Supplementary Material (ESI) for Journal of Materials Chemistry

Supporting information for

Biocompatiable Hollow Silica Microsphere as A Novel Ultrasound Contrast Agent for *In Vivo* Imaging

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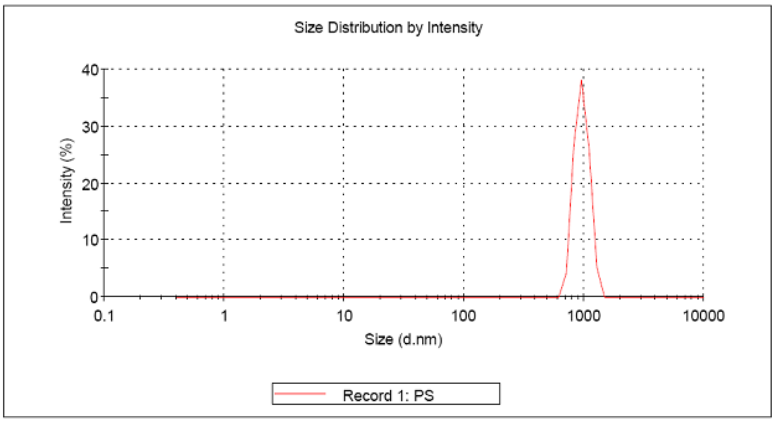
System

Temperature (°C):	25.0	Duration Used (s):	60
Count Rate (kcps):	267.8	Measurement Position (mm):	4.65
Cell Description:	Disposable sizing cuvette	Attenuator:	10

Results

			Diam. (nm)	% Intensity	Width (nm)
Z-Average (d.nm):	1192	Peak 1:	966.0	100.0	135.4
PdI:	1.000	Peak 2:	0.000	0.0	0.000
Intercept:	0.892	Peak 3:	0.000	0.0	0.000

Result quality Refer to quality report



System

Temperature (°C):	25.0	Zeta Runs:	100
Count Rate (kcps):	505.3	Measurement Position (mm):	2.00
Cell Description:	Clear disposable zeta cell	Attenuator:	6

Results

			Mean (mV)	Area (%)	Width (mV)
Zeta Potential (mV):	2.17	Peak 1:	2.17	100.0	26.2
Zeta Deviation (mV):	26.2	Peak 2:	0.00	0.0	0.00
Conductivity (mS/cm):	5.59e-4	Peak 3:	0.00	0.0	0.00

Result quality See result quality report

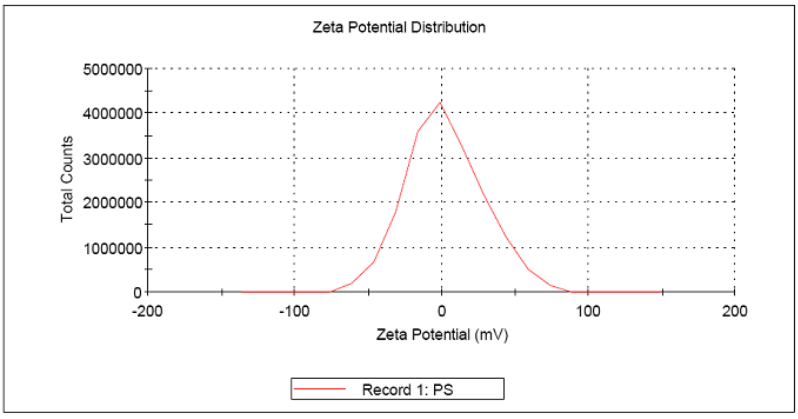


Figure S1. The size distribution and Zeta potential of surface positively charged polystyrene (PS) template.

Figure S2. Zeta potential of (A) pure SiO_2 hollow sphere, (B) SiO_2-NH_2 hollow spheres, (C) PEG-HSS and (D) PEG-HSS-RB.

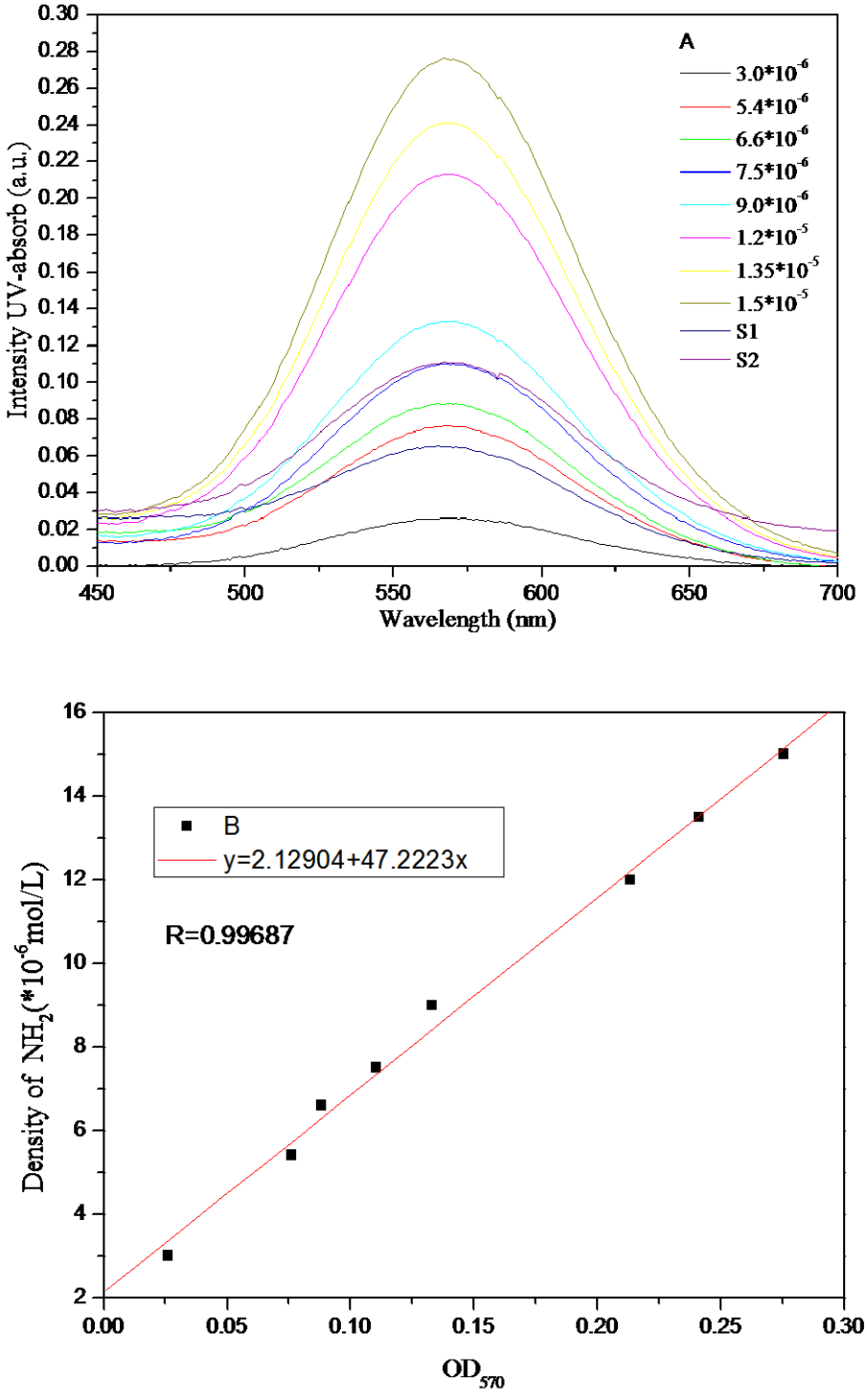


Figure S3. (A) Absorption curve of standard samples and as-prepared samples, (B) Working curve of the standard samples with the max absorption intensity at 570 nm.

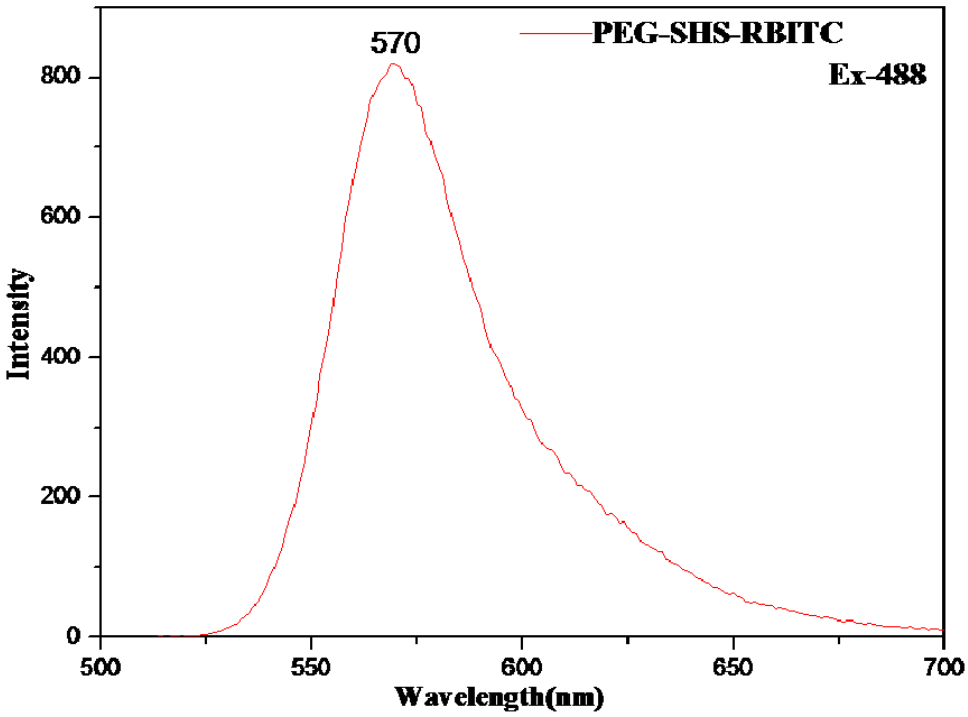


Figure S4. The room temperature photoluminescence (PL) spectra of PEG-HSS-RB with concentration of 100 μg mL^{-1} in aqueous excited at 488 nm.

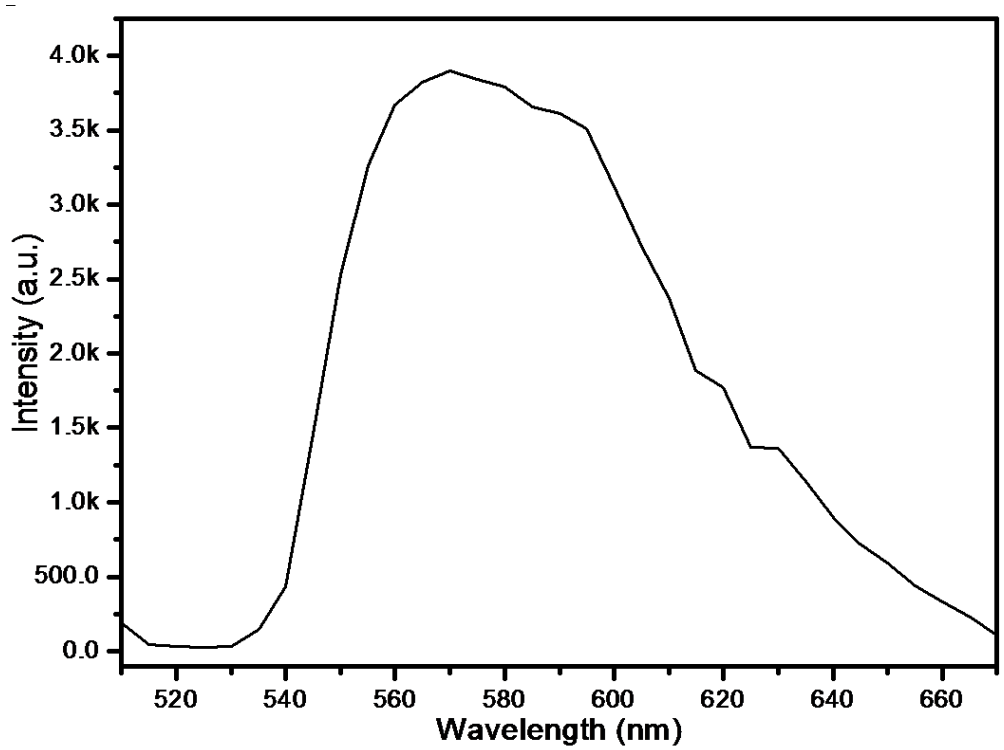


Figure S5. Fluorescent spectra of PEG-HSS-RB in HeLa cells measured during LCSM imaging.

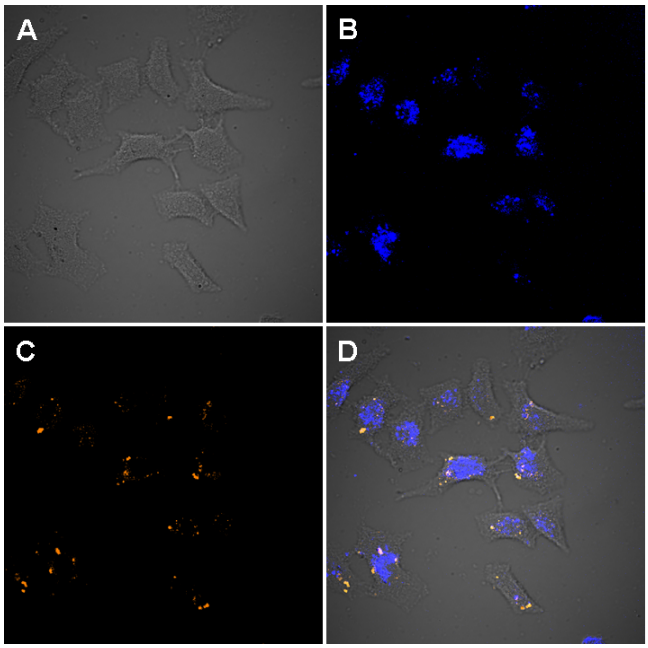


Figure S6. LCSM images of HeLa cells cultivated with 120 μg mL^{-1} PEG-HSS at 4 °C (λ_{ex} = 488 nm) for 20 min as control. (A) Brightfield image of cells, (B) blue fluorescence image of DIPA dye form cell nucleus, (C) orange fluorescence image of RB, (D) overlay imaging of (A), (B) and (C).

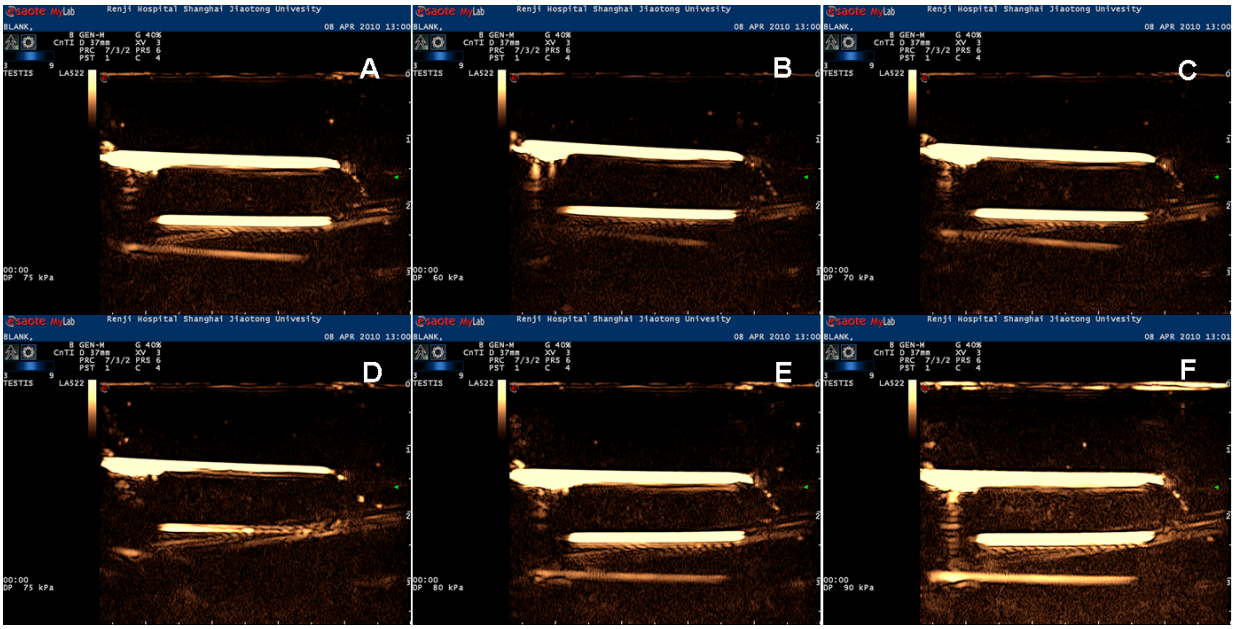


Figure S7. The *in vitro* ultrasound imaging of human blood at different Mechanical Index (MI) of (A) 0.060, (B) 0.070, (C) 0.090, (D) 0.10, (E) 0.11, (F) 0.13 as blank control.

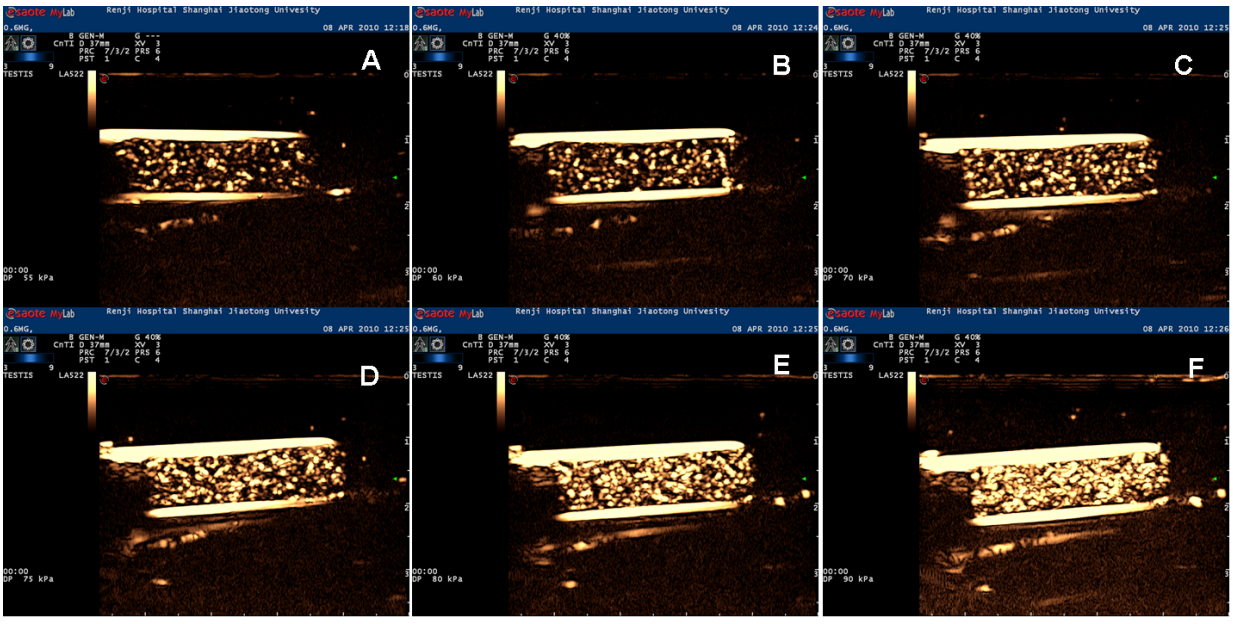


Figure S8. The *in vitro* ultrasound imaging of 0.6mg/mL of PEG-HSS at different Mechanical Index (MI) of (A) 0.060, (B) 0.070, (C) 0.090, (D) 0.10, (E) 0.11, (F) 0.13 in human blood.

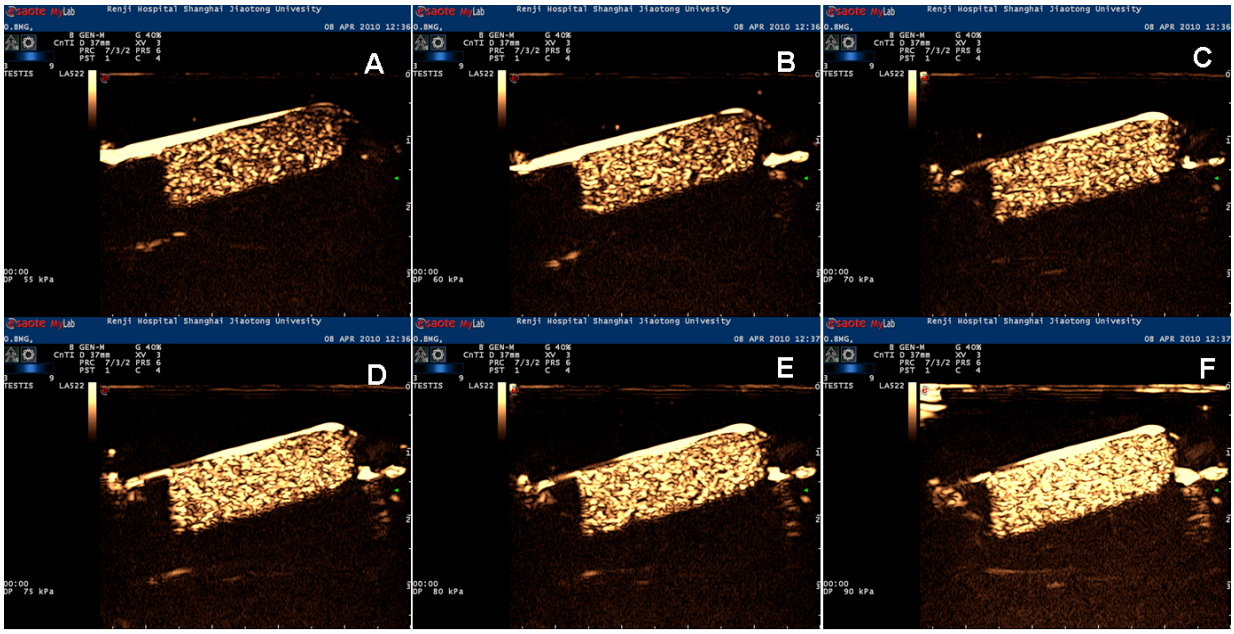


Figure S9. The *in vitro* ultrasound imaging of 0.80 mg/mL of PEG-HSS at different Mechanical Index (MI) of (A) 0.060, (B) 0.070, (C)0.090, (D) 0.10, (E) 0.11, (F) 0.13 in human blood.

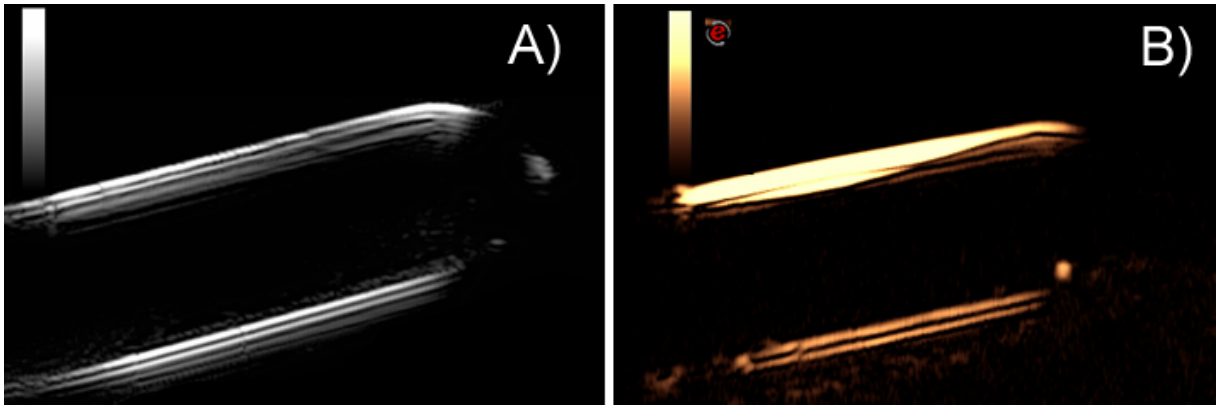


Figure S10. *In vitro* ultrasound images of PS@SiO_2 with concentration of about 5×10^4 particles per 1 mL in physiological saline solution under the 2D (A) and CEUS (B) imaging mode (frequency of 6 MHz and MI of 0.060).

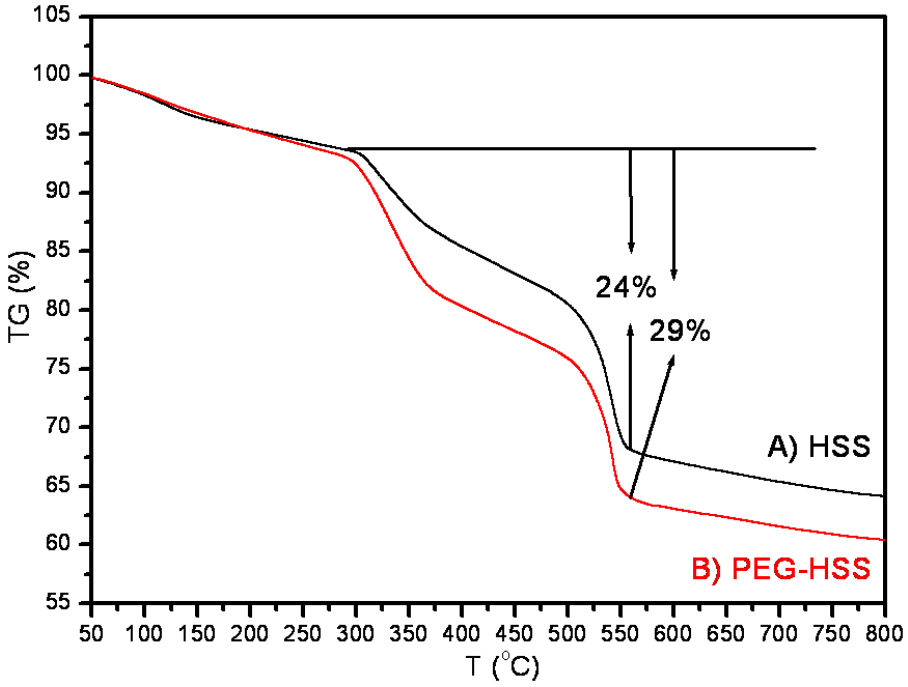


Figure S11. The TGA data of (A) HSS and (B) PEG-HSS.

The early weight loss at temperatures below 150 ^{o}C is seen from TG curve due to loosely bound water, followed by a steady weight loss from about 300 to 550 ^{o}C were mainly due to the decompositions of organics (aminopropyl groups and PEG chains).[1-3] The organics content of HSS and PEG-HSS is 24 % and 29 % respectively. The conjugated PEG was about 5 % according to the TGA data. If the amino-groups conjugated with the PEG-COOH (Mt = 2000) completely, the theoretical content of PEG chains of the PEG-HSS should be 15 % which is calculated based on the density of amino- groups(~ 8.65×10^{-5} mol/g). Then, only one third of amino-groups conjugated with PEG successfully. However, the content of organics (24 %) in HSS may be partly attributed to the incompletely dissolved PS chains stacked in the porous of silica shells.

Reference:

1. Xiong, H.-M.; Liu, D.-P.; Zhang, H.; Chen, J.-S., Polyether-grafted SnO_2 nanoparticles designed for solid polymer electrolytes with long-term stability. *J. Mater. Chem.* **2004,** 14, (18), 2775-2780.

2. Rui Zhang, T.; Feng, W.; Lu, R.; Yan Bao, C.; Jin Li, T.; Ying Zhao, Y.; Nian Yao, J., Thermochromic Organoaminomodified Silica Composite Films Containing Phosphomolybdic Acid. *J. Solid State Chem.* **2002,** *166 (1)*, 259-263.

3. Lay, C. L.; Liu, H. Q.; Wu, D.; Liu, Y., Poly(ethylene glycol)-Graft-Hollow Silica Vesicles for Drug Delivery. *Chem. Eur. J.* **2010,** *16,* 3001-3004.