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A photopolymerized antimicrobial hydrogel coating derived from epsilon-poly-L-lysine

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ABSTRACT

Hydrogels made from epsilon-poly-L-lysine-graft-methacrylamide (EPL-MA) have been found to have impressive wide spectrum antimicrobial activity against both bacteria (specifically *Escherichia coli*, *Pseudomonas aeruginosa*, *Serratia marcescens* and *Staphylococcus aureus*) and fungi (specifically *Candida albicans* and *Fusarium solani*). The EPL-MA hydrogel also possesses *in vitro* biocompatibility and EPL-MA solution is relatively non-hemolytic: the concentration needed for onset of human red blood cell (hRBC) hemolysis is 12,500 μg/mL so that the selectivity for the pathogenic microorganisms over hRBCs is 230–1560. Further, EPL-MA hydrogel can be conveniently ultraviolet-immobilized onto plasma-treated plastic surfaces to form thin highly adherent antimicrobial hydrogel coatings for medical devices and implants.

1. Introduction

Infections associated with medical implants are becoming increasingly common and result in significant morbidity and, in some cases, mortality [1]. Recently, there has been much effort to overcome implant-associated infections through the application of antimicrobial coatings based on antibiotics, antimicrobial chemicals such as silver, antimicrobial peptides (AMPs), inherently antimicrobial polymers *etc.* [2–12]. However, antibiotics, antimicrobial drugs and chemicals should be used cautiously for combating infection because of their toxicity and the possibility of development of resistance in pathogens. For example, *Methicillin-Resistant Staphylococcus Aureus* (MRSA) infection has become a common problem in hospitals. On the other hand, AMPs which are also highly effective for preventing microbial colonization of medical devices cause cell death typically through membrane permeabilization so that it is difficult for pathogens to develop resistance to them [13–19]. However, many AMPs are hemolytic and/or toxic and lose their efficacies when immobilized on a surface as a coating [20]. Cationic polymers such as poly(vinyl-N-hexyl-pyridinium) have also been investigated as contact-active antimicrobial coatings but they usually have poor biocompatibility, high toxicity and/or poor coatability [21], so that they are unsuitable for implant coating. Coating formulations which have high broad spectrum antimicrobial activity, and biocompatibility and are also easy to apply, biodegradable and non-toxic are limited [22,23].

Epsilon-poly-L-lysine (EPL), an AMP typically produced by *Streptomyces albulus*, has been found to possess high antimicrobial activity, especially grades with lengths of 10 residues or more. EPL is active against both Gram-negative and Gram-positive bacteria (such as *Escherichia coli* (*E. coli*), *Pseudomonas aeruginosa* (*P. aeruginosa*), *Serratia marcescens* (*S. marcescens*) and *Staphylococcus aureus* (*S. aureus*)) and fungi (such as *Candida albicans* (*C. albicans*)) [24–28]. EPL kills microorganisms in a similar manner to other AMPs, *i.e.* by its adsorption onto the surface of microbe membrane leading to physiological damage to the cell so that the microorganism does not easily develop resistance to it [24]. Unlike many other AMPs, EPL is edible, non-toxic, biodegradable, and can be produced at a low cost [29–32]. However, to date this material has been mostly exploited as an antimicrobial food additive or gene delivery agent in solution form and not as an antimicrobial coating material [33]. The immobilization should ideally not impede its antimicrobial efficacy.

This paper reports a series of highly effective broad spectrum ntimicrobial hydrogel film and coating based on photopolymerizable, contact-active and non-cytotoxic EPL-*graft*-methacrylamide, *i.e.* CH_2=C(CH_3)CONH-EPL (hereafter referred to as EPL-MA). EPL (Mn = 3000) was reacted with methacrylic acid (MA) using N-hydroxy-succinimide (HOSu) and N,N′-Dicyclohexylcarbodiimide (DCC) to produce EPL-MA polymer. Each hydrogel contains 10% solid which is a blend of EPL-MA, dimethylacrylamide (DMA) monomer

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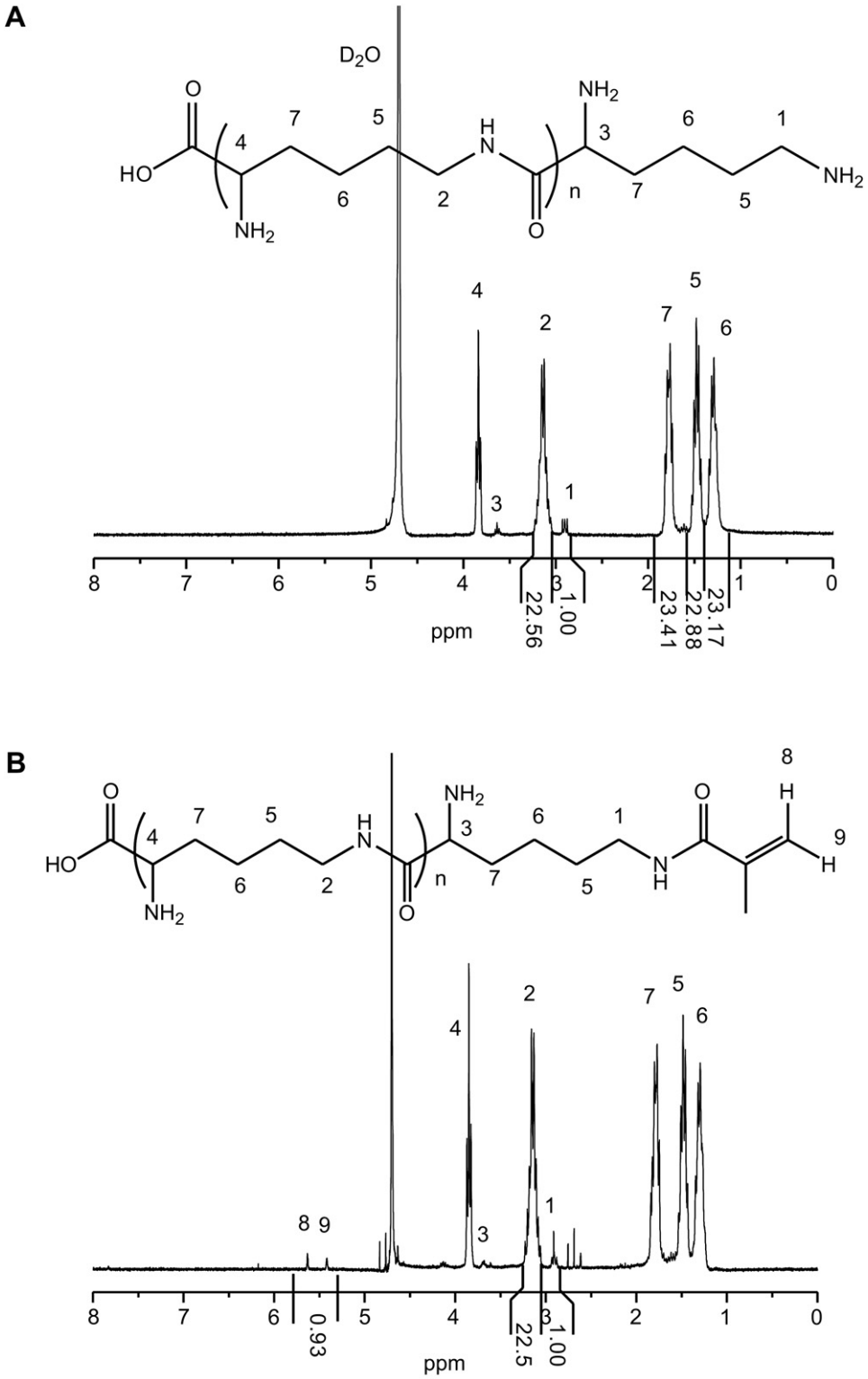

Fig. 1. ^{1}H NMR spectrum of (A) EPL and (B) EPL-MA.

and polyethylene glycol diacrylate (PEGDA) crosslinker (with molecular weight of 700 Da). The EPL-MA content in the hydrogel precursor (solid) was varied from 0, 6.25%, 11.8%, 16.7%, 21.1%–25% (with the remaining being made of PEGDA and DMA kept at the ratio of 2:1) (w/w) and the samples were labeled as A0 (control), A1, A2, A3, A4 and A5 respectively. The minimum inhibitory concentrations (MICs) of the EPL-MA solutions and killing efficacy of the EPL-MA hydrogels against four clinically important bacteria (*i.e. E. coli*, *P. aeruginosa*, *S. marcescens*, *S. aureus*) and two fungi (*i.e. C. albicans* and *Fusarium solani* (*F. solani*)) were evaluated. The hemolytic activities of the EPL-MA solutions and mechanical properties, swelling ability and *in vitro* biocompatibility of the hydrogels were also studied. The morphological changes of the bacteria/fungi in contact with EPL-MA hydrogels were investigated with scanning

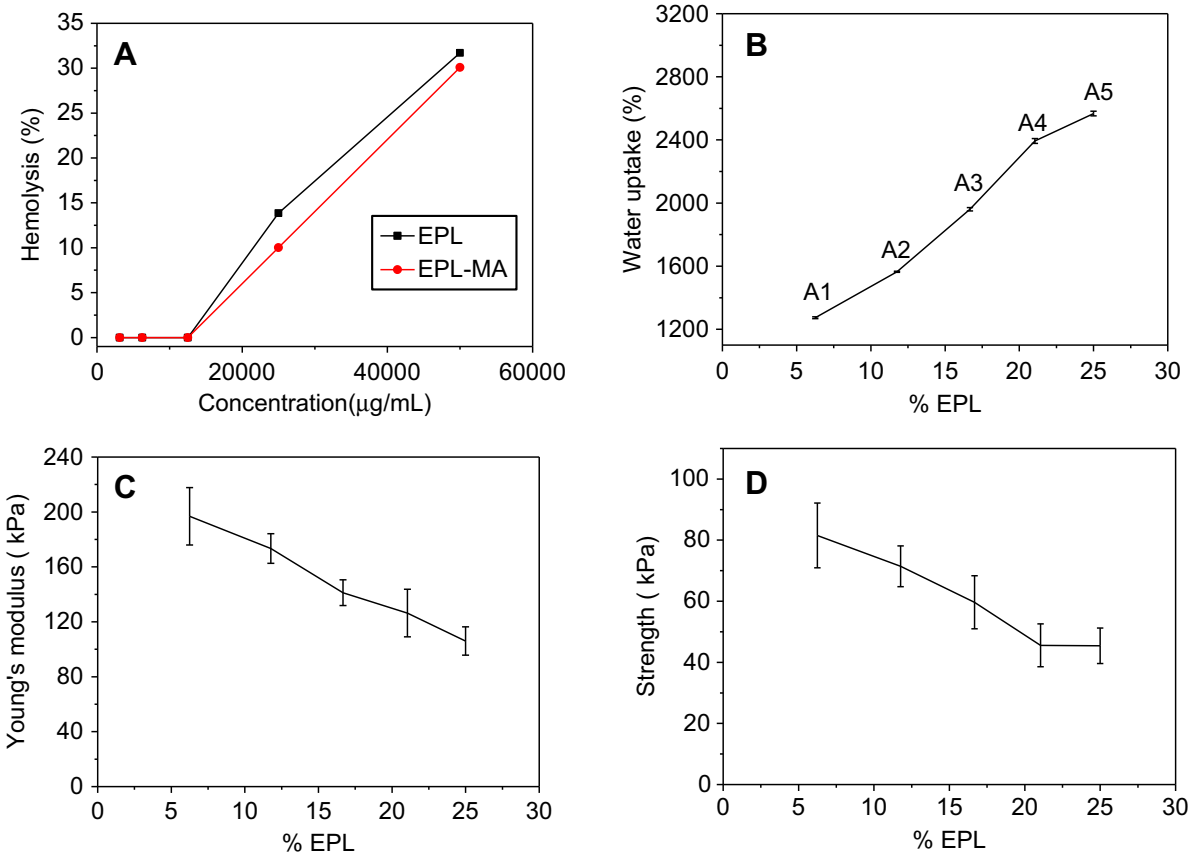

Fig. 2. (A) Hemolysis of human red blood cells by EPL and EPL-MA solutions (B) Water uptake (C) Young's modulus (D) Compressive strength of hydrogels with varying EPL content.

electron microscopy (SEM) and LIVE/DEAD bacterial assay test were also conducted. A hydrogel coating was formed onto a polymer disc by UV irradiation demonstrating the ease of the coating process.

2. Materials and methods

2.1. Materials

Epsilon-polylysine (purified by dialysis, Mn 3000, from Handary Bio-Engineering, Holland), N,N′-Dicyclohexylcarbodiimide (DCC, Sigma–Aldrich), N-hydroxy-succinimide (HOSu, Sigma–Aldrich), N,N-Dimethylformamide (DMF, Sigma–Aldrich), Methacrylic acid (MA, Sigma–Aldrich), N,N-Dimethylacrylamide (DMA, Sigma–Aldrich), Poly(ethylene glycol) diacrylate (PEGDA, Mn = 700, Sigma–Aldrich).

2.2. Modification of EPL by methacrylic acid

0.63 g (7.34 mmol) methacrylic acid (MA) and 0.93g (8.1 mmol) N-hydroxy-succinimide (HOSu) were dissolved in 10 mL DMF. 1.51 g (7.34 mmol) DCC dissolved in 10 mL DMF was gradually added into the MA/HOSu solution over a 30-min-duration and then kept at 0 °C for 2 h followed by stirring at room temperature for 4 h. The mixture was filtered and the solution obtained was added to EPL solution (20 g, (6.67 mmol) dissolved in water/DMF (200 mL: 100 mL)) and stirred for 24 h. After finishing the reaction, the solution was concentrated with a rotary evaporator operated under vacuum, and precipitated in acetone and filtered. The product was dissolved in 100 mL H_20 and filtered to remove undissolved powder. The solution was dialyzed in DI water for two days, evaporated in vacuum and dried in vacuum at 40 °C overnight (yield > 80%).

^{1}H NMR spectra of EPL and EPL-MA were recorded on a Bruker DMX-3000 spectrometer with D_2O as solvent. The average molecular weight (Mn) of the EPL was calculated from Fig. 1A to be 3000. The minimal inhibitory concentrations (MICs) were determined by a standard microtiter dilution method in Luria-Bertani broth (LB) medium. The hemolytic assay using human red blood cells was also performed.

2.3. Solution MIC and hemolysis tests

A series of peptides (EPL and EPL-MA) solution was prepared by two-fold dilution of 2000 μg/mL peptide stock in LB medium to final peptide concentrations ranging from 1000 to 1 μg/mL. Then 100 μL of each diluted peptide from the serial dilutions was added to a 96-well tissue culture polystyrene (TCPS) plate. After that, 100 μL bacterial suspension in LB solution was added to each well to form a final microbial concentration of 10^5 CFU/mL. The 96-well plate was subsequently incubated at 37 °C for 18 h. For the fungal pathogens, the growth medium was RPMI1640 supplemented with 2% glucose and incubated at 28 °C for 48h. MIC is the lowest antimicrobial material's concentration which can inhibit microbial cell growth after a certain time. The test was independently repeated twice.

Different concentrations of peptides (EPL or EPL-MA) were incubated with human blood cells (5%, v/v) in Tris buffer (pH 7.4) for 1 h at 37 °C. Then the tubes were centrifuged. The absorbance value of supernatants was analyzed at 540 nm. The lysis of 0.1% Triton X-100 was determined as positive control. The value of hemolysis (H) for EPL and EPL-MA was calculated from the relation below:

$$H = 100\frac{A_P - A_B}{A_R - A_B}$$

where A_P is the absorbance of peptide solution, A_B is absorbance of buffer, A_R is absorbance of 0.1% Triton X-100.

2.4. Preparation of hydrogels

A series of hydrogels were prepared by mixing EPL-MA, PEGDA and DMA. As an example of the formulation process, to formulate EPL-MA-25 (*i.e.* containing EPL-MA (0.1 g) which is 25 wt% of solid content, DMA (0.2 g) and PEGDA (0.1 g) (Mn = 700)) were blended followed by the addition of water (3.6 mL) and water-soluble photoinitiator Irgacure 2959 (0.05%). The hydrogel precursor solution (10 wt% solids, 90% water) was poured onto a horizontal glass plate. The mixture was then exposed to UV mercury lamp (Honle UV technology machine, 365 nm, 25 mW/cm^2, 15 min). The hydrogels in each series were prepared in a similar manner.

2.5. Characterization of the hydrogels

Compressive properties tests of the hydrogels were performed on swollen gels using an Instron 5543 Single Column Testing System. The hydrogel samples were horizontally sectioned with a sharp knife in order to have flat surfaces before testing. The gels were compressed at strain rate of 0.5 mm/sec. Young's moduli were

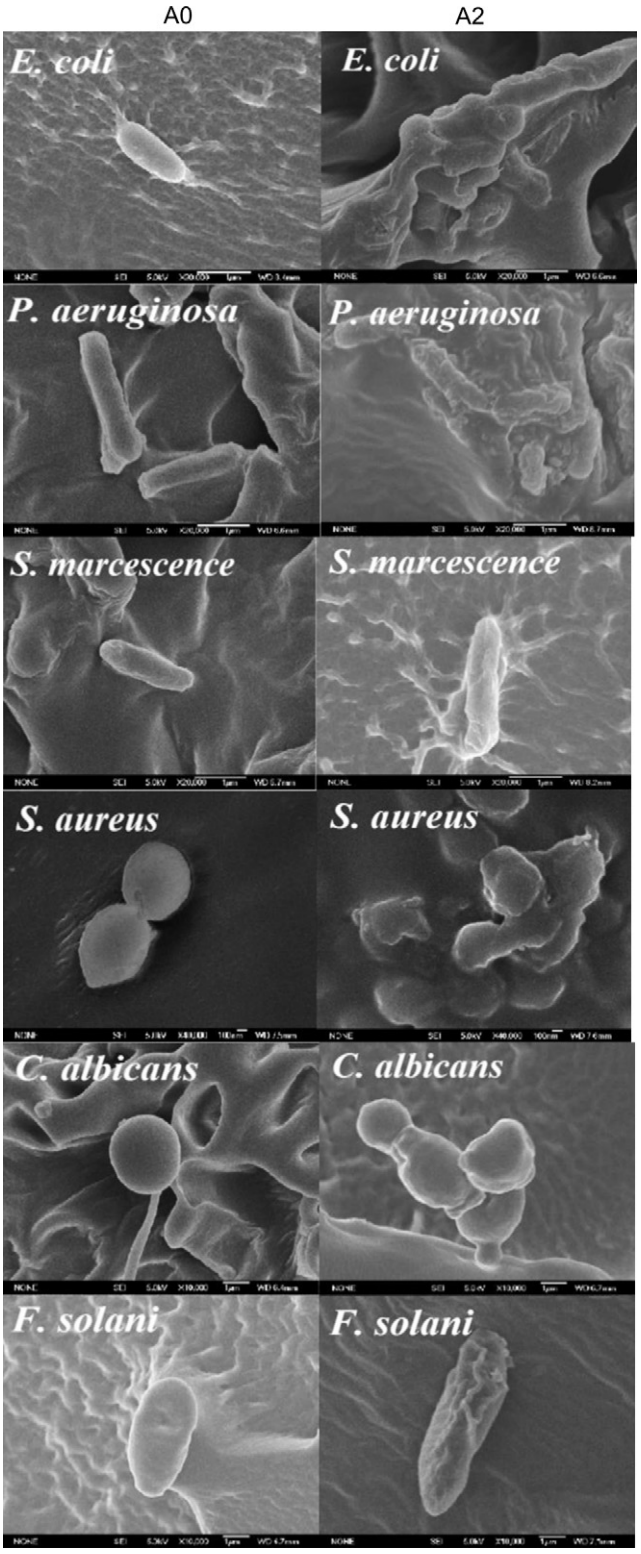

Fig. 3. SEM of various microorganisms seeded on the EPL-MA hydrogels. Left column (control): A0. Right column: A2.

determined by the average slopes of the stress–strain curves over the strain range 0–20%. 10 parallel measurements per sample were performed and the obtained values were averaged.

For the water uptake studies, UV crosslinked hydrogels were washed in DI water for 48 h, the hydrogels were removed from the water, and they were weighed after using the absorbent paper to wipe the surface. Repeat the swelling procedure until no weight increase. Water uptake was calculated as

$$\text{Water uptake \%} = \frac{\text{hydrogel weight} - \text{polymer dry weight}}{\text{polymer dry weight}} \times 100\%.$$

For the hydrogel antibacterial activity tests, the various hydrogels were washed with deionized water for two days. Bacteria were cultured for 24 h in Luria-Bertani broth before being was harvested and centrifuged and then washed with phosphate buffer saline (PBS, pH 7.2); the re-suspension, centrifugation and washing were repeated three times. The bacterial suspensions (10 μL each) in PBS were transferred to the centres of the hydrogel films in tissue culture polystyrene plate wells. The bacteria suspensions were kept in contact with the hydrogels for 2 h (the "exposure time") at 24 °C. A small volume of Luria-Bertani broth was then added to the plate wells to recover any surviving bacteria. The bacteria number was determined by a series of dilutions (10-fold) using Luria-Bertani Agar plates, then cultured for 48 h at 35 °C and counted the colony forming units. The results were computed as:

$$\text{Log reduction} = \text{Log}(\textit{initial count of control}) - \text{Log}(\textit{survivor count at 2 h exposure time})$$

The LIVE/DEAD assay of *P. aeruginosa* was investigated on A2 hydrogel and A0 hydrogel (control). Bacteria cells were cultured in LB overnight, and *P. aeruginosa* suspension in PBS was dispersed on the surface of the hydrogels at 37 °C for 1 h. After that the samples were stained by a combination of dyes (propidium iodide and SYTO 9). Subsequently the samples were analysized by a Zeiss inverted optical microscope which equipped with fluorescein and rhodamine optical filters.

For the microorganism morphology study, 10 μL inoculum was sprayed on 1.5 × 1.5 cm EPL-PEG-DMA hydrogel and PEG-DMA hydrogel (control) films and incubated at 35 °C for 2 h. The gels were then immediately fixed with 5 mL 2.5% glutaraldehyde for 4h and dehydrated in a graded ethanol series (20–100%). The freeze-dried hydrogels were characterized by Field Emission Scanning Electron Microscopy (FESEM, JEOL JSM-6700F) for microbe morphology change.

2.6. Hydrogel coating and characterization

EPL-MA hydrogel was immobilized on the surface of a fluoroalkyl (silicon-containing alkyl) fumarate copolymer disk by plasma-UV induced surface grafting polymerization [34] amphiphilic polynorbornene derivatives. The disk was pre-treated by radio frequency (RF, 13.56 MHz) argon plasma (March PX-500) at 50 W, 100 sccm for 60 s, and then exposed to atmosphere for 15 min. After pretreatment, the A5 macromonomer solution was pipetted onto the surface and covered with polyester film (Melinex 453, DuPont). The hydrogel precursor solution was cross-linked by UV exposure (365 nm, 100 mW/cm^2, and 30 min) through the polyester film. After crosslinking and removal of the polyester film, the construct was rinsed in excess deionized water in ultrasonic bath overnight to remove ungrafted oligomer or homopolymer.

Scanning Electron Microscopy (SEM, JEOL JSM-6390LA) was used to characterize the immobilized EPL hydrogel. For optical microscopy visualization, the EPL hydrogel-immobilized disk was stained with 1% fluorescein (Na salt) for 10 min then washed with deionized water for 1 h. The staining procedure makes the coating easily visible for gross visual evaluation. The antimicrobial activity tests for the hydrogel-coated plastic substrates followed a modified standard method (ISO 22196:2007E, Plastics-Measurement of antibacterial activity on plastics surfaces).

2.7. Antimicrobial test for the hydrogel-coated plastic disk

Bacteria cells were cultured in LB overnight and 1 mL suspension was centrifuged and washed by PBS for three times. And then the bacterial suspension was diluted to 1 mL with PBS. Subesquently 10 μL bacterial suspension were added to the surface of each hydrogel-coated plastic disc, which was then covered with another disk and gently pressed to spread the inoculums over the entire surface of the disk. The discs were incubated at a relative humidity of not less than 90% for 24 h at 37 °C. After the incubation, 2 mL of neutralizing broth were added to the plate to dilute the survived bacterial. The survived bacterial solution was then diluted to different concentrations (10-fold). After that we plate 100 mL of each solution in LB Agar. Then incubate the plate for 1–2 days and 37 °C and the colony forming units were counted. Each concentration incubated for three plates.

3. Results

3.1. Synthesis of EPL-MA

^{1}H NMR spectra of EPL and EPL-MA confirm the successful modification by MA (Fig. 1). Comparing the ^{1}H NMR spectrum of EPL and EPL-MA, the additional signals at 5.40 and 5.64 ppm in Fig. 1B can be attributed to the two hydrogens on the double bond of the methacrylamide group, indicating successful amidation. From the ratio of the Peak 2 to Peak 1 in Fig. 1A, the molecular weight of EPL was calculated to be 3015 (=128 × (peak2/peak1) + 1) Daltons. The

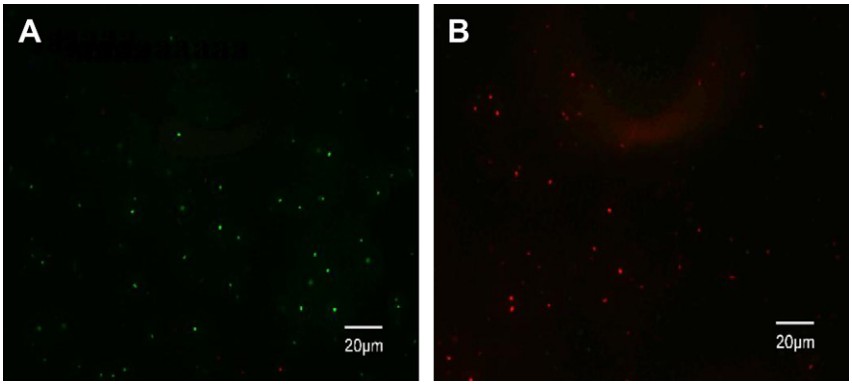

Fig. 4. LIVE/DEAD bacterial viability test results of *P. aeruginosa* on (A) hydrogel A0 (control) and (B) hydrogel A2.

degree of amidation was calculated from the ratio of peak 8 and 9 to peak 1 in Fig. 1B to be 93% (=(peak 8 + peak 9)/peak 1). GPC analysis of EPL (Supporting Information) indicates the Mn of EPL to be 3550 Da, corroborating the NMR results.

3.2. Antimicrobial properties and selectivity

Table 1 shows that the MIC values of EPL and EPL-MA solutions against the various pathogens are very low, typically less than 100 μg/mL, indicating that both EPL and EPL-MA solutions are highly effective at killing the pathogens. This is particularly impressive considering that some of the pathogens like fungi are typically difficult to kill. The results also show that amidation of EPL has no significant detrimental effects on the MICs. The EPL and EPL-MA have almost the same MIC is reasonable since the MA is just a small part on the MA-EPL chain.

Fig. 2A shows there is zero hemolysis (H_{0+}) of human red blood cells (RBCs) at EPL and EPL-MA concentrations up to 12,500 μg/mL. The H_{50} (*i.e.* concentrations causing hemolysis of 50% of RBCs) for both are greater than 50,000 μg/mL which are higher than the typical values of most other antibacterial peptides and polymers (about 500 μg/mL) [35]. The H_{0+}/MIC or selectivity of EPL-MA is hence in the range 230–1560, which is higher than the selectivity of many reported polymers and peptides (typically less than 100) [36–38].

Table 1 shows that A1, A2 and A5 hydrogel films all have high activity against the four tested bacteria (*E. coli*, *P. aeruginosa*, *S. marcescens, S. aureus*), killing more than 99% (2 log reductions) of the bacteria in the 2 h exposure period. A5 has the highest activity against all pathogens, which is attributed to its higher EPL-MA content.

Microbes were seeded on A2 hydrogel for 2 h and observed with SEM (Fig. 3); A0 hydrogel was used as the control. The morphology of the microbes in contact with the A2 hydrogel changed obviously compared with the controls. The bacteria and fungi on the control (A0) hydrogels appear smooth and rounded, whereas those on the A2 hydrogels exhibit wrinkled and withered surfaces. LIVE/DEAD bacterial viability assay was also carried out using *P. aeruginosa* on hydrogel A2 with hydrogel A0 as control. By this assay, bacterial cells that appear green are alive while red bacterial cells are dead ones with damaged membranes. Fig. 4 shows that *P. aeruginosa* cells are alive on hydrogel A0 (control) but dead on hydrogel A2.

3.3. Physical properties

Fig. 2B shows that our A1 to A5 hydrogels are generally highly water swollen with water uptakes of 1200–2400%. However, these hydrogels have fairly high compressive properties with Young's modulus of 196 to 106 kPa and strength of 81 to 40 kPa [39] (Fig. 2C and D).

3.4. In vitro biocompatibility

The biocompatibility of EPL-MA hydrogels was assessed by cytotoxicity test using the method described in the literature [40,41]. The hydrogel (A2) was placed directly on primary human epidermal keratinocytes and cultured *in vitro* for 7 days, with tissue culture polystyrene dish (TCPS) as control. Fig. 5A shows that the MTT absorbance of the cells in contact with A2 hydrogel increases with culture time (though lower than that compared to the TCPS control), indicating that the cells proliferate in the presence of A2 hydrogel. In Fig. 5B, keratinocytes in contact with the A2 hydrogel are mostly stained green after 7 days indicating that they are alive after this period, suggesting that our hydrogel possesses *in vitro* biocompatibility.

3.5. Hydrogel coating on a plastic disc

Fig. 6A shows a pristine disc made of a low surface energy fluoropolymer used for contact lens. Fig. 6B shows a similar contact lens disc coated with a thin (few μm thick) uniform conformal layer of A5 hydrogel. To covalently immobilize the hydrogel coating on the disc, the latter was pre-treated by argon plasma and then immersed into A5 solution which was then UV irradiated; ungrafted loose crosslinked hydrogel would then be washed away with water. Argon plasma generates radicals on the disc surface and these initiate the crosslinking of EPL-MA and DMA and PEGDA on it. Table 1 shows the immobilized A5 coating to also have high activity against bacteria and fungi with log reductions of at least 2 and around 1 respectively.

4. Discussion

EPL-MA hydrogel has been demonstrated to be contact-active against clinically significant bacteria and fungi. The A5 hydrogel killing efficacy (Table 1) is impressive considering that A5 reduces the counts of clinically relevant MRSA and low antibiotic susceptible *P. aeruginosa* by more than 99.9% (3 log reductions). The efficacy of the hydrogels against fungi (*C. albicans* and *F. solani*) is lower than that against bacteria, but was still significant with more than 90% kill ratio (1 log reduction). Further, the EPL-MA hydrogel is non-cytotoxic and has reasonably good mechanical properties.

EPL is a kind of AMP with positive charge and hydrophobic segment of $-(CH_2)_4-$ in each repeat unit of the backbone so that it can interact effectively with the anionic surface and hydrophobic

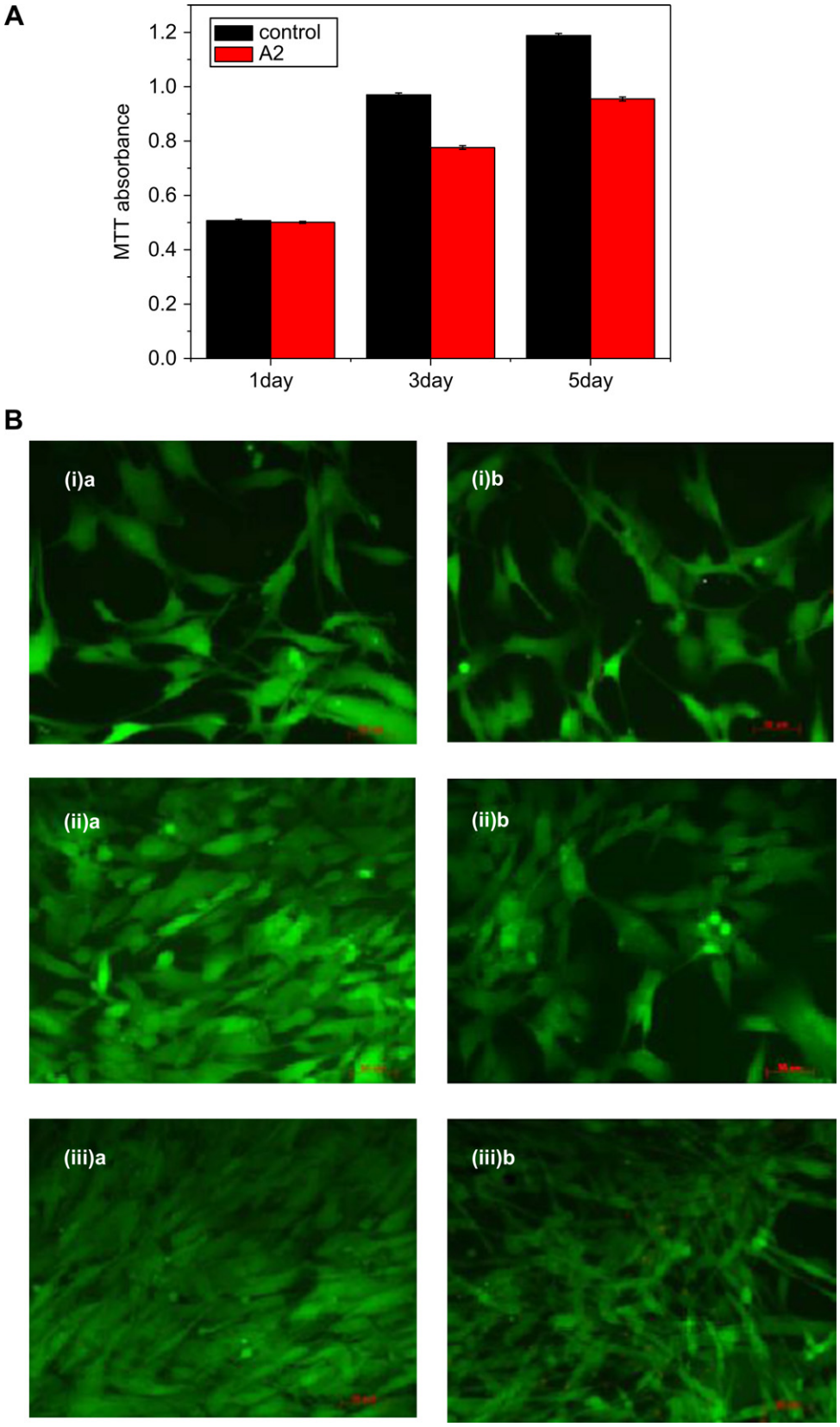

Fig. 5. (A) MTT absorbance (at 490 nm) of primary epidermal kerotinocytes on TCPS control and A2 hydrogel. Error bar represents mean ± standard deviation of mean for $n = 3$. The seeding density is 0.5×10^5 cells/cm^2. (B) LIVE/DEAD cell assay of primary epidermal keratinocytes after (i) 1 day (ii) 3 days and (iii) 5 days (a) on TCPS control and (b) A2 Hydrogel.

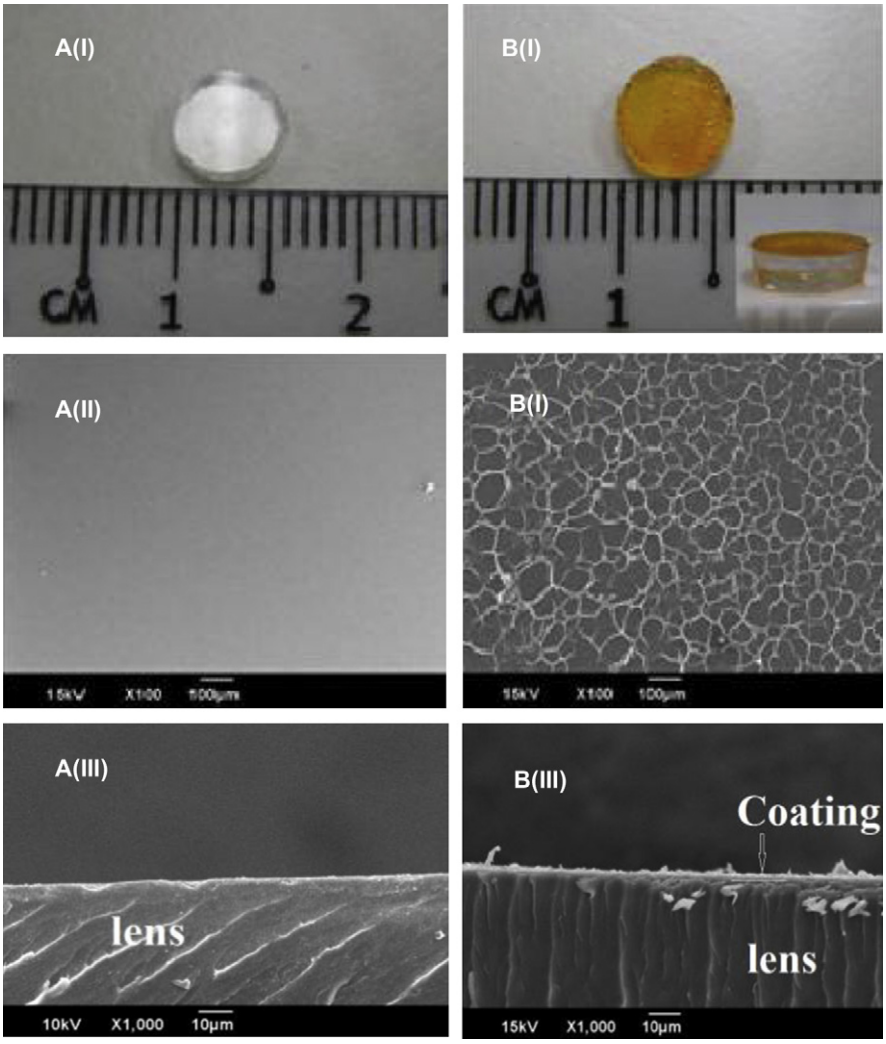

Fig. 6. A5 hydrogel coating on a polymer disc. (A) Uncoated disc (B) Coated disc (I) Phographs (II) Surface (SEM images) (III) Cross-section (SEM images) (A(I) and B(I) were stained with fluorescein).

interior of pathogen membrane. Our SEM images and LIVE/DEAD results suggest that the EPL-MA hydrogel causes damage to the cell membrane. This mechanism agrees with others' finding that EPL kills microbes by adsorbing onto the microbe cell surface and perturbing the outer membrane of the cell, which leads to an abnormal distribution of the cytoplasm and damage to the microbe [23]. The synergistic combination of cell and protein non-adhesion characteristics of our highly swollen hydrogels and the inherent antimicrobial property of EPL-MA makes the killing efficacy of our EPL-MA hydrogels particularly impressive, especially in view of its non-hemolytic behavior.

To retain the antimicrobial efficacy of EPL in the derivative of EPL-MA, the latter was designed and synthesized to have only one MA group per chain so that it will form a brush from the surface. The MA concentration per chain was achieved by controlling the stoichiometry of methacrylic acid (MA) to EPL to a low ratio of 1.1. The effectiveness of our EPL-MA hydrogel in killing pathogens depends critically on MA substitution only on the terminal $-NH_2$ group. In other experiments with increased ratio of MA to EPL, pendant α-NH_2 groups were also modified and the MIC values decreased noticeably (data not shown). These results are consistent with Shima's observation about the solution form of EPL: when the residue length is lower than 9 residues, the antimicrobial ability of the EPL is decreased [23]. Besides the low MA/EPL stoichiometry used, the higher basicity of ε-NH_2 compared to pendant $-NH_2$ (α-NH_2) favors the reaction of the terminal $-NH_2$ group during the amidation reaction: the pKa values of α-NH_2 and ε-NH_2 are 9.06 and 10.54 respectively [42]. α-NH_2, but not ε-NH_2, complexes with its neighboring C═O through hydrogen bond to form a 5-atom ring thereby lowering its activity.

The low density of terminal methacrylamide in our oligomeric EPL-MA (Mn = 3000) makes it difficult to polymerize by itself and the resulting hydrogel to be mechanically weak. Small molecular weight water-soluble DMA monomer and PEGDA crosslinker added increase the efficiency of copolymerization and improve the mechanical properties of the resulting hydrogel. Interestingly, it appears that EPL-MA needs only be few to tens of percent of the solid content (which is 10%) of the hydrogel for the latter to demonstrate high broad spectrum antimicrobial activity (Table 1).

Further, our antimicrobial hydrogel forms a lubricious coating desired for contact lens and many other biomedical devices. The facile coating step and covalent permanent immobilization further accentuates EPL-MA as a highly attractive antimicrobial coating material for biomedical devices. Our EPL-MA hydrogel is inherently antimicrobial so that its effect is long-lasting and there is no diffusible antimicrobial chemicals exuding from the hydrogel.

Table 1
Antimicrobial properties of EPL and EPL-MA (a) MIC (μg/mL) of solutions (b) Log reductions of hydrogel.

Microbes	Solution MIC (μg/mL)		Hydrogel property	
	MIC (EPL) μg/mL	MIC (MA-EPL) μg/mL	sample	Log reduction
E. coli	8	8	A1	2.8
			A2	4.6
			A5	6.0
			A5 coating	3.0
P. aeruginosa	8	8	A1	2.4
			A2	2.7
			A5	3.1
			A5 coating	2.1
S. marcescence	8	16	A1	3.9
			A2	4.9
			A5	5.4
			A5 coating	3.1
S. aureus	16	16	A1	2.0
			A2	2.4
			A5	4.3
			A5 coating	2.6
C. albicans	54	27	A1	1.0
			A2	1.1
			A5	1.3
			A5 coating	0.9
F. solani	109	54	A1	1.6
			A2	1.8
			A5	1.9
			A5 coating	1.0

5. Conclusion

Our EPL-MA can be UV crosslinked with other monomers in water to form hydrogel with excellent antimicrobial activity against clinically significantly bacteria and fungi. EPL-MA hydrogels are highly effective at suppressing the growth of both bacteria and fungi seeded on their surfaces, with log reductions of 3–6 for the Gram-negative and Gram-positive bacteria and above 1.0 for the fungi. The EPL-MA hydrogel shows *in vitro* biocompatibility with primary human epidermal keratinocytes. Further, EPL-MA can be easily UV-immobilized onto plasma-treated contact lens material and other polymeric and organic surfaces to form uniform and highly adherent lubricous antimicrobial coating on surfaces. Further, aqueous solution of EPL-MA has very low hemolytic activity (with H_{0+} greater than 12,500 μg/mL) and very high selectivity of 230–1560 for the pathogenic microorganisms over RBC.

Acknowledgments

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Appendix. Supplementary data

Supplementary data associated with this article can be found in online version at doi:10.1016/j.biomaterials.2010.12.040.

Appendix

Figures with essential color discrimination. Figs. 2, 4–6 in this article are difficult to interpret in black and white. The full color images can be found in the online version, at doi:10.1016/j.biomaterials.2010.12.040.

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