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Supporting Information

Anti-infective Hydrogel Adhesive with Non-swelling and Robust Mechanical Properties for Sutureless Wound Closure

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1. Materials and methods

1.1 Synthesis and characterization of PEGDA

The PEGDA was synthesized by an esterification reaction between PEG and acryloyl chloride. Briefly, 4 g of PEG and 1.4 mL of TEA were dissolved in 20 mL dry CH_2Cl_2. After that, 1 mL of acryloyl chloride predissolved in 5 mL dry CH_2Cl_2 was dropped into the solution in an ice-water bath over 1 h. After reacting for 24 h, the mixture was centrifuged (5000 rpm, 3 min) to remove the undissolved substance. PEGDA was obtained by repeatedly dissolving in CH_2Cl_2 and precipitating in pre-cooled ethyl alcohol/diethyl ether (2/8, v/v) three times. The chemical structure of PEGDA was characterized by nuclear magnetic resonance (^{1}H NMR, Mercury Vx-300) with D_2O as solvent, and fourier transform infrared spectroscopy (FTIR, TENSOR II).

1.2 Synthesis and characterization of F127DA

The F127DA was synthesized by an esterification reaction between F127 and acryloyl chloride. Briefly, 5 g of F127 and 1 mL of TEA were dissolved in 20 mL dry CH_2Cl_2 followed by adding 1 mL of acryloyl chloride predissolved in 5 mL dry CH_2Cl_2 in an ice-water bath over 1 h. After reacting for 24 h, the mixture was filtered and the

solvent was removed by rotational evaporation. The dry crude product was dissolved in 50 mL of deionized water (DIW) and dialyzed using a dialysis membrane (*Mw* cut off 3500) for three days, as well as lyophilized to obtain pure F127DA. The chemical structure of F127DA was characterized by ^{1}H NMR with $CDCl_3$ as solvent, and FTIR. The morphology and size of F127 micelles were characterized by transmission electron microscope (TEM, HITACHI HT7700 Exalens).

1.3 Synthesis and characterization of MAlg

The MAlg was synthesized by the amidation reaction between sodium alginate (SA) and 2-aminoethyl methacrylate hydrochloride. Shortly, 0.3 g of SA was dissolved in 30 mL DIW. Then, 1.2 g EDC.HCl and 0.7 g NHS were added into the solution followed by adjusting the pH to 5.5 with 1M NaOH and 1M HCl. After activation for 2 h, 1 g of 2-aminoethyl methacrylate hydrochloride predissolved in 5 mL DIW was added into the activated solution followed by maintaining pH to 5.5 again. After reacting for 24 h, the solution was dialyzed against DIW using a dialysis membrane (*Mw* cut off 3500) for three days and lyophilized to obtain MAlg. The chemical structure of MAlg was characterized by FTIR.

1.4 Cytocompatibility test

The cytocompatibility was evaluated by both cell counting CCK-8 and live/dead assays. 3T3 fibroblast cells were cultured in DMEM (Gibco Invitrogen, USA) supplemented with 10% fetal bovine serum and 1% penicillin-streptomycin in a CO_2 incubator at 37℃. For the CCK-8 assay, the hydrogel disks were placed into each well in a 48-well culture plate followed by rising with sterilized PBS. Then, 3T3 fibroblast cells (10^5 cells/well) were seeded onto the surfaces of hydrogels and incubated at 37℃. After 1, 3 and 5 days, CCK-8 agent was added into each well and further incubated for 4 h. The optical density (OD) value of cell suspension at 450 nm was measured by a microplate reader for evaluating the proliferation of cells. For the live/dead assay, the hydrogel disks were first placed into each well in a 48-well culture plate and rinsed, and then 3T3 fibroblast cells (10^5 cells/well) were seeded onto the surfaces of hydrogels. After incubation for 1, 3, and 5 days at 37℃, Live/Dead agent was added. After 30 min, the stained cells were observed by a fluorescence microscopy (Zeiss Axio Imager Z1, Germany).

1.5 *In vitro* antibacterial activities

Hydrogel antibacterial properties were evaluated *in vitro* using contact-killing assays. Briefly, hydrogel disks were placed into each well in a 48-well microplate, and then were rinsed with sterilized PBS. Next, 10 μL of bacteria suspension with a concentration of 10^6 colony-forming units/milliliter (CFUs/mL) was dropped onto

each hydrogel surface and incubated for 2 h at 37℃. 200 μL of PBS was then added to each well to re-suspend surviving bacteria. Then, 20 μL of the re-suspended bacterial suspension was picked out and diluted by ten-fold dilution to obtain a final diluting bacterial suspension. 20 μL of the final diluting bacterial suspension was spread onto the surface of an LB agar plate. After incubation for 24 h at 37℃, CFUs on each LB agar plate were counted. As a control, 10 μL of the original bacterial suspension was spread onto a tissue culture plate (TCP) and the above experimental procedures were performed.

2. Results and discussion

2.1 Synthesis and characterization of PEGDA

The PEGDA was synthesized by an esterification reaction between the end hydroxyl groups of PEG and acrylol chloride in the presence of TEA as absorb acid agent (Fig. S1A). As illustrated in Fig. S1B and C, compared to ^{1}H NMR and FTIR spectra of PEG, the appearance of new chemical shifts in 5.8-6.6 ppm region ascribed to the C=C bonds, and the absorption peak at 1721 cm^{-1} attributed to the C=O stretching vibration of ester groups in that of PEGDA indicated the successful synthesis of PEGDA.

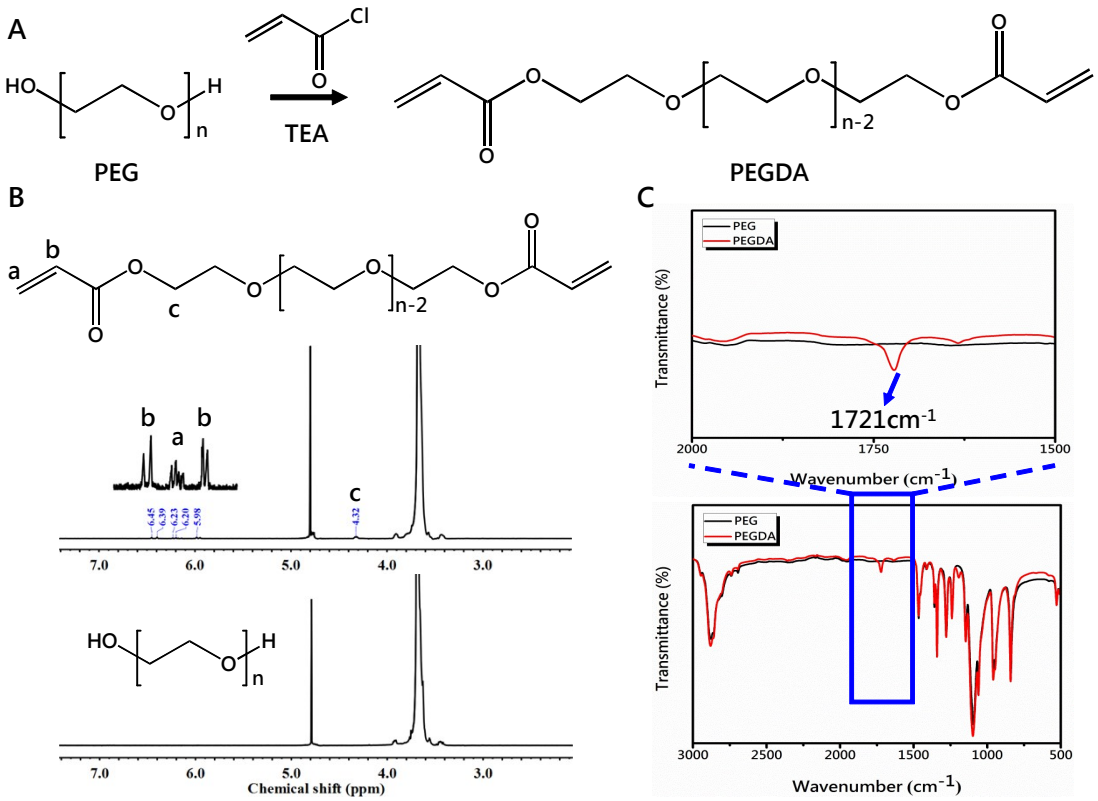

Fig. S1 Synthesis and characterization of PEGDA. (A) Synthetic route of PEGDA. (B) ^{1}H NMR spectra of PEG and PEGDA (D_2O as solvent). (C) FTIR spectra of PEG and PEGDA.

2.2 Synthesis and characterization of F127DA

The F127DA was synthesized by an esterification reaction between the end hydroxyl groups of F127 and acrylol chloride in the presence of TEA as absorb acid agent (Fig. S2A). As shown in Fig. S2B and C, the appearance of additional chemical shifts in 5.8-6.6 ppm region belonging to the C=C bonds, and the absorption peak at 1722 cm^{-1} attributed to the C=O stretching vibration of ester groups in the ^{1}H NMR and FTIR

spectra of F127DA demonstrated the successful synthesis of F127DA. As a typical amphiphilic triblock copolymer, F127DA self-assembled into nanomicelles (Fig. S2D).

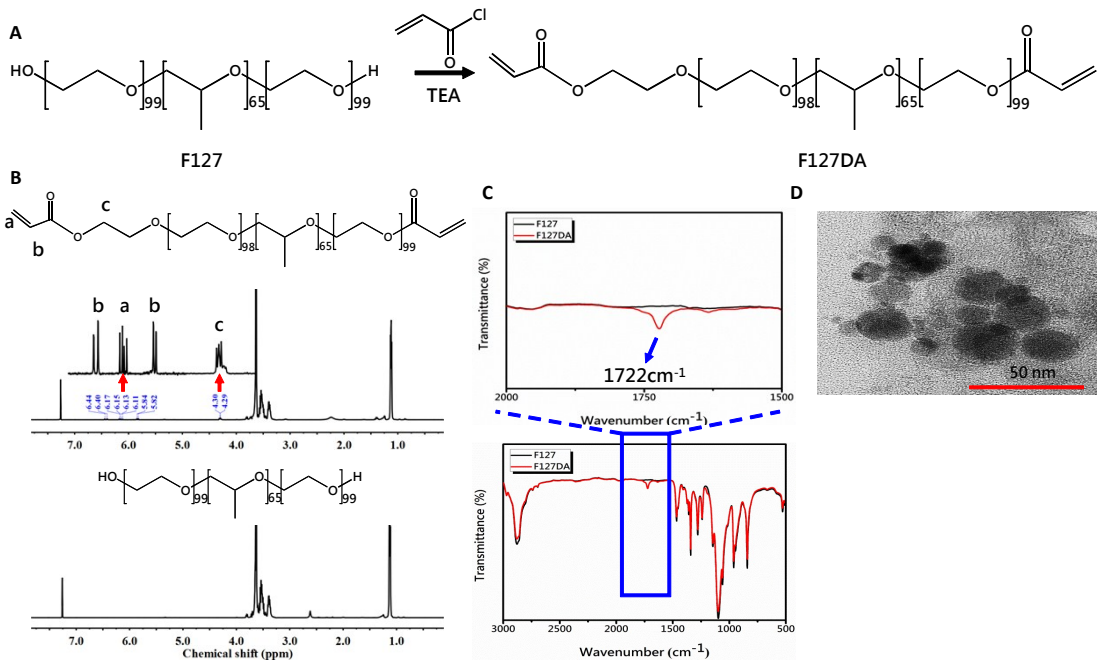

Fig. S2 Synthesis and characterization of F127DA. (A) Synthetic route of F127DA. (B) ^{1}H NMR spectra of F127 and F127DA ($CDCl_3$ as solvent). (C) FTIR spectra of F127 and F127DA. (D) TEM image of micellar F127DA.

2.3 Synthesis and characterization of MAlg

The MAlg was synthesized by the amidation of the carboxylate groups of SA with the amino group of 2-aminoethyl methacrylate hydrochloride (Fig. S3A). As displayed in Fig. S3B, the new absorption peak at 1721 cm^{-1} ascribed to the C=O stretching vibration of ester group of 2-aminoethyl methacrylate hydrochloride could be

observed in FTIR spectrum of MAlg, rather than SA, suggesting the successful synthesis of MAlg.

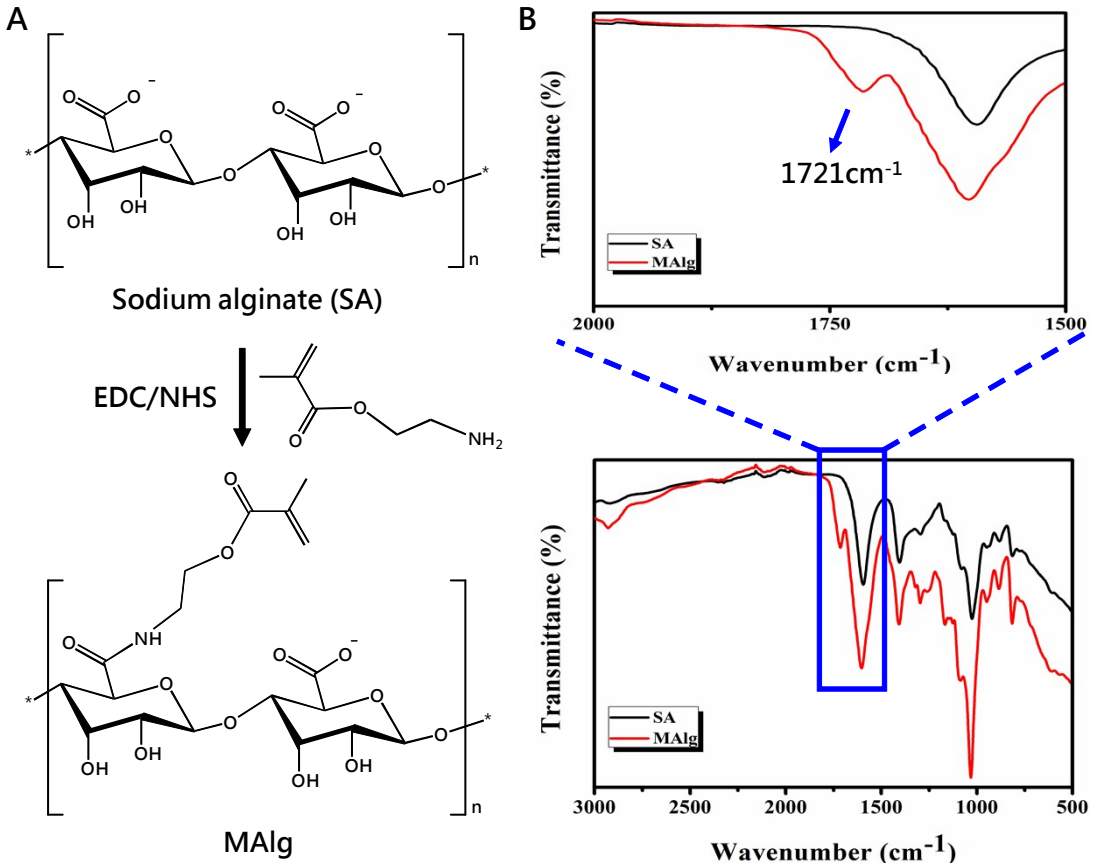

Fig. S3 Synthesis and characterization of MAlg. (A) Synthetic route of MAlg. (B) FTIR spectra of SA and MAlg.

2.4 Molecular structure optimization

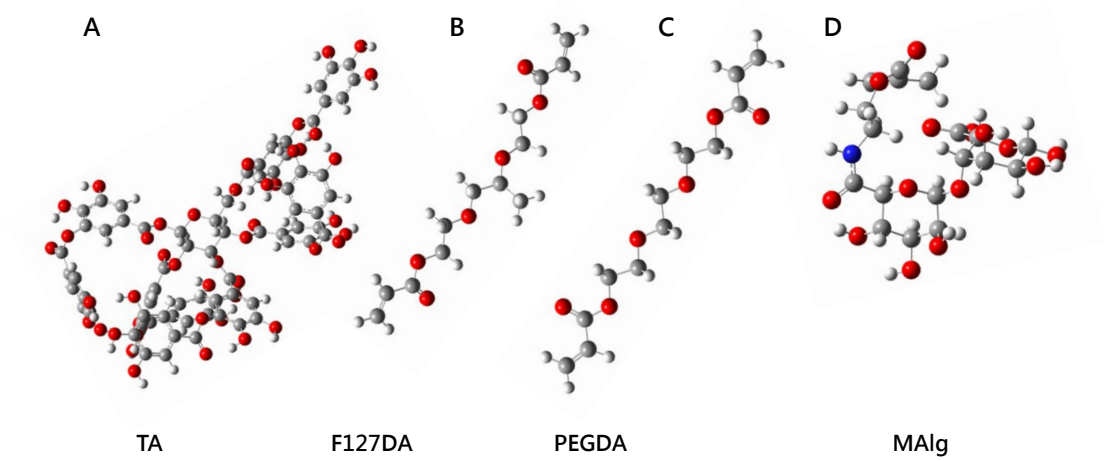

Fig. S4 Molecular structure optimization. Optimized structures of the (A) TA, (B) F127DA, (C) PEGDA, (D) MAlg. Red, gray, white, and blue spheres represent oxygen, carbon, hydrogen, and nitrogen atom, respectively.

2.5 Water contact angle (WCA) test

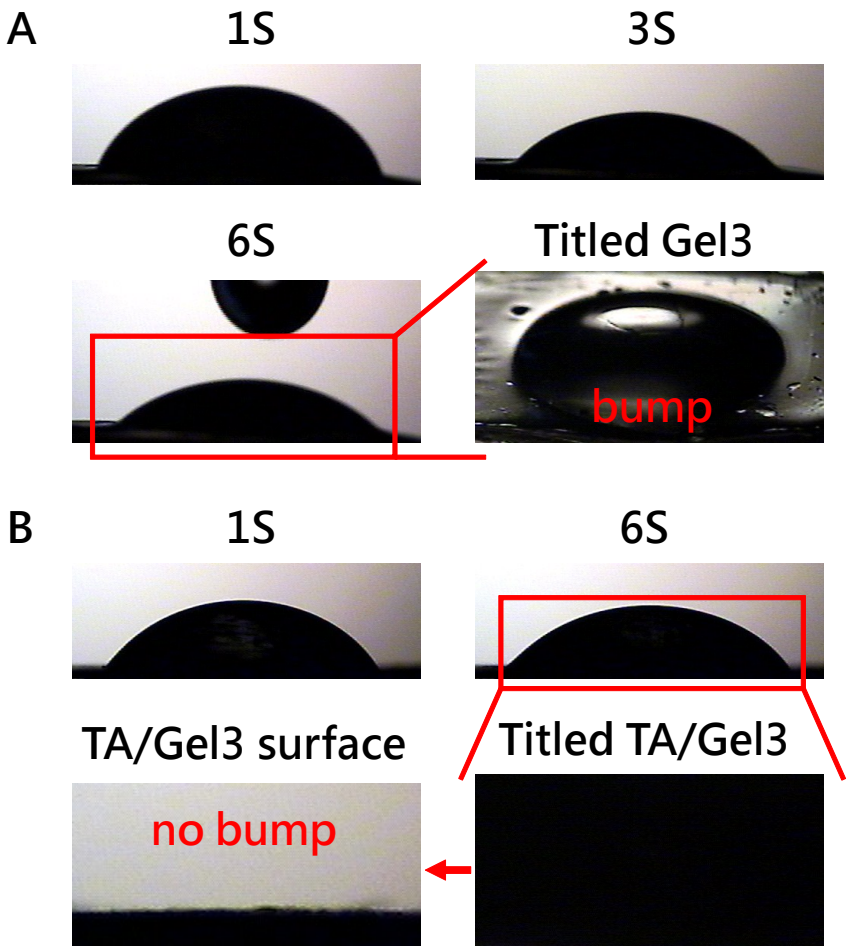

Fig. S5 Swelling behaviors of hydrogels. Images of (A) representative Gel3 and (B) TA/Gel3 during WCA tests.

2.6 Mechanical properties of hydrogels

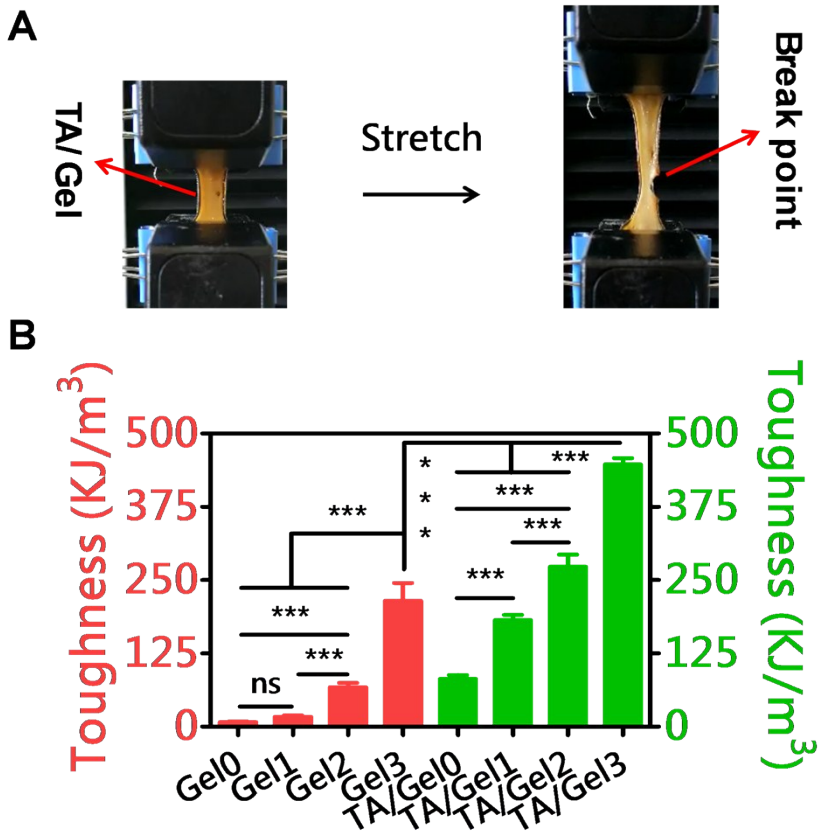

Fig. S6 Mechanical properties of hydrogels. (A) Representative tension images of the TA/Gels. (B) The toughness levels of the Gels and TA/Gels. *n*=3, Data are means ± SD *p<0.05, **p<0.01, ***p<0.001.

2.7 Adhesion properties of hydrogels

Fig. S7 Adhesion properties of hydrogels. Adhesion images of kidney, lung, heart,

spleen, and muscle.