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A versatile UCST-type composite microsphere for image-guided chemoembolization and photothermal therapy against liver cancer

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Preparation of polydopamine coated SPION

To prepare polydopamine coated SPION (SPION@PDA), 30 mg of dopamine was mixed with 25 mg of SPION in 250 mL of 10 mM PBS (pH 8.5). After stirring for 6 hours at room temperature, the SPION@PDA was obtained by centrifugation and was rinsed with water for three times.

Characterizations

X-ray photoelectron spectra (XPS) of the SPION and SPION@PDA nanoparticles were obtained on the Nexsa™ X-Ray Photoelectron Spectrometer System (XPS, Thermo Scientific, USA) with a vacuum level of ~2×10^{-9} mbar, a monochromatic Al Kα X ray source, with the energy of 1486.6eV, 12kV, 720W, Constant Analyzer Energy (CAE) Scan mode and Standard Lens mode. The specimens were pressed into sheets and stuck to aluminum foil, affixing to the test bench with double-sided tape. A scanning electron microscope (SEM, JSM-6330F, JEOL, Tokyo, Japan) and a transmission electron microscope (TEM, JEM-2010HR, JEOL, Tokyo, Japan) were used to characterize the morphology and the microstructure of SPION@PDA. The photothermal effects of SPION and SPION@PDA were determined by laser irradiation of 808 nm and 1.0 W/cm^2 for 10 minutes at a concentration of 3.0 mg/mL.

The diameters of the P(AAm-*co*-AN) microspheres during heating/cooling procedure were observed by an optical microscope equipped with a heating table (DM2500p, Leica, Germany) and statistically analyzed by ImageJ. The heating/cooling rate was 5 ℃/min, and the samples were held for 1 minute under each testing

temperature. It was found that the average diameters of the microspheres were 196.8 μm, 197.5 μm, 201.1 μm, 202.9 μm, and 205.9 μm at 25℃, 37℃, 45℃, 55℃, and 65℃, respectively, during the heating procedure. In the cooling procedure, the average diameters were 205.9 μm, 203.3 μm, 202.5 μm, 198.0 μm, and 196.1 μm at 65℃, 55℃, 45℃, 37℃, and 25℃, respectively. The changes of the microsphere volume along with temperature were derived from the that in diameter. It was calculated that the volumetric alterations of the microsphere as temperature raised were +1.07%, +6.70%, +9.59%, and +14.52% at 37°C, 45°C, 55°C, and 65°C, respectively, when compared with the initial volume at 25°C. During the cooling process, the volumetric alterations when compared with the initial volume were determined as +14.52%, +10.24%, +8.94%, +1.84%, and -1.06% at 65°C, 55°C, 45°C, 37°C, and 25°C, respectively (Figure S1G). These results verified the thermo-responsive behavior of UCST property that the intermolecular interactions between the side groups of crosslinked-polymer became weaker when the temperature increased and *vice versa*.

The encapsulation efficiency of the of P(AAm-*co*-AN) and P(AAm-*co*-AN)/(SPION@PDA) were found to be 27.6 ± 5.1% and 20.1 ± 7.4%, respectively (Figure S1H).

Table S1 Longest viable tumor diameters

Groups	Rabbits	Baseline (mm)	Follow-up (mm)	Changes (mm)	Ratio of changes (%)
Control	1	12.0	24.7	12.7	105.8
	2	14.3	22.3	8	55.9
	3	9.4	18.6	9.2	97.9
	AVG ± SD[a]	11.9±2.45[b]	21.9±3.07	9.97±2.44	86.5±26.8
Treatmen	4	11.2	0	-11.2	-100
t (1 week)	5	14.1	0	-14.1	-100
	6	8.92	0	-8.92	-100
	7	12.6	0	-12.6	-100
Treatmen	8	12.3	0	-12.3	-100
t	9	12.9	0	-12.9	-100
(2 week)	10	10.8	0	-10.8	-100
	11	16.1	10.9	-5.2	-32.3
	AVG ± SD[a]	12.36±2.17[b]	1.36±3.85	-11.0±2.81	-91.5±23.9

[a] AVG: average; SD: standard deviation.

[b] $p > 0.05$

Table S2 Liver enzyme and biochemical examinations

Factors[a]	Day 0	Day 1	Day 4	Day 7	Day 14	*p*[b]
ALT	57.4±31.5	314.9±56.5	459.3±212.8	372.2±305.7	76.6±16.0	0.22
AST	28.4±12.2	354.2±78.9	413.0±341.8	220.0±230.5	35±10.7	0.39
ALP	86.0±27.8	96.5±25.1	108±40.2	115.3±38.0	85.0±17.8	0.96
GGT	8.0±1.8	16.6±6.7	38.0±27.2	38.4±45.2	39.9±38.4	0.19
ALB	34.1±3.0	35.4±1.8	34.0±1.7	36.60±4.6	34.8±4.2	0.83
TBIL	2.65±0.45	1.05±0.64	1.28±0.46	2.25±1.14	2.35±0.48	0.55

[a] Abbreviations and units. ALT, alanine aminotransferase (U/L); AST, aspartate aminotransferase (U/L); ALP, alkaline phosphatase (U/L); GGT, gamma glutamyl transpeptidase (U/L); ALB, albumin (g/L); and TBIL, total bilirubin (μmol/L).

[b] Day 0 *versus* Day 14.

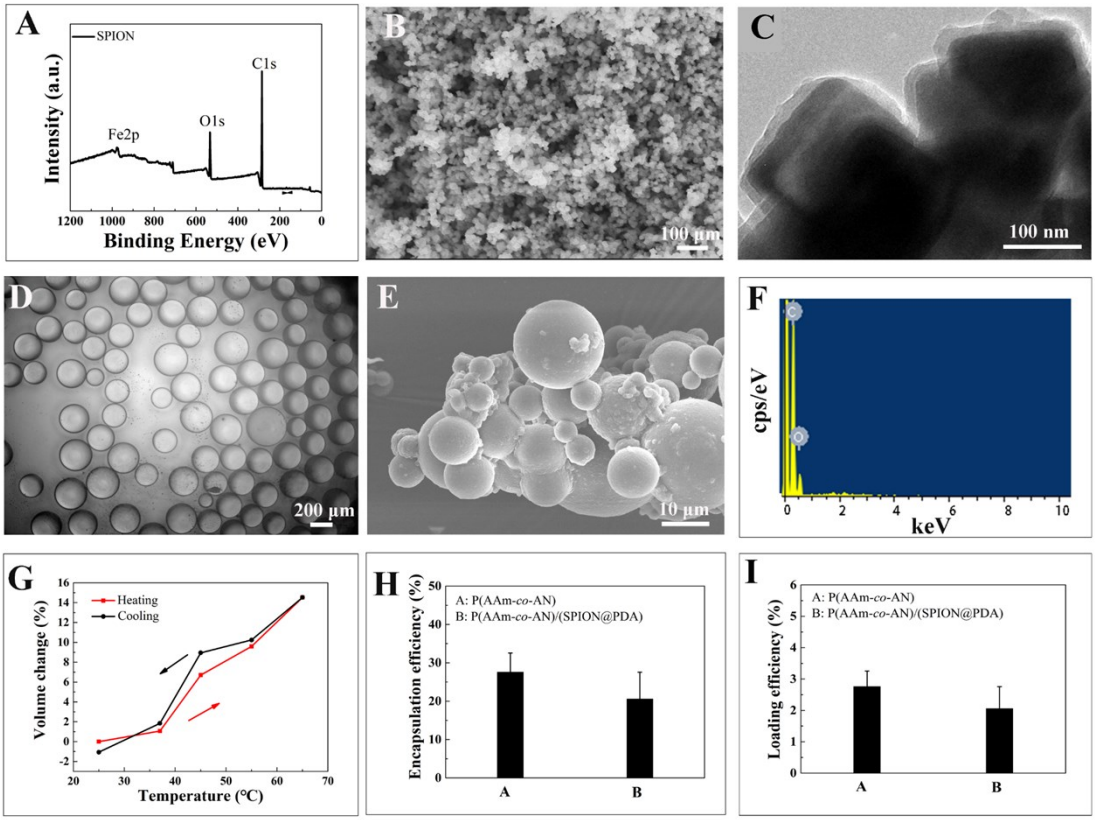

Figure S1. Characterization of microspheres. (A) XPS spectrum of SPION, (B) SEM and (C) TEM images of SPION@PDA. (D) Optical image, (E) SEM image, (F) SEM-EDS spectrum, and (G) volumetric alteration of P(AAm-*co*-AN) microspheres during heating/cooling procedure. (H) Drug encapsulation efficiency and (I) drug loading efficiency of microspheres.

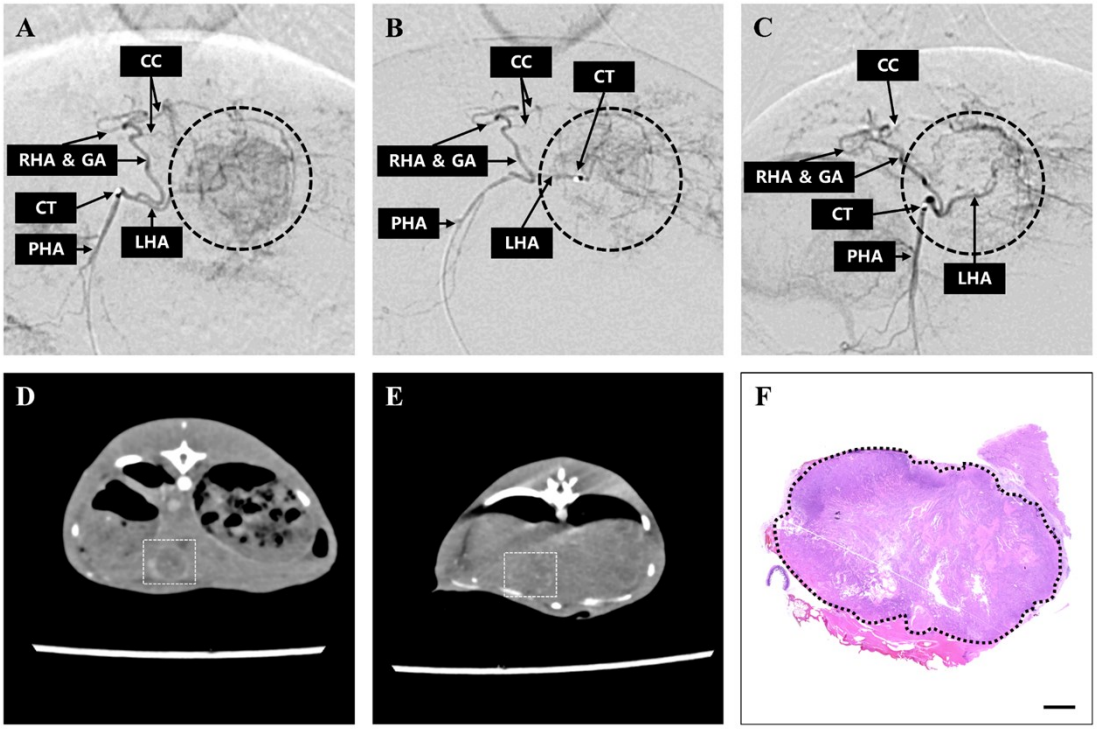

Figure S2. Incomplete embolization due to collateral circulation, leading to a suboptimal treatment outcome. (A) Selective PHA and (B) super-selective LHA angiography before TACE. (C) Selective PHA angiography after TACE. Hepatic arterial phase CT images (D) before and (E) 2 weeks after treatment. White dotted squares indicated tumor locations. (F) HE staining of the tumor with insufficient embolization, scale bar: 2.0 mm. Abbreviations. PHA, proper hepatic artery; RHA, right hepatic artery; LHA, left hepatic artery; GA, gallbladder artery; CC, collateral circulation; CT, catheter tip.