Injectable self-healing hydrogels based on cation-π interactions under physiological pH conditions

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Testing and characterization

The chemical structure of the monomers and polymers was characterized using a nuclear magnetic resonance (NMR) spectrometer (AV III HD 400 MHz, Bruker). The ^{1}H NMR of the samples was observed using deuterium-labeled chloroform ($CDCl_3$) and heavy water (D_2O) as solvents. The hydrodynamic diameter of the copolymer was measured using a Malvern Zetasizer Nano instrument. The polymer concentration was 0.1 wt%, and the experiment was conducted at temperatures ranging from 4 to 45 °C with an accuracy of ±0.1 °C. The sample solution was equilibrated at a constant temperature for at least 3 minutes before data collection. The temperature at which the particle size suddenly increased was determined as the lower critical solution temperature (LCST). The molecular weight was determined using gel permeation chromatography (GPC) with tetrahydrofuran (THF) as the eluent, a flow rate of 0.8 mL/min, and a temperature of 50°C. The rheological properties of different polymer hydrogels were tested using a rheometer (Anton Paar, MCR 302) with a 20 mm parallel plate configuration. Temperature ramp experiment: The LCST of different hydrogels was tested within the temperature range of 4-45°C at a heating rate of 1°C/min. Temperature dynamic strain scan experiment: The gel-sol stability transition of different polymer hydrogels was measured at temperatures above or below the LCST under constant angular frequency (ω = 10 rad/s) and strain (γ = 5%). Strain amplitude scan (γ = 0.1–1000%, ω = 10 rad/s) experiment: The critical strain of different polymer hydrogels was determined by immediately applying a small strain (γ = 5%) after applying a large strain (γ = 1000%), observing the self-recovery of their modulus. Dynamic strain cycle step scan experiment: A step scan was performed between 1% and 300% strain at a constant angular frequency (ω = 10 rad/s). First, 1% strain was applied for 300 s, followed by 300% strain for 50 s, repeated 4 times, to observe the dynamic modulus recovery of different hydrogels.

Self-healing and injectable properties of hydrogels

Simply divide the hydrogel into two parts and then join them together, observing how the state of the joined hydrogel changes over time. Rheological experiments were conducted to test strain amplitude scanning (γ = 0.1–1000%, ω = 10 rad/s) and dynamic strain cycling step scanning

experiments. A 23G × 3/4″ syringe was used to simulate the injectability of the hydrogel. The viscosity of the hydrogel was measured using a rheometer as a function of shear rate.

Anti-bacterial adhesion properties

The hydrogel coating was prepared in a 24-well plate with cell-coated wells. In each well, the hydrogel group and the blank group were immersed in 2 mL of Escherichia coli or Staphylococcus aureus suspension (10^9 CFU mL^{-1}) for 3 hours of co-culture at 37 ºC. The crawling sheets were rinsed three times with PBS solution, then the cell-adhered crawling sheets were heat-fixed and air-dried with a flame. Crystal violet was added and maintained for approximately 30 to 1 min, followed by rinsing with water. The sheets were immersed in Kresyl violet for 1 minute and rinsed with water. Then, they were washed with 95% ethanol or acetone for approximately 10–20 s and rinsed with water. Add vanillin for approximately 1 min and rinse with water. Air-dry, and observe cell adhesion under a microscope.

Cytotoxicity analysis

The cytotoxicity of the hydrogel was assessed using the Cell Counting Kit-8 (CCK-8, Dojindo, Shanghai, China) at 1 day and 7 days. Mouse fibroblasts (L929) were seeded into a 96-well plate at a density of 5,000 cells per well for 1 day and 1,000 cell per well for 7 days. Cell viability was evaluated at 1 day and 7 days. After 24 h of incubation, fresh Dulbecco's modified Eagle's medium (DMEM) cell culture containing the polymer was transferred to each well, with final polymer concentrations of 1, 1.5, 2, and 2.5 mg/mL. Untreated cells served as controls. Proliferation was measured using CCK-8 after 1, 3, 5, and 7 days of incubation. Carefully remove the medium from each well and incubate with fresh medium at a final concentration of 10% and CCK-8 solution at 37°C for 1–2 h. Read the plate using optical absorption spectroscopy at 450 nm and calculate cell viability relative to the control. Perform 6 replicates of the CCK-8 assay for each polymer concentration (n = 6).

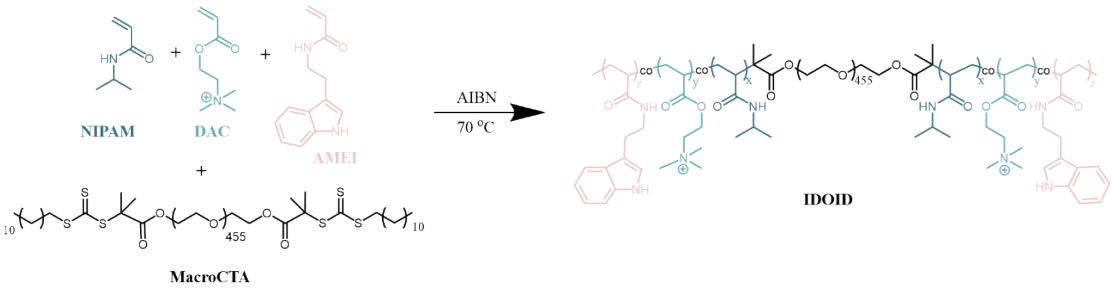

Figure S1. Synthesis scheme of the tri-block copolymer IDOID.

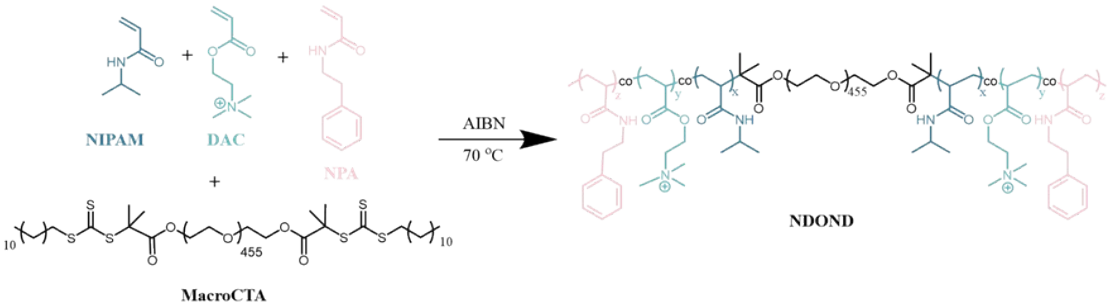

Figure S2. Synthesis scheme of the tri-block copolymer NDOND.

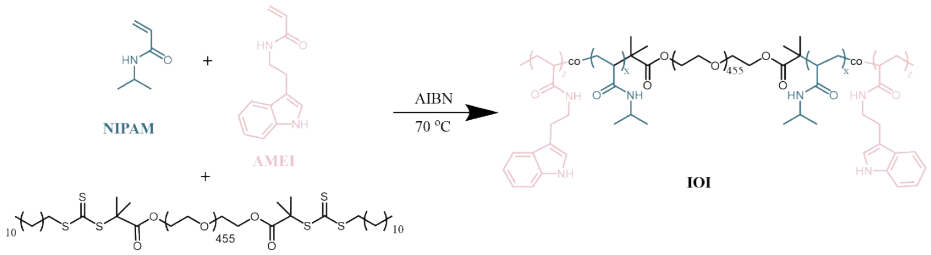

Figure S3. Synthesis scheme of the tri-block copolymer IOI.

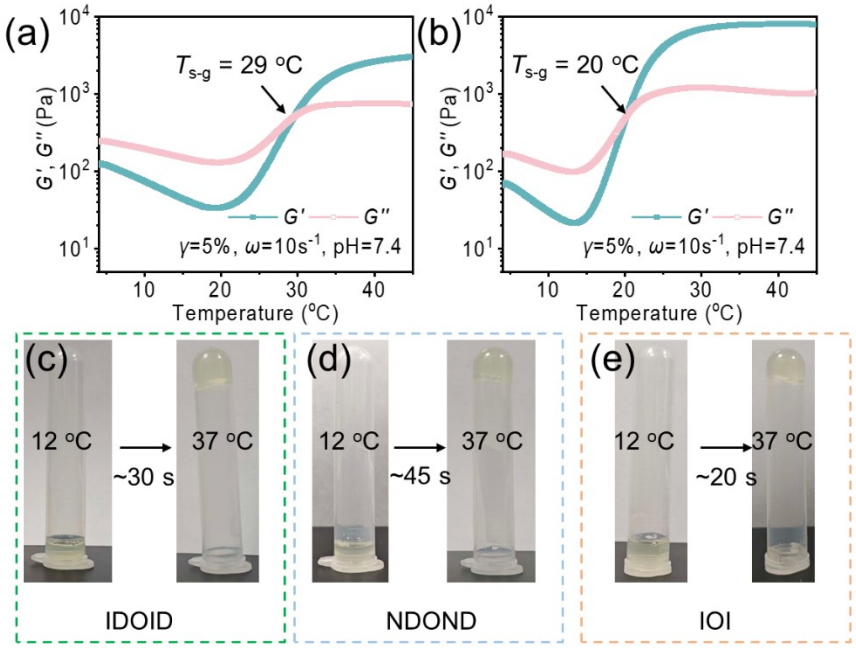

Figure S4. (a) Variation of modulus of NDOND hydrogels with temperature (4 °C ~ 45 °C), the intersection of lines *G′* and *G″* represents the *T*s-g; (b) Variation of modulus of IOI hydrogels with temperature (4 °C ~ 45 °C), the intersection of lines *G′* and *G″* represents the *T*s-g; Testing the gelation times of (c) IDOID hydrogel, (d) NDOND hydrogel, and (e) IOI hydrogel using the inverted vial method.

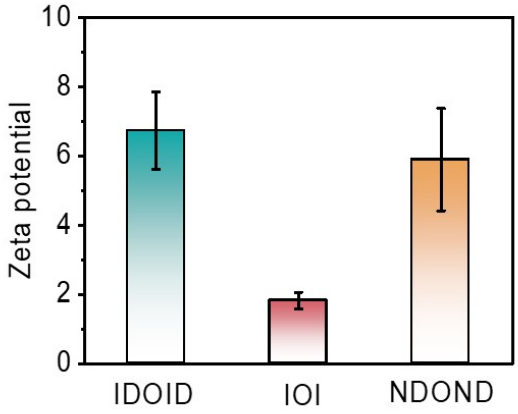

Figure S5. Zeta potentials of various polymers during micelle formation at 37°C.

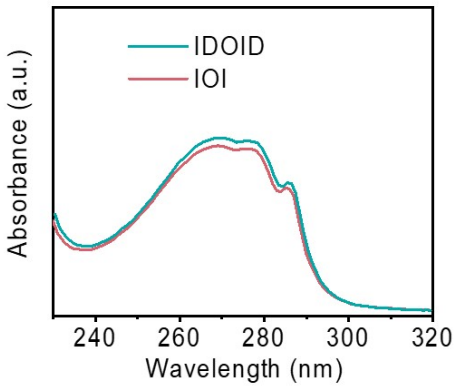

Figure S6. UV absorption spectra of IOI and IDOID in the range of 230-320 nm.

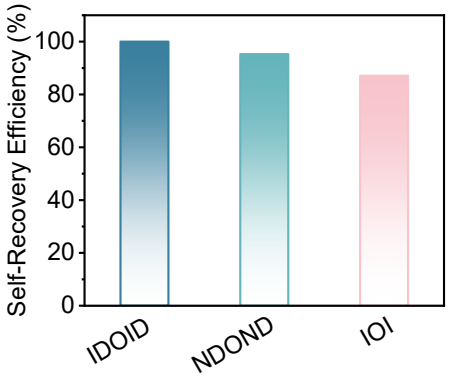

Figure S7. Recovery efficiency of different hydrogels.

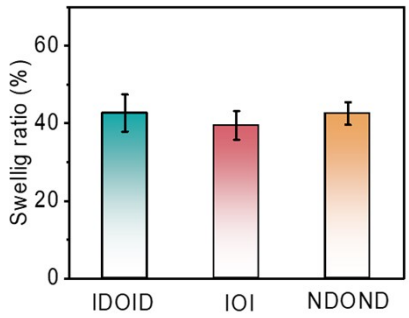

Figure S8. Swelling ratios of the hydrogels.

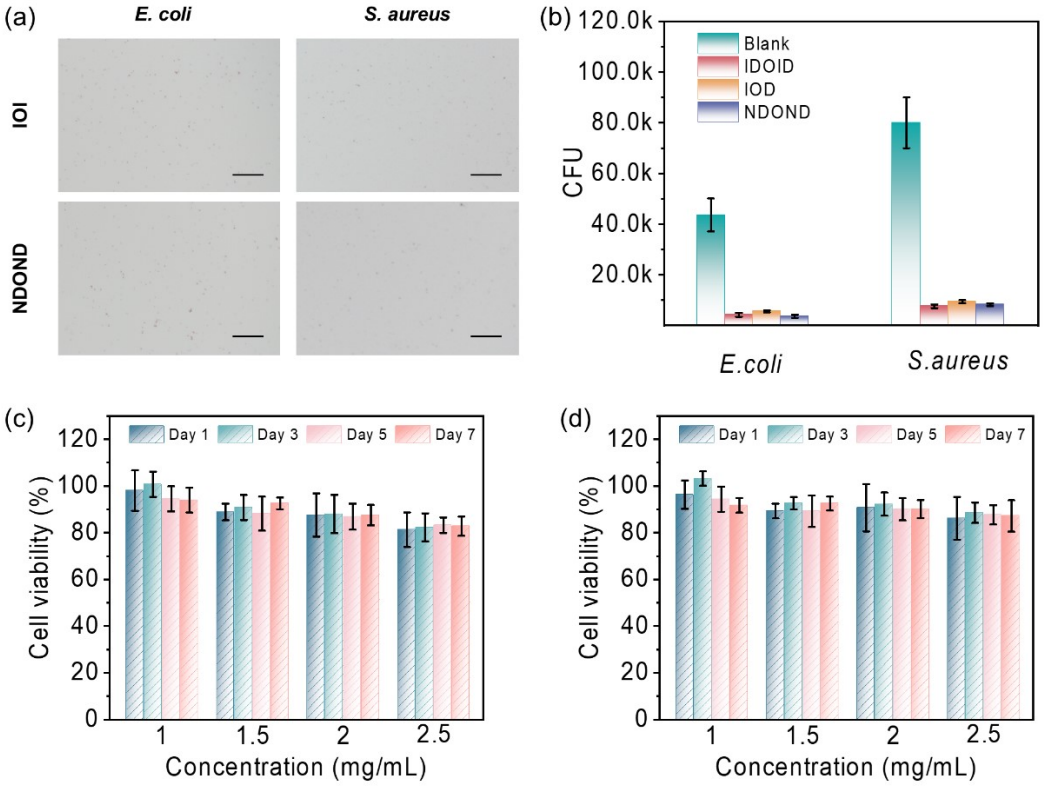

Figure S9. (a) Adhesion of glass plates containing IOI/NDOND hydrogels coating to *E. coli* and *S. aureus*, stained with Gram's reagent. Quantitative assessment of *E. coli* and *S. aureus* adhesion on various glass slides. (c-d) Cell viability of L 929 cells after 1, 3, 5 and 7 days of culture with IOI/NDOND.

Table S1. Molecular characterizations of the synthesized triblock copolymers

Polymer	Mn (kDa)	Mw (kDa)	*Đ*
MacroCTA	26.1	29.3	1.12
IDOID	72.3	89.6	1.24
NDOND	71.6	88.7	1.24
IOI	70.2	82.8	1.18