Sustained Antibiotic Release from an Intraocular Lens–Hydrogel Assembly for Cataract Surgery

Shai Garty,1,2 Rika Shirakawa,1,3 Adelaide Warzen,1,2 Erin M. Anderson,2 Misty L. Noble,2 James D. Bryers,2 Buddy D. Ratner,*2 and Tueng T. Shen*1,2

PURPOSE. To develop a simple, novel polymeric drug-delivery device for prevention of postoperative bacterial infection after cataract surgery in the developing world.

METHODS. A poly(2-hydroxyethyl-methacrylate) (pHEMA) hydrogel was developed to achieve sustained release characteristics of antibiotics. The in vitro antibiotic release kinetics and efficacy of antibiotic function were tested using a silicone biofilm model. In vivo feasibility was investigated using a rabbit model. The control group of rabbits underwent standard cataract surgery with intraocular lens (IOL) implant and postoperative topical antibiotic and steroid. The experimental group received the polymeric device inserted with standard three-piece IOL at the time of surgery and received only topical steroids postoperatively. In vivo intraocular antibiotic levels and outcomes after cataract surgery were evaluated.

RESULTS. The in vitro studies demonstrate the antibiotic release kinetics can be controlled by optimization of the surface coating. The in vivo results showed sustained sufficient antibiotic concentration (above minimum inhibitory concentration for most common bacteria related to endophthalmitis) for >4 weeks. There was minimum toxicity observed in vivo. The device was effective in treating induced intraocular infection after cataract surgery.

CONCLUSIONS. The initial findings of the polymeric drug-delivery device demonstrate the feasibility delivering sufficient antibiotic in the anterior chamber for the immediate postoperative period in a rabbit model. The device is simple to produce and may help alleviate the potential post-surgical infections in the developing nations. (Invest Ophtalmol Vis Sci. 2011;52:6109–6116) DOI:10.1167/iovs.10-6071

Cataracts are the leading cause of treatable blindness worldwide, and the population afflicted with cataracts continues to increase globally.1 The only treatment for cataracts is surgical removal of the cataract and its replacement with a synthetic intraocular lens (IOL).2 Although cataract surgery is remarkably successful in restoring the patients’ vision, it still has potential risks of severe postoperative intraocular infection (such as bacterial endophthalmitis) that may result in devastating permanent vision loss.3 These unfortunate outcomes are more prevalent in the developing nations where suboptimal sterile intraoperative conditions are common and poor patients’ compliance for postoperative antibiotic eye drops (medications are expensive, unavailable in remote rural areas) are difficult to overcome because they require more profound infrastructure improvements. The consequence of severe, preventable blindness is damaging to the economies of developing countries and traumatic for the affected individuals and their families.

The current standard postcataract surgery management requires the use of topical antibiotics (typically fluoroquinolone) as a prophylaxis against bacterial intraocular infection. Topical application of antibiotics has a low level of intraocular penetration (<0.3%). This requires higher concentration of topical applications to achieve the minimum inhibitory concentration (MIC) of the antibiotics within the eye.2 Topical application of the antibiotic is costly and can result in toxicity of the ocular surface. Furthermore, it depends on the patients’ compliance, which may be difficult in elderly population and developing nations.

We therefore designed a novel polymeric system for sustained, rate-controlled release of sufficient intraocular antibiotics during the immediate postoperative period after cataract surgery. The hydrogel construct is compatible with current cataract surgical procedure and IOL implantation techniques. The system is simple to use (it can be easily attached to the haptics of IOL intraoperatively) and inexpensive to produce. Our goal is to develop an affordable and effective device for the developing nations where medication availability and patient compliance are both suboptimal. The antibiotic chosen as a drug model for this study is a broad spectrum fluoroquinolone, norfloxacin.4–5 The detailed synthesis and characterization of the polymeric system are based on our previously published findings.6 In this study the device fabrication, characterization, and in vitro and in vivo testing of the polymeric construct in treatment of bacterial endophthalmitis are reported.

MATERIALS AND METHODS

Hydrogel Synthesis and Device Molding

All materials used were analytical grade and sterile filtered before use. The hydrogel construct is based on poly(2-hydroxyethyl methacrylate) (pHEMA), which consisted of 67.25% (wt/wt) 2-hydroxyethyl methacrylate (HEMA; ophthalmic grade, Polysciences Inc., Warrington, PA) and dissolved into an equal mixture of deionized water (DI water) and ethylene glycol (J.T. Baker Corp., Phillipsburg, NJ). Poly(ethylene gly-
The antibiotic release kinetics of the hydrogel constructs with different coatings thicknesses were examined in situ. The different samples were placed in 1 mL PBS (0.1 M, pH 7.4), in a 48-well plate, and then the plate was placed on an orbital shaker at 100 rpm. At each time point, the samples were weighed and transferred into fresh buffer (n = 8). Aliquots of the solution were analyzed by measuring the maximum absorbance, \( \lambda_{\text{max}} = 277 \) nm, using a UV/vis spectrophotometer equipped with a microplate reader (Benchmark; Bio-Rad, Hercules, CA).\(^{11}\) The concentration of the norfloxacin released was calculated according to the best-fit line to the established calibration curve of drug concentration in PBS. All samples were filtered using 0.22 \( \mu \)m filter before analysis to decrease significantly the background reading noise. The solutions were diluted before measurement to keep the absorbance and corresponding concentration within the linear range.

In Vitro Anti-Bacterial Efficacy

The efficacy of the antibacterial activity of norfloxacin released by the delivery system was examined in vitro using a static biofilm assay. In the study *Staphylococcus epidermidis* (ATCC 35,984) was chosen as the bacterial infection model because it is the most common bacteria associated with endophthalmitis in patients.\(^{12}\) Details of the antibacterial assay are described elsewhere.\(^{6}\) Briefly, colonies of *S. epidermidis* were grown on agar plates supplemented with 10 \( \mu \)L tryptic soy broth (TSB) overnight at 37°C. A single colony is isolated and resuspended in 25 mL of 10 \( \mu \)L TSB medium, and then placed in a shaker at 37°C for 24 hours. Here 1 mL samples of *S. epidermidis* suspension were transferred to individual wells of a 48-well plate containing silicone disks (6 mm diameter and 0.76 mm thickness), which provided a target surface for the bacteria to attach. This approach simulates the currently available IOL optic surfaces (such as the PhacoFLEX II [Abbott Medical Optics Inc., Santa Ana, CA], STAAR Elastic Lens [STAAR Surgical Co., Monrovia, CA], and Foldable Silicone Multi-piece Lens [US IOL Inc., Lexington, KY]) in the setting of endophthalmitis. Antibiotic-containing hydrogel samples were also placed in the wells. After 24 hours, the suspension was collected, and the viability of bacteria was determined using a LIVE/DEAD cell vitality assay kit ( BacLight L113152; Invitrogen, Molecular Probes, Carlsbad, CA).\(^{13}\)

In Vivo Animal Cataract Surgery Model

All animal studies were performed according to the guidelines of the National Institutes of Health for use of laboratory animals, and with the approval of the Institute of Animal Care and Use Committee of the University of Washington following protocol no. UW4139–01. All surgical procedures were performed under general anesthesia. Twelve female New Zealand White rabbits, 5 months of age and weighing 3.2–3.8 kg, were used in the study. All rabbits underwent standard clear corneal cataract surgery with IOL (a three-piece MA60AC AcrySof; Alcon Surgical Inc., Fort Worth, TX) implant. The control group (n = 3) received IOL only and standard topical antibiotics and steroids postoperatively. The experiment group (n = 9) received a drug-loaded hydrogel attached onto the IOL haptics, and folded along with the IOL into the lens injector. The hydrogel device was packed dry for extended shelf time. Immediately before the surgery, saline is added for easy insertion to the IOL haptics. The animals in both control and experimental groups received topical steroid drops to control postoperative inflammation.

In Vivo Assessment of Intracocular Antibiotic Levels

Under general anesthesia and topical anesthetic eye drops, intracocular antibiotic levels were sampled at various time points (daily during the first week, and subsequently every 3 days, for up to 30 days) postoperatively via anterior chamber paracentesis. The anterior chamber fluids collected (100 \( \mu \)L) were then diluted and analyzed by UV/ VIS spectrophotometry over a 200–600 nm wavelength range. The antibiotic concentrations were calculated based on previously established calibration curve with a maximum peak absorbance at the 270–280 nm range optimized for norfloxacin.

Bacterial Endophthalmitis Model

To further evaluate the effectiveness of the drug-delivering device in treating severe intraocular infection, a bacterial endophthalmitis model (using *S. epidermidis*, 30 \( \mu \)L bolus of 10 cfu/mL to inoculate the rabbit anterior chamber) was established in our laboratory. We
hypothesized that because our drug-delivery device releases antibiotics directly inside the eye, it will be more effective in controlling endophthalmitis compared to the standard topical antibiotic drops (with <0.3% penetration into the eye). For this experiment, control group (n = 6) and experiment group (n = 6) both underwent cataract surgery with IOL implants. The control group received topical antibiotics and steroid drops, and the experiment group received IOL–hydrogel construct during surgery and only topical steroid drops postoperatively. At 24 hours after cataract surgery, both groups received bacterial inoculation in the anterior chamber, and the rabbits were followed closely for signs of intraocular infection.

Statistical Analysis
Two-tailed Student’s t-test or analysis of variance (ANOVA) was performed to determine a significant difference between the experimental and control groups, with significance level set at P < 0.05. Results are presented as mean ± SE.

RESULTS AND DISCUSSION
We have developed a novel and simple polymeric intraocular drug-delivery system for efficient release of antibiotics after cataract surgery. Figure 1 shows a prototype produced using the molding technique and the intraoperative attachment to the IOL haptics. This device is designed to be compatible with current cataract surgery and IOL implantation techniques and can be easily used in developing countries. The current standard postoperation prophylaxis includes the application of topical antibiotic eye drops, which have a suboptimum penetration into the eye (0.35%). This approach is also heavily dependent on patient compliance. With increasing number of postcataract surgery infection cases in recent years and the expected significant increase in the aging population (many of them are in the developing countries), improved treatment for the potential blinding infections after routine cataract surgery is essential.14

For this application, the polymerization of HEMA was optimized using a different oxidation-reduction initiator system consisting of APS and a room-temperature accelerator, TEMED. This efficient catalytic pair results in gelation times of less than 1 minute at room temperature, in comparison to the catalytic systems previously used in HEMA polymerization that required gelation times of more than 4 hours to overnight,6,15,16 without compromising the biocompatibility of the resulting polymer.17,18

Verification of Coating Using ESCA and SEM
ESCA was used for determining the surface chemical composition of the coated samples as a function of octadecyl isocyanate reaction time over 60 minutes. Figure 2A shows the survey spectra of coated pHEMA hydrogels over reaction times of 0, 15, 30, 45, and 60 minutes; Figure 2B shows their respective atomic surface composition. It was found that the surface composition of the uncoated control samples to be C, 69.3±0.6%, oxygen (O), 30.7±0.6%, and no detectable nitro-
gen (N), compared with the expected stoichiometric atom percentage of C, 66.6%, and O, 33.3%. After 15 minutes coating reaction, higher C and n content (C, 82.5%/H11006 1.4%; O, 13.6%/H11006 0.9%; n, 3.9%/H11006 0.5%) indicates initial hydrophobic coating formation having a surface composition that is significantly different from the uncoated samples. The C content peaks after coating reaction time of 30 minutes: C, 86.7%/H11006 1.1%; O, 9.9%/H11006 0.8%; n, 3.4%/H11006 1.2%. Composition remains constant for longer reaction times, with no significant difference for 45 minutes coating time (C, 87.3%/H11006 0.6%; O, 9.4%/H11006 0.5%; n, 3.3%/H11006 0.4%) or for 60 minutes coating time (C, 86.0%/H11006 0.8%; O, 10.6%/H11006 0.4%; n, 3.4%/H11006 0.6%). These data suggest that after 30 minutes most of the available hydroxyl groups within this depth have reacted with the octadecyl isocyanate, hence the peaking of the C content. Further, the resulting hydrophobic coating is thicker than ESCA's sampling depth capability, >80 Å.

High-resolution C1s spectra were also obtained from uncoated and coated pHEMA as shown in Figure 3A. Different peaks indicate various C molecular environments. The high-resolution C1s spectrum of uncoated pHEMA was resolved into five distinct peaks, which indicate five different C1s species: -C-H and -C at 285 and 285.7 eV, -CH2-OH and -CH2-O at 286.5 and 286.9 eV, and O=C- at 289.1 eV. Similarly, the spectrum of coated pHEMA was resolved into three distinct peaks, which are -CH and -C at 285 eV, CH2-O at 286.5 eV and O=C- at 289.1 eV. With increasing reaction coating time, as shown in Figure 3B, the proportion of these C1s species changed. Uncoated pHEMA was found to be 46.5% -CH/C-, 37.3% CH2-O-, and 16.2% O=C-, which is close enough to theoretical values of 50%, 33.3%, and 16.7%, respectively. The percentage of -CH/C- on the surface significantly increased even with 15 minutes of reaction coating time and continued to increase with longer reaction coating time. This demonstrates the escalating number of methylene groups on the pHEMA surface that creates a good hydrophobic coating.

The hydrogel surface that would be in contact with tissue and fluids was investigated to determine its surface morphology and roughness using SEM. Figure 4 shows SEM images taken at ×250 magnification of coated and uncoated pHEMA samples. After 15 minutes reaction with octadecyl isocyanate, the surface of pHEMA displays a regular, corrugated pattern that seems to get larger with increasing reaction time. In addition, crevices appear and the surface seemed to get rougher. As indicated by ESCA data, most available surface hydroxyl groups have reacted by 30 minutes, and it seemed that the reaction was proceeding deeper into the bulk of the polymer as the isocyanate groups permeate. At this point the surface starts to get damaged, as shown prominently in Figure 4D.

In Situ Antibiotic Release

The in situ release of norfloxacin from hydrogel constructs with different coatings were determined over a 6-week period. The peak absorbance was measured at various time points, and the cumulative mass of drug released was calculated and plotted as a function of time as shown in Figure 5. In the uncoated and 15-minute coated samples, an initial burst release is seen, which is typical of hydrogel-based drug-delivery systems. A rapid glass-to-rubber transition occurs as water penetrates into the dehydrated hydrogel, resulting in the increased movement of the cross-linked polymer chains and accordingly, the diffusion of the loaded drug out of the ma-

FIGURE 3. (A) High-resolution ESCA C 1s spectra of pHEMA and octadecyl isocyanate and their chemical structures. (B) At different reaction times, the ESCA carbon C 1s peak was resolved into subpeaks. Note the maximum of hydrocarbon components increasing from 46.5% over 80.9% at a reaction time of 30 minutes. This is related to the methylene chain overlayer. The pHEMA peaks, -C=O at 286.5 eV, CH2-O at 286.9 eV, drop substantially at 30 minutes reaction time. The carbonyl group, C=O at 289.1 eV (5), present in both the pHEMA and the octadecyl isocyanate, also decreases in an amount consistent with the stoichiometry of the two components.

FIGURE 4. High-resolution-scanning electron micrograph images of the coated hydrogel (reaction times, 15 minutes; magnification, ×250).
However, the addition of the longer coating, that is, 30, 45, and 60 minutes, on the samples significantly slowed the initial burst release. This thick hydrophobic coating, which is composed of highly organized methylene chains, acted as a rate-limiting barrier that delayed the imbibition of water into the antibiotic-loaded hydrogel matrix, while simultaneously preventing the diffusion of the trapped antibiotic molecules out of the hydrogel. The 30-minute coated samples showed that it can control the further influx of water and allowed for antibiotic release above the MIC, while release from the longer coated samples was below the lethal dosage. Previous work showed that the release mechanism of this *n*-alkyl isocyanate–coated hydrogel drug-delivery system was found to be bordering between anomalous transport (in uncoated hydrogels) and Case II or, time-independent transport (in coated hydrogels). These findings demonstrate that the octadecyl isocyanate-coated pHEMA samples are capable of delivering a clinically relevant dose of drug in situ over the critical 14-day postoperative period.

**In Vitro Antibacterial Efficacy**

The viability of *S. epidermidis* in the presence of our device was compared with control using a LIVE/DEAD cell assay shown in Figure 6. At 24 hours of incubation with pHEMA hydrogel control, there are a significant number of viable cells (stained green in Fig. 6A) where in the case of the norfloxacin-loaded polymer device almost all bacteria are dead (Fig. 6B, staining red). This study confirms that the antibiotic activity of norfloxacin is maintained after being incorporated into the hydrogel. The released level in vitro is above the MIC level to effectively control *S. epidermidis* activity, an important first step to control intraocular infection after cataract surgery.

**Standard Rabbit Cataract Surgery**

Both control and experiment groups of rabbits did well after cataract surgery with an IOL implant. The polymeric device can be easily attached to the IOL haptic and injected into the
posterior chamber at the time of surgery as shown in Figure 7. Both groups recovered from surgery without significant inflammation and any evidence of intraocular infection.

In Vivo Assessment of Intraocular Antibiotic Levels

The intraocular antibiotic levels after cataract surgery are shown in Figure 8. The hydrogel drug depots exhibit some initial burst release and continued releasing antibiotic at rates equal to or higher than the topical drops over the course of 30 days. Note that the amount of antibiotic in the topical drops was 3000 times higher than in the pHEMA device. After this critical early postimplantation period, the measured drug concentration was similar to the topical eye drops. Figure 8B shows the cumulative drug release from the hydrogel samples. The drug was found to continually release from the hydrogel over the course of the in vivo trial for over 1 month.

The in vivo results demonstrate that there was sufficient antibiotic concentration, above the MIC for the bacteria most commonly related to endophthalmitis, for a period of 4 weeks. All measured drug concentrations were below the toxic level of the drug, and, during the implantation period, there was no evidence of drug toxicity. Both groups of animals recovered from surgery without evidence of infection.

The drug release profiles show good correlation between the in situ and the in vivo release. Thus we can predict that the...
in vivo release from the hydrogel beads would continue up to 52 days.

**Bacterial Endophthalmitis Model**

Bacterial endophthalmitis were successfully created in our rabbit model using *S. epidermidis* as the infectious agent delivered directly into the anterior chamber after standard cataract surgery as shown in Figure 9. All rabbits in the control group (treated with topical antibiotics) developed severe intraocular infection, and the animals had to be killed early due to the severity of the infection. The rabbit in the experimental group with the antibiotic hydrogel drug depots was subsequently treated with only the topical steroid eyedrops. After the initial injection of the bacteria, we detected evidence of acute inflammation, including hypopyon and conjunctival injection. These findings continue to improve without any additional antibiotics, and the rabbits fully recovered after 2 weeks. The significance of this result is that the implant, with <0.3% of the total antibiotic compared with the amount administered with the topical drops, demonstrated superior efficacy in the setting of induced bacterial endophthalmitis.

**CONCLUSIONS**

Our polymeric drug-delivery device delivered therapeutically effective antibiotics in the eye. The device is compatible with standard cataract surgery and IOL implantation procedure. Because the antibiotics are released inside the eye (where the infection is most difficult to treat), our device showed superior outcomes in controlling intraocular infection than conventional topical antibiotic drops. This is demonstrated in our bacterial endophthalmitis experiment. Our implantable depot device can be affordable because it is made from low-cost materials familiar to the regulatory agencies and can be produced easily using standard molding techniques. The controlled intraocular release of antibiotics provides better coverage of the bacterial infection at a much lower antibiotic dose and reduced the dependency of patient compliance postoperatively. Our device has the potential to improve the quality of postoperative management while reducing the cost of health care related to postoperative follow-up and the treatment of potential devastating infections, all of which are major challenges in developing countries. The device is simple to produce and can be loaded with a wide variety of medications (such as anti-inflammatory and antiproliferative agents) for further potential treatment of other eye diseases associated with eye surgeries.

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