Increasing Cell Viability Using Cd-Free - InP/ZnS@silica@Layered Double Hydroxide - Materials for Biological Labeling

Experimental

Materials

Anhydrous toluene (99.8%), tetraethyl orthosilicate (TEOS, 99.999% metal basis), cyclohexane, Tergitol (NP7), isopropanol, 3-aminopropyltrimethoxysilane (APTMS), Phosphate Buffer Solution (PBS) and synthetic Mg-Al hydrotalcite were all purchased from Sigma Aldrich. InP/ZnS cadmium-free quantum dots were purchased from Mesolight LLC.

Synthesis of the InP/ZnS@silica nanospheres

The first step in the fabrication of the QDs coated with silica nanospheres involved the synthesis of a primer QD@SiO2 by the reverse microemulsion method.[1, 2] Under vigorous stirring, 0.8 mL QDs and 0.64 mL TEOS were introduced into a liquid system containing 15 mL of cyclohexane and 2.6 mL nonionic surfactant NP7. After 30 min, 0.2 mL ammonia (32%) was injected. The microemulsion was stirred for 4h.

Treatments

A synthetic approach was followed for the preparation of the composite materials, subjecting first the hydrotalcite to delamination in formamide.[3] Subsequently, the resulting LDH nanosheets where put in contact with the InP/ZnS quantum dots solution. After stirring for 3 days, the mixture was left to rest at ambient conditions for another 3 days. The resulting solid was centrifuged at 4400 rpm for 30 min and redispersed in toluene, repeating the washing procedure three times. The final solid, denominated QD@silica@LDH, was dried at 80ºC for 12 h and resuspended in PBS.

Cell Culture

Nanoparticle Preparation

The required amount of nanoparticles was first added to the cell medium and the resulting dispersion was vortexed for 10 s, sonicated for 10 s and finally vortexed again for 10 s prior to the incubation with the cell cultures.

Cell Incubation

HK-2 were grown in DMEM F12 with 200mM glutamine, 5% serum and ITS (insulin, transferrin, selenium), while HeLa cells were grown in Dulbecco's modified Eagle's medium (DMEM; Gibco, Karlsruhe, Germany). For the cell uptake experiments, 50 μL of 15 nM NPs dispersion were added to a culture with 100.000 cells per well and incubated at 37ºC for different times. A previous acidic wash was performed to remove all the remaining NPs outside of the cells. Then, the different samples were fixed and recorded by confocal.

For immunostaining experiments, 50 μL of 15 nM NPs dispersion were added to a culture with 100.000 cells per well and incubated at 37ºC for 10 minutes and then the media were changed for the corresponding one without NPs for another 20 minutes.

Immunostaining

Samples were incubated with primary antibodies against EEA1 ((Anti-EEA1 antibody [1G11] - Early Endosome Marker Cat N. ab70521 from Abcam) and LAMP1 ((LAMP1 (D2D11) XP® Rabbit mAb) Cat N. 9091 from Cell Signaling Technology) for 1 hour, then with secondary antibodies Goat Anti-mouse labeled with Alexa 488 (excitation at 488 nm and emission at 525 nm) and Goat Anti-rabbit labeled with Alexa 633 (excitation at 633 nm and emission at 670 nm) for 45 minutes.

Imaging was performed by confocal microscopy and imaging parameters were kept constant throughout the different samples. To avoid any kind of overlapping due to the multiplex, NPs were excited at 405 nm and the emission was collected in the range 610-640 nm; Alexa 488 was excited at 488 nm and collected in the range 500-550 nm; and Alexa 633 was excited at 633 nm collected in the range 650-690 nm.

Cell uptake

Briefly, using ImageJ selection and drawing tools, a region of interest was chosen on each cell in a background corrected confocal fluorescence image. Thirty cells were analyzed for each condition from a set of three biological replicates and corrected total cell fluorescence was calculated.

MTT test

For assessment of their viability, cells were grown in gelatin-coated 96 well plates (Becton Dickinson; Meylan Cedex, France) for 12 h. For the nanoparticle treatment, 10^5 cells per well were seeded. The cells were stimulated with the described particle suspensions (15 nM) in a dose of 5, 10, 15, 25 and 50 □L in 100 µL of the corresponding medium, without additives, for 24 h. Cells incubated with ultrapure water and untreated cells served as controls. After exposure to the NPs, the medium was removed and the mitochondrial activity of the cells was measured by the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) reduction assay. The cytotoxicity was calculated from the absorbance at 570 nm and expressed as relative values compared with untreated negative controls.

Instrumentation

Spectroscopic measurements were recorded using 1cm path length quartz cell in a Shimadzu UV spectrophotometer 1700. Transmission Electron Microscopy (TEM) was carried out on a JEOL JEM-1011 microscope operating at 100 kV and equipped with a SIS Megaview III CCD camera. Confocal images were recorded using a Zeiss LSM700 and LSM710 Confocal microscope.

References

1. Q. Ma, I. Castelló Serrano and E. Palomares, *Chem Comm*, 2011, **47**, 7071.

2. I. Castelló Serrano, Q. Ma and E. Palomares, *J. Mater. Chem.*, 2011, **21**, 17673.

3. G. Stoica, I. Castelló Serrano, A. Figuerola, I. Ugarte, R. Pacios and E. Palomares, *Nanoscale*, 2012, **4**, 5409.

Results

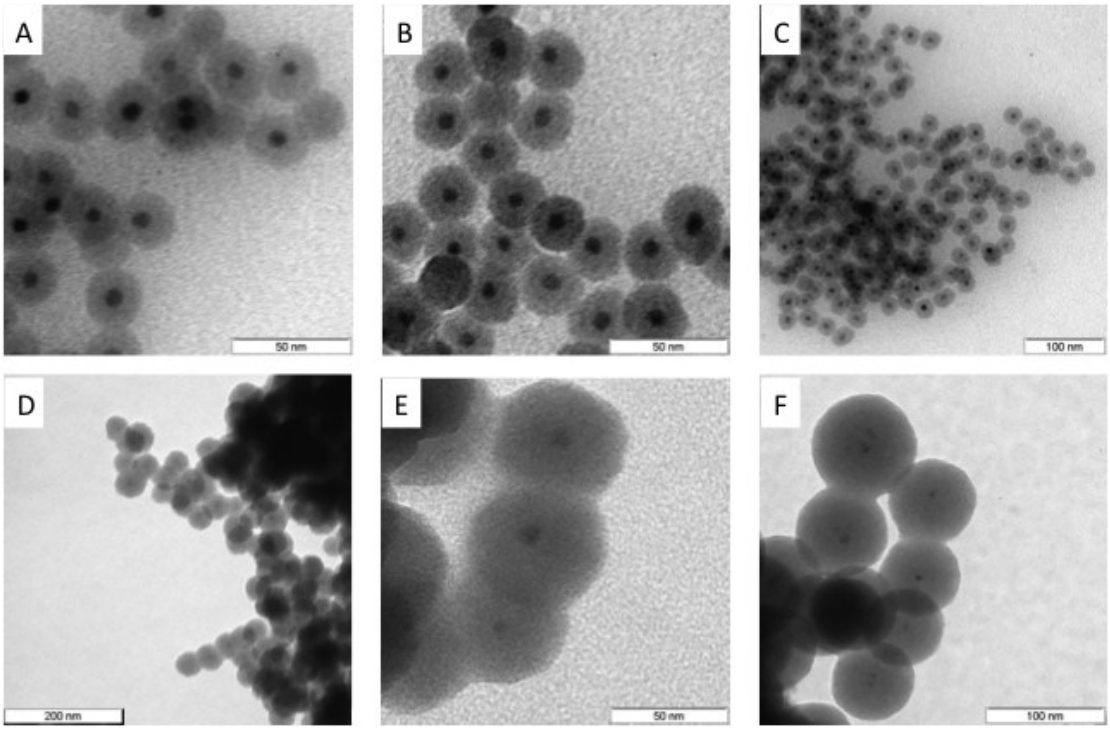

Figure S1. TEM images of different-sized QDs@silica nanospheres: a) 17 nm, b) 27 nm, c) 32 nm, d) 40 nm, e) 55 nm and f) 65 nm.

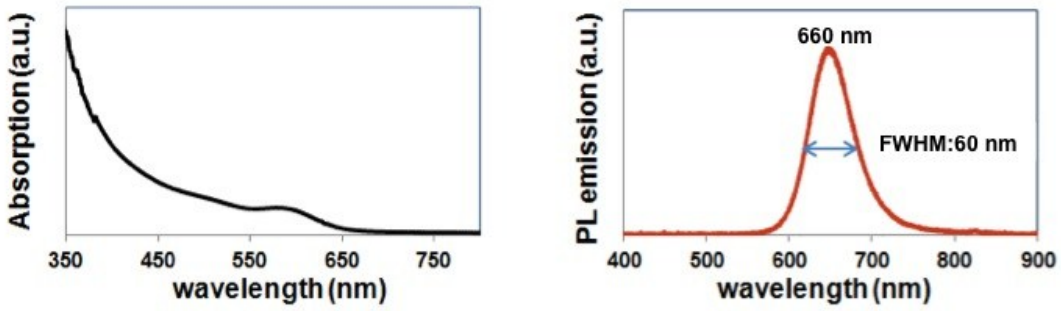

Figure S2. Absorption and emission spectra of InP/ZnS quantum dots.