Supporting information

"Imitative" click chemistry to form a sticking xerogel for portable therapy of bacterial-infected wounds

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Materials.

Lipoic acid (LA) , propargyl alcohol (PA) were purchased from Sigma-Aldrich and used after removing polymerization inhibitor. N,N'-dicyclohexylcarbodiimide (DCC) and 4-dimethylaminopyridine (DMAP) were purchased from Aladdin Chemistry Co. Ltd. Dimethylsulfoxide (DMSO) and tetrahydrofuran (THF) were dehydrated by CaH_2 for 12 h then distilled for use. 5 W ultraviolet lamp was obtained from Counterfeit detector and fitted for use.

Synthesis of TLA.

LA powder (10 mM, 2.06 g) and DCC (10 mM, 2.06 g) were added into a 100 mL round-bottom flask with the solvent of 40 mL THF, and then dissolved sufficiently with magnetic stirring in an ice bath for 30 min. After, DMAP (6 mM, 0.74 g) was added into the flask with nitrogen protection for 5 min, then PA (6 mM, 0.36 mL) in a injector was instilled dropwise followed. The reaction was kept for 12 h with magnetic stirring in low speed. After the reaction, column chromatography (eluent ethyl acetate and petroleum ether=1:4) was used to purified product and the collected effluent was dried in 20 ^{o}C under vacuum for 5 h, finally it yielded 2.12 g (87.6%) of target product TLA (yellow powder).

Preparation of P-TLA.

TLA powder (2 g) was put into a centrifuge tube and the tube was immersed (only above TLA powder) in 90 ^{o}C oil bath for 5 min. After the powder turned into viscous fluid, the mixture was irradiated by a 5 W ultraviolet lamp for 5 min. Subsequently, after cooling down the viscous fluid to room temperature, the P-TLA was obtained.

Preparation of P-LA.

LA powder (2 g) was put into a centrifuge tube and the tube was immersed (only above LA powder) in 90 ^{o}C oil bath for 5 min, then cooling down the viscous fluid to room temperature, the P-LA was obtained.

Characterization.

Fourier transform infrared (FTIR) spectra were determined on a NICOLET 5700 FTIR

spectrometer (KBr disk). Nuclear magnetic resonance (NMR) spectra were obtained on a Bruker Avance 500 spectrometer (DRX 500, 500 MHz). X-ray photoelectron spectroscopy (XPS) was performed with an ESCALAB 250Xi instrument (Thermo Scientific, USA). Scanning electronic microscopy (SEM) images were obtained using a JSM-6510LV electron microscope (JEOL, Japan). TEM images were obtained using a Tecnai G20 transmission electron microscope (USA). Thermo gravimetric analysis (TGA) was performed on a NETZSCH STA 449C analyzerin a nitrogen flow (heating rate: 20 ^{o}C / min). Differential scanning calorimetry (DSC, TAQ2000) was used to measure the lower critical solution temperature of the xerogel. The equipment for recording videos and photographing physical images is Iphone 6plus.

Dynamic thermomechanical analysis, tensile and compress test.

The dynamic thermomechanical analysis of P-LA and P-TLA, including the loss modulus, storage modulus and the loss factor (tanδ), were determined by equipment of TA Q800. the sample was shaped for a piece with the height of 2 mm and the basal diameter of 10 mm, and the frequency is constant by 1 Hz. The tensile and pressure test was measured on a material mechanics machine (CMT4104).For tensile test, the samples was shaped for a piece the width of 20 mm and the thickness of 5 mm, the loading rate was 50 mm/min and the gauge length between the clamps was 20 mm. For pressure test, the sample was shaped for a column with the width of 10 mm and the basal diameter of 8 mm, the maximum compression distance was 4 mm.

Tests of swelling degree.

The P-LA and P-TLA (two samples, kept in 25 ^{o}C and 50 ^{o}C) were cut into blocks with weight of 500 $\pm$ 50 mg (named g_0) after drying, then soaked in a selected solvent (deionized water and DMSO). After a period of time (0, 5, 10, 15, 20 min, respectively), two gels were removed from solvents and weighed for g.

In vitro adhesive bacteria analysis.

S. aureus was used to evaluate the antibacterial activity of the gels by the spread plate method. The experimental devices and samples were sterilized with an ultraviolet lamp for at least 30 min and 200 μL of the diluted bacterial suspension (10^7 CFU/mL) cultured in the sterile Luria-Bertani (LB) medium (100 mL of deionized water with 1 g of Bactotryptone, 0.5 g of Bacto-yeast extract and 1 g of NaCl contained) were added to 96-well plates containing different types of Xerogels

(P-LA and P-TLA). Each sample was cultured for 30 min. The bacterial colony on the plates was photographed and was counted the number of colonies on the plate.

MTT assay.

The in vitro cytotoxicity of the hydrogels was determined by the 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyl tetrazolium bromide (MTT) assay using NIH-3T3 cells. Before the assay, all experimental devices and samples were sterilized with an ultraviolet lamp for at least 30 min. The cells were seeded onto the xerogels (P-LA and P-TLA) on 96-well plates with 200 μL of the medium and cultured for 1, 3, and 7 days with incubation under a humidified atmosphere of 5% CO_2 at 37 °C. 200 μL of the MTT solution with a concentration of 0.5 mg mL^{-1} were added to each well after removing the stock solution and incubated at 37 °C for 4 h. After sucking out the solution, 200 μL of DMSO solution were added to each well and agitated for 5 min . The supernatant fluid was taken to determine the optical density (OD) on a microplate reader (SpectraMax i3, Molecular Devices) at 488 nm or 570 nm. The *in vitro* cytotoxicity of the gels was determined by the MTT assay. The measurements were carried out in triplicate and equation below was used to calculate the inhibition of cell growth.

Cell viability (%) = (OD in experimental group / OD in control group) × 100%

In vivo animal experiments.

Male Wistar rats (180-200 g body weight) obtained from the Hubei Provincial Center for Disease Prevention & Control were used in the animal experiments, and all the animal experiments and procedures were approved by the Hubei Provincial Center for Disease Prevention & Control. All animals were maintained and used in accordance with the Animal Management Rules of the Ministry of Health of the People's Republic of China and the Guidelines for the Care and Use of Laboratory Animals of China.. The rats were individually raised in cages at a standard temperature for 2 days and randomly divided into three groups (stands for 2, 5 and 10 days) with four rats in each group: rats were used for control group, 3 M wound dressing group (Minnesota Mining and Manufacturing Medical Equipment (Shanghai) Co., Ltd), P-LA group , P-TLA group. After anesthesia with 10% chloral hydrate (30 mg/kg), the dorsal area of the rat was totally depilated and one full-thickness circular wound (about 12 mm in diameter) was created on the upper back of each rat. After adding 20 μL of the diluted bacterial suspension

(10^6 CFU/mL) to the wounds and absorption for about 5 min, the wounds of the three groups were treated without any measurement and with 3 M wound dressing, P-LA xerogel pasting and P-TLA xerogel pasting (xerogels were cut to circular pieces with about 15 mm in diameter), respectively, the P-LA xerogel and P-TLA xerogel were pasted for 30 min then were torn off. The wounds of the all groups were tightly wrapped by sterile medical tapes and the rats were individually raised in cages at a mile temperature. After 2, 5 and 10 days, the wounds were examined and photographed. The rats were sacrificed on day 10 and the wound site with the surrounding skin was harvested and fixed with 10% formalin. The central wound sections were fixed on glass slides and stained with Giemsa staining on day 2, and hematoxylin and eosin (H&E) staining on days 2, 5 and 10. These two staining were used to evaluate the amount of adherent bacteria around the wounds and wound-healing progress, respectively.

Statistical analysis.

All of the experiment were evaluated as mean values ± standard deviation of at least three tests. A one-way analysis of variance (ANOVA) program combined with a student t-test was used to evaluate the statistical significance of the variance. Values of *P<0.05, **P<0.01 and ***P<0.001 were considered statistically significant.

Section S1. Characterization of TLA , P-LA and P-TLA.

The changes of characteristic groups between intermediate TLA and LA was showed in Fig. S1. The characteristic peak of ≡C-H stretching vibration and C=O stretching vibration arisen at 3305 cm^{-1} and 1680 cm^{-1}/1530 cm^{-1} respectively after esterification reaction, meanwhile the C-OH stretching vibration (3070 cm^{-1}) and the C=O stretching vibration (1720 cm^{-1}) of carboxyl group was weakened as it was consumed for connection. To further determine whether the TLA was successfully synthesized, ^{1}H MNR was illustrated in Fig. S2 with information of (500 MHz, $CDCl_3$) δ 3.56(s, 1H, ethanol), 3.14(d, J=2.4 Hz, -COCH_2C, 2H), 2.57(ddt, J=13,11.1,5.7 Hz, -SCH_2CH,4H), 2.48(m, -CH_2CH_2COO, 2H), 2.39(s, -CC*H*, 1H), 1.91(dd, J=13.6, 6.9 Hz, -CH_2CH_2CHCH_2, 2H), 1.73-1.62(m, -CH_2C$H_2$$CH_2$, 2H), 1.51(dd, J=13.3,9.0,4.8 Hz, -CHC$H_2$$CH_2$, 4H), which was corresponding to the results of software simulation. X-ray photoelectron spectroscopy (XPS) was measured for P-LA and P-TLA, by means of XPS-peak-differentation-imitating analysis to S element, the result suggested that three types of existence form of S element contained in the P-TLA ,while there were two types of S element in P-LA, which was corresponding to the structure of these two polymers. (Fig. S3 and S4). Differential scanning calorimetry (DSC) showed that the Tg of P-TLA was -72.85 ^{o}C in the test temperature range (Fig. S5), which was a cold-resistant xerogel than other reported before, and the Tc of P-LA was 55.6 ^{o}C, which cound not keep the elastic state in lower temperature. In thermogravimetric analysis(TGA), P-TLA showed its decomposition temperature in 261 ^{o}C while P-LA dropped to 150 ^{o}C (Fig. S6), the reason why the decomposition temperature changed was that the mixture bond of disulfide and thioether were highly crosslinked than the single main chain of disulfide, which resulted in the increase of thermal stability of the xerogel.

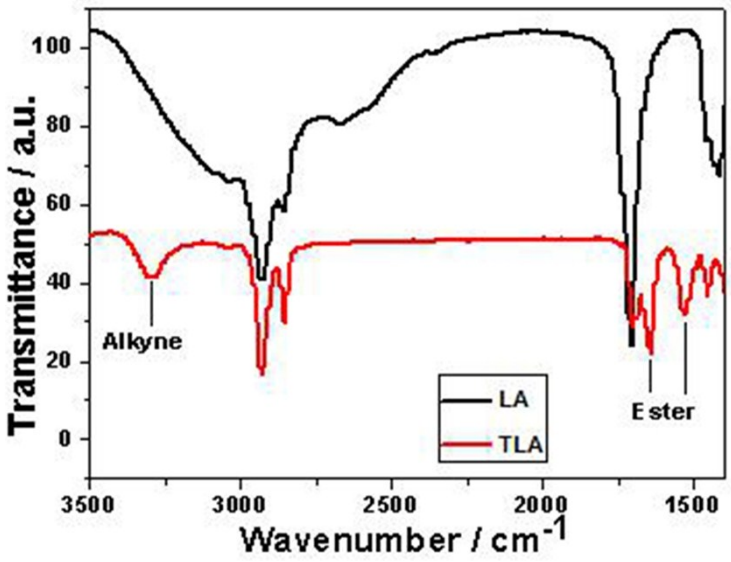

Fig. S1 The FTIR spectrum of LA and TLA.

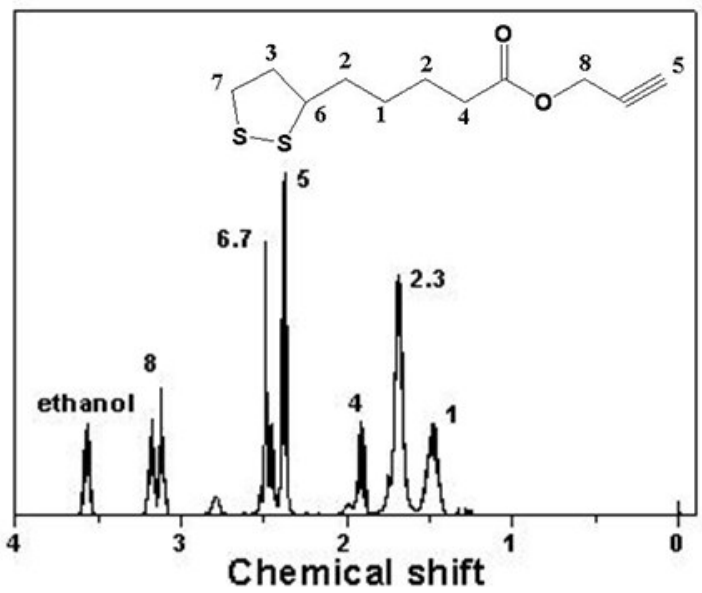

Fig. S2 The H^1NMR analysis of TLA with its simulation in software.

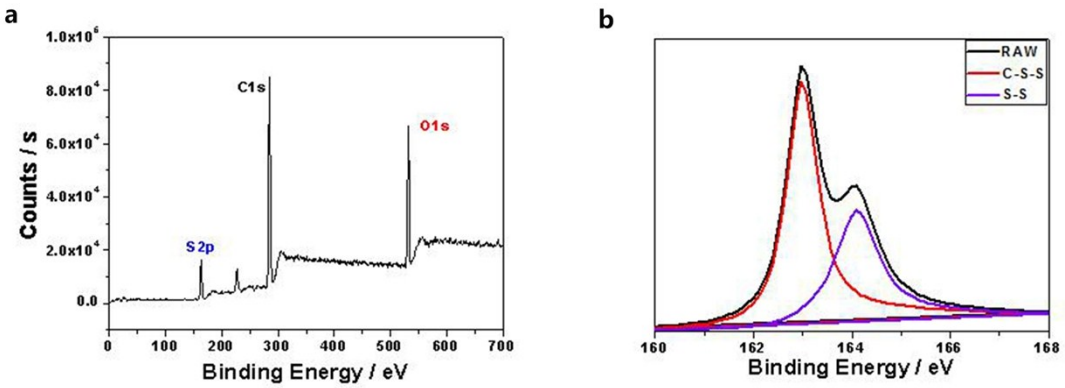

Fig. S3 XPS suvery spectra (a) and S spectra (b) of the P-LA.

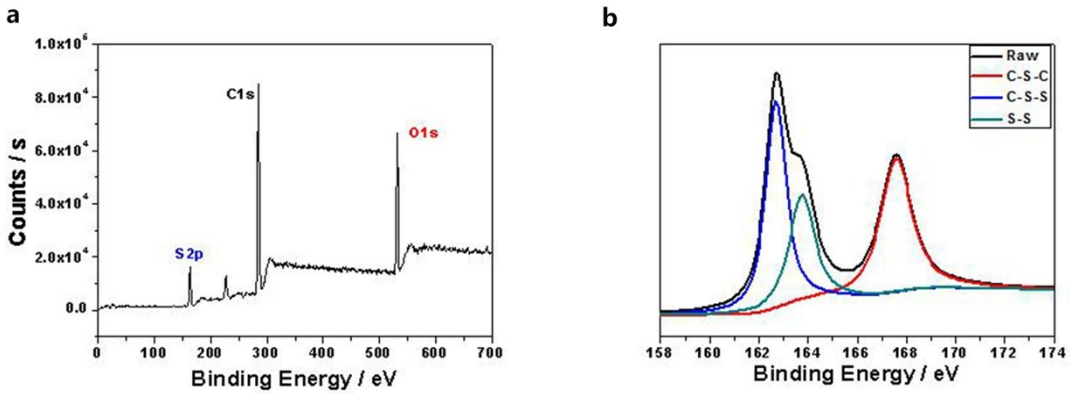

Fig. S4 XPS suvery spectra (a) and S spectra (b) of the P-TLA.

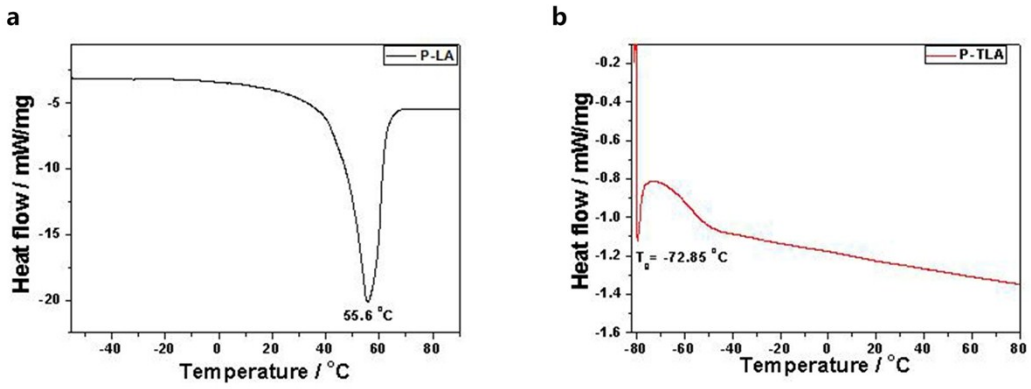

Fig. S5 The DSC spectra of P-LA (a) and P-TLA (b).

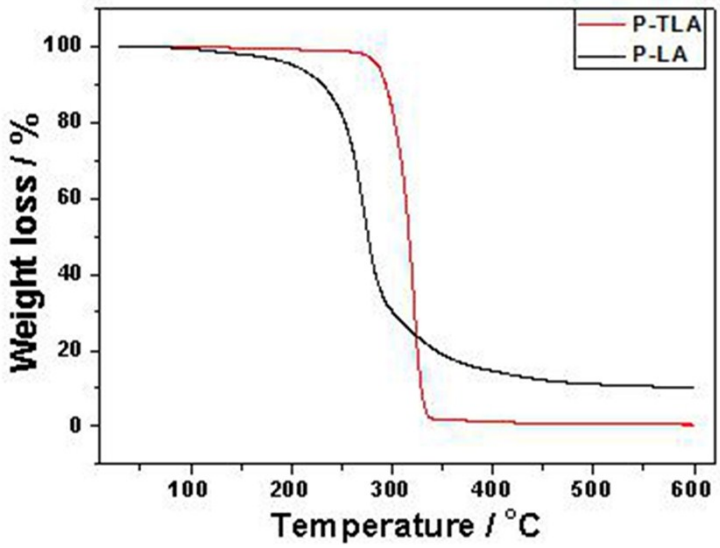

Fig. S6 The TGA spectra of P-LA and P-TLA.

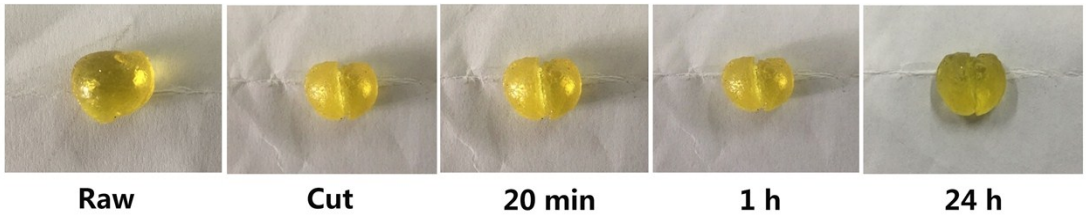

Fig. S7 The self-healing behavior of P-LA.

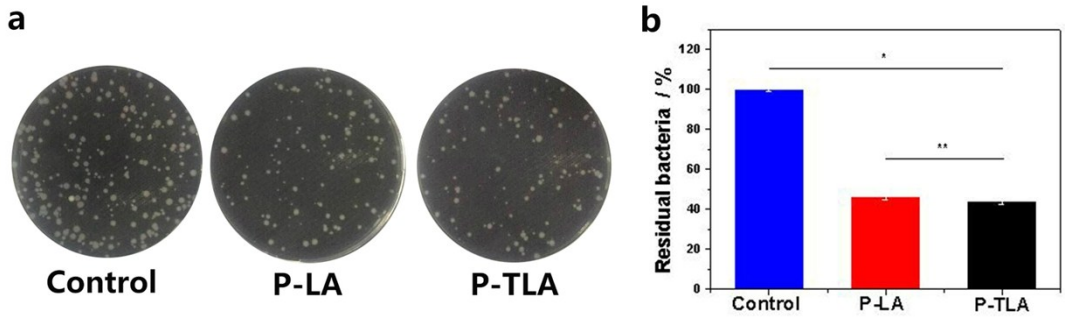

Fig S8. a) *In vitro* bacterial picking-up through spread plate of *E. coli* after being treated with P-LA and P-TLA. b) The bacterial colonies of *E. coli* calculated from spread plate.

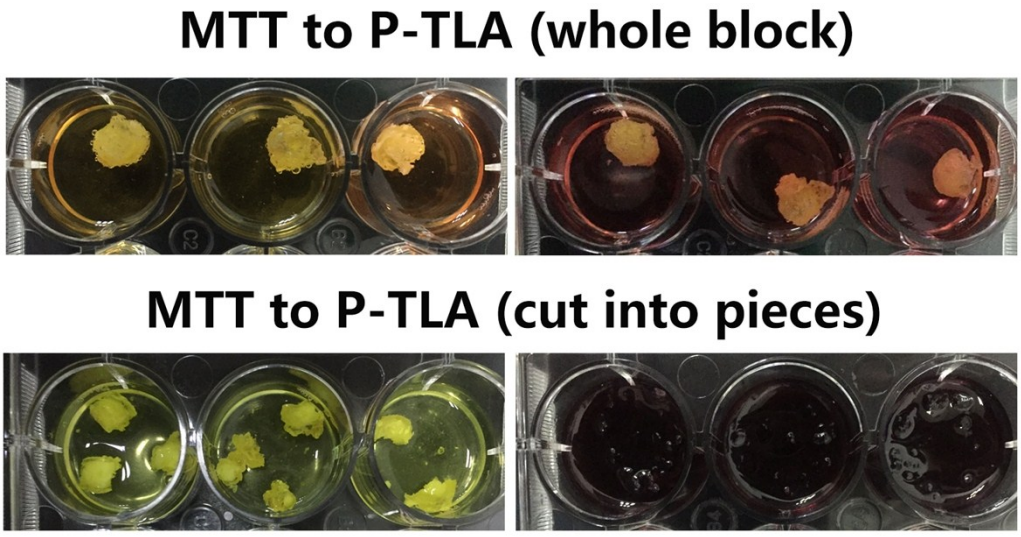

Fig. S9 The moving tendency of NIH cells being treated with P-TLA.

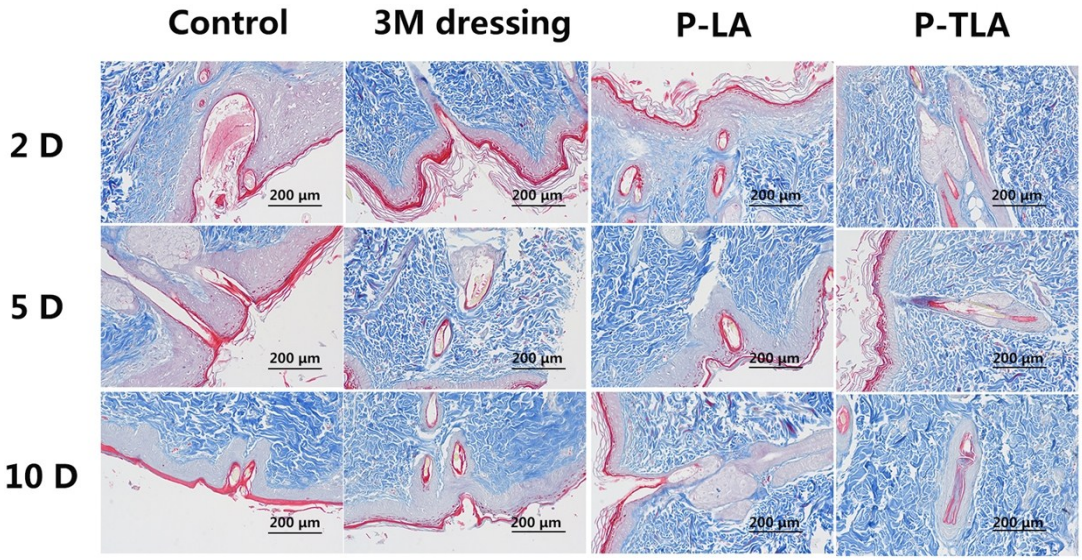

Fig. S10 Masson staining of wound sections after 2, 5 and 10 days of treatment.

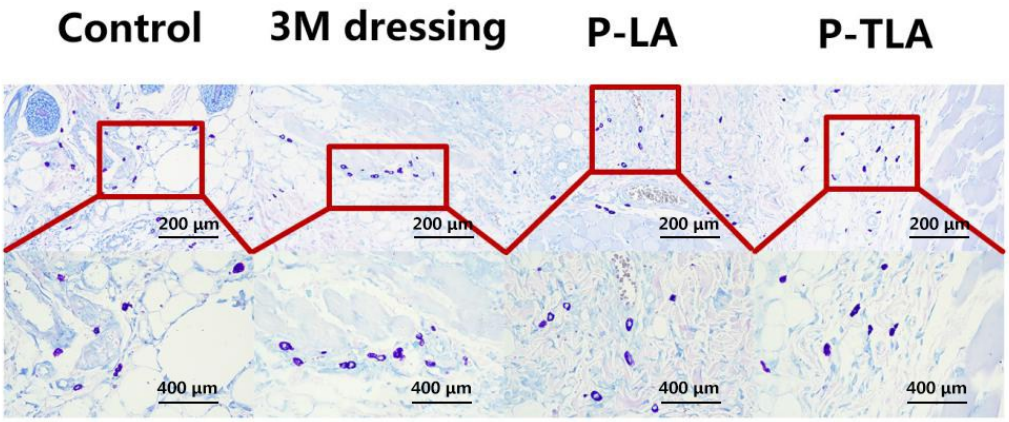

Fig. S11 Giemsa staining of wound sections after 2 days treatment.

Section S2. the plasticity of P-TLA and the adhesion to skin by P-LA and P-TLA.

The plasticity of P-TLA was improved by "imitative" click chemistry due to its highly cross-linked structure, and it could be cut into any shape by a knife. The small piece of P-TLA evenly stretched up to several times then kicked back (Movie. S1). In Movie. S2, P-TLA showed favourable adhesiveness to skin then easily torn off by hand. On the contrary, P-LA could only stick to the skin and was quite difficult to separate due to its unsteady structure caused by lowly cross-linked degree (Movie. S3), this property will limit the practical sticking to P-LA.

Movie. S1 The plasticity of P-TLA.

Movie. S2 The adhesion to skin by P-TLA.

Movie. S3 The adhesion to skin by P-LA.