Self-assembly synthesis, tumor cell targeting, and photothermal capabilities of antibody-coated indocyanine green nanocapsules

Supporting Information

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Discussion of particle size distribution measurement analysis

The autocorrelation function (ACF, g(τ)) fitting of the experimental DLS measurements, $\ln[f|g^{(1)}(\tau)|] = \ln(f) - \bar{\Gamma}\cdot\tau + (\frac{\mu_2}{2!})\cdot\tau^2 - (\frac{\mu_3}{3!})\cdot\tau^3 + (\frac{\mu_4 - 3\mu_2^2}{4!})\cdot\tau^4 + \cdots$, provides $\bar{\Gamma}$ and μ_2, the first and second coefficients of the Taylor series of the natural logarithm of g(τ), respectively. The average translational diffusion coefficient D^* and μ_2 are calculated from $\bar{\Gamma} = D^* q^2$ and $\mu_2 = (D^{2*} - D^{*2})q^4$, where, q is the scattering vector that is defined by the incident light and detected light (90°). The polydispersity index (PDI) was calculated as $PDI = \frac{\mu_2}{\bar{\Gamma}^2}$ or $PDI = \frac{D^{2*} - D^{*2}}{D^{*2}}$.

Particle size (d_i) is related to the translational diffusion coefficient D via the Stokes-Einstein relation, $D = \frac{K_B T}{3\pi\eta(\tau)d_i}$, where K_B is Boltzmann's constant, T is the temperature, $\eta(t)$ is the viscosity of the liquid.

The intensity-based average diameter $D_{average}$ can be estimated by averaging the intensity of the scattered light (I_i) from the suspended particle as I_i is proportional to the number (N_i) and the square of the particle mass (M_i):[1, 2]

$$D_{average} = \frac{\sum_i N_i M_i^{\,2} d_i P_i}{\sum_i N_i M_i^{\,2} P_i}$$

For a monodisperse or a narrowly disperse colloidal system, P_i (the particle form factor) of different particles is very similar so that a constant P_i value can be used. Particle mass is proportional to the third power of the diameter, and so the intensity-based diameter simplifies to

$$D_{h,i} = \frac{\sum_i N_i d_i^{\,6}}{\sum_i N_i d_i^{\,5}}$$

$D_{h,i}$ is affected by the sixth and fifth power of the particle size, and is weighted towards larger particle sizes if the colloidal sample has a broad size distribution. To eliminate the effect of over-weighting by large particles on average diameter calculations, number-weighted average diameter $D_{h,n}$ was calculated.

$$D_{h,n} = \frac{\sum_i N_i d_i}{\sum_i N_i}$$

Discussion of analysis of particle size distribution of Table 1

Two average diameter values were calculated, intensity-based ($D_{h,i}$) and number-based ($D_{h,n}$). The intensity-based and number-based average diameter values are equal if the colloidal system of interest is monodisperse. Otherwise, for broad size distributions, the intensity-based average diameter is larger than the number-based value, because the former is more sensitive to the presence of larger particles. $D_{h,i}$ was very close in value to $D_{h,n}$ at early times. While growing in value, the two parameters also grew apart, indicating a broadening of the size distribution with time.

Relative standard deviation (RSD = standard deviation ÷ mean) is one measure that describes the spread of a distribution. When RSD < 5%, the colloidal system is considered to be monodisperse. When RSD is between 5 and 10%, the system is considered to be narrowly dispersed; above 10%, it is broadly dispersed.[2] Thus, the polymer/salt aggregates had a monodisperse size distribution at 3 min, which became a narrow size distribution and later, a broad distribution by 30 min.

The polydispersity index (PDI) is another measure for size distribution, but it is invariant to how the average diameters are calculated. The size distribution is considered monodisperse, narrow, and broad when the PDI is below 0.05, between 0.05 and 0.08, and greater than 0.08, respectively. The calculations become invalid when PDI > 0.3.[1, 3] According to this PDI scale then, the aggregates were monodisperse at 3 min and at 15 min, which broadened into a narrow size distribution at 30 min.

REFERENCES

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2. Hunter, R. J. *Foundations of Colloids Science*, Oxford University Press: New York, **2001**.
3. Pecora, R. *Dynamic Light Scattering: Applications of Photon Correlation Spectroscopy*, Plenum Press: New York and London, **1985**.

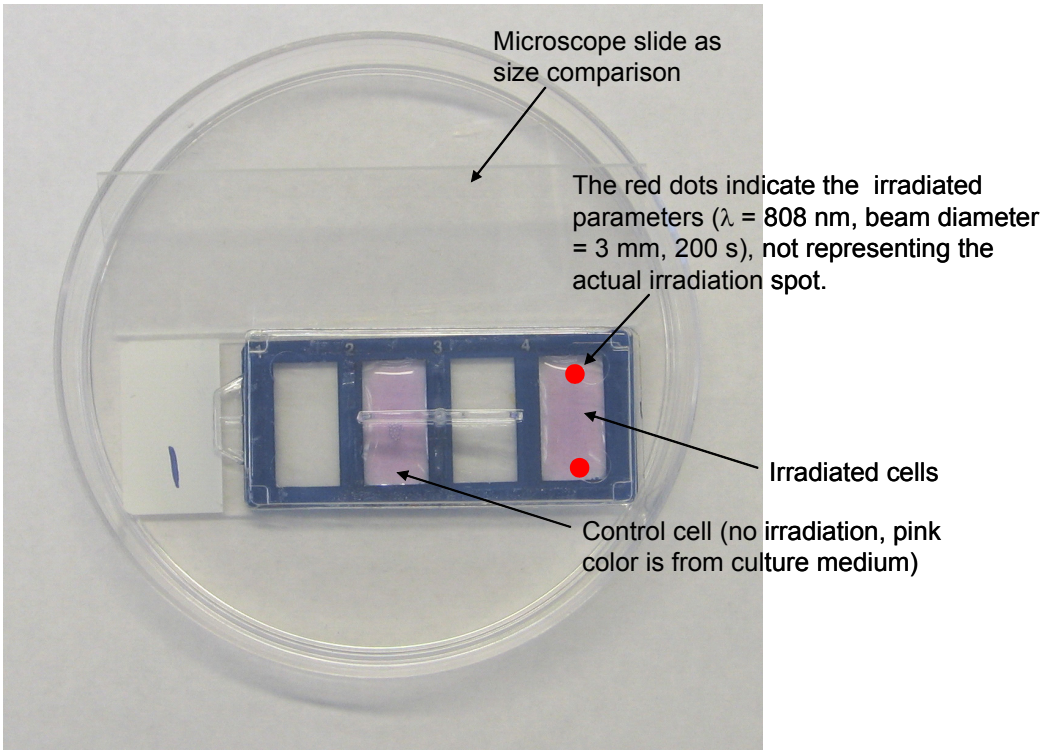

Fig. S1 Schematic of NIR laser irradiation of 1483 cells after the incubation with different formulations of ICG. During the irradiation, the lid was removed.

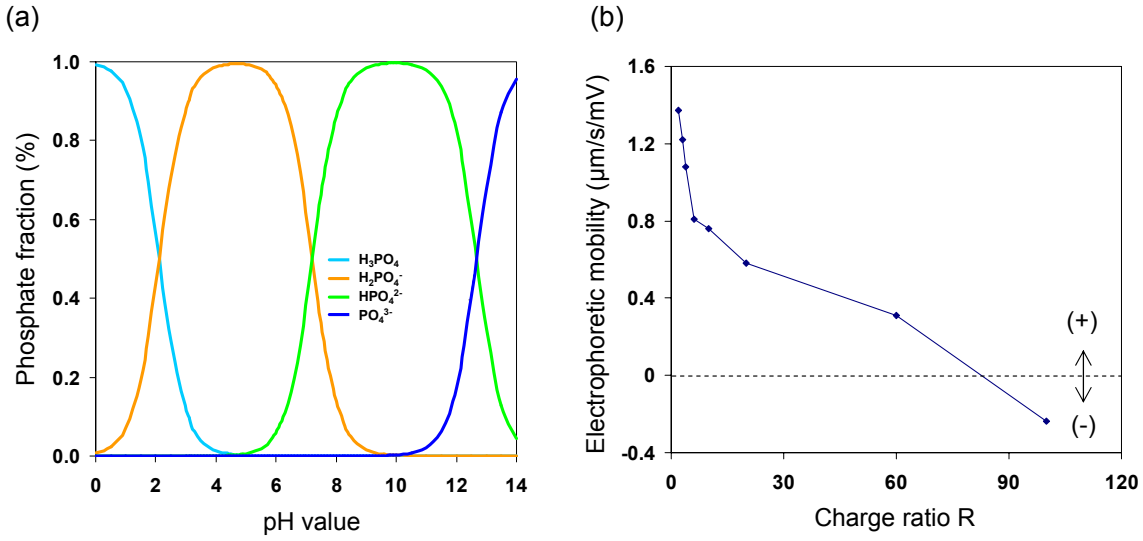

Figure S2. (a) Calculated fractions of phosphate species at different pH values. (b) Measured electrophoretic mobility values for PAH/phosphate$^-$ aggregate suspensions as a function of R ratio.

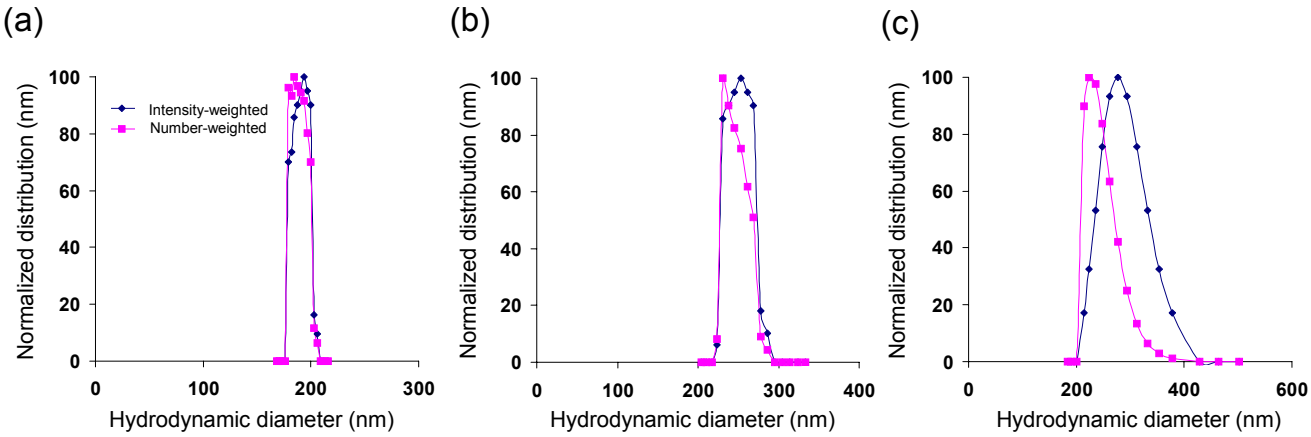

Figure S3. Size distributions of PAH/phosphate aggregates at different aging times: (a) 3 min, (b) 15 min, and (c) 30 min. Aggregate formation conditions: R = 3, temperature = 20 °C, PAH precursor solution concentration of 2 mg/ml, 1:6 volume ratio between PAH and $Na_2HPO_4^{2-}$ precursor solutions.

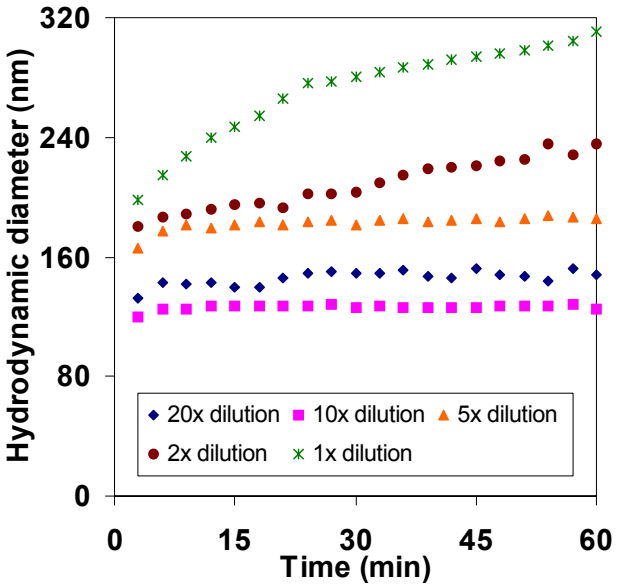

Fig. S4 Effect of deionized water on PAH/phosphate aggregate growth at 20 °C. PAH stock solution was 2 mg/ml, volume ratio of PAH to phosphate salt solution was 1:6 in all the experiments. Dilution factor was based on the volume of phosphate solution.

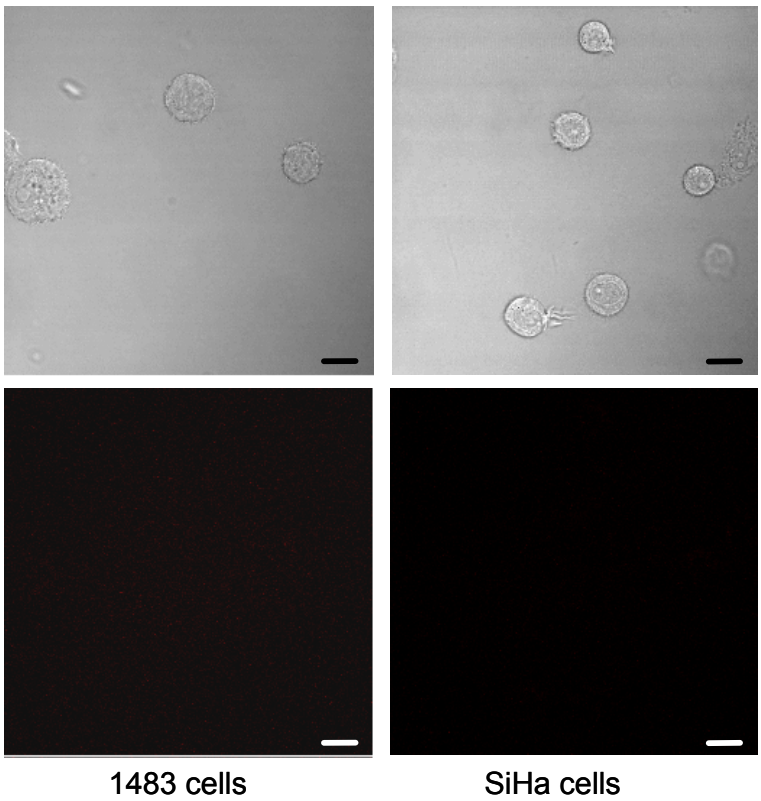

Fig. S5 Confocal microscopy of 1483 and SiHa cells without antibody or nanocapsule incubation. The top two images were taken in bright field mode, and the bottom images were confocal fluorescence images. The faint red dots in the fluorescence images were due to cell autofluorescence. Scale bars: 10 μm.

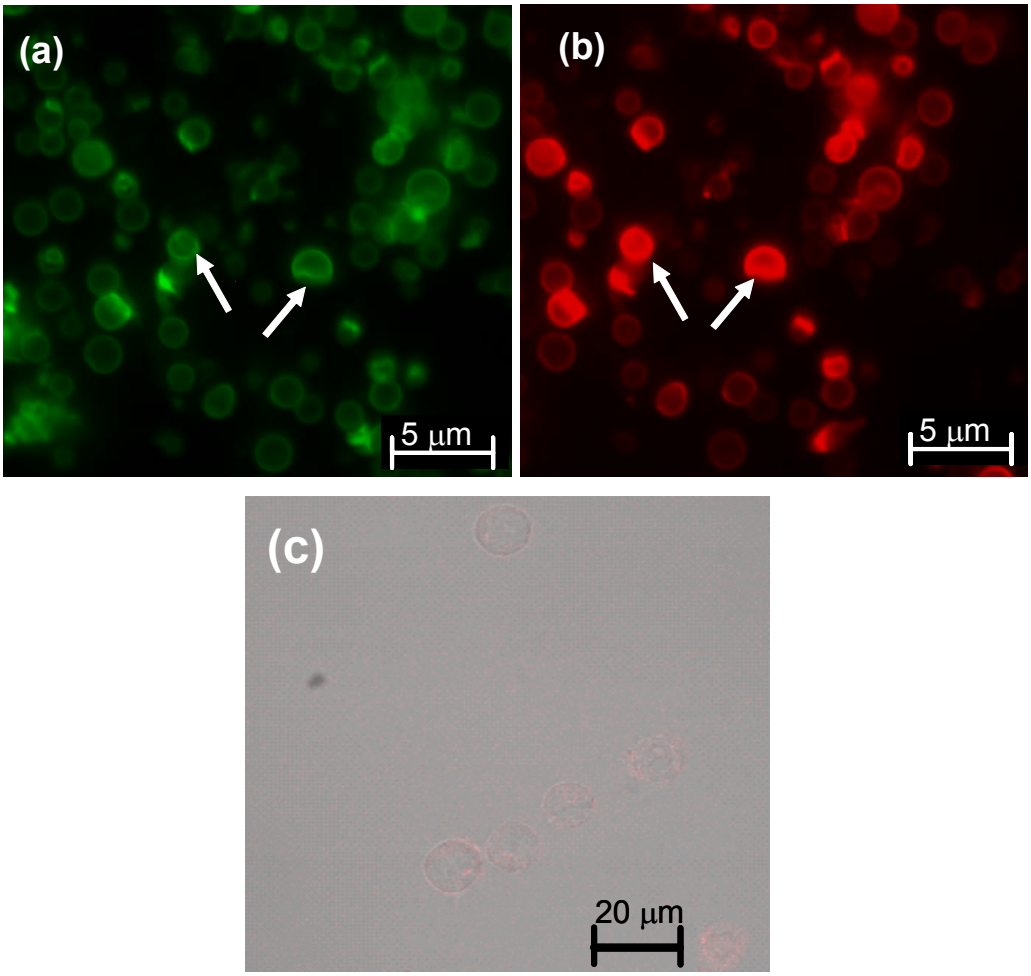

Fig. S6 Fluorescence images of IgG-coated, ICG-containing nanocapsules and microcapsules. The microcapsules were synthesized for optical fluorescence imaging purposes: fluorescence from (a) IgG conjugated with FITC and (b) ICG. The arrows mark the same capsules in both images. (c) Merged bright field-confocal fluorescence image of 1483 cells incubated with IgG-coated nanocapsules (hydrodynamic diameter = 118 nm (RSD = 14%).