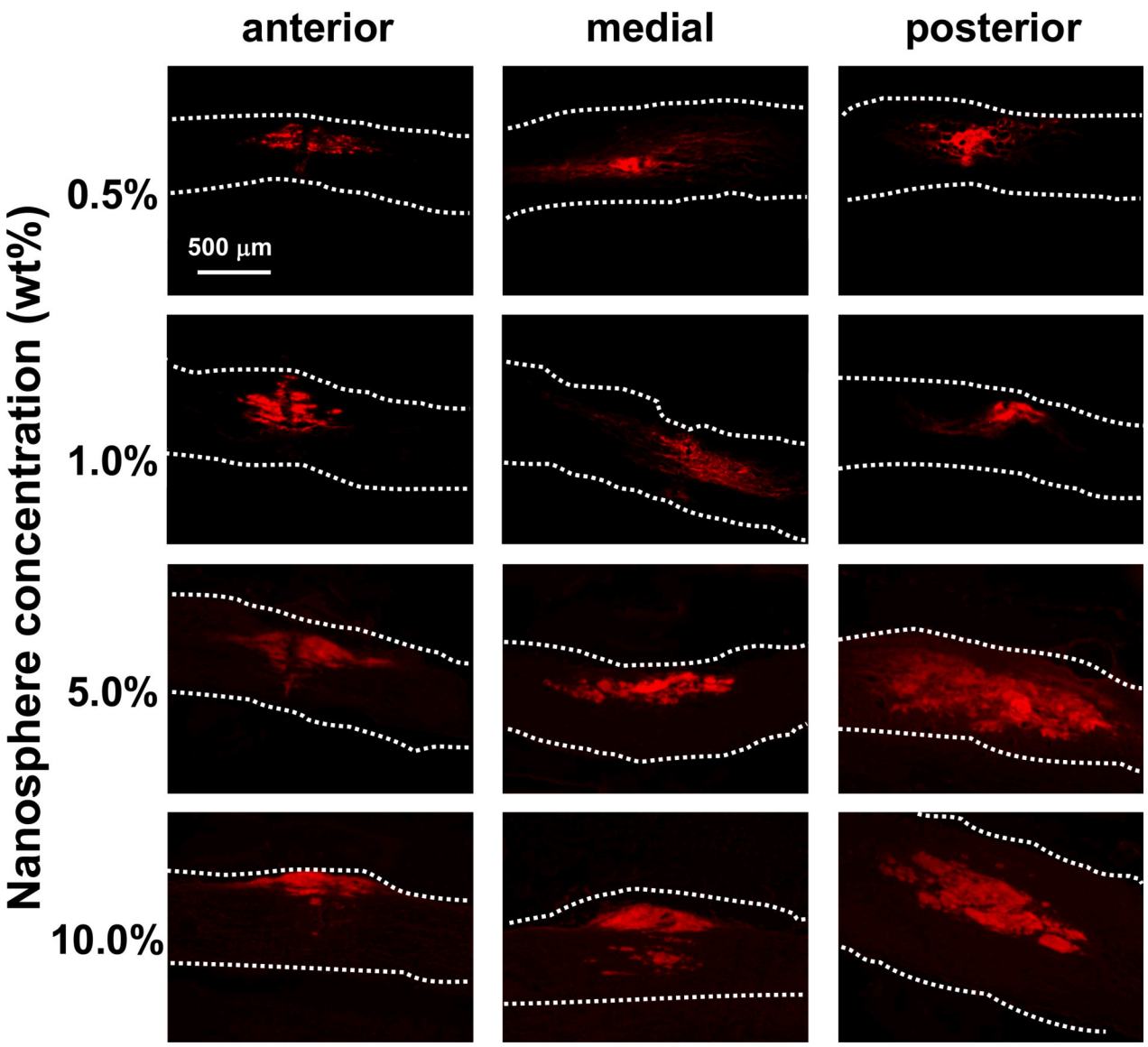

Figure 6.

Representative histological images of human cadaver sclera after infusion of fluorescent nanoparticles as a function of nanoparticle concentration and scleral position. Microneedles were inserted 720 – 1080 μm into anterior, medial and posterior regions of the sclera and then retracted 60 μm every 3 min to a maximum retraction of 240 – 360μm. In each experiment, a 20 μl suspension with a solids content of 0.5, 1, 5 or 10 wt% was infused into the tissue at a pressure of 15 psi. Dotted lines have been added to each image to more clearly indicate the upper and lower edges of the scleral tissue.

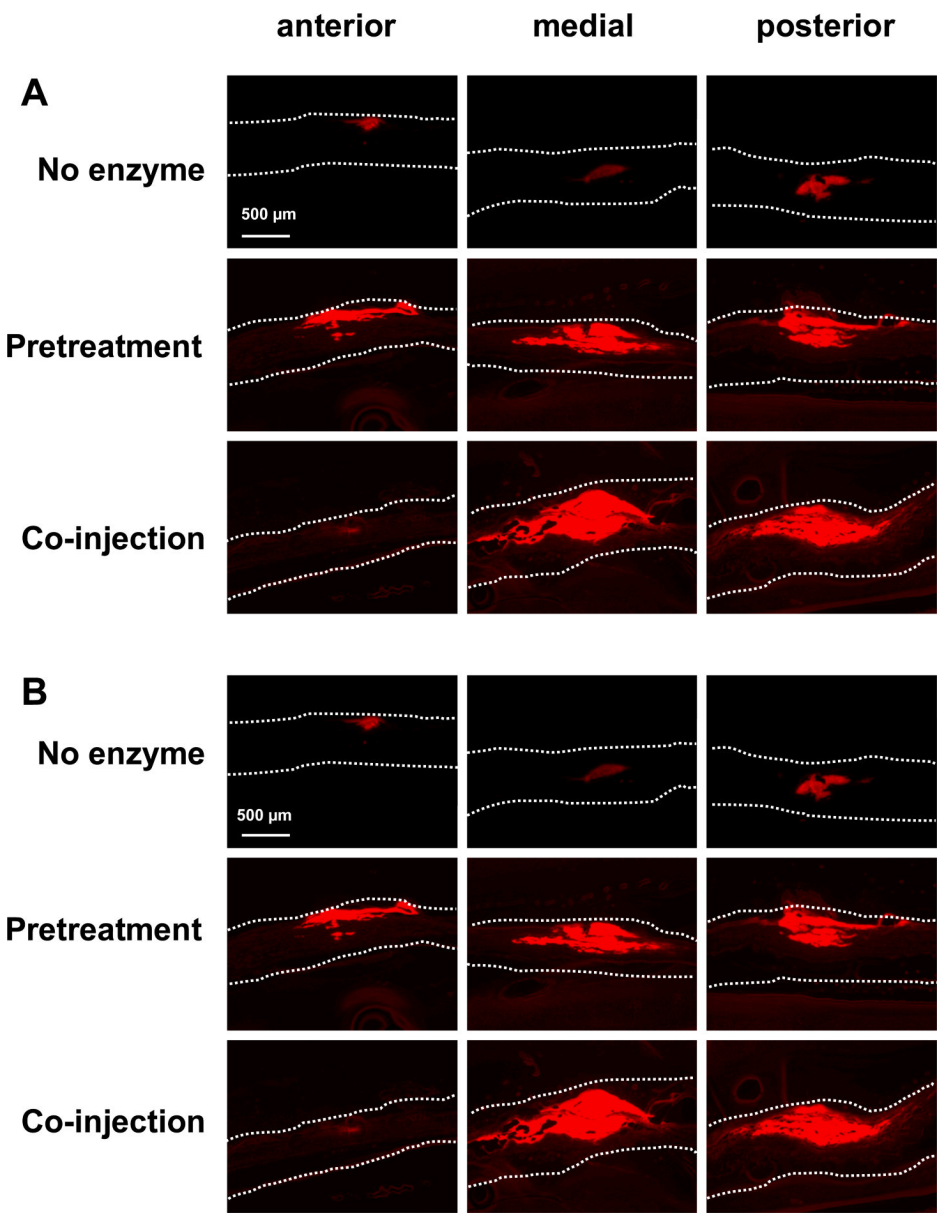

Figure 7.

Representative histological images of human cadaver sclera after infusion of fluorescent microparticles as a function of exposure to the spreading enzymes (A) collagenase and (B) hyaluronidase. Tissues were either untreated (control), incubated in the enzyme for 1 h before microparticle infusion (pretreatment), or co-injected with a mixture of the enzyme microparticles (co-injection). Microneedles were inserted 720 – 960 μm into anterior, medial and posterior regions of the sclera and then retracted 60 μm every 3 min to a maximum retraction of 240 – 300μμm. In each experiment, a 20 μl suspension with a solids content of 1.3 wt% was infused into the tissue at a pressure of 15 psi. Dotted lines have been added to each image to more clearly indicate the upper and lower edges of the scleral tissue.