

Antibacterial Thermoplastic Polyurethane/PL-DOSS Composite Films

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Electronic Supplementary Information

Abstract Medical devices-related infections pose a great threat to patients and cause an increased morbidity and mortality. Herein, we prepare an antibacterial composite (TPU-x) through blending medical grade thermoplastic polyurethane (TPU) and the complex (PL-DOSS) of ϵ -polylysine (ϵ -PL) and docusate sodium (DOSS). >99% reduction of colony forming unit (CFU) can be obtained in TPU-x composite films even at relatively low content of PL-DOSS, e.g. 0.13% for Methicillin resistant *S. aureus* (MRSA) and 0.5% for *E. coli*. The excellent antibacterial activity is mainly attributed to the formation of PL-DOSS nanoparticles that are uniformly dispersed in the TPU matrix with a size of ~100 nm. TPU-x composite films exhibit long-term stability in saline and good biocompatibility, and retain mechanical properties of TPU.

Keywords Nosocomial infections; Antibacterial composite; Solution blending; Thermoplastic polyurethane; Polylysine

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INTRODUCTION

Nosocomial infections are considered as a global health challenge and a fatal threat to patients.^[1,2] In 2011, there were about 7.2×10^5 nosocomial infections in the United States, resulting in about 7.5×10^4 deaths.^[2,3] The bacterial contamination of medical devices is the main cause of nosocomial infections, especially for medical devices inserted into the human body.^[4–6] The medical-grade TPU has not only excellent physical properties, but also excellent biocompatibility, which makes it widely used in medical devices, especially in the catheter.^[7,8] However, the medical-grade TPU itself has no antibacterial properties and bacteria were easy to breed on its surface, posing a great safety risk in the clinical application.^[9] Therefore, it is essential to prevent the colonization of bacteria on the surface of medical devices. Most of the clinically-used TPU materials do not have antibacterial properties. Modification of TPU with antibacterial agents could efficiently reduce the risk of nosocomial infections, especially for immuno-compromised hospitalized patients.

There were several approaches including chain extension,^[10,11] chemical modification through graft polymerization or click reaction,^[9,12–15] surface coating^[16–23] and blending with antibacterial agents^[24–27] for the preparation of antibacterial TPUs. Among these approaches, blending TPU with an antibacterial additive is the most feasible and generalizable

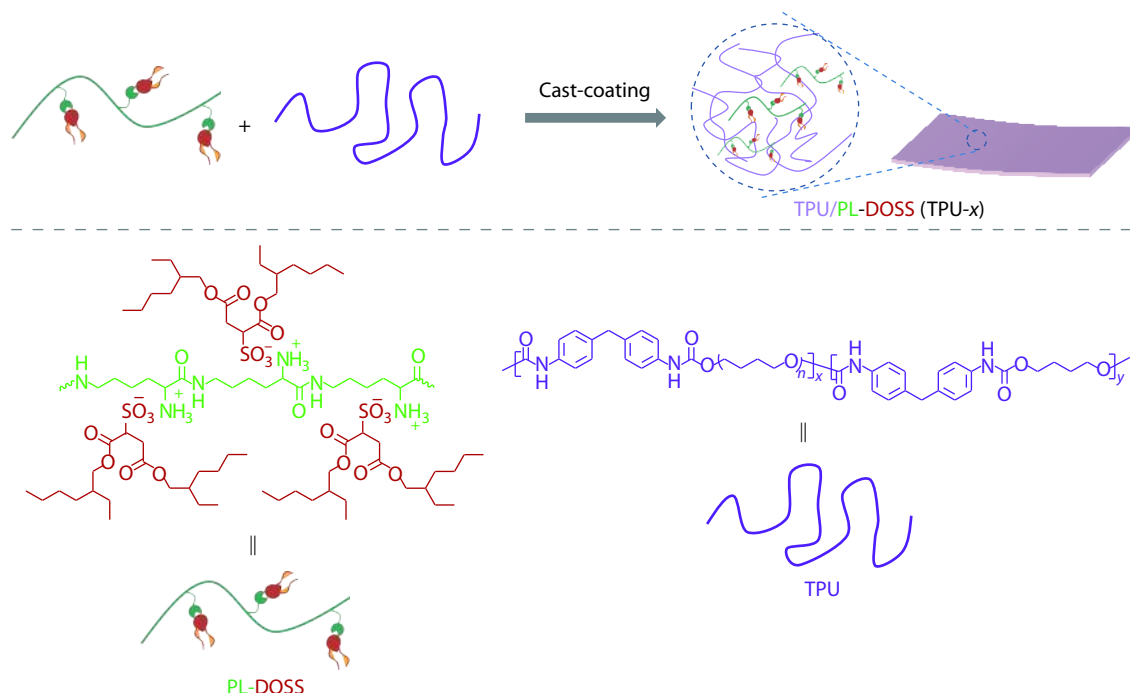
way to prepare antibacterial TPUs. Silver is the most commonly studied inorganic agent for antibacterial TPUs,^[28–32] but the concentration-dependent toxicity and high cost greatly limits its wide application.^[28,33] Blending TPU with antibiotics is another strategy to prepare antibacterial TPUs,^[34–36] while the release of antibiotics from TPU composites could cause the development of antibiotic resistance.^[28] Antibacterial polymers are less susceptible to build up resistance in pathogens,^[37–40] which makes them potential additives for antibacterial TPUs. Maurizio *et al.*^[41] reported that TPU containing 5 wt% chitosan could efficiently inhibit ~50% growth of *S. aureus* and did not show the inhibitory effect against *E. coli*. Furthermore, macroscopic phase separation of TPU and chitosan was observed in the blend. Guo *et al.*^[42] synthesized quaternized chitosan (QCS) and blended it with TPU to prepare antibacterial composites (QCS-TPU). QCS-TPU showed broad-spectrum antibacterial activities under both static and hydrodynamic conditions. Qiao *et al.*^[43] reported for the first time that *N*-halamine modified TPU films had antibacterial activities against both Gram-positive and Gram-negative bacteria. Although the *N*-halamine functional group was not very stable under light or in water environment, the antibacterial function could be regenerated through chlorination. However, it is still challenging to construct TPUs with excellent antibacterial activities that still maintain the thermal and mechanical properties of TPUs.

Herein, we report the use of the PL-DOSS complex of ϵ -polylysine (ϵ -PL) and docusate sodium (DOSS) as the antibacterial additives for the medical-grade TPU (Scheme 1). The TPU/PL-DOSS composite films (TPU-x) were prepared by solu-

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Scheme 1 Schematic illustration for the preparation of TPU-x composite films.

tion blending of TPU and PL-DOSS and showed increased antibacterial activities against *E. coli* and MRSA with the increase of the PL-DOSS content. The excellent antibacterial activities of TPU-x films were attributed to the formation of PL-DOSS nanoparticles in the TPU matrix. Meanwhile, TPU-x films possessed excellent biocompatibility and low cytotoxicity.

EXPERIMENTAL

Materials and Reagents

ϵ -Polylysine with the number average molecular weight (M_n) of ~ 4000 g/mol was received from Silver-Elephant Bio-engineering Co., Ltd., China. Docusate sodium (DOSS, 96%), Triton X-100 and methylthiazolyldiphenyl-tetrazolium bromide (MTT) were purchased from Aladdin Chemistry Co., Ltd., and used as received. Fetal bovine serum (FBS) was purchased from Tianjin Kangyuan Biotechnology Co., Ltd. Dulbecco's Modified Eagle's medium (DMEM) was purchased from Gibco (Grand Island, USA). The medical grade thermoplastic polyurethane (TPU, 1185A) was purchased from BASF Co., Ltd. With regard to all water samples, a Millipore, Milli-Q[®] ultrapure water system was utilized. Tryptic Soy Broth (TSB) and Mueller-Hinton Broth (MHB) were purchased from Qingdao Hopebio-Technology Co., Ltd. *Escherichia coli* (*E. coli*, ATCC 25922) and Methicillin resistant *Staphylococcus aureus* (*S. aureus*, MRSA) (ATCC43300) were obtained from Shanghai Luwei Technology Co., Ltd. All other materials were used without further purification.

Preparation of PL-DOSS Complex

PL-DOSS complex was prepared according to a modified method.^[18,44] The DOSS solution (4% (W/V) DOSS in 50% ethanol) was added dropwisely to the isovolumetric PL solution

(1.5% (W/V) PL in 0.24 mol/L of HCl) at room temperature under vigorous magnetic stirring. The mixture was centrifuged at 1×10^4 r/min for 15 min to obtain a white precipitate which was washed with 200 mL of distilled water for three times and then lyophilized to obtain PL-DOSS.

Preparation of TPU-x Composites Films

The TPU/PL-DOSS composite film, TPU-x, where x is the percentage of PL-DOSS in the composite film, was prepared by solution blending of TPU and PL-DOSS. The TPU pellets were dried in a vacuum oven at 80 °C for 6 h before use. In a typical process of preparing TPU-0.5, 10 mg of PL-DOSS and 2 g of TPU were dissolved in DMF (20 mL) for 48 h under vigorous magnetic stirring. The solution was carefully cast onto a flat glass substrate, and then placed in an oven at 80 °C to dry for 5 h to yield a film with the thickness of ~ 150 μ m.

Antibacterial Activities of TPU-x Composite Films

MRSA or *E. coli* with a deep frozen state (-80 °C) was smeared on an agar plate and incubated at 37 °C for 24 h. Afterwards, a single colony was picked and transferred into 10 mL of TSB and incubated at 37 °C for 12 h to reach exponential bacterial growth. The optical density was measured at an absorbance wavelength of 600 nm (OD_{600}) and the bacterial solution was diluted with fresh MHB medium so that optical density corresponded to $OD_{600}=0.2$ for MRSA and $OD_{600}=0.1$ for *E. coli*. These optical density values could be translated into a bacterial concentration of approximately 10^8 CFU/mL for each strain. The solutions were then further diluted with the LB medium to a final concentration of 5×10^5 CFU/mL for antibacterial test.

The antibacterial activities of TPU-x composite films were quantitatively evaluated using the CFUs count method against *E. coli* and MRSA. Briefly, TPU-x composite films were cut into square samples with the size of 1.5 cm $\times$ 1.5 cm and sterilized by UV-irradiation. The samples were placed in a 6-

well plate and 25 μL of *E. coli* in 1/100 MHB medium or MRSA in MHB medium suspension was added at the center region of each sample. Subsequently, the bacteria suspension was covered with a PE film (1 cm $\times$ 1 cm). Afterwards, the 6-well plate was placed in a moist chamber and then incubated at 37 $^{\circ}\text{C}$ for 24 h. The samples were immersed in 5 mL of MHB medium and vigorously vortexed for 2 min to release the adherent bacteria, and 100 μL of the bacteria suspension was plated onto the LB Agar which was incubated for 24 h at 37 $^{\circ}\text{C}$. The CFUs on the LB Agar were counted.

The Long-term Stability Assay

The long-term stability of TPU and TPU-0.5 in saline was evaluated by monitoring the antibacterial activity over time. The samples were immersed in saline for 0, 7, 14 and 21 days, respectively. The antibacterial assay was carried out on these samples according to the same procedure described above.

Hemolysis Assay

Hemolytic activity was tested by determining the release of hemoglobins in erythrocyte suspensions derived from fresh mice red blood cells (RBCs). Fresh mice blood was washed with PBS buffer for five times and the collected RBCs were diluted to a final concentration of 2 vol%. TPU and TPU-x composite films (0.5 cm $\times$ 0.5 cm) were placed in 1.5 mL microcentrifuge tubes and immersed in the stock solution of RBCs. The tubes were incubated for 1 h at 37 $^{\circ}\text{C}$ with 130 r/min shaking and centrifuged at 2000 r/min for 10 min. 150 μL of the supernatant was transferred into a flat-bottomed 96-well plate and the OD values at a wavelength of 540 nm were collected to calculate the percentage of hemolysis using a multifunctional microplate scanner. The PBS buffer containing Triton X-100 was used as the positive control, while the PBS buffer was used as the negative control. The percentage of hemolysis was calculated from the following equation:

%Hemolysis =

$$\left(\frac{A_{540} \text{ of sample} - A_{540} \text{ of negative control}}{A_{540} \text{ of positive control} - A_{540} \text{ of negative control}} \right) \times 100\%$$

All the experiments were conducted in quadruplicates and the percentage of hemolysis was averaged from four measurements.

Biocompatibility Assay

Standard 3T3 mouse fibroblast cells were cultured in DMEM supplemented with 10% FBS, 100 U/mL of penicillin and 100 U/mL of streptomycin. The cells were incubated at 37 $^{\circ}\text{C}$ in a humidified atmosphere containing 5% CO_2 , detached from the culture dish using trypsin-EDTA solution (0.53 mmol/L), and resuspended in a fresh culture medium for subsequent experiments. The cells were then implanted in a 24-well plate with 1 mL of DMEM at a density of 2×10^4 cells per well and incubated in a humidified atmosphere of 5% CO_2 at 37 $^{\circ}\text{C}$ for 24 h. A TPU-x composite film with the size of 0.5 cm $\times$ 0.5 cm was added into each well of the plate in quadruplets. The control experiment was carried out using the sterile PBS without the composite film. MTT in PBS (5 mg/mL, 100 μL per well) was added and allowed to incubate for additional 4 h. The culture supernatant was aspirated and 1 mL of DMSO was added to dissolve the internalized purple formazan crystal. After 5 min, 200 μL of the DMSO solution was transferred into a 96-well plate

and the absorbance at 490 nm was measured by a microplate reader. The results were expressed as percentages relative to the absorbance obtained in the control experiment.

Characterization

^1H -NMR spectroscopy was carried out on a Bruker AV 300 MHz spectrometer. Fourier transform infrared spectrometer spectra (FTIR) were recorded on a Bruker Vertex-70 instrument in the range of 4000–400 cm^{-1} . The spectra of composites films were collected in the reflection mode. Thermogravimetric analysis (TGA) was performed on a TGA/DSC 1 (Mettler-Toledo, Switzerland) at the rate of 10 $^{\circ}\text{C}/\text{min}$ from room temperature to 800 $^{\circ}\text{C}$ under N_2 atmosphere. Thermal analysis was performed using a NETZSCH Instruments differential scanning calorimeter DSC 214 (Germany) under N_2 atmosphere. The heat enthalpy and temperature of the DSC were calibrated with the indium standard. The measured specimens were firstly heated from -100 $^{\circ}\text{C}$ to 180 $^{\circ}\text{C}$ at a heating rate of 10 $^{\circ}\text{C}/\text{min}$, and maintained at 180 $^{\circ}\text{C}$ for 3 min to erase the thermal history, and then cooled to -100 $^{\circ}\text{C}$ at a rate of 20 $^{\circ}\text{C}/\text{min}$. The samples were further heated to 180 $^{\circ}\text{C}$ at a heating rate of 10 $^{\circ}\text{C}/\text{min}$. The static mechanical properties of the composites were studied using an Instron 1211 tensile-testing machine at a crosshead speed of 50 mm/min at 25 $^{\circ}\text{C}$. All dumbbell-shaped specimens were stored for 24 h at 25 $^{\circ}\text{C}$ before testing, and eight specimens were tested for each sample. The elemental composition of the film surface was determined by an X-ray photoelectron spectroscopy (XPS, Thermo-Electron ESCALAB 250) with an Al K α source ($h\nu=1486.6$ eV). The binding energy ranging from 0 eV to 1200 eV was averaged at each point to identify potential elements on the sample surface. The static contact angle of deionized water was measured at ambient temperature using a KRÜSS model DSA 30 video goniometer. A drop of deionized water of approximately 3 μL was placed on the samples ($n=3$) at different randomly selected locations, and the mean contact angle values were recorded using the sessile drop method by the Kruss Advance software.

The number average molecular weight (M_n) and polydispersity index ($\bar{D}$) of ϵ -PL were determined by gel permeation chromatography (GPC) equipped with a Waters Ultrahydrogel column and a Waters 1515 isocratic HPLC pump equipped with a Waters 2414 refractive index detector. Acetic acid (0.2 mol/L)/sodium acetate (0.1 mol/L) buffer was used as the eluent with a flow rate of 0.6 mL/min at 35 $^{\circ}\text{C}$. The morphology of the composites surface was characterized by field emission scanning electron microscopy (FE-SEM, Zeiss, Merlin) at an accelerating voltage of 1 kV.

RESULTS AND DISCUSSION

Preparation of TPU-x Composite Films

A water-insoluble complex, PL-DOSS, was prepared by mixing ϵ -PL and DOSS. The chemical composition of PL-DOSS was confirmed by ^1H -NMR analysis (Fig. S1 in the electronic supplementary information, ESI). The ratio of the peak area of H_e in ϵ -PL to H_m in DOSS was 1:6, which demonstrated that PL-DOSS was composed of a stoichiometric ratio of ϵ -PL to DOSS. The characteristic peak at ~ 1036 cm^{-1} for O=S bonds from DOSS was observed in the FTIR of PL-DOSS (Fig. S2 in ESI). TG and DSC analyses were performed to evaluate the thermal

properties of PL-DOSS. The temperature of 5% weight loss ($T_{d,5}$) was 244 °C (including the weight loss of absorbed water), indicating that the complex has good thermal stability (Fig. S3 in ESI). The glass transition temperature (T_g) and the melting temperature (T_m) of PL-DOSS were 65.8 and 153.6 °C, respectively, by DSC analysis (Fig. S4 in ESI), in consistent with the results reported in the previous literature.^[44]

Five different TPU-*x* composite films were prepared by solution blending of TPU and PL-DOSS and the percentages of PL-DOSS were 0.06 wt%, 0.13 wt%, 0.25 wt%, 0.5 wt% and 1.0 wt%, i.e. *x* = 0.06, 0.13, 0.25, 0.5 and 1.0, respectively. The control TPU film was also processed under the same conditions as TPU-*x* composite films. All films were transparent with the thickness of ~150 μm.

Thermal and Mechanical Properties of TPU-*x* Composite Films

The effect of PL-DOSS on thermal properties of TPU-*x* composite films is investigated. The T_g values of TPU-*x* composite films were obtained from the DSC curves of the second heating process after erasing the thermal history (Table 1 and Fig. S5 in ESI). T_g was sensitive to the content of PL-DOSS and increased with the increase of PL-DOSS content, from −41.7 °C for TPU to −35.2 °C for TPU-0.06 and −30.6 °C for TPU-1.0. The addition of 1 wt% PL-DOSS led to the reduction of T_g by ~11 °C. The thermal stabilities of TPU-*x* composite films were further studied by TGA measurement. The 5 wt% weight loss temperature ($T_{d,5}$) of TPU-*x* composite films showed a strong

Table 1 Thermal and mechanical properties of TPU and TPU-*x* composites.

Sample	T_g (°C)	$T_{d,5}$ (°C)	E^a (MPa)	σ_b^b (MPa)	ϵ_b^c (%)
TPU	−41.7	296	19.0±2.1	63.3±1.8	948±18
TPU-0.06	−35.2	286	20.9±2.8	58.6±6.0	850±48
TPU-0.13	−34.0	243	22.7±2.7	59.1±4.4	864±27
TPU-0.25	−33.4	243	21.2±1.3	60.9±3.4	870±20
TPU-0.5	−32.5	239	22.9±2.3	62.4±1.8	896±6
TPU-1.0	−30.6	234	26.1±1.1	57.9±1.0	786±18

^a Young's Modulus; ^b Tensile strength; ^c Elongation at break.

dependence on the content of PL-DOSS and decreased from 296 °C for TPU to 286 °C for TPU-0.06 and 234 °C for TPU-1.0 (Table 1 and Fig. S6 in ESI). The PL-DOSS complex might contain residue amine or sulfate groups that might catalyze the degradation of TPU, which lowers the degradation temperature.

The addition of PL-DOSS also affected the mechanical properties of TPU-*x* composites. The moduli (E_s) of TPU-*x* composites were very close and slightly higher than that of pure TPU when the content of PL-DOSS was ≤ 0.5%, i.e. *x* ≤ 0.5, while the E of TPU-1.0 was much higher than that of TPU. Analogously, the elongation at break (ϵ_b) and tensile strength (σ_b) were slightly lower than those of TPU when the content of PL-DOSS was ≤ 0.5%, but TPU-1.0 had much lower ϵ_b and σ_b than TPU. These results suggested that the addition of PL-DOSS slightly strengthened the mechanical properties of TPU

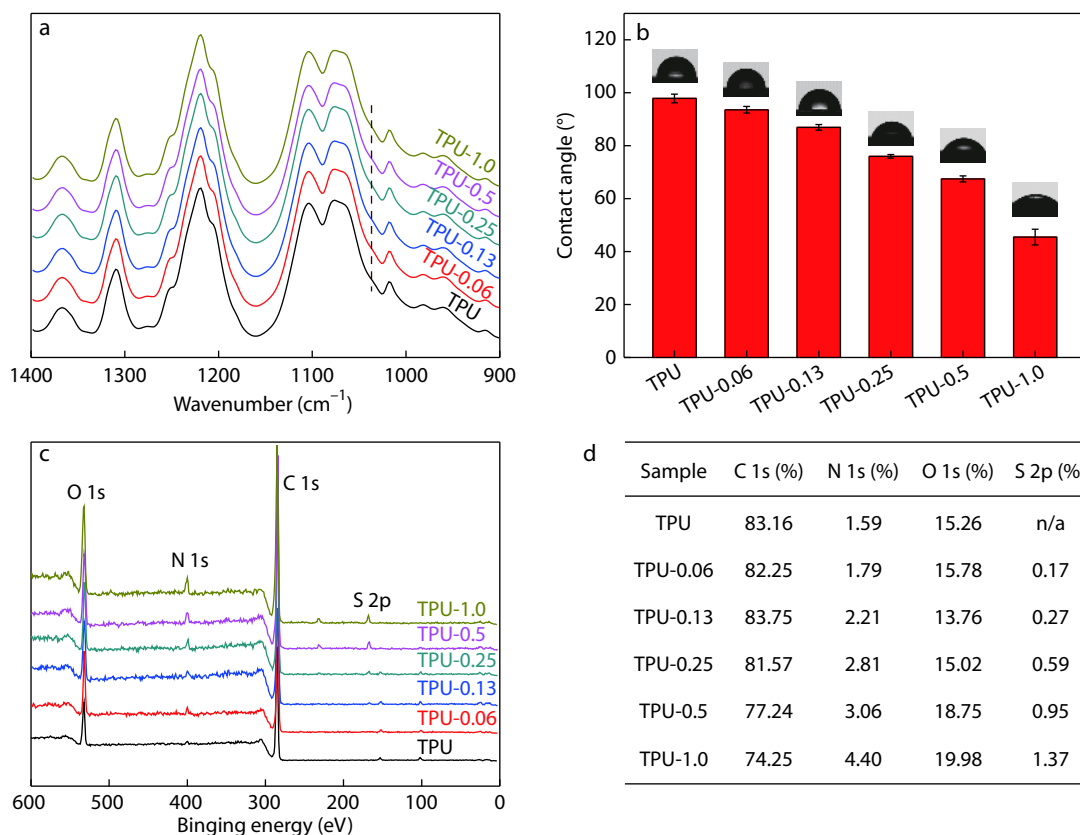


Fig. 1 Surface characterization of TPU and TPU-*x* films: (a) ATR-FTIR spectra, (b) water contact angles, (c) XPS survey spectra, (d) concentrations of C, N, O and S elements on the surfaces of TPU and TPU-*x* films.

even when the content of PL-DOSS was only $\leq 1\%$.

Structural Characterization of TPU-x Composite Films

The surface properties are important for the potential application of composite films in biomedical fields. The wettability of TPU-x composite films was investigated by water contact angle (WCA) analysis (Fig. 1b). TPU was hydrophobic with the WCA of $97.7^\circ \pm 1.7^\circ$. After complexation with PL-DOSS, TPU-x composite films were gradually transformed from hydrophobic to hydrophilic with the increase of PL-DOSS and the WCA of TPU-x composite films decreased from $97.7^\circ \pm 1.7^\circ$ for TPU to $93.6^\circ \pm 1.3^\circ$ for TPU-0.06 and $45.5^\circ \pm 3.0^\circ$ for TPU-1.0. The hydrophilicity of TPU-x composite films was beneficial to the biocompatibility of the functionalized surfaces and could be attributed to the strong protonation of amine and sulfonic acid functional groups. A small shoulder peak at 1036 cm^{-1} , corresponding to the symmetric stretching of sulfonic acid group, was observed in the FTIR spectrum (Fig. 1a and Fig. S7 in ESI), indicating that PL-DOSS was successfully compounded into TPU. The peak intensity was slightly increased with the amount of PL-DOSS. This could explain the transition from the

hydrophobic surface to the hydrophilic surface with the increase of PL-DOSS.

XPS was further used to characterize the surface compositions of TPU-x composite films, as shown in Fig. 1(c). The concentrations of C, N, O, and S elements on surfaces of TPU and TPU-x films were listed in Fig. 1(d). Compared to pure TPU, the concentrations of N and S on the surfaces of TPU-x increased from 1.59% to 4.40% and 0.17% to 1.37%, respectively. Specifically, the surface concentration of S element was almost linear to the amount of PL-DOSS in the composite films.

The morphologies of TPU and TPU-x composite films were investigated by SEM analysis, as shown in Fig. 2. Neat TPU shows a smooth and homogeneous surface. Spherical nanoparticles with a size of $100 \pm 20\text{ nm}$, which was independent on the amount of PL-DOSS, were observed on the surfaces of TPU-x films. The density of PL-DOSS nanoparticles increased with the increase of PL-DOSS. PL-DOSS nanoparticles can barely be observed in the TPU-0.06 film. Cross-section SEM analysis of the cryo-fractured TPU-x films revealed that PL-

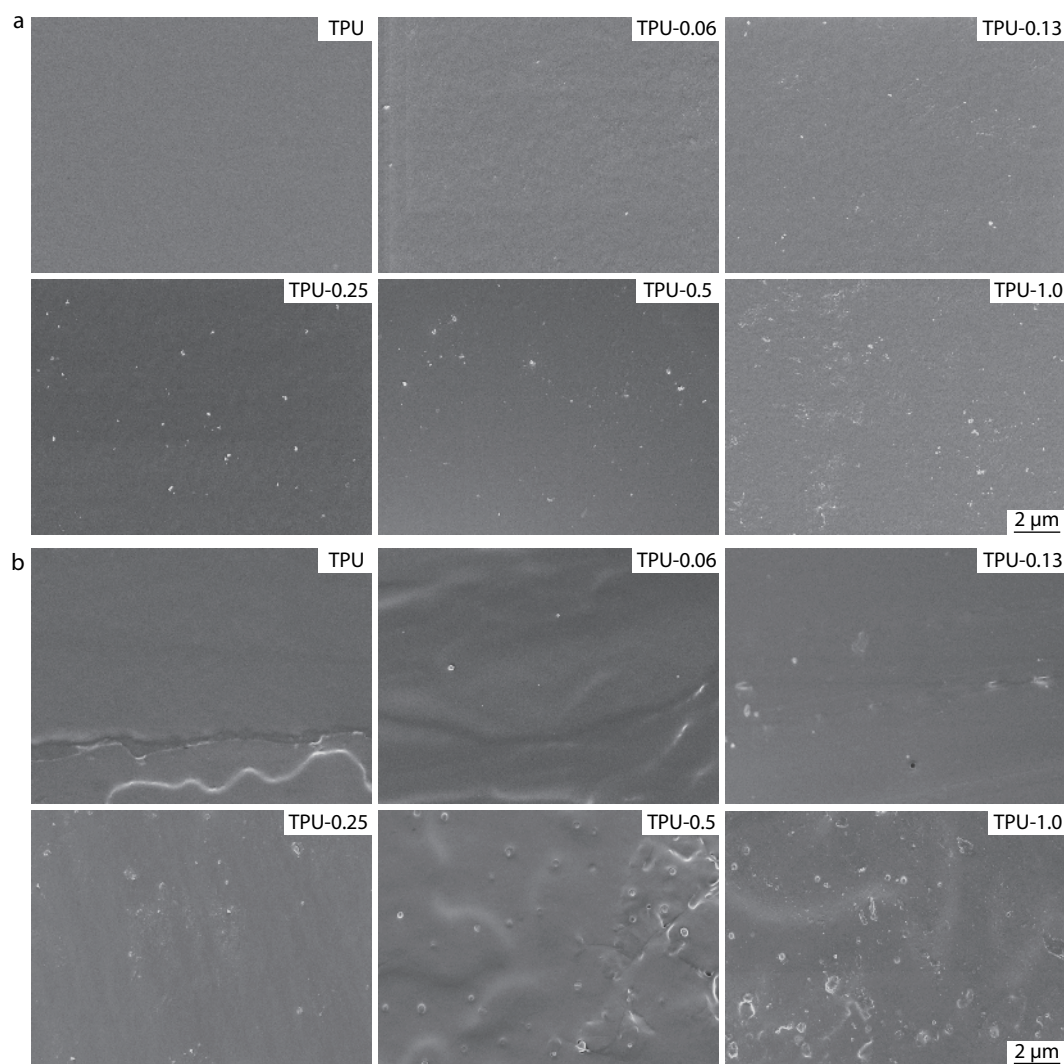


Fig. 2 Top-down (a) and cross-section (b) SEM images of TPU and TPU-x films.

DOSS nanoparticles were uniformly distributed in the TPU matrix without aggregation, and the density of particles was similar on the surface and across the film thickness, indicating good compatibility between PL-DOSS complex and TPU matrix. When the TPU-0.5 composite film was soaked in water, most nanoparticles were intact (Fig. S8 in ESI) and there was no detectable PL-DOSS in the aqueous solution by ^1H -NMR analysis (Fig. S9 in ESI). In comparison, 0.5 wt% ϵ -PL was also directly blended with TPU to prepare the TPU/PL-0.5 composite film using the same protocol, but ϵ -PL phase was separated from TPU in the size of several micrometers, as observed by SEM analysis (Fig. S10 in ESI).

Antibacterial Properties of TPU-x Composite Films

One Gram-positive bacteria, MRSA, and one Gram-negative bacteria, *E. coli*, were used to evaluate the antibacterial performances of TPU-x composite films. Figs. 3(a) and 3(b) show the optical images of bacterial CFUs on the agar plates. Neat TPU films did not display antibacterial activities against either MRSA or *E. coli* within 24 h of contact. The antibacterial activities of TPU-x films showed strong dependence on the contents of PL-DOSS. The density of bacterial CFUs decreased with the increase of PL-DOSS in TPU-x films, indicating the increase of antibacterial activities (Figs. 3c and 3d). About 50% and 2-order reduction of the MRSA CFUs were obtained on TPU-0.06 and

TPU-0.13 films, respectively, within 24 h compared to neat TPU, while TPU-0.25 could kill all bacteria. The antibacterial activities of TPU-x films were slightly weaker for *E. coli* than for MRSA, but the TPU-0.5 film could still kill up to 99.1% of *E. coli*, i.e. 2-order reduction of the CFU, within 24 h compared to neat TPU. The difference in the antibacterial activities against MRSA and *E. coli* was mainly due to the structure difference of the cell membranes.

The excellent antibacterial activities of TPU-x composite films could be attributed to the formation of PL-DOSS nanoparticles in the TPU matrix. Compared to uniformly dispersed antibacterial components, nanoparticles greatly increased the local concentration of active components and facilitated the electrostatic interaction with negatively charged bacteria membranes even at the low concentration. In addition, the size of nanoparticles was much smaller than that of bacteria and one bacteria can interact with multiple nanoparticles, which is conducive to improving the antibacterial activity of TPU-x films. In comparison, Ushimaru *et al.*^[44] blended 10 wt% PL-DOSS into polypropylene (PP) to prepare the antibacterial composite and they found that PL-DOSS was uniformly dispersed into the composite matrix. The amount of *E. coli* on the composite was reduced to 1/30 of that on the control PP.

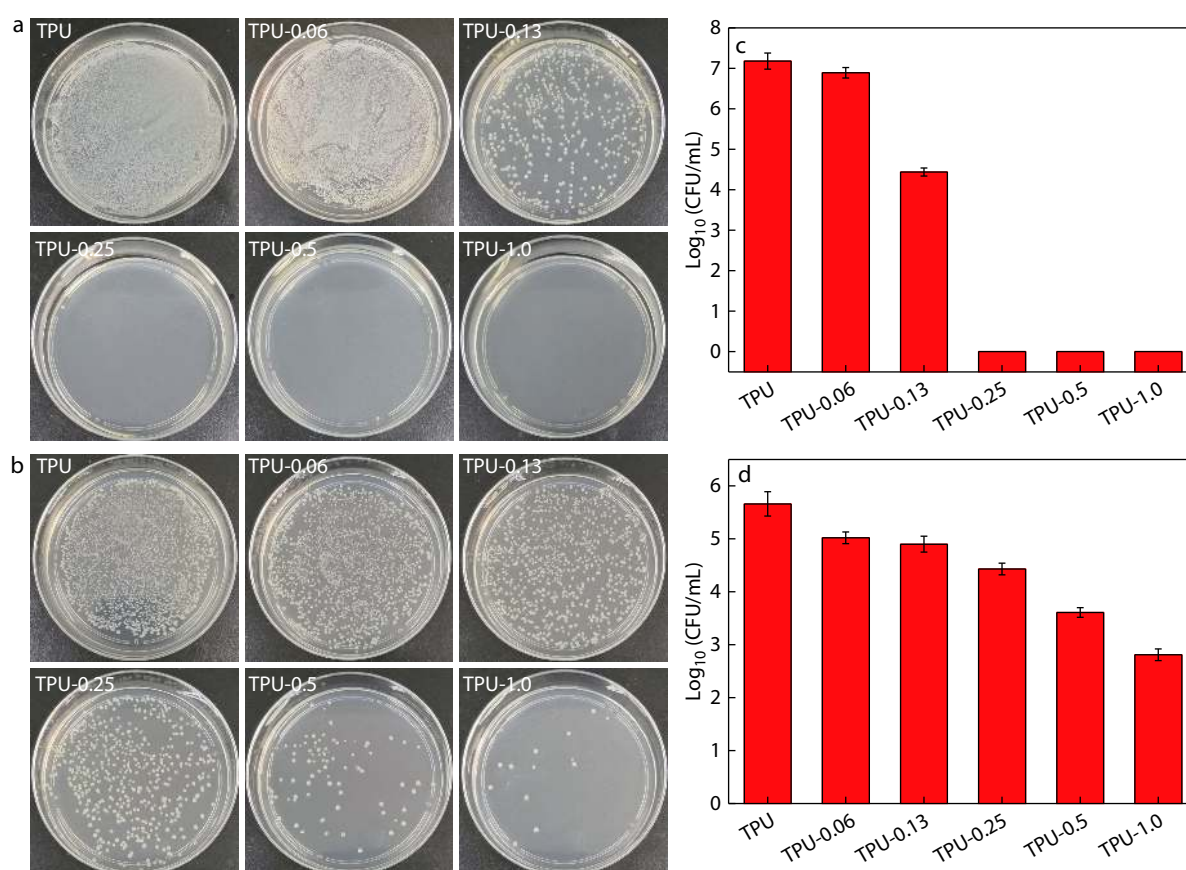


Fig. 3 Antibacterial activities of TPU and TPU-x films against MRSA and *E. coli*. Optical images of (a) MRSA and (b) *E. coli* on the LB agar plates after contact with TPU and TPU-x. Histograms of CFUs of (c) MRSA and (d) *E. coli* of TPU and TPU-x films with different contents of PL-DOSS.

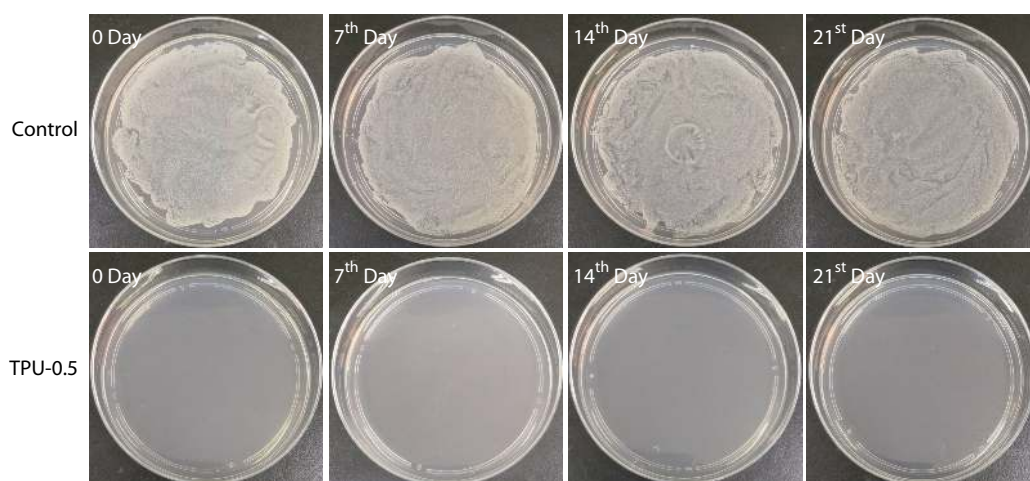


Fig. 4 Antimicrobial activities of TPU and TPU-0.5 against MRSA after being immersed in saline for 0, 7, 14 and 21 days.

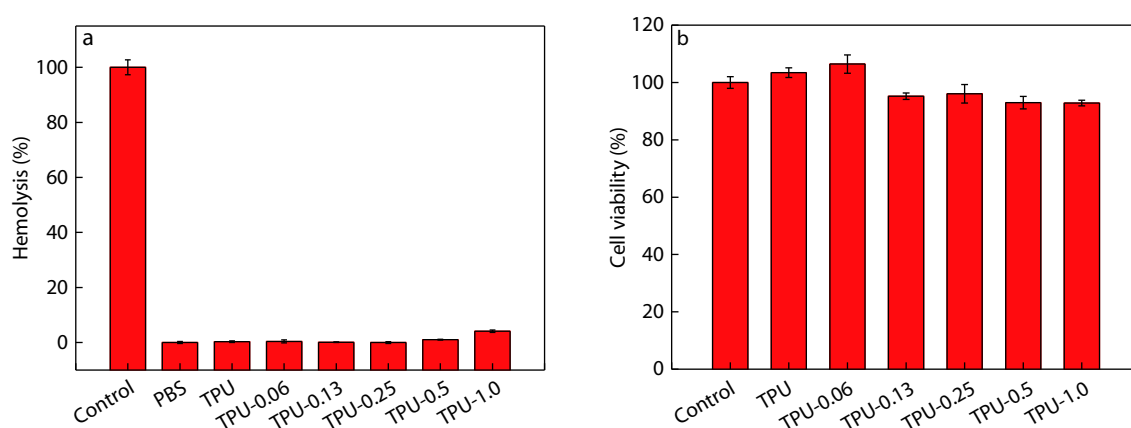


Fig. 5 (a) Hemolysis rate of TPU and TPU-x films and (b) cell viability of NIH-3T3 fibroblast cells in DMEM medium with TPU and TPU-x films. All the experiments were conducted in quadruplicates.

The Long-term Stability of TPU-x Composite Films

The long-term stability of the TPU-x composite film under the physiological condition is of great significance for its potential application. Representative TPU-0.5 films were immersed in saline, a simulated physiological condition, for 7, 14 and 21 days and the antibacterial properties of these TPU-0.5 films were evaluated by the CFU count assay against MRSA to evaluate their long-term stabilities (Fig. 4). The results show that TPU-0.5 films retained the excellent antibacterial activity against MRSA even after being immersed in saline for 21 days, and 6-order reduction of the MRSA CFU was also achieved. The good stability of TPU-x under physiological conditions is mainly attributed to the strong electrostatic interaction between PL and DOSS, which makes PL-DOSS insoluble in saline. In comparison, when the TPU/PL-0.5 composite film was soaked in saline, it did not show any antibacterial activity against MRSA since ϵ -PL is soluble in saline (Fig. S11 in ESI).

In Vitro Biocompatibility

The biocompatibility is very important for biomedical materials. The hemolytic activity of TPU-x composite films was tested to evaluate the toxicity toward mammalian cell membranes by determining the release of hemoglobin in erythrocyte

suspensions derived from fresh mice red blood cells (RBCs). PBS buffer containing Triton X-100 was used as the positive control, while PBS buffer was used as the negative control. As shown in Fig. 5(a), the hemolytic activity of TPU-x increased with the increase of PL-DOSS content and the hemolytic rates gradually increased from 0.29% for TPU-0.06 to 4.09% for TPU-1.0.

The cytotoxicity of the TPU-x composite films was further evaluated by the MTT assay, employing standard NIH 3T3 mouse fibroblast cells as the model mammalian cell line. The results showed that the cell viabilities of the TPU-x composite films were similar to that of the neat TPU after 24 h of incubation, which suggested that the TPU-x did not show obvious cytotoxicity (Fig. 5b), although the cell viabilities were slightly lower for the TPU-x, compared with those for TPU (90.5% for TPU-1.0; 106.4% for TPU).

CONCLUSIONS

In summary, antibacterial TPU-x composite films were successfully prepared by simple solution blending of TPU with an antibacterial additive, water-insoluble PL-DOSS complex. ϵ -PL is an approved food preservative while DOSS is used for relief of

occasional constipation, and both are safe as additives for medical devices. The antibacterial activities of TPU-x composite films increased with the amount of PL-DOSS, and >99% reduction of CFU was obtained in TPU-x films even at relatively low content of PL-DOSS, e.g. 0.13% for MRSA and 0.5% for *E. coli*. SEM analysis confirmed that PL-DOSS complex formed nanoparticles that were uniformly dispersed in the TPU matrix. The excellent antibacterial activities were attributed to the formation of PL-DOSS nanoparticles that provided high concentration of antibacterial agents upon contact with the bacterial membrane. TPU-0.5 films showed long-term stability and their antibacterial activities against MRSA did not change even after they were stored in saline for 21 days. Compared to neat TPU, the thermal and mechanical properties of TPU-x composites were only slightly changed. In addition, TPU-x composite films exhibited low hemolytic activity and cytotoxicity. The present work may provide a promising strategy for the rational design and development of safe antibacterial materials to fight medical device-associated infections.

Electronic Supplementary Information

Electronic supplementary information (ESI) is available free of charge in the online version of this article at <http://doi.org/10.1007/s10118-021-2578-z>.

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