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Lipid bilayer-coated curcumin-based mesoporous organosilica nanoparticles for cellular delivery

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ABSTRACT

Effective and controlled drug delivery systems with on-demand release abilities and biocompatible properties receive enormous attention for biomedical applications. Here, we describe a novel inorganic–organic hybrid material with a strikingly high organic content of almost 50 wt%. The colloidal periodic mesoporous organosilica (PMO) nanoparticles synthesized in this work consist entirely of curcumin and ethane derivatives serving as constituents that are crosslinked by siloxane bridges, without any added silica. These mesoporous curcumin nanoparticles (MCNs) exhibit very high surface areas (ca. 1000 m^2/g), narrow particle size distribution (around 200 nm) and a strikingly high stability in simulated biological media. Additionally, the MCNs show high autofluorescence and were used as a cargo delivery system in live-cell experiments. A supported lipid bilayer (SLB) efficiently seals the pores and releases Rhodamin B as model cargo in HeLa cells. This novel nanocarrier concept provides a promising platform for the development of controllable and highly biocompatible theranostic systems.

1. Introduction

Periodic mesoporous organosilica (PMO) constitutes a new type of inorganic–organic porous hybrid material, which holds great promise in a variety of fields such as chemical sensing [1–7], catalysis [8–12] and biomedical applications [13–15]. Since the independent discovery of this new class of mesoporous materials in the groups of Inagaki, Stein and Ozin in 1999 [16–18], PMO materials, synthesized by using bridged silsesquioxanes as precursors, have recently been prepared at the nanoscale [19–21]. Different approaches were used to synthesize PMO nanoparticles with simple, low-molecular-weight organosilane bridging groups. In a sol–gel process using Pluronic P123 as the template, Landskron et al. synthesized rodlike nanoparticles with adjustable aspect ratios [22]. Using cetyltrimethylammonium bromide (CTAB) as the micellular template and an ammonia-catalyzed sol–gel reaction, Huo et al. prepared highly ordered and dispersible PMO nanoparticles with methane-, ethane-, ethylene- and benzene-based organic bridging groups within the pore walls [23]. In another approach the group of Shi et al. used silica-etching chemistry to obtain hollow PMO nanoparticles that were used for nano-biomedical applications for the first time [24]. Recently, the group of Durand reported the synthesis of biodegradable PMO nanospheres and nanorods with a disulfide-containing organic bridging group. The morphology and size of these nanostructures was controlled by adjusting the ratio of bis(triethoxysilyl)ethane and bis(3-triethoxysilyl-propyl)-disulfide [25]. These mixed PMO nanospheres and rods were used as biodegradable nanocarriers for doxorubicin in breast cancer cell lines. In the group of Kashab et al., enzymatically degradable silsesquioxane nanoparticles were synthesized and used as fluorescent nanoprobes for *in vitro* imaging of cancer cells [26]. Zink and co-workers developed different light-activatable and pH-responsive hybrid materials for drug delivery applications [27–29]. In these studies mostly low-molecular weight organic silsesquioxane bridging groups were incorporated into the pore walls of mesoporous nanostructures. Here, we report the synthesis of a PMO nanomaterial consisting of the biocompatible and large molecule curcumin and ethane organic moieties without the use of additional silica. Curcumin is a natural yellow-colored antioxidant compound extracted from *Curcuma longa* and has been used for centuries in its crude form as dietary supplement and in traditional Asian medicines [30]. Recently, it has been shown that curcumin exhibits an exceptionally large range of biomedical activity against diseases such as Alzheimer, Parkinson, Malaria and many more [31]. In addition, it shows strong anti-inflammatory effects and has potential chemotherapeutic value as it inhibits cell proliferation and induces apoptosis in various cancer

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cell lines [32–35]. However, its bioavailability is limited by its very low aqueous solubility [36,37]. Many different approaches have been investigated to improve the bioavailability and biopharmaceutical properties such as incorporating curcumin into liposomes [38,39], polymeric nanoparticles [40–42], bioactive glasses [43] or amino acid conjugates [44,45]. Various successful *in vitro* [46–48] and *in vivo* [49–51] studies show the exceptional anticancer properties of curcumin nanoformulations. Additionally, it is well tolerated by the human body up to 12 g/day in oral administration as shown in clinical studies, which shows great promise regarding the biocompatibility of curcumin-based nanosystems [52]. Here, we present the synthesis of PMO nanoparticles with curcumin being the main organic constituent of the organosilica framework. Importantly, the synthesis was achieved without the addition of tetraethyl orthosilicate (TEOS), which is often used in other PMO studies for framework stabilization. The nanoparticles obtained in this study exhibit good dispersibility and high porosity parameters, which hold promise for a variety of applications in drug delivery. Furthermore, the incorporated curcumin compounds cause significant fluorescence of the nanoparticles themselves, which implies that no additional dye is necessary to track the NPs in live-cell experiments. The mesoporous PMO nanoparticles were used as a cargo release system with a Supported Lipid Bilayer (SLB) serving as cap in various *in vitro* experiments.

2. Experimental section

2.1. Materials and characterization techniques

Curcumin (60–70%), 3-isocyanatopropyl(triethoxysilane) (IPTES), tetrahydrofuran (dry), triethylamine (97%), cetyltrimethylammonium bromide, ammonium nitrate, ammonium bicarbonate, Rhodamin B, calcein, sodium hydroxide, DMSO-*d6*, dichloromethane, $CDCl_3$ and methanol were purchased from Sigma Aldrich. Bis(triethoxysilyl)ethane (BTSE) was purchased from ABCR. DOPC (1,2-dioleoyl-*sn*-glycero-3-phosphocholine) and DOTAP (1,2-dioleoyl-3-trimethylammonium propane) were purchased from Avanti Polar Lipids. All chemicals were used as received without further purification. Doubly distilled water from a Millipore system (Milli-Q Academic A10) was used for all synthesis and purification steps. All samples were investigated with an FEI Titan 80–300 transmission electron microscope operating at 300 kV with a high-angle annular dark field detector. A droplet of the diluted MSN solution in absolute ethanol was dried on a carbon-coated copper grid. Dynamic light scattering (DLS) measurements were performed on a Malvern Zetasizer-Nano instrument equipped with a 4 mW He–Ne laser (633 nm) and an avalanche photodiode. The hydrodynamic radius of the particles was determined by dynamic light scattering in ethanolic suspension. For this purpose, 100 μL of an ethanolic suspension of MSN (ca. 10 mg/mL) was diluted with 3 mL of ethanol prior to the measurement. Zeta potential measurements of the samples were performed on a Malvern Zetasizer-Nano instrument equipped with a 4 mW He–Ne laser (633 nm) and an avalanche photodiode. Zeta potential measurements were performed using the add-on Zetasizer titration system (MPT-2) based on diluted NaOH and HCl as titrants. For this purpose, 1 mg of the particles was diluted in 10 mL bi-distilled water. Nitrogen sorption measurements were performed on a Quantachrome Instruments NOVA 4000e. All samples (10 mg each) were heated to 100 °C for 12 h in vacuum (10 mTorr) to outgas the samples, before nitrogen sorption was measured at 77 K. Pore size and pore volume were calculated with an NLDFT model of N_2 on silica, based on the adsorption branch of the isotherms. A BET model was applied in the range of 0.05–0.20 p/p_0 to evaluate the specific surface area of the samples. Centrifugation was performed using an Eppendorf centrifuge with an adapter for Falcon tubes or an Eppendorf centrifuge 5418 for small volumes. Raman spectra were recorded on a Jobin Yvon Horiba HR800 UV Raman microscope using a He–Ne laser emitting at $\lambda = 633$ nm with a laser power of 10 mW. IR measurements were performed on a Bruker Equinox 55 FTIR spectrometer in absorbance mode (spectra were background substracted). UV-VIS spectra were recorded with a NanoDrop ND 1000 spectrometer. Usually, 2 μL of sample were used and all presented spectra are background corrected for water absorption. Thermogravimetric analysis (TGA) of the samples (about 10 mg of dried nanoparticles) was performed on a Netzsch STA 440 Jupiter thermobalance with a heating rate of 10 K/min in a stream of synthetic air of about 25 mL/min. Cross-polarized ^{29}Si- and ^{13}C-MAS NMR measurements were performed on a Bruker DSX Avance500 FT spectrometer (11.74 T) in a 4 mm ZrO_2 rotor. The spinning rate was 10 kHz and a total number of 256 scans were recorded. The used contact time was 2 ms and the recycle delay was 1 s.

2.2. Synthesis of curcumin-precursor

Curcumin ((1*E*,6*E*)-1,7-bis-(4-hydroxy-3-methoxy-phenyl)-hepta-1,6-dien-3,5-dion, 2.00 g, 5.43 mmol, 1 eq.) was dissolved in 25 mL dry THF in a three-necked flask. Subsequently, 3-isocyanatopropyl(triethoxysilane) (5.37 g, 21.7 mmol, 4 eq.) and triethylamine (165 mg, 1.63 mmol, 0.3 eq.) were added under stirring and the mixture was refluxed for 24 h at 85 °C in a nitrogen flow. After cooling down to room temperature, the sample was filtered and washed with ethyl acetate. The solvents were evaporated at reduced pressure and the sample was purified with column chromatography on silica gel with a solvent mixture containing 97 v% dichloromethane, 2 v% methanol and 1 v% triethylamine. The compound obtained (named Curcumin-IPTES) was dried under high vacuum for 12 h and used without further purification (yield: 2.29 g, 2.65 mmol, 49%). ^{1}H-NMR (300 MHz, DMSO-d_6): δ [ppm] = 7.72 (t, 2H), 7.62 (d, 2H), 7.44 (s, 2H), 7.26 (d, 2H), 7.09 (d, 2H), 6.95 (d, 2H), 6.16 (s, 1H), 3.80 (s, 6H), 3.73 (qa, 18H), 1.45 (q, 6H), 1.12 (t, 27H), 0.55 (t, 6H). ^{13}C-NMR (400 MHz, $CDCl_3$): δ [ppm] = 183.14, 153.98, 152.00, 141.75, 140.10, 133.36, 124.00, 123.67, 121.10, 111.47, 101.68, 58.49, 51,92, 43.72, 23.61, 18.29, 9.20. MS (ESI): $[M-H]^-$ cal.: 861.36206, found: 861.36672.

2.3. Synthesis of mesoporous Curcumin-PMO nanoparticles (MCNs)

In a two-step sol–gel reaction, cetyl trimethyl-ammonium bromide (CTAB, 0.96 mmol, 350 mg) was dissolved in a mixture containing 120 mL H_2O and 15 mL absolute ethanol in a 250 ml round bottom flask. Subsequently, 875 μL sodium hydroxide solution (2 M) was added and the mixture was stirred at 80 °C for 30 min. In a glass vessel, 400 mg Curcumin-IPTES (0.32 mmol) was mixed with 200 μL bis(triethoxysilyl)ethane (BTSE, 0.51 mmol) and 400 μL ethanol. This precursor solution was preheated to completely dissolve the compounds and afterwards quickly injected into the stirring aqueous template solution. The reaction was maintained for 90 min at 80 °C and 700 rpm. Extraction of the organic template was achieved by heating the ethanol-suspended (80 mg) sample under reflux at 90 °C for 1 h in a mixture of 2 g ammonium nitrate and 100 mL ethanol. Afterwards, the sample was centrifuged for 15 min at 7830 rpm (7197 rcf), redispersed in ethanol and heated under reflux at 90 °C in a solution of 100 mL ethanol for 45 min. After centrifugation, the particles were re-dispersed in 20 mL ethanol, resulting in a colloidal yellow suspension with a concentration of 4 mg/mL.

2.4. Synthesis of reference PMO nanoparticles

The reference PMO nanoparticles consisting exclusively of ethane organic bridging groups were synthesized in a similar