In Vitro and In Vivo Evaluation of Novel Ciprofloxacin-Releasing Silicone Hydrogel Contact Lenses

Alex Hui,[1] Mark Willcox,[2] and Lyndon Jones[1]

[1]Centre for Contact Lens Research, School of Optometry & Vision Science, University of Waterloo, Waterloo, Ontario, Canada
[2]School of Optometry and Vision Science, University of New South Wales, Sydney, New South Wales, Australia

Correspondence: Alex Hui, Centre for Contact Lens Research, School of Optometry & Vision Science, University of Waterloo, 200 University Avenue W, Waterloo, ON, Canada N2L 3G1; a6hui@uwaterloo.ca.

Submitted: May 22, 2014
Accepted: June 29, 2014

Citation: Hui A, Willcox M, Jones L. In vitro and in vivo evaluation of novel ciprofloxacin-releasing silicone hydrogel contact lenses. *Invest Ophthalmol Vis Sci*. 2014;55:4896–4904. DOI: 10.1167/iovs.14-14855

Purpose. The purpose of this study was to evaluate ciprofloxacin-releasing silicone hydrogel contact lens materials in vitro and in vivo for the treatment of microbial keratitis.

Methods. Model silicone hydrogel contact lens materials were manufactured using a molecular imprinting technique to modify ciprofloxacin release kinetics. Various contact lens properties, including light transmission and surface wettability, were determined, and the in vitro ciprofloxacin release kinetics elucidated using fluorescence spectrophotometry. The materials then were evaluated for their ability to inhibit *Pseudomonas aeruginosa* growth in vitro and in an in vivo rabbit model of microbial keratitis.

Results. Synthesized lenses had similar material properties to commercial contact lens materials. There was a decrease in light transmission in the shorter wavelengths due to incorporation of the antibiotic, but over 80% light transmission between 400 and 700 nm. Modified materials released for more than 8 hours, significantly longer than unmodified controls ($P < 0.05$). In vivo, there was no statistically significant difference between the number of colony-forming units (CFU) recovered from corneas treated with eye drops and those treated with one of two modified contact lenses ($P > 0.05$), which is significantly less than corneas treated with unmodified control lenses or those that received no treatment at all ($P < 0.05$).

Conclusions. These novel contact lenses designed for the extended release of ciprofloxacin may be beneficial to supplement or augment future treatments of sight-threatening microbial keratitis.

Keywords: contact lens, microbial keratitis, drug delivery, molecular imprinting, ciprofloxacin

Microbial keratitis (MK), an infection of the cornea by pathogenic microorganisms, represents a true ocular emergency. Unless immediate treatment is initiated with appropriate antimicrobial agents, the probability of retaining normal vision is unlikely.[1] Epidemiological studies have identified certain risk factors for the development of MK, including male sex, younger age, overnight wear of contact lenses (CLs), smoking, poor hygiene, and internet supply of lenses.[2–4] Unfortunately, even with all of the advances in our understanding of MK and implementation of solutions to lower modifiable risk, the incidence of the disease has remained largely unchanged.[5] Contemporary treatment of patients with MK involves the frequent use of topical antibiotic agents, often fortified by a compounding pharmacy.[6] In the early stages of treatment, drop instillation as frequently as every 15 minutes is common to quickly saturate the cornea to therapeutic antibiotic levels. Even with these frequent dosing schedules, practical considerations on the pharmacokinetics of eye drops suggests that the therapeutic windows are reached only for relatively short periods of time, interspersed between times of therapeutic overdose and underdose.[7] Indeed, measurements and modeling suggest that at most only 5% to 10% of an instilled eye drop ultimately exerts therapeutic action, with the remainder flushed away and absorbed systemically.[8] This is disadvantageous economically and therapeutically, as useful molecules are lost without exerting a therapeutic effect, and systemically absorbed agents have the potential to cause side effects. Adherence to strict and frequent treatment regimens for the management of MK is understandably difficult for outpatients; thus, management often requires hospitalization, costing the health care system and individual significant amounts of time and money.[9] Economic analysis of the MK costs in Australia suggests that each case costs upwards of AUD $10,000.[10] Given these therapeutic, practical, and economic challenges, development of alternative MK therapies are warranted and may prove to be beneficial.

Even though CLs are risk factors for developing MK, the use of CLs as vehicles to deliver therapeutics to the eye has been suggested and is not a recent idea. Using hydrogels, such as CLs, as a reservoir for a drug during the treatment of anterior segment disease was proposed as early as 1965 by Sedlácek,[11] and has received renewed research interest of late.[11,12] The appeals of a CL drug delivery device are numerous. The materials have a proven track record of biocompatibility and patient and practitioner acceptance.[13] Modern manufacturing methods have driven the unit cost of each lens to affordable levels. If CLs are used in a drug delivery application, they also can simultaneously correct for refractive error, allowing for continued clarity of vision by the patient undergoing treatment. The oxygen permeability of the lenses also have increased significantly with the introduction of silicone hydrogel materials in the late 1990s, allowing for potentially extended or

overnight treatments with CLs without fear of hypoxic complications.[14] There also exists evidence that combination CL and drug delivery devices would be well accepted by eye care practitioners. In surveys of practicing optometrists and ophthalmologists in North America, a large proportion of practitioners surveyed used CLs as bandages when indicated, and crucially, concurrently also prescribed topical medications, such as antibiotics and anti-inflammatories, and would be willing to accept a lens that did both simultaneously.[15]

The contemporary challenge in the development of a successful drug delivery CL has been the drug release kinetics. Not surprisingly, off-the-shelf commercial CL materials show less than ideal drug release characteristics. The majority of lenses examined showed very rapid release kinetics when tested in vitro. Antibiotics (ciprofloxacin),[16] anti-inflammatories (ketorolac, dexamethasone),[17,18] and antiallergy agents (ketotifen fumarate)[19] have all been tested, and while differences in the absolute amount of the drug being released between commercial lens types are seen, the release time typically is limited to one or two hours. Given this restriction, the focus of research has centered on modifying, extending, and controlling release times. Numerous techniques have been investigated. For example, a group has investigated modification of commercial materials through the incorporation of a Vitamin E coating, to serve as an additional diffusion barrier for drug migration. This technique allowed for extension of release times from several minutes to several hours in vitro, and has been used to investigate release of timolol, an antiglaucoma treatment.[20] The authors were able to demonstrate improved IOP control using the experimental CL system in comparison with eye drops in a glaucomatous dog model.[21] A novel design involving a drug-impregnated film sandwiched between two hydrogel pieces also has been investigated for delivery of antibiotics and antifungals.[22,23] Use of such a system showed a significant increase in the amount of drug released and favorable release kinetics in vitro. Unfortunately, the design was limited by the optical properties of the lens, as the film used as the drug reservoir was opaque, necessitating that the lens require a small, 3-mm pupil cut in the middle of the film to be used for vision, a design that is unlikely to resonate with eye care practitioners or patients.

Molecular imprinting is a strategy that has been derived from work in chromatography. Originally, polymers created by this technique were used to preferentially remove certain components from solutions.[24] In this technique, the molecule of interest ultimately to be released is dissolved in the prepolymerization mixture.[25] Inclusion of a separate small molecule, denoted as the functional monomer, to specifically interact with the molecule of interest through noncovalent interactions, such as hydrogen bonding, creates shape-specific and functional group-specific complexes deemed "cavities" or "molecular memory" within the final polymerized product.[26] This "molecular memory" can significantly slow the movement of the drug of interest from the material; thus, extending drug release times.[12] Previous work has demonstrated that selection of the appropriate functional monomer and the ratio of the functional monomer to the template, are the most crucial aspects in generating materials with desired extended drug release properties.[27] This technique has been used successfully to increase the drug release times observed in vitro for antibiotics, anti-inflammatories, antiglaucoma, and antiallergy medications.[28–35]

In this current study, novel silicone hydrogel CLs were created using a molecular imprinting technique to increase the release times of the fluoroquinolone antibiotic ciprofloxacin. The molecule acrylic acid had been shown previously to be a useful functional monomer to increase fluoroquinolone release times.[28,34] The materials were tested for their CL properties, in vitro drug release characteristics, and sustained antibacterial activity in an in vivo rabbit model of MK.

Materials and Methods

Reagents

2-Hydroxylethlymethacrylate (HEMA), methacryloxy propyl tris (trimethylsiloxy) silane (TRIS), ethylene glycol dimethacrylate (EGDMA), acrylic acid, ciprofloxacin-HCL, Irgacure-1173, polyvinylpyrrolidone (PVP), phosphate buffered saline (PBS), and chloroform were purchased from Sigma-Aldrich (Oakville, ON, Canada). Nutrient Agar was purchased from Sigma-Aldrich PTY (Sydney, Australia). The BBL cation adjusted Mueller-Hinton II Broth and Dey/Engley Neutralizing Broth were purchased from BD Australia (North Ryde, New South Wales, Australia). Polypropelene CL molds were kindly donated from Alcon Vision Care (formerly CIBA Vision, Fort Worth, TX, USA). The polymerizer inhibitor 4-methoxyphenol (MEHQ) was removed from the HEMA and TRIS monomers by passing through a column of Aldrich inhibitor removers. All other reagents were used as received.

Molecular Imprinted CL Synthesis

Filtered HEMA (3.6 g) was mixed with 0.4 g of filtered TRIS, 0.1 g of EGDMA, and 0.3 g of PVP. To this, a 1 mL acrylic acid and ciprofloxacin solution dissolved in chloroform was added so that the final concentration of acrylic acid within the mixture was 100 mM. Control lenses were created by omitting the ciprofloxacin in the acrylic acid solution. Various ratios of acrylic acid to ciprofloxacin solutions were made, ranging from 4:1 moles of acrylic acid:ciprofloxacin, 8:1 moles acrylic acid:ciprofloxacin, and 16:1 moles acrylic acid:ciprofloxacin (hereby denoted as lens "4:1 imprinted," "8:1 imprinted," and "16:1 imprinted," respectively). Isopropanol (1 mL) was added as a diluent, and 0.04 g of the photoinitiator Irgacure 1173 added, and the solution mixed for five minutes at room temperature. Then, 100 μL of the solution were injected into plastic molds, and cured for five minutes using a UV oven (Dymax Silver EC Series UV Light Curing Flood Lamp System; Ellsworth Adhesives Canada, Stoney Creek, Ontario). The cured lenses were removed from the molds, and lenses rinsed daily with acetate buffer (pH 4.0) until no ciprofloxacin could be detected by spectrophotometry. The lenses then were soaked in isopropanol for 1 day to remove any leftover monomers, before being rinsed and stored in PBS.

Determination of Material Properties – Water Content, Wet and Dry Weight, Light Transmission, Center Thickness, Surface Wettability

The water content, and wet and dry weight of lenses were determined using the gravimetric method (Sartorius MA 100; Sartorius Canada, Inc., Mississauga, Ontario), where the change in weight as the lens was heated to 105°C over the course of 7 minutes was correlated to the water content of the lens. The center thickness of a fully hydrated lens was measured using a CL thickness gauge (Vigor Contact Lens Thickness Gauge; Vigor Optical, Carlstadt, NJ, USA). To determine the light transmission, individual lenses and 1 mL of PBS were placed into wells of a 24-well plate, and a wavelength scan from 300 to 750 nm was conducted using a plate reader (Spectramax M5 Microplate reader; Molecular Devices, Sunnyvale, CA, USA). The advancing contact angle, a measure of the surface

wettability, was determined using the sessile drop method employing the Optical Contact Analyzer (OCA; Dataphysics Instruments GmbH, Filderstadt, Germany). A fully hydrated lens was removed from the PBS soaking solution, and the surface dried on lens paper for 20 seconds before being placed on a custom-designed lens holder. Then, 5 μL of high performance liquid chromatography (HPLC) water was dispensed from a syringe, and an image of the contact of the water droplet with the lens surface after settling captured using a high speed camera.[36,37] The contact angle between the settled drop and the lens surface was analyzed using custom software (SCA 20 software, Version 2.04, Build 4; Dataphysics Instruments GmbH).

In Vitro Testing of Ciprofloxacin Release

Prepared lenses were removed from PBS and placed into 4 mL of a 0.3% (3000 μg/mL) ciprofloxacin solution prepared in acetate buffer (pH 4.0). The lenses were autoclaved, and allowed to take up ciprofloxacin from the solution for one week. After one week, the amount of ciprofloxacin loaded into the lenses was determined using fluorescence spectrophotometry in comparison with previously generated standard curves (excitation wavelength 274 nm, emission 419 nm). The lenses then were removed and the surface briefly dried on Lens Paper (VWR Scientific Products, Westchester, PA, USA) before being placed into 2 mL of PBS. Then, 100 μL of PBS was removed at set intervals over the course of 24 hours, and the concentration of ciprofloxacin determined by spectrophotometry. After 24 hours, the lenses were removed, the surface briefly dried on lens paper, and placed into a second vial with 2 mL of fresh PBS, and the time course release was again monitored for another 24 hours. This process was repeated one additional time for a third day to generate release curves.

Bacterial Strain and Growth, Minimum Inhibitory Concentration (MIC) Determination

Pseudomonas aeruginosa strain 6294, a bacterial strain previously isolated in the United States from a human case of MK,[38] was streaked on nutrient agar plates from –80°C frozen stocks and incubated at 34°C for 18 hours. A single colony was picked and grown overnight in Mueller-Hinton Broth before being centrifuged, rinsed in PBS, and resuspended in PBS to an optical density of 0.1 at 660 nm (approximately 1×10^8 colony-forming units [CFU]/mL). The MIC of the test organism was determined using the broth microdilution method.[39] *P. aeruginosa* strain 6294 (5×10^4 CFU) was added to each well of a 96-well plate, with each well containing a doubling dilution concentration of ciprofloxacin in Mueller-Hinton Broth. The plate was incubated overnight, and the turbidity of the solution in individual wells used to determine the minimum concentration of the antibiotic that prevents bacterial growth.

In Vitro Testing of Antimicrobial Activity

Test lenses were removed from the loading solution and briefly dipped in PBS before being added to 2 mL of Mueller-Hinton Broth seeded with 1×10^8 CFU/mL *P. aeruginosa*. Then, 100 μL were sampled hourly into neutralizing broth, and serial dilutions plated on nutrient agar plates. The plates were incubated at 34°C for 18 hours before counting for CFU. The lenses were removed from solution after 24 and 48 hours, briefly dipped in PBS, and placed into fresh Mueller-Hinton bacterial solutions, and the procedure repeated.

In Vivo Testing of Antimicrobial Activity – Rabbit Scratch Model of MK

All animal procedures were approved by the executive of the animal care and ethics committee at the University of New South Wales, and performed in accordance with the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research. New Zealand White rabbits (4 kg) were sourced from S&J Hurrell in Sydney, New South Wales, Australia. After acclimatization for 1 week, the nictitating membrane was removed surgically from both eyes under general anesthesia. Recovery was allowed for a minimum of one week, at which time two 5-mm central corneal scratches on one eye were induced using a 23-gauge needle under general anesthesia, and 20 μL of the *P. aeruginosa* strain 6294 solution placed on the eye (approximately 2×10^6 CFU). The eyes were held closed for 2 minutes, after which the rabbit was allowed to recover from the anesthetic before being returned to the pen. Pain control was achieved through subcutaneous injection of 0.02 mg/kg buprenorphine every 12 hours. At 16 hours after the scratch and bacterial introduction, the rabbits were assigned randomly to one of three intervention groups: intervention by hourly instillation of 3000 μg/mL ciprofloxacin drops for 8 hours, intervention by one of three types of CLs (control, 4:1 imprinted, and 8:1 imprinted) loaded in 30 μg/mL ciprofloxacin solution for 8 hours, or no intervention for 8 hours (three rabbits per treatment condition). The animals were euthanized by lethal injection of 1 mL of sodium pentobarbital intravenously 24 hours after scratch, and the cornea excised. The cornea was homogenized in neutralizing broth, and serial dilutions of the homogenate plated on nutrient agar for 18 hours at 34°C before CFU were counted.

Statistics

All statistics were performed using STATISTICA Version 7 (StatSoft, Tulsa, OK, USA). Analysis of in vitro release curves and bacterial growth curves was done using a repeated measures ANOVA, with lens type as a categorical factor, time as a within effects factor, and μg/g dry weight ciprofloxacin released or CFU as a dependent factor. Comparison of bacteria recovered from rabbit corneas or material properties was done using a 1-way ANOVA, with lens type as a categorical factor, and the measured property as a dependent factor. Post hoc Tukey tests were used as necessary. A P value of less than 0.05 was deemed to be statistically significant.

Results

Material Properties

The water content, wet and dry weight, center thickness, and advancing contact angle are summarized in Table 1.

There was no statistically significant difference in the wet weight and center thicknesses of the lenses. The 4:1 imprinted lenses were found to have dry weights statistically different than the control and 8:1 imprinted lens ($P < 0.05$). The 4:1 imprinted lens was statistically significantly different than all the other lenses in terms of water content ($P < 0.05$). All the lenses were statistically different when compared to each other with respect to contact angle ($P < 0.001$).

The light transmission of the four different lens types tested is shown in Figure 1. Increasing the amount of ciprofloxacin into the lens material led to increased yellow coloration of the lens, and, thus, greater loss of light transmission in the shorter wavelengths.

Table 1. Material Properties of Experimental Lenses

Lens Type	Wet Weight, g	Dry Weight, g	Centre Thickness, μm	Water Content, %	Advancing Contact Angle, deg
Control	0.027 (0.004)	0.016 (0.003)	63 (14)	42.3 (4.5)	94.6 (1.5)
4:1 imprinted	0.029 (0.004)	0.019 (0.003)	64 (15)	36.2 (3.4)	77.4 (2.0)
8:1 imprinted	0.028 (0.006)	0.017 (0.004)	62 (19)	43.3 (3.0)	81.5 (1.3)
16:1 imprinted	0.028 (0.005)	0.016 (0.002)	61 (19)	41.7 (3.0)	89.2 (2.0)

All values are presented as averages (SD) of a minimum of four lenses. The imprinted lenses are denoted by the ratio of the moles of the functional monomer acrylic acid to the moles of the template ciprofloxacin.

In Vitro Testing of Ciprofloxacin Release

After autoclaving and allowing uptake of ciprofloxacin from the loading solution for one week, the amount of ciprofloxacin taken into each lens type is presented in Table 2. There was no statistically significant difference between the amounts taken up by the different lenses.

The in vitro release curves over the course of three days and three releasing solutions are presented in Figures 2A through 2C. On the first release day, the control material reached a plateau concentration after three hours, while the imprinted materials released ciprofloxacin for five hours or more. The plateau concentration of the control material was higher than the imprinted materials, although this difference was not statistically significant ($P > 0.05$). On the second release day in the second release solution, the control and imprinted materials reached a plateau concentration after four hours and there were no statistically significant differences between them. In the third release medium on the third day, the control material reached a plateau concentration after a mere two hours, while the 4:1 and 8:1 imprinted materials continued to release for over eight hours. The plateau concentration reached by the 4:1 and 8:1 imprinted materials also were statistically different than the concentration reached by the control ($P < 0.05$). The plateau concentration reached by the least imprinted material, the 16:1 lens, was not statistically different than the control.

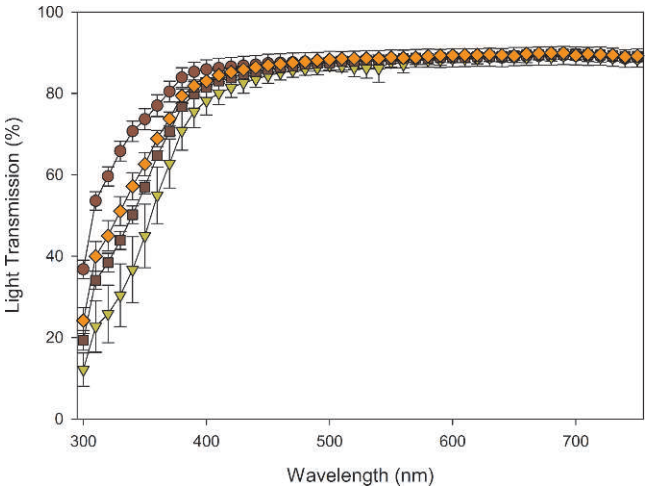

Figure 1. Percentage light transmission curves. Control (●), 4:1 imprinted (▼), 8:1 imprinted (■), and 16:1 imprinted lenses (◆). Increased incorporation of ciprofloxacin into the material leads to increased yellow coloration of the lens, and a decrease in transmission in the shorter wavelengths. *Symbols* represent averages ± SD of 3 lenses.

In Vitro Antibacterial Assays

The as tested MIC of the *P. aeruginosa* strain 6294 was 0.4 μg/mL. All lenses loaded with 0.3% ciprofloxacin were able to inhibit the growth of bacteria completely for the first two days, suggesting that inhibitory amounts of the antibiotic were being released from the lenses. The ability of the lenses to inhibit the growth of *P. aeruginosa* strain 6294 in Mueller Hinton Broth on the third day is presented in Figure 3. There was an initial decrease in concentration of bacteria as the final reserves of ciprofloxacin were released from the lenses. The rate at which the number of viable bacteria were decreasing is indicative of concentration of antibiotic in solution, suggesting that the control lens initially reaches a higher concentration than the two imprinted lenses, which correlates to the released data seen in Figure 2C. As complete inhibitory concentrations were not reached by any of the lenses, by the 8-hour time point the bacteria population began to rebound. There was a statistically significant decrease in the number of bacteria from the beginning to the end of the monitoring period for all three lenses tested ($P < 0.05$). The differences between the lenses, however, was not statistically significant ($P > 0.05$).

In Vivo Model of MK

Corneas scratched and exposed to *P. aeruginosa* strain 6294 began to show an infection response after 16 hours, characterized by development of infiltrates, discharge, and redness as shown in Figure 4B. Left untreated, the severity of the infection increased dramatically over the next 8 hours before euthanasia of the rabbit (Fig. 4C). Treatment intervention with a modified CL at the 16-hour point partially resolved the infiltrate or discharge by the 24-hour point (Fig. 4D).

The number of CFU recovered from excised and homogenized infected corneas are presented in Figure 5. Left untreated, approximately 10^6 CFU per cornea were recovered, while treatment with hourly instillation of ciprofloxacin eye drops led to complete sterilization and lack of any recoverable bacteria from the cornea after only 8 hours. Treatment with lenses soaked in only 30 μg/mL ciprofloxacin solutions (100 times less than the clinical drops) led to differences in bacterial recovery. The number of bacteria recovered from corneas treated with the control (i.e., no molecular imprinting) lenses that had been soaked in ciprofloxacin was not significantly

Table 2. Uptake of Ciprofloxacin Into Each of the Four Tested Lenses

Lens Type	Ciprofloxacin Loaded, μg/lens, Mean (SD)
Control	1383 (144)
4:1 imprinted	1509 (291)
8:1 imprinted	1133 (264)
16:1 imprinted	1234 (295)

All values are presented as averages (SD) of a minimum of three lenses.

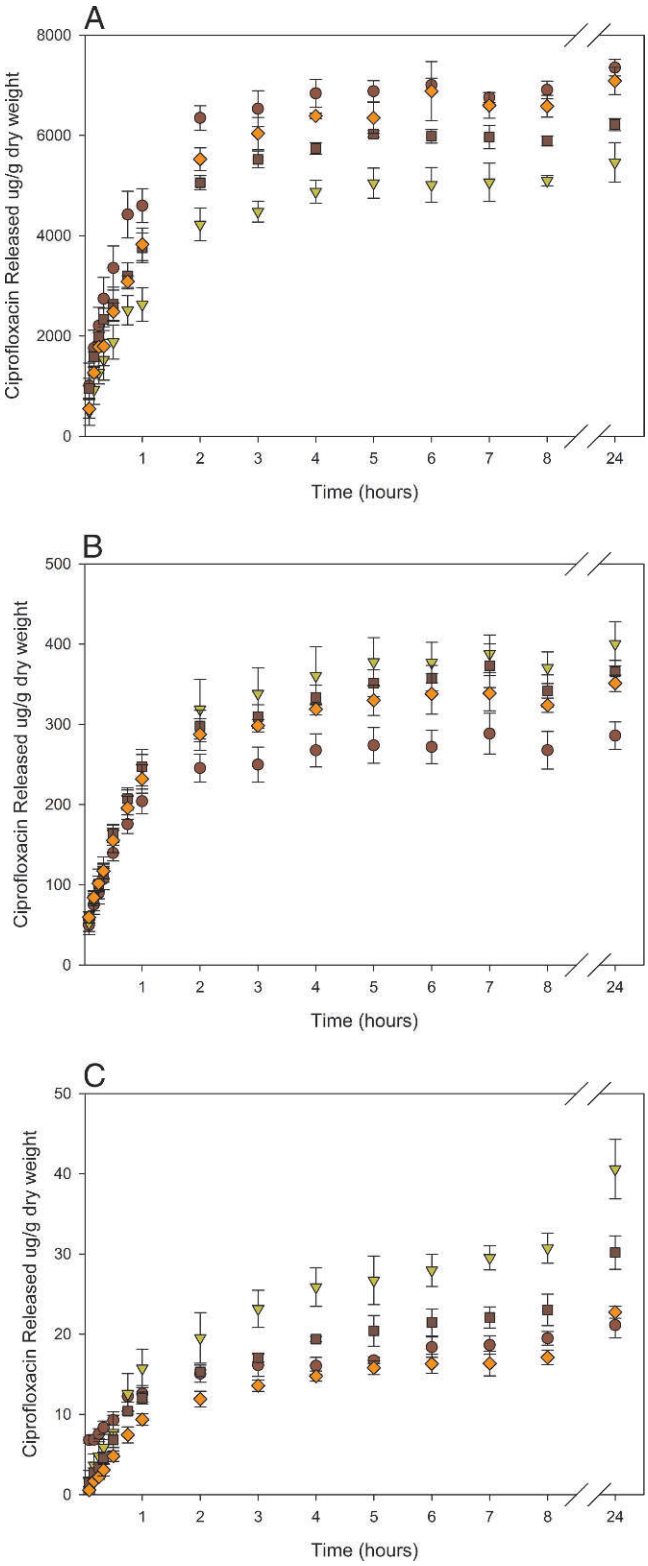

Figure 2. In vitro ciprofloxacin release. Control (●), 4:1 imprinted (▼), 8:1 imprinted (■), and 16:1 imprinted lenses (◆) on Day 1 (**A**), Day 2 (**B**), and Day 3 (**C**) after loading in a 0.3% ciprofloxacin solution for 1 week. *Symbols* represent averages ± SD of at least 4 lenses.

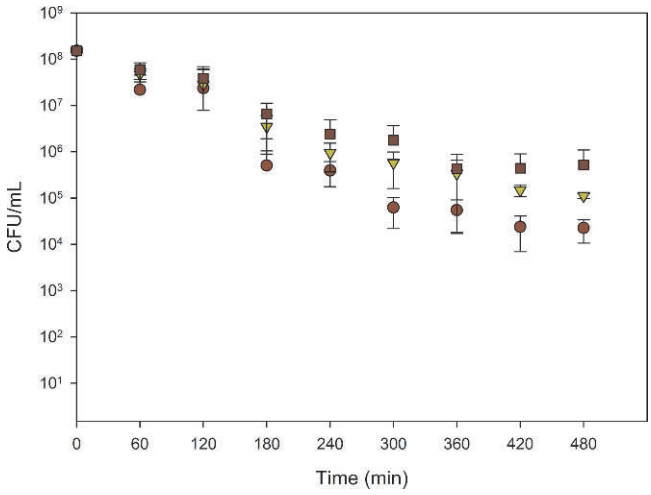

Figure 3. *P. aeruginosa* strain 6294 growth curves in presence of ciprofloxacin releasing CLs. No viable bacteria were recovered from the first two growth media on the first 2 days, as sufficient antibiotic concentrations were reached in solution. The presented *curves* are from the third bacterial solution on the third day, after a significant amount of antibiotic was already released from the lenses. As inhibitory concentrations were not reached, by the 8-hour time point the bacteria numbers are beginning to recover and growth is beginning to increase. Control (●), 4:1 imprinted (▼), 8:1 imprinted (■). Note exponential scale.

different than that of the nontreated control lenses ($P > 0.05$). However, there is a significant reduction in the number of recoverable bacteria from the corneas treated with the slow release, molecularly-imprinted lenses ($P < 0.05$ when compared to untreated control or untreated corneas). Many of the corneas treated with the imprinted lenses were rendered sterile through treatment, and overall no statistically significant difference was found in the number of bacteria recovered from those corneas and corneas treated with antibiotic eye drops ($P > 0.05$).

Discussion

The challenge in the development of CL drug delivery devices remains the relevant drug release kinetics. Previous investigations into drug release from commercially available materials demonstrated less than clinically useful drug release times (Ref. 40 and Karlgard C, et al. *IOVS* 2001;42:ARVO E-Abstract 592), prompting the need for custom design lenses to be developed. As seen from the drug release curves presented in Figure 2, by using a molecular imprinting technique the ciprofloxacin release profiles from CL materials were significantly altered. By incorporating acrylic acid as a functional monomer within the prepolymerization mixture in various ratios to ciprofloxacin, materials were modified to release the antibiotic at various rates, with a ratio of 4:1 functional monomer-to-template molecule showing the greatest extension of release times. The influence of the ratio of functional monomer to the template on the efficiency of molecular imprinting has been presented in the literature.[41] Away from the optimum monomer-to-template ratio, cavities created within the polymerization structure will be inadequately or inefficiently created, and, thus, shift the equilibrium toward disassociation and release of the template, leading to faster release times when release studies are performed in vitro.[41] That the 4:1 ratio was shown to be the most effective in slowing the release of ciprofloxacin is not surprising, as the ratio had been demonstrated

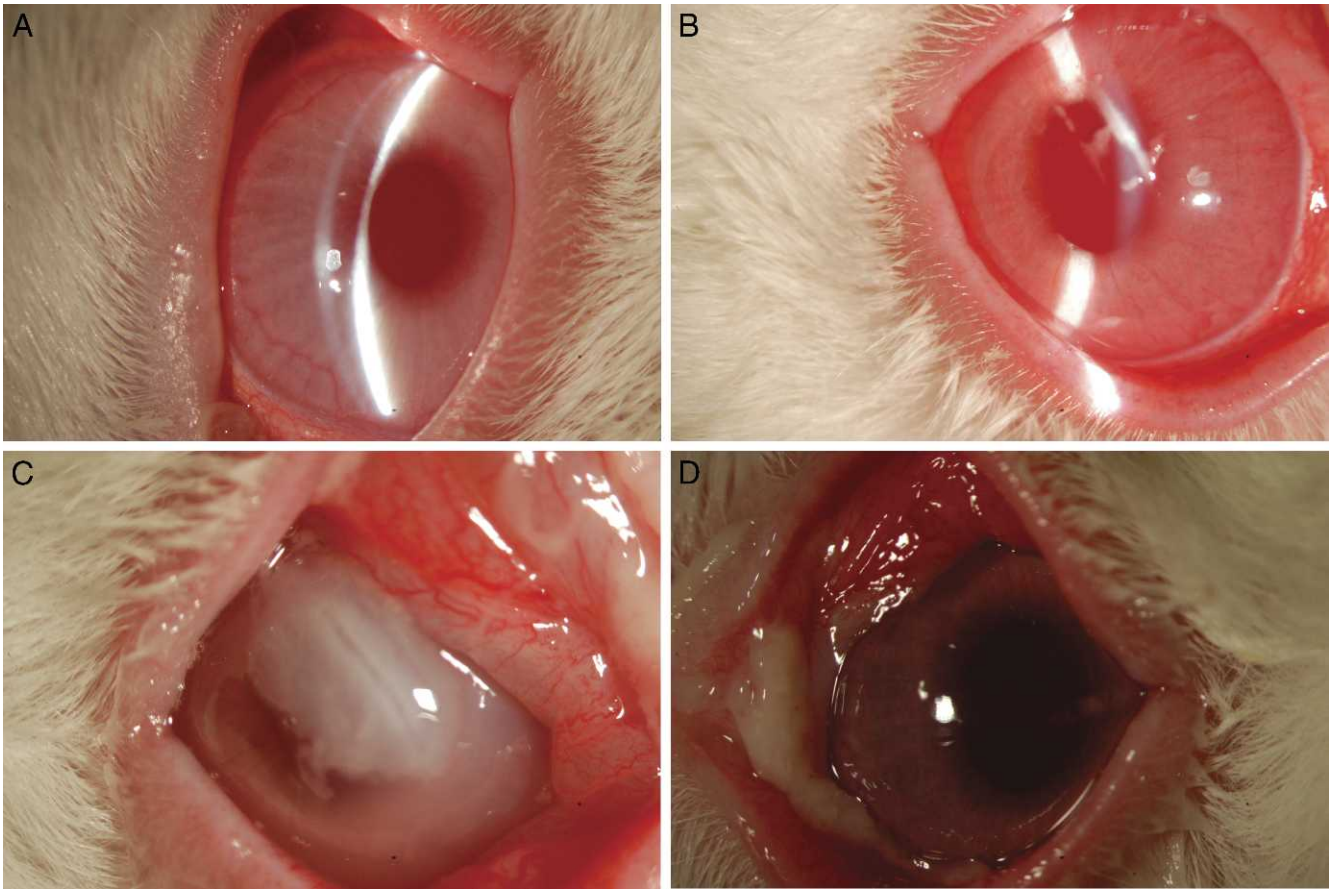

FIGURE 4. Rabbit model of MK. (**A**) Cornea appearance before corneal scratching and bacteria introduction. (**B**) Cornea appearance 16 hours after scratch, showing infiltrate, redness, and discharge. (**C**) Cornea appearance 24 hours after bacteria introduction without treatment showing a large increase in size and severity of the MK. (**D**) Cornea treated with experimental CL for 8 hours 16 hours after bacteria introduction.

previously as most effective in experiments with molecular imprinting and norfloxacin, a first generation fluoroquinolone.[28] Through use of isothermal titration calorimetry, a saturation in the binding of norfloxacin within the hydrogels at a functional monomer-to-template ratio of 4:1 was observed, and, thus, would be the ratio predicted to most perfectly create the imprinted cavities and most prolong release times.[28] This prediction was demonstrated by the norfloxacin release data, as ratios above or below 4:1 did not as effectively control norfloxacin release.[28] This experiment was an improvement to previous in vitro experiments[34] in that the releasing medium was changed on a daily basis to better simulate the changing concentration gradients that are likely to be seen if these materials were placed on the eye. On the initial day, the unmodified control lenses released a very high concentration of drug, while the modified materials released for longer periods, but reached lower final concentrations. As the release medium solutions were changed, the advantage of the molecular-imprinted materials began be more apparent. The control material continued to release extremely rapidly, and reached lower plateaus than the modified materials. This was best exemplified by the data from the third releasing medium on the third day, when the control material reached a fast plateau of ciprofloxacin concentration within 2 hours, while the 4:1 material continued to release for more than 8 hours, and reached a significantly higher concentration in solution.

In vitro testing of the antibacterial activity of the test materials served as a complement to the release of ciprofloxacin in solution. Here, the differences in the recovered bacteria

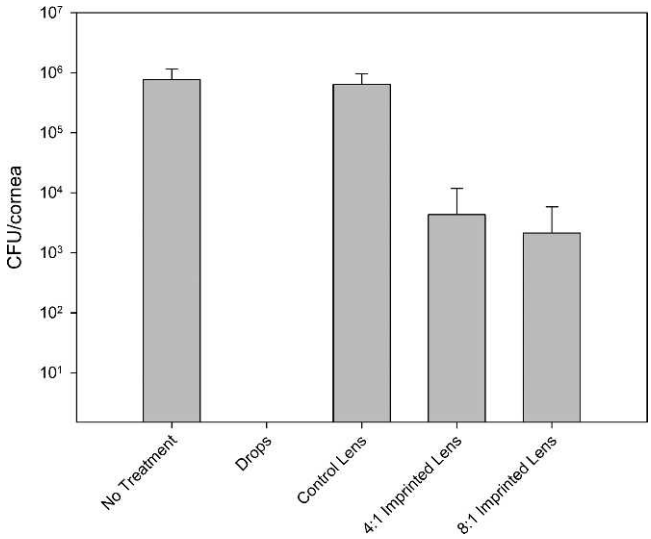

FIGURE 5. Bacteria recovered from excised, homogenized corneas. The bacteria were recovered 24 hours after corneal scratch and introduction of *P. aeruginosa* strain 6294, and 8 hours of different treatment conditions. Corneas treated with drops had 10 μL of a 3000 μg/mL ciprofloxacin solution instilled hourly. Lenses used in the treatment were presoaked and autoclaved in a 30 μg/mL solution of ciprofloxacin. The numbers of bacteria recovered from the no treatment and control lens treatments were significantly different than those treated with ciprofloxacin eye drops or 4:1 or 8:1 imprinted lenses ($P < 0.05$, $n = 3$ for each treatment group). Note exponential scale.

were seen as a surrogate of the amount of ciprofloxacin released. The test organism, *P. aeruginosa* strain 6294, is ciprofloxacin-sensitive, with a MIC of 0.4 μg/mL. The growth of the bacteria within the media is a function of several factors – not only the concentration of ciprofloxacin within the solution, but also the initial seeding concentration of bacteria, the growth phase of the bacteria, and the availability of resources, including nutrients and oxygen.[42–44] The plotted growth curves in Figure 3 reflect all of these factors simultaneously. Several conclusions can be reached by considering the growth of bacteria in the presence of these lenses. First, each of the lenses were initially inhibiting the growth of the bacteria, presumably due to release of the antibiotic. Second, inhibition of growth by these lenses waned over time. By the 8-hour mark, the bacterial population stabilized or started to grow as the limited amount of ciprofloxacin released from the lenses was exhausted or insufficient to prevent multiplication, leaving bacterial growth limited only by available resources. Third, while the differences between the different lenses were not statistically significant, examination of the different growth curves can be suggestive of the effect of imprinting. The rate at which the population growth was reduced by the lenses is reflective of the concentration of the antibiotic in the solution. The control lens, as previously demonstrated, released the majority of the available ciprofloxacin very quickly, and reached higher concentrations of antibiotic in solution faster than the two slow release materials (Fig. 2C). Thus, the decrease in bacterial concentration from systems treated with the control material are expected to be faster than when treated with the two slow release lenses, which is what is seen (Fig. 3). Thus, within a closed, fixed in vitro solution, the control lens released ciprofloxacin, which reached a high concentration quickly and can be considered to be a superior lens used to control bacterial growth.

In vitro testing of antibacterial activity, unfortunately, is an inadequate model for in vivo applications. In the controlled, closed system of a test tube or vial, the bacteria were exposed to all of the antibiotic released from the experimental materials, which would kill the bacteria cells. This was regardless of the rate at which the antibiotic was being released. This is in contrast to what occurs when antibiotic drops or lenses are placed on the ocular surface, where pharmacokinetic factors, including tear production and drainage, epithelial/corneal penetration, and drug metabolism are significant factors in the amount of drug exerting an effect. If the eye was a closed system, then the fast burst release from a control lens could be advantageous and quickly raise drug concentrations to effective levels. Unfortunately, because of tear drainage and corneal cellular barriers, it is likely that much of the antibiotic released in such a burst fashion will very quickly be cleared from the ocular surface, limiting their usefulness, which is why frequent dosing with eye drops is necessary. In contrast, with a sustained release CL supplying the antibiotic, a continual replenishment of the antibiotic is possible. A fast rise to a high concentration is less likely, but over time there is greater potential for therapeutic concentrations to be reached, and more importantly, for them to be sustained over a longer period of time. This is exemplified by the in vivo results seen in Figure 5. Even with the superior performance of the control lens (i.e., normal lenses soaked in ciprofloxacin) against the bacteria in vitro, this superiority did not translate in the in vivo rabbit model. The control lens did not appreciably impact the number of recoverable bacteria compared to no treatment, presumably because all of the antibiotic was released at once, and any of the antibiotic not absorbed was quickly drained away. In contrast, the two imprinted lenses performed significantly better in reducing the number of recoverable bacteria, as the reserves of ciprofloxacin were released slowly over time and could replenish lost drug that was drained away from the surface.

If the field of CL drug delivery is to continue and eventually be accepted by practitioners and patients alike, the wearer experience must be similar to regular CLs on the market. The optical transmission in the visible range must be acceptable for wear in day-to-day life, the water content and wettability must remain within a certain narrow set of parameters to ensure acceptable comfort during wear, and the amount of oxygen being transmitted must be adequate to prevent complications. As shown from the results of our experimental lenses, while not surface-treated, they had acceptable wettability measures in line with other nonsurface-treated silicone hydrogels that incorporate an internal wetting agent, such as polyvinylpyrrolidone.[45] The light transmission in the visible range is acceptable, other than slight tinting of the lenses due to ciprofloxacin drug incorporation causing a mild yellow coloration. The center thickness and water contents also were in line with commercial CLs. The incorporation of silicone monomers into the material will allow for superior oxygen transmission properties. Between the lenses, there were some significant differences in water content and advancing contact angle. The lenses that incorporated the greatest amount of ciprofloxacin in the imprinting process, the 4:1 imprinted lenses, also had the lowest water content. There also were significant differences in the advancing contact angle, with a trend toward lower contact angles as more ciprofloxacin was used. Considering that the only difference in the synthesis of all of the lenses is the amount of ciprofloxacin added, it can be surmised that this difference in water content and advancing contact angle is due to irreversible binding of some ciprofloxacin within the materials during synthesis. The continued yellow coloration of the CLs that had ciprofloxacin incorporation during the molecular imprinting process would lend credence to this theory. The permanently-bound ciprofloxacin is not expected to have had a significant effect on the ciprofloxacin release characteristics from these lenses.

In this study, the ultimate test of the effectiveness of the modified CL drug delivery device was performance in an in vivo model of MK in New Zealand White rabbits. The use of rabbits as a model for MK is well known, as they have an adequate eye size to allow for CL wear.[46] The methods and selection of bacteria for infection also are critical. Classically, to achieve infections of the cornea, animal models of keratitis have required either passing of a silk suture soaked in a bacterial solution into the corneal stroma or direct injection into the corneal stroma of a bacterial solution to get a consistent and repeatable keratitis response.[46] The method chosen in this study involved the creation of a superficial scratch through the epithelium of the rabbit cornea before exposure to a bacterial solution. This method mimicked to some extent the CL rabbit model of Hume et al.,[47] but without the need to add spermidine.[47] Usage of a highly virulent strain of bacteria, *P. aeruginosa* strain 6294 allowed for consistent keratitis responses to be seen under these experimental conditions. The timing of the treatment also was carefully chosen for two separate reasons. A total of 16 hours was allowed to pass so that the MK response could be seen. It also was chosen to mimic a more real-world situation, where a patient may be reluctant to seek treatment after the initial insult, and rather chooses to delay medical attention until the condition and symptoms had significantly worsened. We were limited ethically to an experiment of no more than 24 hours to prevent significant pain, suffering, and distress to the experimental animals. As is evident by the data (Fig. 5), corneas treated with ciprofloxacin eye drops were rendered sterile after only the short 8-hour treatment time frame.

Investigative Ophthalmology & Visual Science

Indeed, this also was seen in treatment trials with the molecularly imprinted CLs, as 2 of the 3 corneas in both of the modified lens trials also were rendered sterile. However, the clinical picture at this time does not reflect the sterility of the cornea as all eyes at the 24-hour time point regardless of treatment type continued to show significant infiltrates, redness, edema, and discharge, although the severity varied between the different treatment conditions. If the study could have continued for longer, an alternative, more clinically relevant outcome measure to recoverable bacteria could have been used, such as time to resolution of the infiltrate and/or re-epithelialization of the corneal surface. The sterility of the corneas also is in contrast to what often is seen in the experimental trials of novel antibiotic drops. For example, in a recent trial testing the efficacy of a new fluoroquinolone antibiotic drop, treatment with the new antibiotic (and other commercially available antibiotics) did not completely sterilize the cornea, rather it merely significantly impacted the number of bacteria recovered compared to nontreatment controls.[48] The difference observed in this trial likely stems from the method of infection used. In antibiotic drop efficacy studies, corneal infection generally are achieved using an intrastromal injection of the offending organism. In contrast, in the current study infection was preceded by a corneal scratch and break in the corneal epithelium. This break in the epithelium can provide an avenue for the antibiotic to reach the microorganisms, while in intrastromal injection models, the antibiotic must traverse through the significant intact epithelial barrier. The performance of the unmodified control lenses in comparison with the treatment with eye drops is illustrative of the dosing needed to eradicate the bacterial organisms. Eye drop therapy was able to sterilize the corneas, but only after repeated instillations over time to ensure that an adequate amount of the antibiotic reaches the ocular structures. Based on its in vitro release kinetics, the control lenses release ciprofloxacin as a very quick initial burst, and any of the drug that is not absorbed is presumably lost. In this manner, the dosing provided by application of a ciprofloxacin-loaded, unmodified control lens behaves much like a single eye drop instillation. Thus, for the control lens to be effective in eradicating bacterial growth, the application of the CL would need to follow the schedule seen with eye drops. Repeated removal of worn lenses and replacement with loaded lenses would have been necessary to provide the proper dose, negating any practical advantages of the drug delivery system.

Recently, there has been a report by a research group demonstrating the feasibility of an extended antibiotic-releasing CL for the prevention of ocular infections.[49] In their model of bacterial endophthalmitis, infection was achieved through anterior chamber injection of a methicillin-resistant strain of *Staphylococcus aureus*.[49] Untreated, after 24 hours approximately 10^5 CFU/mL of bacteria were recovered from the experimental eyes, while treatment with topical fluoroquinolone eye drops only reduced the number of recovered bacteria to approximately 10^4 CFU/mL. In contrast, immediate treatment after bacterial injection with their experimental gatifloxacin-releasing CLs completely prevented growth of microorganisms, proving the utility of their lenses in potentially preventing postoperative infections of the globe. The results of the current study extend the application of antibiotic-releasing CLs even further, with the aim of treatment of infection rather than mere prevention. Delay in treatment of the exposed animals with CLs or eye drops allows for the clinical signs and symptoms of an infection to occur, framing the results from these trials in the context of treatment of ocular infections rather than prevention.

In conclusion, in this study, the development of a slow release ciprofloxacin CL system was achieved using a molecular imprinting strategy. Evaluations in vitro show the potential of these materials to release clinically relevant amounts of the antibiotic while retaining critically important CL material properties, and evidence from in vivo testing show that they can perform similarly to antibiotic drop therapy in models of MK. Application of these materials may be useful for future treatment paradigms of MK.

Acknowledgments

The authors thank Ajay Vijay and Denise Lawler at the University of New South Wales for their aid in performing the animal studies.

Supported by the Natural Science and Engineering Research Council of Canada (NSERC) 20/20 Network for the Development of Advanced Ophthalmic Materials, and by an NSERC Alexander Graham Bell Doctoral Scholarship, the Ezell Fellowship from the American Optometric Foundation, and the Endeavour Research Grant from the Australian Government (AH).

Disclosure: **A. Hui**, CIBA Vision (F), Alcon (C, R), Bausch & Lomb (C, R), Innova (C, R); **M. Willcox**, CIBA Vision (F, C), Allergan (R), Bausch & Lomb (R), CooperVision (R), J&J Vision Care (R); **L. Jones**, CIBA Vision (F, C, R), Abbott Medical Optics (C, R), Advanced Vision Research (C, R), Alcon (C, R), AlgiPharma (C, R), Allergan (C, R), Bausch & Lomb (C, R), CooperVision (C, R), Essilor (C, R), J&J Vision Care (C, R), Oculus (C, R), TearScience (C, R), Visioneering Technologies (C, R)

References

1. Keay L, Stapleton F. Development and evaluation of evidence-based guidelines on contact lens-related microbial keratitis. *Cont Lens Anterior Eye*. 2008;31:3–12.
2. Keay L, Stapleton F, Schein O. Epidemiology of contact lens-related inflammation and microbial keratitis: a 20-year perspective. *Eye Contact Lens*. 2007;33:346–353, discussion 362–343.
3. Stapleton F, Carnt N. Contact lens-related microbial keratitis: how have epidemiology and genetics helped us with pathogenesis and prophylaxis? *Eye (Lond)*. 2012;26:185–193.
4. Edwards K, Keay L, Naduvilath T, Snibson G, Taylor H, Stapleton F. Characteristics of and risk factors for contact lens-related microbial keratitis in a tertiary referral hospital. *Eye (Lond)*. 2009;23:153–160.
5. Stapleton F, Keay L, Edwards K, et al. The incidence of contact lens-related microbial keratitis in Australia. *Ophthalmology*. 2008;115:1655–1662.
6. Young AL, Leung KS, Tsim N, Hui M, Jhanji V. Risk factors, microbiological profile, and treatment outcomes of pediatric microbial keratitis in a tertiary care hospital in Hong Kong. *Am J Ophthalmol*. 2013;156:1040–1044.
7. White CJ, Byrne ME. Molecularly imprinted therapeutic contact lenses. *Expert Opin Drug Deliv*. 2010;7:765.
8. Ghate D, Edelhauser HF. Barriers to glaucoma drug delivery. *J Glaucoma*. 2008;17:147–156.
9. Saeed A, D'Arcy F, Stack J, Collum LM, Power W, Beatty S. Risk factors, microbiological findings, and clinical outcomes in cases of microbial keratitis admitted to a tertiary referral center in ireland. *Cornea*. 2009;28:285–292.
10. Keay L, Edwards K, Dart J, Stapleton F. Grading contact lens-related microbial keratitis: relevance to disease burden. *Optom Vis Sci*. 2008;85:531–537.
11. Sedlácek J. Possibility of the application of ophthalmic drugs with the use of gel contact lenses. *Ceskoslovenska oftalmologie*. 1965;21:509–512.
12. Sellergren B, Allender CJ. Molecularly imprinted polymers: a bridge to advanced drug delivery. *AdvDrug DelivRev*. 2005;57:1733–1741.

13. Sulley A. Practitioner and patient acceptance of a new silicone hydrogel contact lens. *Optician*. 2005;230:15–17.
14. Covey M, Sweeney DF, Terry R, Sankaridurg PR, Holden BA. Hypoxic effects on the anterior eye of high-Dk soft contact lens wearers are negligible. *Optom Vis Sci*. 2001;78:95–99.
15. Karlgard CC, Jones LW, Moresoli C. Survey of bandage lens use in North America, October-December 2002. *Eye Contact Lens*. 2004;30:25–30.
16. Hui A, Boone A, Jones L. Uptake and release of ciprofloxacin-HCl from conventional and silicone hydrogel contact lens materials. *Eye Contact Lens*. 2008;34:266.
17. Boone A, Hui A, Jones L. Uptake and release of dexamethasone phosphate from silicone hydrogel and group I, II, and IV hydrogel contact lenses. *Eye Contact Lens*. 2009;35:260.
18. Karlgard CCS, Wong NS, Jones LW, Moresoli C. In vitro uptake and release studies of ocular pharmaceutical agents by silicon-containing and *P*-HEMA hydrogel contact lens materials. *Int J Pharm*. 2003;257:141–151.
19. Soluri A, Hui A, Jones L. Delivery of ketotifen fumarate by commercial contact lens materials. *Optom Vis Sci*. 2012;89: 1140–1149.
20. Peng CC, Kim J, Chauhan A. Extended delivery of hydrophilic drugs from silicone-hydrogel contact lenses containing vitamin E diffusion barriers. *Biomaterials*. 2010;31:4032–4047.
21. Peng CC, Ben-Shlomo A, MacKay EO, Plummer CE, Chauhan A. Drug delivery by contact lens in spontaneously glaucomatous dogs. *Curr Eye Res*. 2012;37:204–211.
22. Ciolino JB, Hoare TR, Iwata NG, et al. A drug-eluting contact lens. *Invest Ophthalmol Vis Sci*. 2009;50:3346.
23. Ciolino JB, Hudson SP, Mobbs AN, et al. A prototype antifungal contact lens. *Invest Ophthalmol Vis Sci*. 2011;52:6286–6291.
24. Alvarez-Lorenzo C, Concheiro A. Molecularly imprinted polymers for drug delivery. *J Chromatogr B: Analyt Technol Biomed Life Sci*. 2004;804:231–245.
25. Byrne ME, Park K, Peppas NA. Molecular imprinting within hydrogels. *Adv Drug Deliv Rev*. 2002;54:149–161.
26. White CJ, Tieppo A, Byrne ME. Controlled drug release from contact lenses: A comprehensive review from 1965-present. *J Drug Deliv Sci Technol*. 2011;21:369–384.
27. Hiratani H, Alvarez-Lorenzo C. The nature of backbone monomers determines the performance of imprinted soft contact lenses as timolol drug delivery systems. *Biomaterials*. 2004;25:1105–1113.
28. Alvarez-Lorenzo C, Yañez F, Barreiro-Iglesias R, Concheiro A. Imprinted soft contact lenses as norfloxacin delivery systems. *J Controlled Release*. 2006;113:236–244.
29. White CJ, McBride MK, Pate KM, Tieppo A, Byrne ME. Extended release of high molecular weight hydroxypropyl methylcellulose from molecularly imprinted, extended wear silicone hydrogel contact lenses. *Biomaterials*. 2011;32:5698–5705.
30. Malaekeh-Nikouei B, Ghaeni FA, Motamedshariaty VS, Mohajeri SA. Controlled release of prednisolone acetate from molecularly imprinted hydrogel contact lenses. *J Appl Polymer Sci*. 2012;126:387–394.
31. Hiratani H, Alvarez-Lorenzo C. Timolol uptake and release by imprinted soft contact lenses made of N,N-diethylacrylamide and methacrylic acid. *J Control Release*. 2002;83:223–230.
32. Ali M, Byrne ME. Controlled release of high molecular weight hyaluronic acid from molecularly imprinted hydrogel contact lenses. *PharmRes*. 2009;26:714–726.
33. Ali M, Horikawa S, Venkatesh S, Saha J, Hong JW, Byrne ME. Zero-order therapeutic release from imprinted hydrogel contact lenses within in vitro physiological ocular tear flow. *J Control Release*. 2007;124:154–162.
34. Hui A, Sheardown H, Jones L. Acetic and acrylic acid molecular imprinted model silicone hydrogel materials for ciprofloxacin-HCl delivery. *Materials*. 2012;5:85–107.
35. Malaekeh-Nikouei B, Vahabzadeh SA, Mohajeri SA. Preparation of a molecularly imprinted soft contact lens as a new ocular drug delivery system for dorzolamide. *Curr Drug Deliv*. 2013; 10:279–285.
36. Menzies KL, Jones L. The impact of contact angle on the biocompatibility of biomaterials. *Optom Vis Sci*. 2010;87:387–399.
37. Menzies KL, Rogers R, Jones L. In vitro contact angle analysis and physical properties of blister pack solutions of daily disposable contact lenses. *Eye Contact Lens*. 2010;36:10–18.
38. Cole N, Willcox MD, Fleiszig SM, et al. Different strains of Pseudomonas aeruginosa isolated from ocular infections or inflammation display distinct corneal pathologies in an animal model. *Curr Eye Res*. 1998;17:730–735.
39. Tenover FC. Antibiotic Susceptibility Testing. In: Moselio S, ed. *Encyclopedia of Microbiology*, third ed. Oxford, UK: Academic Press; 2009:67–77.
40. Karlgard C, Jones L, Moresoli C. Uptake and release of Acular from silicone-hydrogel and conventional hydrogel contact lens materials. *Optom Vis Sci*. 2000;77:179.
41. Hiratani H, Mizutani Y, Alvarez-Lorenzo C. Controlling drug release from imprinted hydrogels by modifying the characteristics of the imprinted cavities. *Macromol Biosci*. 2005;5:728–733.
42. Drusano GL. Antimicrobial pharmacodynamics: critical interactions of 'bug and drug'. *Nat Rev Microbiol*. 2004;2:289–300.
43. Nightingale J. Clinical limitations of in vitro testing of microorganism susceptibility. *Am J Hosp Pharm*. 1987;44: 131–137.
44. Parry MF, Neu HC. Effect of N acetylcysteine on antibiotic activity and bacterial growth in vitro. *J Clin Microbiol*. 1977;5: 58–61.
45. Menzies KL, Rogers R, Jones L. In vitro contact angle analysis and physical properties of blister pack solutions of daily disposable contact lenses. *Eye Contact Lens*. 2010;36:10–18.
46. Marquart ME. Animal models of bacterial Keratitis. *J Biomed Biotechnol*. 2011;2011:680642.
47. Hume EB, Dajcs JJ, Moreau JM, Sloop GD, Willcox MD, O'Callaghan RJ. Staphylococcus corneal virulence in a new topical model of infection. *Invest Ophthalmol Vis Sci*. 2001; 42:2904–2908.
48. Wu X, Jiang H, Xu Y, et al. Efficacy of gemifloxacin for the treatment of experimental staphylococcus aureus keratitis. *J Ocular Pharmacol Ther*. 2012;28:420–427.
49. Kakisu K, Matsunaga T, Kobayakawa S, Sato T, Tochikubo T. Development and efficacy of a drug-releasing soft contact lens. *Invest Ophthalmol Vis Sci*. 2013;54:2551–2561.

Investigative Ophthalmology & Visual Science