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PEGylated liposome coating QDs/mesoporous silica core-shell nanoparticles for molecular imaging

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Materials and Methods

1. Materials

TOPO coated CdSe/ZnS core/shell QDs were synthesized according to literature procedures.[1] 1,2-Dipalmitoyl-*sn*-glycero-3-phosphocholine (DPPC) and 1,2-Distearoyl-*sn*-glycero-3-phosphoethanolamine-*N*-[methoxy(poly(ethylene glycol))-2000] (DSPE-PEG2000) were provided by Avanti Polar Lipid (USA) as a gift. Cethyltrimethylammonium bromide (CTAB), tetraethylorthosilicate (TEOS), phosphate buffered saline (PBS), Dulbecco's Modified Eagle's Medium (DMEM) medium, antibiotics (penicillin-streptomycin solution), Trition X-100, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-tetrazolium bromide (MTT), diethyl ether, chloroform, cholesterol were purchased from Singma-Aldrich (St. Louis, MO, USA). Fetal bovine serum (FBS) was purchased from Gibco (Life Technologies, AG, Switzerland). MCF-7 breast cancer cells were provided by American Type Culture Collection. Millipore water was produced by the Milli-Q Plus System (Millipore Corporation, Bedford, USA).

2. Synthesis of mesoporous silica coated QDs NPs

Mesoporous silica coated QDs were prepared based on the protocols from Hyeon *et al.*.[2, 3] In a typical procedure, QDs in chloroform was poured into 0.55 mM aqueous cethyltrimethylammonium bromide (CTAB) solution and stirred vigorously for 30 min to obtain transparent black QDs/CTAB solution. (CTAB was used here to enable

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hydrophobic QDs soluble in aqueous solutions and work as templates for the mesopore formation). The formed solution was added to a mixture of 45 mL of water and 0.3 mL of 2M NaOH solution. The mixture was then heated up to 50°C. Subsequently, tetraethylorthosilicate (TEOS) and ethylacetate were dropped into the reaction solution. The resultant solution was allowed to cool down to room temperature. Unreacted precursors and the surfactant can be removed by rinsing with ethanol repeatedly.

3. Preparation of PEGylated liposome coating QDs/mesoporous silica core-shell NPs

Liposomes were prepared using a modified version of the method described by Wafa' T et al.[4] About 14.6 μmol DPPC, 1.6 μmol DSPE-PEG2000, and 8.1 μmol cholesterol (molar ration=1.8:0.2:1) were dissolved in 2 ml of a chloroform: methanol solution (5:1 v/v). Once the lipids were thoroughly mixed in the organic solvent, the solvent in round-bottom flask was removed using an evaporator at 40 ^{o}C under vacuum to yield a lipid film. The lipid film was thoroughly dried to remove residual organic solvent by placing the vial or flask on a vacuum pump overnight. The dry lipid film was hydrated by adding 0.26 μm 1 ml QDs in 4 ml PBS (pH=7.4), followed by agitation for 1 h at 45°C. Finally, small unilamellar vesicles (SUV) were prepared by bath sonication for 10-20 minutes at 50°C.

4. Characterization of PEGylated liposome coating QDs/mesoporous silica core-shell NPs

4.1 Particle size and size distribution

Average particle size and size distribution of the NPs were measured using laser light scattering (LLS, 90 Plus Particle Size, Brookhaven Instruments Co. USA). NPs suspension (2 ml) was added to 3 ml deionized water and sonicated for several minutes. The homogeneous suspension was used to determine the volume mean diameter, size distribution, and polydispersity.

4.2 Surface charge

Zeta potential of paclitaxel-loaded FD NPs was determined with ZetaPlus zeta potential analyzer (Brookhaven Instruments Corporation) at room temperature. The samples were prepared by diluting the NPs suspension with deionized water. The data

were obtained with the average of several measurements.

4.3 Surface morphology

Shape and surface morphology of NPs were investigated by transmission electron microscope (TEM, JEM-2010F, JEOL, Japan). The NPs suspension was dropped on the surface of copper grid with carbon film and dried at room temperature at an accelerating voltage of 200 kV.

4.4 Emission spectra

Suspension solutions of NPs were placed into thin quartz cuvettes (Costar, IL, USA). The emission spectra of the NPs were investigated using a spectroflurophotometer (RF-5301PC, SHIMADZU, Japan) at excitation 480 nm.

4.5 Surface chemistry of PEGylated liposome coating QDs/mesoporous silica core-shell NPs

Surface chemistry of PEGylated liposome coating QDs/mesoporous silica core-shell NPs was analyzed by X-ray photoelectron spectroscopy with an Al Kα X-ray source (1486.6 eV photons) (XPS, AXIS His-165 Ultra, Kratos Analytical, Shimadzu Corporation, Japan). A binding energy ranged from 0 to 1,100 eV was recorded in the survey spectrum with pass energy of 80 eV under the fixed transmission mode..

4.6 In vitro cadmium ions release

PEGylated liposome coating QDs/mesoporous silica core-shell NPs were placed in a PBS buffer (pH 7.4) in a centrifuge tube, which was put in an orbital shaking water bath at 37 °C, and shaken horizontally at 120 rpm. The sample was removed from the shaker bath at the designated time intervals and centrifuged at a speed of 10,000 rpm for 15 min. The supernatant was collected for release analysis. Fresh PBS medium was then added into the tubes, which were put back in the shaker bath for continued measurement. Inductively coupled plasma optical emission spectrometry (ICP-OES, Perkin-Elmer, Optima 3000DV) was utilized to determine the amount of cadmium ions released from NPs.

5. *In vitro* experiment

5.1 Cell cultures

MCF-7 breast cancer cells were obtained from the American Type Culture Collection. The Dulbecco's Modified Eagle's Medium (DMEM) containing 10% fetal bovine

serum (FBS) and 1% penicillin-streptomycin was used for MCF-7 cells as cell culture medium. Cells were cultivated in medium at 37 ^{o}C in humidified environment of 5% CO_2. Before the *in vitro* experiments, the cells were pre-cultured until confluence was reached.[5]

5.2 In vitro cellular uptake of NPs

MCF-7 cells were cultivated in the chambered cover glass system (LAB-TEK®, Nagle Nunc international, Rochester, NY) with 5% CO_2 in their respective medium at 37 ^{o}C as proposed by the American Type Culture Collection. After 24 h incubation time, the adherent cells are washed twice with PBS. NPs diluted in the medium to a concentration of 0.125 mg/ml were added into the chambers. The cells were allowed to be incubated with the NPs and were washed 4 times with PBS after incubation. They were then fixed by 70% ethanol for 15 min. The cells were washed twice with PBS and the nuclei were stained with 4', 6-Diamidino-2-phenylindole dihydrochloride (DAPI) for 30 min. The stained cells are washed twice with PBS and imagined using a confocal laser scanning microscope (CLSM, Olympus Fluoview FV500, Japan).

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