Development and Efficacy of a Drug-Releasing Soft Contact Lens

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Purpose. The purpose of this study was to investigate the uptake and the release of antibiotics from a newly synthesized drug delivery hydrogel soft contact lens (SCL) using an ion ligand mechanism.

Methods. The antibiotics used were Gatifloxacin (GFLX) and Moxifloxacin (MFLX). The uptake amount and the sustained-release kinetics of antibiotics were investigated in vitro, and were also compared with newly synthesized SCLs, etafilcon A and polymacon. The antibiotic concentrations in the cornea, aqueous humor, and crystalline lens, and the effect against bacterial proliferation were investigated in vivo using rabbit subjects. Additionally the drug release efficacy of the new SCL was compared with that of eye drop administrations.

Results. In vitro, antibiotic uptake was increased with the weight percent (wt%) of the anionic group, and the released amount of antibiotics was highest during the initial 1 hour period, which then decreased over the next 72 hours. The released antibiotics volume of the new SCLs was significantly higher throughout 72 hours than that of the other two materials, etafilcon A and polymacon ($P < 0.01$). Whereas in vivo, the concentrations found in the cornea and aqueous humor were higher than those for the eye drop groups ($P < 0.05$ or $P < 0.01$). Antibiotic release at those sites decreased over 72 hours. No bacterial populations were detectable in the group treated with the new SCL presoaked in antibiotics throughout the experimental periods.

Conclusions. The new SCLs released the antibiotics over several days, and showed improved penetration into the eye, along with prevention of bacterial proliferation.

Keywords: soft contact lens, drug delivery, antibiotics, ion ligand

Drug delivery is a difficult task in the field of ocular therapy.[1–3] Owing to the physiologic and anatomic constraints of the eye, achieving the correct therapeutic concentration of a drug at the required site of action can be quite difficult. Eye drops are the most commonly used method of drug delivery to the eye, accounting for approximately 90% of all ophthalmic medical applications.[4,5] However, they are very inefficient as the eye drops are continuously diluted and washed away by reflex tearing and dispersed by blinking. Consequently, only 1% to 7% of the actual medication in a drop is absorbed into the eye,[6] with the remainder either flushed onto the patient's cheek or drained through the nasolacrimal system, where the medication is prone to systemic absorption.[7] This has led to clinicians recommending frequent dosing, which often results in noncompliance by patients. Furthermore, the overdosing of ophthalmic solutions can lead to ocular and systemic side effects with some ophthalmic drugs.[8,9] Because of its small surface area and short contact time with topical drops, the cornea itself only absorbs a fraction of the dose of a drop that is delivered to the surface of the eye.[10]

The concept of delivering drugs specifically through a hydrogel (contact lens) was introduced as early as 1960.[11] There have been and continue to be multiple challenges in developing contact lenses as an ocular drug delivery system.[12–16] The current challenges include incorporation of sufficient amounts of the target drug into the lens matrix, sustaining a controlled drug release for the desired time frame, good optical clarity, patient comfort during prolonged wear, and biocompatibility. Various methods have been proposed for loading the target drug into a lens delivery system. Several key approaches for the preparation of a contact lens–based drug delivery system are discussed in the following, such as lenses soaked in a drug solution, molecularly imprinted polymeric hydrogels, conjugation of nanoparticles, or drug molecules to the contact lens surface, drug-polymer films integrated with contact lenses, and liposome-loaded contact lenses.[3]

We have developed a brand newly synthesized drug delivery hydrogel soft contact lens (SCL) incorporating material commonly used to correct vision.[17–19] In previous studies, a drug delivery hydrogel material consisting of 2-hydroxyethyl methacrylate (HEMA) was used as the main material, ethyleneglycole dimethacrylate (EGDMA) as a crosslinking agent, methacrylamide (MAm) as a functional group, and methacrylic acid or phosphoric acid as an anionic material group.[17,18] The polymer–drug interaction between phosphoric acid as an anionic material, and naphazoline as a cationic drug[19] was also studied, and it was found that HEMA, EGDMA, and MAm were required, since both cationic and anionic monomers are essential to a synthesized drug delivery hydrogel SCL. The reason for this is that the hydrogel material is not able to maintain the strength and the shape necessary for an SCL if either a cationic or anionic monomer was not included. In this

research, the approach of soaking the lenses in a drug solution was taken. Briefly described, the mechanism of drug release is as follows. Initially, a cationic or anionic material in the SCL forms an ionic complex with the drug content in the drug solution. Then, after applying the SCL to the eye, ionic components in the tear fluid, such as sodium, chlorine, or other elements, are gradually replaced with the drug contents. Finally, the drug release is sustained continuously from the SCL by a silyl group in a side chain that inhibits the displacement efficiency of water.

Furthermore, the synthesized drug delivery SCL material has high transparency and consistent shape similar to commercially available disposable SCLs.

Contact lenses may also be used as protective devices or "bandage lenses." Contact lenses used as a bandage typically remain on the eye continuously for several weeks protecting the cornea from rubbing associated with blinking to promote faster corneal healing after disease or trauma.[20–24] Bandage SCLs are typically used in combination with antibiotics or steroid drops to assist in dealing with any inflammation or infection occurring from the corneal trauma. This means that an understanding of the kinetics in the interaction between the drugs and SCL materials remains important.

The purpose of this study was to investigate the uptake and release of antibiotics from a newly synthesized drug delivery hydrogel SCL, as well as to compare the drug delivery SCL with eye drop administration in terms of penetration of the medication to the interior of the eye. In this study, treatment for bacterial corneal ulcers or prevention of postoperative endophthalmitis was targeted.

Materials and Methods

Materials

All materials used were of analytical grade and sterilely filtered before use.

Methacrylic acid (MAA), obtained from Kyoeisha Chemical Co., Ltd. (Osaka, Japan), was used as the anionic monomer. For the cationic monomer, methacrylamido-propyltrimethylammonium chloride (MAPTAC) from Mitsubishi Rayon Co., Ltd. (Tokyo, Japan) was used. The main component, 3-Methacryloxypropyltris(trimethylsiloxy)silane (MPTS) was obtained from Gelest, Inc. (Morrisville, PA), and HEMA was obtained from Mitsubishi Gas Chemical Company, Inc. (Tokyo, Japan). EGDMA from Mitsubishi Rayon Co., Ltd. and 1,9-nonanediol diacrylate (1,9-NDA) from Kyoeisha Chemical Co., Ltd. were used as crosslinking agents. 2,2′-Azobisisobutyronitrile (AIBN) from Wako Pure Chemical Co., Ltd. (Osaka, Japan) was used as the polymerization initiator. The structures are shown in the Table. For application of Gatifloxacin (GFLX) and Moxifloxacin (MFLX), commercially available GFLX eye drops (GATIFLO 0.3%, 3 mg/ml; Senju Pharmaceutical, Osaka, Japan) and MFLX eye drops (VIGAMOX 0.5%, 5 mg/ml; Alcon, Fort Worth, TX) were used. Both eye drops were benzalkonium chloride-free.

Preparation of the Hydrogels

To the liquid monomer (mixtures composed of HEMA and MPTS as the main components), anionic monomers were mixed in with composition ratios of 0, 1, 3, 5, and 10 weight percent (wt%) of MAA. EGDMA and 1,9-NDA (crosslinking agents) were added to prevent high water content levels in the addition of ionic monomers. AIBN (0.4 wt%) was added to the mixture and stirred for 1 hour under exposure to nitrogen. The liquid monomer mixture was poured into a polypropylene mold and heated in the range of 50°C to 100°C for 24 hours to obtain the polymers. The polymers were cooled to room temperature, removed from the mold, and then soaked in distilled water at 60°C for more than 4 hours to hydrate.

Table. Material Details

Anionic monomer	MAA
Cationic monomer	MAPTAC
Main component	MPTS, HEMA
Crosslinking agents	EGDMA, 1,9-NDA
Polymerization initiator	AIBN

Commercially Available Soft Contact Lenses Used in This Study

Commercially available SCLs were prepared for comparison with the newly synthesized drug delivery SCL in terms of uptake and release of a drug. The SCLs used in this study were etafilcon A (1-DAY ACUVUE, Johnson & Johnson Vision Care, Inc., Jacksonville, FL) comprised of HEMA and MAA (an anionic monomer), and polymacon (The BAUSCH & LOMB SofLens 38, Rochester, NY) comprised of HEMA without any anionic monomers.

Sterilization and Uptake of Antibiotic Into the SCL

After hydration, each of the three materials of the SCL was soaked in 3 mL of GFLX or MFLX eye drop solutions with uptake of the antibiotic being conducted by moist heat sterilization (Fig. 1, 121°C, 30 minutes). The antibiotic uptake volumes of hydrogel SCLs with different composition ratios of the anionic group were examined in situ. GFLX and MFLX are both cationic drugs. The composition ratios were 0, 1, 3, 5, and 10 wt% of MAA.

Methods

Measurement of Antibiotic Uptake and Release Amount In Vitro

To measure the uptake amount of GFLX or MFLX by the newly synthesized SCL, each contact lens containing GFLX or MFLX was soaked in methanol for 24 hours. After the GFLX or MFLX had been completely removed, as determined by the absence of its absorption in the UV spectrum, the GFLX and MFLX concentrations were measured by high-performance liquid chromatography (HPLC; JASCO, Tokyo, Japan).

The sustained-release kinetics of GFLX or MFLX from the SCL with an anionic group concentration of 5 wt% of MAA

Figure 1. Newly developed SCLs in MFLX eye drop solutions and antibiotic uptake by moist heat sterilization.

were examined as follows: each contact lens (the newly synthesized SCL, etafilcon A and polymacon) was soaked in 2 mL of PBS solution at 37°C to examine release of the drug. After 1 hour, each contact lens was soaked in a fresh 2 mL of PBS solution under the same conditions. Moreover, each contact lens was continuously resoaked in a fresh solution of PBS for intervals of 2, 4, 8, 24, 48, and 72 hours. The GFLX and MFLX concentrations in each PBS solutions were measured using HPLC. Each sample was filtered using a 0.22-μm pore filter before analysis. The HPLC system used was a model LC-2000Plus series (JASCO) equipped with a LUNA C18 column (4.6-mm internal diameter × 150 mm; Phenomenex, Torrance, CA). Drug concentrations of the released GFLX or MFLX in methanol or PBS were determined using an established calibration curve, and all samples were analyzed in duplicate.

In Vivo Antibiotic Release and the Prevention of Bacterial Proliferation

Animal Model. Eighty-seven Japanese albino rabbits (each weighing approximately 2500 g) were used for this experiment. All animal procedures were performed in compliance with the criteria for Use of Laboratory Animals in Toho University, and in compliance with the ARVO Statement for the Use of Animal in Ophthalmic and Vision Research.

Administration of GFLX or MFLX Eye Drops, and SCLs Presoaked With GFLX or MFLX

The 72 rabbits were divided into four groups. In group 1, topical GFLX 0.3% was administered to the eyes ($n = 9$); in group 2, topical MFLX 0.5% was administered to the eyes ($n = 9$); in group 3, SCLs presoaked with GFLX 0.3% were administered to the eyes ($n = 27$) and in group 4, SCLs presoaked with MFLX 0.5% were administered to the eyes ($n = 27$). SCLs presoaked with antibiotics with an anionic group concentration of 5 wt% were used. Topical administrations of GFLX 0.3% and MFLX 0.5% eye drops were performed three times at 15 minute intervals. The conjunctival sac volume in the human eye is around 0.03 mL, and the volume for one topical administration in the present study was 0.03 to 0.05 mL. Eye drops (0.05 mL) were applied directly into the conjunctival sac in each rabbit eye with a 27-gauge irrigation needle. For the final topical administration, SCLs presoaked with antibiotics were applied to each rabbit eye in groups 3 and 4. The SCLs were matched to the radius of corneal curvature in the rabbit eye (Fig. 2).

Antibiotic Concentrations in the Cornea, Aqueous Humor, and Crystalline Lens of Rabbits

The rabbits were anesthetized in accordance with the animal treatment policies reported previously.[25,26] Simply, anesthesia was achieved by means of an intramuscular injection of 87.5 to 105 mg/kg of ketamine hydrochloride (Ketalar; Daiichi Sankyo Propharma Co., Ltd., Tokyo, Japan) and 5 to 6 mg/kg of xylazine hydrochloride (Celactral; Bayer Japan, Tokyo, Japan) in a 7:1 mixture, for a total of approximately 4 mL administered to one rabbit.

Three rabbits from groups 1 and 2 were selected randomly to obtain samples at 10, 30, and 60 minutes after the final topical administration. Additionally, three rabbits from groups 3 and 4 were selected randomly to obtain samples at 10, 30, and 60 minutes, as well as 2, 4, 8, 24, 48, and 72 hours after the SCL administration. Under aseptic conditions and with a surgical microscope (Takagi, Nagano, Japan), aqueous humor (0.1 mL) was removed from the anterior chamber of the rabbit eyes using a 29-gauge needle placed through the lateral limbal cornea. The rabbits were then put down by intravenous administration of a massive dose of thiopental sodium, and the eyes were enucleated, corneas and crystalline lenses were removed and soaked in methanol after cutting the samples into small pieces with scissors. All samples were immediately frozen. HPLC as described above was used to determine the concentrations of GFLX and MFLX. After homogenization by an ultrasonic homogenizer and filtration with methanol through a 0.22-μm filter, the cornea and crystalline lens samples were examined.

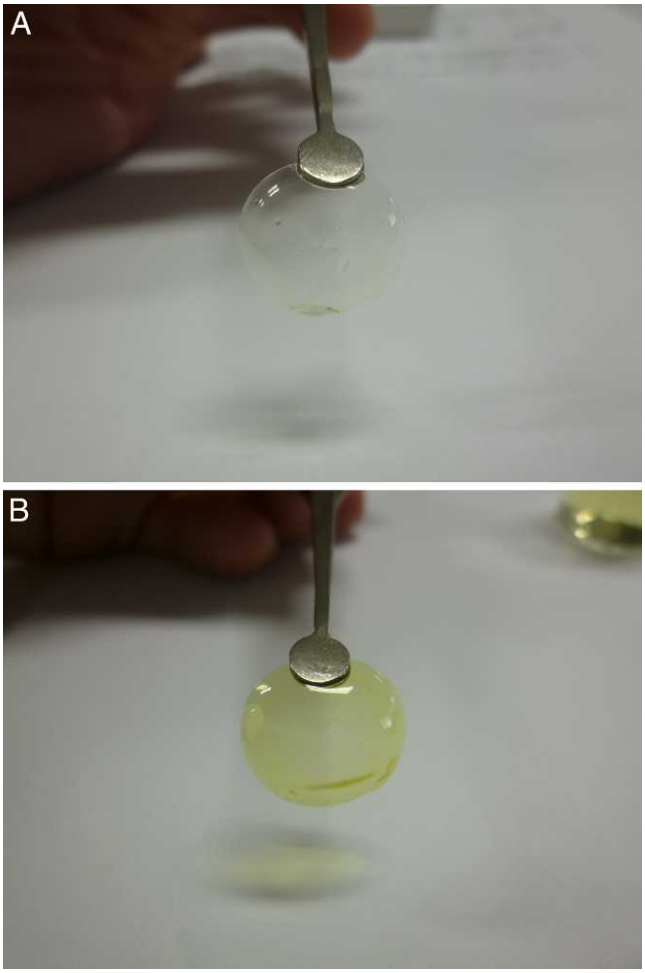

FIGURE 2. SCLs presoaked with antibiotics (GFLX or MFLX). (**A**) Presoaked with GFLX. (**B**) Presoaked with MFLX.

Prevention of Bacterial Proliferation in Rabbits: Comparison Between Antibiotic Presoaked SCL and Eye Drop Administration of 0.3% GFLX

A methicillin-resistant strain of *Staphylococcus aureus* (ATCC 43300) was used in this study. Stock cultures were kept frozen at −75°C in Microbank (Pro-Lab Diagnostics, Round Rock, TX). Before each assay, small quantities of the culture were subcultured in a brain heart infusion broth overnight at 37°C to confirm purity and viability. The levofloxacin (LVFX) and MFLX minimum inhibitory concentrations (MICs) for ATCC 43300 were 0.16 and 0.03 μg/ml.[27,28] Prior to experimentation, each contact lens was soaked in GFLX eye drop solutions for uptake of the antibiotic. Forty-five Japanese albino rabbits were used for this assay. A methicillin-resistant strain of *S.*

aureus (ATCC 43300) was inoculated in the right eyes of all rabbits. Rabbits were randomly assigned to three groups of five animals each. Newly synthesized SCLs not presoaked with antibiotics were administered to eyes (control group, $n = 15$) in group 1; newly synthesized SCLs presoaked with GFLX 0.3% were administered to eyes ($n = 15$) in group 2 and topical GFLX 0.3% was administered to eyes ($n = 15$) in group 3.

Surgical Technique. Preparation and anesthesia were as described above. Initially, aqueous humor (0.1 mL) was removed from the anterior chamber of the rabbit eye using a 29-gauge needle placed through the lateral limbal cornea. *S. aureus* (ATCC 43300) cultures were diluted to 1:10 in PBS, and 0.1 mL of the dilution was injected into the anterior chamber of the right eye. After the bacterial inoculation, newly synthesized SCLs were applied to each rabbit eye in groups 1 and 2, topical GFLX 0.3% was administered to eyes three or four times per day through 72 hours in group 3.

Enumeration of Bacteria in Rabbit Eyes. At intervals of 24, 48, and 72 hours after inoculation, animals were euthanized by intravenous administration of a massive dose of thiopental sodium. The inoculated eyes (right eyes) were then enucleated. Five rabbits from each group were euthanized at each time point. Following the enucleation, each eye was transferred to a sterile 50-mL centrifuge tube (Becton, Dickinson and Company, Franklin Lakes, NJ) containing 3 mL of 1-mm diameter sterile glass beads (SGMT No. 001; Toshinriko Co., Ltd., Tokyo, Japan) in 10 mL of PBS. Several small incisions were carefully made in the cornea and sclera of the enucleated eye in the tube. The tubes were subsequently centrifuged for 2 minutes at 2500 rpm to separate bacterial cells from the eye tissue. The disaggregated bacteria were then enumerated by quantitative track dilution plating as described.[29]

Statistical Analysis

Mann-Whitney *U* analysis and multiple comparison tests (Scheffe, Tukey, and Newman-Keuls) were performed using Mini-StatMate software (version 3, plug-in software for Microsoft Excel; ATMS, Inc., Tokyo, Japan) for Macintosh.

Results

In Vitro Antibiotic Uptake

The uptake amount of GFLX and MFLX was found to increase with the wt% of the anionic group (Methacrylic Acid; Fig. 3). Because both antibiotics were cationic drugs, they formed an ionic complex with the anionic group. Furthermore, each hydrogel SCL was found to be able to uptake up to 4000 μg of GFLX or 6000 μg of MFLX. However, for the new SCLs in which the wt% of the anionic group was higher than 10 wt%, it was found that they were unable to uptake the antibiotics because the new SCL material could not maintain the normal shape necessary to correct vision.

In Vitro Antibiotic Release

Concentrations of GFLX or MFLX released from the newly synthesized SCLs, etafilcon A and polymacon were measured in vitro over 72 hours (Fig. 4). The released antibiotics concentrations of the new SCLs were highest during the initial 1 hour period in both groups (1187.4 μg/mL of GFLX and 1310.7 μg/mL of MFLX), and decreased over the next 48 hours. Approximately 90% of GFLX or MFLX uptake was released in the first 24 hours. Moreover, when the released amounts of GFLX and MFLX are compared, the concentrations of released MFLX were generally higher than those of GFLX, and

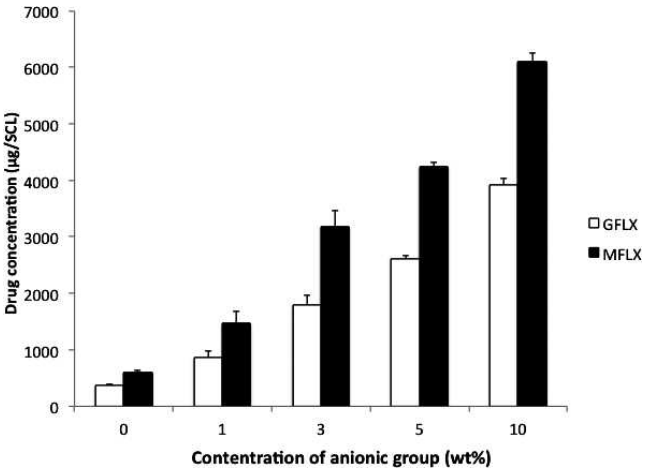

Figure 3. Relationship between antibiotic uptake amount and five different anionic group concentrations. *Bars* and *error bars* represent the mean ± SD.

significant differences appeared at the 24 and 48 hour time points ($P < 0.05$). When comparing the released antibiotics concentrations among the newly synthesized SCLs, etafilcon A and polymacon, those of the new SCLs were significantly highest throughout 72 hours ($P < 0.01$). The peak GFLX or MFLX concentration from the new SCLs was 1187.4 μg/SCL and 1310.7 μg/mL at 1 hour. The peak GFLX or MFLX concentration from the etafilcon A was 663.4 μg/SCL and 1212.5 μg/SCL at 1 hour. The peak concentration from the polymacon was 188.9 μg/SCL and 282.2 μg/SCL at 1 hour. Moreover, etafilcon A had nearly ceased release after 24 hours, while polymacon ceased after 8 hours. The new SCLs continued release throughout 72 hours.

In Vivo Antibiotic Release

Antibiotic Concentrations in the Rabbit Cornea, Aqueous Humor, and Crystalline Lens: Comparison Between Eye Drop Administration of 0.3% GFLX or 0.5% MFLX and Antibiotic-Treated New SCLs. Antibiotic concentrations in the rabbit cornea, aqueous humor, and crystalline lens were determined by HPLC to compare the eye drop administration groups with the new antibiotic-treated SCLs groups.

In the cornea (Fig. 5A), the GFLX concentrations by eye drop administration were found to decrease from 9.2 μg/g to 3.7 μg/g over 60 minutes, whereas those from the new SCLs increased from 45.6 μg/g to 103.3 μg/g. The MFLX concentrations by eye drop administration decreased from 33.4 μg/g to 8.3 μg/g over 60 minutes, while those from the SCL increased from 140.0 μg/g to 260.4 μg/g. The GFLX and the MFLX concentration in the antibiotic-treated SCL groups were higher than those in eye drop groups at each time point ($P < 0.05$ or $P < 0.01$).

In the aqueous humor (Fig. 5B), the GFLX concentrations by eye drops administration were found to increase from 0.66 μg/mL to 1.19 μg/mL over 60 minutes, whereas, those from the SCL increased from 0.87 μg/mL to 12.21 μg/ml. The MFLX concentrations by eye drop administration increased from 4.47 μg/mL to 6.37 μg/mL over 60 minutes, while those from the SCL increased from 7.85 μg/mL to 62.98 μg/ml. The GFLX and the MFLX concentrations in the antibiotic-treated SCL groups were higher than those in eye drop groups at both 30 and 60 minutes ($P < 0.05$ or $P < 0.01$).

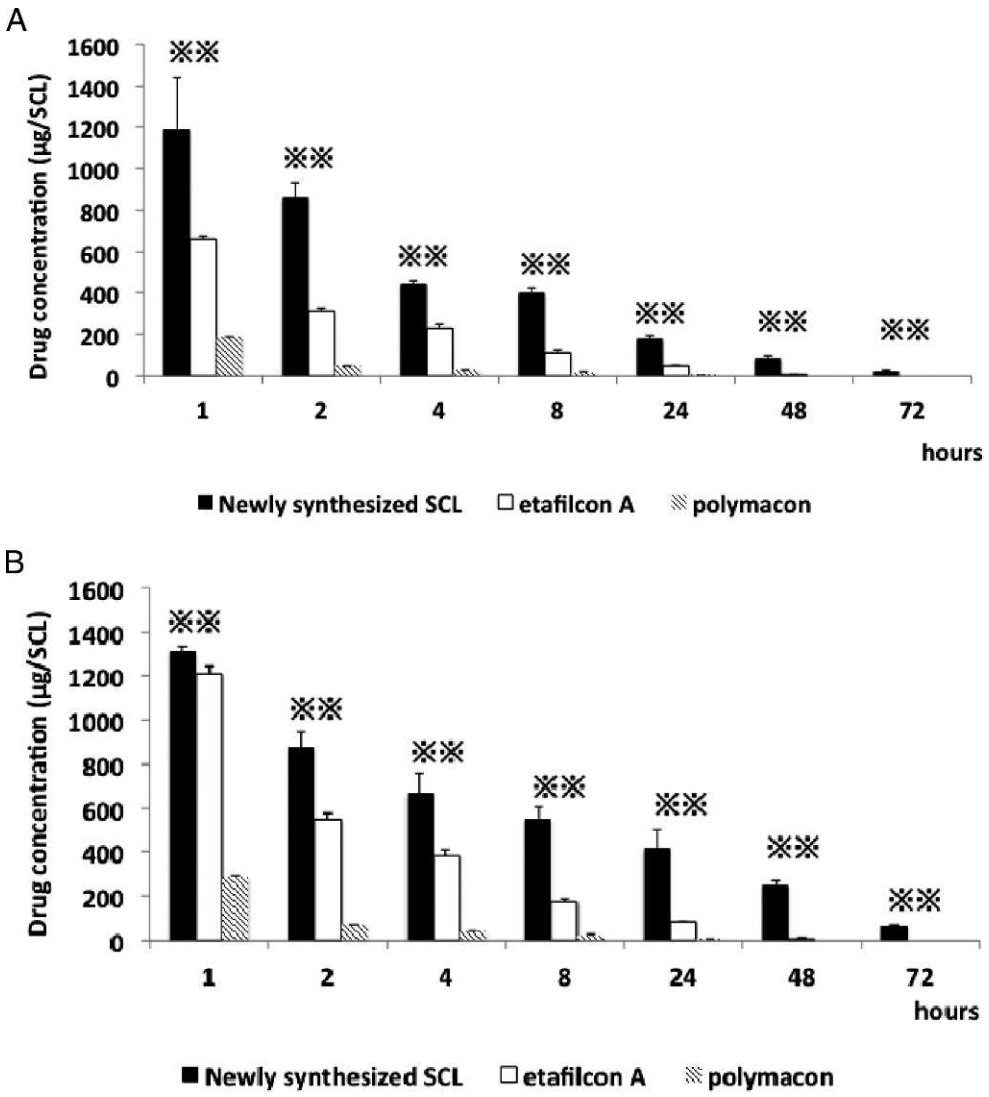

Figure 4. Concentrations of GFLX or MFLX released from the newly synthesized SCL, etafilicon A, and polymacon at 37°C. *Error bars* represent the SD (※※; $P < 0.01$); (**A**) GFLX; (**B**) MFLX.

In the crystalline lens (Fig. 5C), the GFLX concentrations by eye drop administration were found to increase from 0.05 μg/g to 0.07 μg/g over 60 minutes, whereas those from the SCL increased from 0.07 μg/g to 0.16 μg/g. The MFLX concentrations by eye drop administration increased from 0.09 μg/g to 0.87 μg/g over 60 minutes, while those from the SCL increased from 0.09 μg/g to 0.59 μg/g. The GFLX and the MFLX concentrations in the antibiotic-treated SCL groups were only higher than those in the eye drop groups for GFLX at 60 minutes ($P < 0.01$).

Released Antibiotic Concentrations by Antibiotic-Treated SCLs in the Rabbit Cornea, Aqueous Humor, and Crystalline Lens: Comparison Between GFLX With MFLX. Released antibiotic concentrations from antibiotic-treated SCLs in a rabbit cornea, aqueous humor, and crystalline lens were determined by HPLC for comparison with the GFLX and the MFLX groups. The concentrations were found to decrease over time for 72 hours (Figs. 6A–C).

In the cornea, the MFLX concentrations were higher than the GFLX concentrations at 1, 2, 4, 8, 48, and 72 hours (Fig. 6A, $P < 0.05$ or $P < 0.01$). The concentrations of the released antibiotics peaked at 2 hours. The peak GFLX concentration was 120.5 μg/g, and the peak MFLX concentration was 308.5 μg/g. The GFLX concentrations were 10.6, 1.6, and 0.5 μg/g at 24, 48, and 72 hours, respectively. The MFLX concentrations were 26.7, 12.2, and 10.5 μg/mL at 24, 48, and 72 hours, respectively.

In the aqueous humor, the MFLX concentrations were higher than the GFLX concentrations at 1, 2, 4, 24, 48, and 72 hours (Fig. 6B, $P < 0.05$ or $P < 0.01$). The concentrations of the released antibiotics peaked at 2 hours. The peak GFLX concentration was 43.72 μg/mL, and the peak MFLX concentration was 75.66 μg/mL. The GFLX concentrations were 4.11, 0.27, and 0.1 μg/mL at 24, 48, and 72 hours, respectively. The MFLX concentrations were 9.35, 3.92, and 3 μg/mL at 24, 48, and 72 hours, respectively.

In the crystalline lens, the MFLX concentrations were higher than the GFLX concentrations at 1, 4, 8, 48, and 72 hours (Fig. 6C, $P < 0.05$ or $P < 0.01$). The concentrations of the released antibiotics peaked at 8 hours. The peak GFLX concentration was 1.15 μg/g, and the peak MFLX concentration was 2.24 μg/g. The GFLX concentrations were 0.95, 0.37, and 0.26 μg/g at 24, 48, and 72 hours, respectively. The MFLX concentrations were 1.73, 1.14, and 1.02 μg/mL at 24, 48, and 72 hours, respectively.

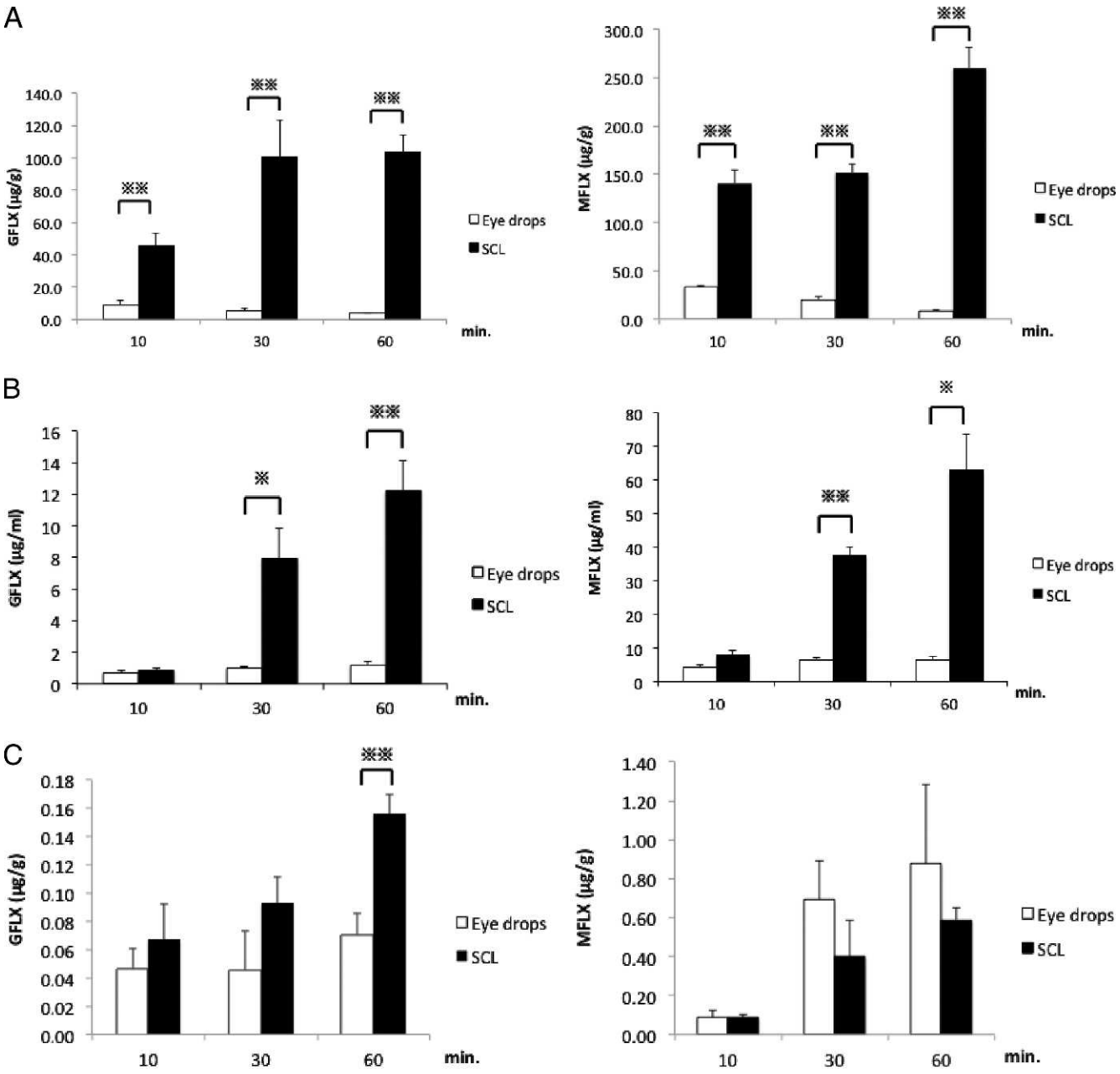

FIGURE 5. Antibiotics release from eye drops or SCL in vivo. *Bars* and *error bars* represent the mean ± SD (※; $P < 0.05$, ※※; $P < 0.01$); (**A**) cornea; (**B**) aqueous humor; and (**C**) crystalline lens.

Prevention of Bacterial Proliferation in Rabbits: Comparison Between Antibiotic Presoaked SCL and Eye Drop Administration of 0.3% GFLX

Administration of *S. aureus* (strain ATCC 43300) to the anterior chamber was initiated with approximately $\log_{10}8.8$ colony-forming units (CFU)/mL. The starting inoculum was $\log_{10}7.8$ CFU/eye. Bacterial endophthalmitis was successfully initiated using *S. aureus* delivered directly into the anterior chamber as shown in Figure 7. All rabbits in the control group developed severe intraocular infection. The bacterial populations were significantly smaller in eyes treated with antibiotic-presoaked SCL and eye drops groups than in the control group through 72 hours after inoculation ($P < 0.01$, Fig. 7). Most importantly, bacterial populations were undetectable in the SCL group throughout the experimental periods. The SCL group populations were significantly smaller than those in the eye drop group at 24 hours. However, there were no significant differences at 48 hours and in the eye drop group the populations were undetectable at 72 hours.

DISCUSSION

Sato et al. have developed a revolutionary contact lens with a drug delivery system applied with an ion ligand.[18,19] An ion ligand is a bond in which an ion binds to a central metal atom to form a coordination complex. Contact lenses with a drug delivery system can be designed to contain the required

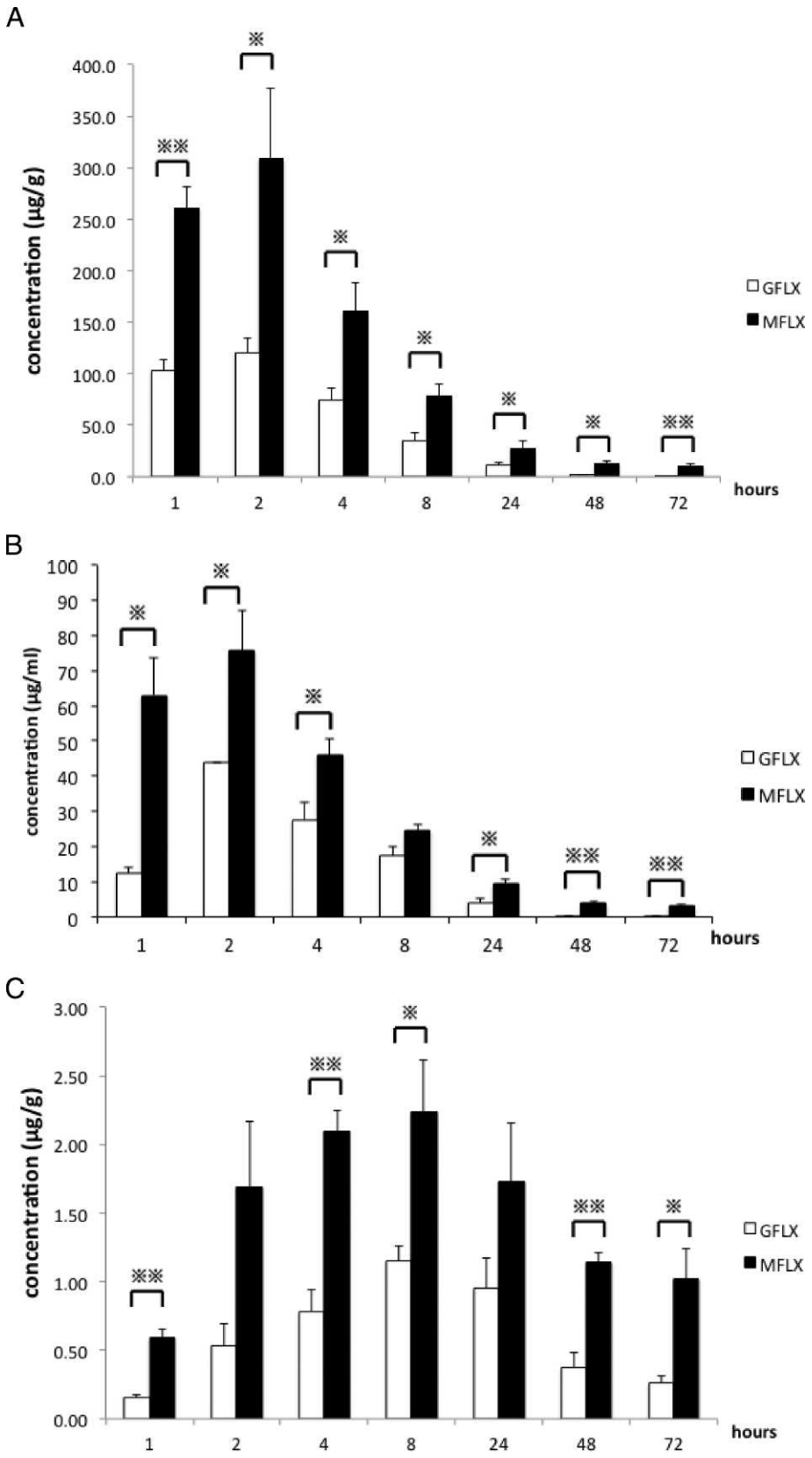

FIGURE 6. Released antibiotic concentrations by antibiotic-treated SCLs in a rabbit cornea, aqueous humor, and crystalline lens: comparison between GFLX with MFLX over 72 hours. *Bars* and *error bars* represent the mean ± SD (※; $P < 0.05$, ※※; $P < 0.01$); (**A**) cornea; (**B**) aqueous humor; and (**C**) crystalline lens.

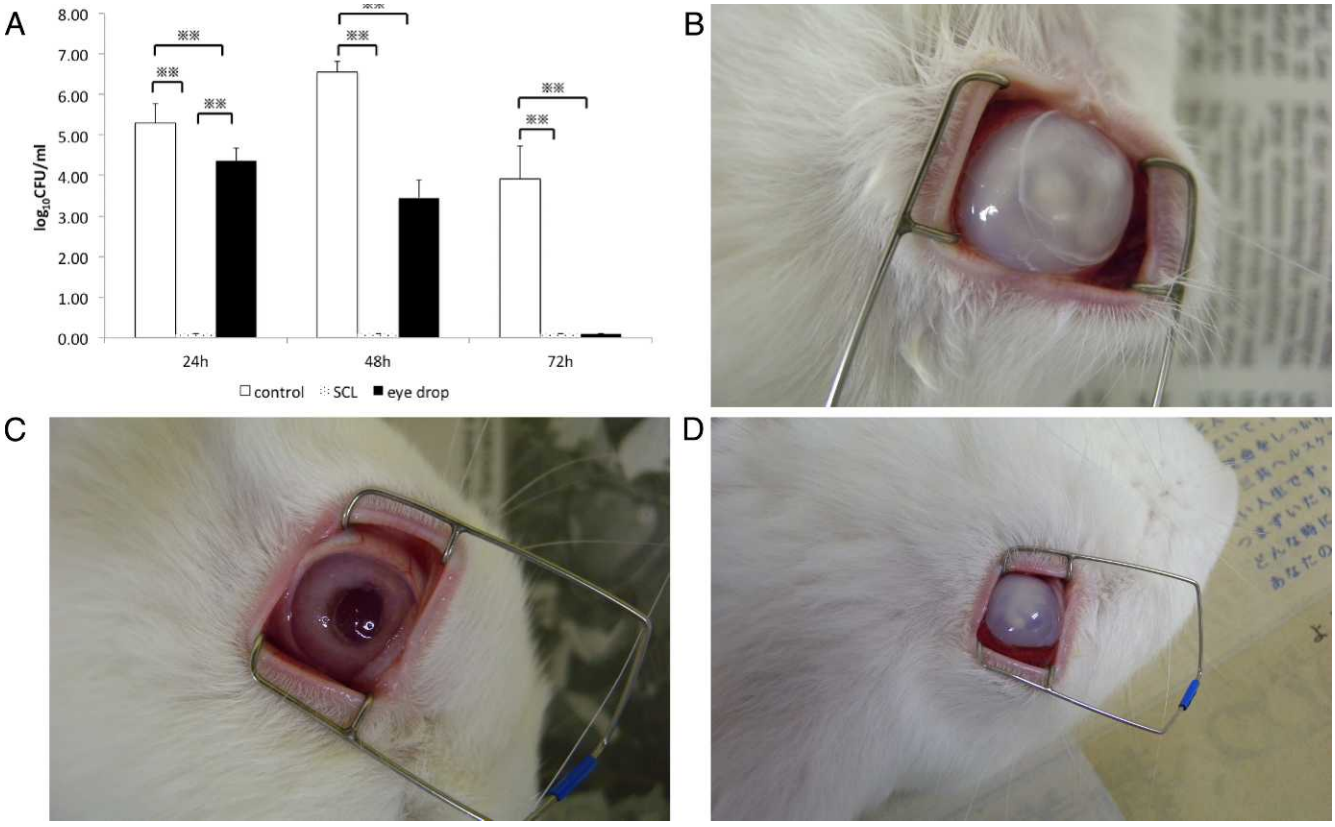

FIGURE 7. Prevention of bacterial proliferation: comparison between antibiotic-presoaked SCL and eye drops administration of 0.3% GFLX. (**A**) Results for bacterial populations in a rabbit endophthalmitis model. *Bars* and *error bars* represent the mean ± SD (※※; $P < 0.01$). (**B–D**) Rabbit eyes at 48 hours after bacterial inoculation. (**B**) Newly developed SCLs not presoaked with antibiotics were administered to eyes. (**C**) Newly developed SCLs presoaked with GFLX 0.3% were administered to eyes. (**D**) Topical GFLX 0.3% was administered to eyes.

amount of a drug through the choice of the ionic group of the ligand.[19] Naphazoline was a model drug for a cationic group, and its content in the contact lens was equivalent to the phosphate group content used as an anionic group.[19] The antibiotics used in this study, GFLX and MFLX, are also cationic drugs. The uptake amounts of GFLX and MFLX were found to increase with the wt% for the anionic group, MAA, up to 10 wt% in a manner similar to that of naphazoline.

The requirements of a drug delivery system using an SCL are the incorporation of sufficient amounts of the drug into the contact lens matrix, and sustained release of the drug for the desired time frame at a controlled rate. The drug amounts contained in the SCLs of this study were approximately 3 to 4 mg per SCL. Garhwal et al. reported that conventional contact lenses incorporated with dispersed ciprofloxacin-containing nanospheres (nanosphere/cipro) released drug amounts of 2.5 mg.[30] Peng et al. reported that vitamin E loaded silicone hydrogel contact lenses released 0.5 mg of lidocaine.[31] Jung et al. reported that temperature sensitive contact lenses released 0.5 to 0.7 mg of timolol.[16] Ciolino et al. reported that their developed drug-eluting contact lens released 4.5 mg of ciprofloxacin.[32] In this study, it was found that the SCLs used, while not able to produce the greatest uptake amounts of antibiotics, were not among the lowest of those reported. The basic concept in this study was to develop SCLs that can correct vision as well as perform as drug delivery device. Compared with commercially available SCLs, the newly synthesized SCLs hold a great advantage with the volume amount and release period of antibiotics.

In regards to the time frame of sustained drug release from the SCLs in vitro, in the early phase an initial burst was seen with a subsequent sustained release over the next 72 hours. This initial burst release is typical of hydrogel-based drug delivery systems.[33–35] SCLs made from poly-HEMA (pHEMA) hydrogels release the majority of their drug content in the first day.[36,37] The material in this study also released 90% of the uptake amounts in the first 24 hours, since they are based on pHEMA. Currently, new contact lenses made from a silicone–hydrogel hybrid have shown a similar uptake but with a lower release of drug when compared with pHEMA lenses.[38,39] In this study, the antibiotics used were fourth generation fluoroquinolone antibiotics whose bactericidal effect is dose dependent. An initial burst is suitable for destroying bacteria. In contrast, if the bactericidal effect of antibiotics is time dependent, for example when using cefuroxime, a lower release rate would be desired. Drug delivery systems for antibiotics, especially whether offering an initial burst or lower, sustained release rate, should be available for an improved bactericidal effect of the delivered antibiotics.

Sano et al. showed that the delivery of levofloxacin to the aqueous humor in albino rabbits was 15 times greater at the end of 4 hours with a drug delivery system using piggyback contact lenses in comparison with eye drops dosed at 30 minute intervals over 4 hours.[3,40] Their developed drug delivery system consisted of a drug plate containing 30 wt% levofloxacin, a hydrophilic SCL and a nonhydrophilic SCL.[40] Their instillation protocol of levofloxacin was performed every 30 minutes through 8 hours. Meanwhile, Fukuda et al. suggested a parameter for the maximum concentration in aqueous humor be called AQCmax to indicate the penetration of an ophthalmic solution into the aqueous humor in animal experimental models.[41]

The original method for obtaining AQCmax was as follows: exactly 50 μL of each ophthalmic solution was measured using a micropipette, and was instilled into the cul de sac of rabbits. This treatment was repeated three times at intervals of 15 minutes. After anesthesia, approximately 100 μL of aqueous humor was collected at 10, 30, 60, 120, and 240 minutes after the final instillation, to be used as samples.[41] AQCmax is referenced as a measure for ocular penetration in this study, because AQCmax is a more practical and clinical parameter. In the above protocol for multiple instillations, the concentration in the aqueous humor reached the maximum from 30 to 60 minutes.[42,43] Based on those results, in this study the drug concentration of SCL and of eye drops were compared until 60 minutes after instillation.

In our results, the drug concentrations in the cornea, and aqueous humor were also 10 times greater at 30 and 60 minutes than those of eye drops in rabbits. As eye drops are always diluted and washed away by tearing and by blinking, a drug delivery system using hydrophilic SCLs has a greater advantage in maintaining the effective drug concentration to the cornea and aqueous humor.

Moreover, this newly developed contact lens was found to release a high concentration of antibiotics over the initial 24 hours to the cornea and aqueous humor. In the cornea, the GFLX and the MFLX concentrations were 0.89 and 2.22 μg/mL at 24 hours. In the aqueous humor, the GFLX and the MFLX concentrations were 4.11 and 9.35 μg/mL at 24 hours.

These results demonstrate that there was sufficient antibiotics concentration above the MIC_{90} to fight the bacteria most commonly related to ocular infection within 4 hours at the cornea and 8 hours in the aqueous humor.[44] It is of particular note that the antibiotics concentrations in the aqueous humor were extremely high, which could be expected to prevent postoperative endophthalmitis. McCulley et al. have reported on GFLX and MFLX penetration into human aqueous humor.[45] Their protocol consisted of administration four times on the day before surgery plus one drop administered 1 hour before surgical entry into the anterior chamber. In their study, the aqueous humor concentration of GFLX was 0.94 μg/mL and that of MFLX was 1.86 μg/ml. Both concentrations were above the MIC required to inhibit the growth of the major ocular pathogens.[45] As for the in vivo endophthalmitis model in this study, the SCL groups exhibited a preventive effect against bacterial proliferation throughout the experimental period, and the large amount of bacteria administered to the interior of the eyes should be taken into account. The MIC of GFLX for the bacterial strain used, ATCC 43300, was calculated to be from 0.03 to 0.16 μg/mL (0.12 μg/mL) in a pilot study. In this study, the GFLX concentration in the aqueous humor was found to reach the MIC for ATCC 43300 within 48 hours, because the concentration was 0.27 μg/mL at 48 hours. Furthermore, the concentration at 24 hours (4.11 μg/mL) was 20 times higher than the MIC for ATCC 43300, which also reach the minimal bactericidal logarithmic (MBC log). This goes to show that the preventive effect against bacterial proliferation of the antibiotic-presoaked SCL was superior to that of antibiotics eye drops administration within 24 hours.

Several researchers reported the ability of a commercially available SCL to deliver antibiotics into the cornea and/or aqueous humor.[46–49] Hehl et al. tested the suitability of Acuvue SCL (etafilcon A) as a drug delivery system for antibiotics.[47] After soaking Acuvue SCLs for 1 hour in antibiotic eye drop solutions, they were applied to patients awaiting cataract surgery 1 to 5 hours prior to surgery.[47] The antibiotics used were gentamicin, kanamycin, tobramycin, ciprofloxacin, and ofloxacin. The antibiotic concentrations in human aqueous humor were measured. From these results, ofloxacin was found to have no significant dependence of aqueous humor concentration to the amount of time worn, and the mean concentration was 55.5 ± 25.3 (20–108.7) μg/mL. Tian et al. soaked Acuvue SCLs for 1 hour in commercially available lomefloxacin, resulting in a mean concentration in aqueous humor of a rabbit eye of 14.95 ± 5.43 μg/mL and 26.6 ± 4.81 μg/mL at 2 and 4 hours after SCL application.[48] In our study, the mean GFLX concentrations in aqueous humor were 12.2 ± 1.89 μg/mL, 43.7 ± 0.17 μg/mL and 27.6 ± 4.81 μg/mL at 1, 2, and 4 hours, respectively. While the mean MFLX concentrations were 63.0 ± 10.87 μg/mL, 75.7 ± 11.5 μg/mL, and 45.8 ± 4.81 μg/mL at the same time periods. The results from the previous two studies are not appropriate for comparison with our results for several reasons. Different antibiotics were used, the samples used by Hehl et al. were human, and the antibiotics concentrations by Hehl et al. suggested the cumulative data. However, when looking at the data for Tian et al., it appears that the amount of released antibiotics from our newly synthetized SCL may be greater than that from Acuvue during the initial 2 hours. Also, in our opinion, the efficacy of commercially available SCLs presoaked in antibiotics was not confirmed by those studies or in ours.

SCLs have the additional benefit of providing a bandage on the cornea. Clear corneal incisions are performed in numerous cataract surgery cases, so antibiotic-treated SCLs may have an important role to play in postoperative management.

However, the antibiotic concentrations of the antibiotic-treated SCLs were found not to differ from those of eye drop administrations in crystalline lenses. The lens capsule is well known to be a barrier protecting the lens from bacterial and most viral infections, and to be selectively permeable to proteins, which are required for proper lens growth and development.[50,51] The newly developed contact lens in this study does not provide for drug penetration into a crystalline lens, therefore, further investigation will be needed.

The concentrations of MFLX were generally higher than those of GFLX at the experimental sites with the MFLX eye drops being 0.5% and the GFLX eye drops 0.3%. This means that the concentration of MFLX was basically 1.6 times higher than that of GFLX. However, the differences between the released concentrations were found to be more than 1.6 times at several time points. It was found that MFLX had a good penetration into the aqueous humor, which suggests that MFLX may have good permeability into the ocular tissue.[52]

In conclusion, this study presents a new concept SCL using an ionic complex to bind a drug for sustained delivery, which can also be used as a conventional SCL. The new SCL could release antibiotics over several days with better penetration to the interior of the eye than from eye drops, particularly to the aqueous humor. Moreover, the new SCL has a bandage effect for the main incision of cataract surgery and may have widespread therapeutic applications.

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