ACS Macro Letters

Letter

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Intrinsically Antibacterial Poly(ionic liquid) Membranes: The Synergistic Effect of Anions

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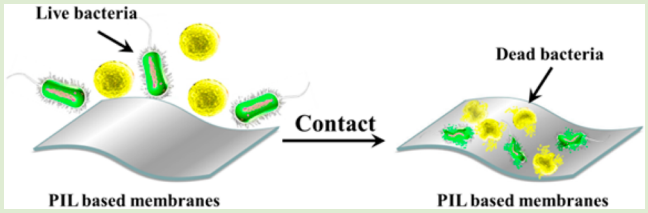
Supporting Information

ABSTRACT: The development of materials with intrinsically antimicrobial activities has attracted great interest. Herein, we report the synthesis of free-standing and robust poly(ionic liquid) (PIL) membranes with high antibacterial activities by in situ photo-cross-linking of an ionic liquid monomer and followed by anion-exchange with an amino acid (L-proline (Pro) or L-tryptophan (Trp)). The resultant PIL-based membranes with excellent robustness exhibit high antimicrobial properties against both Gram-negative *Escherichia coli* (*E. coli*) and Gram-positive *Staphylococcus aureus* (*S. aureus*) and present no significant hemolysis and cytotoxicity toward human red blood and skin fibroblast cells, as well as low adsorption of bovine serum albumin. The synthesized PIL-Trp membranes exhibit the highest antibacterial efficiency due to the synergistic attributes of both imidazolium cation and Trp^- anion. Furthermore, all the PIL-based membranes exhibit long-term antibacterial stability, which demonstrates clinical feasibility in topical applications.



The development of materials with antimicrobial activities has attracted great attention in modern healthcare due to overuse of antibiotics, and rapidly increasing antibiotic-resistance to pathogenic bacteria.[1−3] Antibacterial therapeutics, such as antimicrobial peptides,[4−7] bacteriophages,[8,9] silver,[10,11] carbon-based materials,[12] and cationic compounds and polymers have been reported as having a broad-spectrum of activities.[13−15] From a viewpoint focused on clinical applications, an ideal antibacterial material should possess high efficiency, broad-spectrum of activity, good biocompatibility, long-term activities, low cost, and easy synthesis. Therefore, growing attention has been paid to cationic antimicrobial polymers substituted with quaternary ammonium,[16,17] phosphonium,[18,19] and pyridinium cations.[20]

At present, most cationic antimicrobial polymers reported present antibacterial activities in solution form and exhibit high selectivity for bacterial over mammalian cells.[21−25] However, the antimicrobial efficacy may be highly reduced when the polymers are immobilized as antimicrobial coatings, because the diffusion of cationic polymers into cell membranes is highly hindered.[26] Therefore, polymeric hydrogels with antimicrobial activities against Gram-positive/negative bacteria have been extensively studied.[27−30] For example, chitosan substituted with quaternary ammonium[31] or covalently grafted synthetic polymer hydrogels/films[32] exhibited antimicrobial activities. However, the molecular weight of chitosan usually varies from batch to batch, which greatly affects the antimicrobial properties and biocompatibility of materials.[33] In addition, the covalent immobilization generally requires multistep and (or) postsynthesis and may alter the surface properties (such as transparency and (or) mechanical properties) of the substrates.[34,35] Therefore, it is desirable to develop free-standing polymeric membranes with intrinsically antimicrobial activities (without surface coatings) while maintaining robust mechanical properties during practical application.

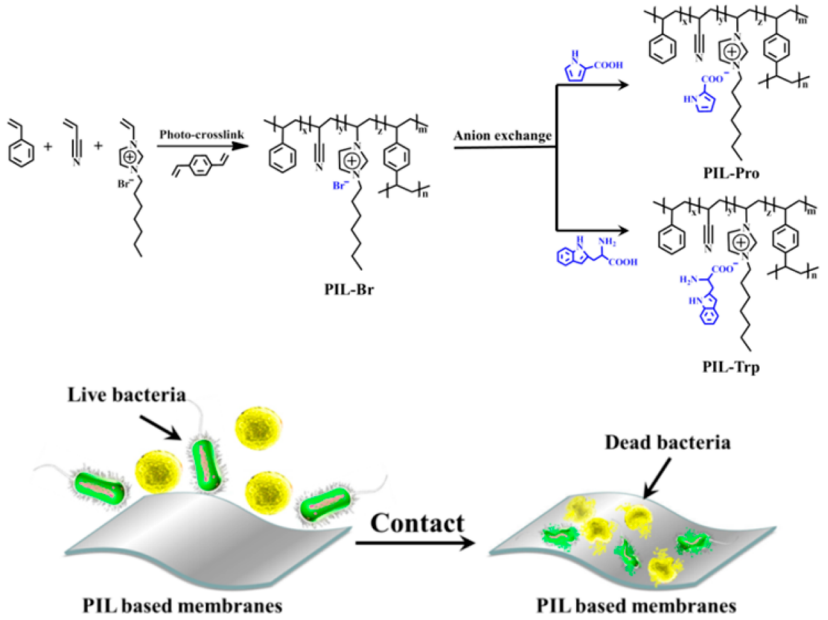
Recently, intensive attention is paid to a novel class of polymers, poly(ionic liquids) (PILs, polymers formed from IL monomers) because they combine the properties of both ILs and mechanical durability properties of polymers.[36−38] Furthermore, the ion-exchange capability of PILs enables the preparation of functional polymers with various counteranions, but the same main chain via polymerization of one IL monomer and followed by anion-exchange reactions.[39,40]

In this work, we present a facile synthetic strategy for the synthesis of robust, recyclable, and biocompatible PIL membranes with inherently antimicrobial activities. An imidazolium-type IL monomer, 3-heptyl-1-vinylimidazolium bromide ([HVIm][Br]), was photo-cross-linked with styrene and acrylonitrile using divinylbenzene as the cross-linking agent

Received: August 26, 2015
Accepted: September 11, 2015
Published: September 15, 2015

DOI: 10.1021/acsmacrolett.5b00609
ACS Macro Lett. 2015, 4, 1094−1098

Scheme 1. Schematic Representation for the Synthesis of PIL-Based Membranes and Their Antibacterial Strategy on the Membranes Surface[a]



[a]The membranes were prepared via in situ photo-cross-linking of an ionic liquid monomer and followed by anion-exchange with an amino acid, L-proline (Pro) or L-tryptophan (Trp).

(Scheme 1). Styrene and acrylonitrile were chosen as the comonomers in this work because poly(styrene-*co*-acrylonitrile) is a type of copolymer material with high chemical resistance and expected ability to form robust membranes.[41] We expected that the hydrophilic region (PIL units) of the membranes facilitates the antimicrobial activities, while the hydrophobic region provides the high mechanical strength.

Figure S1A,B shows the dynamic light scattering (DLS) studies of the monomer mixture containing [HVIm][Br]), styrene and acrylonitrile. It can be seen that micelles with the diameters of 1−20 nm were formed in the solution might due to the very different solubility parameter between the monomers. However, no multiple glass transition temperatures (T_g) were observed for the PIL membranes, indicating that the resultant random copolymers are homogeneous without any (or with very tiny) microphase separation (Figure S1C,D). Figure S2 shows the photographs of synthesized PIL-Br membranes with the thickness of about 100 μm. The membranes are free-standing, flexible, transparent, and can be easily cut into any desired shapes and sizes. The prepared PIL membranes were then immersed in L-proline (or L-tryptophan) aqueous solution to convert the membranes from Br^- to Pro^- (or Trp^-) form. For simplicity, the synthesized PIL-based membranes are abbreviated as PIL-*X*-*Y*% (*X* and *Y* indicate the anion and the molar ratio of imidazolium cations, respectively).

The synthesized PIL membranes were characterized by means of FTIR (see Figure S3). All the membranes show a characteristic peak at 1626 cm^{-1} due to a stretching vibration of imidazolium cation. An absorption band at about 2236 cm^{-1} corresponds to the cyano group (C≡N) stretching, while the peaks at 3027−3128 and 1455−1566 cm^{-1} confirm the existence of polystyrene units. Furthermore, the peaks at 1602 and 1373 cm^{-1} correspond to the carboxyl stretching vibration. The stretching vibration at 1022 cm^{-1} of C−N further represent the existence of Pro^-. The peaks at 1629 and 1302 cm^{-1} are characteristic absorption bands of carboxyl, and the peaks at 1569 and 847 cm^{-1} belong to the stretching vibration of N−H in Trp^-. The results confirm the successful synthesis and anion-exchange of imidazolium-based PIL membranes. The anion-exchange degree of PIL membranes could be determined via the element ratio of Br/C and N/C, based on the energy-dispersive X-ray (EDX) spectra (Figure S4). Here, the anion-exchange degrees of PIL-Pro and PIL-Trp membranes were found to be about 16.7 and 15.0%, respectively. In addition, all the polymeric membranes exhibit tensile strengths at break in the range of 17−40 MPa, which are strong enough to hold a 100 g weight (see Figure S2 A). The morphology of the membranes was investigated by scanning electron microscopy (SEM), which showed that all the membrane surfaces are uniform and smooth without any visible pores (see Figure S5).

To evaluate the antibacterial activities of PIL-based membranes, Gram-positive *S. aureus* and Gram-negative *E. coli* were chosen as model microorganisms. The resultant bacterial suspension was applied for plating and colony counting. Figure 1 shows that PIL-Br membranes could kill or inhibit the growth of *S. aureus* and *E. coli* efficiently once these organisms came into contact with the membrane surfaces.

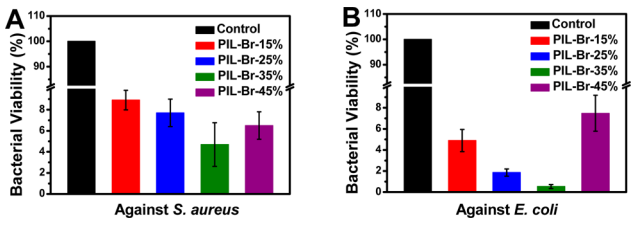


Figure 1. Bacterial viabilities (average of five samples) of (A) *S. aureus* and (B) *E. coli* after contacting with PIL-Br-*Y*% (Y: 15−45) membranes for 6 h, using polyethylene terephthalate (PET) membranes as controls.

DOI: 10.1021/acsmacrolett.5b00609
ACS Macro Lett. 2015, 4, 1094−1098

An increased content of PIL segments (from 15 to 35%) led to a higher antibacterial efficiency, probably due to the enhanced electrostatic interaction of the imidazolium moieties with microbial membrane. However, when the PIL content further increased from 15 to 45% (PIL-Br-45%), the corresponding antibacterial efficiency slightly decreased.

It is hypothesized that the antibacterial mechanism of the PIL-based membranes may involve the electrostatic interaction of the imidazolium moieties with the phosphate groups of the microbial cell wall, and followed by the hydrophobic effect of the hydrophobic segments of the polymer that insert into the hydrophobic regions of the lipid membrane of bacteria, leading to the leakage of the electrolyte out of the cell membrane (poration) and cell death.[42] Here, the very high (45%) PIL content increases hydrophilicity and induces aggregation of hydrophobic segments of polymers, which thus hinder the insertion of polymers into hydrophobic regions of the lipid membrane of bacteria, leading to a decrease of antibacterial activity. Moreover, the antimicrobial activities of the PIL-based membranes to *E. coli* were higher than to *S. aureus* (Figures 1 and 2). The different antibacterial efficiency may be due to the

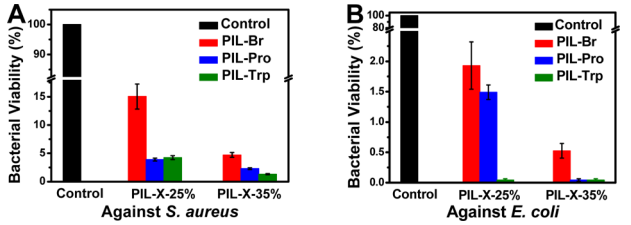


Figure 2. Bacterial viabilities (average of five samples) of (A) *E. coli* and (B) *S. aureus* after contacting with PIL membranes for 6 h, using PET membranes as controls.

different cell membrane structures.[43] As for the Gram-negative bacteria, the cell wall is more anionic and hydrophilic than that of Gram-positive bacteria.[44] Therefore, it is supposed that the stronger electrostatic interaction between the imidazolium cation and anionic cell wall enhance the higher antimicrobial activities.

Figure S6 shows the antimicrobial activities of PIL-based membranes against *S. aureus* and *E. coli*. As can be seen that the surviving colonies of *S. aureus* and *E. coli* decreased sharply after being contacted with PIL-based membranes. Furthermore, among the polymer membranes investigated, the PIL-Trp exhibited the highest antibacterial activities in comparison with PIL-Br and PIL-Pro (Figures 2 and S6). To investigate the effects of anions, antimicrobial activities of small molecules (proline and tryptophan) were studied by measuring the optical density (OD), using *S. aureus* and *E. coli* as model microorganisms. As shown in Figure S7 that tryptophan could efficiently inhibit the multiplication of both *E. coli* and *S. aureus*, while proline cannot suppress the growth of both bacterial strains. The mechanism of these results is still unclear, however, it has already been demonstrated that tryptophon anions may tend to insert into cell walls and destroy the bilayer surface of bacteria and eventually result in cell lysis.[45] Therefore, it can be concluded that these synergistic attributes significantly improve the antimicrobial efficiency of the PIL-Trp membranes against microbes.

The antimicrobial activities of all the PIL-based membranes with various anions (Br^-, Pro^- and Trp^-) were further investigated by analyzing the survival rate of both *S. aureus* and *E. coli* upon contact with membranes at various exposure times (see Figure 3). It can be seen that both *S. aureus* and *E. coli* could be partially killed by PIL-based membranes within 0.5 h. Among the PIL-membranes investigated, PIL-Trp membranes exhibit the highest antibacterial activities, which killed about 80 and 90% of *S. aureus* and *E. coli* within 1 h, respectively. In addition, about 98% of *S. aureus* and *E. coli* were killed and eliminated at 4 h. These results are consistent with the antibacterial activities determined above and further confirm the remarkable synergistic antibacterial activities of the PIL-Trp membranes.

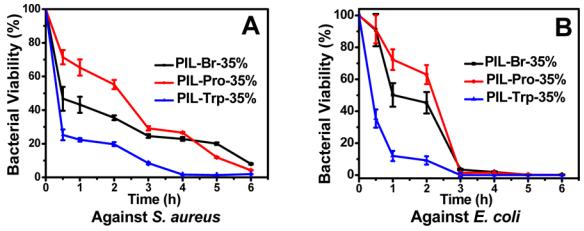


Figure 3. Time course of surviving *S. aureus* (A) and *E. coli* (B) bacteria upon contacting with PIL-Br-35%, PIL-Pro-35%, and PIL-Trp-35% membranes (average of five samples).

SEM images were utilized to observe the morphological changes of bacteria after incubation with the PIL membranes for 5 h. It can be seen from Figure 4A,E that both *S. aureus* and *E. coli* display regular and clear edges and smooth and complete cell walls when on the PET surface (control membranes). However, aggregations of lipid vesicles and collapsed surfaces were observed for both round *S. aureus* (Figure 4B−D) and for rod-like *E. coli* (Figure 4F−H) on the surfaces of PIL membranes, indicating possibly partial or complete membrane lysis or possibly extensive membrane poration of the bacteria. The SEM images further confirm the antimicrobial mechanism of the PIL-based membranes hypothesized above.

The biocompatibility of materials is a requisite for medical application. Here, the cytotoxicity toward mammalian cells and hemolysis was examined to evaluate the biocompatibility of the PIL-based membranes. Fibroblast cells were incubated with PIL-based membranes and their biocompatibility was evaluated by MTT assay. Based on the OD values (at 490 nm) from this MTT assay, the relative growth rates (RGR) of cells in contact with all of the PIL-based membranes were higher than 70%, suggesting a low toxicity to human skin fibroblast cells (Figure S8). The RGR values were determined to be 86 and 80% for PIL-Br-25% and PIL-Br-35%, respectively. While 71 and 85% were observed as the RGR value of PIL-Pro-25% and PIL-Trp-25% membranes, respectively. These results confirmed that the PIL-based membranes synthesized in this work are low-cytotoxic.

Table S1 shows the hemolysis assay of PIL membranes toward the fresh human blood cells (HBC). All the PIL membranes exhibited a low hemolytic activity (<4%) for HBC after 3 h of contact time, which is qualified for nondirect contact biomedical materials (hemolysis rate: <5%).

With the increase of PIL content, the corresponding hemolysis rates were slightly increased due to the increased hydrophilicity of the polymers (see Figure s9). Furthermore, PIL-Trp, with 1.45 ± 0.35 hemolytic activity, showed higher hemolysis than that of PIL-Pro with 1.01 ± 0.53 hemolytic activity, probably due to the higher hydrophobicity of Pro^- (see Figure s9). The results from both cytotoxicity and hemolysis

DOI: 10.1021/acsmacrolett.5b00609
ACS Macro Lett. 2015, 4, 1094−1098

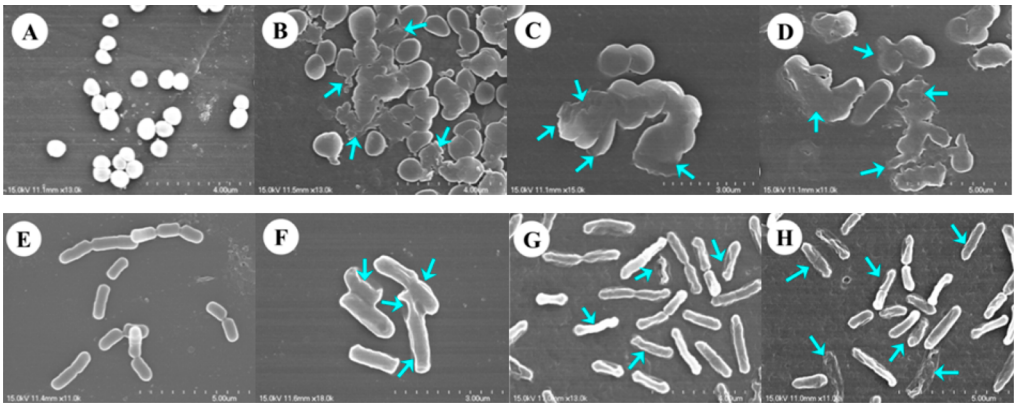


Figure 4. Scanning electron microscopy (SEM) images of *S. aureus* and *E. coli* cultured on the polymer membranes for 5 h. PET (A, E), PIL-Br-25% (B, F), PIL-Pro-25% (C, G), and PIL-Trp-25% (D, H) membranes are displayed in order for *S. aureus* (A–D) and *E. coli* (E–H), respectively. Collapses and fusion of bacterial membrane on the PIL-based membranes are observed (indicated by light green arrows).

assays indicate that all the synthesized PIL-based membranes exhibit good biocompatibility with human cells.[46]

To assess the antifouling properties of the PIL-based membranes, these membranes were immersed in 5 wt % BSA solution for 2 h and 7 days, respectively. The amount of protein absorbed on the membrane surface was measured by a Micro BCA protein assay. The protein absorption on the surface of a PET membrane was determined to be 0.18 $\mu g/cm^2$ after 2 h contact time (Figure 5). Compared with PET membrane, the

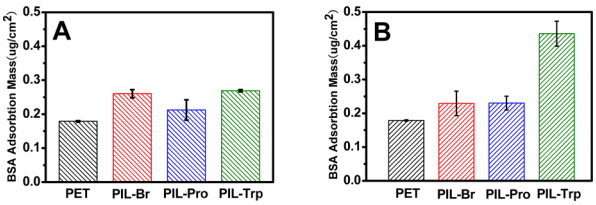


Figure 5. Adsorption mass (average of five samples) of bovine serum albumin (BSA) on the surface of (A) PIL-X-25% and (B) PIL-X-35% membranes with a contact time of 2 h.

absorption masses on the PIL-based membranes were slightly higher, however, but still lower than 0.5 $\mu g/cm^2$ after 2 h contact. The adsorption mass decreased from 0.26 to 0.23 $\mu g/cm^2$ with the increased PIL content from 25 to 35% (Figure 5) because of the increased hydrophilicity of our membranes.[47] Furthermore, the protein adsorption on poly(styrene-acrylonitrile) membrane was determined to be 0.39 $\mu g/cm^2$ under the same experimental conditions. The adsorption mass on PIL-Pro membrane surface (0.21 $\mu g/cm^2$) is slightly lower than that on PIL-Br membrane (0.26 $\mu g/cm^2$) because of the higher hydrophobic surface (see contact angle measurement of the membranes, Figure S9). However, the protein adsorption on the PIL-Pro and PIL-Trp membranes increased after a 7-day test (see Table S2). Our understanding is that the similar chemical structures between amino acid anions (Pro^- and Trp^-) and BSA proteins may tend to increase protein absorption. However, these data are still lower than those reported in the literature.[48−50]

Recyclable antibacterial materials are eco-friendly and environmentally protective, and thus have always been advocated. Here, all the synthesized PIL membranes were stored in a Petri dish in air, without any special protection. *S. aureus* and *E. coli* were used as model bacteria. As shown in Figure S10 that about 83% of *S. aureus* and 87% of *E. coli* were killed by PIL-Br-25% membranes in the fourth cycle. Similar results were observed for PIL-Pro and PIL-Trp membranes. It should be noted that no obvious decrease of antibacterial activity was observed with increasing number of cycles, even after four cycles (by exposure in air for 3 months). These high long-term antibacterial activities demonstrate clinical feasibility in topical applications.

In conclusion, imidazolium type PIL membranes were synthesized by in situ photo-cross-linking, and followed by anion-exchange with L-proline or L-tryptophan. The resultant membranes showed high antimicrobial properties against both *E. coli* and *S. aureus*, good blood compatibility, and low cytotoxicity, as well as low adsorption of bovine serum albumin. The PIL-Trp membranes showed a significant synergistic antibacterial effect against *E. coli* and *S. aureus* in comparison with PIL-Br and PIL-Pro membranes. In addition, these PIL-based membranes could be easily recycled without significant decrease in antimicrobial activities. Therefore, PIL membranes synthesized in this work may find potential applications as the eco-friendly and safe antibacterial materials in the area of healthcare.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acsmacrolett.5b00609.

Further experimental details and spectra (PDF).

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Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

This work was supported by the National Science Foundation for Distinguished Young Scholars (No. 21425417), the Natural Science Foundation of China (Nos. 21274101, 81271725, 81571930), the National Basic Research Program of China (973 Program; Nos. 2012CB825800, 2011CB510100), and the

DOI: 10.1021/acsmacrolett.5b00609
ACS Macro Lett. 2015, 4, 1094−1098

Project Funded by the Priority Academic Program Development of Jiangsu Higher Education Institutions.

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DOI: 10.1021/acsmacrolett.5b00609
ACS Macro Lett. 2015, 4, 1094−1098