Supporting Information

Intrinsically Antibacterial Poly(Ionic Liquid) Membranes: the Synergistic Effect of Anions

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1. Materials

Vinyl imidazole, acrylonitrile, styrene, divinylbenzene, bromoheptane, L-proline, L-tryptophan, benzoin ethyl ether, phosphate buffered saline (PBS, pH=7.4), dimethylsulfoxide (DMSO), polyethylene terephthalate (PET), sodium dodecyl sulfate (SDS), bovine serum albumin (BSA) and ethanol were purchased from Shanghai Chemical Reagents Co. (Shanghai, China). All the reagents were analytic grade and used as received without further purification. The vinyl monomers used in this work were made inhibitor-free by passing the liquid through a column filled with neutral alumina. Deionized water was used throughout all the experiments. *S. aureus* (ATCC 6538) and *E. coli* (ATCC 8099) strains were kindly provided by Dr. Shengwen Shao (Huzhou University School of Medicine, China). The Miro BCA^{TM} protein assay kit was purchased from Pierece Biotechnology Inc.

2. Characterization

^{1}H NMR spectra were recorded on a Bruker Advance 400 MHz NMR spectrometer with the solvent peaks of D_2O as an internal reference. The dynamic light scattering (DLS) measurements were performed using a high-performance particle size HPPS 5001 autosizer (Malvern Instrument, U.K.). The thermal properties of electrolytes were measured on a Perkin-Elmer DSC 4000 at a scanning rate of 5 °C min^{-1} under N_2 atmosphere. Scanning electron microscopy (SEM) images were taken with a Philips Model XL 30 FEG microscope with an accelerating voltage of 10 kV. The energy-dispersive X-ray spectroscopy (EDX) measurements were performed with the spectrometer attached on the Hitachi Model S-4700 field-emission scanning electron microscopy (FESEM) system. The Fourier transform infrared (FT-IR) spectra were recorded on a Varian CP-3800 spectrometer in the range of 4000–400 cm^{-1}. The optical density (OD) values were tested on an Eon microplate spectrophotometers (Bio Tek Instruments, Inc.). The tensile properties of the membranes were measured by using an Instron 3365 at 25 ^oC at a crosshead speed of 2 mm/min. The wettability of the membranes was examined by static contact angle measurements (Drop Shape Analysis System DSA10, KRUESS, Germany). Droplets (1.0 μL) of pure water were placed randomly over the surface.

3. Experimental Section

3.1 Synthesis of 3-heptyl-1-vinylimidazolium bromide ([HVIm][Br])

[HVIm][Br] was synthesized by stirring a mixture containing equivalent molar amount of vinylimidazole and bromoheptane at 0 ^oC for 3 days. The resultant viscous oil was thoroughly washed with ethyl ether and then dried in a dynamic vacuum at room temperature before polymerization. ^{1}H NMR (400 MHz, D_2O, δ): 7.758 (1H, N-CH-N), 7.561 (1H, N-CH-CH), 7.427 (1H, N-CH-CH), 5.764 (1H, CH_2-CH-N), 5.421 (2H, CH_2-CH), 4.219 (2H, N-CH_2-CH_2), 1.877 (2H, CH_2-CH_2-CH_2), 1.249 (8H, CH_2-$(CH_2)_4$-CH_3), 0.84 (3H, CH_2-CH_3).

3.2 Preparation of PIL-based membranes

A mixture containing [HVIm][Br] (15-45%, molar ratio), styrene/acrylonitrile (3:1 molar ratio, 53-73%), divinylbenzene (6 wt%, relative to the formulation, based on the weight of monomer) and benzoin ethylether (1 wt%, as a photoinitiator) was stirred and ultrasonicated 2-3 min at 53KHz to be a transparent solution, which was then cast into a glass mold and photo-crosslinked by irradiation with UV light of 250 nm wavelength for 40 min at room temperature. The resultant membranes were washed and ultrasonicated in ethanol for more than three times to remove the unreacted monomers. The prepared PIL membranes were immersed in a 0.05 M L-proline (or L-tryptophan) aqueous solution for 48 hours to convert the membrane from Br^- to Pro^- (or Trp^-) form.

3.3 Colony assay for the antibacterial activities

The concentrations of viable *S. aureus* (ATCC 6538) and *E. coli* (ATCC 8099) were diluted to 1×10^6 CFU/mL after being routinely grown on Luria–Bertani (LB) agar plates at 37 ^oC with 150 rpm shaking for 24 h. Then 120 μL microbial suspension in PBS was spread onto sterilized PET and PIL-based membranes (1.5×1.5 cm^2), and incubated at 37 ^oC at a relative humidity higher than 90% for 6 h. Afterwards, a volume of 40 μL bacterial suspension was streaked onto a LB agar plate. The number of the colony-forming units (CFUs) was counted after incubated for 12 hours at 37 ^oC. Each colony assay test was repeated three or more times. The antibacterial rate was calculated with the number of colonies from the experimental sample (B) and negative control (A) according to the following formula:

$$\text{Antibacterial rate (\%)} = \frac{A_{\text{negative control}} - B_{\text{sample}}}{A_{\text{negative control}}} \times 100\%.$$

3.4 Hemolysis assay

Fresh human blood (3 mL) from two healthy donors was centrifuged at 1500 rpm for 15 min. The precipitate of red blood cells was diluted to 2 vol% in PBS after washed with PBS till the supernatant was transparent. The sterilized PET and PIL-based membranes (1.5×1.5 cm^2) were dipped into diluted blood (5 mL for each tube) and incubated at 37 ^oC for 3 h, respectively. The treated diluted blood samples were centrifuged at 1500 rpm for 15 min, following by transferred aliquots of 100 μL supernatant from each tube into a 96-well plate. The OD values were recorded at 576 nm to assess hemoglobin release on the Eon microplate spectrophotometers (Bio Tek Instruments, Inc.). The red blood cells with 2 % Triton and in PBS were applied as the positive and negative control, respectively. The hemolysis percentage was calculated according to the following formula:

$$\text{Hemolysis rate (\%)} = \frac{OD_{sample} - OD_{negative\ \text{control}}}{OD_{positive\ \text{control}} - OD_{negative\ \text{control}}} \times 100\%$$

3.5 Cytotoxicity evaluation

Human dermal fibroblasts were kindly provided by Shanghai Ninth People's Hospital, China. The protocols for the isolation and culture of the human dermal fibroblasts follow the processes reported earlier.[1] The toxicity of the PIL-based membranes against normal cell was evaluated via 3-(4, 5-dimethylthiazol-2-yl)-2, 5- diphenyl tetrazolium bromide (MTT) assay. Briefly, human dermal fibroblasts (1×10^4 CFU/mL) in 10% fetal calf serum medium were cultured in a 24-well plate for 48 h. The sterilized PET and PIL-based membranes (1.0 × 1.0 cm^2)were put into the fibroblasts solution and cultured together at 37 ^oC for 72 h. 0.1 mL MTT solution (5 g/L in PBS) was added into each well and incubated at 37 ^oC for 4 h. After the removal of the supernatant, 0.75 mL DMSO was added in each well to dissolve the formazan crystals. The OD values at 490 nm were read to assess formazan release using the Eon microplate spectrophotometers (Bio Tek Instruments, Inc.). All the measurements were

carried out for more than three times. The relative growth rate (RGR) of the human dermal fibroblast cells was calculated according to the following formula: [2]

$$\text{RGR}\ (\%) = \frac{OD_{sample}}{OD_{control}} \times 100\%.$$

3.6 Protein absorption

A Micro BCA™ protein assay was used to determine the adsorption of a model protein bovine serum albumin (BSA) on the surface of PIL-based membranes. The PBS washed PET and PIL-based membranes were immersed into 1 mL BSA (5 wt% in PBS) solution in a 12-well plate at 37 ^{o}C for 2 h. The samples were rinsed with PBS solution three times and transferred into an Eppendorf tube with 1 mL PBS solution containing 1 wt% SDS. The proteins adsorbed on the membrane surface were removed by sonication at 40 KHz for 30 min in 150 μL PBS solution containing 1 wt% SDS. The amount of BSA adsorbed on the membrane surface was measured by the absorbance at 562 nm, and determined by the Micro BCA™ protein assay reagent kit (Pierece, U.S.A.) based on the bicinchoninic acid (BCA) method.

3.7 Morphological changes of bacteria

The field emission scanning electron microscopy (FE-SEM) was employed to observe the morphology of bacteria coated on the surfaces of PET and PIL-based membranes. The microbial suspension (OD reading of 0.1) of *S. aureus* or *E. coli* was dropped on the membrane surface and cultivated at 37 ^{o}C for 5 hours (see the details in colony assay). The membranes were fixed with 2.3% glutaraldehyde for 2 hours and then dehydrated gradiently 10 min with 30 vol%, 50 vol%, 70 vol%, 80 vol%, 90 vol%, and 100 vol% ethanol solution respectively. The morphology of the bacteria was then observed by FE-SEM.

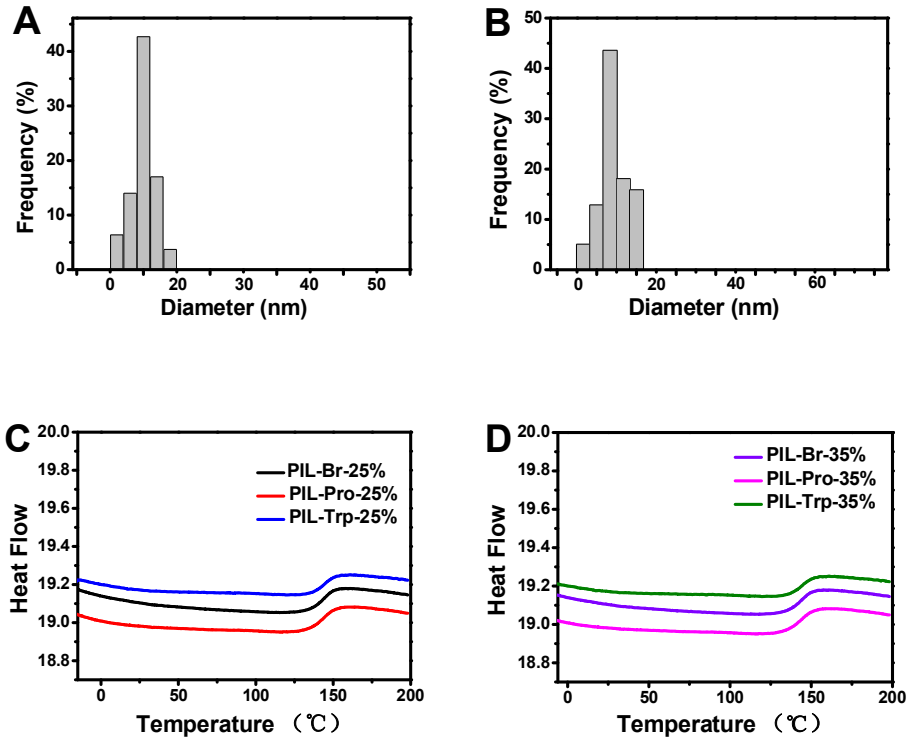

Figure S1 The dynamic light scattering (DLS) studies of the monomer mixture containing [HVIm][Br] (25 and 35%, molar ratio), styrene/acrylonitrile (3:1 molar ratio, 53-73%) A) [HVIm][Br] (molar ratio 25%), (B) [HVIm][Br] (molar ratio 35%). Micelles with the diameters of 1-20 nm were formed in the solution might due the very different solubility parameter between the monomers. Differential scanning calorimetric (DSC) thermograms for (C) PIL-X-25%, (D) PIL-X-35%. 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.

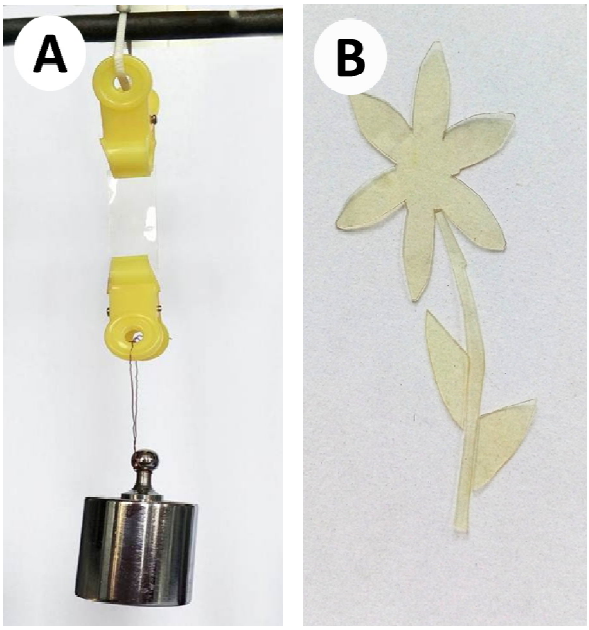

Figure S2 Photographs of PIL-Br-36% membrane (A) holding a 100 g weight; and (B) the polymeric membranes could be easily cut into any desired sizes and shapes.

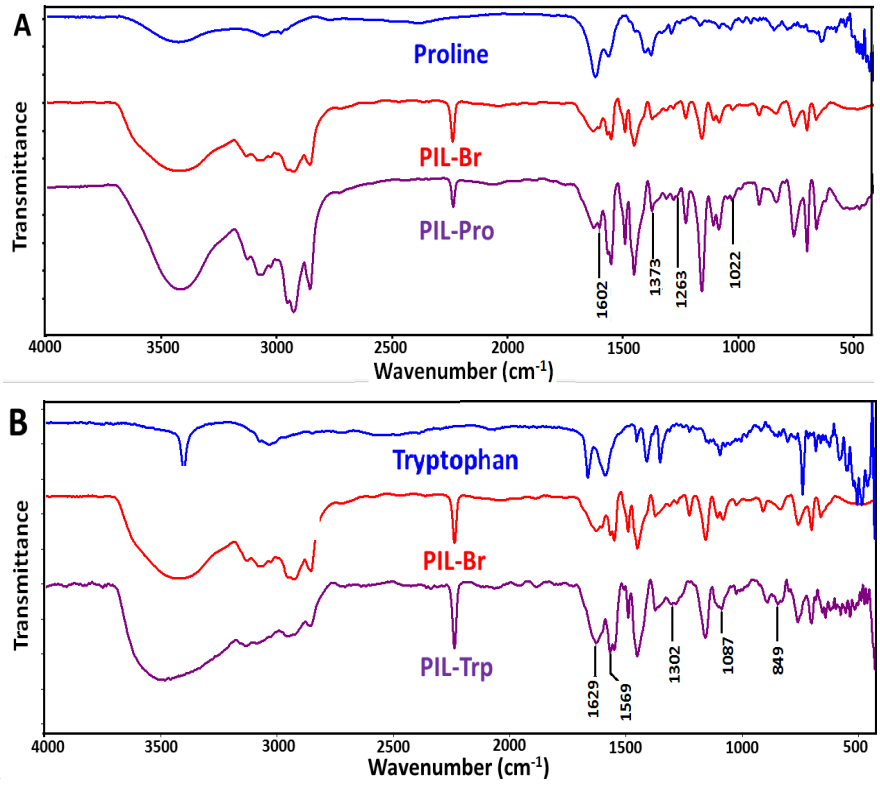

Figure S3 FTIR spectra of A) PIL-Pro-25% (bottom), PIL-Br-25% (middle), and proline (top); and B) PIL-Trp-25% (bottom), PIL-Br-25% (middle), and tryptophan (top). The results confirm the anion-exchange in the membranes.

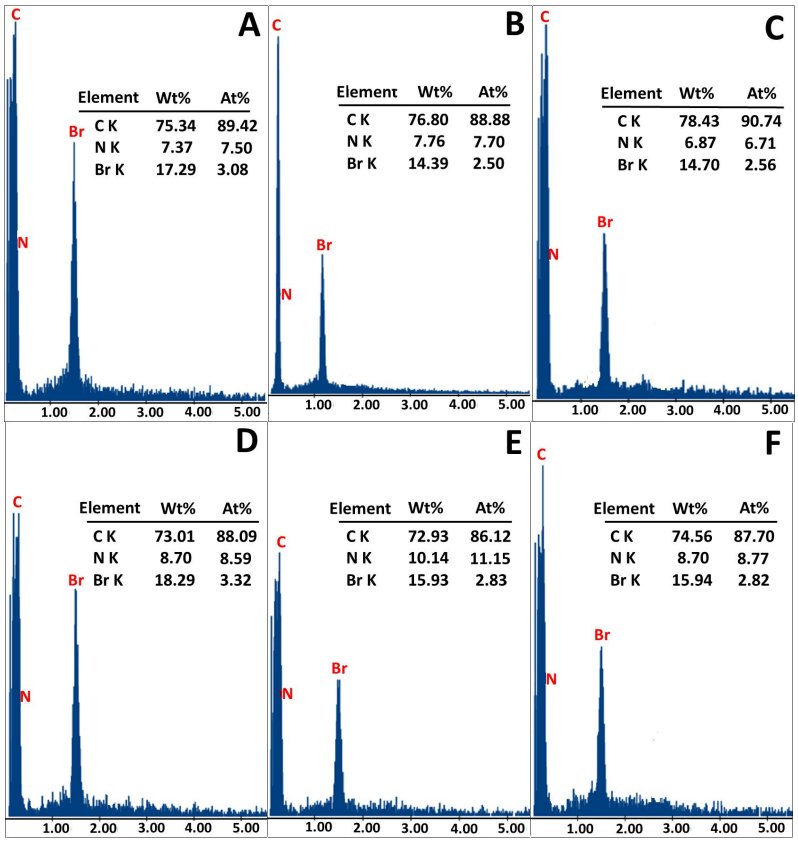

Figure S4 EDX spectra of A) PIL-Br-25%, B) PIL-Pro-25%, C) PIL-Trp-25%, D) PIL-Br 35%, E) PIL-Pro-35%, and F) PIL-Trp-35% membranes. The initial content of Br^- in PIL-Br membranes decreased after being anion-exchanged with proline and tryptophan, which further indicated the Br^- was partially exchanged by Pro^- and Trp^-.

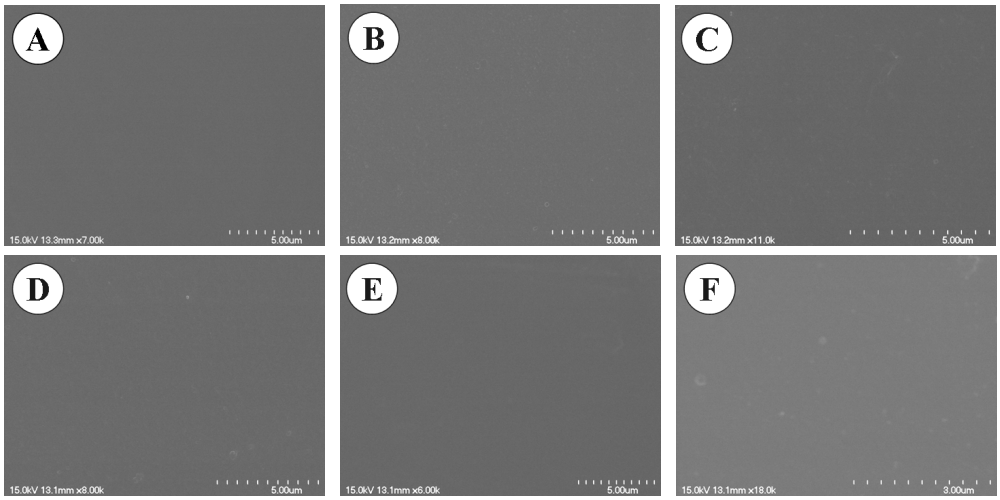

Figure S5 SEM images of surfaces of A) PIL-Br-25%, B) PIL-Pro-25%, C) PIL-Trp-25%, D) PIL-Br-35%, E) PIL-Pro-35% and F) PIL-Trp-35% membranes. All the membrane surfaces are uniform and smooth without any visible pores.

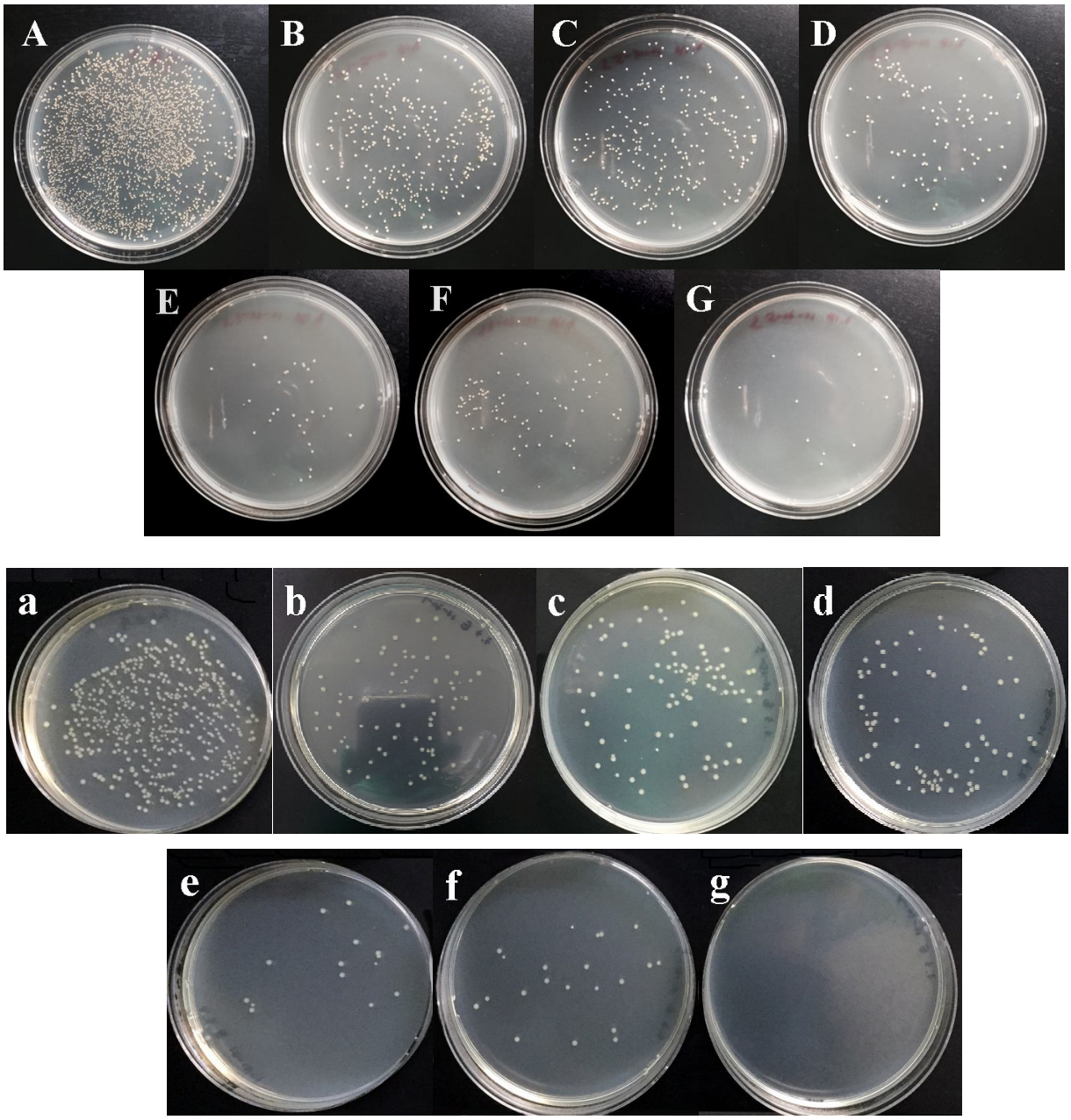

Figure S6 Agar plates of polymeric membranes against *S. aureus* (A-G) and *E. coli* (a-g). (A, a) PET, (B, b) PIL-Br-25%, (C, c) PIL-Pro-25% (D, d) PIL-Trp-25%, (E, e) PIL-Br-35%, (F, f) PIL-Pro-35%, and (G, g) PIL-Trp-35% membranes, respectively. Incubated bacteria strains with polymer membranes at 37 ^{o}C for 6 h, and streaked bacterial suspension onto a LB agar plate. It was found that PIL-based membranes could kill or inhibit the growth of bacteria *S. aureus* and *E. coli* once they were attached to the membrane surfaces.

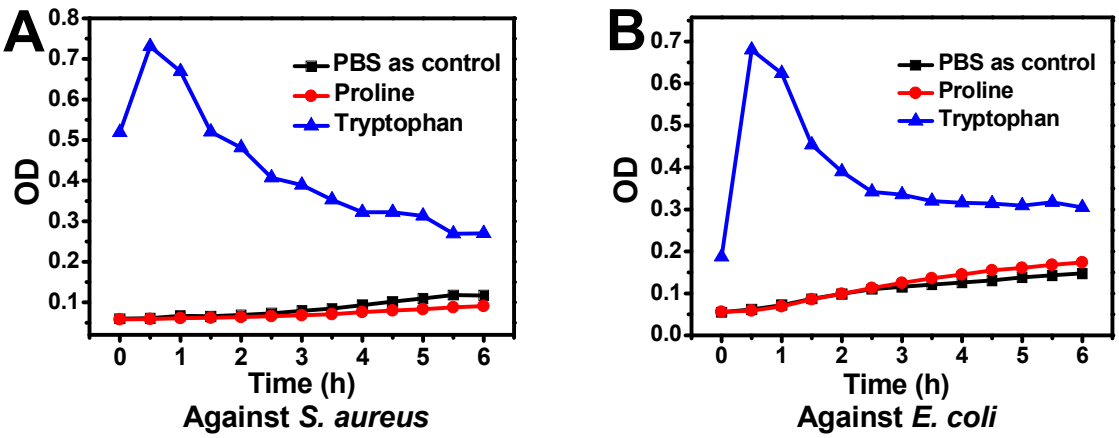

Figure S7 Optical density at 600 nm (OD_{600}) of bacterial suspension of (A) *S. aureus* and (B) *E. coli*, treated with proline and tryptophan, using PBS as control. Only tryptophan could efficiently suppress the growth of both *E. coli* and *S. aureus*.

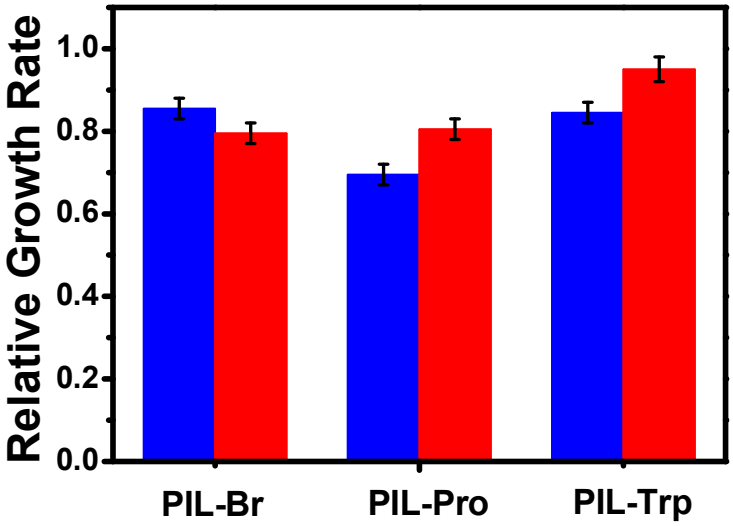

Figure S8 Relative growth rates (RGR) of PIL-X-25% (■) and PIL-X-35% (■) membranes (X: Br, Pro, and Trp) to human dermal fibroblast cells detected by MTT assay. All the RGR values of the membranes are in grade 1 (Except for PIL-Pro-25%, of which the RGR value was determined to be 70.5%). The results indicate that the PIL-based membranes are low-cytotoxic

Table S1 Hemolytic activity of PIL-based membranes. Red blood cells treated with 2 % Triton and PBS were used as positive and negative control, respectively.

Polymer membranes	Hemolysis rate (%)
PET	0.83±0.52
PIL-Br-25%	2.02±0.28
PIL-Pro-25%	1.01±0.53
PIL-Trp-25%	1.45±0.35
PIL-Br-35%	2.14±0.34
PIL-Pro-35%	1.59±0.73
PIL-Trp-35%	3.77±0.33

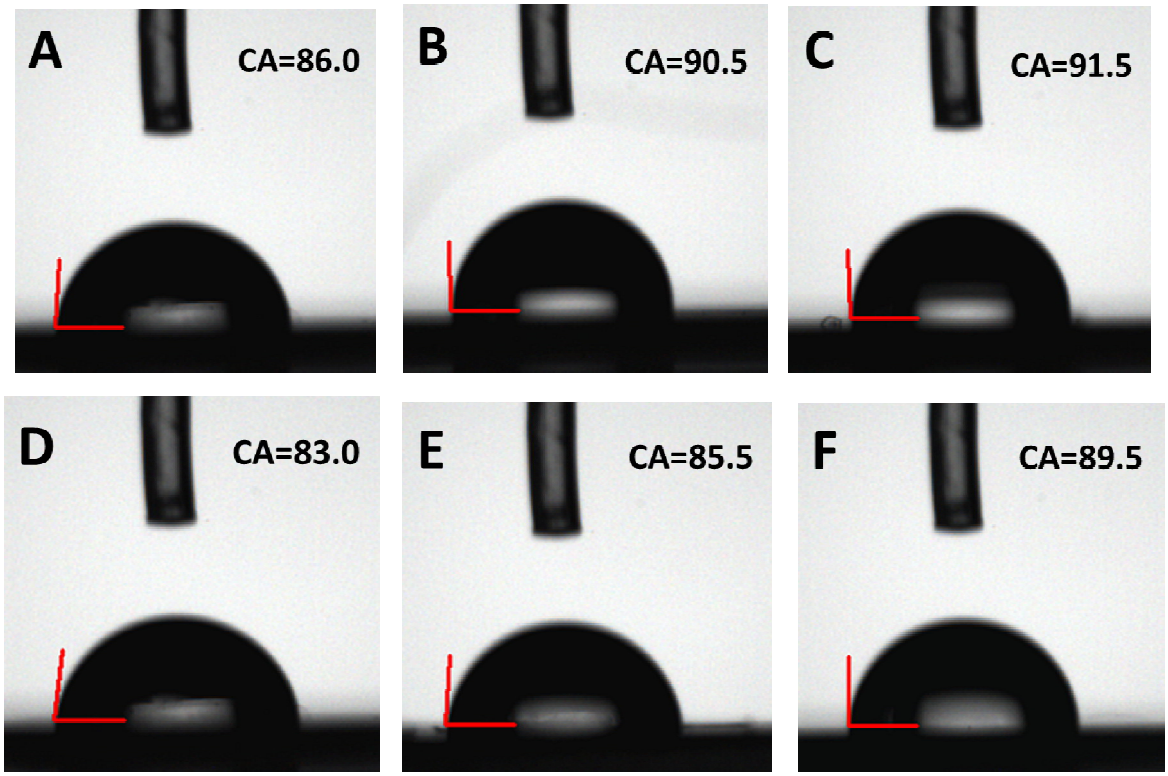

Figure S9 The contact angle measurement of (A) PIL-Br-25%, (B) PIL-Pro-25%, (C) PIL-Trp-25%, (D) PIL-Br-35%, (E) PIL-Pro-35%, (F) PIL-Trp-35%, which demonstrate that the anion-exchange of Br^- with Pro^- and Trp^- increased the hydrophobicity of PIL membranes.

Table S2 The adsorption mass of bovine serum albumin (BSA) on the PIL-X-25% and PIL-X-35% membranes surface with the contact time of 7 days.

Polymer membranes	BSA adsorption mass (μg/cm^2)
PET	0.168±0.02
PIL-Br-25%	0.159±0.026
PIL-Pro-25%	0.203±0.033
PIL-Trp-25%	0.888±0.023
PIL-Br-35%	0.571±0.081
PIL-Pro-35%	1.161±0.150
PIL-Trp-35%	1.263±0.230

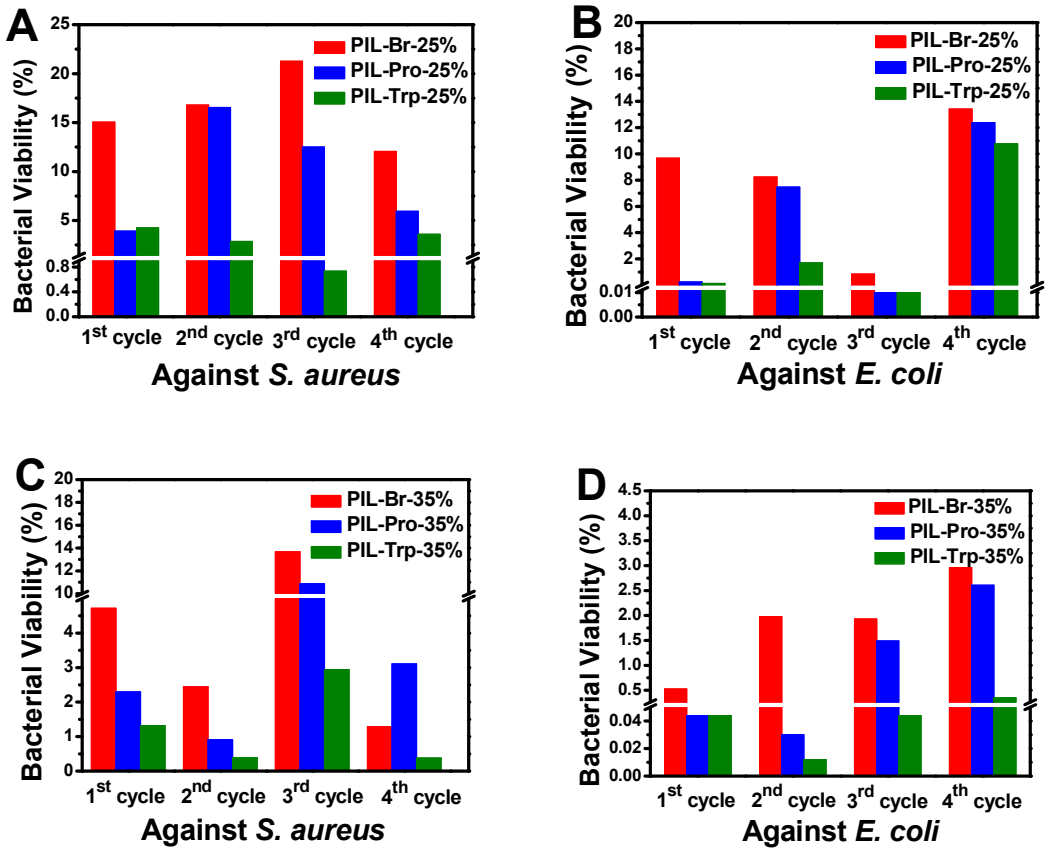

Figure S10 Recyclable antibacterial use of PIL-X-25% membranes against A) *S. aureus*, and B) *E. coli*, and PIL-X-35% membranes against C) *S. aureus*, and D) *E. coli*, respectively.

References

[1] a) Liu, W. Chen, B. Deng, D. Xu, F. Cui, L. Cao, Y. L. *Tissue Eng.* **2006,** *12*, 775-788;

b) Cao, D. J. Liu, W. Wei, X. Xu, F. Cui, L. Cao, Y. L. *Tissue Eng.* **2006,** *12*, 1369-1377.

[2] Luo, L. Li, G. Luan, D. Yuan, Q. Wei, Y. Wang, X. *ACS Appl. Mater. Interfaces* **2014,** *6*, 19371-19377.