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Novel Antimicrobial Coating on Silicone Contact Lens using Glycidyl Methacrylate and Polyethyleneimine based polymers^a

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Abstract:

Contact lenses are medical devices commonly used to correct refractive errors and to maintain ocular health. Microorganisms such as bacteria that grow on the lens surface cause irritation to the eyes and can even cause loss of vision. In this paper, two different coating strategies are employed to form an efficient antimicrobial coating on contact lenses. In the first method, pre-synthesized copolymer of polyethyleneimine-*graft*-polyethylene glycol methacrylate (PEI-PEGMA) was used and the coated lenses show antimicrobial activity (*in vitro*) against Methicillin-resistant *Staphylococcus aureus* (MRSA) bacteria with killing efficacy > 99.99 % and log reduction of 5.1 and proxy host cell viability of 79 %. In the second method, commercially available monomers/polymers such as glycidyl methacrylate (GMA), sulfobetaine methacrylate (SBMA) and polyethyleneimine (PEI) were used. A typical formulation consisting of 1 % GMA shows antibacterial activity against MRSA with killing efficacy > 99.99 % and log reduction of 6.3. Proxy host cell viability for the coated lenses was found to be 90 % indicating that the coating was non-toxic. Antibacterial coating reported here

are very effective in killing gram positive bacteria such as MRSA and *S. aureus*. The second method using commercially available monomers/polymers involving a simple coating procedure is also easily scalable.

1. Introduction:

People worldwide use contact lenses as an alternative to regular spectacles for various reasons such as wide field of vision with no frame to obstruct vision, resistance to moisture condensation, utility in sport and other physical activities and cosmetic appearance. Contact lenses are medical devices used primarily for correcting refractive errors but also at times for ocular health. They normally float on the tear film layer on the surface of the cornea. Contact lens performance is mostly determined by various physical properties such as oxygen permeability, water content, light transmittance and biocompatibility.^[1] The use of contact lens increases the risk of ocular bacterial infection. With the insertion of contact lenses into the eye, microorganisms such as bacteria can attach and grow on the surface of the lenses causing irritation.^[2] These infections are difficult to treat and can even cause vision loss. Contact lens use can lead to adhesion and colonization of bacterial strains which can develop to conditions like contact lens related acute red eye,^[3] peripheral ulcers^[4] and microbial keratitis.^[5, 6] In spite of the availability of new contact lens materials and better cleaning solutions, the risks of the above conditions have remained the same for many years. Bacterial adhesion and colonization are the major challenges for the use of contact lens. Once the bacteria have colonized and formed biofilm on a lens surface, they will be difficult to remove. Modifications which can prevent bacterial colonization are needed.

Depending on the wear time, contact lens market is classified into two main areas of daily wear and extended wear.^[7] The daily wear contact lenses must be cleaned daily for reuse or else disposed of and replaced with a new lens. The extended wear lenses can wear continuously for 30 days and then are either cleaned or disposed of and replaced. The cornea

requires oxygen and insufficient oxygen due to wearing of contact lens can result in swelling of the cornea and associated complications that can lead to vision loss.^[8] This requires that contact lenses be oxygen permeable. High oxygen permeability is not required for daily wear lenses, since they are not continuously worn. Hydrogel lenses contain water and hence they are oxygen permeable. Gaseous oxygen dissolves in water within the lenses and will diffuse to the cornea. Oxygen permeability will be higher for the lenses with higher water content.^[9] Hydrogel lenses prepared using 2-hydroxyethyl methacrylate (HEMA) have only about 38 % of water content which limits oxygen diffusion to the cornea. The water content in the hydrogel lenses can be increased by adding more hydrophilic or ionic monomers. Oxygen permeability can also be increased by reducing the lens thickness.^[7] There is a limit to the water content in the hydrogel lenses beyond which the mechanical properties of the lenses are adversely affected. At high water content, the mechanical properties of the lenses are compromised for higher oxygen permeability. Silicone hydrogels were great innovations to overcome the shortcomings of HEMA based hydrogel lenses.^[10] Oxygen permeability of silicone hydrogel contact lenses are higher compared to other types of hydrogel lenses and hence widely used for both daily and continuous wear.^[11] The design of the contact lenses is constrained by the necessity that surface and bulk properties be compatible with maintenance of corneal health.

Antibacterial properties of silver coating on contact lens have been widely studied, but the material can leak out of the lenses and its antimicrobial effect fades over time.^[12] Martinez *et al.*^[13] reported the use of silver nanoparticle against various microorganisms. A review was recently published by Amy Xiao *et al.*^[14] on various chemical strategies and techniques used for the design of antimicrobial contact lenses. Spallholz *et al.*^[15] reported bacterial inhibition on poly HEMA contact lenses by organoselenium coating through wet chemistry. Though organoselenium technique for antibacterial contact lens is promising, more systematic studies are required to confirm the clinical efficacy. Antimicrobial peptides (AMPs) have been used to control various types of bacterial and fungal infections. Electrostatic interaction between the

AMPs and bacterial membranes play a significant role for controlling these infections.^[16] Willcox *et al.*^[17] designed an antimicrobial peptide, melimine and tested for its ability to reduce bacterial adhesion on contact lens. They found about 80 % reduction of *P. aeruginosa* and *S. Aureus* by using melimine as antimicrobial agent. Another approach is to use passive antimicrobial agents such as 2-methacryloyloxyethyl phosphorylcholine (MPC) that can reduce the adhesion of bacteria to the lens surface.^[18] A zwitterionic coating on polydimethylsiloxane elastomer was reported by Yeh *et al.*^[19] which prevent adherence of *P. aeruginosa* and *S. epidermidis*. Dhand *et al.*^[20] found that poly vinyl alcohol films modified with polymerized hydroquinone (HQ) and epinephrine (EP) shows antibacterial activity against Gram-positive bacterial strains. Though, the antibacterial properties of HQ and PG modified PVA film are acceptable, no coating was made on contact lenses using these films. Recently, Hoyo *et al.*^[21] reported their coating on contact lens with zinc oxide (ZnO) nanoparticles, and chitosan with good antibacterial activity against *S. aureus*. Retention of antibacterial activity in germanium plasma polymer thin films was reported by Ahmed Al-Jumaili *et al.*^[22] These polymers can be used as antibacterial coating for contact lenses. Tuby *et al.*^[23] reported efficient antibacterial nano coatings of zinc doped copper oxide on contact lens. A new way to deposit biocompatible nanocomposite coating on silicon hydrogel contact lens to minimise UV exposure and biofouling was reported by Huang *et al.*^[24]

In this work, we adopted a simple coating strategy to impart antibacterial properties to contact lenses. We used poly glycidyl methacrylate/-poly SBMA or -AMPTMA/polyethylene imine coating to effectively kill the bacteria on contact lens surface. There are many advantages of this coating process such as commercial availability of the materials as well as the simplicity of the process itself. The solution concentration used for the coating process is small, less than 5 wt. %. The coating process can be easily scale-up. We also synthesized PEI-PEGMA polymer and used that for formation of an antibacterial coating on contact lens surface in order to prevent bacterial infection and reduce the risk of extended wear.

2. Experimental Section

2.1. Chemicals and Materials:

Polyethylenimine (PEI, $M_w = 25,000$ Da) was purchased from Sigma-Aldrich and freeze-dried before use. Isopropyl alcohol (IPA), sodium hydroxide (NaOH), potassium carbonate (K_2CO_3), poly(ethylene glycol) methacrylate (PEGMA, $M_n = 360$ Da), iodoethane, glycidyl methacrylate (GMA), methylene chloride, Acryloylaminopropyl trimethylammonium chloride solution (AMPTMA, 75 wt.% in water), *N*-(3-Sulfopropyl)-*N*-(methacryloxyethyl)-*N,N*-dimethylammonium betaine (SBMA), PEI with M_w 2000 Da and 800 Da were purchased from Sigma-Aldrich. Chloroacetyl chloride ($C_2H_2Cl_2O$), ethanol, chloroform and toluene were purchased from Merck Pte Ltd. Polyethylene glycol dimethacrylate (PEGDMA, M_w 1000 Da), Polyethylene glycol diacrylate (PEGDA, M_w 700 Da), *N,N'*-Methylenebis (acrylamide) (NMBA) were purchased from Sigma Aldrich. **Figure S1** shows the chemical structures of the monomers and PEI used for series II coating.

2.2. Synthesis of Chloro- functionalized Poly(ethylene glycol) methacrylate (Cl-PEGMA):

PEGMA with $M_n = 360$ Da (11.7 mL, 35.2 mM) (**Scheme S1 (a)**) was dissolved in 100 mL toluene followed by the addition of $C_2H_2Cl_2O$ (11.2 mL, 141.2 mM) at 23 °C and refluxed for 24 hours. The solvent (toluene) was separated with a rotavapor. The gum thus obtained was re-dissolved in 100 mL methylene chloride, added 1 gm K_2CO_3 and stirred for 10 min. Solid K_2CO_3 was then separated by filtration and solvent removal, affording the required Cl-PEGMA. 1H NMR (300 MHz, $CDCl_3$) (**Scheme S1 (b)**) at 25 °C was used to confirm the product: δ (ppm) 6.06 and 5.50 (m, 1H), 4.29 - 4.21 (m, 2H), 4.02 (d, 2H), 3.69 - 3.67 (m, 2H), 3.66 - 3.56 (m, ethylene protons) and 1.89 -1.87 (m, 3H).

2.3. Synthesis of PEI-PEGMA (1:16):

PEI with molecular weight 25,000 Da was grafted with PEGMA in 1:16 ratio (**Scheme S2 (a)**). PEI (25K)-PEGMA (1:16) prepared as follows. Polymer solution was prepared by dissolving 5 gm PEI in 50 mL deionized water (DI), and 2 mL NaOH (1 M) solution was added at 23 °C.

Previously synthesized Cl-PEGMA (3.51 g) in 5 mL IPA was added drop by drop to the solution. The solution mixture in a round bottom flask (RB) was stirred for 24 hours at ambient temperature followed by dialysis (MWCO 12K-14K) in DI water for 72 hours. The excess water was evaporated with a rotavapor and the product was obtained via lyophilization. ^1H NMR (300 MHz) (**Scheme S2 (b)**) in D_2O at 25 °C: δ (ppm) 5.53 and 5.22 (d, 1H), 3.59-3.11 (m, protons from ethylene glycol), 2.81-2.56 (m, protons from PEI ethylene), 1.80-1.76 (m, 3H), 1.04 (m, protons from PEI NH/NH₂).

2.4. Preparation of Antibacterial Coating with PEI –PEGMA using Thermal Method:

In this method, the silicone contact lenses were treated with ozone to activate the surface and form peroxide groups. After the ozone treatment, the lenses were put under vacuum for 1 hour to remove the residual atmospheric oxygen attached on the lens surface. Formulations with different concentrations of PEI-PEGMA and SBMA were dissolved in DI water and purged with Argon gas for 45 min to remove oxygen from the coating solution. The ozone treated contact lenses were removed from the vacuum chamber by venting the chamber with Argon and immediately transferred to the coating solution. The solutions with contact lenses were kept stirring at 90 °C for 4 hours. The radical initiation was activated thermally. The coated lenses were washed 5-6 times and sonicated with DI water to remove the un-grafted polymer.

2.5. Preparation of Antibacterial Coating with GMA, SBMA and PEI using Thermal Method:

In this method, GMA, SBMA and PEI were used to form antimicrobial coating on the lens surface via a two step process. **Figure 1** shows the schematic of this procedure. In the first step, different concentrations of GMA and SBMA were prepared in 5 ml of DI water and isopropyl alcohol (IPA) solution (1:1 ratio). The solution mixture in 5 ml RB flask was degassed using Argon bubbling for about 45 min to remove all the dissolved oxygen. The silicone contact lenses were treated with ozone to activate the surface and form peroxide groups. Ozone treatment of the contact lens was carried out in an ozone generator with air flow rate fixed at

around 15 L/min for 20 min at room temperature (23°C). The ozone gas was passed through sodium hydroxide crystals to remove moisture. After the ozone treatment, the lenses were put under vacuum for 1 hour to remove residual atmospheric oxygen attached on the lens surface. The ozone treated lenses were then transferred to the formulation solution and kept in oil bath at 90 °C for about 3 hours with stirring. Poly GMA grafted lenses were washed 5-6 times with 20 minutes duration for each wash cycle in DI water and IPA to remove the un-grafted polymer and residual monomers. In the second step, different concentrations of PEI in DI water were prepared and the poly GMA grafted lenses were immersed in the PEI solutions. The lenses with PEI solutions were kept in oil bath at 90 °C for about 3.5 hours. The polymer coated lenses were finally washed and sonicated in DI water to remove all the un-grafted PEI and residual monomers and polymers from the contact lens surface.

2.6. Equipment and Characterization:

¹H NMR spectra of the synthesized polymer were collected with an ADVANCE 300 spectrometer (Bruker, USA). Static contact angle measurements were performed with a Kruss DSA 25 contact angle analyser using sessile drop method. Infrared absorption spectra of coated lenses were performed using an FT-IR spectrometer with ATR accessory (Nicolet, Thermo Fisher Scientific, USA). The surface morphologies of the coated contact lenses were performed with Scanning Electron Microscopy (SEM, JSM 6390LA). The coated lenses were sputtered with a platinum layer before loading to SEM analysis. Surface roughness of the coated lens surfaces was measured using an MFP3D AFM microscope in tapping mode. Transmittance spectra for the coated lens and unmodified lenses were measured using a Varian Cary 5000 spectrophotometer.

2.7. In Vitro Antibacterial Test:

Contact lenses were washed by immersion in 3 mL of phosphate-buffered saline (PBS) and gentle shaking. The lenses were then placed on a small petri dish and UV sterilized for 15 min. The lenses were thoroughly rinsed with DI water and dried to remove any remaining PBS.

Bacteria inoculum (MRSA BAA-40, $\sim 1 \times 10^8$ CFU in 10 μ L) was spread evenly on the contact lens surfaces (concave side). Contact lenses were then incubated at 37 °C with 95 % relative humidity for 2 hours. After 2 hours, the lenses were removed from the petri dish and placed in 2 mL Eppendorf microtubes with the addition of 1 mL of PBS. The contact lenses were then vortexed for 3 min to release MRSA bacteria. PBS containing bacteria were diluted 10x serially in a 96-well plate. After serial dilution, the bacteria were laid on LB agar and incubated at 37 °C with 95% relative humidity for 16 hours. Bacteria colonies were counted and log reduction was calculated with respect to the control. Log reduction was calculated using the formula

$$\text{Log reduction number} = \text{Log}_{10} \left[\frac{CFU_{\text{control}}}{CFU_{\text{sample}}} \right]$$

2.8. In Vitro MTT Cytotoxicity Test by Extraction Method:

The cellular compatibility of the contact lenses was tested by MTT assay using fibroblasts (NIH-3T3 cells) received from ATCC, and cultured in DMEM medium enriched with 1 % antibiotics and 10 % FBS (fetal bovine serum). Incubated the cells at 37 °C and 5 % CO₂ until a monolayer, with greater than 80 % confluence, was obtained. The cultured cell lines were harvested and plated at a concentration of 10 K cells per well (200 μ l) into 96-well microtiter plate for 24 hours. Contact lenses were incubated in the same medium as cells for 24 hours to extract soluble materials from the lenses. After 24 hours, the culture media were discarded from the cell culture plates and the cells were cleaned with PBS. The extracted materials from the lenses were added into the cells and incubated for another 24 hours. After the addition of 200 μ L of MTT (5 mg/ml) into each well, the plates were incubated for 4 hours. The MTT was aspirated and dimethyl sulfoxide (DMSO) was added into each well and the plates were shaken at 150 rpm for 10 min. The absorbance (OD) at 570 nm was read using a microplate reader spectrophotometer (BIO-RAD, Benchmark Plus). All the assays were performed in triplicate. The percentage of the cell viability was calculated using the formula:

$$\% \text{ cell viability} = \frac{\text{Average absorbance of treated cells}}{\text{Average absorbance of control cells}} \times 100\%$$

3. Results and Discussion:

3.1. Polymer Coating Synthesis and Characterization:

Table 1 shows two series of different formulations used for silicone contact lens coating. Series I formulation contains PEI-PEGMA (1:16) and zwitterion monomer SBMA. PEI-PEGMA polymer was employed as the series I coating since it is known to have antibacterial properties.^[25] The ¹H NMR characterization spectra of the polymer PEI-PEGMA is shown in **Scheme S2 (b)**. The resonance at 5.53 and 5.22 ppm (a, a') are assigned to the methylene protons and the resonance at 1.80-1.76 ppm (b) is assigned to the methyl proton of methacrylate. The resonance at 3.59-3.11 ppm (c) is assigned to ethylene glycol protons of PEGMA. The resonance at 2.81-2.56 ppm is assigned to PEI ethylene protons and the resonance at 1.04 ppm is assigned to PEI NH/NH₂ protons. These assigned resonance peaks of the NMR spectrum indicate successful synthesis of PEI-PEGMA. The actual graft ratio of polyethylene glycol methacrylate to polyethyleneimine (mol/mol) were obtained via titration^[25] and is found to be 6.20 to 7.27 for different batches (Table S1).

Since the synthesis of cationic polymer such as PEI-PEGMA is non-productive, time consuming and not industry viable, we also adopted an alternative coating strategy. For series II coating, we used different ratios of commercially available chemicals such as GMA, SBMA and PEI. There are two steps involved in series II coating. Step 1 is adhesive layer formation on silicone contact lens surface and step 2 is grafting of PEI on the adhesion layer. Series II-i formulation used 1wt% of GMA and 1wt% of SBMA for step 1 and 0.5 wt% of PEI (M_w 2 kDa) for step 2 as shown in Table 1. Series II-ii formulation used 2 wt% GMA and 1 wt% SBMA for step 1 and 4 wt % PEI (M_w 800 Da) for step 2. Series II-iii formulation used 5 wt % of different cation monomer (AMPTMA) and 2 wt% of GMA for step 1 and 1 wt% of PEI (M_w 2 kDa) for step 2. Series II-iv formulation used 1 wt% GMA and 1 wt% SBMA for step 1 and

1 wt% PEI (M_w 2 kDa) for step 2. Series II-v formulation used 2 wt% GMA and 1 wt% SBMA for step 1 and 1 wt% PEI (M_w 2 kDa) for step 2.

Figure 2 shows the FT-IR-ATR spectra of polymer coated contact lens by series I-i and series II-i coated contact lenses along with uncoated lens as control. The broader peaks for the coated samples at 3500 cm^{-1} corresponds to -NH_2 group due to PEI-PEGMA or PGMA-PSBMA-PEI coating. The new peak at 1560 cm^{-1} in Figure 2 (b) and (c) is attributed to the C-N stretching in the polymers. The more intense peak at 1651 cm^{-1} in Figure 2 (b) corresponds to N-H bending of primary amine in the PEI-PEGMA coating and peak at 1720 cm^{-1} in Figure 2 (c) correspond to the ester carbonyl group from GMA. In Figure 2 (c), the peaks identified at 1147 cm^{-1} and 1040 cm^{-1} attribute to the SO_3^- of SBMA. FT-IR spectra for series II-ii and II-iii formulation are given in **Figure S2**. The FT-IR spectra of these samples likewise confirm the presence of antibacterial coating on the contact lenses.

Figure 3 shows the SEM images of uncoated contact lens and lens coated using series I-i with formulation PEI-PEGMA (15 %) and SBMA (5 %). Figure 3(a) shows the SEM image of uncoated lens and Figures 3 (b) to (d) show the SEM images of coated lens at different magnifications. The surface of uncoated lens is basically smooth and no specific morphology. Compared to uncoated lens, the SEM images of the series I-i coating shows pebbled structure on the lens surface. Since we use thermal method to graft the PEI-PEGMA polymer on the lens surface and also the total concentration of the polymer together with SBMA in the solution is 20 wt%, the resulting coating formed on the surface is quite dense. **Figure 4** shows the SEM images of the formulation GMA (1 %), SBMA (1 %) and PEI 2 kDa (0.5 %) using thermal method. SEM images were made of different locations of the contact lens surface and the coating was found to be uniform and to have porous structure. In series II type coating, the total concentration of the monomers and PEI used is low (<5 %) and this might have resulted in the porous structure of the coating. The porous structure arise from cross linking of polymer chains on the lens surface. SEM images of the coating for other formulations (series II-ii to II-v) are

shown in **Figures S3 to S5**. Surface morphology for most of the series II type coatings is porous with varying degree of porosity. In Figure S3 (series II-ii), with the PEI concentration increased to 4 wt%, the coating is less porous. Also, in Figure S5 (series II-v), as the GMA concentration increased to from 1 % to 2 %, the coating morphology became less porous. The samples for SEM studies were freeze dried before loading to SEM instrument. Freeze drying the samples keeps the surface morphology of the coating intact. Air drying and thermal drying normally change the surface morphology because of shrinkage. Freeze drying can avoid the problem of surface force at the coating/lens interface and keep the surface morphology unaffected. Coating on the lens surface was also characterized by contact angle measurement and the data are shown in **Figure S6**. Uncoated silicone contact lens is highly hydrophobic with contact angle of 108° while the contact angle for the coated lens (series II-i) is 66° ; the coating makes the lens surface hydrophilic. Surface morphology and roughness of the coating was also measured by AFM and are shown in **Figure S7**. The AFM image of uncoated lens surface is quite smooth with average surface roughness 2.5 nm. The surface morphology for series II-iii coating is much higher, with average surface roughness of 65 nm, confirms the presence of coating on lens surface.

There will be different types of coating failures such as (1) reduction of coating adhesion to the substrate and (2) disintegration of interface between coating and substrate. To improve coating formulations and to resist failures caused by the applied normal load with fingers of users on contact lens, monomer AMPTMA or SBMA was incorporated in the coating formulation. These monomers help to improve the hydrophilicity of coating. The polymer PEI-PEGMA is used for the coating is because of the presence of double bonds that can link with the radicals on the activated contact lens surface and form an effective antibacterial coating. PEI-PEGMA is a hydrophilic polymer, and the PEI has higher density of protonated amines ($-\text{NH}_3^+$). Degassing the polymer solution plays a crucial role in the coating process. If there is oxygen present in the solution, it quenches the reactive radicals on the contact lens surface and coating will not happen or will not be uniform. The free

radicals on the lens surface ensures higher grafting efficiency together with formation of covalent bonds between the lens and the coating layer.

For industrial applications, coating process using metal catalyst is quite dangerous due to environmental pollution. Thermal initiation is an alternative choice for creating radicals for the grafting of antibacterial coating on the lens surface. UV radiation or a metal containing catalyst can also be used as energy sources to create radical species at the lens surface for covalent reactions. After the ozone treatment, peroxide groups form on the surface of the contact lens. Heating will generate radicals on the peroxide groups. These generated radicals will react with the chemical species in the formulation and form covalent bonds with the polymer or monomeric species to form an antibacterial coating layer on the contact lens. For series I and II, we used thermal initiation for the radical generation and coating formation.

The method we report here is based on free radical macromolecular grafting. The two approaches reported here ensure the formation of covalent bonds between the contact lens and polymer coating. The coatings are designed to improve the antibacterial property of the contact lens. The formation of coating is done in two steps for series II coating. In the first step, the epoxy groups are attached on the contact lens surface using GMA through free radical reaction at the contact lens surface. Polymerization of GMA and SBMA and their adhesion to the lens surface happen in the first step. Step 1 process leaves plenty of epoxy groups at the lens surface. The second step is the ring opening polymerization of epoxy groups by amine groups in PEI. We used branched PEI for the step 2 reaction process. In series II coating, we tried different formulations and the details are given in Table 1. We used only small concentrations of the monomers and PEI for coating. GMA concentration is from 1-2 %, SBMA/AMPTMA concentration is 1 to 5 %. We used PEI with two different molecular weights such as PEI 800 Da and PEI 2 kDa. Concentration of PEI used in the second step reaction process is less than 5 %. In combination with GMA, SBMA or AMPTMA forms a crosslinked network on the contact lens surface. It is observed that if we increase the concentration of GMA to above 5 %,

the coating becomes thicker which causes the lens to become opaque. In the second step, epoxy ring opening occurs and the amine groups in PEI react with epoxy to form PGMA-PSBMA-PEI cross linked polymer coating. The active hydrogen of the amino group is consumed by the epoxide group and forms hydroxyl groups through ring opening reaction.

PEI has strong antibacterial activity. Covalent attachment of poly-cations on the surface is very effective for killing bacteria. The hydrophilic and porous coating on the lens surface that contain PEI with higher density of protonated amines helps to kill the bacteria. The presence of pores on the coating surface promotes the absorption of bacteria. Cationic PEIs have been widely utilized as drug carriers in biomedical application due to its ability to permeabilize cell membranes.^[26] PEIs are positively charged and highly basic aliphatic polymers, containing primary, secondary and tertiary amino groups. Amino nitrogen atoms that are present in the polymeric chain may undergo protonation and ethylene backbone serves as hydrophobic groups. Gibney *et al.*^[27] reported the antibacterial activity and cytotoxicity of unmodified PEI. PEI can be attached to negatively charged bacteria cells.

3.2 In Vitro Antibacterial Assay

Figure 5 shows the antibacterial test and cell compatibility results for the series I and three of the series II coatings. MRSA BAA40 bacteria was used to study the killing efficacy of the coating. The series I type produces an average log reduction of 5.1 and has cell viability of 79 %. Different formulations of series II type coating also gives log reductions of more than 4. Series II-i coating has average *in vitro* log reduction of 6.3 (>99.99%) for MRSA and cell viability of 90 %. For series II-ii formulation with 2 % GMA and 1 % SBMA and with 4 % PEI (M_w 800 Da), the log reduction is 4.1 and cell viability is 88 %. For series II-iii formulation with 2 % GMA and 5 % AMPTMA and with 1 % PEI (M_w 2kDa), log reduction is 5.9 and cell viability is 94 %. It is very difficult to treat the antibiotic resistant bacterial infections such as microbial keratitis (MK).^[28] In a case study by Gorscak *et al* ^[29] reported that about 40 % of *S. aureus* strains isolated from MK cases were resistant to ciprofloxacin. Our contact lens coating

can kill MRSA bacteria effectively. The coating is very effective in preventing MRSA growth on the lens surface. The high cell viability data (>80 %) is achieved by using PEI with lower molecular weight such as 2 kDa or 800 Da. The high cell viability results indicates that the coatings are non-leachable in DMEM media and are non-toxic.

Antibacterial test results for the coated lenses using series II-iv and II-v are shown in **Figure S8**. Here we have done the coating as a batch of 5 lenses. These coated lenses were tested against two bacteria strains, MRSA and *S. aureus*. We measured log reduction of 4.2 for *S. aureus* and 3.4 for MRSA with series II-iv formulation containing 1 % GMA and 1 % SBMA and with 1 % of PEI (M_w 2kDa). The series II-v formulation, with 2 % GMA, 1 % SBMA and 1 % PEI (M_w 2kDa) had log reductions of 4.8 for *S. aureus* and 3.1 for MRSA. The slight decrease in the log reduction for series II-iv and II-v compared to series II-i to II-iii is because of the batch processing, which may need further design of facilities and equipment. The series II-iv coated contact lens were tested for antimicrobial activity against MRSA before and after rubbing for 20 cycles on each side of the lens. The rubbing test was carried out by placing the coated contact lens in the wet state at the center of the left hand palm and rub the lens surface with right hand forefinger. The results shows log reduction of the lens after rubbing is 2.8 (99.87 %) and is similar to the log reduction of 3.4 (99.96 %) before rubbing. This shows the coating is quite robust and remains on the lens surface after 20 cycles of rubbing. In order to check whether the coating has affected the transparency of the lens, we have measured the transmittance of the coated lens in the wavelength range 400 nm to 800 nm using UV-Vis spectrometer and compared with unmodified lens. The transmittance data for the coated and uncoated lens is shown in **Figure S9**. The transmittance data shows that the antibacterial coating has insignificant effect on the optical properties of the lens. Optical properties of the contact lenses were not affected by the ozone treatment (**Figure S10**). Özçam *et al.* ^[30] reported the effect of ozone treatment on the surface of siloxane network and did not found any deviation in the optical properties such as absorbance and transmittance in the visible and infrared range.

In the gross visual appearance of the lenses used in the log reduction studies, many bacteria colonies can be plainly seen on the control sample and almost no bacterial colonies were found on the lens sample coated with series II- i formulation (**Figure S11**). We found significant improvement in log reduction for the coated lens using the formulations with different concentrations of the GMA, SBMA monomers and PEI. We observed from the log reduction results that PEI with M_w 2 kDa can form more effective antibacterial coating than PEI with M_w 800 Da. This may be because PEI (M_w 2 kDa) has more amine groups in the coating film that gives better log reduction than with PEI (M_w 800 Da). We used low M_w PEI due to its less toxic nature and good antibacterial properties. The advantage of the series II formulations is that the monomers GMA, SBMA or AMPTMA and PEI are commercially available, inexpensive and the coating procedure is facile.

Our coating incorporates SBMA or AMPTMA, which contribute to hydrophilicity, GMA, which provides of the epoxy functional groups and PEI (linked to GMA), which provides the antibacterial properties of the coating. Bacteria contacted to the coating are attracted by cationic surface and killed by membrane perturbation by polycationic PEI chains (**Figure 6**). Porous structure of the coating increases the efficacy of killing. The porous structure have empty space to receive bacterial strains and can get attract to coating surface. Milovic *et al.* [31] reported their study on antibacterial activity of quaternarized *N*-alkyl-PEI (long chained hydrophobic) against various types of gram positive and gram negative bacteria. Detailed structure and activity relationship studies of PEIs with wide range of molecular weights were reported by Gibney *et al.* [27] Unmodified PEI itself provides necessary cationic amphiphilic structure needed for the bacterial cell membrane disruption. The MIC of branched PEI for Gram-positive bacteria *S. aureus* was found to be 16-31 $\mu\text{g/ml}$ but for Gram-negative bacteria *E.coli* was more than 250 $\mu\text{g/ml}$ indicates that branched PEI is more selective to *S. aureus* than *E.coli*. Novel antimicrobeal agents for optimal activity can be designed by understanding the intrinsic antimicrobial mechanism of unmodified PEIs.

In our coating, we have used monomers/polymers such as glycidyl methacrylate (GMA), sulfobetaine methacrylate (SBMA) and polyethyleneimine (PEI). The resultant coating has good antimicrobial activity against Methicillin-resistant *Staphylococcus aureus* (MRSA) bacteria. We have checked the fluorescence microscopy imaging to assess the nature of adhesion of bacteria to the lens surface. To check the adhesion effect of bacteria to the lens surface, the control (uncoated) and surface coated lenses (series II-v) were inoculated with MRSA bacteria and incubated at 37 °C for 2 hours as mentioned in section 2.7. The control and coated lenses were washed with 0.85 % of NaCl for about 2 min, 120 rpm to remove the unattached bacteria. Lenses were stained with 6 μ M of SYTO 9 (stain both live and dead bacteria) and 15 μ M of Propidium Iodide (PI, stain dead bacteria) for 15min at room temperature with gentle shaking and viewed under confocal. **Figure 7** shows the Fluorescent microscopy images of bacterial aggregation on uncoated silicone contact lens and contact lens with series II-v (GMA (2 %) + SBMA (1 %) + PEI 2 kDa (1 %)) coating. The control lens showed bacteria adhesion on the surface, but no bacteria was found on the coated lens surface. Hence, dead bacteria will not remain on the coated lens surface.

Zeta potential of uncoated contact lens and surface coated lens (series II-v) were measured in 5 mM NaCl solution with electrolyte's neutral pH (5.6-5.8) using surface zeta potential measurement system (Anton Paar, SurPASS 3). Samples were loaded to the sample holder and rinsed 6 times with test electrolyte to wet the samples and to adjust the gap height to about 100 μ m. Test electrolyte were replaced with fresh electrolyte and measured the surface zeta potential. The zeta potential for the uncoated lens is found to be -6.7 ± 0.28 mV and for series II-v coated lens is $+8.05 \pm 0.76$ mV. The confocal image and zeta potential results shows low bacterial adhesion to the coated lens. Strong adhesion of bacteria on the control lens is due to hydrophobic interaction between the bacteria and lens surface. Presence of PEI and zwitterion polymer poly SBMA causes the killing and resistance of the bacterial adhesion to

the coated lens surface. Bin Ran *et al.*^[32] has reported a bacterial adhesion resistant coating by polydopamine - assisted zwitterionic polyethylenimine-g-poly(sulfobetaine methacrylate) polymers on silicon substrate and evaluated the adhesion of bacteria by SEM images. The study reported less number of bacteria on the coating surface compared to large number of bacteria on silicon surface without coating. Our coating on contact lens surface have similar molecular structure of PEI and Poly SBMA, adhesion of bacteria to the coated lens surface is weak. The presence of poly SBMA resist the bacterial adhesion on the coating surface.

4. Conclusion:

Two series of antibacterial nontoxic coatings on silicone contact lens were prepared by the ozone-activation and thermal-polymerization method. The coatings were thoroughly characterized by FT-IR, SEM, AFM, contact angle and light transmittance measurements. The characterization data show the coatings were uniform. Series I (PEI-PEGMA) coating shows good *in vitro* antibacterial property against MRSA bacteria with average log reduction of 5.1 (>99.99 % kill) and high mammalian cell viability of 79 %. Series II coating used monomers GMA, SBMA or AMPTMA, and PEI polymer. A typical formulation of series II coating with 1 wt% GMA, 1 wt% SBMA and 0.5 wt% PEI (M_w 2kDa) shows *in vitro* antibacterial activity for MRSA with log reduction of 6.3. Cell viability for the coated lens with the above formulation is 90 % indicates that the coating is non-leachable in DMEM media and nontoxic. All the coatings on contact lens shows high cell viability and good log reduction and hence these coatings can be used as potential coating materials for contact lenses and other medical devices. An advantage of the series II coating is that the raw materials are commercially available and the coating procedure is simple and scalable. Silicone contact lenses are widely used and effective antibacterial coating on silicone contact lenses can prevent many ocular problems caused by colonization of bacteria on contact lenses.

Supporting Information

Supporting Information is available from the Wiley Online Library

Acknowledgements

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Figure 1: Schematic of the typical procedure for the series II antimicrobial coating of poly glycidyl methacrylate- poly *N*-(3-Sulfopropyl)-*N*-(methacryloxyethyl)-*N,N*-dimethylammonium betaine- polyethyleneimine (PGMA-PSBMA-PEI) polymer on the surface of silicone contact lens.

Figure 2: FT-IR spectra of (a) uncoated lens, (b) PEI-PEGMA (15 %) + SBMA (5 %) coating (series I-i) and (c) GMA (1 %) + SBMA (1 %) + PEI (2 kDa, 0.5 %) coating (series II-i)

Figure 3: SEM image of (a) uncoated silicone contact lens, (b-d) lens coated with series I-i: (PEI-PEGMA (15 %) + SBMA (5 %)) with different magnifications.

Figure 4: SEM image for Series II-i: GMA (1 %) + SBMA (1 %) + PEI 2 kDa (0.5 %) coating with different magnifications.

Figure 5: Log reduction and cell viability results for series I- i (PEI-PEGMA (15 %) + SBMA (5 %)); series II-i (GMA (1 %) + SBMA (1 %) + PEI 2 kDa (0.5 %)); series II-ii (GMA (2 %) + SBMA (1 %) + PEI 800 Da (4 %)) and series II-iii (GMA (2 %) + AMPTMA (5 %) + PEI 2kDa (1 %)).

Figure 6: Schematic diagram showing the killing mechanism of antimicrobial coating of the contact lens

Figure 7: Fluorescent microscopy images of bacterial aggregation on (a) uncoated silicone contact lens and (b) contact lens with series II-v: GMA (2 %) + SBMA (1 %) + PEI 2 kDa (1 %) coating.

Table 1: Formulation details for different series of coating using ozone and thermal method.

Coatings	PEI- PEGM A (wt. %)	AMPT MA (wt. %)	GMA (wt. %)	SBMA (wt. %)	PEI 2 kDa (wt. %)	PEI 800 Da (wt. %)
Series 1-i	15	-	-	5	-	-
Series II-i-step 1	-	-	1	1	-	-
Series II-i-step 2	-	-	-	-	0.5	-
Series II-ii-step 1	-	-	2	1	-	-
Series II-ii-step 2	-	-	-	-	-	4
Series II-iii-step 1	-	5	2	-	-	-
Series II-iii-step 2	-	-	-	-	1	-
Series II-iv-step 1	-	-	1	1	-	-
Series II-iv-step 2	-	-	-	-	1	-
Series II-v-step 1	-	-	2	1	-	-
Series II-v-step 2	-	-	-	-	1	-

Supporting Information

(a)

(b)

Scheme S1: The synthesis strategy for a) Cl-PEGMA (b) ¹H NMR (300 MHz) in CDCl₃ at 25 °C.

(a)

(b)

Scheme S2: The synthesis strategy for a) PEI-PEGMA (1:16) (b) ¹H NMR (300 MHz) in D₂O at 25 °C.

(a)

GMA monomer

(b)

SBMA

(c)

AMPTMA

(d)

PEI

Figure S1: Chemical structures of (a) GMA monomer (b) SBMA (c) AMPTMA and (d) PEI.

Figure S2: FT-IR spectra of (a) uncoated lens, (b) GMA (2 %) + AMPTMA (5 %) + PEI (2 kDa, 1 %) coating (series II-iii) and (c) GMA (2 %) + SBMA (1 %) + PEI (800 Da, 4%).

Figure S3: SEM image for Series II-ii: GMA (2 %) + SBMA (1 %) + PEI 800 Da (4 %).

Figure S4: SEM image for Series II-iii: GMA (2 %) + AMPTMA (5 %) + PEI 2 kDa (1 %).
coating with different magnifications using thermal method

Figure S5: SEM image for Series II-v: GMA (2 %) + SBMA (1 %) + PEI 2 kDa (1 %).

Figure S6: Contact angle of (a) uncoated lens (control) and (b) coated lens with series II-i formulation (GMA (1 %) + SBMA (1 %) + PEI 2 kDa (0.5 %))

Figure S7: AFM surface image for (a) uncoated lens and (b) coated lens with series II-iii formulation (GMA (1 %) + AMPTMA (5 %) + PEI 2 kDa (1 %))

Figure S8: Log reduction results for two different bacteria types (MRSA BAA40 and SA 29213) using the formulation series II-iv (GMA (1 %) + SBMA (1 %) + PEI 2kDa (1 %)) and series II-v (GMA (2 %) + SBMA (1 %) + PEI 2 kDa (1 %)).

Figure S9: Transmittance data for uncoated lens and coated contact lens with series I-i (PEI-PEGMA (15 %) + SBMA (5 %)) and series II-v formulation GMA (2 %) + SBMA (1 %)+1% PEI (M_w 2kDa, 1 %)

Figure S10: Transmittance data for the control lens and lens after ozone treatment for 20 min at room temperature.

Figure S11: Visual appearance of antimicrobial activity of (a) uncoated control and (b) coated lens series II-i Formulation (GMA (1 %) + SBMA (1 %) + PEI 2 kDa (0.5 %))

Table S1: Double bond content for different batches of PEI-PEGMA by titration

Batch Name	Ratio	PEGMA per PEI molecule by titration (mol/mol)
PEI-PEGMA batch 1	1:16	6.20
PEI-PEGMA batch 2	1:16	6.86
PEI-PEGMA batch 3	1:16	7.27