Supporting Information

Nanoinitiator Triggered Enzymatic Polymerization and Reinforcement of Gelatin-based Printable Hydrogel

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1. Materials

Gelatin, acrylamide (AAM), N-Acryloxysuccinimide (NAS), tetraethyl orthosilicate (TEOS), *N*-Hydroxy-5-Norborene-2, 3-Dicarboximide (HONB), and 1,4-dithio-D-threitol (DTT) were obtained from Sigma-Aldrich (USA). KH-590 was obtained from Energy Chemistry. Glucose oxidase was obtained from Aladdin Industrial Inc. (Shanghai, China). Ethyl alcohol, ammonium hydroxide and glucose were purchased in Sinopharm chemical reagent Co., Ltd. NIH-3T3 cells were obtained from ATCC, Manassas, USA. Dulbecco's Modified Eagle's Medium (DMEM), Fetal Bovine Serum(FBS), Pancreatin (0.25% EDTA, GIBCO), Penicillin-Streptomycin solution were purchased from Thermo Fisher Scientific (China) Co. Ltd. Fluorescein diacetate (FDA), Propidium iodide (PI), PBS buffer and Cell Counting Kit-8 were purchased from 7sea biotech (China) Co. Ltd. All materials were used as received without further purification.

2. Characterizations

FTIR Spectrum

SiO_2 NPs and *N*-hydroxyimide@SiO_2 were free-dried into powders before detection. FTIR spectra were recorded on a Nicolet 5700 Fourier spectrophotometer (Thermo Electron Corporation, USA) using KBr pellets.

EPR Spectrum

Two solutions: Solution A: 100 μL GOx $_{(aq)}$ (1000 U/mL) and 400 μL *N*-hydroxyimide@SiO_2 aqueous dispersion (10 mg/mL), Solution B: 100 μL glucose $_{(aq)}$ (100 mM), 400 μL H_2O were mixed with equal volumes, and then the mixture was rapidly transferred to a standard capillary and placed into the EPR spectrometer (JES FA200) with DMPO as the capturer.

SEM and TEM Images

The SEM samples of *N*-hydroxyimide@SiO_2 were placed on the silicon wafer, dried in vacuum overnight, and treated by spray-gold before testing with a field emission scanning electron microscopy (Hitachi S-4800) at a 3 KV voltage. The samples of S-TEM were prepared in the same way with SEM. The measurements were conducted on FEI Magellan 400L system. The TEM samples were coated on the carbon-coated copper grid and dried in vacuum overnight. The TEM pictures were acquired with a transmission electron microscopy (JEM-2010) at an 80 KV voltage.

Mechanical Measurements

The compressive properties of Gelatin-PAAM hydrogels were evaluated by compressive stress-strain measurements using a tensile-compressive tester (Farui Co.,

China) with 1mm•min^{-1} operated speed at room temperature. The diameter of the gels is about 14 mm and the thickness is 4-5mm. The tensile properties of Gelatin-PAAM hydrogels were evaluated under 10 mm•min^{-1} operated speed at room temperature. The diameter of the gels is about 3 mm and length is 10 mm. The compressive and tensile modulus was both calculated by the average slope of stress-strain curve in the strain range of 5%-15%.

GPC Testing Condition

The chromatographic column is Ultrahydrogel™Linear 300 mm×7.8 mmid×2. The column temperature is 30 °C. The mobile phase is 0.1 mol/L NaNO3 solution with 0.8 mL/min flow rate.

Rheological Measurements

The kinetics of gel formation was evaluated using a RS6000 rheometer (Thermo Scientific, Karlsruhe, Germany) with parallel plate geometry (25 mm diameter, 0.3 mm gap). The strain amplitude sweeps of gels were taken at 37 °C in the dynamic oscillatory mode with a constant deformation of 1% and frequency of 1 Hz. The measurements of frequency-dependent sweep were measured as a function of angular frequency at fixed strain of 0.03 %.

3D Printing Process

The precursor solution containing 8 wt % acryloylated gelatin, 1 wt % N-hydroxyimide@SiO_2, 1 wt % AAM, glucose (10 mM) and GOx (100 U) was prepared, and incubated at 37 °C for 30 min. Then predesigned 3D structures were printed in the position set-point on a valve-based printer at isothermal model (37 °C)

by 3D printer (Nano-Plotter NP 2.1, GeSiM, Grosserkmannsdorf, Germany of Shanghai Institute of Ceramics, Chinese Academy of Sciences).

Confocal Microscopical Analysis

NIH-3T3 cells (5×10^4/mL, 1mL) were seeded onto confocal dishes and incubated for 12 h (5% CO_2, 37 °C). Then, the culture medium was removed, 1mL fresh culture medium containing 1, 3, 5 mg/mL hydrogel powder was added to each dish and the cells were further incubated for 24 h. The culture medium was then removed and the cells were stained with FDA (40 μg/mL) and PI (10 μg/mL) in serum-free DMEM media for 5 min. The cells were washed with 1mL PBS once, and 1mL serum-free medium was added. Images were acquired using Leica TCS SP8 confocal laser scanning microscope, and illumination was provided by an argon gas laser at 488 nm or a 561nm diode laser.

3. Figures

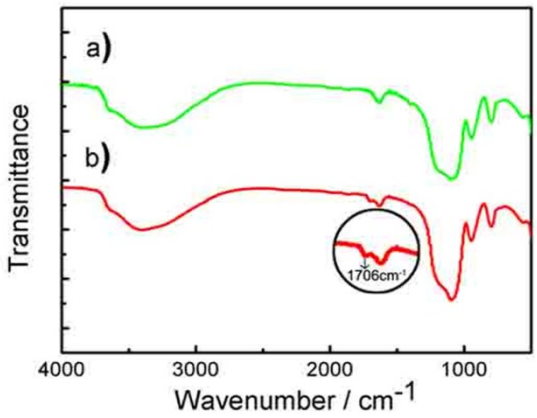


Figure S1. FTIR spectrum of (a) silica NPs and (b) *N*-hydroxyimide@SiO_2 NPs.

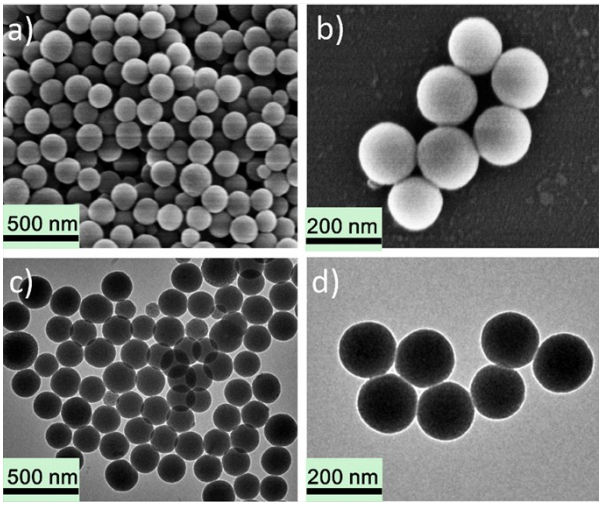


Figure S2. Scanning electron microscope (SEM) images (a, b) and transmission electron microscopy (TEM) images (c, d) of *N*-hydroxyimide@SiO_2 NPs.

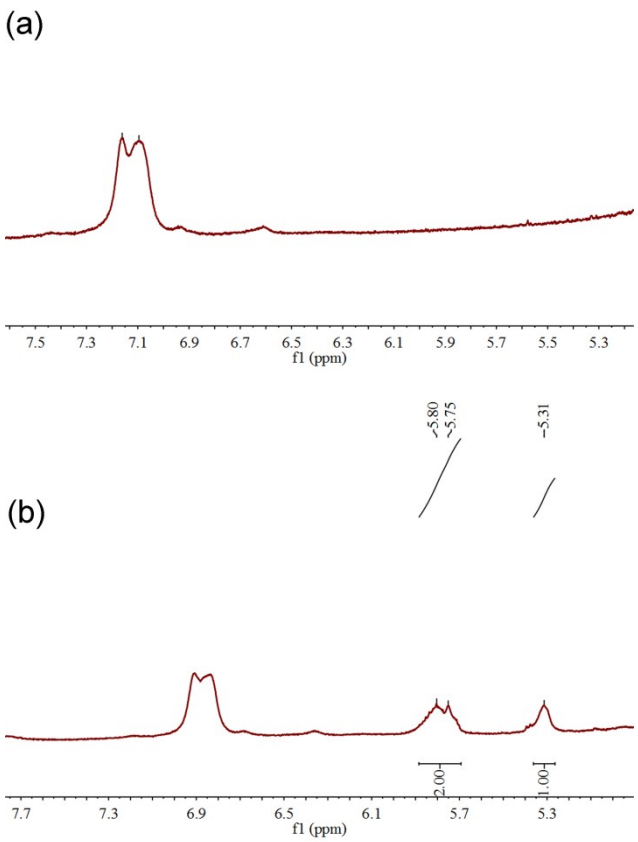


Figure S3. [1] NMR spectrums of (a) Gelatin and (b) Acryloylated Gelatin.

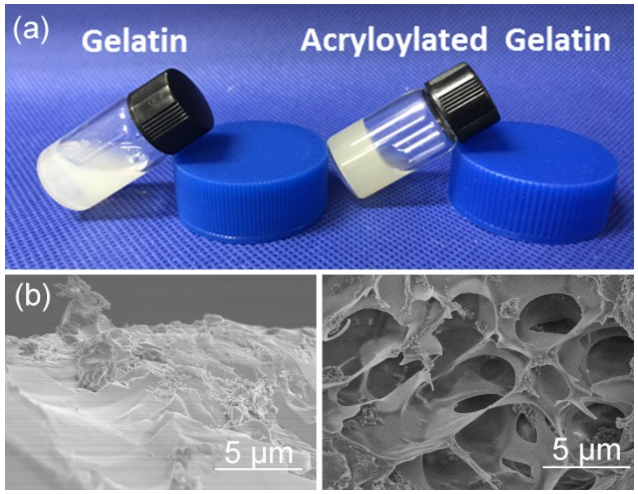


Figure S4. (a) Enzymatic gelation test using gelatin (10 wt %) and acryloylated gelatin (10 wt %), respectively, initiated by 0.5 wt % N-hydroxyimide@SiO_2, glucose (10 mM) and GOx (100 U) at 37 °C and (b) SEM images before (left) and after (right) enzymatic gelation.

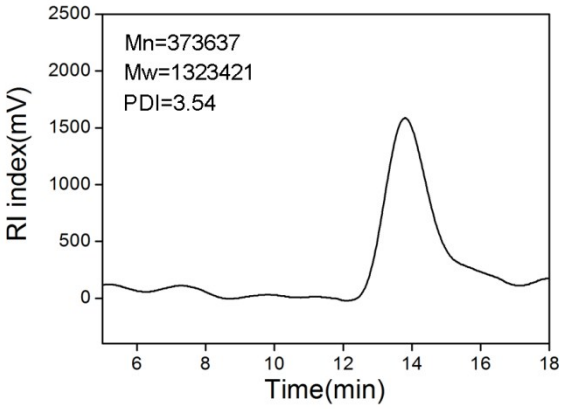
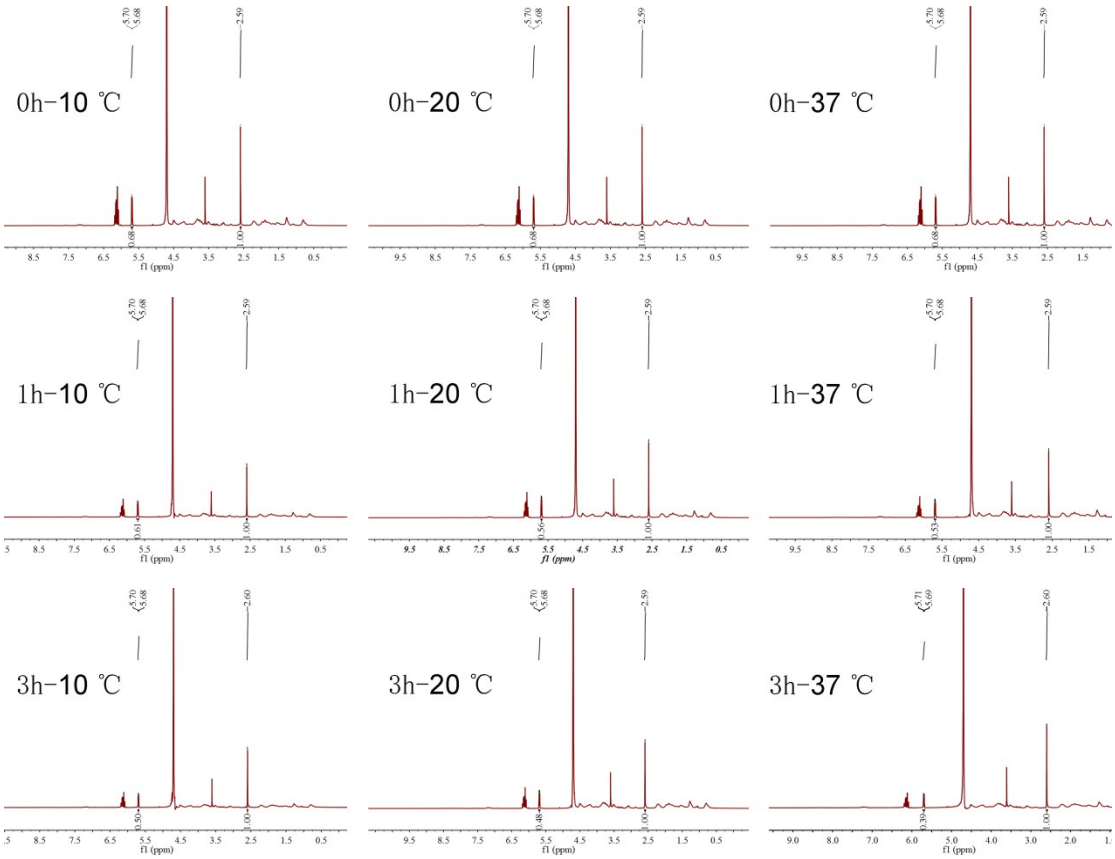


Figure S5. GPC spectra of PAAM (5 wt %) initiated by initiated by 0.5 wt % *N*-hydroxyimide@SiO_2, glucose (10 mM) and GOx (100 U). Sample was well mixed and 10-fold diluted before testing.



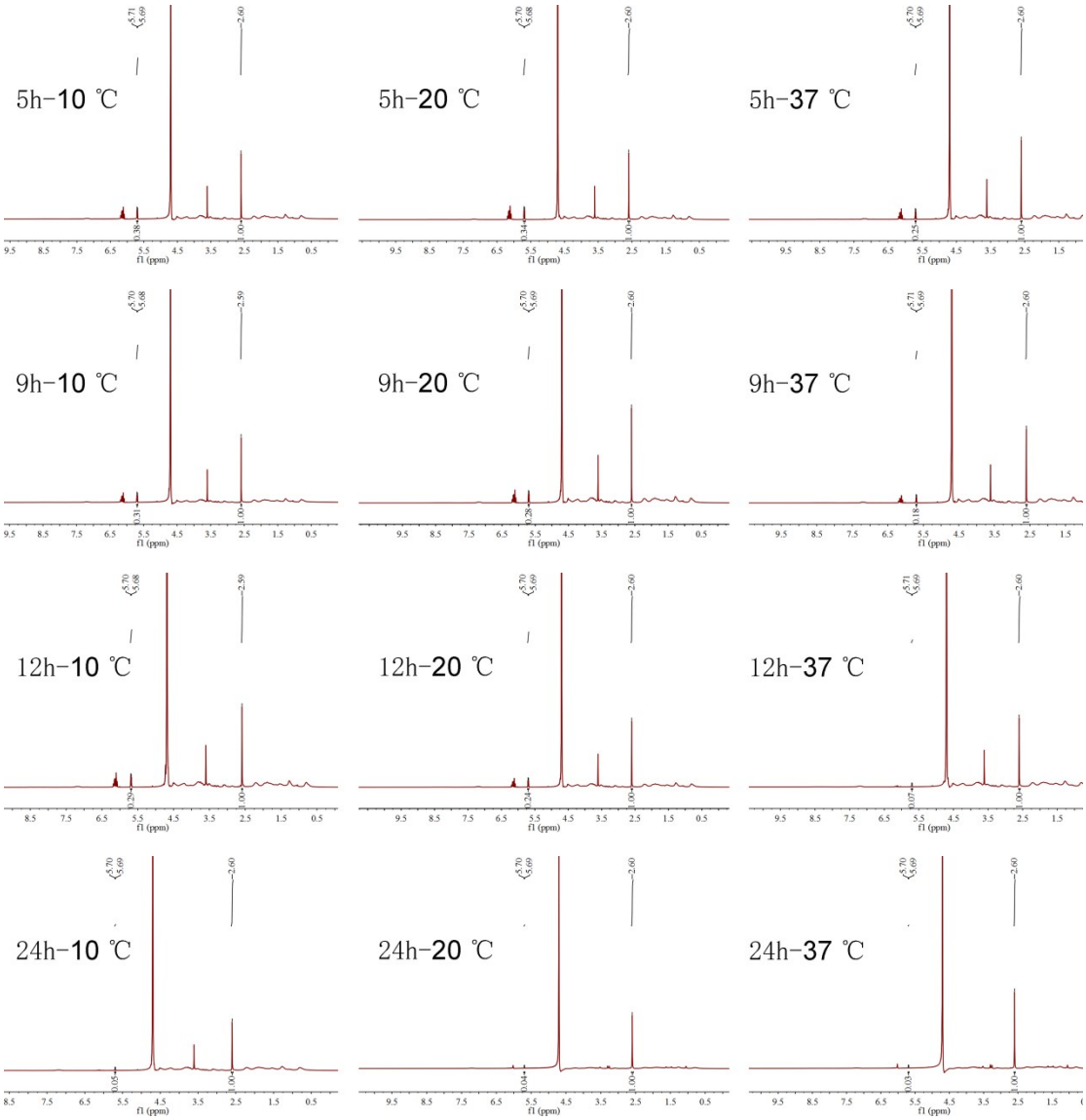


Figure S6. [1] NMR spectrums of Gelatin (8 wt %)/AAM (1 wt %) system at 37 ℃, 20 ℃ and 10 ℃, respectively, using DMSO (5 μL) as internal standard during the polymerization process.

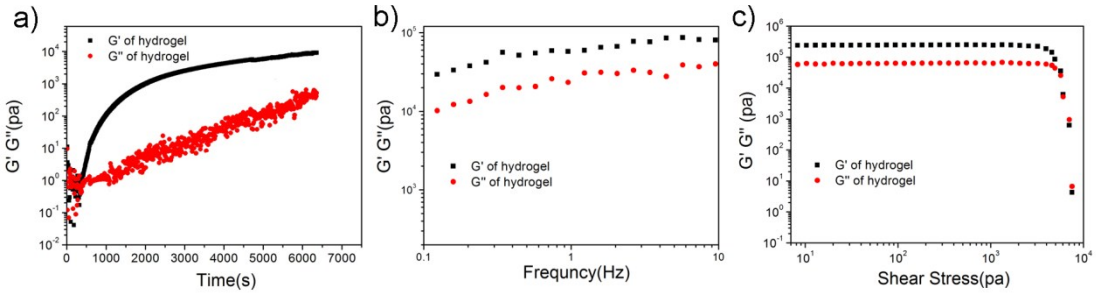


Figure S7. Spectrums of rheological measurements. (a) Time scan (b) Frequency sweep (c) Stress sweep of Gelatin-PAAM hydrogels containing 5 wt % acryloylated gelatin and 5 wt % AAM.