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Research paper

A drug refillable device for transscleral sustained drug delivery to the retina

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ABSTRACT

Continuous drug administration with better adherence to treatment and less invasive procedures is important in treating retinal diseases such as age-related macular disease. In this study, we report a drug-refillable device consisting of a silicone reservoir and an injectable gelatin/chitosan gel (iGel). The silicone reservoir was fabricated with polydimethylsiloxane (PDMS) using a computer-aided design and manufacturing to have micropores at a releasing side for uniaxial release to the sclera. A stainless steel wire and sheet were combined in the side and bottom of the reservoir to ensure flexibility and to fit on the curvature of the eyeball and prevent irritation to the sclera through the bottom of the reservoir. The drug was injected and formulated in the reservoir by *in situ* crosslinking of gelatin/chitosan gel with the crosslinker; 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride. The *in vitro* release study using fluorescein molecules showed that the release rate from encapsulated iGel in the reservoir was slower than that from the original iGel. After reinjecting the iGel into the reservoir, the same release profile as the first injection was observed. The reservoir containing iGel was placed on the sclera of a rabbit and the distribution of 150 kDa fluorescein isothiocyanate-dextran (FD150) in the retina and choroid/retinal pigment epithelium (choroid/RPE) was studied. The cryosections showed that FD150 was observed in the choroid/RPE. Homogenates of the retina and choroid/RPE showed fluorescence during 12 weeks implantation, indicating the drug could be delivered to the retina by using the device. The drug filling was successful into the reservoir implanted on the sclera through the conjunctiva by using a needle. In conclusion, the refillable drug delivery device is a promising tool to administer drugs long-term by reinjection with less invasiveness to intraocular tissues.

1. Introduction

Retinal diseases such as age-related macular degeneration (AMD) cause impaired vision loss and blindness. AMD results from pathological alterations of retinal pigment epithelium (RPE) as well as its related tissues such as the choroid and photoreceptors [1]. The increase in vascular endothelial growth factor (VEGF) expression caused by ischemia and hypoxia in the related areas generates neovascularization, leading to retinal degeneration [2]. Intravitreal injection of anti-VEGF reagents can improve visual acuity in some patients [3]. However, the treatment requires repeated injections, usually once a month, potentially causing side-effects such as endophthalmitis [4]. Therefore, the development of an optimal drug administration system as well as the discovery of new therapeutic agents are important in AMD treatment [5].

The design of an intraocular drug delivery system is a challenging ophthalmological task. In the case of posterior segment eye diseases, topical eye drops may be less effective due to the barrier function of the corneal epithelium and tear fluid turnover [6]. Therefore, intravitreal injection is clinically used and successful for AMD treatment. However, as mentioned herein, repeated injections are needed to maintain the effective dose in the eye. To reduce the invasive procedure risks in the eye, we have developed a transscleral drug delivery device [7,8]. We reported the protective effects of the device against light-induced [9,10], laser-induced [11], and transgenic retinal degeneration in rats and rabbits [12,13]. However, the device should be replaced when the drug release was finished, and this required surgical procedures to open the conjunctiva and remove the device to then implant a new device. Therefore, it is desirable to refill a reservoir with additional drug when the infused drug has been depleted, without device removal.

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Several refillable devices for intraocular drug delivery have been reported. Lo et al. reported that a microelectromechanical system (MEMS) device made of polydimethylsiloxane (PDMS) and consisting of a reservoir, release tube, check valve, and suture tabs was used for drug delivery to the anterior chamber through an incision at the limbus [14,15]. Molokai et al. reported that a capsule inserted into the lens was refillable and capable of delivering the anti-VEGF reagent, Avastin, for 2 days [16]. Genentech is currently investigating a phase 3 study for AMD patients using a port deliver system (PDS) with ranibizumab, which is surgically implanted in the vitreous cavity and can be refilled through an external port spanning the sclera [17]. Although the devices reported previously could deliver drugs into the eye and were refillable, treatments inside the eye sometimes induce adverse side effects, such as retinal detachment and infection [18]. In spite of the advantages of episcleral implant in terms of being less invasive in the eye [19], few studies have reported using refillable episcleral devices, and there has been no evidence showing the device could be refillable and capable of long-term drug delivery to the retina *in vivo*.

In this study, we evaluated the combination of a silicone reservoir with micropores and a gelatin/chitosan gel for injectable and sustained drug release formulation, that could release drugs for several weeks and with the capability of refilling *in vivo*, for transscleral sustained drug delivery to the retina. The gelatin/chitosan gel was prepared as an injectable gel (iGel) with a refillable formulation. The devices were placed and fixed with sutures on the sclera of rabbits and *in vivo* drug distribution into the retina and choroid/RPE were evaluated.

2. Materials and methods

2.1. Mold fabrication

The shape of the reservoir with nine pores (diameter; 0.5 mm) was designed using software (With Right; PMT Co., Fukuoka, Japan), and the shape was milled on an acrylic board (100 mm × 100 mm × 5 mm) using an endmill (diameter: 0.5 mm) operated by a CAD/CAM-based microprocessing machine (MicroMC2; PMT Co.). Polydimethylsiloxane (PDMS; Silpot 184 W/C; Dow Corning Toray, Tokyo, Japan) was degassed under vacuum, cast into the acrylic mold and cured at 80 °C for 3 h. Polymerized PDMS was carefully peeled off the acrylic master, oxidized in oxygen plasma (1 Torr, 100 W, 5 min), and silanized with 1H, 1H, 2H, 2H-perfluoro octyltrichlorosilane (FOTS; Wako, Tokyo, Japan) vapor for 1 h to aid in the subsequent release of PDMS. PDMS prepolymer was cast into the PDMS replicas, cured at 80 °C for 3 h, oxidized, and silanized to make a PDMS master mold.

2.2. Device fabrication

The drug-refillable device consisted of a PDMS reservoir with nine pores and a stainless steel sheet and PDMS cover with stainless steel wire (Fig. 1). The PDMS reservoir was molded by heat-curing at 80 °C for 3 h. A stainless steel sheet (0.03 mm thick, 2.76 mm wide, 5.36 mm length; SUS316; Nilaco Co., Tokyo, Japan) was attached to the bottom of the reservoir inside with PDMS prepolymer at 80 °C for 3 h. A PDMS sheet (0.38 mm thick) combined with stainless steel wire (φ 0.25 mm; SUS316; Asone, Osaka, Japan) was attached to the reservoir with PDMS prepolymer at 80 °C for 3 h. The dimensions of the reservoir are shown in Fig. 1 and the maximum loading volume was 150 μL. The device was sterilized by autoclaving.

2.3. In situ cross-linking of the gelatin/chitosan gel

Gelatin from porcine skin (Sigma-Aldrich, Tokyo, Japan) was dissolved at a concentration of 5.1 wt% in water at 40 °C. Chitosan (deacetylation rate was 98%; Dainichiseika Color & Chemicals Mfg. Co. Ltd., Tokyo, Japan) was dissolved at a concentration of 2.55 wt% in water at 40 °C. The 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDC; Wako) was dissolved at a concentration of 50 wt% at room temperature. Fluorescein (FL; Wako) or fluorescein isothiocyanate-dextran (average molecular weight 150,000; FD150; Sigma-Aldrich) was mixed at a concentration of 50 mg/mL in the gelatin/chitosan mixture. The mixtures were filtered with a membrane filter (Millex-HV, 0.45-μm pore size; Millipore, Tokyo, Japan) for sterilization. The 50 wt% EDC was added to the gelatin/chitosan mixture at a final concentration of 1.0 wt% EDC. Immediately after mixing, the mixture was used to assess mechanical strength, *in vitro* degradability, *in vitro* release, or for *in vivo* animal studies. The final concentrations of gelatin, chitosan, and EDC in the iGels are listed in Table 1. A 25G needle (Terumo, Tokyo, Japan) was used to fill the device with the injectable gel (iGel).

2.4. Mechanical strength of iGel

The mechanical strength of the iGel was measured by using a combination of a vertical motorized test stand (EMX-1000N, Imada, Aichi, Japan) and a digital force gauge (ZTS-5N, Imada). The iGels (660 μL) were prepared in a 48-well plate (Iwaki, Tokyo, Japan) with a height of 8 mm, using the procedure described herein. The compressive stress was measured using a circular probe (diameter, 3 mm) moved into the gel at a speed of 1 mm/min [20].

2.5. In vitro degradability of iGels

The iGels containing FD150 (1 mL) were prepared in a 5 mL conical tube, then 4 mL of phosphate-buffered saline (PBS) was added to the gels and incubated at 37 °C overnight. After discarding the PBS, the gels were dried for 1 h and weighed. The gels were incubated in 4 mL of 1 mg/mL lysozyme (Wako) in PBS at 37 °C. After discarding the lysozyme solution, the gels were dried for 1 h and weighed. The *in vitro* degradability was evaluated by the weight reduction after lysozyme incubation.

2.6. In vitro release study

The iGels containing FD150 (1 mL) were prepared in a 5 mL conical tube and incubated in 4 mL of PBS at 37 °C. To estimate the amount of FD150 that had diffused out of the gels, the FD150 content in the PBS solution was measured using a fluorescent plate reader (Infinite F200PRO; Tecan Systems, San Jose, CA, USA). The PBS was replaced with new PBS during the course of the release study to ensure that the concentration of fluorescent molecules or drugs was below 20% of its saturation value at all times. For the release assessment of the drug-refillable device, the PDMS capsules loaded with iGel (G3/C1, listed in Table 1) including FL and FD150 were immersed in 10 mL of PBS in a 25 mL conical tube at 37 °C, and fluorescent release was estimated by using the aforementioned method. After the release was finished, the device was washed with PBS and new iGel (G3/C1) including FL was reinjected into the device using a 25G needle. The *in vitro* release study after reinjection was performed by the same method as described for the first injection. This refilling process was repeated three times. The results were expressed as the amount released, as determined using a standard curve.

2.7. Animal experiments

Male Japanese white rabbits (Kumagai Shigeyasu Co., Sendai, Japan) weighing 2 kg each were used in this study. All animals were handled in accordance with the Association for Research in Vision and Ophthalmology Statement for the Use of Animals in Ophthalmic and Vision Research after receiving approval from the Institutional Animal Care and Use Committee of the Tohoku University Environmental & Safety Committee.

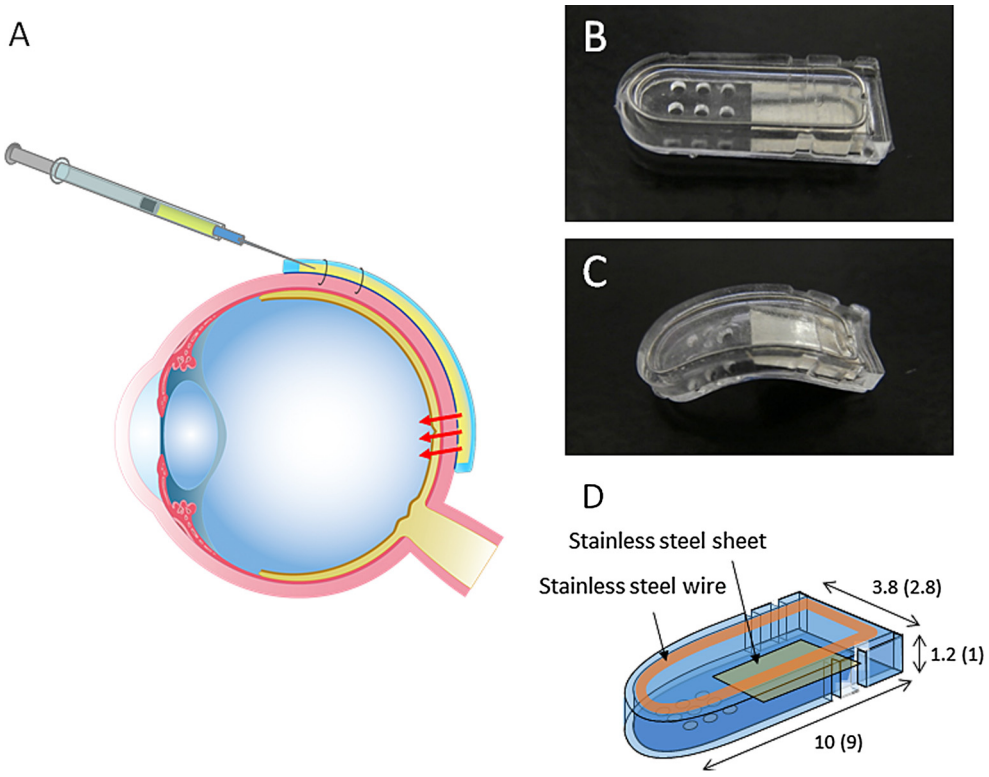

Fig. 1. (A) Schematic image of transscleral refillable drug delivery device placed on the sclera. Small red arrows indicate release of drug from the device. (B) Photograph of the refillable device showing its configuration. (C) Photograph of the refillable device after bending to fit the curvature of the rabbit eyeball. (D) Schematic image of the device. The numbers indicated external dimensions (mm) of the reservoir. The numbers in parentheses are internal dimensions (mm).

Table 1
Final concentrations of gelatin, chitosan, and EDC in the iGels.

Name	Volume in the mixture (mL)			Final concentration (%)		
	5.1% Gelatin	2.55% Chitosan	50% EDC	Gelatin	Chitosan	EDC
G0/C2.5	0	5	0.102	0	2.5	1
G1/C2	1	4	0.102	1	2	1
G3/C1	3	2	0.102	3	1	1
G4.5/C0.25	4.5	0.5	0.102	4.5	0.25	1
G5/C0	5	0	0.102	5	0	1

2.8. Implantation

The rabbits were anesthetized with ketamine hydrochloride (90 mg/kg) and xylazine hydrochloride (10 mg/kg). Their ocular surfaces were anesthetized with a topical instillation of 0.4% oxybuprocaine hydrochloride. A 4 × 4 mm paralimbal conjunctival incision was made at the upper temporal limbus. The device was inserted between the conjunctiva and sclera and the front head of the device was placed just beside the optic nerve head. The peripheral region was sutured onto the sclera with 7–0 silk to tightly fix the device onto the sclera. The conjunctival incision was closed with 9–0 silk and antibiotic ophthalmic ointment was inserted into the conjunctiva. The devices were placed onto the left eyes. The right eyes remained untreated.

2.9. Measurement of FD150 distribution in the retina and choroid/RPE

For *in vivo* evaluation, the devices loaded with iGel (G3/C1) including FD150 were used. To evaluate the intraocular fluorescence distribution after device treatment, the concentration of FD150 in the tissue homogenates was measured. One, 4, 8, and 12 weeks after device implantation, the eyes were enucleated. Photographs and fluorescent images of the eyeballs were captured using a handheld retinal camera for fluorescein angiography (Genesis-D; Kowa, Aichi, Japan) to document the fluorescence distributions around the devices. Then, the retina and choroid/RPE tissues were carefully separated and lysed in 0.1 M NaOH, and 1% Triton X100 in PBS for 15 min on ice. The lysate was homogenized using a homogenizer (Bioruptor II; CosmoBio, Tokyo, Japan). The lysate was neutralized with 0.9 M HCl, 1% Triton X100 in PBS, and centrifuged at 15,000 rpm for 10 min at room temperature. The fluorescent intensity of supernatants was measured using a fluorescent plate reader (Infinite F200PRO; Tecan). The results were determined using a standard curve of FD150. For histological evaluations, the eyeballs were enucleated and frozen in liquid nitrogen. After mounting the cryostat sections using Vectashield (Vector Labs, Burlingame, CA, USA), the distribution of fluorescein was observed by fluorescent microscopy (BZ9000; Keyence, Osaka, Japan).

2.10. In vivo injection of iGel into the device implanted on the sclera

The empty device was implanted onto the sclera of the rabbits. Three weeks after implantation, ocular surfaces were anesthetized with a topical instillation of 0.4% oxybuprocaine hydrochloride and the implanted device was held by a tweezers. The iGel (G3/C1) including FD150 was injected into the device using a 25G needle. The needle insertion was carefully conducted and injection was performed when the edge of the needle touched the stainless sheet in the bottom of the device. After injection, antibiotic ophthalmic ointment was applied onto the conjunctiva.

3. Results

3.1. Device fabrication

The device consisted of two components, and the bottom and top layers were fabricated separately. A bottom layer included nine pores and a stainless steel sheet and the top layer included a stainless steel wire (Fig. 1). A stainless steel sheet played the roll of a barrier when injecting the drug *in vivo*. This sheet further acted as an indicator for ensuring the needle was inserted into the reservoir, which was under the conjunctiva. A stainless steel wire provided flexibility to fit any

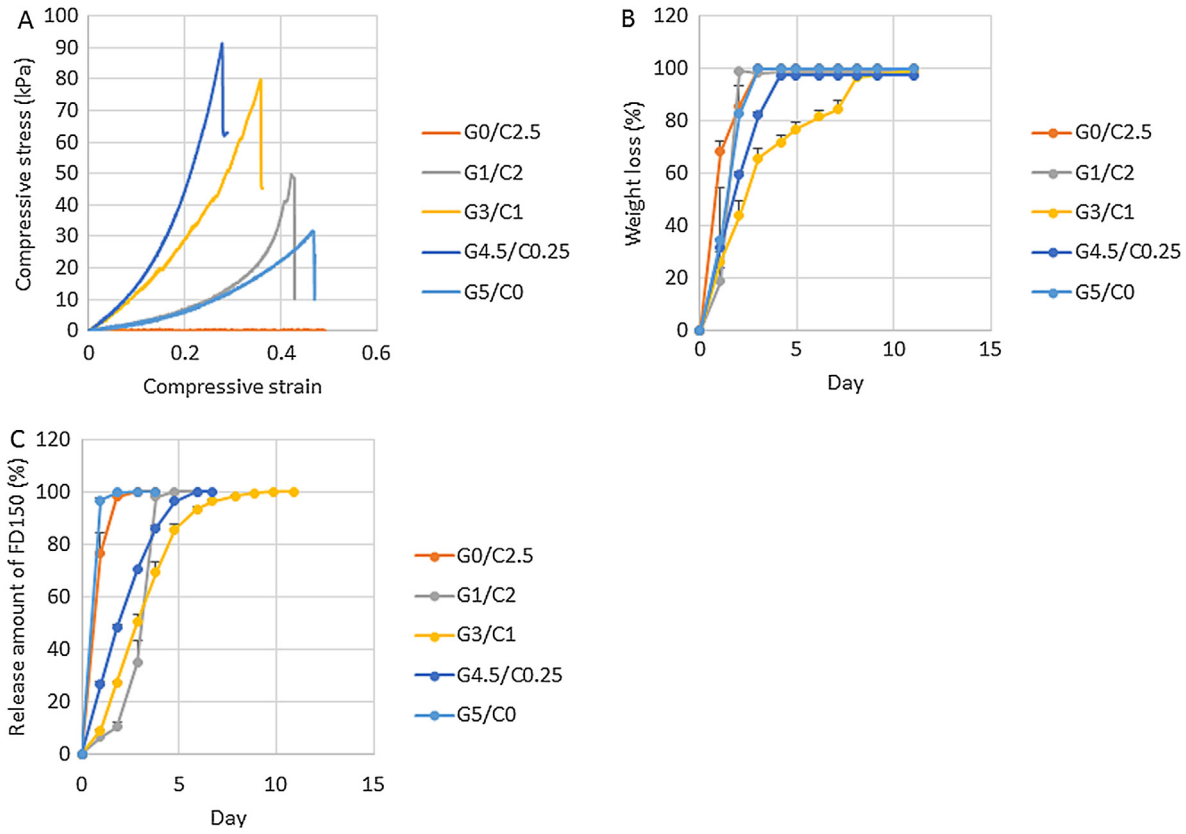

Fig. 2. Mechanical strength (A), *in vitro* degradability in lysozyme solution (B), and *in vitro* release profile of FD150 (C) from iGels. The final concentration of gelatin and chitosan in iGels was 0%/2.5% (G0/C2.5), 1%/2% (G1/C2), 3%/1% (G3/C1), 4.5%/0.25% (G4.5/C0.25), and 5%/0% (G5/C0). Values are the mean ± standard deviation; n = 4. G = gelatin; C = chitosan. FD150 = fluorescein isothiocyanate-dextran average molecular weight 150,000.

curvature of the eyeball. The device was made of rubber-like silicone enabling needle insertion into the device at any position. The reservoir had indentations at the side to enable fixing it onto the sclera by a suture.

3.2. Mechanical strength, in vitro degradability, and in vitro FD150 release of iGel

The mechanical strength of the iGels is described in Fig. 2A. Chitosan-only iGels (G0/C2.5; G, gelatin; C, chitosan; numbers mean 0 gelatin:2.5 chitosan, in %) were brittle. The strength of the iGels increased with an increase in gelatin concentration and reached a maximum at a ratio of G4.5/C0.25 iGel. Gelatin only iGels (G5/C0) showed less strength than the G4.5/C0.25 iGels.

Fig. 2B shows the *in vitro* weight loss of iGels after lysozyme digestion. A faster degradation rate was observed for the chitosan only (G0/C2.5) and gelatin only (G5/C0) iGels. The combination of gelatin and chitosan showed a slower degradation rate and the G3/C1 iGels showed the slowest degradation rate.

Fig. 2C shows the *in vitro* release study results of FD150-loaded iGels. A faster release was observed for the chitosan only (G0/C2.5) and gelatin only (G5/C0) iGels. The combination of gelatin and chitosan slowed the release rate and the G3/C1 iGels showed the lowest release rate.

3.3. In vitro release study of iGel-loaded devices

Fig. 3 shows the *in vitro* release profiles of FL and FD150 from iGels and iGel-loaded devices. The iGel showed a burst-like release and the duration of release was less than 5 days. However, the iGel-loaded device showed a longer release time than the iGel only, and the duration of release was more than 24 days and 14 days for FL and FD150, respectively, which was about five times or two times longer, respectively, than that of the iGel alone. The release rate of the iGel-refilled devices showed almost the same profile as that of the first and second refills (Fig. 3A).

3.4. In vivo release study of iGel-loaded devices

Fig. 4 shows the photographs and fluorescent images of rabbit eyeballs before and after device implantation. All devices were observed at the implantation site and were attached tightly onto the sclera. There were no abnormal findings on the sclera after removing the devices. Fluorescent images of the eyeballs showed intense fluorescence from released FD150 on the sclera and the FD150 could be observed during 16 weeks implantation with a decrease of fluorescent intensity with time.

Fig. 5 shows cryo-sectioned images of the eyeballs after device implantation. The released FD150 fluorescence was observed locally around the implantation site. There was no fluorescence observed at the opposite site across the optic nerve. Magnified images around the retina and choroid showed that fluorescent FD150 was observed in the choroid and RPE. After 16-week implantation, there was no fluorescence around the choroid and retina.

Fig. 6 shows the quantitation of FD150 in the retina and choroid/RPE as determined by measuring fluorescence intensity in the tissue homogenates. The FD150 concentrations reached a maximum at 1 week, then gradually decreased during the next 12 weeks. The FD150 in the retina was 301.3, 35.1, 18.0, and 38.9 ng/g at 1, 4, 8, and 12 weeks, respectively, after implantation (Fig. 6A). The FD150 in the choroid/RPE was 3,750, 1,748, 722, and 235 ng/g at 1, 4, 8, and 12 weeks, respectively, after implantation (Fig. 6B).

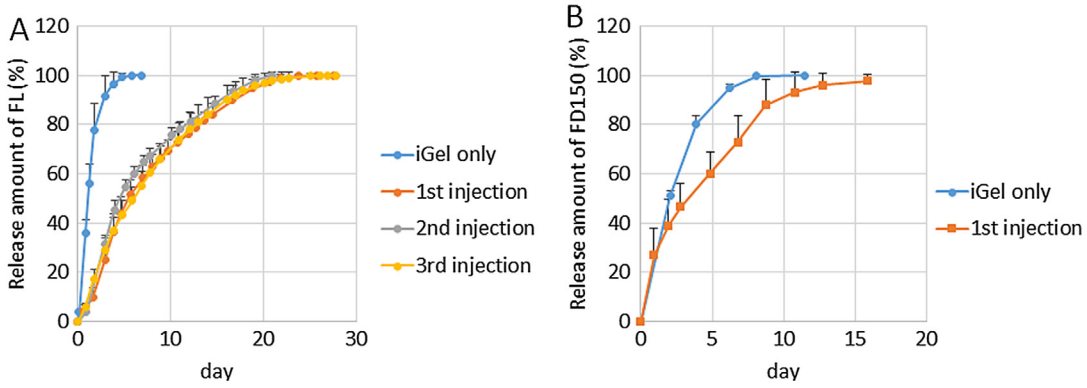

Fig. 3. *In vitro* release profile of fluorescein (FL) (A) and FD150 (B) from an iGel (G3/C1)-loaded device. After FL release, iGel (G3/C1) including FL was reinjected into the device. Data points are the mean ± standard deviation; n = 4. G = gelatin; C = chitosan. FD150 = fluorescein isothiocyanate-dextran average molecular weight 150,000.

3.5. *In vivo* injection of iGel into the device implanted under the conjunctiva

Three weeks after implantation of an empty device, the FD150-loaded iGel (G3/C1) was injected into the device. The iGel injection was conducted 3 weeks after implantation when the inflammation due to implantation surgery had subsided. We could visually identify the device position under the conjunctiva (Fig. 7B). Holding the device with tweezers, the iGel was injected with a syringe and 25G needle into the device implanted on the sclera (Fig. 7C, Movie S1). There was no leakage of iGel from the device because the iGel immediately formed a crosslinked gel in the device (Fig. 7D). Fig. 7E shows that the iGel was successfully loaded into the device, and fluorescence photography images of the removed device showed the device filled with iGel (Fig. 7F).

4. Discussion

We have developed an EDC-crosslinked gelatin/chitosan gel for injectable and sustained drug release formulation. EDC, a water soluble carbodiimide, forms covalent crosslinks between amino- and carboxyl-groups by the formation of amide bonds. EDC is a non-cytotoxic reagent and we have reported that EDC-crosslinked collagen materials are biocompatible *in vitro* and *in vivo* [21,22]. EDC does not crosslink to chitosan directly because chitosan does not have a free carboxyl group; chitosan-only iGels (G0/C2.5) have low mechanical strength. However, the carboxyl groups in the gelatin could crosslink to the amino groups in the chitosan, providing higher mechanical strength, slow degradability, and sustained release ability in iGels. We found that the G3/C1 iGels had the best properties in terms of these physical properties. Recently, efforts have been made to develop *in situ* forming hydrogels for ophthalmic use [23]. Those used to form *in situ* gel systems can be categorized according to the environmental stimuli that triggers their conversion from sol to gel, including temperature-responsive [24], pH-dependent [25], or ion-responsive gelling systems [26]. In contrast to these gels, iGel forms irreversible covalent bonds between gelatin and chitosan polymers, which have the advantages of high mechanical strength and low biodegradability. Due to their superior physical properties, gelatin and chitosan composite biomaterials have attracted increasing interest for use in tissue engineering such as for use as scaffolds [27]. In the present study, we demonstrated the application of the *in situ* gelatin/chitosan gelling system as an ocular injectable drug carrier for refillable drug delivery systems.

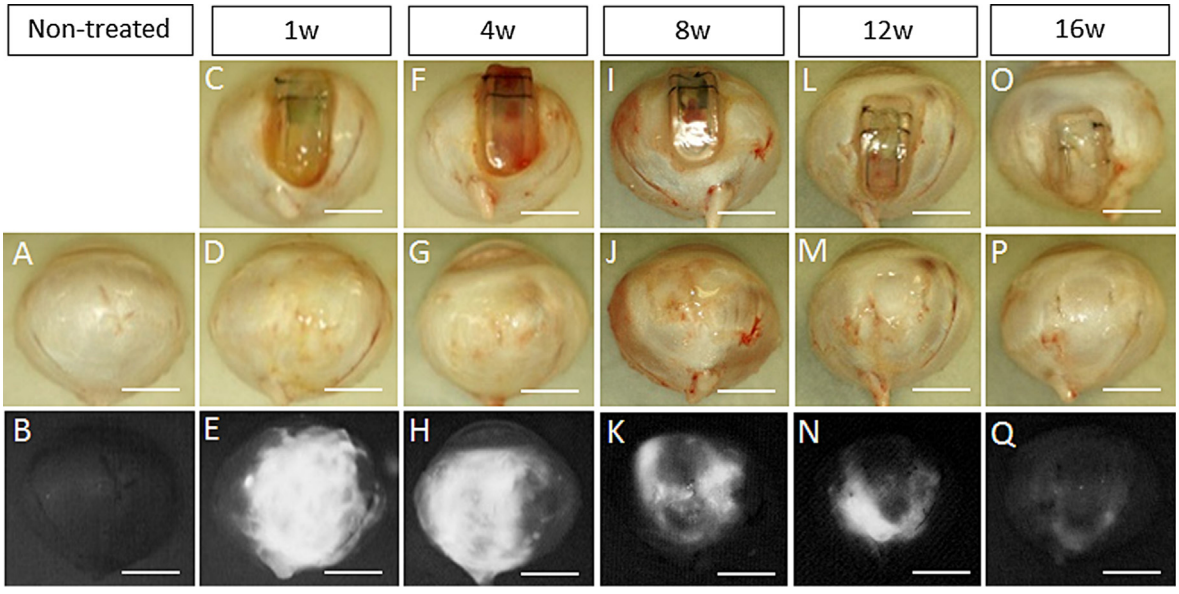

Fig. 4. Photographs and fluorescent images of enucleated eyes before (A and B) and after 1 (C, D and E), 4 (F, G and H), 8 (I, J and K), 12 (L, M and N), and 16 weeks (O, P and Q) after implantation. Devices were observed at the implantation site (C, F, I, L and O). There was no abnormal finding on the sclera after removing the devices (D, G, J, M and P). Fluorescence images of the eyeballs showed released fluorescent FD150 (white area) on the sclera (E, H, K, N and Q). Bars; 5 mm. FD150 = fluorescein isothiocyanate-dextran average molecular weight of 150,000.

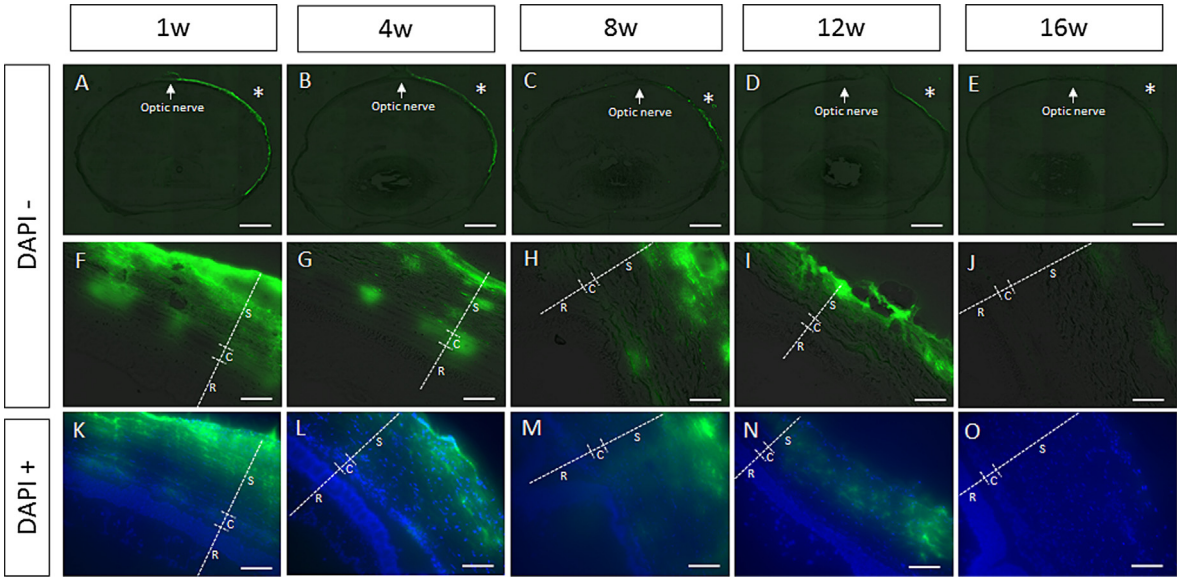

Fig. 5. The distribution of FD150 (green) in the retina, choroid, and sclera around the implantation site at 1 (A, F and K), 4 (B, G and L), 8 (C, H and M), 12 (D, I and N), and 16 weeks (E, J and O) after implantation (asterisks: device implantation site). Magnified images of the retina under device implant (F–J). Cell nuclei were stained with DAPI (K–O). Scale bar; 1 mm (A–E), 100 μm (F–O). Abbreviations; R: retina, C: choroid, S: sclera. FD150 = fluorescein isothiocyanate-dextran average molecular weight of 150,000.

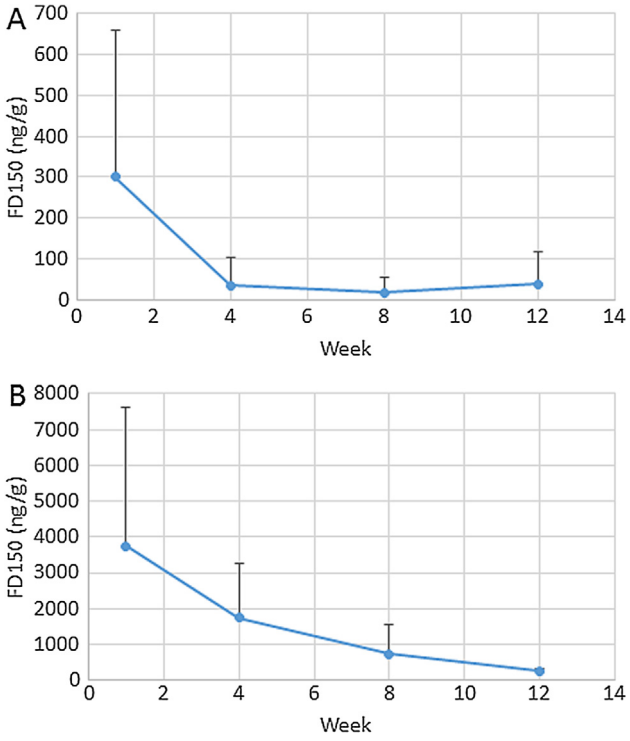

Fig. 6. Intraocular distribution of FD150 during 12 weeks in rabbits. Concentration in the retina (A) and choroid/RPE (B). Values are the mean ± standard deviation; n = 4. FD150 = fluorescein isothiocyanate-dextran average molecular weight of 150,000.

The silicone reservoir made of PDMS was molded using a computer-aided design and lithography. The micropores located on the bottom and front edge of the device provided controlled release of the drug and unidirectional release to the scleral side at the posterior eye segment. We previously reported that the release rate of basic FGF could be fine tuned by the density of the micropores in the subcutaneous implant [28]. PDMS is impermeable to most small and large molecules; therefore, the fluorescent compounds (FL and FD150) were released via pores. Degraded gelatin/chitosan residues from the iGels could be eliminated through the pores, enabling multiple reinjection of iGel into the reservoir, as shown in Fig. 3A. By using the iGel formulation, many types of drugs, ranging from small compounds (FL) to large molecules (FD150), and possibly with differing solubility (water-soluble or lipophilic) and forms (powder or liquid) could be loaded into the reservoir, which is an advantage of our device.

Although the fluorescent FD150 release showed a plateau at 14 days in the *in vitro* release study, fluorescence was detected *in vivo* even at 12 weeks after implantation. The difference in release profiles between the *in vitro* and *in vivo* experiments might have been caused by the different environment around the device. The content of water around the device *in vivo* could be lower than that *in vitro*, resulting in lower solubility of the fluorescent compounds in the surrounding tissues. In addition, *in vivo* reactions against the iGel and/or PDMS reservoir might have affected the release rate. In a separate study, the biodegradation of iGel in the rat sclera required 12 weeks (Supplementary Fig. S1), which was slower than that of the *in vitro* degradation rate. PDMS was not biodegradable during the 12 week implantation period (Supplementary Fig. S1), however, there was scar tissue formation around the PMDS, which might have provided a barrier against drug permeation. The *in vivo* drug diffusion mechanisms from the device to the retina under episcleral implantation should be further studied in the future.

Compared to the refillable episcleral devices reported previously, long-term release of more than 12 weeks is a primary advantage of our device. Few refillable drug delivery systems with controlled drug release have been reported; therefore, the previously reported devices only released drug for several days. To prolong the drug release, the iGel was developed for injectable sustained release formulation. The properties of the iGel were optimized for multiple injection use, high gel strength, slow degradability, and slow drug release. Our device could extend the release time by a combination of iGel formulation with controlled release pores in the PDMS capsule. In addition, the refilling method by using a needle inserted through the conjunctiva was less invasive to the eye than intravitreal injections. The results indicated that an every 3-month injection of iGel would be needed to deliver a constant dose of drug to the retina. Therefore, the refillable device could reduce the burden in patients from monthly repeated intravitreal injections.

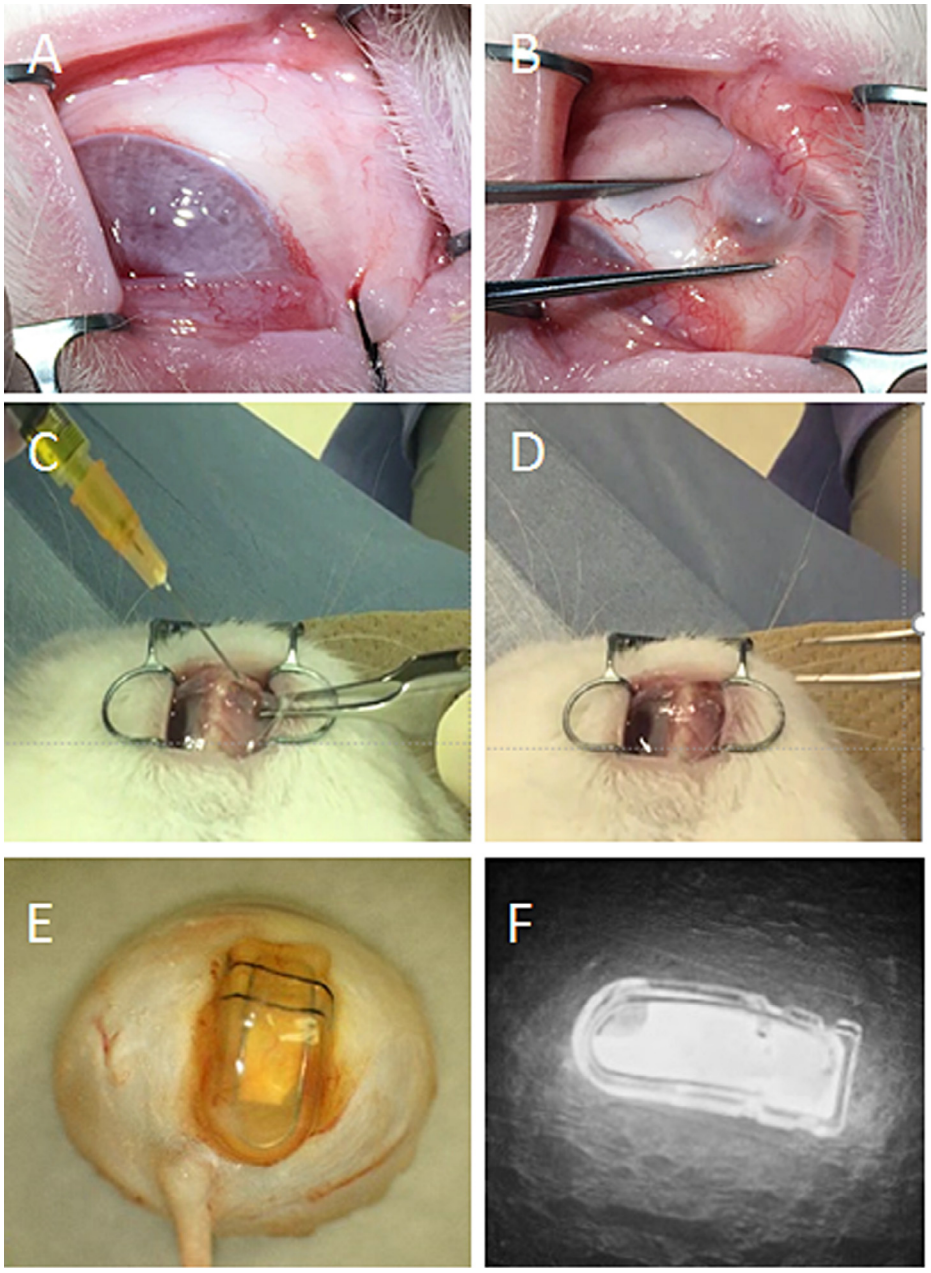

Fig. 7. Photographs of the implantation site of a rabbit eye before (A) and 3 weeks (B) after implantation. Photographs of the device during iGel (G3/C1) injection using a 25G needle (C) and after the injection (D). There was no leaking of the iGel. Arrows show the device. An enucleated eye with device immediately after injection (E) and fluorescent image of the removed device (F).

The limitation of the study was the lack of efficacy studies of the device loaded with anti-VEGF drugs in an AMD animal model. FD150 (150 kDa) was used as a model of anti-VEGF drugs, such as Avastin (149 kDa), Lucentis (48 kDa), and Aflibercept (96 kDa), and fluorescence could be measured in the retina for 12 weeks. The pharmacokinetics of actual anti-VEGF drugs in the retina using our device should be studied in the future. Laser-induced choroidal neovascularization (CNV) models are popular and we have reported laser-induced CNV experiments in mouse, rats, and monkeys [11,29,30]. We plan to evaluate the efficacy of the device loaded with anti-VEGF drugs on the reduction of CNV in monkeys in the future. In addition, the long-term efficacy and safety after repeated refilling should be evaluated in the future. It is possible that fibrous tissues could grow around the device, making it difficult to access the device for repeated injections. Our device has a stainless steel sheet to identify the bottom of the device when injecting to prevent irritation or damage to the sclera, thus providing an easier and safer repeated injection process for patients. However, long-term studies are needed to determine how many repeated injections would be tolerated for effective intraocular drug delivery.

5. Conclusions

We demonstrated that the drug-refillable device was refillable *in vitro* and *in vivo* and delivered the drug to the retina over 12 weeks. Intravitreal injections and intravitreal/transscleral implants have been effective and safe for intraocular treatment; however, they require repeated injections and replacement with new implants to maintain their efficacy. Furthermore, surgery to replace the implant is more invasive to ocular tissues. However, our refillable device has no need of replacement for up to 12 weeks, and could maintain a sustained release by repeated injections of iGel into the device in a less invasive manner

to ocular tissues. Therefore, the system could be a promising device for long-term treatment of posterior segment eye diseases.

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Competing financial interests

The authors declare no conflict of interest.

Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ejpb.2019.01.024.

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