Supplementary Materials for

Modular penetration and controlled release (MP-CR): improving the internal modification of natural hierarchical materials with smart nanoparticles

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Materials and Methods

1. Synthesis of the template terpolymer PPAAG

Terpolymer poly(PEGMA-co-AA-co-AM) (PPAA) was obtained by copolymerizing three monomers: poly (ethylene glycol) methyl ether acrylate (PEGMA, $M_n \sim 480$, Aladdin), acrylic acid (AA, General-Reagent), and acrylamide (AM, General-Reagent). Briefly, PEGMA (0.02 mol), AA (0.02 mol), AM (0.02 mol), and azobisisobutyronitrile (AIBN, General-Reagent, 250 mg) dissolved in methanol were added into a 250 mL flask and the solution was purged with nitrogen for 30 min. The flask was then placed in a preheated oil bath. After reacting at 70 °C for 24 h, the PPAA terpolymer was purified by precipitating the concentrated mixture into ether twice to remove unreacted monomers.

In the second step, PPAA was post-functionalized with glyoxal to obtain PPAAG. In detail, PPAA (12 g) and glyoxal (4.35 g, 40 wt. % solution in water, Adamas Reagent) were mixed in 40 mL of water in a beaker, and NaOH solution (1 M, General-Reagent) was used to adjust the pH to 8.0. After reacting for 4 h, the mixed solution was dialyzed against deionized water for 48 h, and the flocculant product was obtained after freeze-drying.

2. Fabrication of Al-loaded nanoparticles (Al-NPs)

Using PPAAG as a template, Al-NPs were fabricated by an in-situ mineralization technique according to the literature.[1] First, 0.3 g of PPAAG and 0.55 g of aluminum chloride hexahydrate (AlCl$_3$·6H$_2$O, 99%, General-Reagent) were dissolved in 50 mL and 25 mL of deionized water, respectively. Then, the AlCl$_3$ solution was added dropwise into the PPAAG solution. After stirring for 4 h, 25 mL of NaOH (0.28 g, 7.00 mmol) aqueous solution was added dropwise to the mixture in 5 min. The molar ratio of [Al^{3+}]/[OH$^-$]/[AA] was set at 1:3:1. Lastly, the mixture was stirred at room temperature for 24 h with a speed at 600 rpm. For comparison, nanoparticles without aldehyde groups were also prepared [named as Al-NPs(PPAA)] by using PPAA as a template according to the method mentioned above.

3. Determination of the aluminum loading efficiency

After the loading experiment, unencapsulated Al^{3+} in the solution was removed via dialysis against deionized water. The dialysate was reacted with a solution mixture of nitric acid (2 mL) and hydrogen peroxide solution (8 mL, 20%). The content of aluminum ions in the nitration solution was analyzed based on the aluminum calibration curve by inductively coupled plasma atomic emission spectroscopy (ICP-AES, model JY38S, France). The aluminum loading efficiency was calculated by the following formula (1):

$$\text{Aluminum loading efficiency} = \frac{A}{A_0} \times 100\% \qquad (1)$$

where A denotes the content of aluminum ions in the nitration solution, and A_0 is the total aluminum in the solution of Al-NPs.

4. Determination of Al content in treated leather samples

The Al quantity in leather samples processed by $Al_2(SO_4)_3$, Al&PPAAG, Al-NPs(PPAA), and Al-NPs was determined. Different leather samples were treated in a mixture solution of nitric acid (2 mL) and hydrogen peroxide solution (8 mL, 20%). The content of aluminum ions in the supernatant was analyzed based on the aluminum calibration curve by inductively coupled plasma atomic emission spectroscopy (ICP-AES, model JY38S, France). The aluminum content in leather was calculated by the following formula (2):

$$\text{Aluminum content} = \frac{M}{M_0} \times 100\% \qquad (2)$$

where M denotes the weight of aluminum (calculated by Al_2O_3) in the nitration solution, and M_0 is the weight of leather sample.

5. Characterization

Fourier transform infrared spectroscopy (FT-IR, Nicolet 6700, Thermo Scientific, America), proton nuclear magnetic resonance (^1H NMR) spectroscopy (Bruker AV400, D_2O) were used to characterize terpolymers PPAA and PPAAG. The molecular weight and PDI of PPAAG determined by size exclusion chromatography (SEC, waters 410, Waters Corporation) were 13600 g mol^{-1} and 3.76. Transmission electron microscopy (TEM, Tecnai G2 F20 S-TWIN, USA) with the accelerating voltage of 300 kV was used to observe the morphology of Al-NPs, and dynamic light scattering (DLS, Nano ZS system, MalverZEN3600n, England) was used to measure the size. The chemical composition of Al-NPs was determined by energy dispersive X-ray spectrometry (EDX, ProX, Netherlands). XRD measurements were performed with an X-ray diffractometer (XRD, EMPYREAN, Netherlands) with Cu Kα ($\lambda = 1.542$ Å) radiation. Data were collected from 10° to 90° with a step size of 0.05 and a step time of 5 s.

6. Colloidal stability and pH-responsiveness of Al-NPs

The colloidal stability of Al-NPs in sodium chloride (1 M, NaCl, General-Reagent) or bovine serum albumin (BSA, 5 mg·mL^{-1}, Aladdin) solution was studied by monitoring the particle size of Al-NPs. Data were collected from 0 to 24 h at pH 7.0. To investigate the pH-responsiveness, the size change and Al^{3+} release of Al-NPs under different pH values in 24 h were also monitored.

7. pH-controlled release of Al^{3+} from Al-NPs in solution

The amount of aluminum ions (Al^{3+}) released from Al-NPs was tracked at gradually increased pH values from 3.0 to 5.0 and 7.0. Briefly, 5 mL of Al-NPs solution was placed in a dialysis bag (MWCO = 3500), which was then placed in 30 mL of ultrapure water with the same pH. At intervals of set time points, 5 mL of samples was taken out and replaced with 5 mL of ultrapure water. The concentration of aluminum ions in samples extracted at different time points was determined by the above-mentioned ICP-AES method.

8. Internal crosslinking of animal skin with Al-NPs

Four pieces of pickled sheep skin (Figure S3) were treated with Al-NPs, Al-NPs(PPAA), Al&PPAAG, and 2% $Al_2(SO_4)_3$, respectively. The aluminum (III) content in Al-NPs was the same with that in 2% $Al_2(SO_4)_3$. The details of the procedures are summarized in Figure 4A and Figure S9.

9. Characterization of the leather samples treated by different tanning agents

9.1 The distribution of Al-NPs in leather

The treated leather was freeze-dried and then sliced from the middle. The cross-sectional distribution of Al-NPs in the leather was observed by the field-emission scanning electron microscope (SEM, JSM-7500F, Japan). EDX spectra were collected to analyze the elemental distribution in the entire cross-section of the leather matrix.

9.2 Determination of crosslinking rate of aluminum ions

After treatment, the wastewater was collected and nitrated. The concentration of aluminum ions in 5 mL of nitrated waste was measured by ICP-AES. The crosslinking rate of aluminum ions can be calculated by formula (3):

$$\text{Crosslinking rate of Al}^{3+} = \frac{C_0 - C}{C_0} \times 100\% \qquad (3)$$

where C_0 represents the total aluminum content be fed in the tanning process and C represents the aluminum content in tanning wastewater.

9.3 Determination of shrinkage temperature (T_s) and thickening rate

The leather samples after crosslinking were cut into a standard shape by a die forming knife, and then the T_s of each sample was measured with a shrinkage tester (HY-852; Heng Yu

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Instrument, China) following ISO 3380: 2002 standards. The thickening rate of leather samples was measured with a leather thickness tester (MH-YDI, Sunshine Electronic Research Institute, China). The thickness before crosslinking was marked as d_1 (mm), and the thickness at the same position after crosslinking was marked as d_2 (mm), the thickening rate can be calculated by the following formula (4):

$$\text{Thickening rate} = \frac{d_2 - d_1}{d_1} \times 100\% \qquad (4)$$

9.4 Physical and mechanical properties

The leather samples were cut into rectangular strips and placed under standard atmospheric conditions for 48 h. Afterwards, mechanical properties including the tear strength, tensile strength, and elongation at break were analyzed by a universal testing machine (AI-7000 SN, Gotech Testing Machines Limited, China) following the ISO 3376:1976 standard.
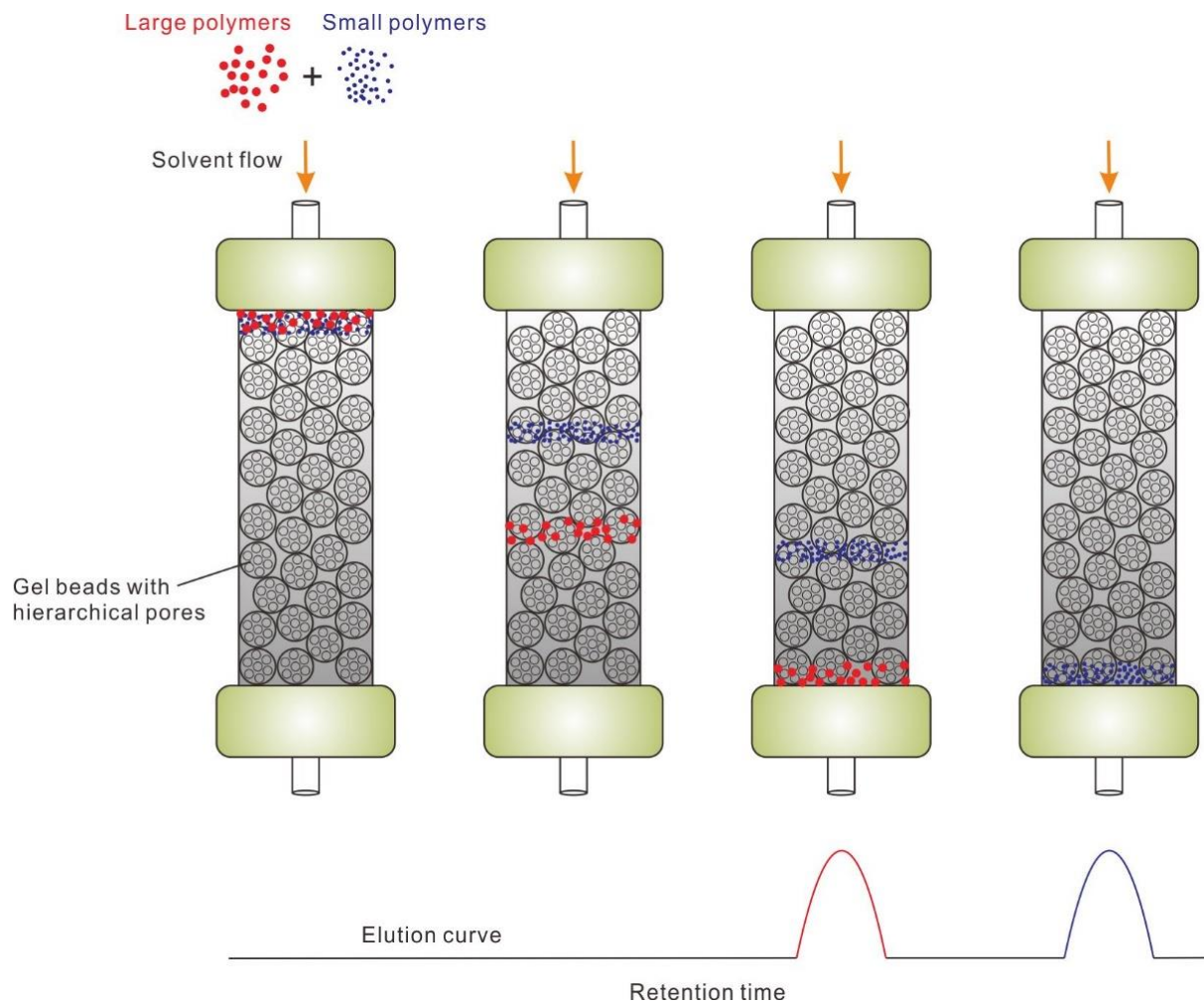
Figure S1 The working principle of size exclusion chromatography (SEC).
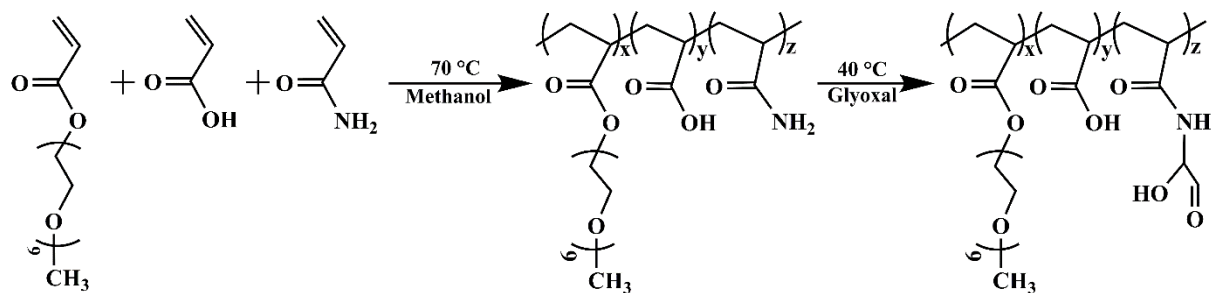
Figure S2. Synthesis of terpolymer poly(PEGMA-co-AA-co-AM) (PPAA) and its post-modification with glyoxal to obtain terpolymer PPAAG.
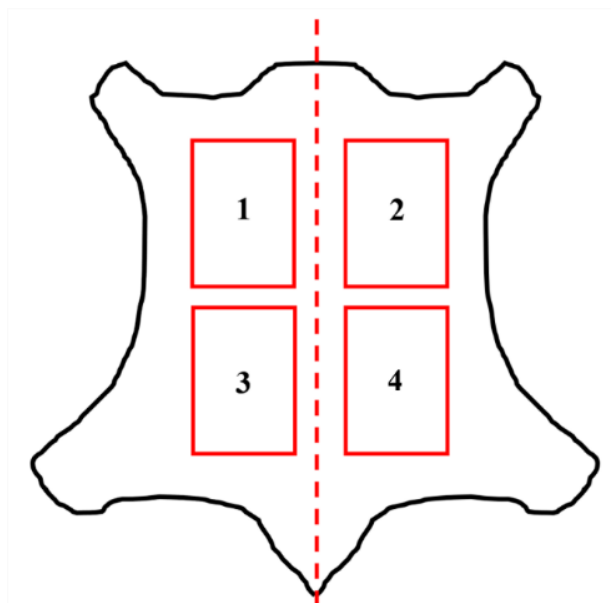
Figure S3. Leather sampling diagram for different tanning agents: (1) Al-NPs, (2) Al&PPAAG, (3) 2% $Al_2(SO_4)_3$, and (4) Al-PPAA NPs.

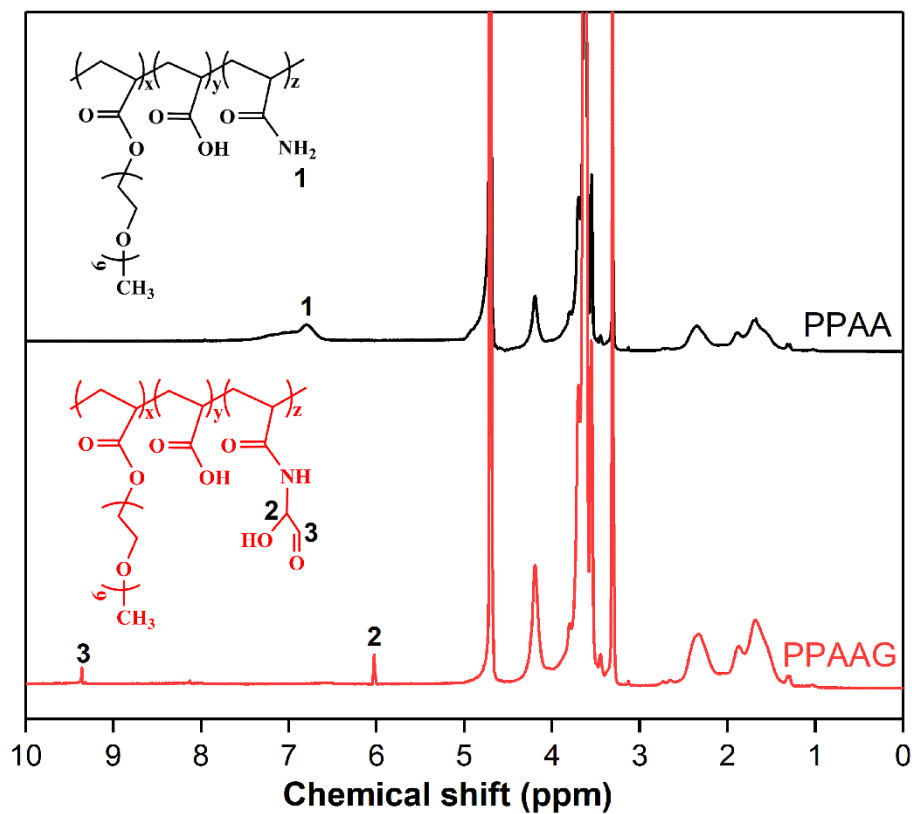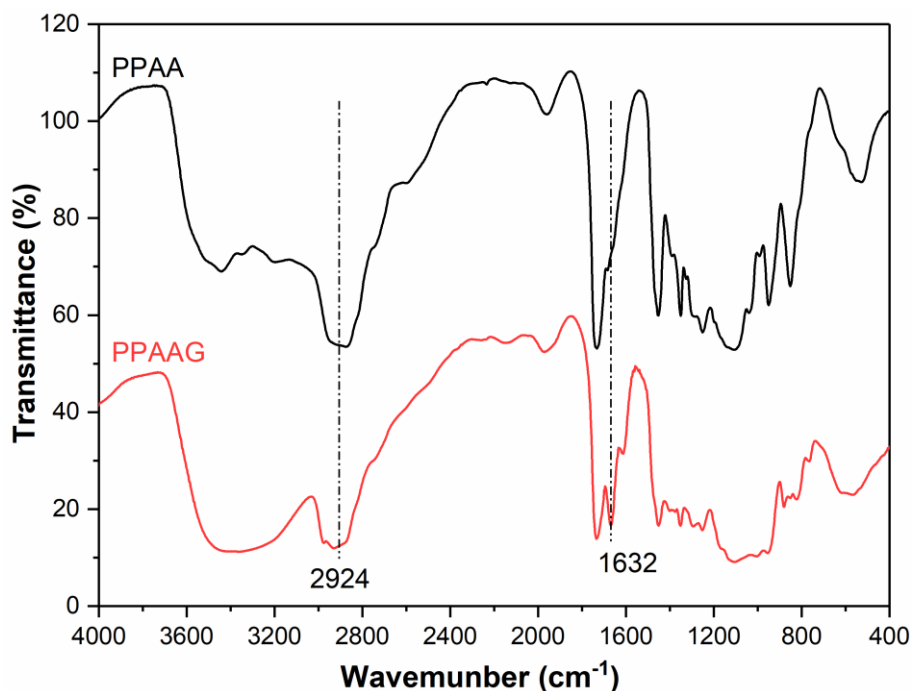**Figure S4.** ^{1}H NMR spectra of PPAA and PPAAG.
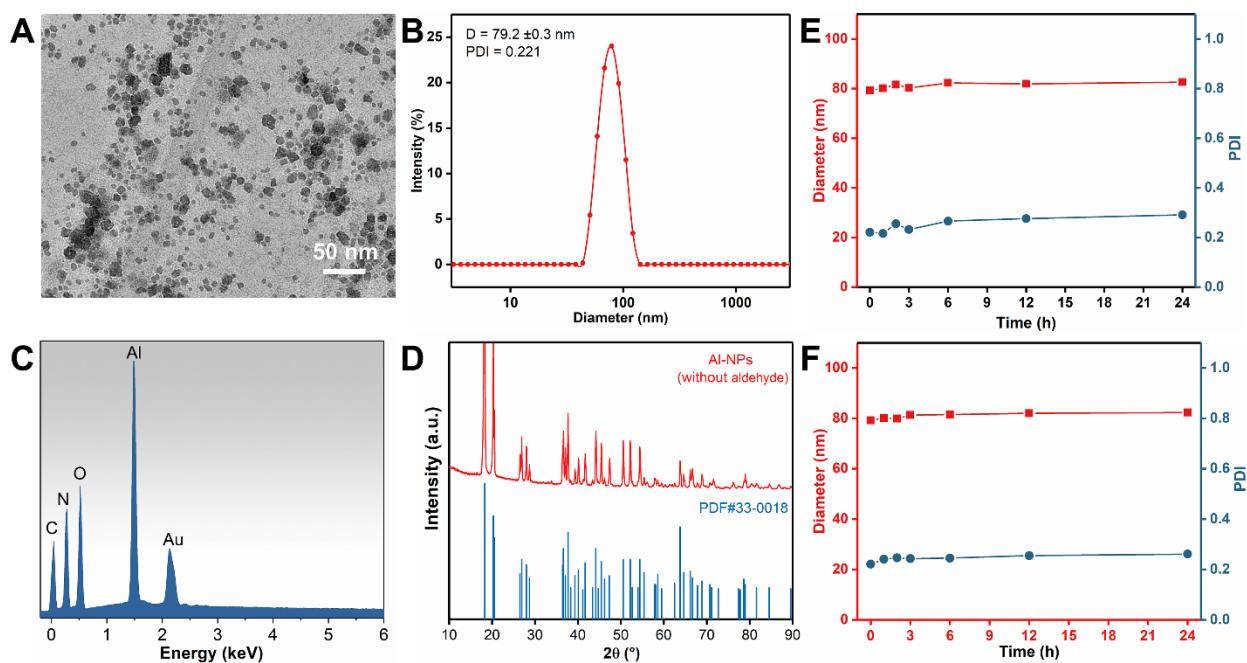
Figure S5. FT-IR spectra of PPAA and PPAAG.

Figure S6. Characterization of Al-NPs(PPAA). (A) TEM image. (B) DLS size distribution. (C) EDX spectrum of Al-NPs(PPAA). (D) XRD patterns of Al-NPs(PPAA) and PDF#33-0018. (E, F) Stability test in the aqueous solution of 1000 mM NaCl (E) or 5 mg·mL^{-1} of BSA (F) over 24 h.
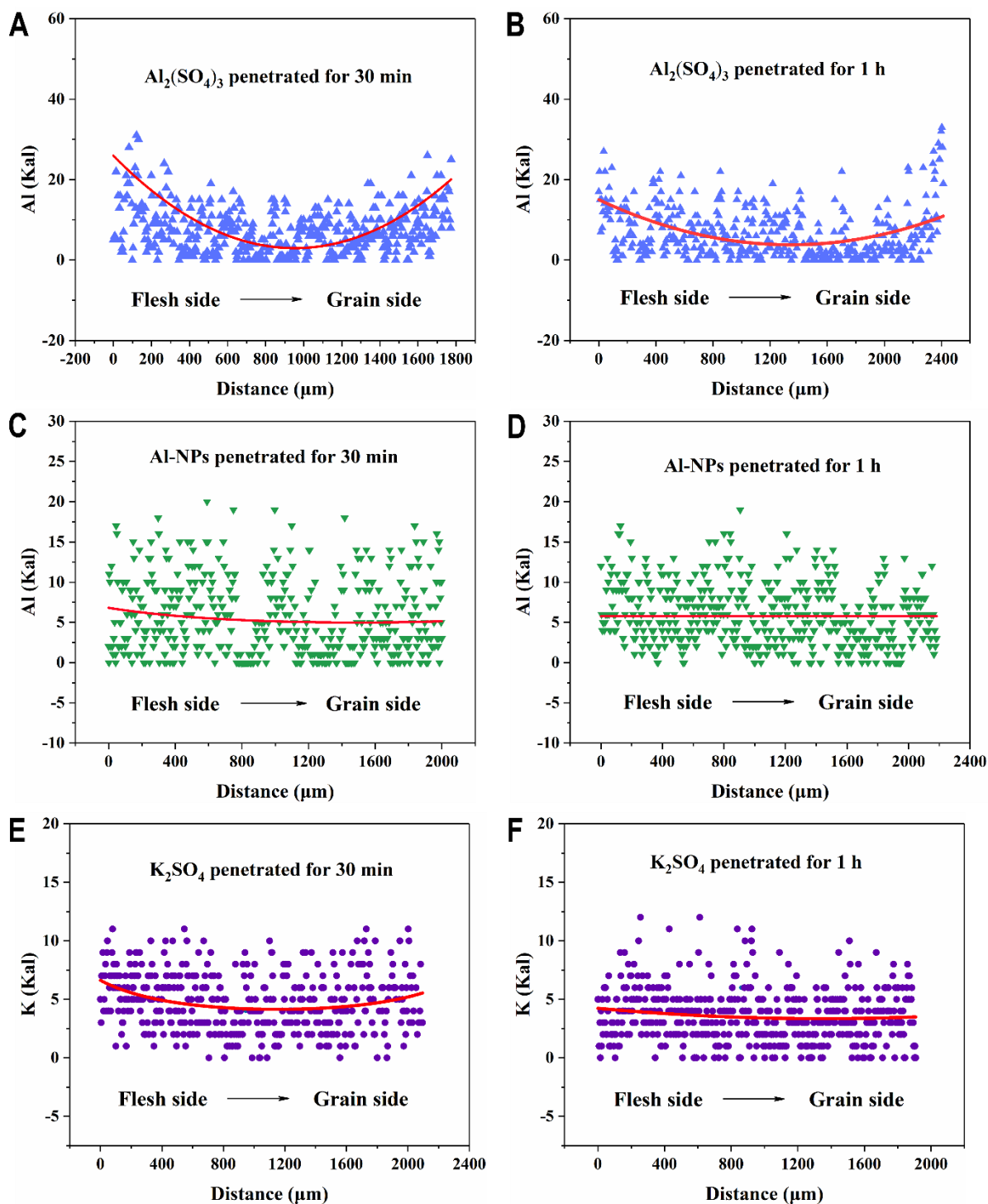
Figure S7. The cross-sectional metal distributions of the leather samples treated by different agents: (A, B) $Al_2(SO_4)_3$, (C, D) Al-NPs, and (E, F) K_2SO_4 for 30 min and 1 h.
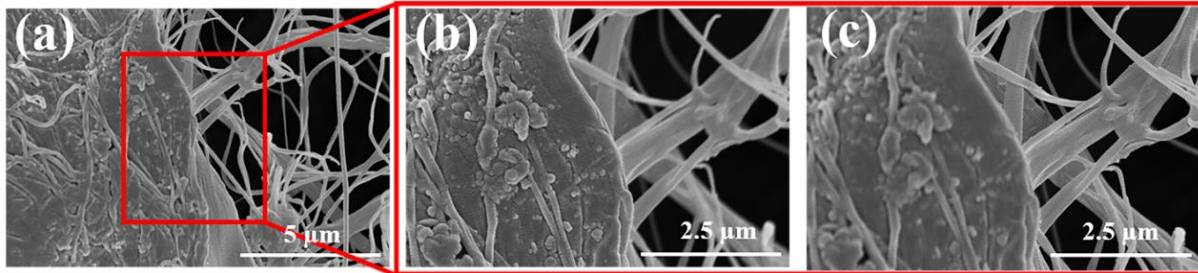
Figure S8. SEM images focused on different heights showing the distribution of Al-NPs on collagen fibers and collagen fibrils.
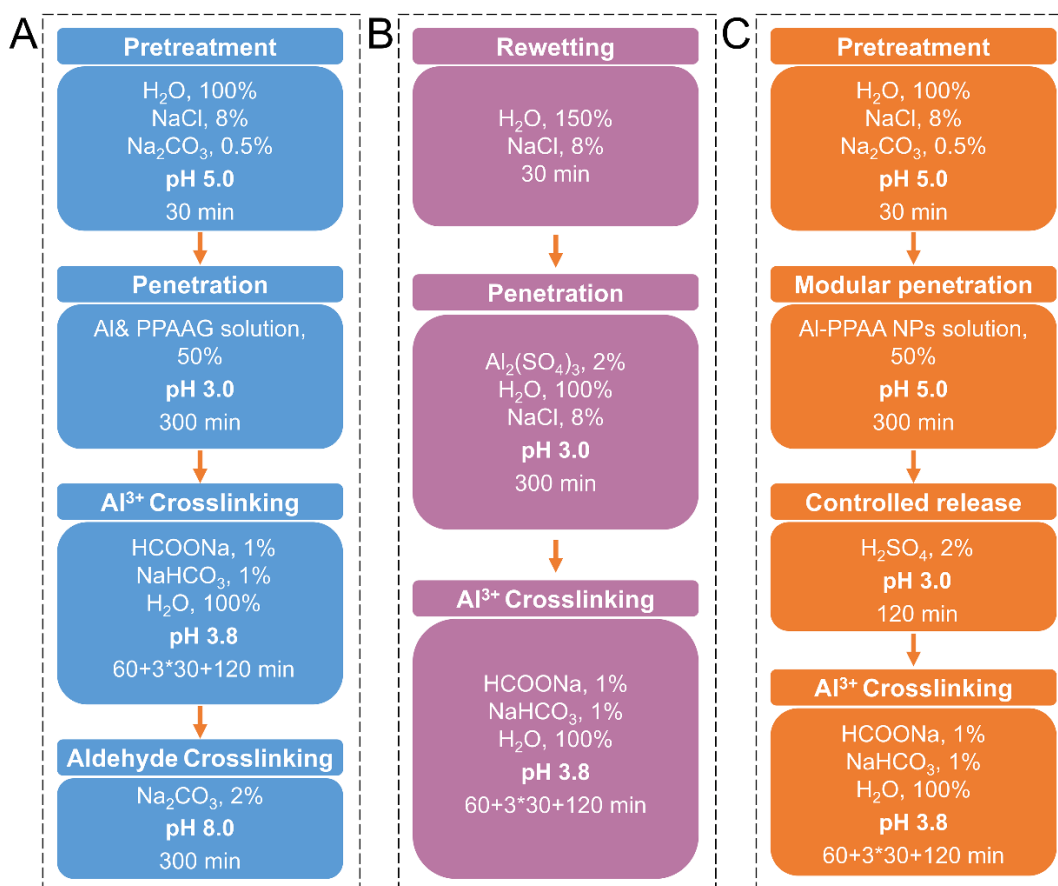

Figure S9. Crosslinking procedures for the sheep skin samples using (A) Al&PPAAG, (B) $Al_2(SO_4)_3$ and (C) Al-NPs(PPAA). During the Al^{3+} crosslinking step, the pH value of the reaction system was adjusted to 3.8 by adding 1% sodium formate for 60 min and 2% $NaHCO_3$ in three times at 30 min intervals, after that heated at 40°C for 2 h.
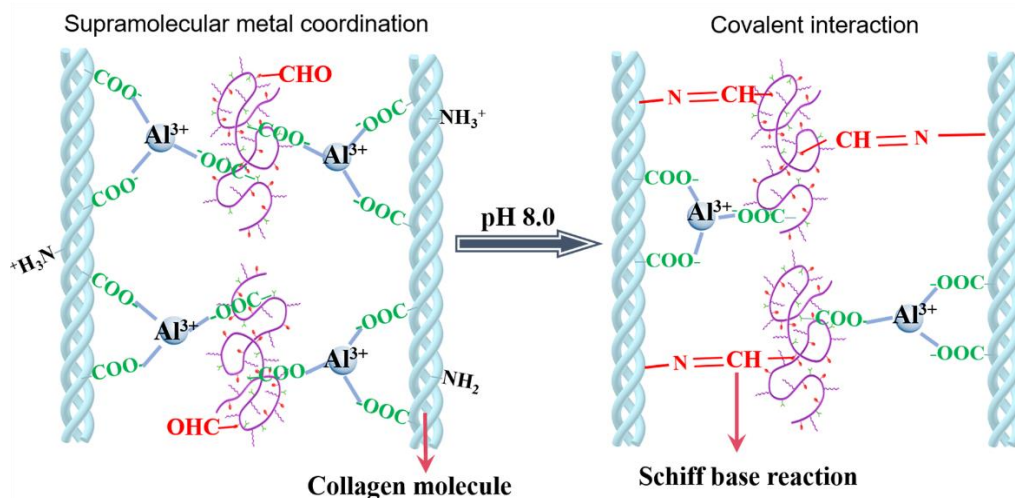
Figure S10. Schematic illustration of the synergistic crosslinking via supramolecular metal coordination and covalent reaction enabled by the pH-responsive Al-NPs.
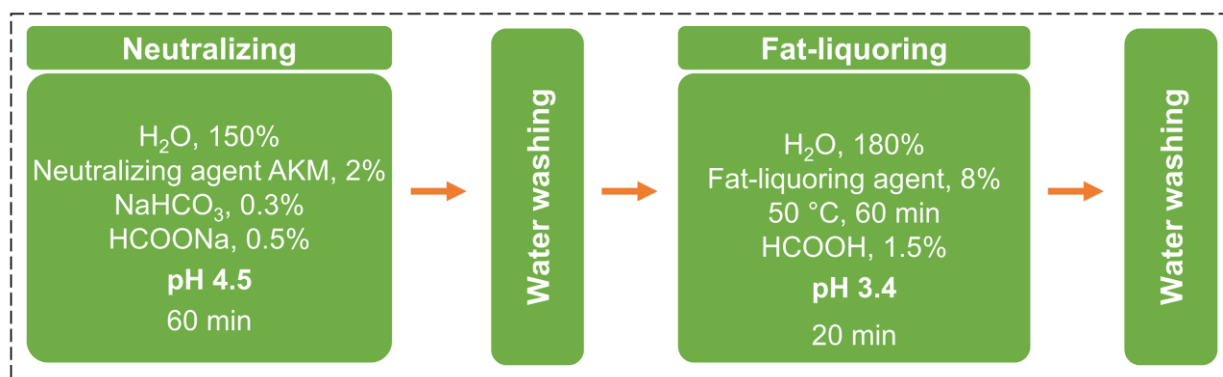

Figure S11. The fatliquoring process for different leather samples treated by Al-NPs, Al&PPAAG, $Al_2(SO_4)_3$, and Al-NPs(PPAA).
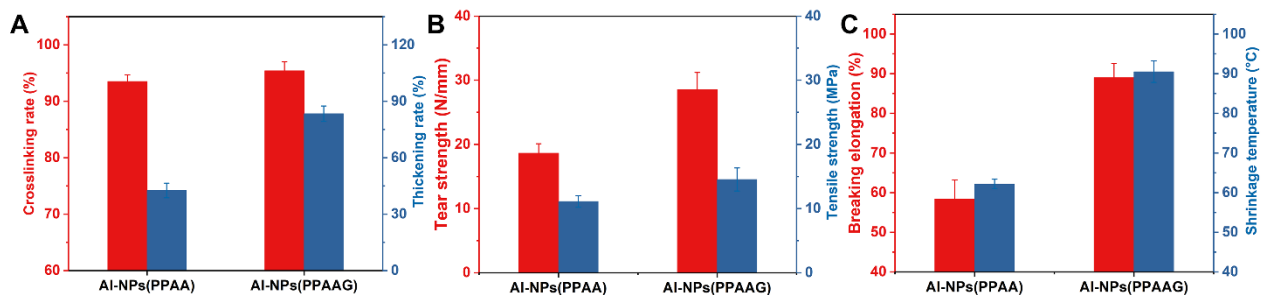

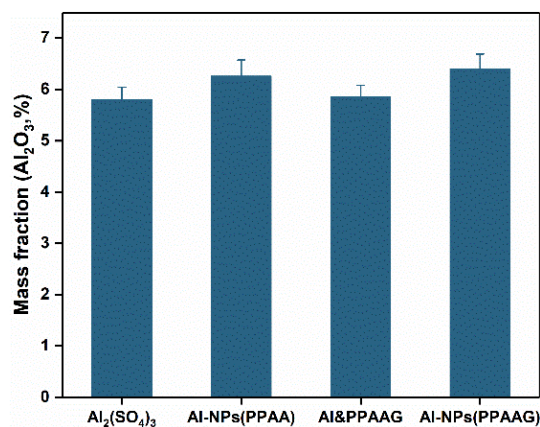**Figure S12.** Various properties of the leather samples cross-linked by Al-NPs(PPAA) and Al-NPs.

Figure S13. Aluminum quantity calculated by Al_2O_3 remaining in leather treated by $Al_2(SO_4)_3$, Al&PPAAG, Al-NPs(PPAA), and Al-NPs.

Supplementary Reference

1. B. J. Kim, K. H. Min, G. H. Hwang, H. J. Lee, S. Y. Jeong, E.-C. Kim and S. C. Lee, *Macromolecular Research*, 2015, 23, 111-117.