Synthesis of poly-tetrahydropyrimidine antibacterial polymers and research of their basic properties

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Preparation of culture medium and bacteria cultivation:

Dissolve 2.5 g of LB broth in 100 mL of deionized water completely, and then adjust the pH to 7.2-7.4 with NaOH solution (1M, 0.2%). Sterilize the culture medium under 120℃ and high pressure for 2 hours to obtain the liquid culture medium. Dissolve 0.14 g of agar powder and 0.25 g of LB broth in 10 mL of deionized water, adjust the pH to between 7.2 and 7.4 with NaOH solution (1M, 0.2%), and sterilize at 120°C for 2 h to obtain the lower solid medium; Dissolve 0.07 g of agar powder and 0.2 g of LB broth in 5 mL of deionized water, adjust the pH to between 7.2 and 7.4 with NaOH solution (1M, 0.2%), and sterilize at 120°C for 2 h to obtain the upper solid medium.

Pour the sterilized lower solid medium into a petri dish evenly. After solidification, inoculate the bacteria on the medium and place it in a 37℃ constant temperature and humidity incubator for 24 hours. Take a single point of the colony in a 20mL culture medium, culture for 24 hours under the condition of constant temperature and humidity, and obtain the bacterial solution.

Cell culture:

Thaw the frozen cells in a 37°C constant temperature shaker and transfer them to a centrifuge tube, add 5 mL of 1640 nutrient solution, centrifuge and remove the supernatant. Add 5 mL of 1640 nutrient solution again, blow the cells away with a pipette gently, transfer them to the cell culture box, and put it into the cell culture box for 24 hours.

Purification of P-THP polymers:

The hydrophobic polymers were purified by extraction. Stop the polymerization and remove THF from the system by rotary evaporation. Then dissolve the concentration solution with 100mL dichloromethane and poured it into the separating funnel. Add the saturated $NaHCO_3$ solution in equal amount, shake, place it in layers, and

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collect the lower polymer solution. Repeat this procedure three times. Next, excess anhydrous $MgSO_4$ was added to the polymer solution after three times extraction to remove the water in the system. Finally, dichloromethane was removed by rotary evaporation, and the P-THP polymers were further purified by freeze-drying.

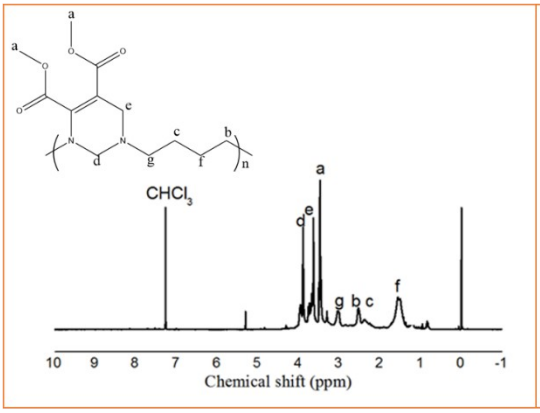
For the hydrophilic polymers, we purified them via dialysis and freeze-drying. Stop the polymerization and remove THF from the system by rotary evaporation. Dissolve the concentrate solution with deionized water, transfer the polymer solution to a 3500M dialysis bag, dialyzed with deionized water for 48 hours, and change the water frequently during dialysis. After that, the final products were obtained by freeze-drying.

Steps for testing the stability of coatings:

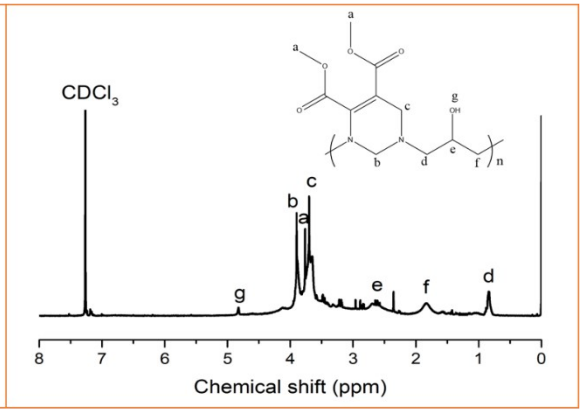
Dissolve P-THP-3, P-THP -8, and P-THP-12 with THF, P-THP-4 with DMSO respectively, and dilute their concentrations to 0.01mg/mL. Sterilize and dry four slides, drop 1ml of different polymer solutions onto the four slides, then let them dry in natural conditions. After that, soak four slides in the same amount of deionized water respectively, and take the solutions in contact with the coatings for UV test to observe their absorbance.

Detailed steps of wound healing assays:

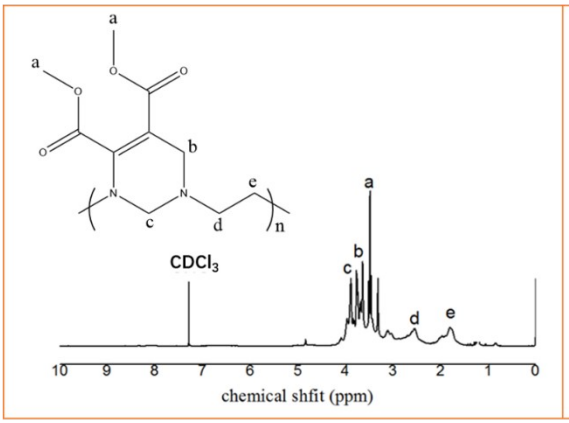
Dilute a single spot colony to 2×10^6 CFU/mL with LB broth. Four adult male mice aged 6-8 weeks were bred for one week at a constant temperature for 12 hours of light/dark exposure. The mice were injected intraperitoneally with chloral hydrate (50 mg/kg) for anesthesia, remove their hair on the back, and make an open wound with a diameter of 6 mm on the back skin to the depth of subcutaneous tissue.



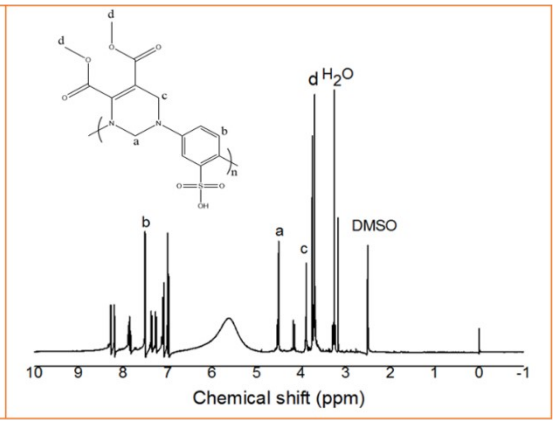
P-THP-1



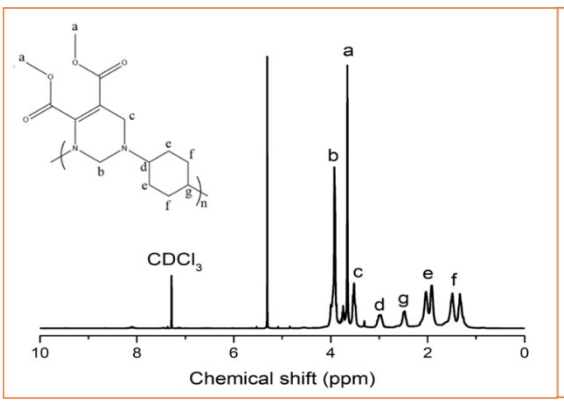
P-THP-2



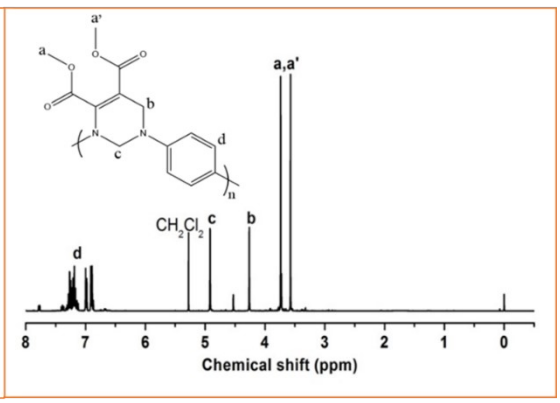
P-THP-3



P-THP-4



P-THP-5



P-THP-7

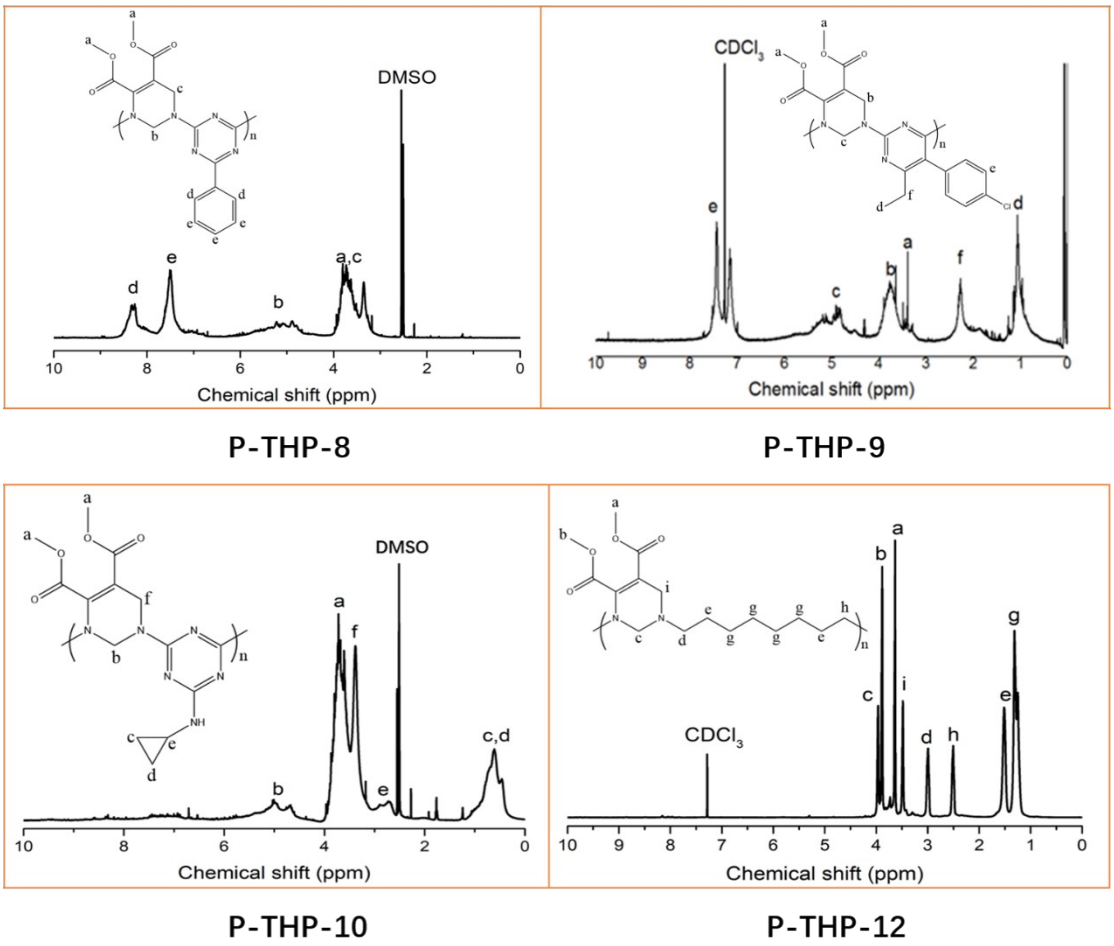


Fig. S1 ^{1}H NMR spectrum of P-THP polymers.

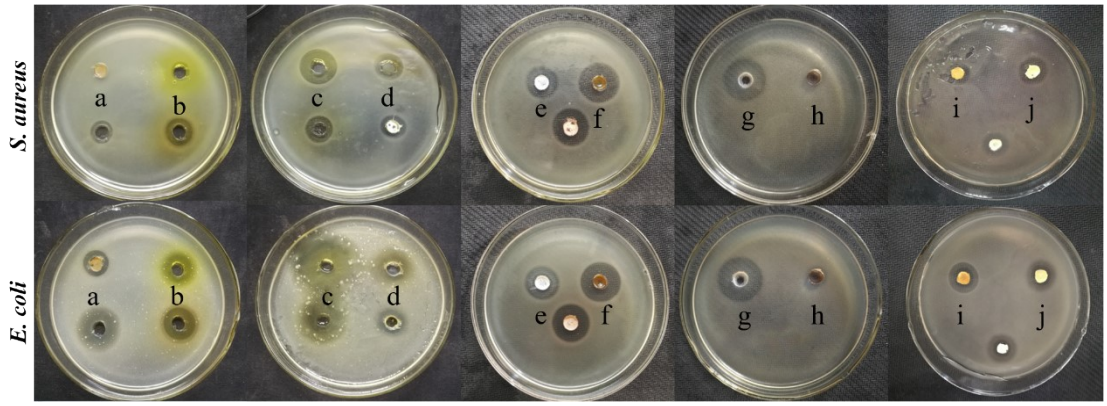
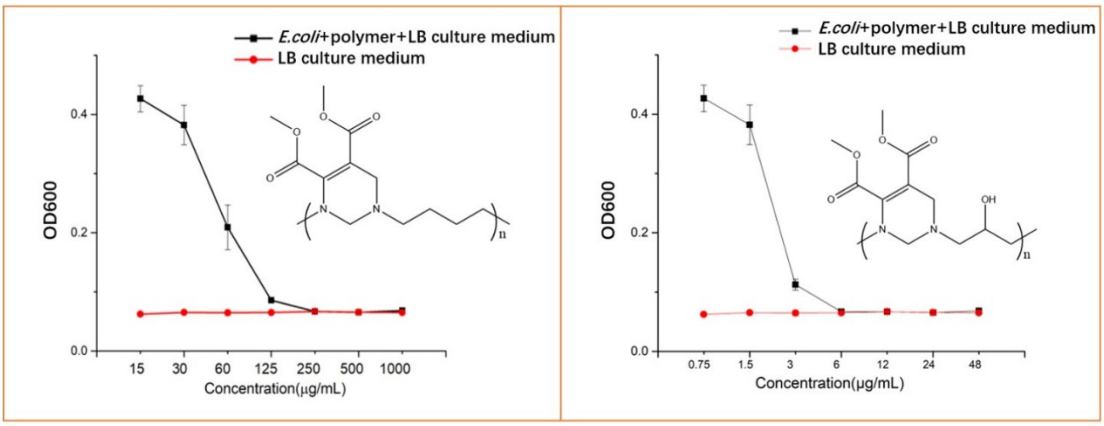
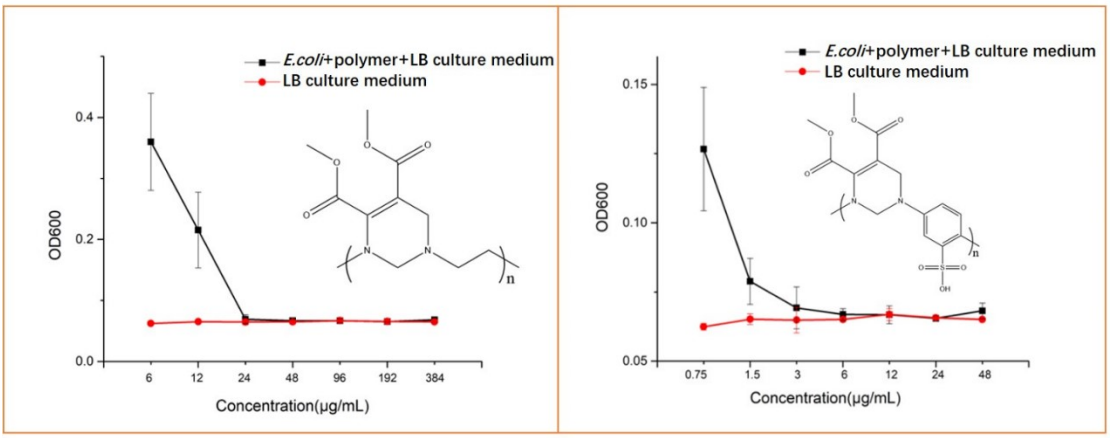


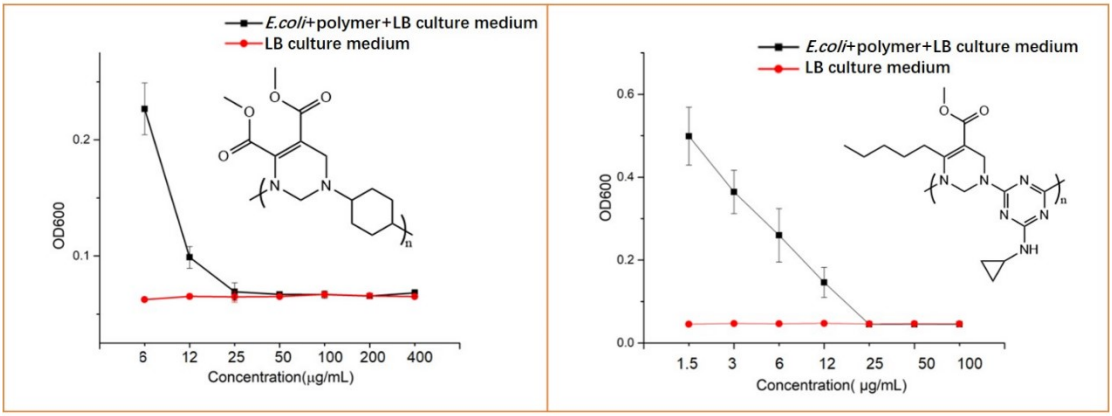
Fig. S2 Inhibition zone experiment of P-THP polymers (a to j represent P-THP-3 to P-THP-12 respectively).



P-THP-1 P-THP-2



P-THP-3 P-THP-4



P-THP-5 P-THP-6

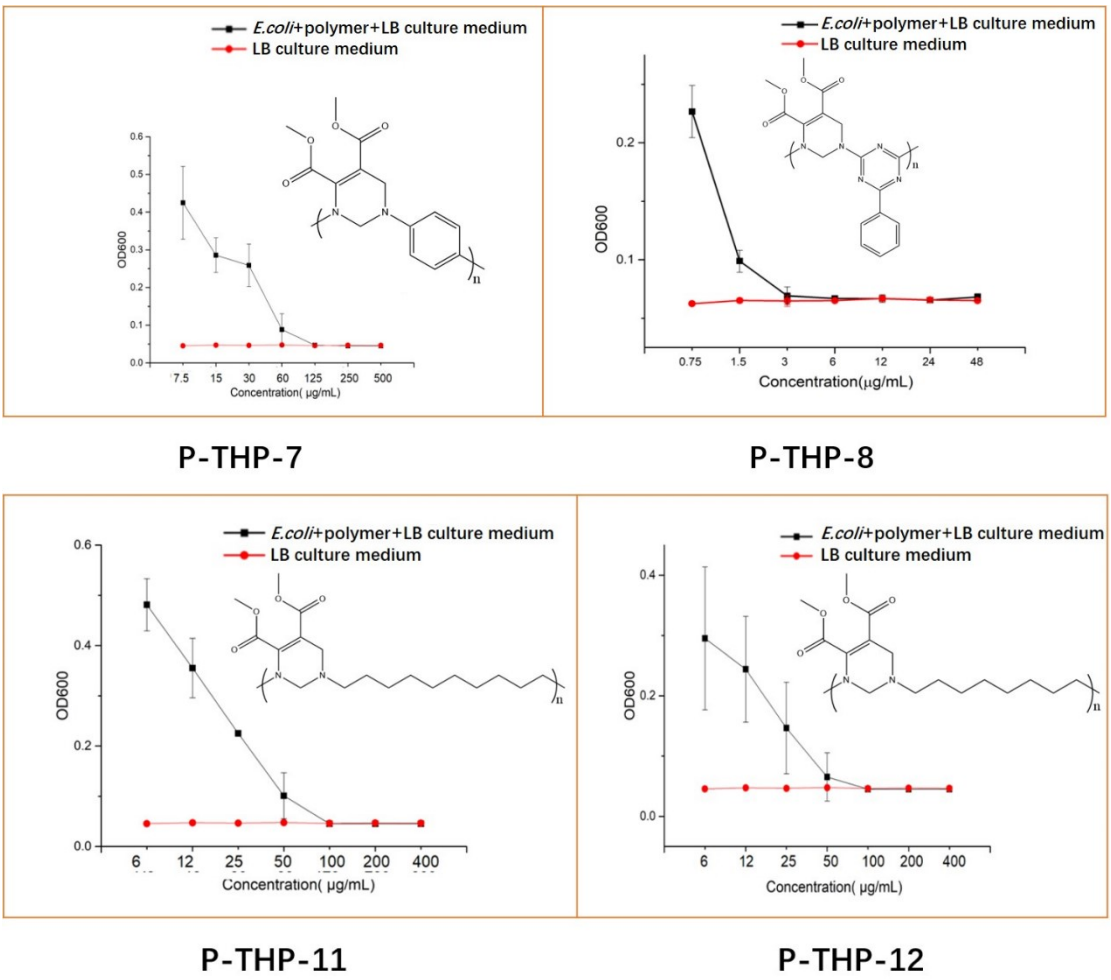
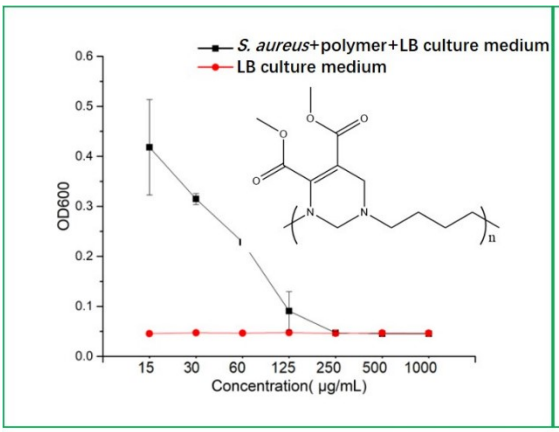
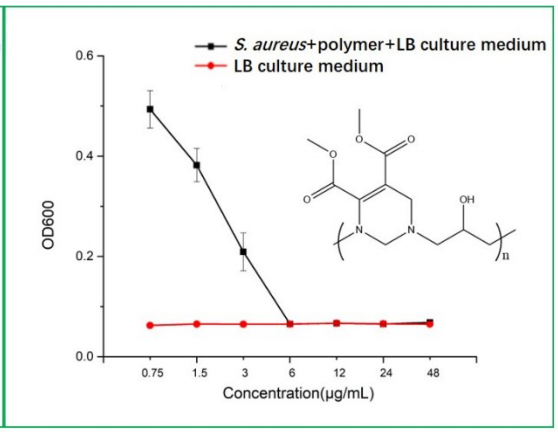


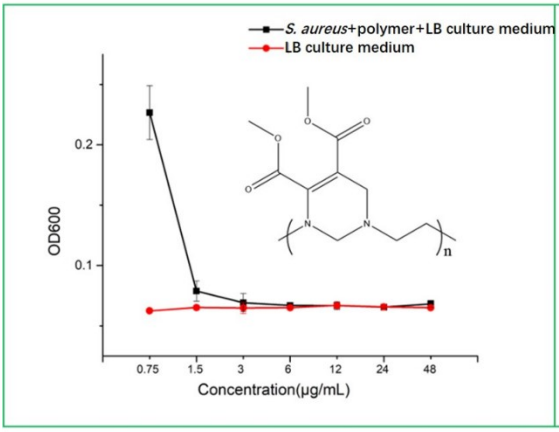
Fig. S3 The MICs of P-THP polymers against *E. coli*.



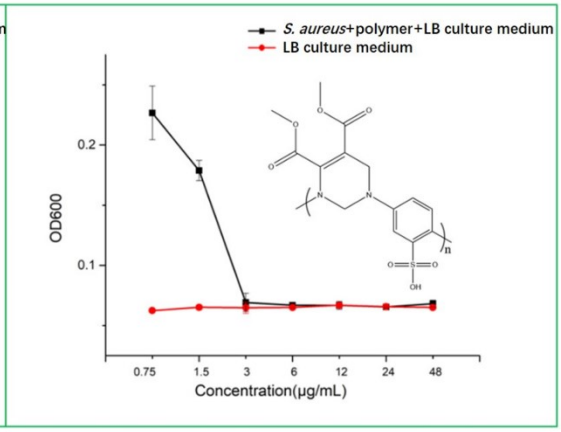
P-THP-1



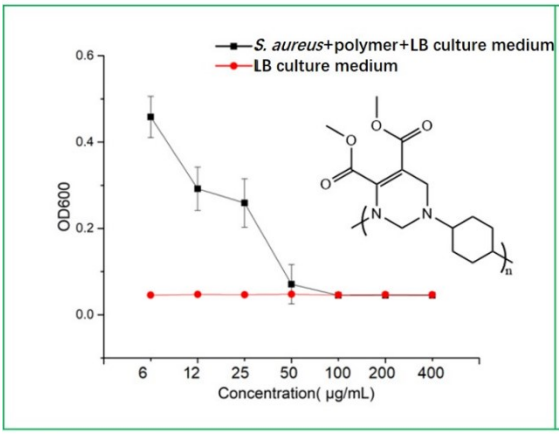
P-THP-2



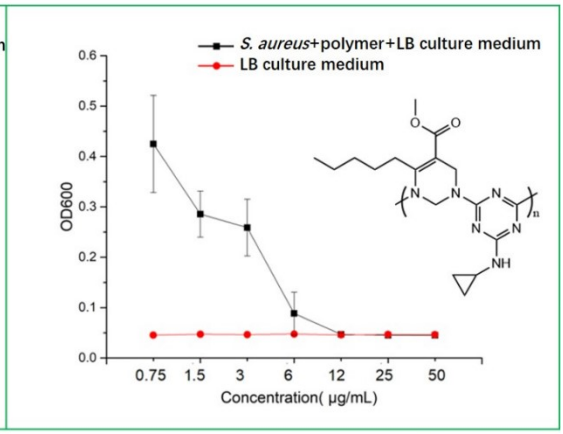
P-THP-3



P-THP-4



P-THP-5



P-THP-6

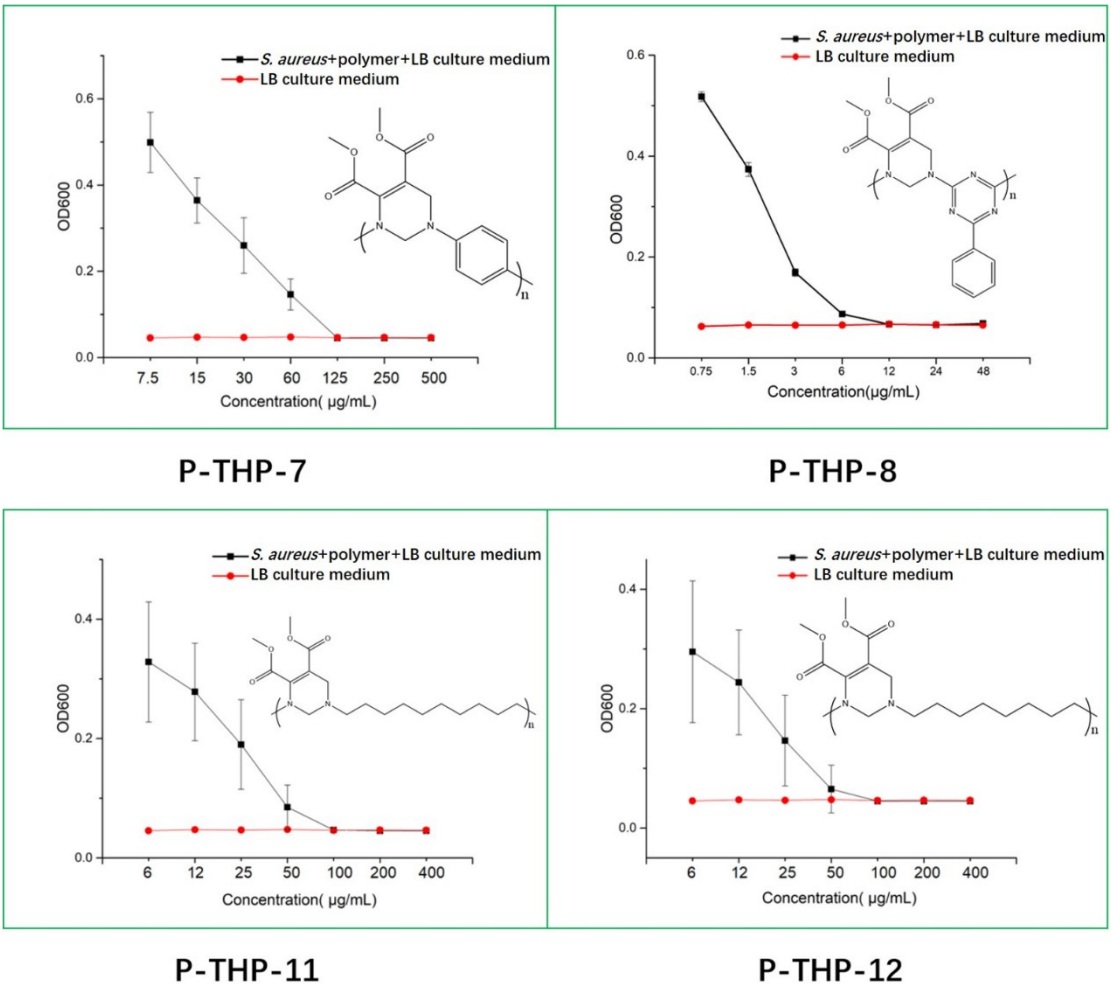


Fig. S4 The MICs of P-THP polymers against *S. aureus*.

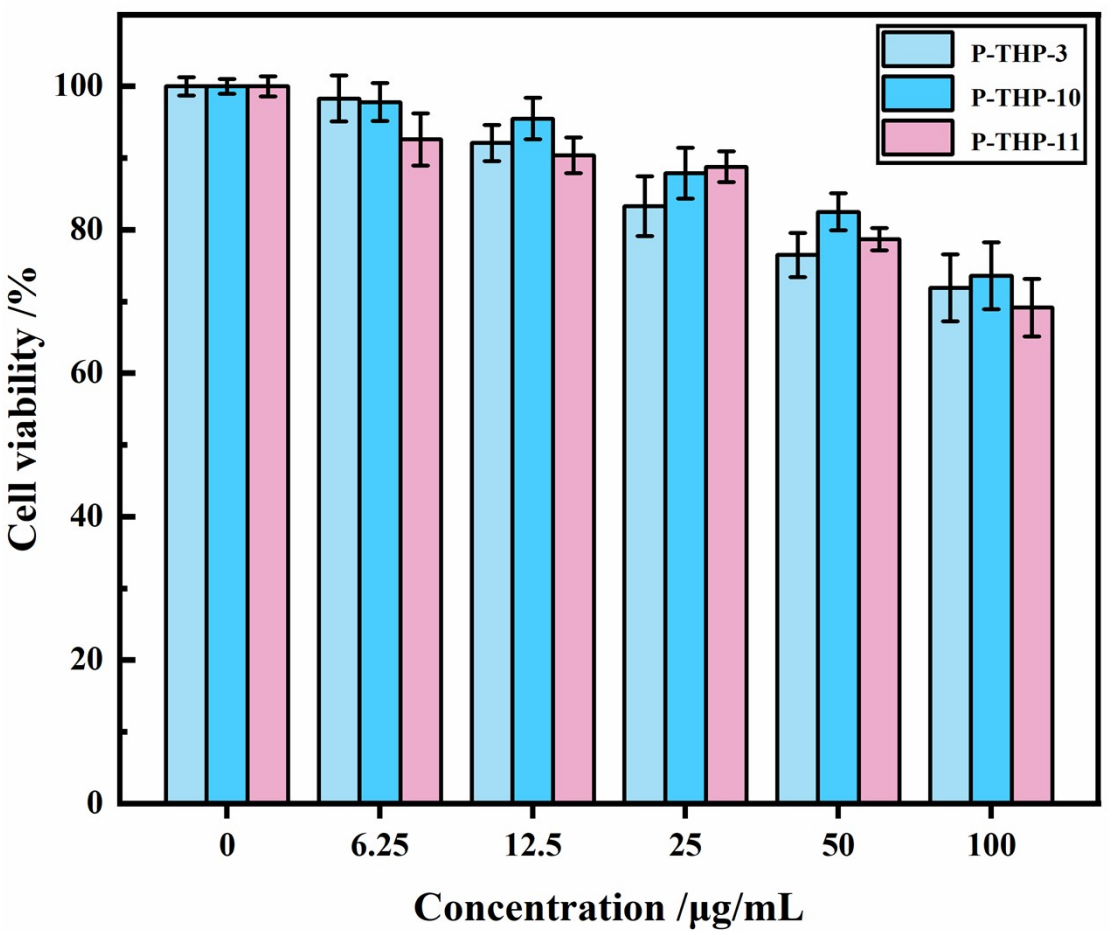


Fig. S5 Cytotoxicity histogram of P-THP-3, P-THP-10 and P-THP-11.