

Sol–Gel Nanocomposite Coatings for Preventing Biofilm Formation on Contact Lens Cases

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Purpose: To evaluate the efficacy of a nanosilver-based sol–gel coating in preventing biofilm formation on contact lens cases.

Methods: An organic–inorganic hybrid silica–zirconia sol formulation with immobilized silver nanoparticles was deposited on contact lens case coupons. The coated and uncoated coupons were subjected to biofilm formation to Gram-negative and Gram-positive keratitis isolates and ATCC strains using a standard protocol. The biofilms were evaluated using crystal violet, MTT assay, and scanning electron microscope (SEM) examination. The duration of efficacy of the coating was evaluated by exposing the coated and uncoated coupons to a multipurpose lens cleaning solution for various durations up to 30 days and comparing their biofilm characteristics. The cytotoxicity of the coated surface was assessed using cell culture studies.

Results: Cross-hatch tests and SEM confirmed the presence of a uniform, well-adhered coating on the surface. The coating resulted in a nearly 95% reduction in biofilm formation of the tested bacteria and was effective despite exposures of up to 30 days to a multipurpose lens cleaning solution. The coating did not exhibit cytotoxicity to human corneal epithelial cells.

Conclusions: The silver nanoparticle-based coating exhibits a good antibiofilm property for both Gram-negative bacilli and Gram-positive cocci and is promising for commercial use in preventing contact lens-related infections.

Translational Relevance: Biofilm formation on lens cases continues to be an important concern. The proposed coating will help reduce such formations, thus reducing the risk of lens-associated microbial keratitis.

Introduction

Contact lens-related microbial keratitis (CL-MK) is the most devastating ocular infectious condition associated with lens wear.^{1,2} The annual incidence of CL-MK varies from 2.2 to 6.9 per 10,000 wearers with daily-wear contact lenses and from 9.3 to 20.9 per 10,000 wearers with extended-wear contact lenses.^{3,4} With an estimated 140 million contact lens wearers globally,³ this complication is a major concern. Various risk factors of infection with contact lens wear have been identified and include overnight use,^{5–13} age,^{8,14,15} male gender,¹⁶ living in a warm climate,¹⁷ and noncompliance with lens or lens case hygiene practices.^{5–10,13,18}

The moist environment of lens cases promotes bacterial colonization and the formation of biofilm, which in turn is transferred onto contact lenses and becomes a source of infection.

A variety of efforts have been made to prevent biofilm formation in contact lens cases, including copolymerization of silver,^{19–21} selenium,²² or furanones²³ along with the polymer of the lens case material. These are all labeled active chemical strategies, as they employ microbicidal chemicals. Another set of strategies, referred to as passive strategies (anti-adherent or anti-wetting), employ chemical modifications to change the surface properties so as to make them hydrophobic, thereby reducing microbial attachment and biofilm formation.^{24–27} A third approach is a

combination of two strategies. In a recent study, Ellinas et al.²⁸ observed that the antibacterial actions of a superhydrophobic surface are dependent on bacterial concentration and are compromised beyond a threshold level. The authors suggested that metal-enriched superhydrophobic surfaces are the ultimate hybrid antibacterial surfaces.²⁸ To this end, silver is a well-studied material for creating antibacterial surfaces by a variety of methods, including physical deposition and chemical reduction; however, in most of these methods, silver particles are weakly bound to the surface and easily leach out in the solution, body fluids, and tissues. To circumvent this, Shen and associates²⁹ produced an anti-adhesive coating incorporating antibacterial material by covalently bonding specially designed silica microspheres with polydimethylsiloxane. Bare silver nanoparticles are spontaneously generated on the surface of these silica microspheres through the reduction process of silver ions by thiol groups. The authors reported that such a surface treatment inhibited the growth of *Escherichia coli* and *Bacillus subtilis*.²⁹ However, the procedure reported is a multistep, time-consuming process; therefore, the quest to find an antibiofilm coating continues.

In this study, we evaluated the antibiofilm performance of a combined strategy employing the bactericidal properties of silver and antiwetting properties of an organic-inorganic hybrid nanocomposite formulation derived by a simple wet chemical route referred to as sol-gel technology.

Methods

Materials

Methacryloxypropyl trimethoxy silane (MPTMS; purity, >99%), zirconium *n*-propoxide (*ZrnP*; 70% in *n*-propanol), methacrylic acid (MAA; purity, >99%; Alfa Aesar, Ward Hill, MA), and silver nitrate (purity, >99.5%; S D Fine-Chem Ltd., Mumbai, India) were used as received without further purification. Hydrochloric acid (HCl) and isopropanol (IPA) supplied by Thermo Fisher Scientific India were used for sol synthesis.

Synthesis of Silver Modified Hybrid Sol

ZrnP and MAA were used in a 1:1 molar ratio, and an equimolar amount of IPA was used to dilute the mixture to avoid precipitation of *ZrnP* into zirconium hydroxide. This mixture was labeled as Part A. Part B was obtained by homogenizing MPTMS and H₂O in a molar ratio of 1:0.7, and 0.1-M HCl

was used as the catalyst to promote hydrolysis and condensation of MPTMS. Part A was added slowly to Part B under vigorous stirring in an ice bath, as the reaction is highly exothermic. The resultant mixture was stirred for 3 hours at room temperature. The sol was diluted with IPA in 1:1 weight ratio and stirred for 8 hours, and 1% (w/w) of silver nitrate was then added to this sol and stirred until the silver nitrate dissolved completely. Prior to coating lens cases, 1% (w/w) of the photo-initiator IRGACURE 184 (Ciba Specialty Chemicals, Tarrytown, NY) was added to the sol and stirred until it dissolved completely. This silver-modified hybrid sol was used for the coating trials.

Coating of Contact Lens Case Material

Acrylonitrile butadiene styrene (ABS) thermoplastic contact lens cases (Crescent Vision Care, Hyderabad, India) were cut into small-sized circular coupons 10 mm in diameter (Supplementary Fig. S1). Prior to coating, these circular discs were surface treated using an atmospheric air plasma treatment system (Plasma-treat GmbH, Steinhagen, Germany). This step of plasma treatment was necessary to improve adhesion of the coating on the substrate. Plasma was created with compressed air at a supply pressure of 3 bar and 100 L/h flow rate. Plasma power was fixed at 5 kW. During treatment, the two nozzles (80 mm apart) of the plasma head generated a plasma zone 80 mm in diameter. The substrates were picked by a vacuum pad held by a Motoman robot (Yaskawa Motoman Robotics, Miamisburg, OH) and moved over the plasma jet at a speed of 100 cm/min to activate the surface. The substrate-to-plasma nozzle distance was fixed at 10 mm throughout the experiment.

Applying the sol to the plasma-treated lens cases (coupon) was performed by dip-coating that employed 1-mm/s withdrawal speed. Subsequently, the coated coupons were air dried for 15 minutes followed by ultraviolet (UV) curing (120 W/cm with total wattage/lamp = 12 kW) of both sides using a conveyorized UV curing unit (Advance Curing System, Bangalore, India). During curing, the belt speed was maintained at 2 m/min. The light dose as measured by UV radiometer (EIT LLC, Leesburg, VA) was 871 mJ/cm² in the UV-C region. UV curing helped to generate uniformly embedded silver nanoparticles in the hybrid sol-gel silica matrix. The UV cured coupons were then thermally cured in air at 80°C for 6 hours.

The surface of the coupons was examined under a scanning electron microscope (SEM; S-3400N; Hitachi, Tokyo, Japan) after gold sputtering as per the

protocol provided in the Supplementary Material.³⁰ Hydrophobicity was determined by measuring the water contact angle using a Krüss DSA100 drop shape analyzer (Krüss Scientific, Hamburg, Germany). The adhesion of the coating was evaluated by cross-hatch test carried out as per ASTM D3359-17.

Induction of Biofilm on Uncoated and Sol–Gel-Coated Coupons

Both uncoated coupons (positive controls) and sol–gel-coated coupons (test surfaces) were exposed to an identical protocol of biofilm formation. Uncoated coupons and sol–gel-coated coupons exposed to culture media without bacteria served as negative controls. Details regarding the selection of bacteria for the biofilm and the protocol for biofilm production on the lens case material are given in the Supplementary Material. Briefly, a set of 10 isolates (clinical and ATCC) each of *Staphylococcus aureus* and *Pseudomonas aeruginosa* were tested for biofilm-forming capabilities. Bacterial strains producing optimal biofilm on contact lens cases were identified by crystal violet assay (Supplementary Fig. S2). Two such isolates, *P. aeruginosa* (L-2026/16) and *S. aureus* (ATCC 25923), produced well-characterized biofilms and were used throughout the study for all experiments. The resultant biofilms on the coupons were evaluated by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay,³¹ as well as by SEM.

Log Reduction Studies

The aspirated inoculum of each well after 24 hours of incubation with the coated or uncoated coupons was diluted serially in 1 mL of 1× PBS sterile buffer, and 10 µL of the resultant solution was spotted on a blood agar plate to count the number of colonies. The CFU/mL count was determined for both coated and uncoated coupons using the formula $\text{CFU/mL} = \text{number of colonies} \times \text{dilution factor/volume of culture plated (mL)}$.

Cytotoxicity Studies

The cytocompatibility assay was carried out by using the human corneal epithelial (HCE) cell line HCE-P3.³² The HCE cells were cultured in Dulbecco's Modified Eagle's Medium/Ham's F-12 Nutrient Mixture (DMEM/F-12; Sigma-Aldrich, St. Louis, MO) supplemented with 10% fetal bovine serum (FBS).

HCE cells were seeded at a density of 0.6×10^5 cells per well of a 12-well Petri plate with either a coverslip or sol–gel-coated coverslip. DMEM/F-12 medium with 10% FBS was used as a nutritional supplement. Cell morphology and growth were assessed by phase-contrast images obtained over 3 days. Cytotoxicity was analyzed using LIVE/DEAD assays (LIVE/DEAD Cell Imaging Kit, 488/570 nm; Thermo Fisher Scientific, Waltham, MA) to estimate the effect of the sol–gel coating on the cell toxicity when compared to control. After 3 days, the MTT assay was performed to evaluate the cell growth rate compared to control.

For SEM analysis on coupons, 10^4 cells/mL were added to each well, which included a coverslip and either uncoated or sol–gel-coated coupons, and were allowed to adhere for 30 minutes. The cells were cultured for 3 days in the DMEM/F-12 medium and fixed for SEM analysis.

Stability of Coatings by SEM

Under normal circumstances, daily-wear contact lenses are stored in lens cases immersed in a multipurpose solution. The solution is exchanged frequently by contact lens users. To understand the stability of the test coating under such conditions, both uncoated and sol–gel-coated coupons were exposed to Bausch + Lomb Biotrue multipurpose solution (Bausch + Lomb, Rochester, NY). Test and blank coupons were transferred to a fresh 24-well plate containing 1 mL of the solution. The solution was exchanged daily for a variable periods of no exposure (0 day), 7 days, 14 days, and 30 days. After each time point, select groups of coated and uncoated coupons were removed aseptically and exposed to optimum biofilm forming conditions of *P. aeruginosa* (L-2026/16) and *S. aureus* (ATCC 25923) for 24 hours. The coupons were processed for SEM analysis as described above. The biofilm quality was compared between coated and uncoated coupons, as well as at different time points of exposure to the solution.

Statistical Analysis

Statistical analyses were performed using the Student's unpaired *t*-test to determine the significance of differences between the coated and uncoated groups of coupons. SigmaStat 3.5 (Systat Software, San Jose, CA) was used for analysis, and significance was obtained at $P \leq 0.05$. All of the experiments were performed in duplicate, with at least three independent repeats, unless indicated otherwise.

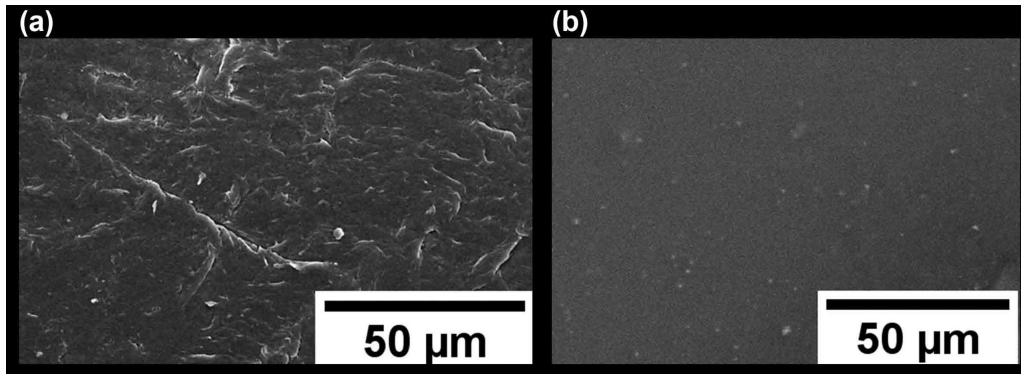


Figure 1. SEM of an uncoated coupon (a) and a sol-gel-coated coupon (b). Each image represents scanning of at least two coupons independently and represents an average scan of three scan areas.

Results

Properties of Sol-Gel Coatings on Lens Case Material

SEM analysis showed a uniform coating of coupons. The control coupon without any coatings

showed a rough surface (Fig. 1). The adhesion as determined by the tape adhesion test (per ASTM D3359-17) was ranked as 5B (no peeling off). The water contact angle on the sol-gel-coated surface was determined to be $85^\circ \pm 5^\circ$, compared to the water contact angle of $70^\circ \pm 5^\circ$ exhibited by the uncoated contact lens surface, suggesting that the coating had increased hydrophobicity.

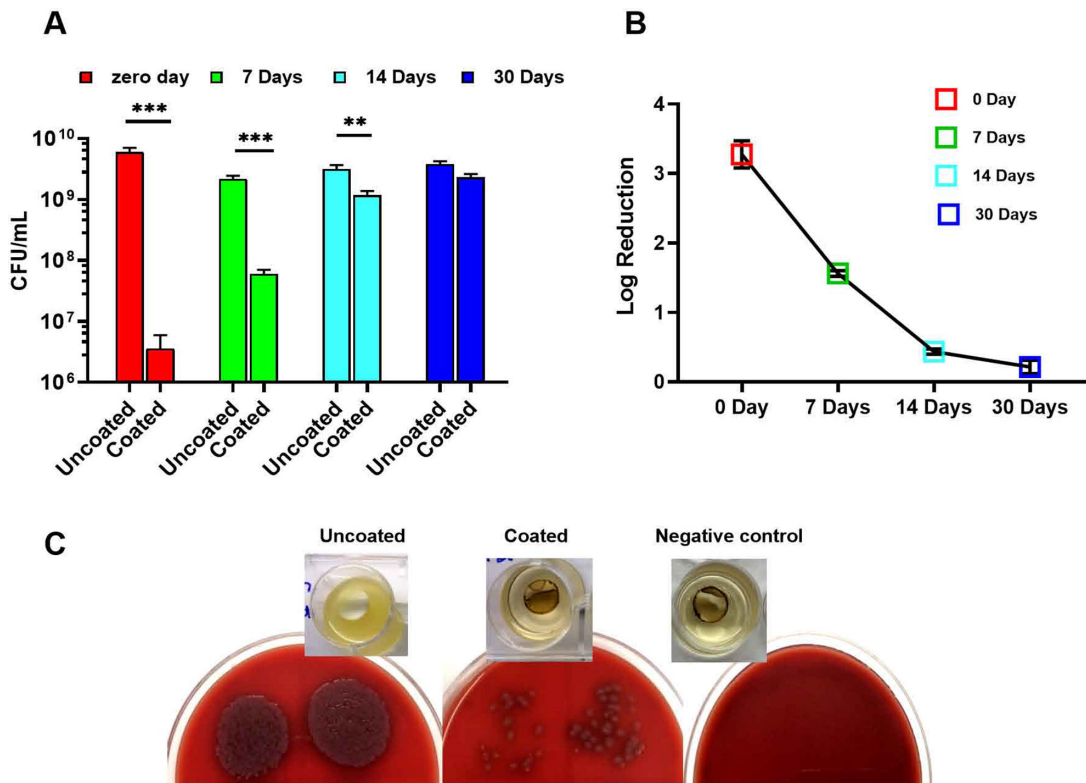


Figure 2. Inhibition activity of the sol-gel coatings against *P. aeruginosa* (ocular isolate) by log reduction studies. (A) The colony forming units per milliliter (mean \pm SD; $n = 4$) of bacteria recovered from wells containing coated and uncoated coupons in BHI media after soaking them in multipurpose solution for different periods of time. (B) Corresponding log reduction values for the same. (C) The bacterial colonies on blood agar plates after 24 hours of growth in BHI.

Table 1. Bacterial Inhibition Activity of Sol–Gel Coatings Compared to Uncoated Coupons by Log Reduction Studies

Day	CFU/mL, Mean \pm SD				
	Uncoated Coupons	Coated Coupons	Log Reduction	Percent (%) Reduction	<i>P</i>
0	6.00E+09 \pm 1.00E+09	3.58E+06 \pm 2.32E+06	3.276 \pm 0.196	99.94 \pm 0.028	0.0005
7	2.17E+09 \pm 2.89E+08	6.00E+07 \pm 1.00E+07	1.559 \pm 0.039	97.23 \pm 0.252	0.0002
14	3.17E+09 \pm 4.73E+08	1.17E+09 \pm 2.08E+08	0.4350 \pm 0.037	63.18 \pm 3.104	0.0026
30	3.77E+09 \pm 4.51E+08	2.30E+09 \pm 3.00E+08	0.2146 \pm 0.097	37.94 \pm 14.53	0.0094

Inhibition of Bacterial Growth (Log Reduction)

Compared to the uncoated coupons, 24 hours of exposure to the coated coupons resulted in a 2- to 3-log reduction of bacteria for the *P. aeruginosa* inoculum, amounting to a reduction of approximately 99% in the growth of *P. aeruginosa* (Fig. 2, Table 1). This biocidal property of the coating was observed for up to 7 days of exposure to the multipurpose solution; after this time period, the biocidal activity declined. However, this biocidal activity was not observed against *S. aureus*, as the colony counts at 24 hours of exposure did not differ between the coated and uncoated coupons. Table 1 shows the means \pm standard deviations (SDs) for the colony forming units determined in the inoculum of *P. aeruginosa* after 24 hours of exposure to uncoated and sol–gel-coated coupons. The differences are represented as log reductions and their correspond-

ing percent reduction values. The experiments were repeated after exposing coated and uncoated coupons to the Biotrue multipurpose lens cleaning solution for 7, 14, and 30 days.

Quantification of Biofilm on Lens Case Coupons by MTT Assay

The results of the MTT assays of the coated and uncoated coupons are shown in Figure 3 and Table 2. Compared to positive controls (uncoated coupons), the sol–gel-coated coupons exhibited a reduction in metabolic activity of 95.67% \pm 2.05% for *S. aureus* and 94.69% \pm 2.22% for *P. aeruginosa*.

Table 2 provides quantification of the biofilms on the coated and uncoated coupons as determined by MTT assay. The metabolic activity of biofilms on uncoated coupons was expressed as maximum activity; the test results were normalized to the uncoated

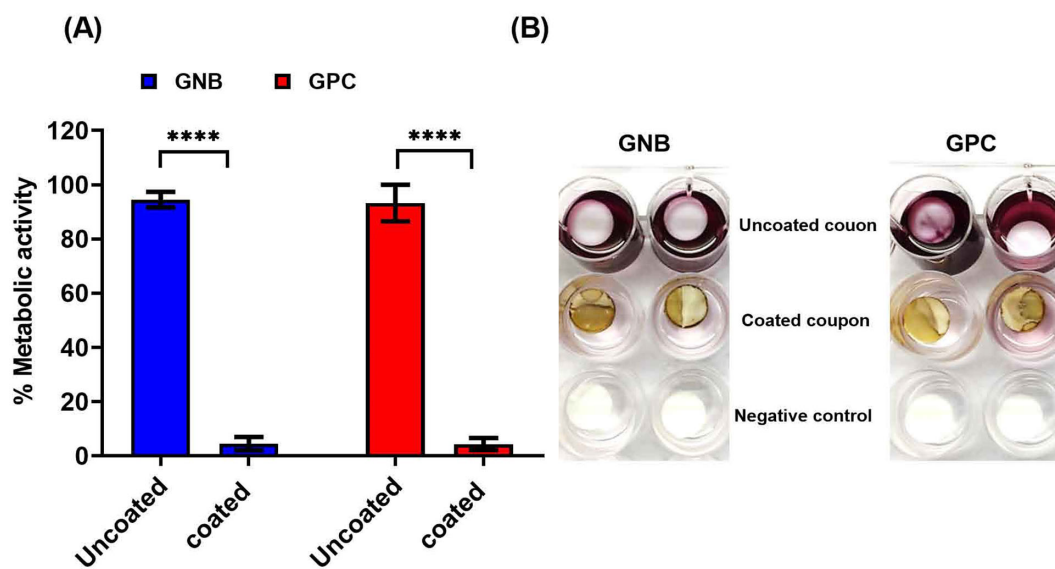


Figure 3. Quantification of biofilm by MTT assay. (A) Metabolic activity in percent (mean \pm SD; *n* = 6) of biofilm on coated and uncoated coupons after 24 hours of exposure to bacteria (*P. aeruginosa* and *S. aureus*); Student's *t*-test, *P* < 0.0001. (B) MTT-stained coupons after solubilizing the biofilm in dimethylsulfoxide.

Table 2. Quantification of Biofilm on Coated and Uncoated Coupons by MTT Assay

	Percent (%) Growth on Coupons, Mean \pm SD		<i>P</i>
	Uncoated	Coated	
Gram-negative bacilli	94.47 \pm 2.89	5.31 \pm 2.43	<0.0001
Gram-positive cocci	93.24 \pm 6.77	4.33 \pm 2.24	<0.0001

Table 3. Quantification of Biofilm by MTT Assay on Coated (With or Without Silver) and Uncoated Coupons

Coupons	Mean \pm SD		<i>P</i>
	Percent (%) Growth	Percent (%) Reduction	
Uncoated	90.17 \pm 11.78	—	—
Coated with silver	4.33 \pm 2.24	95.67 \pm 2.05	<0.0001
Coated without silver	15.68 \pm 5.6	84.32 \pm 5.65	<0.0001

coupons and are expressed as percent growth on the coupons.

Further, to determine why the coating did not inhibit *S. aureus* growth but did prevent biofilm formation, we performed a control experiment with coatings having no silver. The results of the MTT assays are shown in Figure 4 and Table 3. The coatings without silver showed 84.32% \pm 5.65% reduction for *S. aureus* ($P < 0.0001$); however, this reduction was lower than

that observed in the experiments with silver-containing coatings (95.67% \pm 2.05%).

SEM Analysis of Biofilms on Coated and Uncoated Coupons

SEM results are shown in Figure 5. The images of the coated coupons show significantly fewer bacteria, which were scattered over the surface. The findings were similar for both *S. aureus* and *P. aeruginosa*. In contrast, the uncoated coupons (positive controls) showed dense colonies for both organisms. At lower resolution, the bacterial colonies (biofilm) had an appearance like flakes, and higher resolution microscopy revealed a large number of bacteria enclosed in a complex matrix of extracellular material.

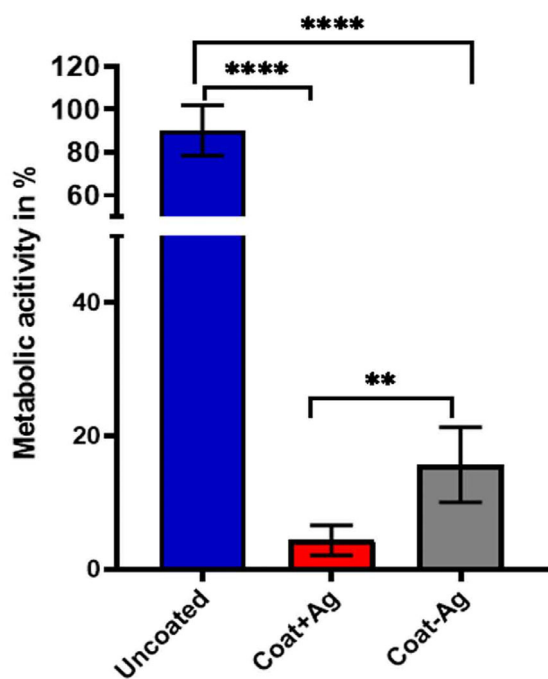


Figure 4. Quantification of biofilm by MTT assay. (A) Metabolic activity of biofilm in percent (mean \pm SD; $n = 6$ coupons coated with or without silver, $n = 12$ for uncoated coupons) on coated and uncoated coupons after 24 hours of exposure to bacteria (*S. aureus*) ($P < 0.0001$).

Cytotoxicity Studies of the Sol-Gel Coating

Figure 6 shows SEM images of HCE cells grown in the presence of uncoated coupons (control) and sol-gel-coated coupons (test). The SEM images clearly show good growth of the epithelial cells and formation of a uniform monolayer over the coverslips with no difference whatsoever between those exposed to uncoated and sol-gel-coated coupons, suggesting that the sol-gel coating exhibits no cytotoxicity on corneal epithelial cells.

Study of Impact of Exposure to Lens Cleaning Solution on Antibiofilm Property of the Coating

Figure 7 shows SEM images of biofilms on uncoated and sol-gel-coated coupons pretreated with lens cleaning solution for various durations (7, 14, or

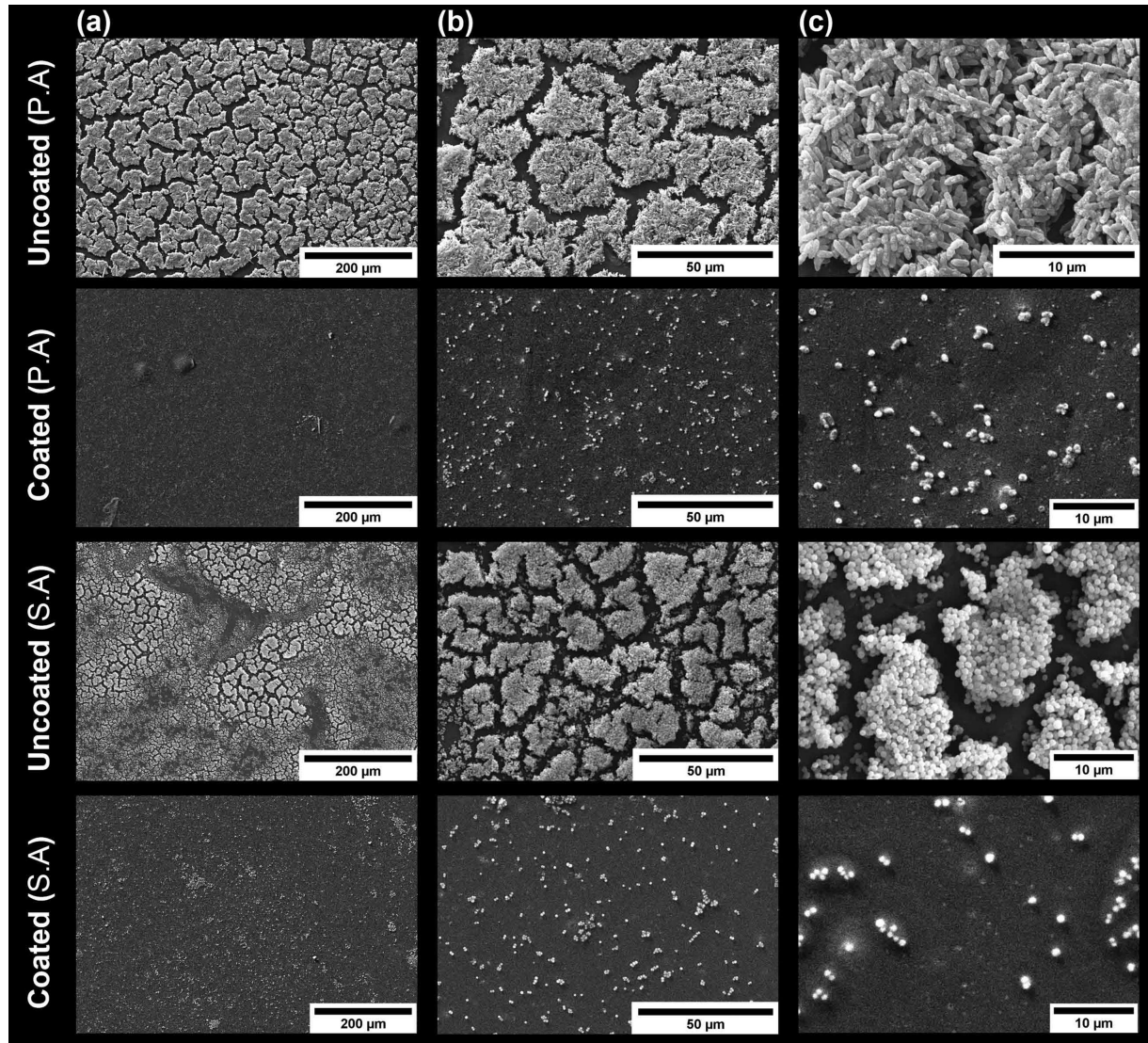


Figure 5. SEM of uncoated and sol-gel-coated coupons after 24 hours of exposure to bacteria. The upper two panels show coupons exposed to *P. aeruginosa*, and the lower two panels show coupons exposed to *S. aureus*. The micrographs were taken at 200 \times , 1000 \times and 3000 \times magnifications. Each image represents scanning of two coupons independently and represents an average scan of at least three scan areas.

30 days). Compared to the uncoated coupons (positive controls), which showed a well-formed biofilm at all time points, the sol-gel-coated coupons showed very few scattered bacteria (*S. aureus* and *P. aeruginosa*) even after 30 days of exposure to the multipurpose solution. The finding clearly suggests that the coating is active in preventing biofilm formation up to and beyond 30 days of exposure to lens cleaning solutions.

Discussion

A biofilm is defined as an aggregation of microorganisms embedded within a self-produced matrix of

extracellular polysaccharide substances adhered to each other and/or to a surface. Biofilms are implicated in a wide variety of infections, including contact lens-associated microbial keratitis.³³ Various epidemiological studies have found a strong association between microbial keratitis events and poor lens hygiene and contact lens case contamination.³⁻¹⁶ Further, even among users adhering to good lens care practices, a high percentage of lens cases were found to harbor disease-causing microbes.³⁴ The moist environment of lens cases and the solid-liquid interface between the contact lens case surface and the aqueous medium provides an ideal environment for the attachment and growth of microorganisms.^{35,36} When a contact lens is

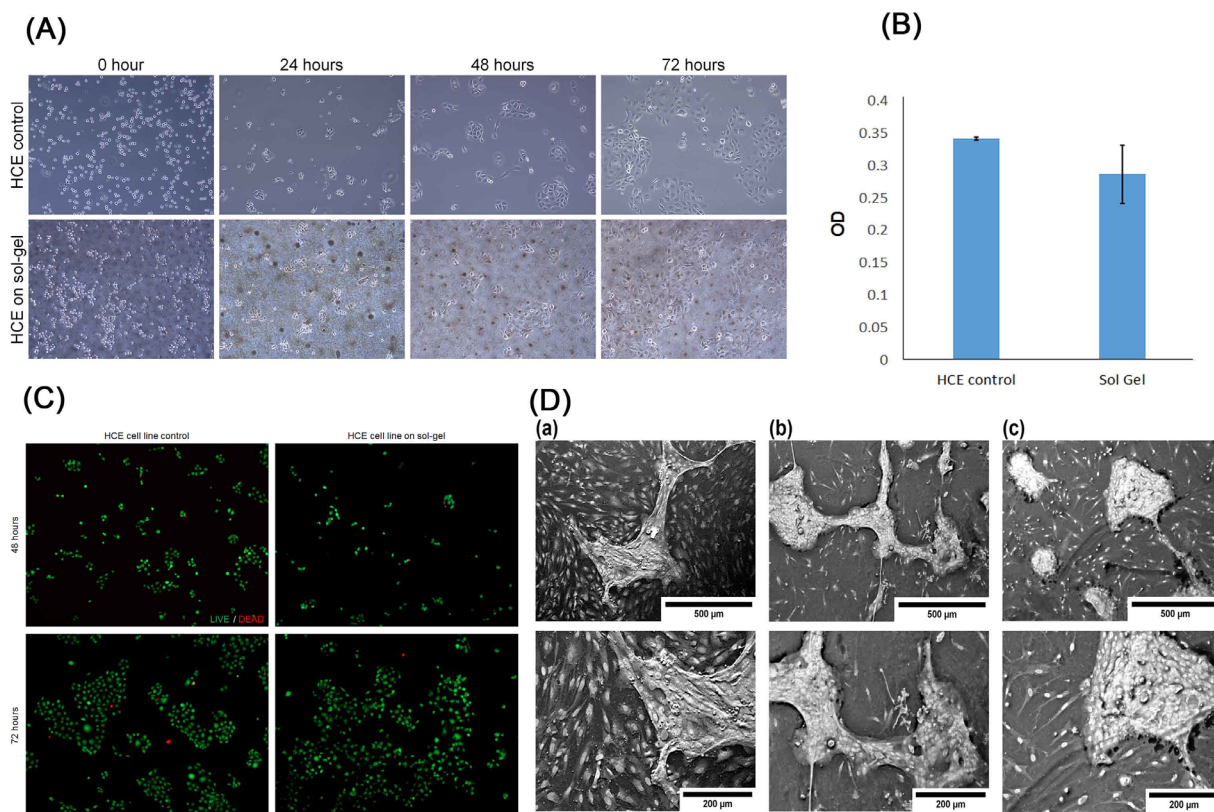


Figure 6. Cytotoxic studies of the sol-gel coatings. (A) Phase contrast images of HCE cells on a Petri dish (control) and sol-gel-coated plate grown for 72 hours at 10 \times magnification. (B) Cell proliferation of HCE cells, as assessed by MTT assay, on sol-gel-coated plates after 72 hours. Error bars indicate SD. (C) LIVE-DEAD staining of HCE cells on sol-gel-coated plates was similar to control after 48 and 72 hours; fluorescent images were taken at 10 \times magnification. (D) HCE cells were grown on coverslips (a), uncoated coupons (b), and sol-gel-coated coupons (c) for 72 hours and subjected to SEM. Upper lane magnification, 100 \times ; lower lane magnification, 200 \times . Each image represents scanning of two coupons independently and represents an average scan of at least three scan areas.

placed in a contaminated lens case, the microorganisms in the biofilm adhere to the lens surface and are transferred to the cornea when the lens is placed in the eye.

Despite several innovations related to superior lens material and biocidal lens care solutions, the incidence of microbial keratitis has largely remained unchanged. Therefore, there is continued interest in interventions such as antimicrobial contact lenses and lens cases that can potentially prevent microbial adhesion and growth at the source.

In this article, we have described a unique sol-gel-derived organic-inorganic hybrid silica-zirconia matrix material that produced a stable and uniform coating on lens case material and in turn effectively prevented bacterial attachments and biofilm formation for both Gram-positive and Gram-negative bacteria. The material is an UV polymerizable, organically modified silane (MPTMS), such that the acrylic groups become polymerized when exposed to UV radiation (Fig. 8).³⁷ Further, the UV exposure simultaneously

generates silver nanoparticles, which become uniformly distributed and strongly bound to the hybrid silica-zirconia matrix.

The organic moieties (acrylic functional groups) provide increased hydrophobicity and thus an antibiofilm effect that is supplemented by the bactericidal properties of the nanosilver. This synergism of surface modification and bactericidal activity results in overall improved antibiofilm efficiency as demonstrated by both MTT assay and SEM studies. Demonstrable antibiofilm activity was seen both for Gram-positive (*S. aureus*) and Gram-negative (*P. aeruginosa*) bacteria.

Further, this composite coating inhibited the growth of ocular isolate of *P. aeruginosa*, as indicated by the up to 3-log reductions (99.9%) of bacteria in solutions aspirated from wells containing coated coupons compared to the control coupons after 24 hours of exposure. The antibacterial activity remained unchanged even after 7 days of soaking with a

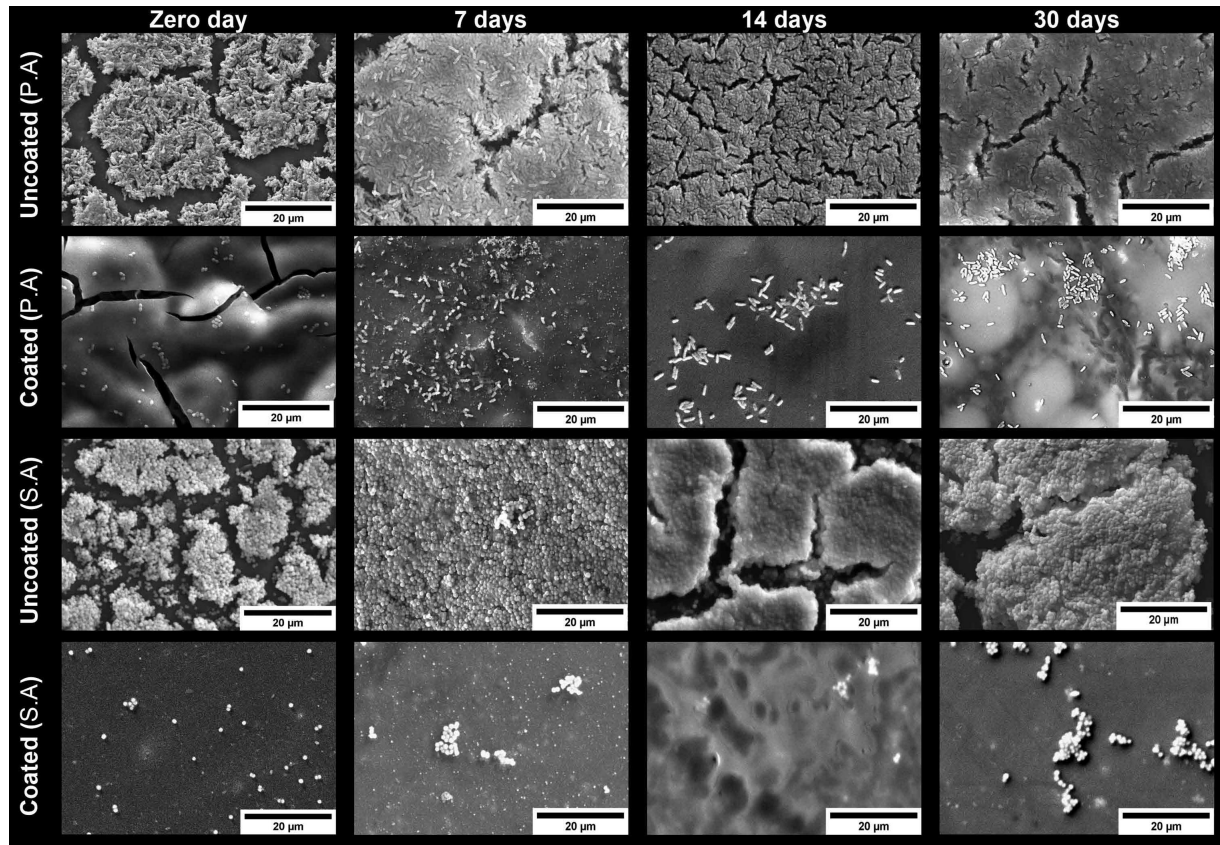


Figure 7. SEM of uncoated coupons and sol-gel-coated coupons soaked in multipurpose solution for 7, 14, and 30 days and then treated with bacteria for 24 hours. The upper two panels show coupons exposed to *P. aeruginosa*, and the lower two panels show coupons exposed to *S. aureus*. The micrographs were taken at 2000× magnification. Each image represents scanning of two coupons independently and represents an average scan of at least three scan areas.

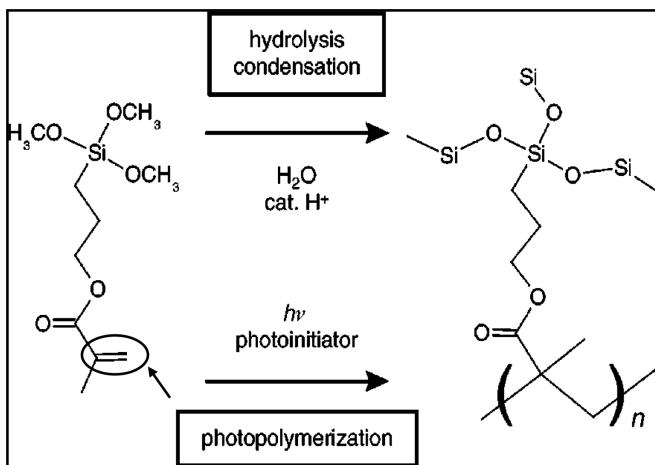


Figure 8. Schematic diagram showing crosslinking of the networks by photothermally induced polymerization.³⁷

multipurpose cleaning solution. However, upon further soaking for 14 or 30 days, this antibacterial activity declined to 0.435 ± 0.037 log reductions, or a reduction of $63\% \pm 3\%$ ($P = 0.0026$). On the other

hand, the log reduction experiments did not exhibit inhibition of growth of *S. aureus*. The results of the experiments with coatings having no silver showed lower but significant ($84.32\% \pm 5.65\%$) reduction of metabolic activity for *S. aureus*, based on MTT assays, indicating that the prevention of biofilm formation is due to a property of the coating itself. Furthermore, the difference in results between coatings containing silver and those not containing silver suggest that the silver-containing coatings inhibited *S. aureus*, as well. However, this effect could not be detected by the log reduction method, as the log reduction test would be positive only if the reduction was 90% or greater. Understanding this differential behavior of the silver coating will require more work, especially in light of previous investigations comparing the antibacterial activities of silver-impregnated contact lens cases that have reported significant differences in the activity among different lens cases.^{19,20} A silver-impregnated contact lens case sold under the name I-Clean (Cooper-Vision, Lake Forest, CA) showed a 5.4-log reduction in *S. aureus* but no effect against *P. aeruginosa*.²⁰

Despite the reduction in bactericidal activity past 1 week, the sol–gel coating still retained some antibiofilm activity and continued to prevent the development of biofilm for up to 30 days. Further, because all of the experiments were performed with higher concentrations of inoculum (10^6 CFU/mL for *P. aeruginosa* and 10^7 CFU/mL for *S. aureus*) and in nutrient rich media (brain–heart infusion [BHI] and trypticase soy broth + glucose), we expect that under real-life situations the coatings would be far more effective. We observed that, although biofilms developed on uncoated contact lens cases even after exposure to multipurpose solution, the same did not happen with coated coupons.

In addition to being effective, the technology has the following additional properties: (1) Unlike the silver-impregnated storage cases, in which the inclusion of silver is achieved through copolymerization with the monomer used in making the lens cases, ours is only a surface modification. (2) Sol–gel matrices are promising for immobilizing bactericidal materials such as nanosilver which in turn prevents leaching of the material while providing bactericidal properties. (3) The formulation adheres very well to any surface due to the formation of covalent bonds and can be easily applied on a wide variety of surfaces. (4) It is multifunctional, in that it offers antibacterial activity, scratch resistance, increased hydrophobicity (from low surface energy), and corrosion resistance. In our earlier study, the coatings generated from the same matrix (MPTMS and ZrnP) yielded a pencil scratch hardness of H when deposited on polymethylmethacrylate (a plastic substrate such as ABS) after testing as per ASTM D3363-05.^{38,39} This is three orders of magnitude higher than that of the bare poly(methyl methacrylate) substrate, which exhibited a pencil scratch hardness of B. The abrasion resistance of the coated substrate was found to improve by more than 50% compared to uncoated substrate. (5) The coatings can be cured using UV radiation when applied on temperature-sensitive substrates such as plastics.

The study has certain limitations. We tested the sol–gel coatings against keratitis-causing bacteria, and further studies are necessary to test the antimicrobial and antibiofilm properties of sol–gel coatings against fungal species. Also, throughout the study, we used only one cleaning solution to test the stability and durability of the coatings. It would be desirable to test the performance using different types of cleaning solutions, as well as with repeated contact lens insertion and removal.

In conclusion, a new type of sol–gel coating has been developed using silver nanoparticles to reduce the burden of microorganisms on contact lens cases. The new coating has biocidal activity against Gram-

negative bacteria and antibiofilm activity against both Gram-negative and Gram-positive bacteria. The coating is not toxic to corneal epithelial cells. These properties, combined with ease of application and sterilization using UV irradiation, clearly indicate that this coating has potential for commercial applications for coating contact lens cases. Prevention of biofilm formation will go a long way toward reducing the risk of contact lens-associated microbial keratitis.

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References

1. Cheng KH, Leung SL, Hoekman HW, et al. Incidence of contact-lens-associated microbial keratitis and its related morbidity. *Lancet*. 1999;354(9174):181–185.
2. Nilsson SE, Montan PG. The annualized incidence of contact lens induced keratitis in Sweden and its relation to lens type and wear schedule: results of a 3-month prospective study. *CLAO J*. 1994;20(4):225–230.
3. Stapleton F, Keay L, Jalbert I, Cole N. The epidemiology of contact lens related infiltrates. *Optom Vis Sci*. 2007;84(4):257–272.

4. Stapleton F, Carnt N. Contact lens-related microbial keratitis: how have epidemiology and genetics helped us with pathogenesis and prophylaxis. *Eye (Lond)*. 2012;26(2):185–193.
5. Dart JK, Stapleton F, Minassian D. Contact lenses and other risk factors in microbial keratitis. *Lancet*. 1991;338(8768):650–653.
6. Stapleton F, Edwards K, Keay L, et al. Risk factors for moderate and severe microbial keratitis in daily wear contact lens users. *Ophthalmology*. 2012;119(8):1516–1521.
7. Stapleton F, Keay L, Edwards K, Holden B. The epidemiology of microbial keratitis with silicone hydrogel contact lenses. *Eye Contact Lens*. 2013;39(1):79–85.
8. Lim CH, Carnt NA, Farook M, et al. Risk factors for contact lens-related microbial keratitis in Singapore. *Eye (Lond)*. 2016;30(3):447–455.
9. Booranapong W, Prabhasawat P, Kosrirukvongs P, Tarawatcharasart Y. Risk factors for contact lens related microbial keratitis: a case-control study. *J Med Assoc Thai*. 2012;95(5):693–698.
10. Khater MM, El-Shorbagy MS. Contact lens-related microbial keratitis in Egypt: 5y epidemiological study. *Int J Ophthalmol*. 2015;15(10):1675–1679.
11. Sauer A, Meyer N, Bourcier T, French Study Group for Contact Lens-Related Microbial Keratitis. Risk factors for contact lens-related microbial keratitis: a case-control multicenter study. *Eye Contact Lens*. 2016;42(3):158–162.
12. Cheung N, Nagra P, Hammersmith K. Emerging trends in contact lens-related infections. *Curr Opin Ophthalmol*. 2016;27(4):327–332.
13. Becmeur PH, Abry F, Bourcier T, Meyer N, Sauer A. Risk factors for contact lens-related microbial keratitis: a multicenter case-control study [article in French]. *J Fr Ophthalmol*. 2017;40(3):224–231.
14. 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(1):153–160.
15. Chalmers RL, Keay L, Long B, Bergenske P, Giles T, Bullimore MA. Risk factors for contact lens complications in US clinical practices. *Optom Vis Sci*. 2010;87(10):725–735.
16. Poggio EC, Glynn RJ, Schein OD, et al. The incidence of ulcerative keratitis among users of daily-wear and extended-wear soft contact lenses. *N Engl J Med*. 1989;321(12):779–783.
17. Efron N, Wohl A, Toma NG, Jones LWJ, Lowe R. *Pseudomonas* corneal ulcers associated with daily wear of disposable hydrogel contact lenses. *Int Contact Lens Clin*. 1991;18(3–4):46–52.
18. Lam DS, Houang E, Fan DS, et al. Incidence and risk factors for microbial keratitis in Hong Kong: comparison with Europe and North America. *Eye (Lond)*. 2002;16(5):608–618.
19. Amos CF, George MD. Clinical and laboratory testing of a silver-impregnated lens case. *Cont Lens Anterior Eye*. 2006;29(5):247–255.
20. Dantam J, Zhu H, Stapleton F. Biocidal efficacy of silver-impregnated contact lens storage cases in vitro. *Invest Ophthalmol Vis Sci*. 2011;52(1):51–57.
21. Datta A, Willcox M, Stapleton F. In vitro antimicrobial efficacy of silver lens cases used with a multipurpose disinfecting solution. *Transl Vis Sci Technol*. 2019;8(3):52.
22. Tran PL, Huynh E, Pham P, et al. Organoselenium polymer inhibits biofilm formation in polypropylene contact lens case material. *Eye Contact Lens*. 2017;43(2):110–115.
23. Baveja JK, Willcox MD, Hume EB, Kumar N, Odell R, Poole-Warren LA. Furanones as potential anti-bacterial coatings on biomaterials. *Biomaterials*. 2004;25(20):5003–5012.
24. Yao K, Huang XD, Huang XJ, Xu ZK. Improvement of the surface biocompatibility of silicone intraocular lens by the plasma-induced tethering of phospholipid moieties. *J Biomed Mater Res A*. 2006;78(4):684–692.
25. Qu W, Hooymans JM, Qiu J, et al. Nonadhesive, silica nanoparticles-based brush-coated contact lens cases—compromising between ease of cleaning and microbial transmission to contact lenses. *J Biomed Mater Res B Appl Biomater*. 2013;101(4):640–647.
26. Yan S, Luan S, Shi H, et al. Hierarchical polymer brushes with dominant antibacterial mechanisms switching from bactericidal to bacteria repellent. *Biomacromolecules*. 2016;17(5):1696–1704.
27. Jing X, Guo Z. Biomimetic super durable and stable surfaces with superhydrophobicity. *J Mater Chem A*. 2018;6:16731–16768.
28. Ellinas K, Kefallinou D, Stamatakis K, Gogolides E, Tserepi A. Is there a threshold in the antibacterial action of superhydrophobic surfaces? *ACS Appl Mater Interfaces*. 2017;9(45):39781–39789.
29. Shen Q, Shan Y, Lü Y, Xue P, Liu Y, Liu X. Enhanced antibacterial activity of poly (dimethylsiloxane) membranes by incorporating SiO₂ microspheres generated silver nanoparticles. *Nanomaterials (Basel)*. 2019;9(5):705.
30. Bozzola JJ. Conventional specimen preparation techniques for scanning electron microscopy

- of biological specimens. *Methods Mol Biol.* 2014;1117:133–150.
31. Mosmann T. Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. *J Immunol Methods.* 1983;65(1–2):55–63.
 32. Araki-Sasaki K, Ohashi Y, Sasabe T, et al. An SV40-immortalized human corneal epithelial cell line and its characterization. *Invest Ophthalmol Vis Sci.* 1995;36(3):614–621.
 33. Costerton JW, Stewart PS, Greenberg EP. Bacterial biofilms: a common cause of persistent infections. *Science.* 1999;284(5418):1318–1322.
 34. Dart J. The inside story: why contact lens cases become contaminated. *Cont Lens Anterior Eye.* 1997;20(4):113–118.
 35. Elder MJ, Stapleton F, Evans E, Dart JK. Biofilm-related infections in ophthalmology. *Eye (Lond).* 1995;9(pt 1):102–109.
 36. Fleiszig SM, Evans DJ. Pathogenesis of contact lens-associated microbial keratitis. *Optom Vis Sci.* 2010;87:225–232.
 37. Soppera O, Croutxé-Barghorn C, Lougnot DJ. New insights into photoinduced processes in hybrid sol-gel glasses containing modified titanium alkoxides. *New J Chem.* 2001;25:1006–1014.
 38. Gururaj T, Subasri R, Soma Raju KRC, Padmanabham G. Effect of plasma pretreatment on adhesion and mechanical properties of UV-curable coatings on plastics. *Appl Surf Sci.* 2011;257:4360–4364.
 39. Soma Raju KRC, Subasri R, Jyothirmayi A, Telasang G, Padmanabham G. *UV Curable Primer-cum-Paint System for Mild Steels Based on Sol-Gel Coating Technology, SAE Technical Paper 2009-28-0052.* Pune, India: Automotive Research Association of India.