1040-5488/14/9105-0570/0 VOL. 91, NO. 5, PP. 570–581
OPTOMETRY AND VISION SCIENCE

ORIGINAL ARTICLE

Biocompatibility of Antimicrobial Melimine Lenses: Rabbit and Human Studies

Debarun Dutta*, Jerome Ozkan*, and Mark D. P. Willcox†

ABSTRACT

Purpose. Covalent immobilization of antimicrobial peptide melimine onto contact lenses can produce broad-spectrum antimicrobial lenses. The purpose of this study was to investigate the performance of melimine-coated contact lenses in an animal model and human clinical trial.

Methods. Melimine was covalently attached onto the surface of contact lenses via EDC (1-ethyl-3-[3-dimethylaminopropyl] carbodiimide hydrochloride) coupling. A rabbit model of daily contralateral wear of lenses for 22 days was conducted to assess the lens safety. A prospective, randomized, double-masked, one-day human clinical trial was used to evaluate subjective responses and ocular physiology during contralateral wear of melimine-coated (test) and uncoated (control) lenses. Delayed reactions were monitored during follow-up visits after 1 and 4 weeks. *Ex vivo* retention of antimicrobial activity of worn lenses was assessed by reduction in numbers of viable *Pseudomonas aeruginosa* and *Staphylococcus aureus*.

Results. Melimine-coated lenses produced no ocular signs or symptoms that would indicate cytotoxicity during the lens wear of rabbits. No histological changes were found in rabbit corneas. During the human trial, no differences were observed in wettability, surface deposition, lens-fitting centration, movement, tightness, and corneal coverage between test and control lenses ($p > 0.05$). There were no significant differences in bulbar, limbal, or palpebral redness or conjunctival staining ($p > 0.05$). Mean corneal (extent, depth, and type) staining was higher for test lenses compared with that for control lenses ($p < 0.05$). There was no significant difference in subjective responses for lens comfort, dryness, and awareness ($p > 0.05$). No delayed reactions were associated with the test lenses. Worn test lenses retained more than 1.5 log inhibition against both bacterial types.

Conclusions. Melimine-coated contact lenses were worn safely by humans. However, they were associated with higher corneal staining. The melimine-coated lenses retained high antibacterial activity after wear.

(Optom Vis Sci 2014;91:570–581)

Key Words: peptide, contact lens, biocompatibility, melimine, antimicrobial activity

Microbial contamination of contact lenses during wear is closely associated with ocular inflammation such as contact lens–induced acute red eye (CLARE),[1,2] contact lens peripheral ulcer (CLPU),[3] and infiltrative keratitis.[4] Although rare, microbial keratitis (MK) is a sight-threatening contact lens–related infection.[5–7] These continue to be an ongoing problem with contact lens wear for wearers and practitioners alike. A contact lens with high antimicrobial activity may inhibit microbial adhesion and consequently reduce these contact lens–related adverse events.

Antimicrobial peptides (AMPs) are small peptides and a part of the innate immune system of all multicellular organisms, with the native ability to inhibit microbial growth.[8–13] Although more than 800 to 1000 AMPs have been discovered to date,[14,15] only a few have been tested on animals and humans.[16] Lipsky et al.[17] evaluated pexiganan acetate cream to treat mildly infected diabetic foot ulcers in comparison with systemic ofloxacin and showed that the topical AMP cream was an effective alternative. Another phase III trial demonstrated that the use of omiganan was associated with significant reductions in catheter-related infections.[18]

Previous studies have shown that melimine, prepared by combining active regions of protamine (from salmon sperm) and melittin (from bee venom), is a broad-spectrum AMP.[19,20] Covalently bound melimine on contact lenses has demonstrated high activity against a range of microorganisms, including fungi, *Acanthamoeba*, and various strains of multidrug-resistant bacteria.[21] The coating is heat stable and not toxic to mammalian cells *in vitro*.[19,21] In addition to its broad-spectrum antimicrobial activity,[21] the coating was also capable of reducing the severity and incidence of CLPU and CLARE in animal studies.[22] Thus, it would be worthwhile to

*BOptom
†PhD, FAAO
School of Optometry and Vision Science, The University of New South Wales, Sydney, New South Wales, Australia (DD, JO, MDPW); and Brien Holden Vision Institute, Sydney, New South Wales, Australia (DD, JO).

investigate the *in vivo* biocompatibility of the broad-spectrum antimicrobial contact lens in a rabbit model following the guidelines of the International Organization for Standardization (ISO)[23] and in a human clinical trial.

METHODS

Covalent Attachment of Melimine to Contact Lenses

Melimine (T-L-I-S-W-I-K-N-K-R-K-Q-R-P-R-V-S-R-R-R-R-R-R-G-G-R-R-R-R) was synthesized by conventional solid-phase peptide synthesis by the American Peptide Company (CA, USA). Peptides with more than 80% purity were used in this study. Detailed procedures for covalent attachment of melimine onto contact lenses have been explained by Dutta et al.[21] Briefly, etafilcon A lenses (base curve, 8.7 mm; diameter, 14.0 mm; Johnson & Johnson Vision Care Inc., Jacksonville, FL) were used for this study. Melimine was covalently bound to the contact lenses via EDC (1-ethyl-3-[3-dimethylaminopropyl] carbodiimide hydrochloride) coupling. Lenses were regularly washed with phosphate buffered saline (PBS) pH 7.4 (NaCl 8 g L^{-1}, KCl 0.2 g L^{-1}, Na_2HPO_4 1.15 g L^{-1}, KH_2PO_4 0.2 g L^{-1}) and incubated overnight with 10% wt/vol NaCl followed by soaking in PBS for 2 hours to remove any dissolved melimine remaining in the lens matrix. Subsequently, lenses were autoclaved (121°C) in PBS for 15 minutes. This covalent technique was able to attach 152 μg melimine onto contact lenses.[21] Uncoated etafilcon A lenses were used as controls. Ten unused melimine-coated contact lenses were used as comparator to worn melimine-coated lenses during testing for retention of antimicrobial activity. To facilitate masking of the contact lens types during the trials, control lenses were carefully removed from the blister packets, washed three times in PBS, and autoclaved in 5 mL of PBS in a glass vial that is visually identical to the melimine contact lens vial. All the contact lenses were stored in a cold room (5°C) until required.

Animal Model for Assessing the Safety and Ocular Irritation of Melimine-Coated Lenses

This was a prospective, masked, randomized, controlled study conducted following the guidelines of ISO 9394.[24] All animals were treated strictly in accordance with the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research, and before the study commencement, approval from University of New South Wales Animal Care and Ethics Committee was obtained.

A total of six female New Zealand white rabbits were allocated for contralateral melimine and control contact lens wear. They were acclimated for at least 1 week. Only rabbits in good general health, weighing more than 3.5 kg, and having eyes free of clinically significant ocular irritation were used in the study. The nictitating membrane was not removed from the rabbits' eyes. The test and control eyes were randomly assigned using Microsoft Office Excel (Microsoft, Redmond, WA).

Lens Wear and Assessments

Rabbits wore contact lenses for 7 to 8 hours daily for 21 consecutive days. Biotrue (Bausch & Lomb, Rochester, NY) multipurpose disinfecting solution was used for overnight storage, pre–lens insertion, and rinsing. Day 22 was the last day, and lenses were worn for at least 4 hours on this day. All rabbits were monitored daily for any indication of stress by examining their movements, alertness, gait, behavior, vocalizations, and respiration. Rabbits were weighed at baseline and days 8, 15, and 22 of contact lens wear. Special attention was given to observing any scratching or pawing of eyes, which might indicate ocular irritation. Detailed slit lamp ophthalmic examinations were performed at baseline and immediately after lens removal on days 8, 15, and 22 following the McDonald-Shadduck Score System[24] by a masked observer. Conjunctival congestion/swelling/discharge, aqueous flare, iris involvement, corneal cloudiness, vascularization, and fluorescein staining were determined at each observation time. Baseline examinations were performed within 24 hours of starting the study. Slit lamp biomicroscopy (Nikon FS-3V, Tokyo, Japan) and detailed anterior segment examinations were carried out, including sodium fluorescein (Chauvin Pharmaceuticals Ltd, Surrey, United Kingdom) staining. Wratten no. 12 filter (Bausch & Lomb) was used in conjunction with cobalt blue filter to excite fluorescence. Contact lenses that fell out of the eye during the treatment period were thoroughly examined, rinsed, and reinserted. If the lenses were lost or damaged, new lenses were inserted as replacements. A maximum of four replacement lenses were allowed during the entire study. Lens retention on the rabbit eyes was checked frequently by visual inspection. After 8 hours of contact lens wear, the lenses were removed from each eye, inspected for damage, rinsed, and soaked overnight in the designated storage cases with solutions. On day 22, after the final ophthalmic observations, contact lenses were removed and all rabbits were euthanized.

Histopathology

Twelve corneas of six rabbits were collected in 4% formaldehyde (BDH Chemicals, Victoria, Australia) for histopathology. Corneal samples were placed in cassettes and then loaded into a Shandon Excelsior ES Tissue Processor (Thermo Fisher Scientific, Waltham, MA) for overnight processing (infiltration with paraffin). Samples were then removed and embedded in wax molds on a Shandon Histocentre 3 (Thermo Fisher Scientific, Pittsburgh, PA). Wax blocks were trimmed, and sections were cut on the Leica RM 2165 Microtome (Leica Microsystems, Rijswijk, The Netherlands) at 4-μm thickness. Slides were placed in a laboratory oven at 56°C for 1 hour and stained with hematoxylin and eosin using a Leica XL Autostainer (Leica Microsystems Inc., Bannockburn, IL). Slides were then coverslipped using the Dako CR 100 Coverslipper (Dako, Produktionsvej, Denmark) and allowed to dry overnight. Processed slides were stored at 4°C before microscopic examination.

Biocompatibility and Retention of Antimicrobial Activity in a Human Clinical Trial

A prospective, randomized, double-masked, contralateral, 1-day clinical trial was conducted using melimine-coated and control contact lenses. The participants' comfort, dryness, and lens awareness with lenses and corneal health were evaluated, and the lenses were collected on completion of the study to determine the retention of antimicrobial activity.

Seventeen participants were enrolled to demonstrate a statistically significant difference in corneal staining score of 0.5 ± 0.7 at the 5% level of significance and 80% power. The study was

approved by the Human Research Ethics Committee of the University of New South Wales and followed the tenets of the Declaration of Helsinki 1975, as amended in 2000, including local regulations as applicable such as Therapeutic Goods Administration, Australia. The clinical trial was conducted under the clinical trial notification scheme following the regulations of the Therapeutic Goods (Medical Devices) Regulations 2000. The clinical trial was registered in the publicly accessible Australian New Zealand Clinical Trial Registry (trial ID ACTRN 12613000369729).

Inclusion criteria required participants to be older than 18 years, in good health, not taking any medications, and correctable vision to 6/12 or better in each eye; both experienced and non–contact lens wearers were included in the study. Exclusion criteria were any preexisting ocular irritation; injury or condition (including infection or disease) of the cornea, conjunctiva, or eyelids that would preclude contact lens fitting and safe wearing of contact lenses; any systemic disease; eye surgery; systemic or topical medication up to 12 weeks before or during the trial that may adversely affect ocular health; and/or being pregnant or having had corneal refractive surgery.

Subjects were recruited from the subject population at Brien Holden Vision Institute and School of Optometry and Vision Science, University of New South Wales. Participants were screened for general clinical trial suitability by way of a routine eye examination that included refraction, visual acuity, and general eye health. Informed consent was obtained from all the participants before the trial.

Study Visits and Clinical Techniques

A baseline visit was conducted to assess the suitability of the participants, and baseline measurements were taken for the trial. A total of four visits were undertaken: lens dispensing (visit 1), lens collection after 8 hours (visit 2), and follow-ups after 1 and 4 weeks. Because both the follow-up visits included no assigned contact lens wear, participants were free to wear own lenses or glasses if needed. A follow-up visit after 4 weeks was conducted to rule out any delayed toxicity of melimine-coated lenses.

Visual acuity was measured at each visit using computer letter charts.[25] Slit lamp biomicroscopy (Zeiss SL-120, Carl Zeiss Meditec, Jena, Germany) was performed by a single masked observer. All the clinical grading was conducted using the CCLRU[26] grading scales (0 to 4 units) interpolated into 0.1 increments. Bulbar and limbal redness, palpebral redness and roughness, and corneal and conjunctival staining were assessed at all visits. Examination of corneal and conjunctival staining and lens-induced conjunctival indentation was conducted with fluorescein (Fluorets ophthalmic strips, 1 mg; Chauvin Pharmaceuticals Ltd) with the help of Wratten no. 12 filter (Bausch & Lomb, Rochester, NY) in conjunction with cobalt blue filter. Examination with fluorescein was conducted before and after contact lens wear in the lens dispensing and collection visit, respectively. Type, extent, and density of corneal staining were recorded in each of the five corneal zones according to the CCLRU staining criteria,[26] as shown in Fig. 1. Fluorescein was carefully washed from the eyes completely before the lens insertion. Lenses were inserted and removed using aseptic gloves (DermaClean Sterile, Ansell Ltd, Richmond, Australia). Lens surface deposits and wetting, back surface debris, centration, tightness, fluting, primary gaze movement and gaze lag, corneal coverage, and overall acceptance were assessed at the lens dispensing and collection visits. Slit lamp photographs were taken using a Nikon photographic slit lamp (Nikon FS-3V; Nikon, Tokyo, Japan), which provided up to 32× magnification. Subjects were asked to rate the comfort of the lenses based on their overall impression of ocular comfort, ocular dryness, lens awareness, and lens edge awareness at the time of contact lens collection using a 1 to 10 scale using whole number steps (1 = very uncomfortable, dry, or aware; 10 = comfortable, not dry, or not aware). Participants were asked for the preference of either eye (forced preference: either right or left eye) based on contact lens wear experience. After wear, lenses were collected in glass vials containing 2 mL of sterile PBS.

Retention of Antimicrobial Activity

Worn and unworn contact lenses were processed for evaluation of retention of antimicrobial activity against *Pseudomonas aeruginosa* strain 6294 (isolated from a case of MK) and *Staphylococcus aureus* strain 31 (isolated from a case of CLPU) within 48 hours after a procedure detailed earlier.[21] Briefly, bacteria were grown overnight in Tryptone Soya Broth (Oxoid, Basingstoke, United Kingdom) and

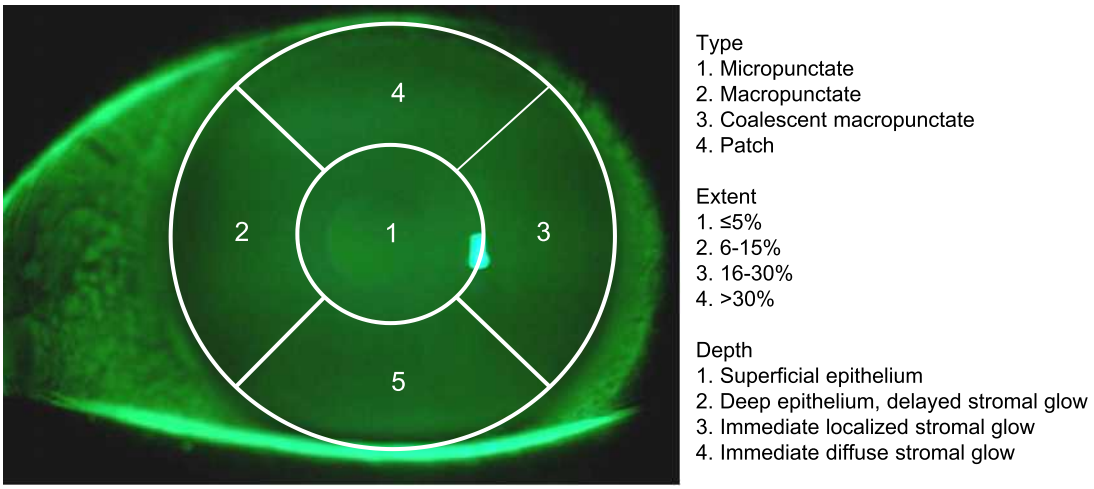

FIGURE 1.
Example of the corneal fluorescein staining. Each of the five corneal zones was graded for staining type, extent, and density.

then resuspended in 1/10 Tryptone Soya Broth (*S. aureus*) or PBS (*P. aeruginosa*) to an OD_{660nm} of 0.1 (1.0×10^8 colony-forming units [CFUs] per milliliter). The bacterial cell suspensions were then serially diluted (1/10) to 1.0×10^6 CFU mL^{-1} for adhesion assays. Worn and unworn melimine-coated and uncoated contact lenses were transferred to 1 mL of bacterial suspensions in wells of 24-well tissue culture plates (CELESTAR, Greiner Bio-One, Frickenhausen, Germany). To allow bacterial adhesion, lenses were incubated for 18 hours at 37°C with shaking (120 rpm). Contact lenses were then washed three times with PBS to remove nonadherent cells and then stirred rapidly in 2 mL of PBS containing a small magnetic stirring bar. After log serial dilutions in Dey Engley neutralizing broth (DE; Becton, Dickson and Company, USA), 3×50 μL of each dilution were plated on a tryptic soy agar (Oxoid) containing Tween 80 and lecithin for recovery of cells. After 24 hours of incubation at 37°C, the viable bacteria were enumerated as CFUs per square millimeter. Results are expressed as the reduction in adherent viable bacteria. Triplicate measurements were performed on a minimum of three separate occasions.

Data Analysis

Data were analyzed using Microsoft Office Excel and Statistical Package for the Social Sciences software for Windows software version 21.0 (SPSS, Inc., Chicago, IL). Percent of lens retention for rabbits was calculated as [(actual wear time for duration of study)/(total possible wear time for duration of study)] × 100. Analytical manipulation of the data, such as the sum or frequency of scores, was calculated where appropriate. Human clinical and subjective ratings were summarized using descriptive statistics. Differences between lens types were determined at each visit using paired *t* test or Wilcoxon signed rank test based on the type of variable. Frequency and percentage of participants preferring any of the contact lens types were reported for each preference category. The bacterial adhesion data were $\log_{10}(x+1)$ transformed before data analysis where *x* is the number of adherent bacteria in CFUs per square millimeter. Differences in bacterial adhesion were analyzed using independent two-sample *t* test. Statistical significance was set at 5%.

RESULTS

Melimine-Coated Contact Lens Wear in the Animal Model

All six animals included in the study maintained good health, and no abnormal behavior was observed during the study period indicative of ocular discomfort. Contact lens loss during the study was infrequent. Lens retention was 94% for melimine-coated contact lenses and 96% for control contact lenses.

Ocular Response

Gross Ocular Examination

Gross ocular scores are presented in Table 1. Mild conjunctival redness (score 1) was observed twice with melimine lenses. Mild conjunctival discharge (score 1) and redness were observed once each with control contact lenses. The remaining eyes appeared normal (score 0) for both melimine and control lenses throughout the study.

Ophthalmic Observation by Slit lamp Biomicroscopy

Slit lamp biomicroscopy scores are presented in Table 2. Unless detailed in Table 2, all eyes appeared normal. Scores of "1" for fluorescent retention by the cornea are commonly noted in healthy rabbits' eyes; thus, this score was not considered clinically significant. At baseline, corneal fluorescein staining (score 1) was observed in two eyes in each treatment group. Mild conjunctival congestion (score 1) and mild corneal fluorescein staining (score 1) were the only two other signs occasionally observed in the study in both the treatment groups.

Diffuse and fluorescent slit lamp photographs of melimine and control contact lenses worn by the same rabbit at baseline, day 8, day 15, and day 22 are shown in Fig. 2. The fluorescein photographs in this figure (pictures 1C to 4C and 1D to 4D) confirm the absence of corneal staining of control and test eyes. Neither melimine-coated nor control contact lens wear was associated with any other slit lamp biomicroscopy signs of ocular irritation, such as conjunctival chemosis or swelling, discharge, iris changes, corneal cloudiness, or vascularization. During the study period, observations made by slit lamp biomicroscopy indicated no significant clinical signs that might suggest ocular irritation induced by melimine coatings. None of the rabbits were discontinued from contact lens wear during the trial.

Histopathology

Histopathology of corneal sections stained with hematoxylin and eosin indicated no major structural differences between corneas exposed to melimine or control contact lenses (Fig. 3). All the sections from 12 corneas showed normal central and peripheral structures. All the three layers of corneal epithelium (basal layer, intermediate layer, and flattened cells) were intact and identical in all sections observed with high (40× objective) magnification.

Melimine-Coated Contact Lens Wear in Humans

A total of 17 participants were enrolled in this study, of which eight were experienced wearers. There were no disqualifications, and data from all the enrolled participants were included in the analysis. There were 10 females in this study, and the mean (±SD)

TABLE 1.
Gross ocular observation by Drazie Scale

	Rabbit	Eye	Response	Grade	Study day
Melimine lenses	1	OS	Conjunctival redness	1	9th
	2	OD	Conjunctival redness	1	9th
Control lenses	3	OD	Conjunctival discharge	1	14th
	1	OD	Conjunctival redness	1	9th

Unless mentioned above, all eyes appeared normal (score = 0).

TABLE 2.
Ophthalmic observation by slit lamp biomicroscopy

Lenses	Day	Ophthalmic observation	No. eyes	Score
Melimine	Baseline	Corneal fluorescein staining	2	1
	8	Conjunctival congestion	1	1
		Corneal fluorescein staining	1	1
	15	Appeared normal	0	0
	22	Conjunctival congestion	2	1
		Corneal fluorescein staining	2	1
Control	Baseline	Corneal fluorescein staining	2	1
	8	Conjunctival congestion	1	1
		Corneal fluorescein staining	1	1
	15	Conjunctival congestion	2	1
	22	Conjunctival congestion	1	1

Unless mentioned above, all eyes appeared normal (score = 0).

age of the participants was 30.9 (±9.4) years. The mean (±SD) lens wearing time was 6.9 (±0.9) hours. Table 3 shows refractive error and keratometry readings at baseline visit (n = 34).

Clinical Signs and Symptoms

There were no significant differences seen in wettability or surface deposition between melimine-coated and control contact lenses during both lens dispensing and collection visits ($p > 0.05$). Melimine lenses showed clinically acceptable centration, movement, and tightness at all times. Overall fitting acceptance for both the lens types at both time points was rated highly (>3.0), which indicated complete corneal coverage, good centration, adequate primary gaze movement, and acceptable tightness. None of the contact lenses needed to be refitted, and no lens loss was reported.

There were no significant differences in different areas of bulbar redness, limbal redness, palpebral redness, and palpebral roughness

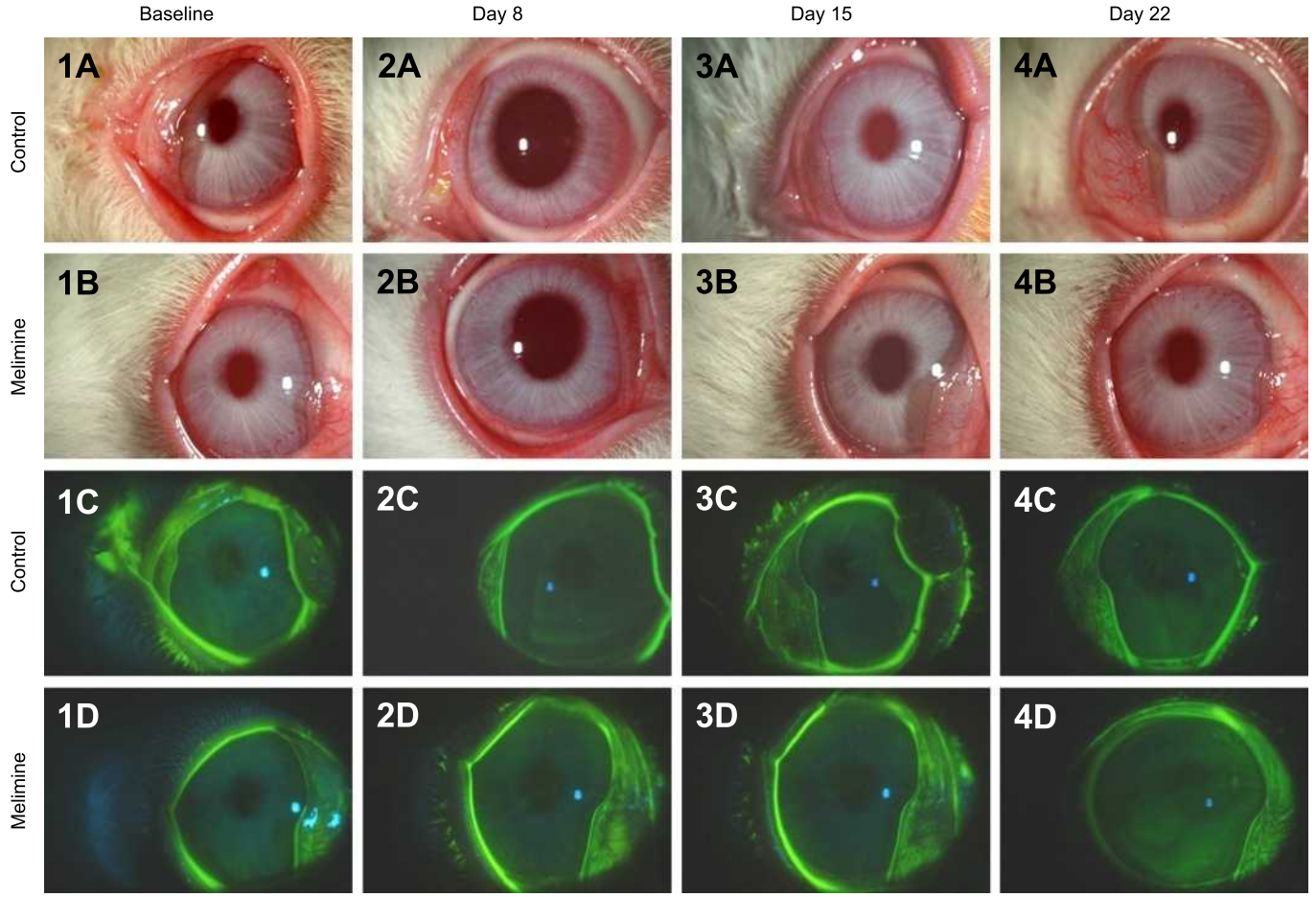

FIGURE 2.
Representative images of diffuse and fluorescent slit lamp photographs of a control and melimine contact lens worn by a rabbit eye for 22 days. A and B, diffuse; C and D, fluorescent slit lamp photograph; 1, baseline; 2, day 8; 3, day 15; 4, day 22 observations. Captured using slit lamp biomicroscope at 32× magnification.

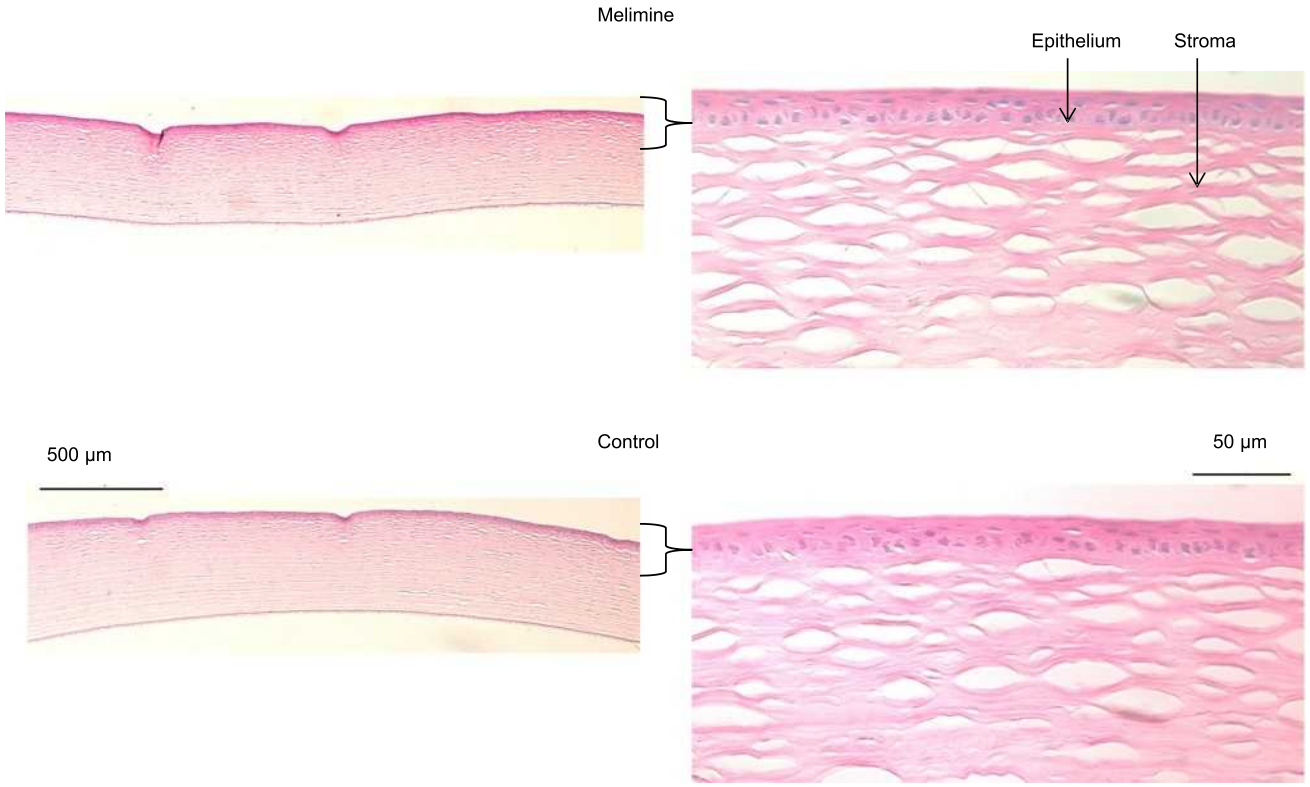

FIGURE 3.
Representative light micrographs of comparative rabbit corneal histology sections after melimine and control contact lens wear for 22 days. The empty spaces in the stroma are artifacts produced during histopathology processing.

between the melimine-coated and control lenses ($p > 0.05$). One participant with melimine-coated lenses showed slightly higher conjunctival staining in all four quadrants (mean difference in grade of 0.7). Overall, melimine lenses did not show any significant difference in conjunctival indentation and staining when compared with control lenses ($p > 0.05$). Melimine-coated contact lens wear was associated with significantly higher levels of corneal staining (Fig. 4) in all areas compared with the control lenses ($p < 0.05$; extent, depth, and type). Fig. 5 shows the extent, depth, and type (median; mean ± SD) of fluorescein staining associated with melimine and control lenses in all the corneal areas. Both corneal staining mean and median were higher in corneas with melimine lenses than controls ($p < 0.05$).

Overall, 65% participants preferred the control contact lenses. Distribution of comfort scores during melimine-coated and control contact lens wear is presented in Fig. 6 using box plots. One participant was uncomfortable with the melimine-coated lens and reported high levels of dryness, lens awareness, and lens edge awareness that are represented as the outliers in Fig. 6. Although there was no significant difference in overall comfort ($p = 0.07$), dryness ($p = 0.10$), lens awareness ($p = 0.06$), or lens edge awareness ($p = 0.20$), the mean responses were slightly lower with melimine-coated lenses. Standard deviations of comfort ratings for melimine lenses (range, 1.9 to 2.5) were higher than those for control lenses (range, 1.7 to 2.0).

TABLE 3.
Refractive error and keratometry readings at baseline for study participants

Variables	Mean	SD
Refractive error – sphere, Ds	−1.15	1.70
Refractive error – cylinder, Ds	−0.29	0.43
Keratometry – flat, D	43.30	1.42
Keratometry – steep, D	43.86	1.58

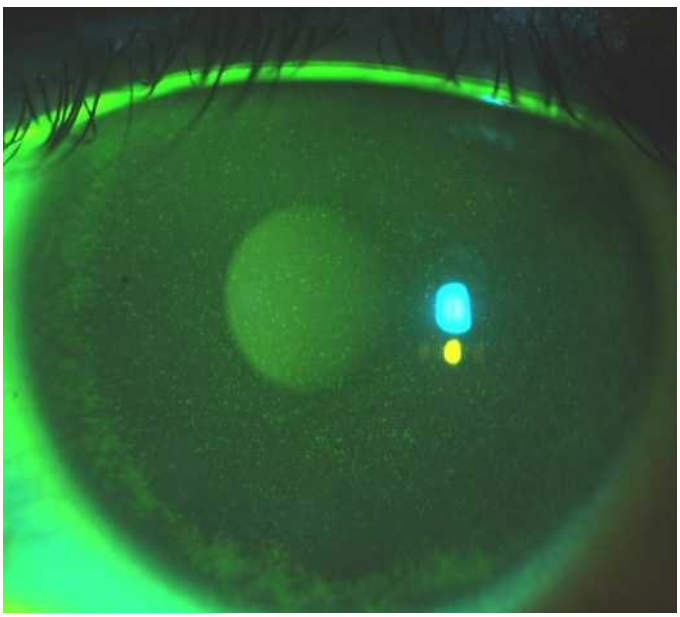

FIGURE 4.
Diffuse corneal staining after melimine-coated contact lens wear.

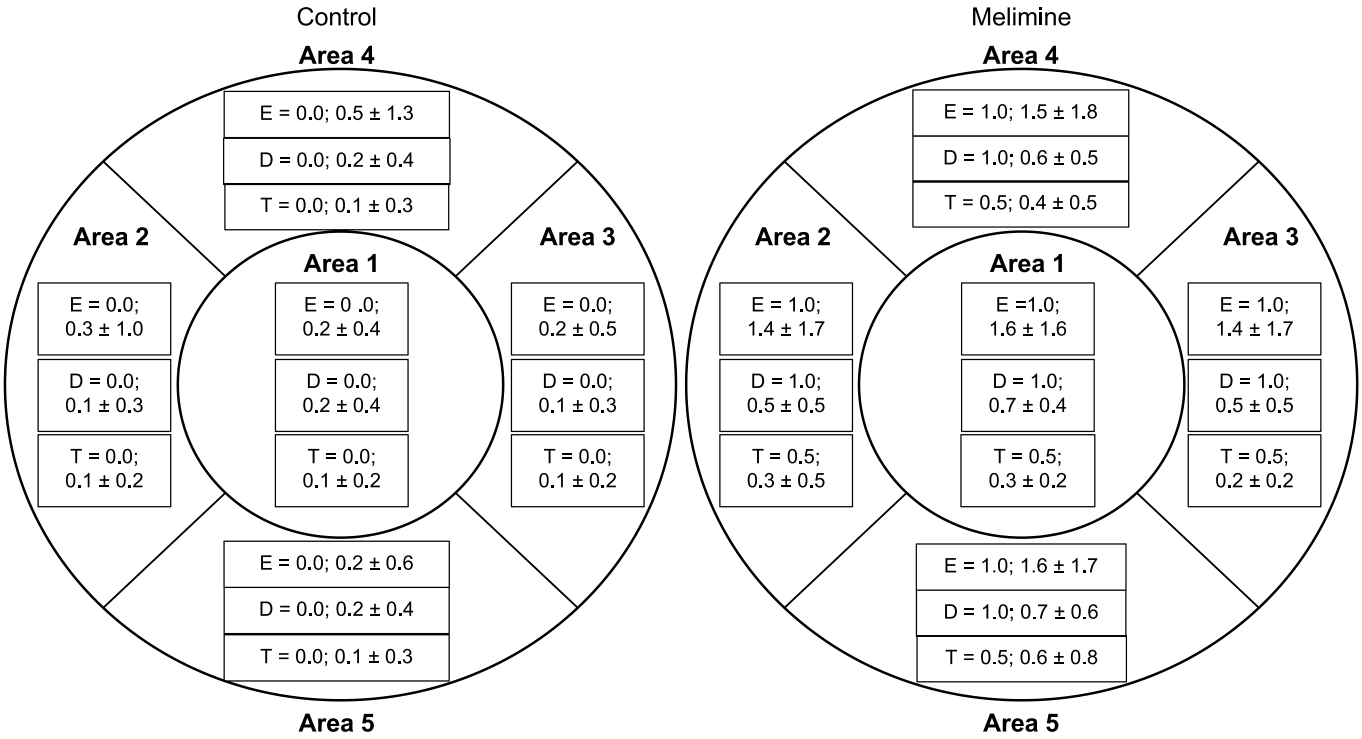

FIGURE 5.

Fluorescein staining scores (median; mean ± SD) in melimine-coated and control contact lens corneal areas.

Retention of Antimicrobial Activity

When incubated with *P. aeruginosa* 6294 and *S. aureus* 31, worn melimine contact lenses showed significantly lower adhesion ($p < 0.05$) when compared with worn control lenses, resulting in 1.5 ± 0.5 log and 1.5 ± 0.4 log inhibition in adhesion, respectively. Worn melimine lenses showed 0.5 ± 0.3 log ($p = 0.05$) and 0.8 ± 0.5 ($p > 0.05$) log higher *P. aeruginosa* 6294 and *S. aureus* 31 adhesion than unworn melimine lenses (Fig. 7). *Pseudomonas aeruginosa* 6294 and *S. aureus* 31 adhesion to contact lenses collected from each of the 17 participants is presented in Fig. 8.

DISCUSSION

This study provides the first evidence to indicate that AMP-coated contact lenses can be worn by humans without any major side effects. Although the contact lenses covalently coated with melimine showed increased human corneal staining, they retained antibacterial activity after 1 day of wear.

All the animals during this trial remained healthy and behaved normally, and no ocular irritation–related symptoms such as eye scratching or pawing of eyes were observed. Slit lamp and gross ocular observation of the cornea, conjunctiva, and ocular adnexa did not show any ocular signs that might indicate irritation. Corneal fluorescein staining indicated no difference between eyes during melimine or control contact lens wear. This was supported by the histopathological investigation that confirmed the absence of toxicity to corneal tissue, especially epithelium. A previous study has shown that melimine-coated contact lenses are able to reduce the clinical manifestations of CLPU and CLARE, arising from both gram-positive and gram-negative bacterial contaminations in rabbit and guinea pig models, respectively.[22] Considering the results presented here and those of previously reported studies, it may be concluded that, at least in animal models, melimine-coated contact lenses are safe in the wear modalities that have been investigated and have the capacity to reduce the severity and or incidence of bacterially driven adverse events.

During the human trial, melimine-coated lenses performed similarly to the control lenses for lens surface characteristics, including wettability, deposition, and debris. This result is in agreement with the high *in vitro* hydrophilicity of melimine-coated lenses reported earlier.[21] Similar to the antimicrobial fimbrolide-coated contact lenses,[27] melimine lenses showed acceptable fit with optimum movement/tightness and centration. This finding is in agreement with our previous study determining that covalent immobilization of melimine does not change lens parameters *in vitro*.[21]

This study, for the first time, investigated biocompatibility of synthetic AMP in human eyes and is one of the few studies that have evaluated human responses of antimicrobial lens in a clinical trial.[27–29] Melimine-coated lenses were not associated with conjunctival staining, bulbar and limbal redness, and palpebral redness and roughness. The melimine lenses were not associated with any delayed ocular toxicity. However, when compared with controls, melimine-coated lens wear was associated with significantly higher corneal fluorescein staining mean and median. Ten of 17 participants wearing melimine-coated lenses had clinically significantly (difference in corneal staining >0.5 grading) higher corneal staining. However, the time taken to resolve these stainings was not determined with an unscheduled visit, and participants were doing well after 1 week. The observed corneal staining was similar to that of solution-induced corneal staining (SICS) reported with the use of contact lens care solutions.[30]

Solution-induced corneal staining generally represents as diffuse corneal staining in at least four of the five regions.[30] Similarly,

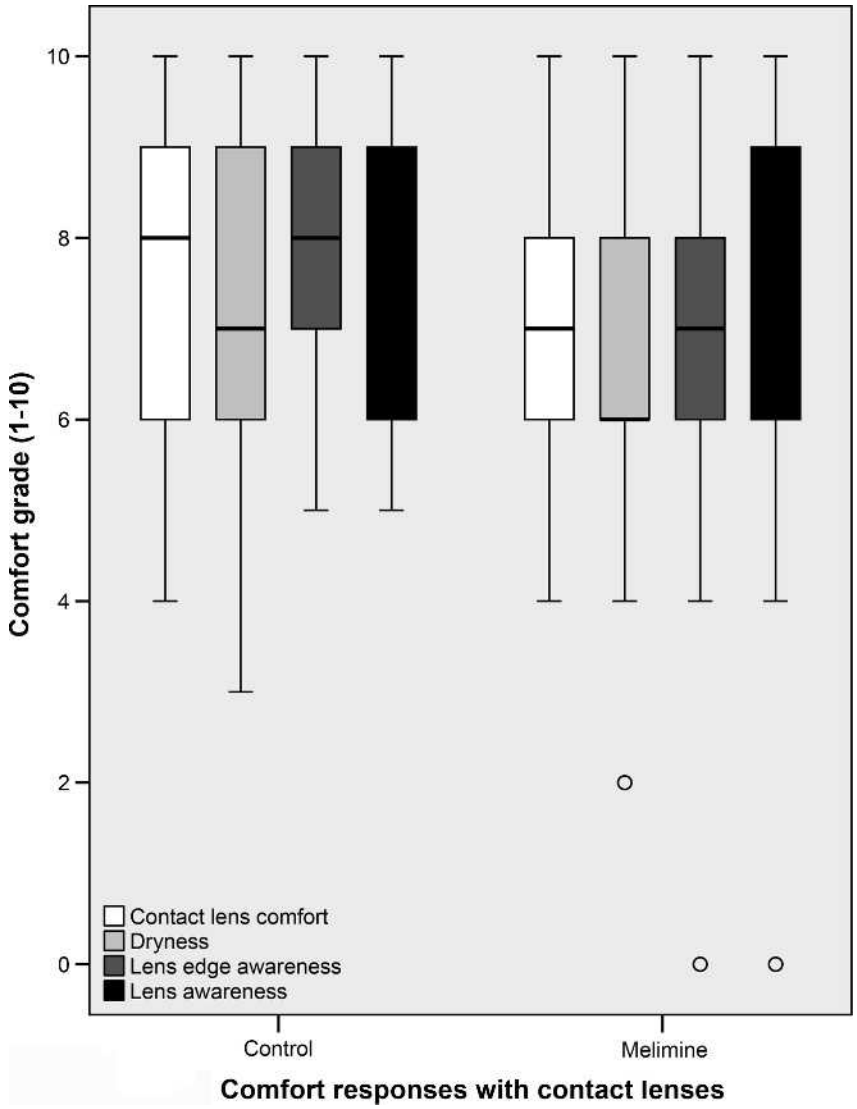

FIGURE 6.

Distribution of comfort scores during melimine-coated and control contact lens wear. Data are presented as box plots showing median and 25th and 75th percentile ranges.

fluorescein staining associated with melimine-coated lenses were greater in all the five corneal areas. Uptake of cationic biocides including polyhexamethylene biguanide, other quaternary ammonium compounds such as benzalkonium chloride, and polyquaternary ammonium compounds such as polyquaternium-1[31] has been strongly associated with the incidence of SICS.[32–35] However, the exact mechanism of fluorescein interaction with corneal epithelial cells during SICS is not well understood.[32,36–38] Fluorescein pooling,[36,39] ionic interaction with negatively charged fluorescein,[40] uptake by apoptotic cells,[41] staining of dead/damaged cell contents with compromised membranes,[36] and accumulations in the intercellular space on the ocular surface[42] are various theories that have tried to explain this. However, Bandamwar[41] has shown that accumulation of fluorescein solutions in the voids on ocular surface or in the intracellular space is unlikely to be the mechanism of corneal staining. Given that melimine is covalently coupled and not released from lenses, other hypotheses such as ionic interactions with cationic surfactants bound to epithelial cells and fluorescein molecules or adhesion of cationic compounds to cell membranes are unlikely to be applicable here. Fluorescein staining of dead cells is controversial, and a few studies have shown that dead cells were actually responsible for lowest staining intensities.[41] In addition, Morgan et al.[36] suggested that corneal staining cannot be explained by its uptake onto damaged epithelial cells. Apoptotic cells have demonstrated much higher fluorescein staining than live or dead cells.[41] Perhaps the bound melimine might be inducing apoptosis in these cells. This effect was not reported earlier with the *in vitro* cytotoxicity assay.[21] It should be noted however that the *in vitro* assay used mouse fibroblast cells and not human corneal epithelial cells. Interestingly, the fluorescein staining observed with melimine-coated lenses in human corneas was not detected in rabbit corneas. Rabbit tears have higher divalent cations such as Ca^{2+} and Mg^{2+} than those of humans.[43] It appears that there may not be any ionic difference between rabbit tears and melimine-coated contact lens surface, whereas significant ionic difference with human tears may have stimulated corneal fluorescein staining.

Solution-induced corneal staining with the use of polyhexamethylene biguanide and polyquad-based multipurpose disinfecting solution has

FIGURE 7.

Bacterial adhesion to worn melimine-coated and control contact lenses. The * represents significantly reduced adhesion to worn or unworn melimine-coated lenses compared with worn or unworn uncoated lenses, whereas # represents significantly higher adhesion to worn melimine lenses compared with unworn melimine lenses.

been associated with higher corneal infiltrative events.[44] Whether melimine-coated lenses would be associated with inflammation because of the SICS-like response cannot be ruled out and needs further exploration. However, Szczotka-Flynn et al.[45] showed that corneal staining is frequent during continuous contact lens wear and not associated with the development of corneal infiltrative events. This was a contradictory finding with the previous work by the same investigators[46] and was a consequence of fluorescein staining grades

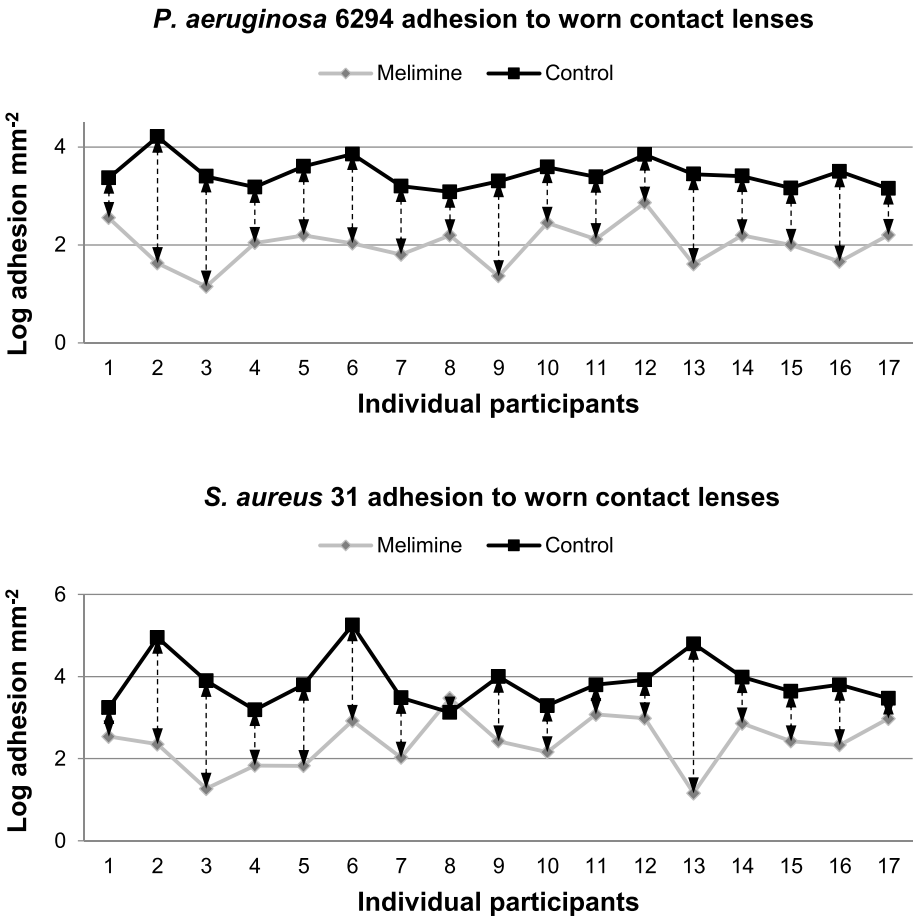

FIGURE 8.

Bacterial adhesion to melimine-coated and control contact lenses collected from each participant. The dotted vertical lines show inhibition in bacterial adhesion to melimine lenses when compared with controls after lens wear for each individual.

being used in the earlier study[46] that underreported corneal staining. Perhaps the incidence, mechanism, type, duration, and consequence of corneal staining with melimine-coated contact lenses should be minutely investigated in a larger clinical trial, especially considering the uncertainty to the causative mechanism behind corneal staining.

The median of comfort scores indicated that control lenses were associated with marginally higher comfort when compared with melimine-coated lenses. Mean grades of overall comfort scores, lens-related dryness, lens awareness, and lens edge awareness were also slightly higher with control lenses, but the differences with those for melimine-coated lenses were not detected to be clinically significant. This finding is in agreement with the finding that clinical relevance of SICS is not known and often not associated with patient symptoms.[32,47,48] Comfort results of melimine-coated contact lens wear were consistent with the results with fimbrolide-coated antimicrobial contact lenses,[27] showing slightly less comfort and increased dryness and lens edge and lens awareness. Although this study was not designed to evaluate statistical difference in participants' preference, 65% of the participants preferred control lenses, indicating that 15% more participants ($p = 0.22$) felt better with control lenses than hypothesized (50%). It is difficult to correlate these subtle differences in comfort score, as the melimine covalent coupling procedure involved several additional laboratory steps that could have affected the comfort or preference responses.

Zhu et al.[27] have shown that fimbrolide-coated antimicrobial contact lenses are safe in humans; however, they did not evaluate retention of antimicrobial activity. The current study showed that melimine-coated lenses retained 1.5 log (96.8%) inhibition against *P. aeruginosa* and *S. aureus* after contact lens wear. When compared with unworn melimine lenses, there was increased bacterial adhesion to worn melimine lenses, but the difference was not statistically significant. The opposite trend was seen with the control lenses, which showed 0.4 ± 0.2 ($p > 0.05$) log higher *S. aureus* adhesion compared with worn lenses, but that was not the case for *P. aeruginosa*. Contact lens wear has different effects on bacterial adhesion partly because of differences in tear components and microorganisms present in the ocular biota of wearers.[49,50] Comparative *ex vivo* bacterial adhesion to worn and unworn etafilcon A lenses varies considerably between studies.[51,52] Negatively charged methacrylic acid of etafilcon A lenses encourage *S. aureus* adhesion[53] and deposition of the cationic protein lysozyme from tears.[54–56] However, the attachment of the cationic peptide melimine is likely to result in an increased positive charge on the lens surface, perhaps making the surface either positive or neutral. The human tear film consists of various negatively charged components, such as phospholipid,[57] mucins, and mucin-like proteins such as lubricin[58] or the protein lipocalin,[59,60] which may interact with the surface-bound melimine and perhaps may affect activity. This requires further investigation.

Susceptibility of AMPs to *in vivo* proteolytic degradation is possible and may limit the pharmacokinetics and functions of AMPs.[61–63] These interactions may make AMPs unsuitable for certain applications. Trotti et al.[64] investigated an AMP called "iseganan" in a mouthwash to reduce oral mucosis during radiotherapy treatment for head and neck cancer. The peptides failed to effectively reduce ulcerative events and subsequent morbidity possibly because the presence of various proteases and enzymes in the oral cavity may have reduced the activity of the AMP. An effective way to increase the stability of AMPs to degradation by proteolytic enzymes is to modify the *C*-terminus by amidation.[65] Surface-attached melimine has been shown to retain activity after exposure to the proteolytic enzyme trypsin,[66] indicating that this lens surface–immobilized melimine may be resistant to proteases at the ocular surface. The current study showed that melimine-coated contact lenses are active after 8 hours of human lens wear, indicating that melimine-coated contact lenses may have a permanent activity. Whether melimine could reduce contact lens–related adverse events during wear, especially extended wear, requires more extensive clinical trials. Given the incidence of CLARE, CLPU, and infiltrative keratitis, prospective clinical trials with melimine-coated lenses may be able to demonstrate a reduction. However, MK is relatively rare and postmarket studies may be required to demonstrate a reduction in incidence and severity.

In conclusion, this study has shown that melimine-coated contact lenses can be safely worn by humans without any major side effects. It is supported by animal study, and the antimicrobial benefit could be achieved without any adverse effect on mammalian eye health. Although melimine lenses were less preferred, subjective comfort scores were broadly comparable to those of uncoated control lenses. Melimine lens wear was associated with a higher corneal staining and retained antibacterial activity against *P. aeruginosa* and *S. aureus* after wear. Given the period of human contact lens wear, melimine lenses were biocompatible and retained antibacterial activity.

ACKNOWLEDGMENTS

The authors thank Ms. Denise and Ms. Robyn Lawler from the University of New South Wales for assistance in the animal study, Dr. Thomas John for help with the statistical analysis, and Dr. Judith Flanagan for help with scientific writing.

This work is original, has not been published, and is not being considered for publication elsewhere. This work was partly presented as a paper at the American Academy of Optometry annual meeting 2013. There are no conflicts of interest for any of the authors that could have influenced the results of this work. The first author is supported by the University International Postgraduate Award and Maki Shiobara scholarship from the University of New South Wales, the Ezell fellowship (Bausch & Lomb) from the American Academy of Optometry, and the Brien Holden Vision Institute.

Received December 3, 2013; accepted January 23, 2014.

REFERENCES

1. Sankaridurg PR, Vuppala N, Sreedharan A, Vadlamudi J, Rao GN. Gram-negative bacteria and contact lens–induced acute red eye. Indian J Ophthalmol 1996;44:29–32.
2. Holden BA, La Hood D, Grant T, Newton-Howes J, Baleriola-Lucas C, Willcox MD, Sweeney DF. Gram-negative bacteria can induce contact lens–related acute red eye (CLARE) responses. CLAO J 1996; 22:47–52.
3. Wu P, Stapleton F, Willcox MD. The causes of and cures for contact lens–induced peripheral ulcer. Eye Contact Lens 2003;29:S63–6.
4. Willcox M, Sharma S, Naduvilath TJ, Sankaridurg PR, Gopinathan U, Holden BA. External ocular surface and lens microbiota in contact lens wearers with corneal infiltrates during extended wear of hydrogel lenses. Eye Contact Lens 2011;37:90–5.
5. Willcox MD, Holden BA. Contact lens–related corneal infections. Biosci Rep 2001;21:445–61.

6. Dart JK, Stapleton F, Minassian D. Contact lenses and other risk factors in microbial keratitis. Lancet 1991;338:650–3.
7. Ormerod LD, Smith RE. Contact lens–associated microbial keratitis. Arch Ophthalmol 1986;104:79–83.
8. Ganz T. The role of antimicrobial peptides in innate immunity. Integr Comp Biol 2003;43:300–4.
9. Kolar SS, McDermott AM. Role of host defence peptides in eye diseases. Cell Mol Life Sci 2011;68:2201–13.
10. McDermott AM. The role of antimicrobial peptides at the ocular surface. Ophthalmic Res 2009;41:60–75.
11. McDermott AM. Defensins and other antimicrobial peptides at the ocular surface. Ocul Surf 2004;2:229–47.
12. Hancock RE. Cationic peptides: effectors in innate immunity and novel antimicrobials. Lancet Infect Dis 2001;1:156–64.
13. Hancock RE, Lehrer R. Cationic peptides: a new source of antibiotics. Trends Biotechnol 1998;16:82–8.
14. Yount NY, Bayer AS, Xiong YQ, Yeaman MR. Advances in antimicrobial peptide immunobiology. Biopolymers 2006;84:435–58.
15. Brogden KA. Antimicrobial peptides: pore formers or metabolic inhibitors in bacteria? Nat Rev Microbiol 2005;3:238–50.
16. Brandenburg LO, Merres J, Albrecht LJ, Varoga D, Pufe T. Antimicrobial peptides: multifunctional drugs for different applications. Polymers 2012;4:539–60.
17. Lipsky BA, Holroyd KJ, Zasloff M. Topical versus systemic antimicrobial therapy for treating mildly infected diabetic foot ulcers: a randomized, controlled, double-blinded, multicenter trial of pexiganan cream. Clin Infect Dis 2008;47:1537–45.
18. Yeung AT, Gellatly SL, Hancock RE. Multifunctional cationic host defence peptides and their clinical applications. Cell Mol Life Sci 2011;68:2161–76.
19. Willcox MD, Hume EB, Aliwarga Y, Kumar N, Cole N. A novel cationic-peptide coating for the prevention of microbial colonization on contact lenses. J Appl Microbiol 2008;105:1817–25.
20. Rasul R, Cole N, Balasubramanian D, Chen R, Kumar N, Willcox MD. Interaction of the antimicrobial peptide melimine with bacterial membranes. Int J Antimicrob Agents 2010;35:566–72.
21. Dutta D, Cole N, Kumar N, Willcox MD. Broad-spectrum antimicrobial activity of melimine covalently bound to contact lenses. Invest Ophthalmol Vis Sci 2013;54:175–82.
22. Cole N, Hume EB, Vijay AK, Sankaridurg P, Kumar N, Willcox MD. *In vivo* performance of melimine as an antimicrobial coating for contact lenses in models of CLARE and CLPU. Invest Ophthalmol Vis Sci 2010;51:390–5.
23. ISO 10993-5. Biological Evaluation of Medical Devices–Part 5: Tests for *In Vitro* Cytotoxicity. Geneva, Switzerland: International Organization for Standardization; 2009.
24. ISO 9394. Ophthalmic Optics–Contact Lenses and Contact Lens Care Products–Determination of Biocompatibility by Ocular Study Using Rabbit Eyes. Geneva, Switzerland: International Organization for Standardization; 2012.
25. Ehrmann K, Fedtke C, Radic A. Assessment of computer generated vision charts. Cont Lens Anterior Eye 2009;32:133–40.
26. Cornea and Contact Lens Research Unit. CCLRU grading scales. In: Phillips AJ, Speedwell L, eds. Contact Lenses. Oxford, UK: Butterworth-Heinemann; 1997:863–7.
27. Zhu H, Kumar A, Ozkan J, Bandara R, Ding A, Perera I, Steinberg P, Kumar N, Lao W, Griesser SS, Britcher L, Griesser HJ, Willcox MD. Fimbrolide-coated antimicrobial lenses: their *in vitro* and *in vivo* effects. Optom Vis Sci 2008;85:292–300.
28. Lakkis C, Anastasopoulos F, Slater J, May L. The effect of silver-infused silicone hydrogel contact lenses on the ocular biota during daily wear. Cont Lens Anterior Eye 2011; 34:S10.
29. Ozkan J, Zhu H, Willcox MD. Efficacy and clinical performance of selenium antibacterial silicone hydrogel contact lenses. Invest Ophthalmol Vis Sci 2009;50:ARVO E-Abstract 5632.
30. Carnt N, Willcox MD, Evans V, Naduvilath T, Tilia D, Papas E, Sweeney D, Holden BA. Corneal Staining: The IER Matrix Study. Contact Lens Spectrum 2007. Available at: http://www.clspectrum.com/articleviewer.aspx?articleid=100843. Accessed December 18, 2013.
31. Jones L, Powell CH. Uptake and release phenomena in contact lens care by silicone hydrogel lenses. Eye Contact Lens 2013;39:29–36.
32. Stiegemeier MJ, Friederichs GJ, Hughes JL, Larsen S, Movic W, Potter WB. Clinical evaluation of a new multipurpose disinfecting solution in symptomatic contact lens wearers. Cont Lens Anterior Eye 2006;29:143–51.
33. Lebow KA, Schachet JL. Evaluation of corneal staining and patient preference with use of three multipurpose solutions and two brands of soft contact lenses. Eye Contact Lens 2003;29:213–20.
34. Jones L, Jones D, Houlford M. Clinical comparison of three polyhexanide-preserved multipurpose contact lens solutions. Cont Lens Anterior Eye 1997;20:23–30.
35. Lipener C. A randomized clinical comparison of OPTI-FREE EXPRESS and ReNu MultiPLUS multipurpose lens care solutions. Adv Ther 2009;26:435–46.
36. Morgan PB, Maldonado-Codina C. Corneal staining: do we really understand what we are seeing? Cont Lens Anterior Eye 2009;32:48–54.
37. Ward KW. Superficial punctate fluorescein staining of the ocular surface. Optom Vis Sci 2008;85:8–16.
38. Fonn D, Peterson R, Woods C. Corneal staining as a response to contact lens wear. Eye Contact Lens 2010;36:318–21.
39. Ladage PM, Petroll WM, Jester JV, Fisher S, Bergmanson JP, Cavanagh HD. Spherical indentations of human and rabbit corneal epithelium following extended contact lens wear. CLAO J 2002; 28:177–80.
40. Bright FV, Merchea MM, Kraut ND, Maziarz EP, Liu XM, Awasthi AK. A preservative-and-fluorescein interaction model for benign multipurpose solution–associated transient corneal hyperfluorescence. Cornea 2012;31:1480–8.
41. Bandamwar KL. Mechanism and Clinical Significance of Superficial Micropunctate Fluorescein Staining of the Cornea [PhD thesis]. Sydney, Australia: University of New South Wales; 2011.
42. Feenstra RP, Tseng SC. Comparison of fluorescein and rose bengal staining. Ophthalmology 1992;99:605–17.
43. Wei XE, Markoulli M, Millar TJ, Willcox MD, Zhao Z. Divalent cations in tears, and their influence on tear film stability in humans and rabbits. Invest Ophthalmol Vis Sci 2012;53:3280–5.
44. Carnt N, Jalbert I, Stretton S, Naduvilath T, Papas E. Solution toxicity in soft contact lens daily wear is associated with corneal inflammation. Optom Vis Sci 2007;84:309–15.
45. Szczotka-Flynn L, Lass JH, Sethi A, Debanne S, Benetz BA, Albright M, Gillespie B, Kuo J, Jacobs MR, Rimm A. Risk factors for corneal infiltrative events during continuous wear of silicone hydrogel contact lenses. Invest Ophthalmol Vis Sci 2010;51:5421–30.
46. Szczotka-Flynn L, Debanne SM, Cheruvu VK, Long B, Dillehay S, Barr J, Bergenske P, Donshik P, Secor G, Yoakum J. Predictive factors for corneal infiltrates with continuous wear of silicone hydrogel contact lenses. Arch Ophthalmol 2007;125:488–92.
47. Jones L, MacDougall N, Sorbara LG. Asymptomatic corneal staining associated with the use of balafilcon silicone-hydrogel contact lenses

disinfected with a polyaminopropyl biguanide–preserved care regimen. Optom Vis Sci 2002;79:753–61.
48. Garofalo RJ, Dassanayake N, Carey C, Stein J, Stone R, David R. Corneal staining and subjective symptoms with multipurpose solutions as a function of time. Eye Contact Lens 2005;31:166–74.
49. Miller MJ, Wilson LA, Ahearn DG. Effects of protein, mucin, and human tears on adherence of *Pseudomonas aeruginosa* to hydrophilic contact lenses. J Clin Microbiol 1988;26:513–7.
50. Dutta D, Cole N, Willcox M. Factors influencing bacterial adhesion to contact lenses. Mol Vis 2012;18:14–21.
51. Borazjani RN, Levy B, Ahearn DG. Relative primary adhesion of *Pseudomonas aeruginosa*, *Serratia marcescens* and *Staphylococcus aureus* to HEMA-type contact lenses and an extended wear silicone hydrogel contact lens of high oxygen permeability. Cont Lens Anterior Eye 2004;27:3–8.
52. Boles SF, Refojo MF, Leong FL. Attachment of *Pseudomonas* to human-worn, disposable etafilcon A contact lenses. Cornea 1992; 11:47–52.
53. Arciola CR, Maltarello MC, Cenni E, Pizzoferrato A. Disposable contact lenses and bacterial adhesion. *In vitro* comparison between ionic/high-water-content and non-ionic/low-water-content lenses. Biomaterials 1995;16:685–90.
54. Senchyna M, Jones L, Louie D, May C, Forbes I, Glasier MA. Quantitative and conformational characterization of lysozyme deposited on balafilcon and etafilcon contact lens materials. Curr Eye Res 2004;28:25–36.
55. Bruinsma GM, Rustema-Abbing M, de Vries J, Stegenga B, van der Mei HC, van der Linden ML, Hooymans JM, Busscher HJ. Influence of wear and overwear on surface properties of etafilcon A contact lenses and adhesion of *Pseudomonas aeruginosa*. Invest Ophthalmol Vis Sci 2002;43:3646–53.
56. Vijay AK, Zhu H, Ozkan J, Wu D, Masoudi S, Bandara R, Borazjani RN, Willcox MD. Bacterial adhesion to unworn and worn silicone hydrogel lenses. Optom Vis Sci 2012;89:1095–106.
57. Losche M, Mohwald H. Electrostatic interactions in phospholipid membranes: II. Influence of divalent ions on monolayer structure. J Colloid Interface Sci 1989;131:56–87.
58. Schmidt TA, Sullivan DA, Knop E, Richards SM, Knop N, Liu S, Sahin A, Darabad RR, Morrison S, Kam WR, Sullivan BD. Transcription, translation, and function of lubricin, a boundary lubricant, at the ocular surface. JAMA Ophthalmol 2013;131: 766–76.
59. Greiner JV, Glonek T, Korb DR, Leahy CD. Meibomian gland phospholipids. Curr Eye Res 1996;15:371–5.
60. Berta A, Torok M. Soluble glycoproteins in aqueous tears. In: Holly FJ, ed. The Preocular Tear Film in Health, Disease, and Contact Lens Wear. Lubbock, TX: Dry Eye Institute; 1986:506–20.
61. McDermott AM. Cationic antimicrobial peptides. A future therapeutic option? Arch Soc Esp Oftalmol 2007;82:467–70.
62. Jenssen H, Hamill P, Hancock RE. Peptide antimicrobial agents. Clin Microbiol Rev 2006;19:491–511.
63. Dutta D, Willcox MD. A laboratory assessment of factors that affect bacterial adhesion to contact lenses. Biology 2013;2:1269–81.
64. Trotti A, Garden A, Warde P, Symonds P, Langer C, Redman R, Pajak TF, Fleming TR, Henke M, Bourhis J, Rosenthal DI, Junor E, Cmelak A, Sheehan F, Pulliam J, Devitt-Risse P, Fuchs H, Chambers M, O'Sullivan B, Ang KK. A multinational, randomized phase III trial of iseganan HCl oral solution for reducing the severity of oral mucositis in patients receiving radiotherapy for head-and-neck malignancy. Int J Radiat Oncol Biol Phys 2004;58:674–81.
65. Svenson J, Stensen W, Brandsdal BO, Haug BE, Monrad J, Svendsen JS. Antimicrobial peptides with stability toward tryptic degradation. Biochemistry 2008;47:3777–88.
66. Rasul R. Novel Antimicrobial Biomaterials. [PhD Thesis] Sydney, Australia: University of New South Wales; 2010. Available at: http://www.unsworks.unsw.edu.au/primo_library/libweb/action/dlDisplay.do?vid=UNSWORKS&docId=unsworks_10024&fromSitemap=1&afterPDS=true. Accesssed February 20, 2014.

Debarun Dutta
School of Optometry and Vision Science
Rupert Myers Bldg, Gate 14, Barker St
The University of New South Wales
Sydney New South Wales 2052
Australia
e-mail: debarun.dutta@unsw.edu.au