

Supporting Information

Hydrogel-Coated Near Infrared Absorbing Nanoshells as Light-Responsive Drug Delivery Vehicles

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S1. Batch-to-Batch Variability

The variability in thickness of the hydrogel coating obtained after the SI-ATRP reaction was assessed by DLS measurements (Malvern Zetasizer Nano ZS) of three different batches of hydrogel-coated nanoshells synthesized in three different SI-ATRP reactions. Average hydrodynamic diameters of the hydrogel-coated nanoshells are displayed in Figure S1, with coating thicknesses of ~99 nm, ~90 nm, and ~84 nm observed for the three batches.

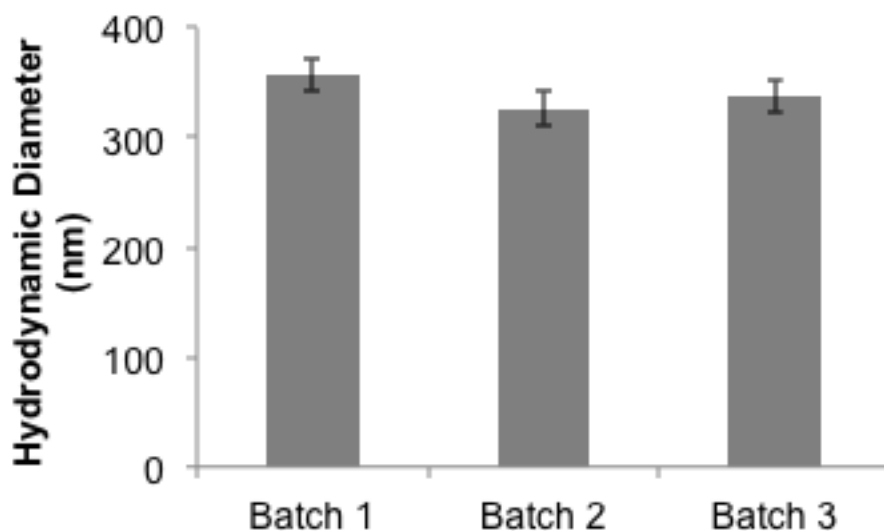


Figure S1. Z-Average hydrodynamic diameter measurements of three separate batches of hydrogel-coated nanoshells. Bars represent the average \pm standard deviation, $n=3$.

S2. Particle Diameter vs. Temperature

DLS measurements were used to investigate changes in hydrogel-coated nanoshell size in response to increased temperatures. Measurements were taken at various temperatures (25 °C, 37 °C, 45 °C, 50 °C, 55 °C, and 60 °C). Temperatures were maintained for at least 15 min before a reading was taken, with Z-average hydrodynamic diameters being reported in Figure S2 below.

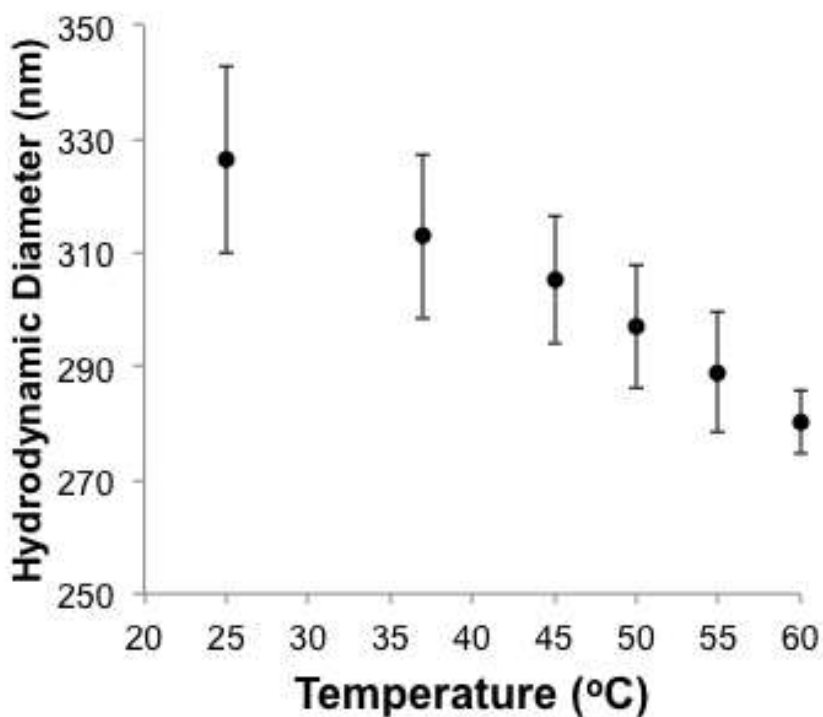


Figure S2. Hydrogel-coated nanoshell hydrodynamic diameter as a function of temperature.

S3. Material Cytotoxicity

For material cytotoxicity analysis, cells were seeded at 4500 cells/well (3T3s, ATCC) or 9000 cells/well (HepG2s, ATCC) in 96-well plates. Both cell types were cultured in Dulbecco's Modified Eagle Medium (DMEM, ATCC) with 10% FBS

(Atlantic Biologicals), 100 U/L penicillin, and 100 mg/ml streptomycin. Cultures were maintained at 37 °C and 5% CO₂. A lower concentration of 3T3 cells were seeded, as these fibroblasts are much larger in size than the HepG2 cells. The next day, cells were exposed to particle concentrations of 0, 3750, 7500, or 15000 hydrogel-coated nanoshells/cell with media/particle volumes of 120 µl/well, with each condition tested in triplicate. After 48 hr, 26 µl of MTS reagent (Promega) was added to each well and the cells were incubated for 45 min at 37 °C and 5% CO₂. Media samples were then centrifuged (735 x g, 5 min) in order to pellet any nanoparticles in the suspension. Absorbance readings of the supernatant were measured at 490 nm. Cell viability was then determined by measuring the average OD₄₉₀ value for each treatment group and expressing the values as a percent of the average OD₄₉₀ for the cell-only controls.

Figure S3 displays cell viability at 48 hr in terms of percent viability as compared to untreated controls. Cell viability was greater than 90% in all groups, and no statistical differences were seen among any groups (ANOVA with Tukey's HSD), indicating that hydrogel-coated nanoshells do not exhibit any material cytotoxicity.

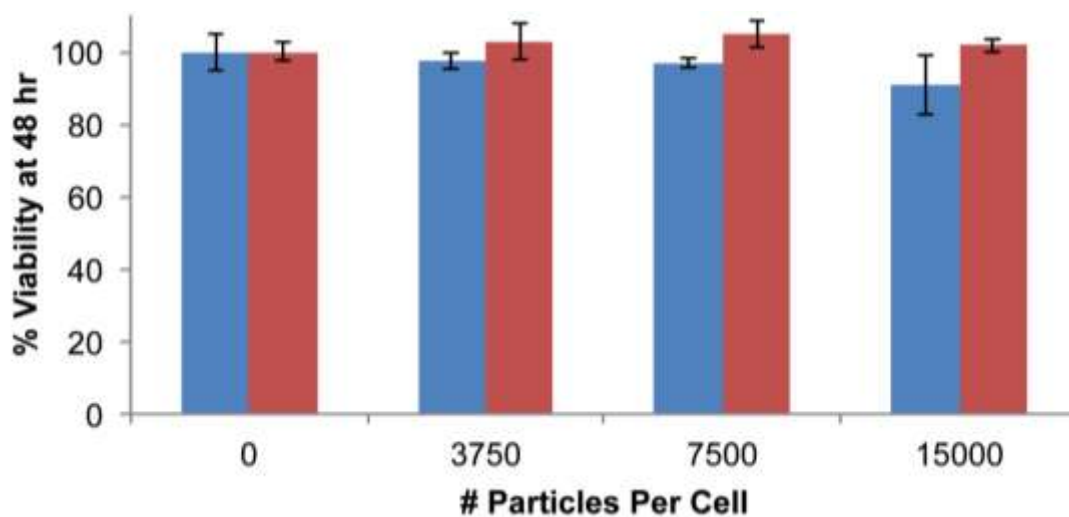


Figure S3. Material cytotoxicity of hydrogel-coated nanoshells. Viability of 3T3 fibroblasts (blue) and HepG2s (red) exposed to increasing particle concentrations. Cell

viability remained >90% in all groups and no statistical significance was observed among any groups.

S4. Doxorubicin loading calculations

Spectra of hydrogel-coated nanoshells at a concentration of 1.2×10^9 particles/ml and hydrogel-coated nanoshells loaded with doxorubicin are shown in Figure S4A. The spectrum of the hydrogel-coated nanoshells loaded with doxorubicin contains two distinct peaks: one at 785 nm (as seen in unloaded hydrogel-coated nanoshells) and one at 485 nm (as seen in the spectrum of doxorubicin). Furthermore, these two peaks can be separated by subtracting the hydrogel-coated nanoshells spectrum from the hydrogel-coated nanoshells + doxorubicin spectrum, yielding a spectrum with one peak which closely matches the spectrum of doxorubicin in solution (Figure S4B). From these two peaks in the hydrogel-coated nanoshells + doxorubicin spectrum, both the number of particles (estimated using a Mie theory simulation based on the peak at 785 nm)¹ and the amount of doxorubicin loaded into the particles (estimated from the peak at 485 nm and using the concentration curve in Figure S4C) can be calculated.

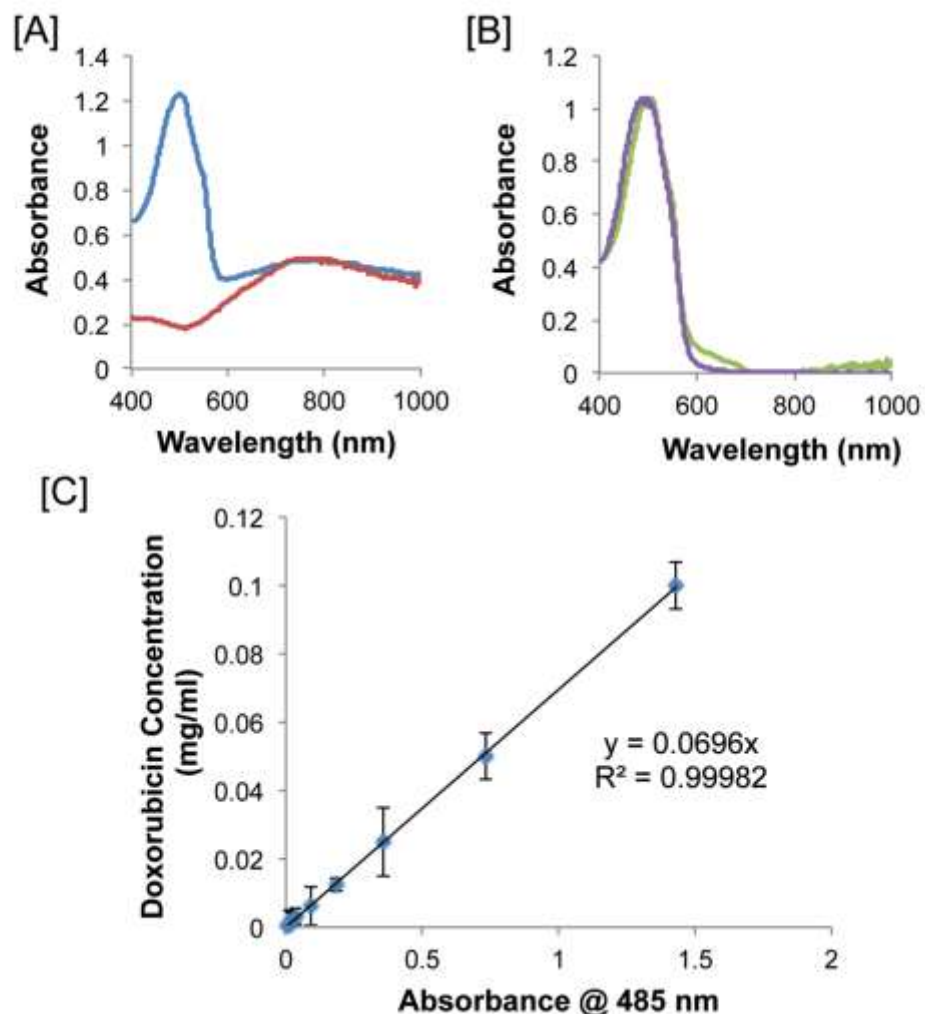


Figure S4. Loading of doxorubicin hydrogel-coated nanoshells. [A] Spectrum of hydrogel-coated nanoshells + doxorubicin (blue) and hydrogel-coated nanoshells at an OD of 0.5 (1.4×10^9 particles/ml, red). [B] The result of subtracting the hydrogel-coated nanoshell spectrum from the hydrogel-coated nanoshell + doxorubicin spectrum (green) closely matches the spectrum of doxorubicin at 0.07 mg/ml (purple). [C] Standard curve relating absorbance at 485 nm to concentration of doxorubicin in solution.

S5. Doxorubicin release at 37 °C in the presence and absence of serum

Release of doxorubicin from hydrogel-coated nanoshells was assessed at 37 °C in the presence and absence of serum. Hydrogel-coated nanoshells loaded with doxorubicin ($\sim 7.1 \mu\text{g}$ doxorubicin/ 10^9 particles) were suspended at an OD of 0.5 (1.2×10^9 particles/ml) in TBS or TBS + 10% fetal bovine serum (FBS, Atlantic Biologicals) and incubated at 37 °C. After 48 hr, the suspension was filtered through a 0.22 μm

polyethersulfone membrane (Genesee Scientific) to separate the doxorubicin-loaded hydrogel-coated nanoshells from the free doxorubicin in the sample. Doxorubicin content of the samples was then determined by measuring absorbance at 485 nm. Approximately 25% of the loaded doxorubicin was released from the particles over the 48 hr period, with the presence of serum not influencing the amount released (Figure S5).

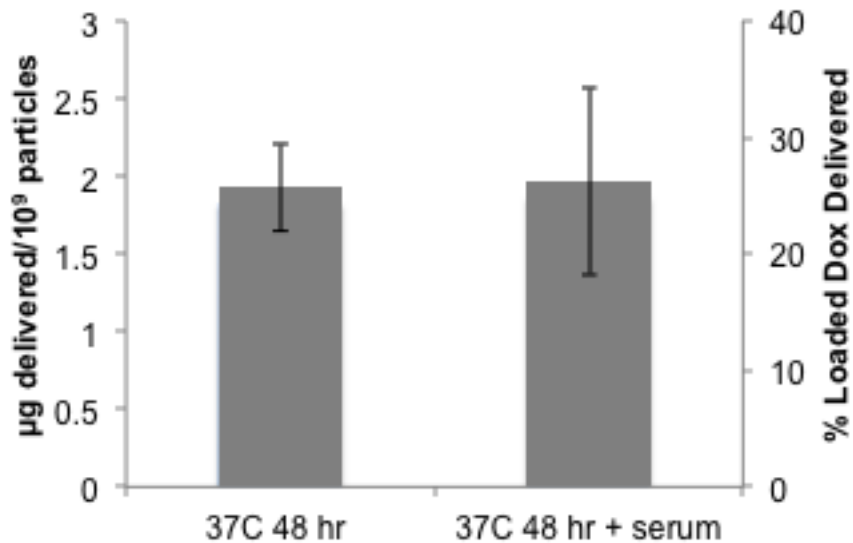


Figure S5. Release of doxorubicin from hydrogel-coated nanoshells at 37 °C. Particles were incubated for 48 hr in the presence and absence of serum. Approximately 25% of the loaded doxorubicin was released from both groups.

References

(1) Averitt, R. D.; Westcott, S. L.; Halas, N. J. Linear optical properties of gold nanoshells. *J. Opt. Soc. Am. B.* **1999**, *16*, 1824-1832, DOI:10.1364/JOSAB.16.001824.