Supporting information

Polymer Post Modification via the Biginelli Reaction to Prepare Water-soluble Polymer Adhesives

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Experiment Section

1. Materials

2-(Acetoacetoxy)ethyl methacrylate (AEMA, Shanghai Aladdin Bio-Chem Technology Co.,Ltd., 95%), poly(ethylene glycol) methacrylate (PEGMA-950, $M_n \sim$ 950 g·mol⁻¹, Sigma Aldrich), benzyl methacrylate (BMA, J&K Scientific, 98%), 4hydroxybenzaldehyde (Sigma Aldrich, 95%), 4-formylphenyl boronic acid (Accela Inc., 98%), benzaldehyde (J&K Scientific, 98%) were used as purchased. 2,2'-Azobis(2-methylpropionitrile) (AIBN, J&K Chemical, 99%) was recrystallized from acetone prior to use. 4-Cyano-4-(ethylthiocarbonothioylthio) pentanoic acid was synthesized as literatures^{1, 2} and used as the chain transfer agent (CTA). Solvents were purchased from Sinopharm Chemical Reagent and used directly without further purification.

2. Instruments and analyses

Gel permeation chromatography (GPC) analyses of polymers were performed using N, N-dimethyl formamide (DMF) as the eluent. The GPC system was a Shimadzu LC-20AD pump 45 system consisting of an auto injector, a MZ-Gel SDplus 10.0 μ m guard column (50 × 8.0 mm, 10² Å) followed by a MZ-Gel SDplus 5.0 μ m bead-size column (50 – 10⁶ Å, linear) and a Shimadzu RID-10A refractive index detector. The system was calibrated with narrow molecular weight distribution polystyrene standards ranging from 200 to 10⁶ g mol⁻¹. ¹H NMR spectra were obtained using a JEOL JNM-ECA400 spectrometer for all samples. The tensile shear strength was measured using a MTS SYSTEMS (CHINA) Co. Ltd. SANS CMT6503 electromechanically universal testing machine. Single-Molecule Force Spectroscopy (SMFS) measurements were carried out utilizing a commercially available molecular force probe 3D (Asylum Research, Santa Barbara, CA).

The *in vivo* tests were performed under the technical guidelines for non-clinical study issued by CFDA, and authorized by the ethics committee of Cancer Hospital, Chinese Academy of Medical Science.

3. Methods

3.1 Synthesis of poly(acetoacetoxyethyl methacrylate-co-polyethylene glycol maleate) (poly(AEMA-co-PEGMA))



AEMA (5.14 g, 24 mmol), PEGMA-950 (11.4 g, 12 mmol), CTA (47 mg, 0.18 mmol) and AIBN (6 mg, 0.036 mmol) were charged into a Schlenk tube with 25 mL

of toluene. The tube went through three cycles of freeze–pump–thaw to remove oxygen, then was sealed and kept in a 60°C oil bath. After 24 h, the polymerization was quenched in an ice-water bath. The mixture was precipitated in diethyl ether for 3 times, and then dried under vacuum to obtain the pure polymer for further characterizations and use.

¹H NMR (400 MHz, d₆-DMSO, δ/ppm): 4.27 (s, C<u>H</u>₂OCOCH₂COCH₃), 4.11 (s, C(CH₃)COOC<u>H</u>₂), 3.62 (s, COC<u>H</u>₂COCH₃), 3.23 (s, C<u>H</u>₃O), 2.21 (s, C<u>H</u>₃CO), 2.00-0.60 (m, C<u>H</u>₂C<u>H</u>(CH₃)CO).

The polymerization conversion (93.3%) was calculated by comparing the integral ratio of the peaks of the vinyl protons and the terminal methyl protons of the PEG side chain. The molecular weight (DP ~ 66, M_{nUV} ~31000) was obtained by measuring the UV absorbance of the trithiocarbonate group in the polymers compared to the native CTA ($\varepsilon_{EtOAc} = 6082 \text{ L cm}^{-1} \text{ mol}^{-1}$). GPC was also used to test the PDI and molecular weight of the final polymer ($M_{nGPC} \sim 52700$, PDI: 1.09, Fig. S1).

3.2 PPM of poly(AEMA-co-PEGMA)



The poly(AEMA-co-PEGMA) was modified through the Biginelli reaction. Typically, poly(AEMA-co-PEGMA) (1.55 g), urea (0.26 g, 4.4 mmol), benzaldehyde (0.35 g, 3.3 mmol) and magnesium chloride (21 mg, 0.22 mmol) were dissolved in 5 mL of acetic acid, then the mixture was stirred in a 100°C oil bath for 4 h. The mixture was then dialyzed against methanol (MWCO: 3500) for 2 days to obtain the pure polymer (PPM-1) after solvent evaporation (1.03 g, yield: 73.0%).

Through the same process, other polymers with different functional side chains

were achieved respectively by varying the aldehyde substrates, and named as PPM-2 (4-hydroxybenzaldehyde as the reactant) and PPM-3 (4-formylphenyl boronic acid as the reactant).

SCMP-1:

¹H NMR (400 MHz, d₆-DMSO, δ /ppm): 9.27 (s, N<u>H</u>CONHCH), 7.73(s, NHCON<u>H</u>CH), 7.35-7.15 (m, Ar<u>H</u>), 5.16 (s, ArC<u>H</u>), 4.50-3.70 (m, COOC<u>H</u>₂C<u>H</u>₂O), 3.23 (s, C<u>H</u>₃O), 2.25 (s, C<u>H</u>₃C), 1.30-0.60 (m, C<u>H</u>₂C (CH₃)CO).

SCMP-2:

¹H NMR (400 MHz, d₆-DMSO, δ/ppm): 9.31 (s, ArO<u>H</u>), 9.18 (s, N<u>H</u>CONHCH), 7.60 (s, NHCON<u>H</u>CH), 7.03 (s, Ar<u>H</u>), 6.67(s, ArH), 5.05 (s, ArC<u>H</u>), 4.50-3.70 (m, COOC<u>H</u>₂C<u>H</u>₂O), 3.23 (s, C<u>H</u>₃O), 2.23 (s, C<u>H</u>₃C), 1.30-0.60 (m, C<u>H</u>₂C (CH₃)CO).

SCMP-3:

¹H NMR (400 MHz, d₆-DMSO, δ/ppm): 9.23 (s, N<u>H</u>CONHCH), 7.94 (s, <u>H</u>OBOH), 7.71 (s, NHCON<u>H</u>CH and HOBO<u>H</u>), 7.25 and 7.21(s, ArH), 5.15 (s, ArC<u>H</u>), 4.50-3.70 (m, COOC<u>H₂CH₂O), 3.23 (s, CH₃O), 2.23 (s, CH₃C), 1.30-0.60 (m, CH₂C (CH₃)CO).</u>

The structure of post modified polymer was determined using ¹H NMR by comparing the peaks between the terminal methyl group at PEG moiety (3.23 ppm, CH₃O) and the specific methine group of Biginelli product (5.16 ppm, ArCH), and the result suggested 98-100% of the dione groups in the parent polymer were converted. The PDI and molecular weight of the final polymer were calculated through GPC analyses as PPM-1: $M_{nGPC} \sim 50600$, PDI = 1.19 (Fig. S2); PPM-2: $M_{nGPC} \sim 68900$, PDI = 1.17 (Fig. S2); PPM-3: $M_{nGPC} \sim 77700$, PDI = 1.13 (Fig. S2).

3.3 Synthesis of poly(benzyl methacrylate-co-polyethylene glycol maleate) poly(BMA-PEGMA)



Benzyl methacrylate (BMA, 4.23 g, 24 mmol), PEGMA-950 (11.4 g, 12 mmol), CTA (47 mg, 0.18 mmol) and AIBN (6 mg, 0.036 mmol) were charged into a Schlenk tube with 25 mL of toluene. The tube went through three cycles of freeze–pump–thaw to remove oxygen, then was sealed and kept in a 60°C oil bath. After 24 h, the polymerization was quenched in an ice-water bath, and the mixture was precipitated in diethyl ether for 3 times, then dried under vacuum to obtain the pure polymer for further characterizations and use.

¹H NMR (400 MHz, d₆-DMSO, δ/ppm): 7.31 (s, Ar<u>H</u>), 4.90 (s, ArC<u>H</u>₂), 3.23 (s, C<u>H</u>₃O), 2.00-0.60 (m, C<u>H</u>₂C<u>H</u>(CH₃)CO).

The polymerization conversion (99.2%) was calculated through ¹H NMR by comparing the peaks of the vinyl protons and the terminal methyl protons of the PEG side chain. The PDI and molecular weight of the final polymer were calculated through GPC analyses as $M_{nGPC} \sim 51000$, PDI = 1.17 (Fig. S2).

3.4 Tensile shear measurements

3.4.1 Preparation of specimens.

Metal specimens (brass, aluminum) and poly ethylene (PE) specimens were cut into uniform slides ($100 \times 10 \times 0.1$ mm). Bovine bone specimen was prepared in different size ($25 \times 20 \times 0.4$ mm) due to its low physical strength. Metal specimens were polished with sandpaper (P800) and cleaned with acetone. PE specimens were sonicated in deionized water for 5 min and dried in air. Bovine bone specimen was polished with sandpaper (P2400) and cleaned with acetone prior to measurement.

3.4.2 Preparation of test joints.

The aqueous solution of modified polymer adhesive was prepared in deionized water (100 mg mL⁻¹), then 15 μ L of solution was spread on two pieces of specimens

separately. The coated specimens were overlapped and clamped together, then kept in a 70°C oven or 24 h (37°C for bovine bone specimens). The same conditions were applied to prepare test joints for commercial glues.

3.4.3 Tensile shear measurement procedure.

The tensile shear strength was measured according to the ASTM D1002 standard method with slight modifications. The overlap length was set as 10 mm, and the free crosshead speed was set to 2.0 mm min⁻¹. Ten samples were tested in parallel for each polymer adhesive, and the ranges of data are shown in the graphs as error bars.

3.5 The SMFS experiment

The details of SMFS experiment have been described elsewhere³. In brief, when an AFM tip (spring constant: 0.010-0.040 N/m) is brought into contact with a gold substrate, the polymer absorbed onto the gold substrate ahead of the time have chance to absorb stably onto the AFM tip. When the tip is separated from the substrate, the polymer chain will be desorbed gradually from the substrate, resulting in the bending of AFM tip. The bending extent of the AFM tip were recorded and converted to force curves, which can offer information about the interaction between the polymer chain and the substrate. All the force curves were obtained on gold surface in the ethanol environment. The typical signal events for all the four kinds of polymer were single or multiple peaks (Fig. S3). The heights of the peaks and their distribution were analyzed to obtain the data in the main text.

3.6 Biological safety evaluation

All *in vivo* tests were performed under the technical guidelines for non-clinical study issued by CFDA and authorized by the ethics committee of Cancer Hospital, Chinese Academy of Medical Science. *In vivo* tests were carried out to verify the biological safety of the water-soluble adhesive (PPM-3). Typically, 200 µL of PPM-3 aqueous solution (100 mg mL⁻¹) was injected into the left femurs of female nude mice (Bal/c-Nu) and followed with a 72h-observation. For a parallel control, same volume of commercial glue was induced by the same method. After 72 hours, all mice were sacrificed and skinned for further observation.

Supporting Data

Polymer	Feeding ratio	Exact ratio ^b	Substituent group	conversion ^c	$\mathbf{M}_{\mathbf{n}\ \mathrm{designed}}$	M _{n GPC} ^d	PDIe
Poly(AEMA-	2:1	2.04:1	-	93.3%	45500	52700	1.09
co-PEGMA)							
Poly(BMA-co-	2:1	1.98:1	-	99.2%	41200	51000	1.17
PEGMA)							
PPM-1	-	-	Phenyl	96.7%	52500	50600	1.19
PPM-2	-	-	Hydroxyl	96.1%	53600	68900	1.17
PPM-3	-	-	Boronic acid	98.0%	55500	77700	1.13

Table S1. Summary properties of synthetic polymers^a

a. All polymers were synthesized following the protocol in section 3.1-3.3.

b. Calculated by the ¹H NMR.

c. Calculated by the ¹H NMR.

d. Measured by GPC.

e. Measured by GPC.



Figure S1. GPC trace of poly(AEMA-co-PEGMA) and PPMs



Figure S2. GPC trace of poly(BMA-co-PEGMA)



Figure S3. Typical force curves of four kinds of polymer (curves have been shifted vertically for clarity).

References

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- 3. T. Hugel and M. Seitz, *Macromol. Rapid Commun.*, 2001, 22, 989-1016.