Electronic Supplementary Material (ESI) for Journal of Materials Chemistry B

SUPPORTING INFORMATION

1. Liposomes Preparation

For liposome preparation, the lipids and DOTAP were dissolved in chloroform to prepare a stock solution which was used according to the desired final concentration. Typical 2 mM final phospholipid concentration was used to prepare the vesicles. For PC:DOTAP (8:2 molar ratio or 5:5 molar ratio) and DOPE:DOTAP:DPPE-Rh (1:1:0.1 molar ratio), aliquots of the respective stock solutions were sampled according to their initial concentration. All lipid vesicles were first prepared by film hydration. The lipids in chloroform were placed in a small vial glass and the solvent was evaporated under N_2 to form the lipid film. Any residual solvent was removed by a vacuum pump for approximately 4 h. The film was hydrated with ultrapure water and vortexed until complete detachment of the lipids from the vial wall (usually for 2 min).

2. Stem Cells and Red Blood Cells

Human-derived umbilical stem cells were cultivated in cover glasses placed in well culture plates with Modified Eagle's medium (DMEM) at 37°C under 5% CO_2 saturated atmosphere. Blood samples were collected from healthy donors in EDTA tubes and washed 3 times, by centrifuging the sample 1500xg, 5 min. with 0.9% (w/v) saline to obtain the RBCs.

Before we started the experiments with liposomes of any type, some control experiments have been carried out. Stem cells were incubated with bare MPA CdTe QDs at 10 fold higher concentration than that used for liposome encapsulation. As shown in Figure S1 A and B, no labeling has been observed under conventional fluorescence microscopy by using the same parameters as described in the manuscript. Analogously, we incubated type A RBCs with bare MPA CdTe QDs at higher concentration (up to 25 fold higher that that used in the liposomes experiments) and no labeling had occurred when cells were monitored under conventional fluorescence microscopy and by flow cytometry (Figure S1 C). The RBCs were analyzed by a FACSCalibur cytometer (Becton Dickinson) and the software used for data processing was Cell Pro (Cell QuestTM Software, Becton Dickinson). The amount of 10,000 events per samples was acquired. The fluorescence was excited at 488 nm and measured in FL1 filter (530 nm/15 nm). Therefore, taken together these findings show that, at the conditions tested in this work, bare MPA QDs are not able to bind these cells.

Figure S1. In A and B, live stem cells incubated with 10 fold higher bare QDs concentration than that used for encapsulation in liposomes showing the cell morphology by phase contrast microscopy and no QDs signal under fluorescence microscopy, respectively, bars: 25 μm. In C, flow cytometry data plot showed no labeling of red blood cells incubated with 25 fold higher bare QDs concentration than that used for encapsulation in liposomes. Data plot shows that only 0.7% of cells were labeled in this condition. FL1 and FL2 represent green and orange channels respectively.

3. Optical and Fluorescence Microscopy of Liposomes

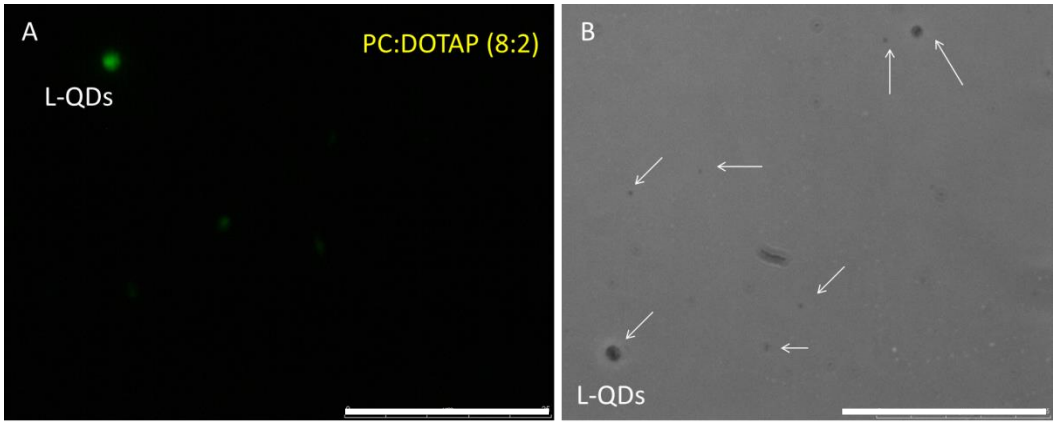

Figure S2. Some vesicles seen by the fluorescence of the encapsulated QDs in A are shown in B under phase contrast. The more fluorescent they are in A, the darker they appear in B. The vesicle entitled L-QDs is the same on these images. Arrows indicates small vesicles. The differences in position comparing both images are due to Brownian motion. Bars: 25 µm.

4. Estimative of Encapsulation Efficiency

By using QDs-loaded Egg PC liposomes, we estimated the efficiency of encapsulating by applying two types of methodology, one by using ultrafiltration tubes and another by using G25 sephadex column. For the first one, 300 µL of the loaded PC liposomes (at 2 mM) plus 300 µL of ultra pure water were added to 100 kDa ultrafiltration tubes (AMICON, Millipore) and centrifuged with 4500 rpm for 7 minutes. After each centrifugation, the pellet was separated. The amount of 300 µL of ultra pure water and the centrifugation procedures were repeated until the pellet became completely transparent and showed no fluorescence. After this process, the encapsulation efficiency was estimated by evaluating the QDs' amount in the initial liposomal preparation and in the pellets by UV-Vis spectroscopy. We also observed the pellet by light microscopy to confirm that there were no liposomes in the precipitate (which could have passed through the membrane). For the second procedure, 300 µL of the unloaded PC liposomes (at 10 mM) were added to a G25 sephadex column to saturate the column with the lipids and avoid interactions between the lipids and the column. Afterwards, QD-loaded PC liposomes (at 10 mM) were added and it was possible to observe the separation of liposomes from free QDs by the color of the samples within the column. The first collected fraction corresponded to the liposomes and the second to the free QDs. After, UV-Vis spectrophotometric analysis was performed by using the same procedure used to the ultrafiltration tubes.

5. Transmission Electron Microscopy of Liposomes

Liposomes at 1 mg/mL were centrifuged (3000 RPM, 8^{o}C) for 30 min prior to fixing with Glutaraldehyde (2.5%), *p*-Formaldehyde (4%) and Cacodylate Buffer (0.1 M). After this step, samples were washed with the same buffer three times, for 10 min each wash. Then, liposomes were fixed with a solution containing cold 2% Osmium Tetroxide (OsO_4), 5 mM Calcium Chloride and 0.8% Potassium Ferrocyanide in 0.1 M Cacodylate buffer. All suspensions were washed twice with Cacodylate buffer 0.1 M for 10 min, and once with distilled water, for 10 min. The samples were stained with 5% Uranyl Acetate at 5% for 1 h and then washed three times with distilled water for 10 min. They were dehydrated in a series of increasing strength of acetone solutions (50%, 2 x 70%, 95%, 2 x 100%) for 15 min. Fixed liposomes were then infiltrated with three increasing concentrations of Epon LX 112 resin in acetone (2:1; 1:1; 1:2), ending in 100% resin overnight. All samples were embedded in Epon LX112 embedding medium at 60 ^{o}C for three days. Embedded liposomes were trimmed and sectioned on an Ultracut E-Reichert-Jung ultramicrotome. Thin sections (90 nm thickness, approximately) taken from each sample and retrieved to copper grids were allowed to dry, and then stained with 5% Uranyl Acetate for 35 min plus Lead Citrate for 1 min. These grids were examined with a Hitachi H-300 electron microscopy using Kodak 4489 electron microscopy film.

TEM images were acquired to further confirm the liposomes encapsulation with QDs. Figure S3 shows TEM images of PC and PC:DOTAP liposomes. The electron dense pattern of Figure S3 C and Figure S3 D confirms the encapsulation. Since there is a reasonable

polydispersivity presented in the liposomes preparation, we can observe in Figure S3 some pictures with larger liposomes when compared to DLS analysis. Nevertheless, it is also possible to see some liposomes with sizes of approximately 250 nm in Figure S3 D. As the protocol, used to prepare the samples for TEM, employs consecutive centrifugations steps, it was observed preferentially larger liposomes, since the smaller one were probably eliminated in this process.

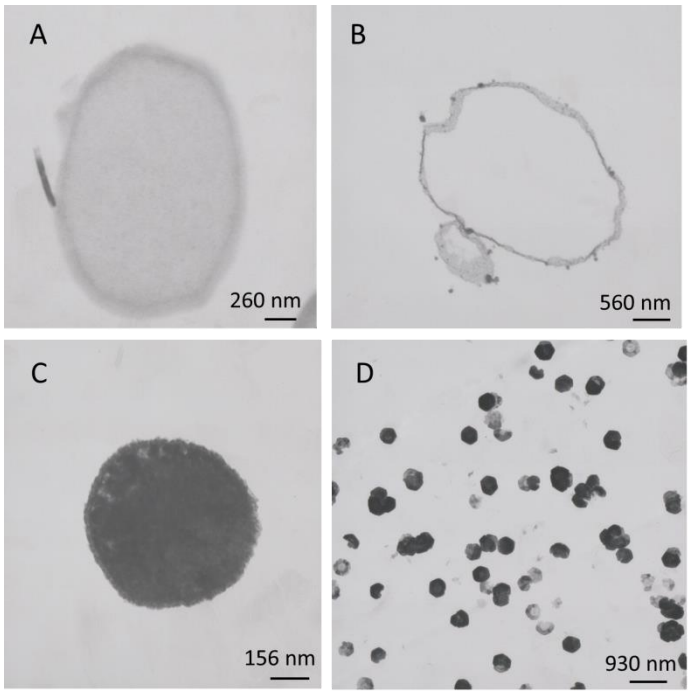

Figure S3. Transmission electron microscopy images of liposomes. In A and B, empty PC and PC:DOTAP liposomes. In C, a single PC liposome containing electron dense nanoparticles, corresponding to QDs. In D, an image of cationic PC:DOTAP (20% of DOTAP) liposomes with variable amount of encapsulated QDs.

6. Giant Vesicle Preparation

Giant Unilamellar Vesicles (GUVs) were made using a simplified protocol described by Akashi et al. (1) without the use of negative lipids. Lipid film was formed as described above for PC:DOTAP vesicle, and hydrated with ultrapure water or with 1.8 µM QD suspension to make a 0.5 mM lipid concentration. The pH of the QDs suspension was close to its original value ~ 10. A sample of the cloudy region of this resultant GUV suspension was then collected and mounted in glass/coverglass for microscopic observation. Conventional fluorescence image were collected using the same microscope and parameters as described for cells. Confocal images were acquired using a confocal multispectral fluorescence microscope Olympus F-1000 under excitation at 473 nm and green fluorescence was detected for 500 – 550 nm band through an oil-immersion 40X objective.

REFERENCES

(1) Preparation of giant liposomes in physiological conditions and their characterization under an optical microscope. Akashi, K.; Miyata, H.; Itoh, H.; Kinosita, K. Biophysical Journal 1996, 71, (6), 3242-3250.