Supplemental Information: Hard shell gas-filled contrast enhancement particles for colour Doppler ultrasound imaging of tumors

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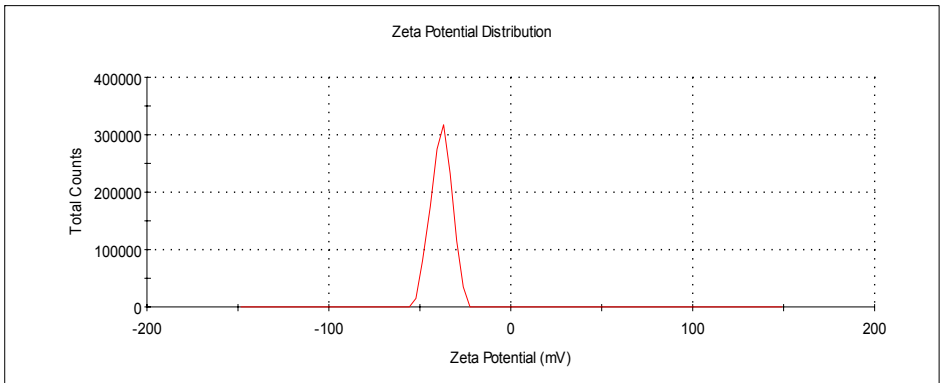
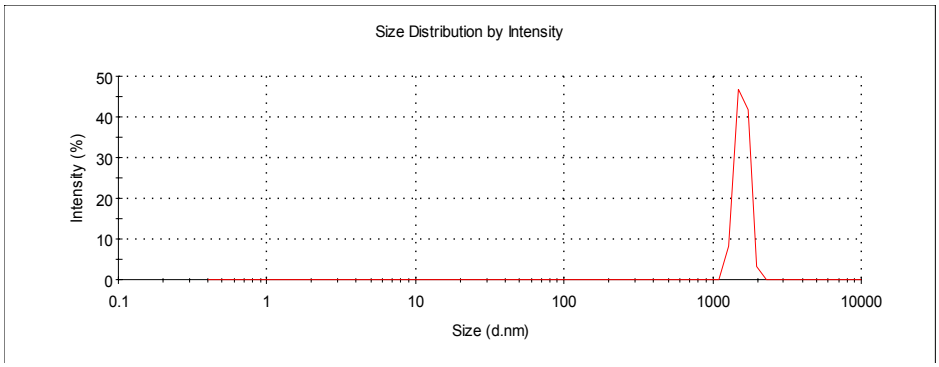
Zeta potentials and size distributions by DLS were measured using a Malvern Zetasizer Nano series instrument. Zeta potentials were measured using a Malvern disposable zeta potential cuvette with ethanol as a dispersant. DLS sizing was done using Malvern disposable sizing cuvettes and phosphate buffered saline solutions (PBS). Samples were sonicated in their respective solvents for 30 mins prior to measurements.



Zeta potential = -38.2mV Zeta deviation = 5.65mV

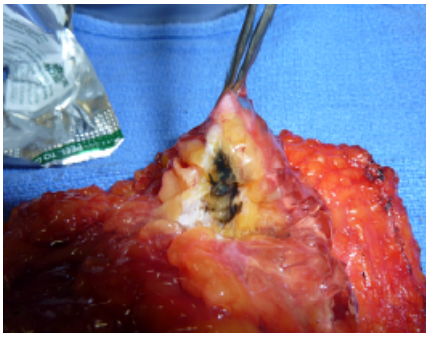


Average size 2.2µm PDI: 0.27

Tissue images

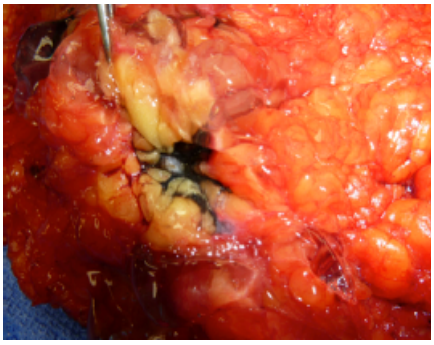
Images are below are photographs of breast tissue post-injection with microshell suspensions that also contained India ink added for injection tracking and particle location during histological analysis.

2 uM



100uL injection 7.9 x 10^8

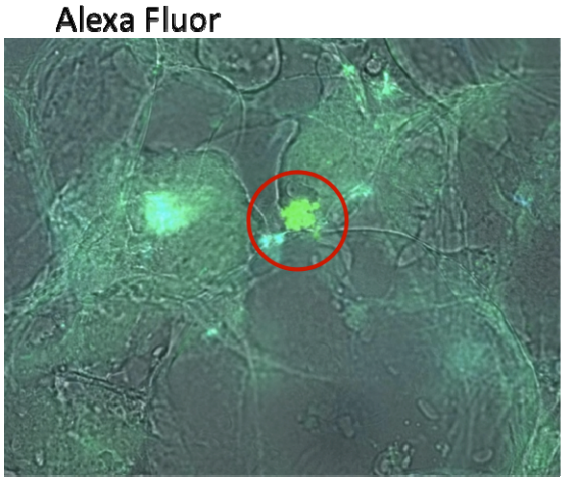
200 nm



100µL injection ~4.0 x

Fluorescence images.

Bright green spots are the Alexafluor-488 dye labeled microshells in breast tissue, as seen in a histological analysis. The image was taken at 40x (Ziess fluorescence microscope) and overlaid on the brightfield image using ImageJ software.



Composite 2um_C1_40x

The equation below is based on the known weights and number of particles for a given mass of solid porous silica microparticles, as obtained from Polysciences Inc.

The weight factor equation allows for a reasonable mass estimation of a hollow particle. This was done by the determination a weight factor expression that estimates the percentage of mass that the shell accounts for by subtraction of the internal volume using the inner and outer radius from TEM images. This ratio is also allows for an estimate of the number of particles for a given size and mass.

Calculation of Weight factor

$$V = \frac{4}{3}\pi r^3$$

$$V = \frac{4}{3}\pi r_{solid}^3 - V = \frac{4}{3}\pi r_{shell}^3$$

$$V_{shell}/V_{solid} = Weight\ fraction$$

$$WF = \left(\frac{r_{solid}^3 - r_{Shell}^3}{r_{solid}^3}\right)$$

2.0 µM Particles

$$WF = \left(\frac{1\mu M^3 - 0.990\mu M_{Shell}^3}{1\mu M^3}\right) = 0.0297$$

200 nm Particles

$$WF = \left(\frac{100nm^3 - 90\mu M_{Shell}^3}{100nm^3}\right) = 0.297$$

Mass per particles

2.0 µM Particles

Solid Particles

$$1.183 \times 10^{8}\ Beads/mg \rightarrow 1/x = 8.453 \times 10^{-9}\ mg/Beads$$

Hollow Particles

$$8.453 \times 10^{-9}\ mg/Beads \times 0.0297 = 2.51 \times 10^{-10}\ mg/Beads$$

200 nm Particles

Solid Particles

$$1.19 \times 10^{11}\ Beads/mg \rightarrow 1/x = 8.40 \times 10^{-12}\ mg/Beads$$

Hollow Particles

$$8.40 \times 10^{-12}\ mg/Beads \times 0.297 = 2.49 \times 10^{-12}\ mg/Beads$$

Eq **1**.

Ultrasound instrument and parameters used:
Ultrasound Doppler color imaging of phantoms (chicken livers and agar gel) was performed using a Phillips iU22 ultrasound. In the color mode setting parameters were as follows: L12-5 transducer, color Doppler at 5.75-7.5MHz, mechanical index (MI) 0.7 (max setting), and pulse repetition frequency (RPF) 500Hz.