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Polyglycerol mediated covalent construction of magnetic mesoporous silica nanohybrid with aqueous dispersibility for drug delivery

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ABSTRACT

Construction of nanohybrids with chemical and colloidal stability is of great importance for the exploration of their potential applications in biomedical field. In this work, a versatile strategy based on polyglycerol (PG) mediated covalent linkage is developed to fabricate a core-satellite nanohybrid, termed MMSN, consisting of a mesoporous silica nanoparticle (MSN) as a core and many superparamagnetic iron oxide nanoparticles (SPION) on the outer surface. In this synthetic strategy, the PG grafted SPION is derivatized to convert partial periphery hydroxyl groups to carboxyl moieties, followed by attachment to aminated MSN through amide bonds. The PG layer accounting for ~17 wt% of MMSN not only serves as a tether to connect the two nanoparticles but also greatly enhances the colloidal stability of the nanohybrid, resulting in no significant change in hydrodynamic diameter and zeta potential during four months. Taking advantage of the combined porosity and magnetic property of the nanohybrid, a photosensitizer chlorin e6 (Ce6) is loaded on MMSN and efficiently delivered into target cells under magnetic guidance, leading to an enhanced efficacy of photodynamic therapy (PDT). The versatile strategy presented here opens up a new route to rational design and fabrication of multifunctional nanohybrids for various biomedical purposes.

1. Introduction

Nanohybrids containing two or more entities with different functions in a single particle have attracted considerable interest across diverse research areas due to their combined features of each component as well as synergistic properties [1–5]. In biomedical field, multifunctional nanohybrids have been demonstrating their great potential as biosensors, imaging agents, drug carriers, and therapeutic tools [6–19]. Inorganic nanoparticles such as iron oxide [20–21], quantum dots [22], and nanocarbons [23–25] possess unique physical and chemical properties (magnetism, fluorescence, large specific surface area, *etc.*), are among the most popular building blocks for constructing the multifunctional nanohybrids for biomedical applications. Currently, most inorganic nanohybrids are fabricated by growing heterogeneous nanostructures on a nanoplatform [26–30] or noncovalent attachment [31–35]. However, these methods suffer from either high cost or chemical instability. In addition, given the hydrophobic nature of inorganic nanoparticles, enhancing the aqueous dispersibility of the nanohybrids by surface modification (*e.g.*, PEGylation) is also essential for biomedical applications [36–39]. Therefore, it is needed to develop new synthetic strategies to construct nanohybrids with enhanced chemical stability and aqueous dispersibility.

In recent years, polyglycerol (PG) functionalization has emerged as a convenient and versatile surface modification technique to enable the biomedical applications of various inorganic nanoparticles [40–43]. The PG layer can be directly grafted on the nanoparticles bearing reactive functional groups such as hydroxyl and carboxyl groups through surface-initiated ring-opening polymerization of glycidol [44]. The PG layer is composed of hyperbranched backbone of polyethylene glycol and numerous hydroxyl groups on the periphery, which tremendously increase the aqueous dispersibility and stability of coated nanoparticles. When applied in biological systems, the PG layer shows antifouling behavior to suppress the adsorption of a variety of proteins [45–46], excellent shielding effect to prevent non-specific uptake of the nanoparticles by cells [12,24,47], and stealth effect to help the nanoparticles to escape the capture by reticuloendothelial system [48]. Moreover, through stepwise organic transformations and reactions, the hydroxyl groups on the PG layer can be readily converted to more reactive functional groups like azido, amino, and carboxyl groups, enabling further functionalization with a range of functional moieties including targeting

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ligands, imaging probes and anticancer drugs [20,23–24,49]. Inspired by these findings, researchers have rationally engineered PG layer as a versatile linker to integrate different nanoparticles into multifunctional nanohybrids. For example, Zhou and coworkers reported that the carboxylated PG layer grafted on magnetic nanoparticles served as an ideal template to anchor various metallic nanocatalysts; the nanohybrids were stabilized by the complexation between the carboxyl groups on the PG layer and the metal nanoparticles [50]. However, PG mediated construction of nanohybrids through more stable covalent bonding has been rarely investigated so far [51].

In this work, we developed a versatile strategy that is based on PG mediated covalent linkage to construct a core-satellite nanohybrid, termed MMSN, consisting of a mesoporous silica nanoparticle (MSN) as a core and many superparamagnetic iron oxide nanoparticles (SPION) on the outer surface. Specifically, the PG grafted SPION was derivatized to convert partial periphery hydroxyl groups to carboxyl moieties, followed by attachment to aminated MSN through amide bonds. The PG layer accounting for ~17 wt% of MMSN not only served as a tether to connect the two nanoparticles but also greatly enhanced the colloidal stability of the nanohybrid, enabling its biomedical applications without PEGylation. Combining the intrinsic porosity of MSN and magnetic property of SPION, MMSN suggested great promise as a nanocarrier for drug delivery. As a proof-of-concept application, a photosensitizer chlorin e6 (Ce6) was loaded on MMSN and efficiently delivered into cancer cells under the guidance of an external magnetic field, leading to an enhanced efficacy of photodynamic therapy (PDT).

2. Experimental

2.1. Materials

Succinic anhydride, ferric acethlacetonate, tetarethyl orthosilicate (TEOS), *N*,*N*-dimethyl-4-aminopyridine (DMAP), hexadecyl trimethyl ammonium bromide (CTAB) were purchased from Energy Chemical (Shanghai, China). Ce6 was purchased from MedKoo Bioscience. (3-aminopropyl)triethoxysilane (APTES), *N*-hydroxy-succinimide (NHS), 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (EDC) were provided by TCI. Gelatin was obtained from Macklin (Shanghai, China). Glycidol was provided by aladdin (Shanghai, China) and distilled prior to use.

2.2. Instruments

UV-vis-NIR absorption measurements were carried out on a Shimadzu UV-3600 spectrophotometer. FTIR spectral measurements were conducted on a Nicolet iS50 FT-IR spectrometer (Thermo Scientific) using reflectance mode. Hydrodynamic diameter and zeta potential were determined in water using a Malvern Zetasizer Nano ZS90. Thermogravimetric analysis (TGA) was carried out using a NETZSCH STA 449F3 simultaneous thermogravimetric analyzer under nitrogen in the temperature range from 30 to 600 °C with a heating rate of 10 °C/min. Small angle X-ray diffraction (SAXRD) was recorded using a Bruker D8 Advance X-ray diffractometer (CuKα irradiation, $\lambda = 1.5406$ Å) operated at 40 kV and 40 mA. Scanning transmission electron microscopy (STEM) was performed on a JEOL JSM-7500F field emission scanning electron microscope. All samples for electron microscopy were prepared by evaporating one drop of samples on ultrathin carbon-coated copper grids.

2.3. Synthesis of MSN

MSN was prepared according to the procedure reported previously [52]. Briefly, CTAB (0.6 g, 1.65 mmol) and gelatin (0.6 g) were dissolved in the mixture of MilliQ water (50 mL) and ethanol (50 mL) at 45 °C. Next, 14.5 mL of aqueous ammonia (26% ~ 28%) and TEOS (0.85 g, 4.1 mmol) were added into the solution successively, and then vigorously stirred at 38 °C for 2 h to generate a white precipitate. The precipitate was collected by centrifugation at 4000 rpm for 5 min, washed with ethanol and MilliQ water three times, and dried *in vacuo* at 60 °C overnight. Finally, the sample was calcined in air at 600 °C for 6 h to remove the templates under a heating rate of 10 °C/min, yielding a white powder.

2.4. Synthesis of MSN-NH_2

MSN (80 mg) was dispersed in anhydrous toluene (30 mL) by bath sonication. Subsequently, APTES (3 mL) was added into the dispersion, and then stirred at 80 °C for 20 h under nitrogen atmosphere [53]. After being cooled down, the excess toluene was evaporated and the residue was dialyzed in methanol/water (v/v = 1/1) to remove any unbounded APTES, and then in pure water to replace the solvents. The purified product was well dispersed in water and stored at 4 °C prior to use.

2.5. Synthesis of SPION-PG-COOH

PG-grafted iron oxide nanoparticles (SPION-PG) were prepared according to the method reported previously [20]. To introduce carboxyl groups, SPION-PG (15 mg) was well dispersed in dry pyridine (5 mL), followed by adding with succinic anhydride (15 mg, 0.15 mmol) and DMAP (2 mg, 0.013 mmol). The mixture was stirred at room temperature for 24 h. The resulting solid was collected by ultracentrifugation (Beckman Coulter JA-21, 21,000 rpm) and purified in DMF/water by repeated redispersion/ultracentrifugation cycles. The purified product was dispersed in water and stored at 4 °C prior to use.

2.6. Synthesis of MMSN nanohybrid

SPION-PG-COOH (14.3 mg), NHS (149 mg, 1.3 mmol), and EDC (148 mg, 0.77 mmol) were mixed in water (5 mL), and then stirred for 1 h at room temperature to activate the carboxyl groups. Subsequently, the mixture was added into MSN-NH_2 (40 mg) dispersed in water (5 mL), and then stirred for 24 h. The resulting nanohybrid was readily collected by a permanent magnet, whereas the unbound SPION-PG-COOH possessing excellent dispersibility was hardly attracted. Therefore, the nanohybrid was isolated and purified in water by repeated magnetic separation/redispersion cycles until the supernatant became clear. The purified product was dispersed in water and stored at 4 °C prior to use.

2.7. Synthesis of Ce6@MMSN nanohybrid

Ce6 (3.8 mg, 0.006 mmol) was first activated by treating with NHS (52 mg, 0.45 mmol) and EDC (114 mg, 0.6 mmol) in 2 mL of PBS for 1 h in the dark. The mixture was subsequently added into MMSN (20.4 mg) dispersed in 2 mL of PBS for 24 h in the dark at room temperature. The nanohybrid was collected by magnetic separation, and then purified in PBS by repeated magnetic separation/redispersion cycles until the supernatant became colorless. The product was redispersed in PBS and stored at 4 °C prior to use.

2.8. Calculation of Ce6 concentration

Ce6 concentration was calculated from the UV-vis-NIR spectra of dispersions using a calibration formula: $Y = 0.0247 + 0.223X$, where Y is the absorbance at 402 nm, and X represents the concentration of Ce6 (μg/mL). To determine the Ce6 content of Ce6@MMSN dispersion, the absorbance contributed by MMSN was deducted prior to the calculation.