Supporting Information

for

Dual responsive hydrogel based on functionalized mesoporous silica nanoparticles as an injectable platform for tumor therapy and tissue regeneration

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1. Materials

Doxorubicin hydrochloride (DOX.HCl), bovine serum albumin (BSA), rhodamine 6G, FITC labeled BSA, 3-(aminopropyl)triethoxysilane (APTES), 3-(trimethoxysilyl) propyl methacrylate (TMSPMA), 2-aminoethanethiol hydrochloride, aldrithiol-2, hyaluronic acid (HA, M_w = 10kDa), tetraethyl orthosilicate (TEOS), N-cetyltrimethylammonium bromide (CTAB), 1,3,5-trimethylbenzene (TMB) and polyacrylic acid (PAA, Mw = 45kDa), were purchased from Sigma-Aldrich, USA. All the chemicals were analytical grade and used without further treatment. N-isopropylacrylamide (NIPAM) (97 %, Aldrich) was purified by recrystallization from hexane and dried in a vacuum prior to use.

2. Synthesis of mesoporous silica nanoparticles (MSNs)

MSNs were prepared by the classical CTAB-templated, base-catalyzed sol-gel method[1]. The pH value of 1000 mL deionized water was adjusted to approximately 11

with 52.8 mL ammonium hydroxide (29 wt% NH3 in water). The temperature was raised to 323 K, and then 1.12 g CTAB and subsequent 5.8 mL TEOS were added with rapid stirring. After 2 h, the mixture was aged overnight, then centrifuged and washed thoroughly with distilled water and ethanol. As-synthesized silica nanoparticles were dispersed in ethanol by sonication for 30 min, followed by the addition of 20 mL of 1:1 mixture (v/v) of water and TMB. The mixture was placed in the autoclave, and kept at 140 °C for 4 days without stirring. The resulting white powder was washed with ethanol and water five times each. The surfactant templates were then removed by extraction using acidic methanol (9 mL of HCl/400 mL of methanol, 36 h) at 70 °C, which were further centrifuged, washed several times with ethanol and dried under vacuum for 20 h.

3. Modification of as-synthesized MSNs with functional groups[2]

Amino groups and C=C double bonds functionalized MSN (NH2-MSN–C=C) was obtained by refluxing 1 g of the resultant MSN-T with 2 mL of APTES and 0.1 ml MPS in 240 mL of ethanol for 12 h, followed by filtration, washing with copious ethanol, and drying overnight in a vacuum at 45 °C.

4.Cargos loading and polymer functionalization of MSNs

100 mg of as-synthesized NH_2-MSN–C=C, 300 mg of PNIPAM, and 7.5 mg of AIBN were successively added into 40 mL of acetonitrile. After 30 min of bubbling, the mixture was refluxed at 80℃ for 2 h. The resultant was separated by filtration and washing with copious ethanol. Then the product was dried in a vacuum at 45℃ for 12 h, to give PNIPAM-MSN-NH_2 as a white powder.

100 mg of as-synthesized PNIPAM-MSN-NH_2 and 25 mg of FITC-BSA were dispersed in 0.01 M sterile PBS (pH 7.4). The mixture was incubated at 37 ℃ under gentle shaking at 60 rpm for 24 h. After that, the suspension was washed with copious distilled water and then lyophilized for one day.

The growth factors loaded PNIPAM-MSN-NH_2 (90 mg) was dispersed in 10 mL of water, and then 150 mg of PAA was dissolved into the mixture. The mixture was stirred at room temperature for 24 h to form hydrogen bond between PAA and the amino group at the surface of PNIPAM-MSN-NH_2. After the reaction, the mixture(PNIPAM-PAA-MSNs)was centrifuged and washed with water for 5 times.

50 mg of as-synthesized growth factors loaded PNIPAM-MSN-NH_2 and 25 mg of doxorubicin (rhodamine 6G) was dispersed in 10 mL of water. The mixture was stirred at room temperature for 24 h. The resultant was separated by filtration and washing with copious water. Then the product was lyophilized for one day to give anti-tumor drug and growth factors co-loaded PNIPAM-PAA-MSNs.

5. **Stimuli-responsive hydrogel formation and structure transformation**

100 mg PNIPAM-PAA-MSNs nanoparticles were dispersed in 1 mL of PBS buffer solution (pH 7.4),then the heating (> 36 ℃) and/or cooling (< 36℃) were introduced to trigger the switch between hydrogel and nanoparticles solution, respectively.

In order to simulate the difference of physiological conditions between tumor tissue and healthy tissue, the PNIPAM-PAA-MSNs based hydrogel was incubated in PBS buffer with pH 6.8 and/or 7.4 at body temperature (36.5℃) for 12 h to investigated the structure transformation between loose network and compact network.

6. Stepwise cargo release

In the pH triggered cargo release experiment, a certain amount of PNIPAM-PAA-MSNs nanoparticles based hydrogel with FITC-BSA and Dox loading was immersed in 50 mL of 2 different types of PBS buffer (i: pH 6.8 and ii: pH 7.4) at 36.5 ℃. Subsequently, 2 mL of supernatant were taken at predetermined time intervals from the release medium, and replaced with the same volume of the fresh release medium. After centrifugation (15 000 rpm, 20 min), the UV-Vis spectroscopy was used to quantitatively analyze the release amount and release percentage.

7. Preparation of simulated tumor tissue (SCC cell spheroids)

The three dimensional culture systemswereestablished to resemble the biological and pathophysiological situation in human tissue. 96-well plates were pre-coated with 50 μL 1.5% agarose (Sigma-Aldrich, USA). SCC cells were cultured in Dulbecco's modification of Eagle's medium (DMEM, Invitrogen, USA) supplemented with 10% fetal bovine serum (FBS, Gibico, USA) and penicillin-streptomycin (100 U/mL and 100 μg/mL, Gibico, USA), and incubated at 37^{o}C in 5% CO_2. For cell cytotoxicity analysis, the cells were seeded at 3×10^3 per well onto agarose-coated 96-well plates. After incubating plates for 4 days, using a phase-contrast microscope to monitor the formation of mutlcelluar spheriods.

8. *In vitro* cytotoxicity analysis

After SCC cell spheroids were cultured in agarose-coated 96-well plates for 4 days, the spheroids were exposed to PNIPAM-PAA-MSNs hydrogel, Dox-loaded PNIPAM-PAA-MSNs hydrogel and BSA/Dox co-loaded PNIPAM-PAA-MSNs hydrogel, with

equal concentrations of Dox (0.5 μg/mL). All the samples were sterilized before cell experiments by UV irradiation (254 nm) for 30 min. Cell viability was measured by using a DNA quantification kit (Sigma-Aldrich, USA) according to the manufacture's protocol at 12h, 24h, 48h, 72h and 96h. The fluorescence of DNA content was read by using Varioskan Flash multimode reader (Thermo Fisher Scientific, USA).The excitation wavelength was 360 nm and emission wavelength was 460 nm.

9. Cell/hydrogel interactions *in vitro*.

We used rabbit BMSCs for the cell and hydrogel interaction examination in different physiological conditions (36.5 with pH 6.8 and pH 7.4). Rabbit BMSCs were obtained from tibiae bone marrow using density gradient centrifugation and then suspended in α-minimum essential medium with 10% fetal bovine serum (FBS, Gibco BRL, Gaithersburg, MD) and incubated at 37 °C with 5% CO_2 in humidity. The BMSCs were split, purified, and re-suspended for passage until they reached 90% confluence. Cells of passage 3 were used for the following *in vitro* study.

To examine BMSC cellular morphology on the PNIPAM-PAA-MSNs hydrogel at pH 6.8 and pH 7.4, cell cytoskeleton and nucleus was stained for fluorescent visualization. Briefly, the BMSCs were seeded at a density of 5×10^4/mL in the hydrogel solution. And then, the mixture was incubated in α-minimum essential medium with 10% FBS at 37 °C with pH 6.8 or pH 7.4. After 2 d of incubation, the cell contained hydrogel were fixed in 4% formaldehyde for 30 min at room temperature and washed twice with PBS. Next, the samples were treated with trinitrotoluene in PBS for 30 min and rinsed twice with PBS. The samples were incubated with rhodamine-phalloidin (diluted as

1:100, Thermo Fisher Scientific, USA) to stain the F-actin of cytoskeleton for 15 min, and the nucleus were subsequently stained with 4', 6-diamidino-2-phenylindole (DAPI, Life Technologies, USA). Prior to visualization under a fluorescence microscope(IX70; Olympus, Tokyo), the samples were washed four times with PBS.

10. **Characterization**

Transmission electron microscopy (TEM) images were recorded on a Philips CM200 transmission electron microscope operated at 200 kV. For the TEM observation, samples were obtained by dropping 5 μL of solution onto carbon-coated copper grids. All the TEM images were visualized without staining. The ultraviolet-visible (UV-Vis) spectra were measured with dilute aqueous solution in a 2 mm thick quartz cell using a HitachiU-2910 spectrophotometer. Dynamic light scattering (DLS) were measured by a Malvern Zetasizer Nano Series running DTS software and using 4 mW He–Ne laser operating at a wavelength of 633 nm and avalanche photodiode (APD) detector. The infrared (IR) spectra were measured by AVATAR 320 FT-IR using KBr pellets. All pH value measurements were carried out on a Sartorius BECKMAN F 34 pH meter. The cargos distribution and *in vitro* cell/hydrogel interactions was respectively monitored by fluorescence microscopy using an Olympus BX51 microscope equipped with a fluorescent lamp.

[1] aM. Vallet-Regi, A. Ramila, R. P. del Real, J. Perez-Pariente, *Chem Mater* **2001**, *13*, 308-311;bM. H. Kim, H. K. Na, Y. K. Kim, S. R. Ryoo, H. S. Cho, K. E. Lee, H. Jeon, R. Ryoo, D. H. Min, *Acs Nano* **2011**, *5*, 3568-3576;cX. Chen, A. H. Soeriyadi, X. Lu, S. M. Sagnella, M. Kavallaris, J. J. Gooding, *Adv Funct Mater* **2014**, *24*, 6999-7006.

[2] H. W. Li, J. Z. Zhang, Q. Q. Tang, M. Du, J. H. Hu, D. Yang, *Mat Sci Eng C-Mater* **2013**, *33*, 3426-3431.

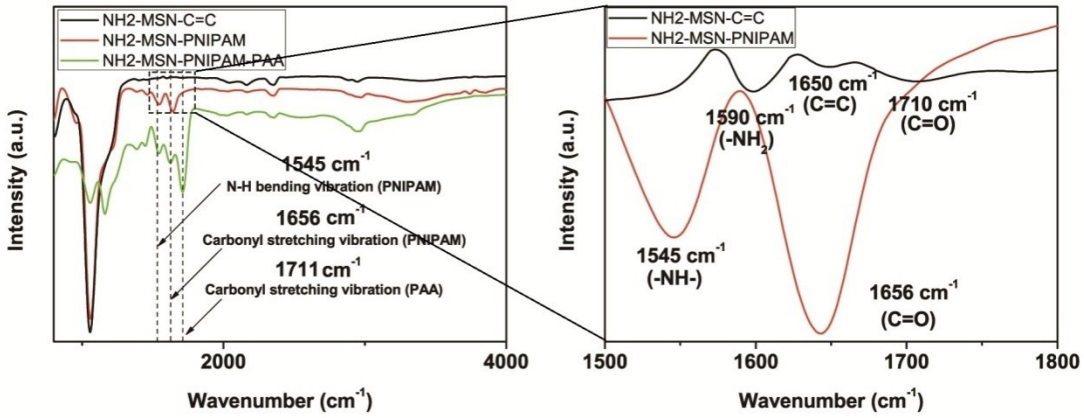

Figure S1. The fourier transform infrared spectroscopy （FTIR）of amino groups and unsaturated linkage functionalized MSN vehicles (NH2-MSNs-C=C, black curve), PNIPAM polymerized MSN vehicles (PNIPAM-MSN, red curve), and PAA/PNIPAM co-modified MSN vehicles (PNIPAM-PAA-MSNs, green curve).

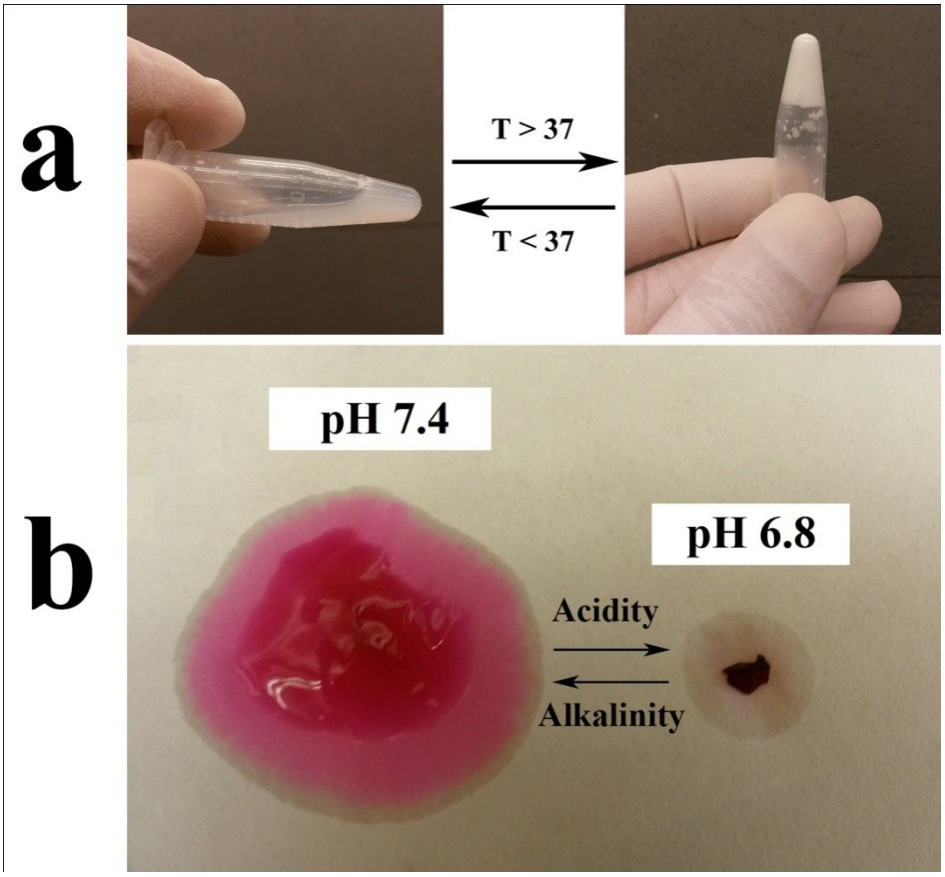

Figure S2. (a) Photographs of PNIPAM-PAA-MSNs nanoparticles solution and PNIPAM-PAA-MSNs nanoparticles based hydrogel presenting the thermal-induced structure transformation. (b) Photographs of PNIPAM-PAA-MSNs nanoparticles based hydrogel under different pH value indicating pH-induced swelling and shrinking of the

hydrogel.

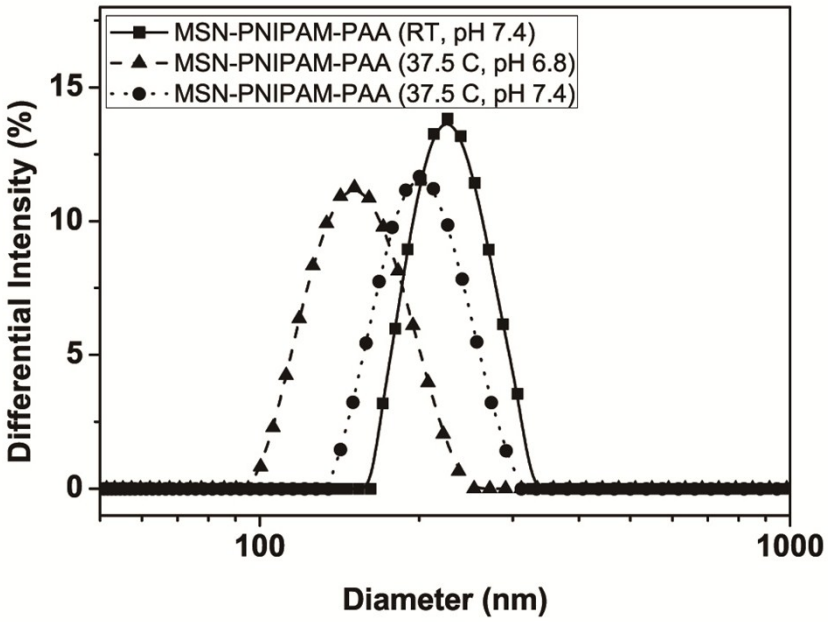

Figure S3. Dynamic light scattering (DLS) of PNIPAM-PAA-MSNs nanoparticles in PBS at pH 7.4 under room temperature (square curve), at pH 6.8 under body temperature (triangle curve) and at pH 7.4 under body temperature (circle curve).

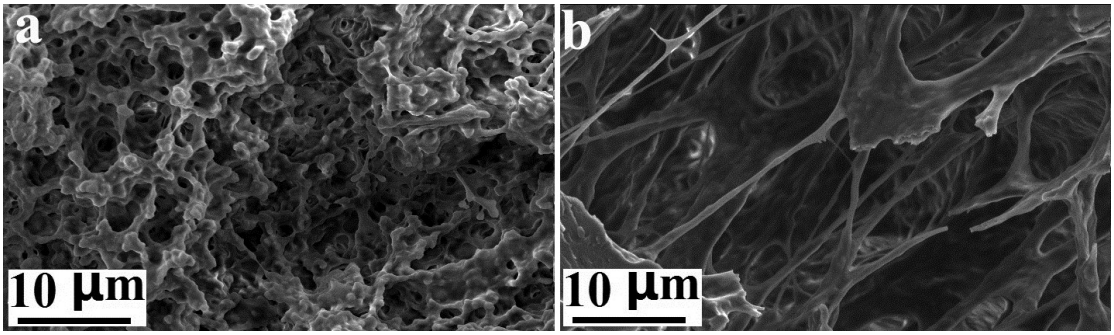

Figure S4. Scanning electron microscopy images of hydrogel formed by PNIPAM-PAA-MSNs nanoparticles in 10% fetal bovine serum at pH 6.8 (a) and pH 7.4 (b).

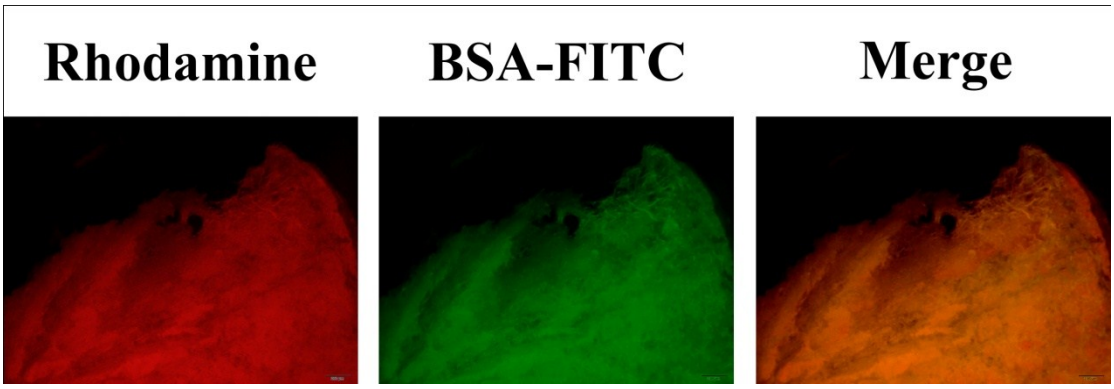

Figure S5. Fluorescent microscope images of rhodamine (model drug) and FITC-BSA (model growth factor) co-loaded PNIPAM-PAA-MSNs hydrogel, indicating the homogeneous distribution of rhodamine and FITC-BSA within the gel structure.

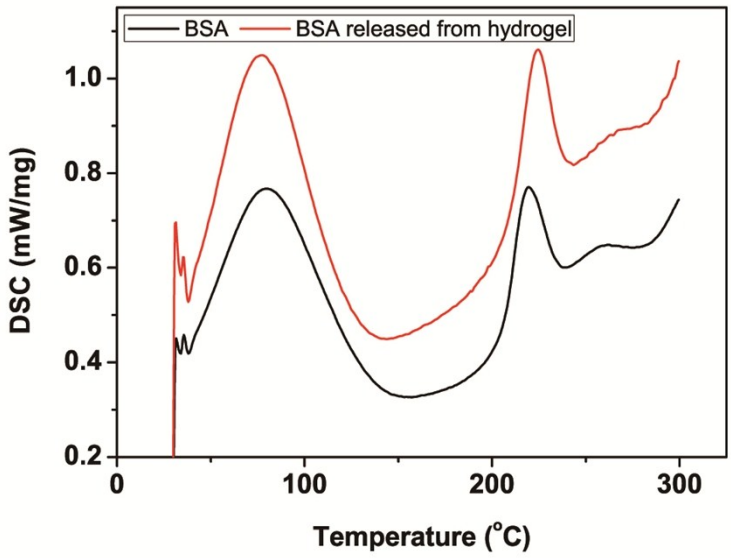

Figure S6. Differential scanning calorimetry results of commercially available BAS before loading (Black curve) and after release (Red curve) from the PNIPAM-PAA-MSNs nanoparticles/hydrogel.

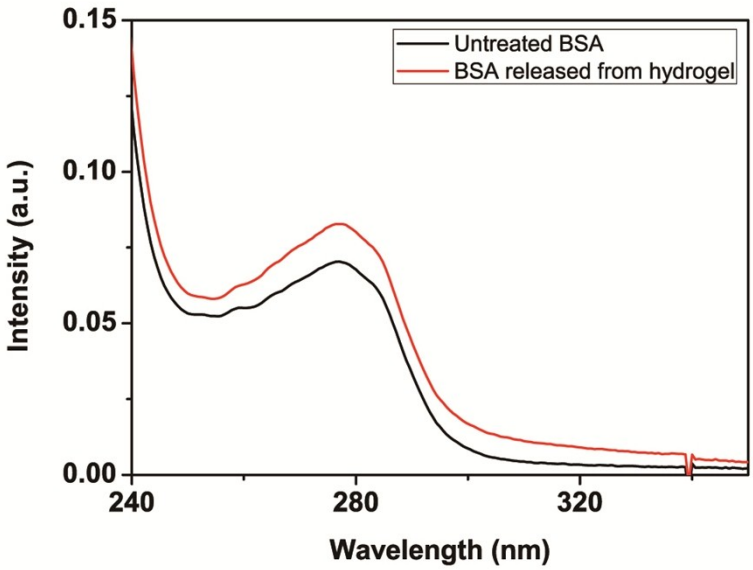

Figure S7. UV-Vis spectroscopy of commercially available BAS before loading (Black curve) and after release (Red curve) from the PNIPAM-PAA-MSNs nanoparticles/hydrogel.

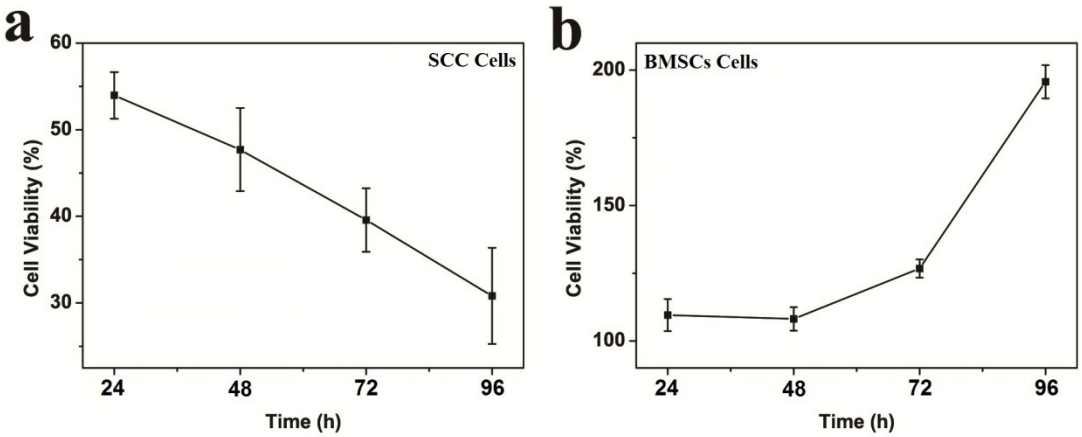

Figure S8. a) Time-dependent cytotoxicity of Dox/BSA co-loaded PNIPAM-PAA-MSNs hydrogel on the viability of human squamous carcinoma cell spheroids (SCC, simulated tumor tissue) with 0.5 μg/mL Dox. b) Time-dependent proliferation of bone mesenchymal stem cells (BMSCs) on the cell viability promoted by the Dox/BSA co-loaded PNIPAM-PAA-MSNs hydrogel after 10 days incubation with SCC cells.