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Hybrid Gold/Silica/Quantum-Dots supramolecular-nanostructures encapsulated in polymeric micelles as potential theranostic tool for targeted cancer therapy

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ABSTRACT

Efficient theranostic tools are today more and more frequently represented by the nano-sized systems. In this paper, polymeric micelles were produced and exploited to encapsulate both an antitumor drug (Doxorubicin) and gold core–shell quantum dots nanoparticles (Au-SiO_2/QDs). α,β-poly(N-hydroxyethyl)-DL-aspartamide (PHEA) was functionalized with lipoic acid (LA), polyethylenglycol (PEG), and folic acid (FA) pendant moieties to obtain a synthetic derivative (PHEA-LA-PEG-FA) able to self assemble in aqueous medium giving rise to the formation of polymeric micelles exposing on their surface both targeting groups (FA) and hydrophilic chains (PEG). The drug carrying ability of PHEA-LA-PEG-FA micelles was here studied along with the cytotoxity of the obtained nanostructures toward breast cancer cells employing doxorubicin as a model anticancer drug. Finally, the properties of the gold-shell QDs incorporated into the micelles as cells imaging agent and photothermal anticancer treatment tool were explored.

1. Introduction

Theranostic agents have been gaining increasing attention in the field of controlled drug delivery thanks to their ability to release drugs while acting as contrast agents for the visualization of cells and targeted tissues, such as tumors, by single or multiple imaging techniques. Merging various distinct elements into a single multifunctional nanoparticle could be advantageous in many biomedical applications [1]: for instance, the combination of fluorescent quantum dots (QDs) with plasmonically active gold nanoshells is particularly interesting and has been exploited so far for immunofluorescence labeling of cells and tissues, intracellular sensing, tumor imaging, photothermal therapy and, lately, for the study of nanoparticle-mediated drug delivery [2–7]. Indeed, QDs–gold core–shell exhibit outstanding features. First, QDs–gold core–shell are more eligible for biological applications than QDs alone (which typically are not water soluble), as the gold shell passivates the core, improves fluorescent properties and significantly enhance photostability against photobleaching [8–12]. Second, the QDs–gold core–shell show both an intense surface plasmon absorption band tunable in the visible/near infrared (VIS-NIR) region and a strong light scattering due to their gold shell. This renders QDs–gold NPs an excellent dual-modality imaging probe [1]. This new class of multimodality nanoprobe provides imaging with both fluorescence and scattering modalities and at the same time enables light-triggered photothermal treatment (particularly when the surface plasmon band is tuned towards the NIR region), useful, for instance, in cancer treatment [1]. These modes of imaging and therapy cannot be simultaneously achieved with the traditional silica-coated QDs since these last do not show photothermal features, but rather excellent photoluminescence properties [13,14]. Furthermore, another important advantage over the traditional QDs is the simplified bioconjugation of the surface due to the well-established thiolate–gold chemistry [15–17]. Due to their striking properties, it is particularly in the areas of *in vivo* imaging, intracellular labeling and anticancer treatment that QDs–gold

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core–shell hold the most promise. For almost all of these applications, the ability to achieve intracellular delivery is a key issue; in this context, the attachment on QDs–gold core–shell surface of various chemicals/biological molecules can enhance cell uptake and provide a desired specific biorecognition and/or targeting function. For example, these nanosystems can be functionalized with a variety of tumor-recognition antibodies for both specific binding and *in vivo* fluorescent localization [18,19], drugs for therapy [20], and coating polymers to improve stability and biocompatibility in physiological environments, besides increasing drug dosage and potency [21]. Manifold QDs surface functionalization approaches have been devised within the recent past including ligand exchange with thiol-containing molecules [22,23] or more complex ones such as oligomeric phosphines [24], dendrons [25], and peptides [26]; encapsulation by a layer of amphiphilic diblock or triblock copolymers [27], phospholipid micelles [28], polymer beads [29], polymer shells [30], or amphiphilic polysaccharides [31,32]. To date, QDs functionalization technology is going to rapidly evolve and stable functionalization of QDs is still a challenge. Taking into account the above considerations, in this work the synthesis of a new amphiphilic polyaminoacid based copolymer, henceforth named PHEA-LA-PEG-FA, able to self-assemble into a micelle-like structure and to encapsulate QDs-gold core–shell (Au-SiO_2/QDs) as well as load the anticancer drug Doxorubicin is reported. For this aim, the synthetic polyaminoacidic polymer α,β-poly(N-hydroxyethyl)-DL-aspartamide (PHEA) [32,33] was chemically modified in the side chain with hydrophobic molecules, particularly with lipoic acid tails, and with hydrophilic chains such as poly ethylene glycol (PEG) bearing folate residues as tumor-targeting ligand. The conjugation of folate onto PHEA polymeric backbone can potentially enhance the selective drug delivery to tumors expressing high levels of folate receptor (FR), such as breast cancer [34–39]. Furthermore, PEGs chains with molecular weight of 2–5 KDa constitute the hydrophilic shell of the polymeric micelles [40] which endows the micelles with effective steric protection, prevents recognition by the reticuloendothelial system (RES) and prolongs bloodstream circulation [41]. On the other hand, lipoic acid tails bear disulfide groups necessary to chemically functionalize the gold-shell of QDs NPs through the well documented thiolate-gold chemistry [15,16].

2. Matherials and methods

2.1. Materials

α,β-Poly(N-2-hydroxyethyl)-D,L-aspartamide (PHEA) was prepared following the previously reported method [42–44]. Spectroscopic data (^{1}H NMR) were in accordance with attributed structure: ^{1}H NMR (300 MHz, D_2O, 25 °C, δ): 2.82 (m, 2H, −CH−C<u>H</u>$_2$−CONH−), 3.36 (t, 2H, −NH−C<u>H</u>$_2$−CH_2−OH), 3.66 (t, 2H, −CH_2− C<u>H</u>$_2$−OH), 4.72 (m, 1H, −NH−C<u>H</u>−CO−CH_2−). Bis(4-nitrophenyl)carbonate (BNPC), N-ethyl-N′-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDC-HCl), N-hydroxy-sulfosuccinimide (NHSS), Folic acid, Poly(ethylene glycol) diamine 2 KDa, (±)-α-Lipoic acid, Dicyclohexylcarbodiimide (DCC), 4-(Dimethylamino)pyridine (DMAP), doxorubicin hydrochloride (doxorubicin HCl), hydrogentetrachloroaurate trihydrate ($HAuCl_4$·H2O, ≥99.9%), were purchased from Aldrich. Milli-Q water (resistivity 18.2 MΩ·cm at 25 °C) was used in all experiments. Sephadex G-25, anhydrous dimethylformamide (DMFa), acetone, methanol, diethyl ether, were purchased from Fluka (Switzerland). All reagents were of analytic grade, unless otherwise reported.

2.2. Cell cultures and reagents

Human breast cancer cells (MCF7), purchased from ATCC, were grown in Dulbecco's modified Eagle's medium (DMEM) with 10% fetal bovine serum (FBS) and 1% of penicillin/streptomycin (100 U/mL penicillin and 100 mg/mL streptomycin), 1% glutamine and 0.5% of amphotericin B, at 37 °C in 5% CO_2 humidified atmosphere. DMEM and other constituents were purchased from Euroclone. Cell Titer 96 Aqueous One Solution (MTS reagents for cell proliferation assay) was purchased from Thermo Scientific.

2.3. Apparatus

^{1}H NMR spectra were recorded using a Bruker Avance II 300 spectrometer operating at 300 MHz.

UV–Vis spectra were recorded using an Shimadzu UV-2400 spectrophotometer. Fluorescence spectra were recorded using a Shimadzu RF-5301 PC spectrofluorophotometer. Zeta-potential measurements (mV) were performed at 25 °C using a Malvern Zetasizer NanoZS instrument. Transmission electron microscopy (TEM) images were acquired on a JEOL 2010 field electron gun microscope operated at 200 keV. Scanning electron microscope (SEM) images were taken using a FEI Magellan (conditions: 15 kV, 6.3 pA).

The confocal fluorescence microscopy experiments were performed with an Olympus FluoView FV10i. The laser excitation was set at 405 nm and emission was recorded at 604 nm.

2.4. Synthesis of hybrid Gold/Silica/Quantum-Dots (Au-SiO_2/QDs)

The synthesis of silica encapsulated quantum dot was done as previously reported [45]. Briefly, the preparation of 12 nm diameter core–shell CdSe-CdS quantum dots (of 1.5 nm core radius) was done accordingly to the high temperature protocol developed as in Ji et al. [46]. The formation of a silica shell around the QDs was performed by an inverse water-in-oil microemulsion micelles method. In particular, 1.9 g of the surfactant Triton X-100 and 1.5 g of 1-Hexanol as co-surfactant were mixed and dissolved in 15 mL of cyclohexane. Then, ~0.15 nmol of QDs dispersed in hexane were injected (typical volume of 60 μl) and after 10 min of stirring with a magnetic bar, 380 μl of water and 60 μl of ammonia (29% in water) were added. After thermalization at 4 °C in a cold room, 60 μl of TEOS were added to start the reaction of silica formation around the dots. After 6 h, another amount of 320 μl of TEOS was added to have final 100 nm diameter silica nanoparticles after 140 h of total time of growth (~40 nm silica thickness). The microemulsion was broken by adding acetone and after centrifugation (4 min@4000 rpm) the nanoparticles were washed by centrifugation and sonication in different solvents (50% n-butanol-50% hexane, 50% isopropanol-50% hexane, 50% ethanol-50% hexane, three times in ethanol and methanol) and finally dispersed in 15 mL of methanol with a final concentration of the order of 10 nM.

2.5. Synthesis of PHEA-LA-PEG-FA

PHEA-LA-PEG-FA copolymer was synthetized following a two-step procedure. First, PHEA-LA was synthetized adding a solution of DCC (78 mg, 0.38 mmol) and DMAP (46 mg, 0.38 mmol) in 1 mL of DMFa to a solution of LA in the same solvent (63 mg, 0.316 mmol, in 1.5 mL of DMFa). The reaction mixture was kept 1 h at 25 °C and then added to a solution of PHEA (200 mg, 1.265 mmol of repeating units) in 2.5 mL of DMFa, and left to react for 18 h at 30 °C. After that, the reaction mixture was filtered and DMFa was removed by means of a rotary evaporator at 30 °C under vacuum. The obtained product, PHEA-LA, was precipitated and washed three times in acetone/diethyl ether mixture 1:1 v/v and then stored as dried powder (yield: 75% w/w based on the starting PHEA). In the second step, BNPC (38 mg, 0.126 mmol) was added to a PHEA-LA solution (200 mg, 1.265 mmol of repeating units in 2.5 mL of DMFa). The reaction mixture was kept 2.5 h at 40 °C. Then, it was added drop-wise to a PEG-FA solution obtained as previously reported (302.4 mg, 0.126 mmol in 1.5 mL of DMFa) [47]. The reaction mixture of PHEA-LA and PEG-FA was kept under stirring for 18 h at 25 °C. The obtained product was precipitated in acetone and washed three times in acetone/methanol mixture 1:1 v/v centrifuging 10 min at 9800 rpm. Then, the solid product was dissolved in 2 mL of milli-Q water and