Gelling hypotonic polymer solution for extended topical drug delivery to the eye

Yoo Chun Kim1,2, Matthew D. Shin1,2, Sean F. Hackett2, Henry T. Hsueh1,3, Raquel Lima e Silva1,2, Abhijit Date1,2,11, Hyounkoo Han1,2, Byung-Jin Kim2, Amy Xiao1,4, Youngwook Kim1,5, Laolu Ogunnaikel1,3, Nicole M. Anders6, Avelina Hemingway6, Ping He6, Albert S. Jun2, Peter J. McDonnell2, Charles Eberhart2, Ian Pitha2, Donald J. Zack1,2, Peter A. Campochiaro2, Justin Hanes1,2,3,4,6,7,8,9 e-mail: hanes@jhmi.edu; lensign@jhmi.edu

Eye-drop formulations should hold as high a concentration of soluble drug in contact with ocular epithelium for as long as possible. However, eye tears and frequent blinking limit drug retention on the ocular surface, and gelling drops typically form clumps that blur vision. Here, we describe a gelling hypotonic solution containing a low concentration of a thermosensitive triblock copolymer for extended ocular drug delivery. On topical application, the hypotonic formulation forms a highly uniform and clear thin layer that conforms to the ocular surface and resists clearance from blinking, increasing the intraocular absorption of hydrophilic and hydrophobic drugs and extending the drug–ocular-epithelium contact time with respect to conventional thermosensitive gelling formulations and commercial eye drops. We also show that the conformal gel layer allows for therapeutically relevant drug delivery to the posterior segment of the eyeball in pigs. Our findings highlight the importance of formulations that conform to the ocular surface before viscosity enhancement for increased and prolonged ocular surface contact and drug absorption.

Eye drops are the dominant dosage form for the ocular route, and are used for a wide range of indications, including glaucoma, dry eye, inflammation, infection and allergy. However, as with all exposed epithelial surfaces, there are innate protective mechanisms that hinder efficient drug delivery to the eye. Tears continuously bathe the ocular surface before draining into the nasolacrimal duct and the blinking of the eyelids frequently wipes the ocular surface clean of debris and excess fluids. Thus, only a small fraction of drugs administered by eye drops is typically absorbed into the eye1, often necessitating multiple doses per day. As the number of eye-drop doses per day increases, so does the potential for ocular surface irritation and systemic side effects, while patient compliance to the treatment regimen decreases2. There is a compelling need for approaches towards more efficient and longer-lasting topical ocular drug delivery.

To increase ocular drug absorption without disrupting the ocular epithelium, an eye-drop formulation should hold as high concentration of soluble drug in contact with the tissue for as long as possible. Poorly soluble drugs are often formulated as milky suspensions, which can blur vision on application. For highly potent insoluble or poorly soluble drugs, solubility enhancers may be used, but these approaches still have the issue of limited retention on the ocular surface. Viscosity-enhancing polymers can increase retention time by resisting lacrimal drainage, but they typically result in uneven coating that blurs vision and difficulty in dispensing single, reproducible volume drops from dropper bottles can be an issue. Ointments are also sometimes used, but they are messy and their effects on vision typically limit their use to bedtime. Another option is in situ gelling systems, which are liquids that undergo a sol–gel transition triggered by temperature, polymerization, ions or some other physical stimulus on application to the eye3. However, there are only a handful of eye-drop products available that gel on application, partly because immediate gelation upon contact with the ocular surface typically leads to clumpy gels that can also be messy and blur vision.

Preclinically, a variety of in situ gelling systems has been studied for topical ocular drug delivery, with thermosensitive polymers being one of the most extensively tested1. Here we used Pluronic F127, a triblock copolymer that has thermoreversible gelling properties above the critical gel concentration (CGC) of about 15–16 % (w/w) under physiological conditions1. Pluronic F127 is an ingredient in a variety of US Food and Drug Administration-approved products1 at concentrations typically less than 2% (w/w) in eye drops. The conventional approach for formulating thermoreversible polymers for in situ gelation is to use a polymer concentration above the CGC, such that a gel forms immediately as temperature increases past the sol–gel transition temperature1,4. By contrast, our aqueous formulation contains F127 at a concentration below its CGC, such that it will not gel in vitro. This is particularly important for a polymer such as F127, which has a sol–gel transition
temperature in a range at which it may prematurely gel at room temperature. The key to our formulation approach is that the polymer solution contains a lower than physiological ion content, so it is hypotonic to the ocular surface and causes osmotically-induced water absorption upon application. Water absorption spreads and concentrates the polymer right at the ocular surface, resulting in a thin, uniform, clear gel that holds the drug on the ocular surface and fits comfortably under the eyelids to resist clearance from blinking. By contrast, conventional thermogelling formulations, which are isotonic to the eye and contain polymer above the CGC, immediately form clumpy, uneven gels on contact with the eye, which may be wiped away by the eyelids, leading to suboptimal drug delivery. We demonstrate that our hypotonic, lower concentration thermogelling formulations outperform both conventional isotonic thermogelling formulations and commercial eye drops for drugs that lower intraocular pressure (IOP), both hydrophilic (brimonidine tartrate (BT)) and hydrophobic (brinzolamide (BRZ)), as well as a hydrophobic peptide drug (cyclosporine) used for treating dry eye. Importantly, our hypotonic gelling formulation caused no signs of ocular irritation or toxicity with twice-daily administration for up to five weeks in rabbits, as measured by corneal staining, blink-rate test and corneal histology. Further, characterization of the uniformity, refractive index and absorbance suggest that visual clarity is maintained and the shear-thinning properties are similar to those of commercial lubricating eye drops. We also provide data to support the hypothesis that by holding the drug at high concentration at the ocular surface, drug delivery to the posterior retina and choroid can be increased in large animals with eyes similar in size to the human eye, leading to the prevention of laser-induced choroidal neovascularization (CNV).

Results

Hypotonicity leads to uniform surface coating and gelation. In contrast to liquid eye drops, gels may reside longer on the ocular surface to provide increased intraocular drug absorption. The conventional formulation approach for thermoreversible polymer eye drops includes polymer at or above the CGC (~15–16% w/w for F127) in aqueous solution isotonic to the ocular surface (~290 mOsm kg\(^{-1}\)), where 18% F127 is commonly used in numerous prior topical eye-drop studies. Immediate gelation on contact would result in uneven, clumpy gels that may be removed from the ocular surface by the eyelids, get caught up in the lids and lashes, and obscure vision. We hypothesized that the potential advantages of a thermoreversible gel in terms of duration on the ocular surface could be better realized by creating a more uniform gel layer that would conform to the contours of the ocular surface and fit comfortably under the eyelids during blinking. Further, both the polymer concentration and tonicity could be lowered to enable increased surface coverage and uniformity driven by water absorption that also acts to concentrate the polymer to gel on the ocular surface. We first sought to determine whether there must be a balance between the polymer dilution and the extent of water absorption that occurs to achieve gelation on the ocular surface. Thus, we evaluated the pharmacodynamic effect of hypotonic eye drops containing one of two common ocular drugs with 10–18% F127. Reduction of IOP after a single dose of BT, and tear production after a single dose of cyclosporine A (CsA) was measured over time in normotensive rabbits. For both drugs, there was an increase in cumulative drug concentration at the ocular surface, drug delivery to the posterior retina and choroid can be increased in large animals with eyes similar in size to the human eye, leading to the prevention of laser-induced choroidal neovascularization (CNV).

We then sought to visualize the eye-drop behaviour upon application in vivo using optical coherence tomography (OCT) imaging in rats. The hypotonic 12% F127 formulation (12% hypo) was compared with an isotonic 12% F127 formulation (12% iso), which should behave as a viscous liquid that does not induce water absorption by the ocular tissues, and a conventional isotonic 18% F127 formulation (18% iso). The immediate gelation of the conventional 18% iso formulation resulted in a thick, irregular coating on the ocular surface that was variable between animals (Supplementary Fig. 1c) and more readily cleared upon blinking (Fig. 1a, top row). By contrast, the 12% hypo formulation formed uniform surface coatings that were consistent between animals (Supplementary Fig. 1c) and that persisted on the ocular surface after blinking (Fig. 1a, middle row). The 12% iso formulation, which does not concentrate upon application to the eye to undergo a sol–gel transition, was cleared after blinking (Fig. 1a, bottom row). To visualize the macroscopic behaviour of the formulations, F127 was fluorescently labelled (Supplementary Fig. 2a–d) (imparted blue colour) and video was recorded to capture eye-drop application to conscious rabbits. Fluorescent labelling and adjustment of salt content did not affect the F127 gelling transition temperature (Supplementary Fig. 2e). The immediate gelation of the conventional 18% iso resulted in limited spreading and rapid clearance due to the blinking of the eyelids, which is also illustrated in the schematic representation to the left of the real-time recording in Supplementary Video 1. By contrast, the 12% hypo formulation spread to coat the eye (Supplementary Video 1).

To visualize the residence time of the 12% hypo formulation on the surface of the eye, fluorescently labelled F127 was used. The 12% hypo formulation was still clearly evident by fluorescent imaging of cryosections of the rat eye 3 h after administration, whereas the 12% iso formulation was undetectable due to clearance by blinking (Supplementary Fig. 2f). We then sought to determine whether the prolonged residence time of the 12% hypo formulation relative to the 12% iso formulation was due to concentration of the polymer sufficient to cause gelation. As the 12% hypo formulation cannot gel in vitro, the observation of the gelation phenomenon can only be performed after administration to an intact eye. To do this, we used real-time multiple-particle tracking (MPT) to visualize the motion of fluorescent probe nanoparticles within the eye-drop formulations in vitro, as well as on the surface of the eye of rats after administration in vivo. Nanoparticles diffuse freely within liquids due to thermal motion, and their diffusion rate, expressed here as $\langle \text{MSD} \rangle$ at a time scale of 1 s, is directly related to the viscosity and physical pore structure of the surrounding environment. Thus, MPT facilitates rheological measurement on a micro scale, where traditional bulk rheometric analysis is not feasible. Here, we synthesized nanoparticles with non-adhesive surface coatings to ensure that any effects on nanoparticle mobility would reflect steric entrapment. In vitro at $37^\circ C\text{,}$ 200 nm nanoparticles were effectively trapped within the 18% iso gel polymer micelle network, exhibited by the ~130-fold lower ($\langle \text{MSD} \rangle$) compared with unobstructed nanoparticle diffusion in the viscous 12% hypo and 12% iso fluids (Fig. 1b). However, when the nanoparticles were administered in the 12% hypo formulation to the intact eye in vivo and then visualized ex vivo, the nanoparticles exhibited a lower ($\langle \text{MSD} \rangle$), similar to nanoparticles administered in the 18% iso formulation (Fig. 1b). By contrast, nanoparticles administered in the 12% iso formulation diffused relatively unobstructed (Fig. 1b), demonstrating that a gel did not form in vivo. Representative ex vivo trajectories demonstrate the contrast between the nanoparticles sterically trapped within the gel formed on the ocular surface in vivo by 12% hypo and 18% iso compared with freely diffusive nanoparticles in the 12% iso viscous liquid (Fig. 1c). As increasing the viscosity of the vehicle fluid is a common approach to increase residence time on the ocular surface, we further compared the 12% hypo formulation to 12% iso. The in vivo gelation behaviour of the 12% hypo formulation led to prolonged levels of BT in rat ocular tissues (cornea, conjunctiva and aqueous humour) for at least 8 h after the last dose, compared...
with the 12% iso formulation (Supplementary Fig. 2g–i). Further, increased intraocular delivery of BT with the 12% hypo formulation led to a sustained decrease in IOP in normotensive rabbits that was significantly lower than for the 12% iso formulation for at least 6–10 h (Supplementary Fig. 2j).

The hypotonic gel enhances intraocular drug absorption. We then formulated several commonly used topical anterior-segment drugs with different physicochemical properties (Supplementary Table 1) to compare ocular delivery in the 12% hypo gelling formulation with the conventional gelling formulation approach (18% iso) and the corresponding commercially available formulations. BT is commercially available as an aqueous solution at 0.15% w/v (Alphagan P), which was readily soluble in the 12% hypo and 18% iso vehicles. We found that BT dissolved in the 12% hypo formulation (12% hypo(BT)) provided significantly higher drug concentration in the rat cornea 1 and 8 h after the last dose compared with 18% iso(BT) and 1, 4 and 8 h for Alphagan P (Fig. 2a). We observed a similar trend in the conjunctiva, although the increased drug concentration in the 12% hypo(BT) group was only significant at 8 h (Fig. 2b). In the aqueous humour, the 12% hypo(BT) formulation provided significantly increased drug concentration compared with 18% iso(BT) at 4 and 8 h, and significantly increased drug concentration compared with Alphagan P at 1, 4 and 8 h (Fig. 2c). We then evaluated each formulation for IOP reduction with a single dose in normotensive rabbits. The 12% hypo(BT) formulation provided the largest and most sustained decrease in IOP, which was still significant compared with both the 18% iso(BT) and Alphagan P at 10 h (Fig. 2d). As expected, the 12% hypo vehicle (vehicle) did not affect IOP (Fig. 2e). Surprisingly, despite the prolonged intraocular absorption provided by the 12% hypo(BT) formulation compared with Alphagan P, the systemic BT exposure after a single dose in rabbits was similar (Fig. 2f). Systemic drug absorption after topical dosing is a key factor that limits intraocular drug absorption\(^{1}\), and achieving the same efficacy with less frequent dosing would reduce overall systemic drug exposure and potential side effects. Indeed, equivalent efficacy with reduced systemic exposure was the rationale for approval of Allergan's most recent iteration of brimonidine eye drops\(^{11}\).

We next formulated two anterior-segment drugs with relatively low water solubility, BRZ and CsA (Supplementary Table 1). The commercial formulations for both drugs are milky suspensions, whereas both 12% hypo formulations were optically clear at the time of formulation (vehicle) did not affect IOP (Fig. 2e). Surprisingly, despite the prolonged intraocular absorption provided by the 12% hypo(BT) formulation compared with Alphagan P, the systemic BT exposure after a single dose in rabbits was similar (Fig. 2f). Systemic drug absorption after topical dosing is a key factor that limits intraocular drug absorption\(^{1}\), and achieving the same efficacy with less frequent dosing would reduce overall systemic drug exposure and potential side effects. Indeed, equivalent efficacy with reduced systemic exposure was the rationale for approval of Allergan's most recent iteration of brimonidine eye drops\(^{11}\).

We then evaluated each formulation for IOP reduction with a single dose in normotensive rabbits. The 12% hypo(BT) formulation provided the largest and most sustained decrease in IOP, which was still significant compared with both the 18% iso(BRZ) and Azopt (Fig. 3a). The trend was similar in the conjunctiva, but was significant only at 4 h (Fig. 3b). In the aqueous humour, the drug concentrations were similar for all formulations (Fig. 3c), which is reflective of the low drug concentrations and lack of BRZ accumulation in the aqueous humour observed in humans\(^{12}\). In normotensive rabbits, a single dose of 12% hypo(BRZ) provided the largest and most sustained decrease in IOP, which was still significant compared with both the 18% iso(BRZ) and Azopt at 8 h (Fig. 3d). For CsA, we found that 12% hypo(CsA) provided significantly increased drug concentrations in the rat cornea compared with 18% iso(CsA) and Restasis at 4 and 8 h after the last dose (Fig. 3e). The trend was similar in the conjunctiva, but was statistically significant only at 4 h (Fig. 3f).

Enhanced delivery to the back of the eye in mice. We next sought to determine whether providing enhanced intraocular drug absorption with the hypotonic gelling formulation would also provide therapeutically relevant drug delivery to the posterior segment. We formulated two water-soluble drugs with markedly different partition coefficients (log \(P\)) (Supplementary Table 1), acriflavine hydrochloride (ACF) and sunitinib malate (SM), which have been demonstrated to have anti-angiogenic properties in the eye\(^{13,14}\). As shown in Fig. 4a, once-daily dosing with 0.5% ACF in the 12% hypo formulation (12% hypo(ACF)) resulted in significant suppression

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**Fig. 1 | Hypotonicity drives water absorption that leads to uniform ocular surface coating and gelation.** **a**, Representative OCT images from rat eyes taken immediately after topical application (left) of 12% hypo, 12% iso and 18% iso eye drops and after manually blinking the eyelids (right). Images are false-coloured to differentiate the eye structures (blue) and the topical formulations (red). \(n = 5–6\) independent eyes and three images were obtained per eye. White arrows point to the eye-drop layer. Scale bar, 100 \(\mu m\) (applies to all images). **b**, Ensemble-averaged mean square displacement (\(\text{MSD} \pm \text{s.e.m.}\)) of 200 nm nanoparticles in indicated media at 37 °C in vitro and after application in vivo (particles visualized ex vivo). Each data point represents the ensemble average of 50–100 nanoparticles tracked in an individual sample. \(n = 4–7\) independent animals. Data are mean ± s.e.m. Statistical analyses conducted by one-way ANOVA with multiple comparisons with respect to 12% hypo in vivo group. The in vivo data groups with boxes around them correspond to those shown in **c, c**, Representative schematics showing the ex vivo tracked trajectories (~20 s) of nanoparticles in the 12% hypo (left) formulation compared with 12% iso (middle) and 18% iso (right) after ocular application in vivo.
of laser-induced CNV compared with the fellow eye treated with 12% hypo vehicle only. By contrast, ACF dosed in water did not significantly suppress CNV compared with the fellow eye treated with vehicle only (Supplementary Fig. 4a). Once-daily dosing of 0.4% SM in the 12% hypo formulation (12% hypo(SM)) also significantly suppressed laser-induced CNV compared with the fellow eye treated with the 12% hypo vehicle only (Fig. 4b). However, SM dosed in saline did not significantly suppress CNV compared with the fellow eye treated with vehicle only (Supplementary Fig. 4b).

We observed increased delivery to the posterior segment using the 12% hypo gel-forming vehicle, so we attempted to gain some mechanistic insight into this effect in vitro. As SM is currently in clinical trials for treating wet age-related macular degeneration (ClinicalTrials.gov NCT03953079), we used it as our model drug in subsequent experiments. One major challenge for any in vitro characterization is that the 12% hypo formulation does not form a gel at 37 °C, and it is difficult to model the epithelial water-absorption effect that concentrates the polymer to form a gel upon administration in vivo. Thus, we had to use 18% F127 to investigate the effect of the gel on corneal drug permeability and drug release. Using excised rabbit cornea tissue, we found that the average apparent corneal permeability coefficient measured at 30 min for SM in F127 gel (18% hypo(SM)) was not different than for SM in saline (Supplementary Fig. 5), which means the gel itself does not make the cornea inherently more permeable to the drug. However, others have reported similar or lower in vitro corneal permeability of drugs formulated as gels, micelles or other types of sustained-release systems, probably because there is no appropriate method for modelling the relative clearance in vitro. In other words, in vitro permeability often does not reflect the improvement in ocular drug delivery in vivo, especially when comparing an immediate release formulation (for example, aqueous solution) to a sustained-release formulation (for example, 12% hypo or 18% iso). Indeed, SM release from F127 gel (18% hypo(SM)) in vitro was prolonged compared with the viscous liquid (12% iso(SM)), reflecting the sustained release of drug from gels compared with viscous liquids (Supplementary Fig. 6). While the formation of a gel did not significantly change SM permeability through corneal tissue in vitro, gels provide prolonged residence time and drug release on the ocular surface, which probably contributed to the increased intraocular drug absorption observed with the 12% hypo formulation in vivo.

Enhanced delivery to the back of the eye in rabbits and pigs. We next sought to test whether the hypotonic gelling formulation can deliver therapeutically relevant drug concentrations to the posterior segment in larger animals with eyes that are more similar in size to human eyes. We measured levels of both sunitinib and its major active metabolite, N-desethyl sunitinib, which has been shown to have similar potency to sunitinib. The combined concentrations of sunitinib and N-desethyl sunitinib in various ocular tissues are
Fig. 3 | Hypotonic gelling formulation (12% hypo) solubilizes and provides improved intraocular delivery of two insoluble drugs, BRZ (1% w/v) and CsA (0.05% w/v), compared with the conventional gelling formulation (18% iso) or the commercially available formulations (Azopt and Restasis). a–c, BRZ concentrations in the rat cornea (a), conjunctiva (b) and aqueous humour (c) measured at 1, 4 and 8 h after the last dose (twice-daily dosing for 5 d, n = 5–10 independent animals). d, ΔIOP in normotensive rabbits measured for 8 h after a single dose, comparing 12% hypo(BRZ) with 18% iso(BRZ) or Azopt (n = 5–6 independent animals). Data are mean ± s.e.m. Dotted lines mark the starting average IOP (ΔIOP = 0). e,f, CsA concentrations in the rat cornea (e) and conjunctiva (f) measured at 1, 4, and 8 h after the last dose (twice-daily dosing for 5 d, n = 6–9 independent animals). Statistical analyses conducted by one-way ANOVA with multiple comparisons.

shown after daily dosing with 12% hypo(SM) for 14 d in rabbits (Fig. 5a) and 5 d in pigs (Fig. 5b). Concentrations in homogenates of the retina, choroid and retinal pigment epithelium (Ch/RPE) were well above the inhibition constant (K_i) for SM inhibition of vascular endothelial growth factor receptor (VEGFR) or platelet-derived growth factor receptor (PDGFR) in rabbits (Fig. 5a) and pigs (Fig. 5b). However, sunitinib is known to bind to ocular melanin, which reduces the amount of available drug. Thus, we then tested whether the potentially therapeutically relevant level of drug in the posterior tissues may provide suppression of neovascularization in pigs. Once-daily dosing with 12% hypo(SM) resulted in significant suppression of laser-induced CNV compared with dosing with the same concentration of SM formulated in saline (SM in saline) or dosing with 12% hypo vehicle (vehicle) alone (Fig. 5c).

Compatibility with the ocular surface and visual acuity. We next evaluated the effect of chronic topical administration of the hypotonic gelling formulation vehicle in rabbits. Rabbits were dosed two times per day for five weeks, and the 12% hypo formulation was compared with the 12% iso vehicle, commercially available balanced salt solution (BSS) and no treatment (untreated). For all the treatment groups, no significant differences in corneal staining and blinking frequency were observed compared with untreated rabbits (Fig. 6a,b). Similarly, no significant histological differences were observed in corneas obtained at the end of the five weeks of treatment across all groups (Fig. 6c).

Since the hypotonic gelling formulation has a prolonged residence time on the ocular surface, we sought to evaluate material properties relevant to the potential effects on vision and comfort. We found no significant differences in the visual light absorbance of F127 solution and gel compared with water at 37 °C (Supplementary Fig. 7a). Similarly, the refractive index of F127 solution and gel was between that of human tear fluid and common soft contact lens materials (Supplementary Fig. 7b). Further, F127 gel showed significant shear-thinning (lubricating) behaviour, which is necessary for ocular comfort when blinking. The viscosity of F127 gel at high shear rates was similar to that of commercially available lubricating eye gel (GenTeal Severe Dry Eye Relief Lubricant Eye Gel) (Supplementary Fig. 8a,b).

Discussion

More than 90% of ophthalmic products are eye drops. However, despite the noninvasive administration and relative simplicity of eye drops, there is substantial room for improvement of these products.
Low intraocular drug absorption, rapid clearance, intensive dosing regimens and ocular surface toxicity resulting from repeated administration contribute to poor patient adherence and even discontinuation of medication. These limitations can be especially problematic for chronic diseases, such as dry eye and glaucoma. Numerous glaucoma medications, including BT and BRZ, require dosing up to three times per day, increasing the risk of side effects. Further, adherence rates for eye drops that require dosing more than two times a day are as low as 39%. Eye drops can only effectively treat disease when used properly; for glaucoma, poor adherence results in uncontrolled IOP and higher rates of vision loss.

Here we describe an approach to use thermosensitive gelling polymers as eye drops that provide increased and sustained intraocular drug exposure. The eye-drop vehicle was compatible with a variety of ophthalmic drugs with different physicochemical properties, suggesting a potential for broad versatility. Key features for tailoring the formulation for maximum compatibility with the unique physiological properties of the ocular surface were hypotonicity and polymer concentration below the CGC. Upon application, the eye-drop spread uniformly over the surface of the eye, while water absorption concentrated the polymer at the ocular surface to form a thin, clear gel layer that was resistant to clearance from blinking. The conventional approach for increasing the residence time of drugs on the ocular surface is the use of excipients, including cellulose derivatives, hyaluronan, polycarboxophil and carbopol that provide enhanced viscosity and/or mucoadhesion. One of the few examples of a commercially available in situ gelling eye drop, Timoptic XE, contains gelnam gum, which undergoes a gel transition when exposed to cations in the tear film. Timoptic XE was shown to provide similar efficacy in lowering IOP with once-daily administration compared with twice-daily administration of timolol solution. However, significantly more patients reported blurred vision and tearing with the gel-forming eye drop compared with the solution eye drop. By contrast, in this study we describe a thin, uniform, clear gel with similar absorbance and refractive index as water or contact lens materials that is not expected to negatively impact vision. It is possible that formulating other viscosity-enhancing and gel-forming materials hypotonically with lower polymer concentration may further increase residence time and reduce vision distortion in a similar manner.

Drugs are typically delivered to the posterior segment via injections, such as intravitreal injection of anti-VEGF biologics. Although injections are performed routinely, there is a small but significant risk for serious adverse events that endanger the patient’s vision. Further, treatment may require returning to the clinic every four to eight weeks to achieve the best visual outcome. Eye drops for treating retinal diseases could potentially be safer, simpler and cheaper. However, efforts toward clinical development have not been successful. One potential factor is that drugs that perform well in preclinical rodent studies may not be as effective in humans due to the large size difference between human and rodent eyes. Thus, larger animals such as rabbits, pigs or non-human primates are needed to assess whether similar drug biodistribution can be achieved in larger eyes. For example, topical administration of pazopanib three times per day successfully suppressed retinal vascular leakage in mice, but topical dosing four times per day did not show efficacy in a rabbit model.

Efficacy in reducing the prevalence of grade IV lesions in a primate laser CNV model was demonstrated with topical administration of regorafenib suspension, leading to a phase 2 clinical trial that failed due to inadequate efficacy. It was subsequently reported that levels of regorafenib and pazopanib in the posterior tissues were quite low after topical dosing in monkeys, and that the levels in both monkeys and rabbits were much lower than in rats. They went on to show that processing the regorafenib suspension such that the particulates were nano-sized rather than micron-sized could improve delivery to the posterior segment in rabbits. Another formulation approach that has shown promise for insoluble drugs is complexation with cyclodextrins, which provided delivery of dexamethasone to the retina in rabbits that translated to efficacy similar to subtenon triamcinolone in a clinical trial for treating diabetic macular oedema.

In this study, we unexpectedly observed enhanced drug delivery to the posterior segment of the eye. We achieved robust ocular biodistribution of water-soluble SM with daily topical delivery in both rabbits and pigs, which have similar corneal and scleral thickness and vitreous volume to the human eye. We speculate that holding the drug in contact with the ocular surface for longer periods of time can result in increased drug delivery to the posterior segment, although the efficiency of delivery to the posterior tissues is likely to be dependent on individual drug properties, such as solubility and log P. The drug distribution in rabbits and pigs shown in Fig. 5 showed higher levels in the cornea and conjunctiva and Ch/RPE, with lower levels in the vitreous, suggesting that...
enhanced delivery may occur through transscleral diffusion and/or uveoscleral outflow. We demonstrated that daily administration of 12% hypo(SM) effectively prevented laser-induced CNV in both rodents and pigs. A daily eye drop that could potentially delay the time between visits to the clinic, or even replace intravitreal injections, may improve patient quality of life. We also anticipate that absorption-induced gelation can be applied more broadly to other absorptive mucosal tissues, such as the nasal51, vaginal52 and gastrointestinal epithelia53,54.

Methods

Materials. Lutrol F127 (F127), tetrahydrofuran, carbonyldimidazole, BRZ, Span 20, dimethylsulfoxide and Alexa Fluor 488-labelled Griffonia simplicifolia (GSA) isoelectin were purchased from Vector Laboratories. Fluorescent carbosyyl-modified yellow-green polystyrene nanoparticles (200 nm), and lissamine green were purchased from CARBOsynth. ACF was purchased from TCI chemical. SM was purchased from Carbosynth. Alphagan and Restasis was purchased from Allergan. Azopt was purchased from Fisher Scientific. Optimal cutting temperature embedding medium was purchased from Toronto Research Chemicals. Amine-modified polyethylene glycol (PEG) (5kDa) was purchased from Creative PEGworks. Regenerated cellulose dialysis membrane with 1 kDa and 10kDaMW cut-off was purchased from Spectrum Labs. Phosphate buffered saline and 4% paraformaldehyde were purchased from Thermo Fisher Scientific. Optimal cutting temperature embedding medium was purchased from Fischer Scientific. Alphagan and Restasis was purchased from Allergan. Azopt was purchased from Allergan. A Franz cell was purchased from the Permeagear. Excised rabbit eyes were purchased from Pel-freez Biological.

Eye-drop formulations. To make hypotonic F127 solution ranging from 12–20% (w/w), F127 powder was added to water using appropriate weight ratios and stored at 4°C until fully dissolved. For the hypotonic BT formulations, BT powder was weighed and dissolved into hypotonically prepared 12–18% (w/w) F127 to the final BT concentration of 0.15% (w/v). To make hypotonic BRZ formulations, 4 mg BRZ, 0.02 mg Span 20 and 0.0068 mg F127 were dissolved in 1.1 ml acetone. After completely dissolving the materials, 0.4 ml water was added. Acetone was evaporated using a rotary evaporator and an additional amount of F127 powder was added to make 10–18% (w/w) of F127 at a BRZ concentration of 1% (w/v). To prepare hypotonic CsA formulations, 20 mg of CsA and 200 mg of F127 powder were added into 4 ml of dimethylsulfoxide. The obtained solution was then dialysed in water with regenerated cellulose dialysis membrane with 1 kDa molecular weight cut-off overnight at room temperature and stirred in deionized water for 12h. Additional water and F127 powder was added to achieve a final CsA concentration of 0.05% (w/v) in F127 solutions with final concentration ranging from 12–18% (w/w). To prepare 12% hypo(ACF), ACF was dissolved (0.5% w/v) in 12% (w/w) F127 dissolved in deionized water. To prepare ACF in water, ACF was dissolved in deionized water. To prepare 12% hypo(SM) solution, SM was first dissolved in water at 1% (w/v) using an ultrasonic probe sonicator (Sonics). The prepared solution was then mixed with 20% (w/w) F127 in deionized water at 46 ratio (w/w) to make a final solution with 0.4% (w/v) SM in 12% (w/w) F127. To prepare SM in saline solution, SM was dissolved in sterile normal saline at a concentration of 0.4% (w/v) using an ultrasonic probe sonicator. For the BT, BRZ and CsA formulations, pH was tested using pH strips (VWR Chemicals) and adjusted, if necessary, using 10 M NaOH (<1% total volume) to reach pH 7 ± 0.5. To prepare isotonic F127 solutions, tonicitics of the hypotonically prepared samples were adjusted by adding concentrated (up to 100-fold) BSS. Concentrated BSS was prepared by lyophilizing the commercial formulation and re-dissolving it in water. The pH values of SM and ACF formulations were measured using a pH probe (Thermo Fisher Scientific). The osmolality of the formulations (Supplementary Table 2) was measured using a vapour pressure osmometer (Wescor). For the measurements that were below the linear calibration range (100–1,000 mOsm kg⁻¹), samples were mixed with an equal volume of BSS (300 ± 10 mOsm kg⁻¹) before the measurement. The osmolality of the measured mixture was used to calculate the sample osmolality by assuming a linear contribution from each component in the mixture.
A specific sex was not specified when ordering any species, and roughly equivalent numbers of both male and female animals were used. C57BL/6 mice, Sprague Dawley rats, New Zealand White rabbits and Dutch belted rabbits were obtained from Charles River. Yorkshire pigs were obtained from Archer Farm. Animals were anaesthetized before euthanasia.

Optical coherence tomography imaging. Corneal clearance of various formulations after blinking was determined by real-time in vivo imaging using spectral domain OCT. Sprague Dawley rats were anaesthetized and placed in a holding cylinder for stable image acquisition. Each image acquisition was processed with Envisu XHR 4110 ophthalmic imaging system (Leica Microsystems) with a 10 mm telecentric lens. All images were obtained using a rectangular scanning mode (3 mm × 3 mm, 1,000 A-scans × 100 B-scans and 1.4 × 0.78 um pixel size). After topical administration of a single eye drop (5 μL) of various formulations (12% hypo, 12% iso, 18% iso), all images were obtained within 1 min and also immediately after each manual blinking of the eyelids twice.

In vivo multiple-particle tracking. For the in vivo MPT, non-adhesive nanoparticles (0.0004% w/v) were added to 12% hypo, 12% iso and 18% iso formulations. Sprague Dawley rats were anaesthetized before administration of an eye drop (5 μL). After 1 min, rats were euthanized by cervical dislocation and the eyes were immediately enucleated, taking care not to perturb the ocular surface. All the following procedures were done in a humidified environmental chamber at 37°C. Surgical tools were preheated before starting the experiment. Excised eyes were placed in a custom-made microwell on a glass cover slide with the corneal side facing down towards the optical side of the inverted microscope. A custom-made cap was placed above the eye and sealed with superglue to prevent evaporation. Imaging was started within 2 min after euthanasia. Particles on the ocular surface were imaged and particle motion was characterized as described above.

Pharmacokinetics studies. Sprague Dawley rats 6–8 weeks of age were anaesthetized using isoflurane. Eye drops (5 μL) in various formulations were delivered unilaterally, and the rats were placed back in an isoflurane chamber for an additional 5 min before allowing them to awaken. Eye drops were delivered twice per day for 5 d. After the last dose, rats were sacrificed at 1 h, 4 h and 8 h post-dose. While anaesthesia was used to reduce variability in dosing between rats, the lack of reflexive blinking response would also mean reduced clearance in the initial minutes after dosing. Dosing of large animals was conducted without anaesthesia to better recapitulate eye-drop dosing in humans. Dutch Belted rabbits (2–3 kg, n = 6) were given 12% hypo(SM) eye drops (50 μL), unilaterally once per day while gently restrained, for a total of 14 d. Various ocular tissues were obtained 6 h after the last dose. Yorkshire pigs (20–30 kg, n = 4) were gently restrained and fed dried fruit as a distraction in order to unilaterally dose 12% hypo (SM) daily (~50–100 μL approximate drop volume dosed with a commercially available eye dropper bottle) for 5 d. Various ocular tissues were obtained 1 h after the last dose. The reported K value for SM inhibition of VEGFR and PDGFR (~8–9 nM) was converted from molar concentration to mass concentration (~3.5 mg mL−1) by assuming tissue density of 1 g ml−1. Measurements of drug concentrations were conducted by liquid chromatography–tandem mass spectrometry as described in the Supplementary Methods.

IOP measurements. For the IOP measurements in a normotensive rabbit model, New Zealand White rabbits (2–3 kg) were used. IOP was measured with a hand-held rebound tonometer (TonoVet) in the awake and lightly restrained rabbit. Each rabbit was acclimatized to the IOP measurement procedure for at least 5 d to obtain a stable background IOP reading. Average IOP measurements for an individual eye were taken every other day for 6 d (3 times in total) and used as a baseline value. After single topical administration (50 μL), IOP was measured every 2 h and change in IOP from the baseline (ΔIOP) was reported.

Laser-induced CNV studies. C57BL/6 mice (4–6 weeks of age) were anaesthetized and the pupils were dilated with 1% tropicamide and 2.5% phenylephrine. A cover slip was placed on top of the well and sealed with superglue to prevent evaporation and movement. The slides were then incubated in a temperature-controlled chamber enclosing the microscope for 15 min at 37°C. The slide was placed on an inverted epifluorescence microscope (Axio Observer D1; Carl Zeiss) and imaged with a ×100, 1.46 numerical aperture oil-immersion objective. Videos of nanoparticle motion were recorded over 20 s at an exposure time of 66.7 ms by an Evolve 512 EMCCD camera (Photometrics). Obtained videos were analysed using in-house code in MATLAB (MathWorks). The x and y coordinates of the centroids of hundreds of nanoparticles were identified and tracked over time (example nanoparticle trajectories are shown in Fig. 1c) to calculate individual-particle MSD. (MSD) was calculated as the geometric mean of individual-particle MSDs at a time scale of 1 s.

Animal studies. Animal welfare statement. All experimental protocols were approved by the Johns Hopkins Animal Care and Use Committee. All animals were handled and treated in accordance with the Association for Research in Vision and Ophthalmology Statement for Use of Animals in Ophthalmic and Vision Research.

Fig. 6 | Hypotonic gelling formulation (12% hypo) is indistinguishable from untreated with twice-daily dosing for 5 weeks in rabbits. a–c. Treatment with 12% hypo was compared with an isotonic formulation (12% iso), isotonic BSS and no treatment (untreated) with bilateral administration of twice-daily dosing for 5 weeks in rabbits. Ocular surface damage, as assessed by lissamine green staining and scoring (a) and counting the number of blinks in a 3 min period 3 h after the first dose on a given day (b), as well as haematoxylin and eosin staining of cornea tissues collected at 5 weeks (n = 3 independent eyes and one image was obtained per group) (c), was indistinguishable across all groups. Scale bar, 100 μm (applies to all images).

**ARTICLES**

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phenylephrine and sterilized with a drop of 1% providone. A fundus contact lens was placed on the cornea. A portable diode laser with a slit lamp delivery system was used to rupture Bruch’s membrane in 9–11 locations equidistant from the disc using a 75 µm spot size, 0.1 s duration, and 430 mW power in each eye. The pig was then given another bilateral topical dose and kept warm with blankets until recovering from anaesthesia. Topical eye drops were dosed bilaterally daily for an additional 9 d using gentle restraint and food as a distraction. The next day after the last dose (10 d after rupture of Bruch’s membrane), pigs were euthanized and the choroid was fixed, stained with Alexa Fluor 488-labelled GSA lectin and flat mounted. The area of CNV at each Bruch’s membrane rupture site was measured by a blinded observer using Image Pro Plus. Statistical analysis was performed using linear mixed model with each laser-induced CNV spot as a separate experimental value.

Safety study. Topical eye drops (12% hypo, 12% iso or BSS) were given to New Zealand white rabbits (n = 3) twice a day (8 h apart) for 5 weeks. Eye drops were given bilaterally, but each rabbit was given a different treatment in each eye (n = 3 eyes per group). At the end of each week, corneal staining and blink rates were assessed. These measures are used both preclinically and clinically to establish potential for ocular surface irritation/toxicity10. For the blink-rate measurements, rabbits were mildly restrained using a towel and placed in a small container. The blink rate was determined by counting the number of blinks in a 3 min period. All the blink-rate measurements were done 3 h after the topical administration. Following the blink-rate measurement, the corneal staining test was performed. A single eye drop of lissamine green (1% in sterile saline) was topically administered, and corneal staining was scored using a previously reported grading scheme11 by a board-certified ophthalmologist in a blinded manner. For the histological evaluation, the eyes were enucleated and placed in 4% paraformaldehyde before paraffin embedding, sectioning and haematoxylin and eosin staining by the Johns Hopkins Reference Histology Laboratory. Histological sections were evaluated by a board-certified ophthalmologist in a blinded manner for the signs of inflammation and ocular surface damage. Statistical analysis. Statistical analyses of two groups were conducted using two-tailed Student’s t-test, two-tailed Mann–Whitney test or two-way analysis of variance (ANOVA). For the comparison of multiple groups, one-way ANOVA with Dunnett’s multiple comparison test was used. Statistical analysis was done using GraphPad Prism 8. For the statistical analysis in the CNV model in pigs, we checked for the potential correlation among measurements of CNV size from the same animal were analysed using linear mixed-effects models with random intercepts at the level of eye and the level of animal. The intra-class correlation coefficient was close to zero, indicating low correlation among the measurements and therefore the linear regression model was used and each CNV measurement was treated as an independent sample. Differences were considered to be statistically significant at a level of p < 0.05.

Reporting summary. Further information on research design is available in the Nature Research Reporting Summary linked to this article.

Data availability
The main data supporting the findings of this study are available within the paper and its Supplementary information. The associated raw data are too large to be readily shared publicly but are available from the corresponding author on reasonable request.

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Author contributions

Y.C.K., M.D.S., S.H., A.S.J., P.M., D.I.Z., P.A.C., J.H. and L.M.E. designed experiments. Y.C.K., M.S., S.H., H.T.H., R.L.e.S., A.D., H.H, B.-J.K., A.X., Y.K., L.O., N.M.A., A.H., P.H., C.E. and I.P. performed experiments and/or analysed experimental data. Y.C.K., M.S., S.H., B.-J.K., N.M.A., J.H. and L.M.E. wrote sections of the manuscript. All authors read and approved the final version of the manuscript.

Competing interests

Y.C.K., A.D., L.M.E. and J.H. are inventors on US patent nos. US10892509B2, US10646434B2 and US10485757B2; Australian patent no. AU2016211696B2; Canadian patent no. CA2974715C; and on 11 pending patent applications related to this technology. J.H. and L.E. are founders and have equity in NovusBio LLC. NovusBio LLC intends to develop products using the technology described in this manuscript. The terms of this arrangement are being managed by the Johns Hopkins University in accordance with its conflict of interest policies.

Additional information

Supplementary information is available for this paper at https://doi.org/10.1038/s41551-020-00606-8.

Correspondence and requests for materials should be addressed to J.H. or L.M.E.

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### Software and code

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| Description                                                                 | Details                                                                      |
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| The optical-coherence-tomography images                                    | were taken using software (InVivoVue v2.4) provided by the manufacturer.    |
| Liquid-chromatography-tandem-mass-spectrometry data                         | were collected by using the manufacturer’s provided software (Analyst v1.6 or v1.7). |
| Multiple-particle-tracking data                                             | were collected with MetaMorph software v7.1.                                |
| Light-absorbance data                                                       | were collected via the manufacturer’s provided software (Gen5 v 1.1).        |
| Rheometre-data                                                              | were collected by using the manufacturer’s provided software (TRIOS v.4.3).  |
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**Data analysis**

Statistical testing was performed using Prism v8.

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- **Sample size**: Sample sizes were determined on the basis of prior experimental work or a pilot study. No statistical calculations were made to determine sample size.
- **Data exclusions**: All exclusion criteria were pre-established. For the in vivo studies, the criteria for exclusion were failure in administration, mistakes in tissue dissections, and health concerns unrelated to the procedure requiring euthanasia and exclusion.
- **Replication**: All experiments were carried out with at least 3 replicate samples for each experimental group.
- **Randomization**: Samples were allocated into experimental groups at random.
- **Blinding**: The study was blinded where noted in Methods. The acquisition of optical-coherence-tomography imaging and of data from liquid chromatography–tandem mass spectrometry was blinded. Corneal staining score and histological examination were done in a blinded manner.

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| 🗙️ | ChIP-seq |
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- **Laboratory animals**: A specific sex was not specified when ordering any species, so both male and female animals were used as provided. C57BL/6 mice (6–8 weeks old), Sprague Dawley rats (6–8 weeks old), New Zealand White rabbits (2–3 kg), and Dutch Belted rabbits (2–3 kg) were obtained from Charles River. Yorkshire pigs (20–30 kg) were obtained from Archer Farm.

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