Supporting Information

Multifunctional inverse opal particles for drug delivery and monitoring

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Experimental

Materials

Eight kinds of SiO_2 nanoparticles with the size of 200, 215, 235, 255, 268, 277, 285 and 298 nm were purchased from NanJing DongJian Biological Technology Co., Ltd. N-Isopropylacrylamide (NIPAM, 97%), Fluorescein isothiocyanate-dxetran (FITC-dextran, average molwt 150,000), Calcium chloride, Poly (ethylene glycol) diacrylate (PEGDA, average molecular weight of 700) and 2-hydroxy-2-methylpropiophenone photoinitiator were all purchased from Sigma-Aldrich (St. Louis, MO, USA) and used as received. Hydrofluoric acid and N-Methylolacrylamide were purchased from Aladdin Industrial Corporation (Shanghai, China). Sodium alginate was purchased from Alfa Aesar China Ltd (Heysham, Lancs). All other reagents were of the best grade available and used as received.

Generation of template colloidal crystal beads

The silica colloidal crystal beads (SCCBs) were prepared by the droplet template method. The aqueous suspension and silicon oil were injected into the microfluidic device. In consequence, the aqueous suspension was sheared into droplets by the oil flows in the microfluidic channel. The droplets were collected in a container filled with silicon oil. The silica nanoparticles self-assembled into ordered lattices during the evaporation of water in the droplets at 75°C in an oven. After solidification overnight, the silica colloidal crystal beads were gently and thoroughly washed with hexane to remove the silicon oil. Finally, the silica colloidal crystal beads were calcined at 800°C for 3h to improve their mechanical strength. The concentration of the used silica nanoparticles was 20% (w/v). The injection speeds of the oil and dispersed phase were 0.5mL/h and10mL/h, respectively. Photographs of the SCCBs were taken with a light microscope (OLYMPUS BX51) equipped with a color CCD camera (Media Cybernetics Evolution MP 5.0). Reflection spectra of the SCCBs were recorded by the microscope (OLYMPUS BX51) equipped with a fiber optic spectrometer (Ocean Optics, QE65000).The microstructures of the beads were characterized by a scanning electron microscopy (SEM, Hitachi, S-300N).

Fabrication of thermo-responsive inverse opal particles

The pre-gel solution used for the thermo-responsive inverse opal particles fabrication was composed of N-isopropylacrylamide and N-methylolacrylamide (with different w/t), 2-hydroxy-2-methylpropiophenone (1% v/v) and poly (ethylene glycol) diacrylate (5% v/v). The mass ratio of N-isopropylacrylamide and N-methylolacrylamide was 9:1. The temperature responsive inverse opal particles were replicated from the voids of the template SCCBs. To ensure the pre-gel solution could fill the void entirely, the SCCBs were previously treated with piranha solution (30% v/v hydrogen peroxide and 70% v/v sulfuric acid) for 6h. After washing with water and drying by nitrogen flow, the SCCBs were immersed in the pre-gel solution for 2h. They were then dispersed between two quartz disks separated by a 300-mm-thick spacer, and exposed to UV light (365 nm, 100 W, 10 minutes) for the polymerization of the pre-gel solution in and out of the SCCBs. Broad-band excitation in the near-UV range (330–385 nm) was provided by a 100W mercury lamp. The prepared film

containing the SCCBs was immersed in the buffer solution for 1h in succession. After stirring the hydrogel film into pieces by a stirrer, the hybrid beads were filtered from the buffer solution. Finally, the thermo-responsive inverse opal particles were obtained after removing the silica template by immersed in hydrofluoric acid (4%, v/v) for 2h. Photographs, reflection spectra and microstructure pictures of the inverse opal particles were measured as previously mentioned.

Characterization of the inverse opal particles

The fabricated inverse opal particles were kept in a transparent glass holder filled with deionized water and placed on a heating stage. Before the dynamic swelling experiments, the inverse opal particles had been equilibrated at 55 °C for more than 30 minutes to reach a full shrinking state. In the dynamic swelling process, the ambient temperature is gradually decreased from 55 °C to environment temperature. The volume and color change as well as the reflection spectra shifts of the particles were recorded during the cooling process. In order to investigate the temperature-dependent equilibrium volume change, the ambient temperature of the particles was set in the range from 26 to 55°C. Before each measurement, particles at every predetermined temperature had been kept for more than 30 minutes to reach an equilibrium phase state. Photographs and reflection spectra of the samples were measured as previously mentioned. Diameters of fifteen beads were measured at each temperature to get an average value.

Drug encapsulation and in vitro dextran release

Sodium alginate pre-gel solution with different concentration of 0.25%, 0.5%, 0.75%, 1.0%, 1.5%, 2.0%, 3.0% (w/v) were previously prepared and mixed with 2.5mg/ml FITC-dextran. The inverse opal particles with characteristic reflection at 620 nm were immersed in the mixture for more than 2h. Then 2.0% (w/v) calcium chloride was added into the pre-gel solution contending dextran and particles to realize the gelatin of calcium alginate hydrogel. The prepared hydrogel containing the dextran loading particles were immersed in the buffer solution for 30minutes in succession. After stirring the hydrogel into pieces by a stirrer, the particles were filtered from the buffer solution. For each concentration from 0.75% to 3.0% (w/v) of calcium alginate

hydrogel, the samples of fifteen beads were suspended in 1.5ml release buffer consisting of PBS (pH 7.4 ± 0.05). These samples were incubated for more than twenty days at room temperature with shaking (200 rpm). At various time points, 1.0 ml supernatant was removed and replaced with fresh media in order to maintain constant condition. Fluorescence pictures of drug loading inverse opal particles were taken by the microscope (OLYMPUS BX53) with a high resolution CCD (OLYMPUS DP73) and were processed by Cell Standard software to characterize the fluorescence intensity through the obtained green grey value. Fluorescence cross-sectional images were obtained using a confocal microscope (OLYMPUS FV500-IX81).

Controlled release and monitoring experiments

For the temperature depending release experiment, the samples consisted of approximately fifteen drug loading beads at each concentration from 0.25% to 2.0% (w/v) of calcium alginate hydrogel were suspended in 1.5ml release buffer consisting of PBS (pH 7.4 ± 0.05).Then they had been equilibrated at 45 °C for 5 minutes to reach a shrinking state. After heating, they were kept at room temperature for more than 15 minutes to recover back to swelling state. Also 1.0 ml supernatant was removed and replaced with fresh media in order to maintain constant condition after each temperature change. Fluorescence pictures of the drug loading particles with concentration from 0.25% to 2.0% (w/v) were detected, and reflection spectra of the samples at the concentration of 0.25%, 0.5% and 0.75% (w/v) were recorded as results after every release procedure. All the drug release experiments were carried out under dark condition.

Supporting Figures:

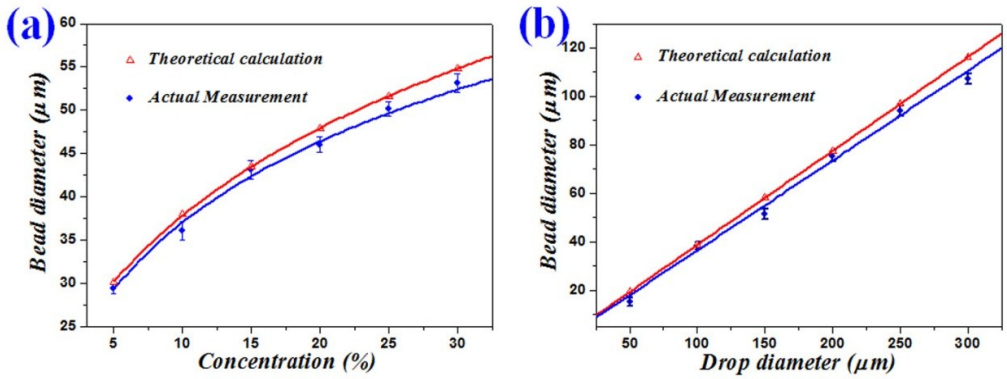

Figure S1. The SCCBs were derived from aqueous droplet templates that contain silica nanoparticles, their size could be customized from several to hundreds of microns, not only by changing the flow rate of microfluidic water or oil phases, but also by using different concentrations of the silica nanoparticles for droplet generation. Figure S1 (a) showed the relationships of the SCCB sizes and concentrations of the silica nanoparticles in 100 μm droplet templates; Figure S1 (b) showed the relationships of the SCCB sizes and droplet sizes with 10% w/v concentration of the silica nanoparticles.

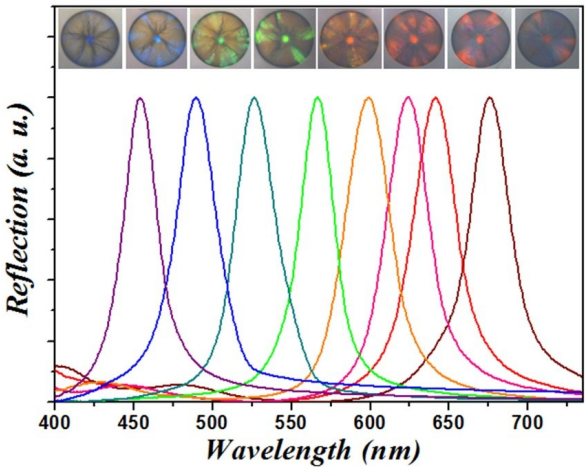

Figure S2. The reflection images and reflection spectra of eight kinds of the template SCCBs, these SCCBs were derived by using different size of silica nanoparticles for assembling in droplets.

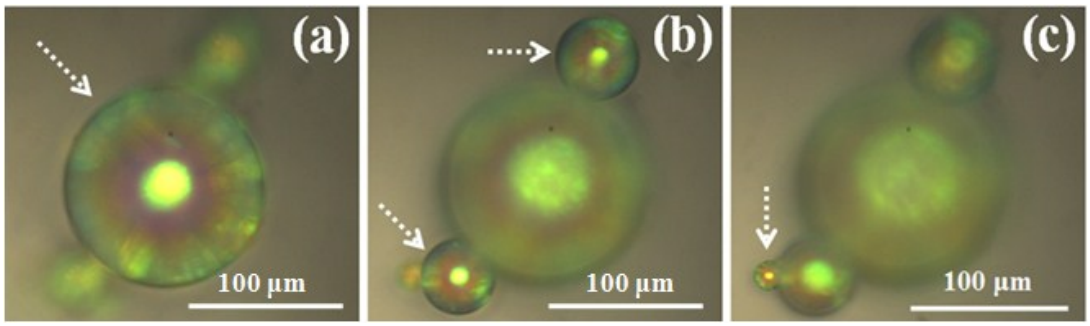

Figure S3. The reflection images of the opal particles with different sizes. By adjusting the focal length of the microscope, the structural colors of the opal particles with the size from hundred microns to several microns (from a to c) could be observed.

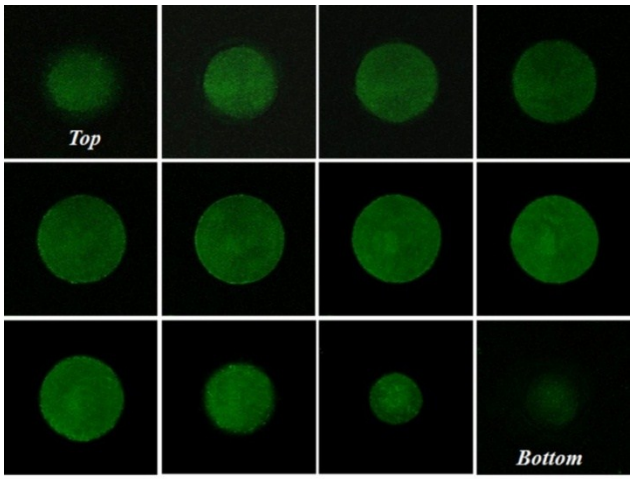

Figure S4.LSCM images of the FITC-dextran loaded pNIPAM hydrogel inverse opal particles. Optical slices 1–12 (parallel to horizontal) are indicative of the images taken in the z-direction from the top of the beads to the bottom.

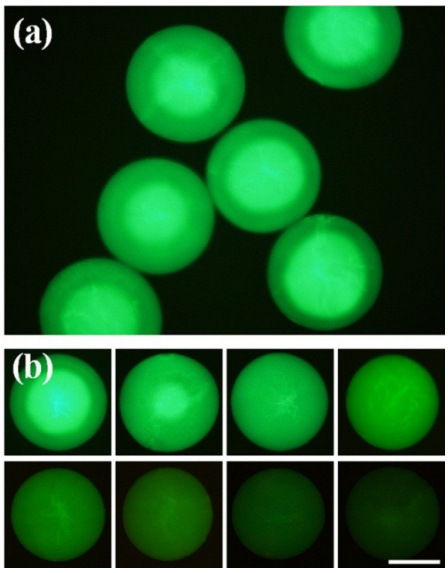

Figure S5.(a) Fluorescent image of the FITC-dextran loaded pNIPAM hydrogel inverse opal particles. (b) In vitro release of the FITC-dextran from the particles, the pictures were captured after drug encapsulating at 1, 4, 7, 10, 15, 19, 21 and 22 days, the encapsulated calcium alginate hydrogel concentration was 2% (w/v). Scale bar is 200 μm.

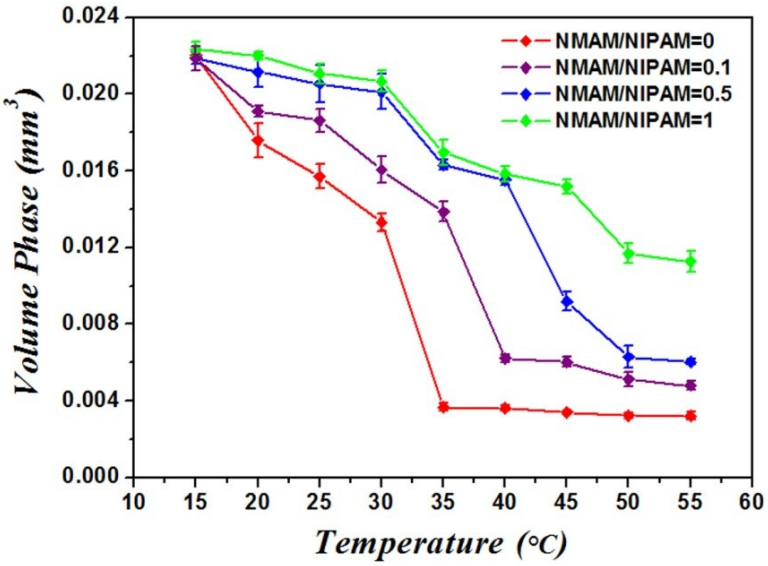

Figure S6. Volume size of the pNIPAM hydrogel inverse opal particles at different temperature.The LCST of PNIPAM could be tailored by adding different concentration of N-methylolacrylamide (NMAM) monomer into the hydrogel.

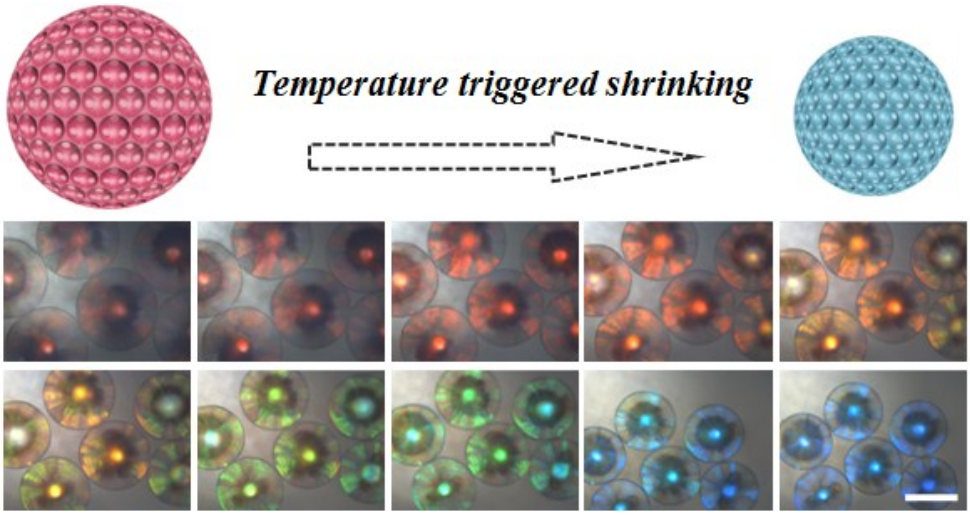

Figure S7. Temperature responsive characterize of the pNIPAM hydrogel inverse opal particles, the particles trend to shrink and generate a blue shift of their structural colors during a dynamic temperature increase process from room temperature to 55°C. Scale bar is 200μm.

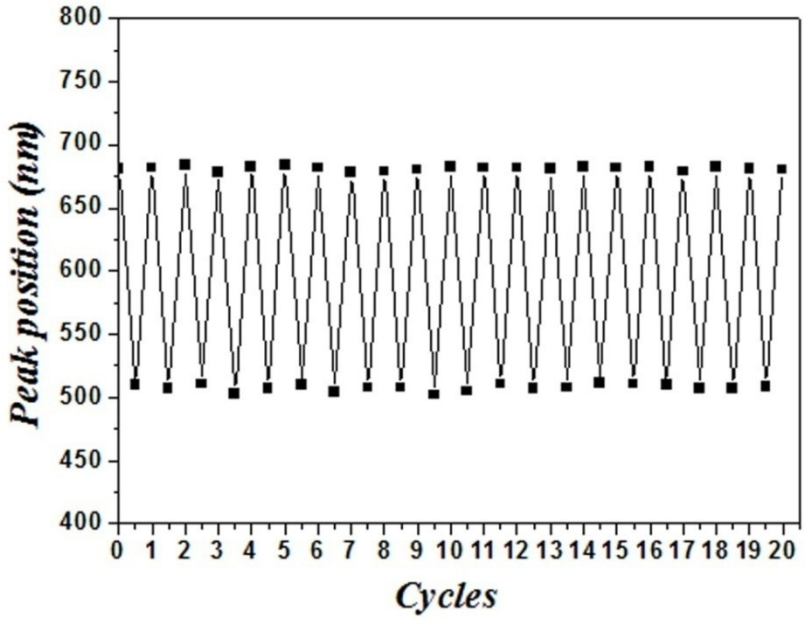

Figure S8. Reversible changes of the reflection peaks of the pNIPAM hydrogel inverse opal particles, they had good durability during more than fifty temperature cycles, twenty of them were shown in this figure.

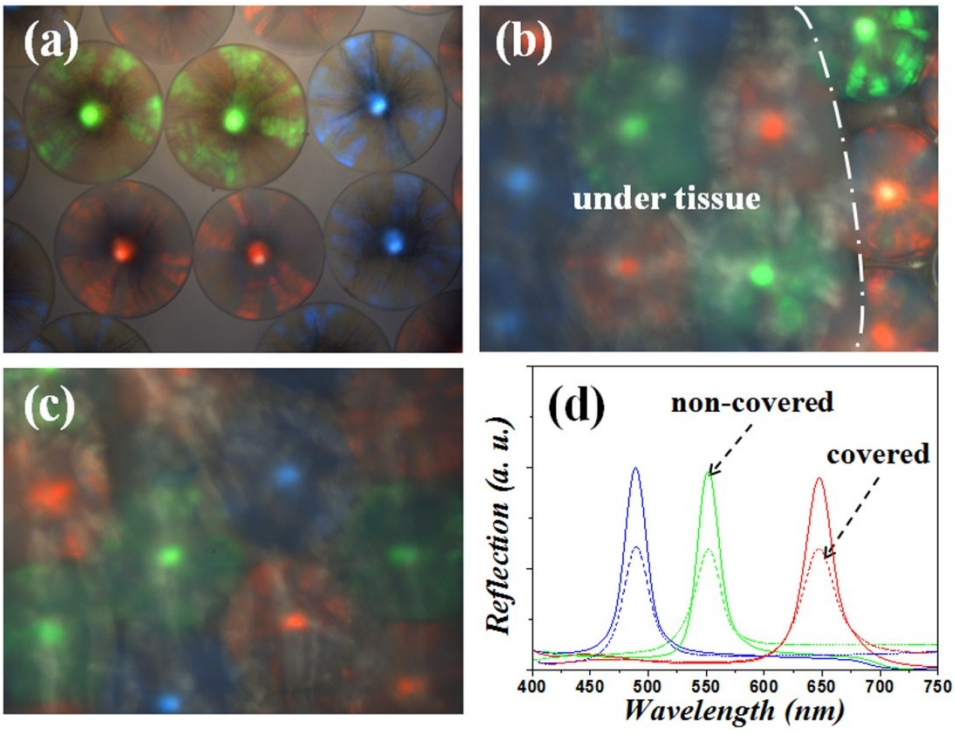

Figure S9.Reflection images and reflection spectra of opal particles: (a) uncovered particles; (b, c) particles covered with 2 mm and 5 mm of tissues; (d) reflection spectra of opal particles in (a) and (c). The reflections were decreased due to the high scattering of the tissue, while the peak positions were consistent.