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In-situ Formed Thermogelable Hydrogel Photonic Crystals Assembled by Thermosensitive IPN Nanogels

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Experimental Section

Materials

N-Isopropylacrylamide (NIPAm, 97%, Aldrich); *N,N*'-Methylenebisacrylamide (Bis, 99%, Aldrich); Acrylic acid (AA, 99%, Aldrich); Sodium dodecylsulfate (SDS, 99%, Aldrich); Ammonium persulfate (APS, 98%, Aldrich); Sodium metabisulfite ($Na_2S_2O_5$, 97%, Adamas); Bovine serum albumin (BSA, 98%, Aldrich), Hemoglobin from bovine blood (85%, Aldrich), Myoglobin from equine skeletal muscle (98%, Aldrich)

Synthesis of PNIPAm nanogels

A series of crosslinked PNIPAm nanogels was prepared by free radical precipitation polymerization in an aqueous solution. Typically, 1.9 g of NIPAm and 0.033 g of Bis were dissolved in 140 g of ultrapure water. Following, different amounts of SDS (SDS: PNIPAm mol%=5.0%, 2.5%, 1.25%) were added to obtain nanogels with various particle sizes that were labeled as PNIPAm-1, PNIPAm-2, PNIPAm-3. The solutions were heated to 70 °C and a radical initiator solution consisting of 0.073 g of APS was added to the mixture. The reactions lasted for 4h under nitrogen atmosphere at 70 °C. The resultant PNIPAm nanogels were dialyzed for one week at room temperature.

Synthesis of PNIPAm/PAA IPN nanogels

A series of PNIPAm/PAA IPN nanogels was synthesized by in situ polymerization of AA and Bis inside the PNIPAm network. Each series of IPN nanogels was synthesized by one type of PNIPAm suspension but with different amounts of AA monomer. Specially, 20 g of PNIPAm suspension was dilute six times with ultrapure water. 0.5 g/ 1.0 g/ 1.5 g of AA and certain amount of

crosslinker with constant molar ratio of 10% (Bis:AA) were then added, continuously stirring for 30 min under the nitrogen atmosphere. Following, the initiator (0.1 g of APS) and accelerator (0.1 g of $Na_2S_2O_5$) was separately dissolved in water and added into the dispersion. The reactions lasted for 20-45 min under a nitrogen atmosphere at 10 ^oC and was suspended by adding 1 mL of 5M NaOH aqueous solution. The actual loaded-PAA content was calculated from the loaded-COOH content which was measured by potentiometric titration. The resultant IPN nanogels were dialyzed for one week at room temperature.

Particle size measurement

The particle sizes of nanogels in a dilute solution were measured by a dynamic light scattering spectrometer (DLS, Nano-ZS, Malvern). The measurements were carried out at a scattering angle of 90 ^oC. The test temperature was controlled by a refrigerator circulating water bath.

Component and SEM measurement

The amounts of carboxyl groups in IPN nanogels were determined by conductivity titration (Mettler Toledo S230). The SEM images for 1.5 wt% IPN1-3 hydrogel powder which was dried under lyophilizer after thermogelling were recorded on a Field Emission Scanning Electron Microscope (S-4800)

Rheological Characterization

Dynamic rheological analysis was carried out in a stain-controlled ARES rheometer. The parallel plate geometry with the plate diameter of 50mm was used, and the sample gap was adjusted to 0.65 mm. Temperature-dependent changes in storage modulus, G', and loss modulus, G", were recorded in a dynamic temperature ramp test from 20 ^oC to 60 ^oC, with a heating rate of 1 ^oC/min. The temperature was controlled by a Peltier system in the bottom plate connected with a water bath. Dynamic frequency sweep test at a constant temperature of 25 ^oC was carried out to study the viscoelastic behavior of nanogels over a wide range of frequencies (0.1−100 rad/s). The gelation time and the reversibility of sol-gel transition were measured by the repeated dynamic time sweep tests that were conducted at 25 ^oC and 37 ^oC. All the tests were performed within the linear viscoelastic region and the temperature/time sweep test were performed at an angular frequency of 10 rad/s

Cell culture

Helf cells were cultured in DMEM (Hyclone, USA) culture media containing 10% fetal bovine serum, 50 units/mL penicillin and 50 units/mL streptomycin at 3 ^oC, 5% CO_2/ 95% air (v/v) environment.

Cell viability

Cell viability was measured by MTT assay. Helf cells were seeded in 96-well plates at a density of 8×103 cells per well and cultured for overnight. Different formulations were added with predetermined nanogel concentrations and incubated for another 24/ 48 h. Thereafter, 20 µl of

3-[4, 5-dimethylthiazol-2-yl]-3, 5 diphenyltetrazolium bromide solution (MTT, 5 mg/mL in PBS solution) was added to each well. After incubating for another 4 h at 37 °C, the media was removed and 150 µl of DMSO was added to dissolve formazan crystal. The absorbance at 490 nm was measured by ELIASA of BioTek®.

Confocal laser scanning microscope

Confocal laser scanning microscope was used to visualize the cellular viability. Helf cells were incubated with different nanogel concentrations. After incubating for 48 h, the media was removed and the cells were washed with PBS solution for 3 times. The cells were stained with calcein for 30 min and Hoechst 33342 for 15 min. The cells were visualized under a laser scanning confocal microscope (Leica TCS SP8 STED 3X).

Protein loading and release in vitro

Three kinds of proteins with various molecular weight (Bovine serum albumin (BSA, 133 kD), Hemoglobin (65 kD), Myoglobin (17 kD) were chosen to investigate the potential ability of drug sustained release under physiological conditions. Briefly, proteins (final concentration: 1 mg/g) were mixed with IPN nanogels (1.5 wt%, 3.0 wt%) at room temperature. After incubating at 37 °C for 20 min, 1 mL of PBS was added to each sample. At each time point, 500 µL of the upper solution was collected for protein measurements by using UV-vis spectra (Lambda 35, Perkin-Elmer, USA) analysis at 278 nm (BSA), 408 nm (hemoglobin) and 403 nm (myoglobin). Particularly, another contrast loading-release experiment was carried out as follows: BSA (final concentration: 1 mg/g) was dispersed into 0.5 wt% IPN nanogel dispersion and continuously stirred for 20 min at 37 °C. Following, the dispersion was centrifuged (35000 rpm, 20 min) to remove the unloaded BSA. And the precipitant was re-dispersed in fresh PBS and transferred to a dialysis bag (MWCO: 8000-12000 Da). At desired time intervals, 1mL of external PBS was taken out for UV-vis spectra analysis and replenished with an equal volume of fresh medium. All release measurements were carried out in duplicate.

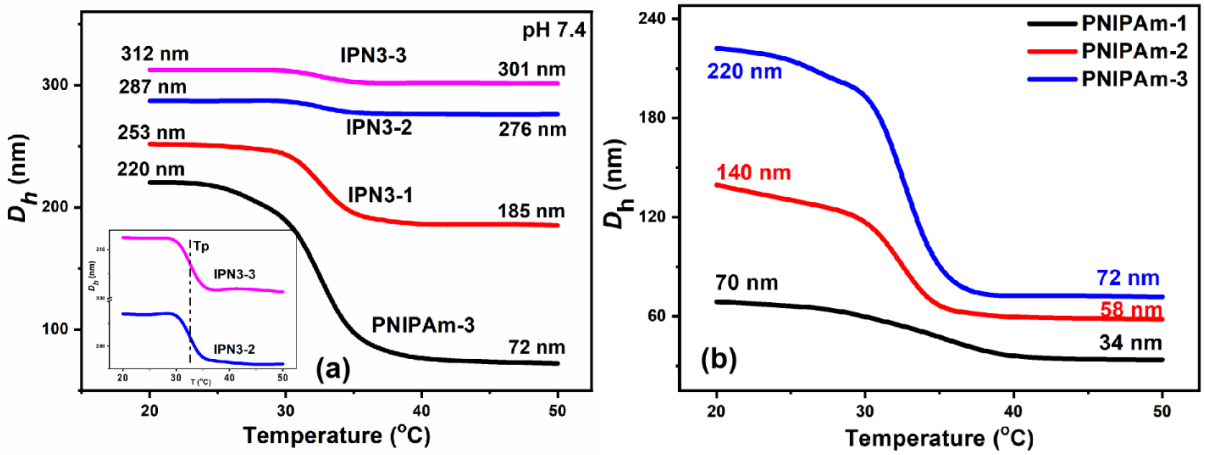

Figure S1 Temperature-induced phase transitions of (a) IPN nanogels and (b) PNIPAm nanogels. These transitions showed the same phase transition temperature of 32 °C

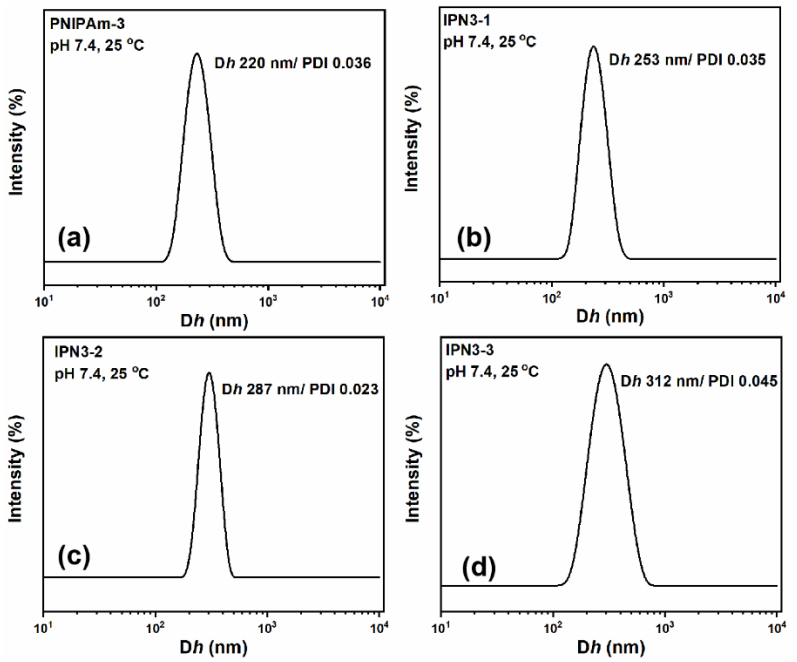

Figure S2 The size distributions of (a) PNIPAm nanogel (b) IPN3-1 nanogel (c) IPN3-2 nanogel and (d) IPN3-3 nanogel

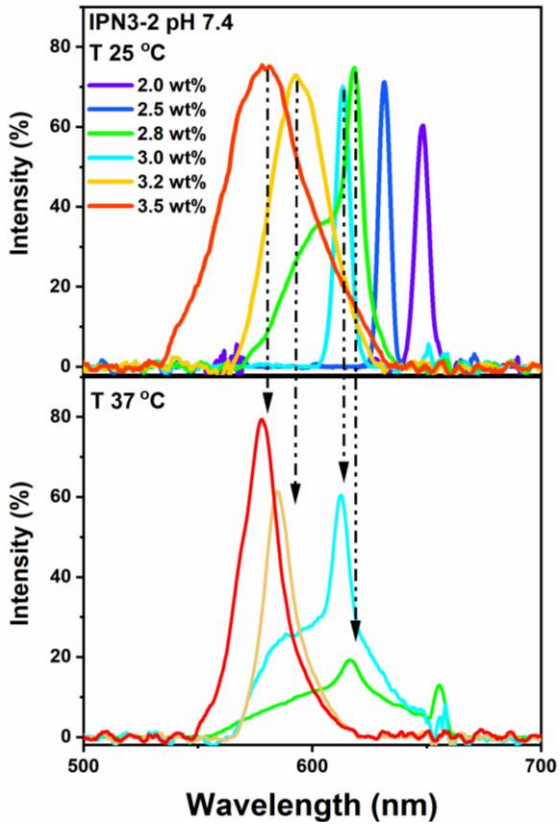

Figure S3 Reflection spectra of photonic crystals assembled by IPN3-2 nanogel dispersion with different concentrations at 25 °C and 37 °C (above the Tp)

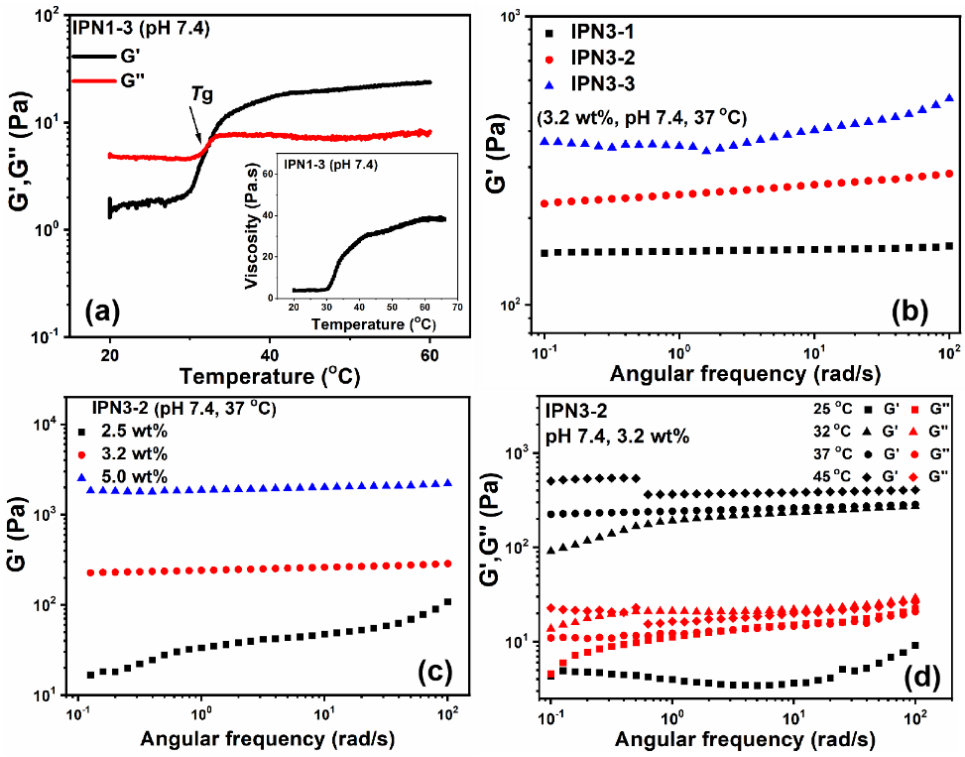

Figure S4 (a) Temperature dependence of G' and G'' for IPN1-3 nanogel. Insert: complex viscosity of IPN1-3 nanogel during sol-gel heating process (b) G' of IPN S-3 nanogels (3.2 wt%) loaded with various amount of PAA content as a function of angular frequency (ω) at 37 °C (c) G' of IPN3-2 nanogel as a function of concentration at 37 °C (d) G' (black symbol) and G'' (red symbol) of IPN3-2 nanogel (3.2 wt%) as a function of angular frequency (ω) at various temperature