One-Pot Ultrasonic Synthesis of Multifunctional Microbubbles and Microcapsules Using Synthetic Thiolated Macromolecules

Francesca Cavalieri,^{a,b,c} Meifang Zhou,^b Frank Caruso,^c* and Muthupandian Ashokkumar^b*

Materials

Materials. Poly(methacrylic acid, sodium salt) (PMA), Mw 15 kDa, was purchased from Polysciences. Cystamine hydrochloride, dithiothreitol (DTT), *N*-hydroxysuccinimide (NHS), and N-(3-dimethylaminopropyl)-N'-ethylcarbodiimide hydrochloride (EDC), tris(hydroxymethyl)amino methane (Tris buffer), perfluorohexane, doxorubicin hydrochloride were purchased from Sigma-Aldrich and used as received. High-purity water with a resistivity greater than 18 M Ω cm was obtained from an in-line Millipore RiOs/Origin system.

Polymer Synthesis (PMA_{SH}). PMA samples with varying thiol content (mol %) were synthesized by the modification of PMA with cystamine. Briefly, the PMA solution (475 mg of

30 wt % solution) was diluted with 5 mL of potassium phosphate buffer (0.1 M, pH 7.2). The resulting solution was charged with EDC (100 mg) and NHS (65 mg), and the mixture stirred for 15 min. After this time, cystamine hydrochloride (14–72 mg) was added to the mixture and the reaction was allowed to proceed overnight. The resulting mixture was dialyzed extensively against distilled water and the polymer was isolated via freeze-drying. The degree of functionalization was estimated from ¹H NMR, and corresponds to 5, 10, and 30 mol % modification. ¹H NMR (D₂O) δ (ppm): 0.8 -CH₃ from PMAA; 1.6 -CH₂ from PMAA; 1.9; 2.5-3.4 -CH₂ from cystamine.

Microbubbles and Microsphere Preparation. An aqueous solution of thiolated polymethacryclic acid (PMA_{SH}) (1 mL at 4% w/v) was treated in 50 mM of trishydroxymethylaminomethane-Tris-HCl buffer (pH 8) containing 60 mg DTT for 2 min. The pH of the solution was adjusted to \sim 3 and then 50 µL of perfluorohexane (PFH) was added to the solution. A 20 kHz ultrasound generator

(Branson) with a microtip (3 mm in diameter) horn and adjustable power intensity was used for microbubbles and microcapsule preparation. The tip of a high-intensity ultrasonic horn was positioned at PFH-water interface and the sample was sonicated at an applied power of 160 W for

30 s. The microbubbles and microspheres were separated from the unreacted polymer by flotation and precipitation, and repeated washing with MilliQ water. After sonication, the reaction mixture was transferred to a separation funnel, MilliQ water was added and the contents were mixed by shaking the funnel. The mixture was then left standing for a few hours for the oil-filled microcapsules to settle down and the air-filled microspheres to float to the surface of the liquid. The bottom and top layers were then separated and the microcapsules and microspheres were collected from the bottom and top layers of the liquid, respectively.

Microbubbles and Microspheres Characterization

An inverted Olympus IX71 wide field fluorescence microscope with a 60× objective lens was used to view the microbubbles (Figure S1). Microbubbles average diameter and standard deviation were determined over a set of 100 microparticles using optical microscope images (Figure S2). The free thiol contents were determined by using Ellman's reagent, DTNB 5-5'-dithiobis(2-nitrobenzoic acid).



Fig. S1. Optical microscope image of PMA_{SH30} microbubbles.



Fig. S2. Size distribution of aggregates formed upon PMA_{SH30} and PMA_{SH10} phase separation at pH 3 prior to sonication. Ultrasound treatment causes these aggregates to be broken down into smaller sized nanoparticles by the physical forces generated during acoustic cavitation.

Zeta-potential measurements were carried out on a Malvern Zetasizer. Dynamic light scattering (DLS) analysis of PFH- filled microcapsules and PMA_{SH} aggregates was performed on a Malvern Zetasizer equipped with a single angle backscatter system and a temperature controller.

A scanning electron microscope (SEM) (FEI Quanta) operated at an acceleration voltage of 10 kV was used to examine the morphology of the microbubbles. SEM images were recorded on air-dried microbubbles sputter-coated with a thin gold film.

Sonication at 1 MHz at 1 W/cm² was used to test the ultrasound response of a 0.5 mg/mL microbubble suspension. The collapsed microbubbles were collected at the bottom and analysized by SEM (Figure S3).



Fig. S3. SEM image of collapsed PMA_{SH30} microbubbles destruction upon ultrasound irradiation (1 MHz , 1 W/cm²)

Drug Loading on PMA_{SH} microbubbles and microcapsules. In a typical preparation process, 1 mL of suspension (0.4 mg/mL) were incubated with doxorubicin at a concentration of 0.09 mg/mL overnight. After washing, the microparticles were washed several times with Milli-Q water and the doxorubicin (DX) loading (50 μ g/mg) was quantified spectrophotomerically using the appropriate extinction coefficient, ε_{485} = 11500 M⁻¹ cm⁻¹. DX release was monitored by measuring the time dependence of the absorbance at 485 nm.