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Kinetics-mediate fabrication of multi-model bioimaging lanthanide nanoplates with controllable surface roughness for blood brain barrier transportation

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ABSTRACT

Effective delivery of imaging agents or therapeutics to the brain has remained elusive due to the poor blood-brain barrier (BBB) permeability, resulting in the apparent risks of inefficient diagnosis and therapeutic agents for brain disease. Herein, we report on the surface roughness mediated BBB transportation for the first time. The lanthanide-based core/shell/shell structured $NaYF_4$:Yb,Er@$NaGdF_4$:Yb@$NaNdF_4$:Yb nanoplates with controllable surface roughness and multi-model bioimaging features were synthesized and used to evaluate the surface roughness dependent BBB permeability without any surface bio-functionalization. By controlling the kinetics of the shell coating process, the hexagon-disc, multi-petals and six-petals nanoplates with different surface roughness can be obtained. Comparing with the NPs with less Ra and receptor-conjugated NPs, the obtained six-petals nanoplates with highest roughness exhibit excellent performance in BBB transportation and tumor targeting, which lay solid foundation for the diagnosis and the therapy of brain tumor.

1. Introduction

The blood–brain barrier (BBB) is built up by polarized layer of brain capillary endothelial cells (BCECs) that physically separates blood from brain tissue [1], which hamper the permeating of theranostic agents for the brain-related diseases, resulting in the apparent risks of inefficient diagnosis and and therapeutic agents for brain disease [2,3]. Only un-ionized, lipophilic, and low molecular weight molecules can diffuse freely through the BCECs membrane and may thus passively cross the BBB [4]. Polar molecules and small ions are almost totally excluded by the tightly closed intercellular cleft [5]. Many attempts in using nanoparticles as blood brain theranostic delivery systems were performed with certain success thus demonstrating the feasibility of drug delivery to overcome the BBB using these nanocarriers [6–8]. However, the strategies for transporting nano-agents from the blood into the brain usually need specific surface bio-functionalization for the BCECs endocytosis and crossing, such as the transferrin-mediated transcytosis [9,10], siRNA-dominated extensive inhibition of BCECs P-glycoprotein [11] and modulation of tight junction structure in BCECs by using chitosan [12].

Recently, it has been demonstrated that nanoparticle geometry, especially the surface roughness have obvious influence on both their binding with biomolecules (*e.g.*, protein, genetic molecules) and afterwards cellular uptaking without any assist of surface bio-conjugation [13,14]. Therefore, we hypothesize that the enhanced BCECs endocytosis and subsequent transcytosis across of BBB may be generally realized by controlling the surface roughness of nanoparticles. Herein, the core/shell/shell structured $NaYF_4$:Yb,Er/$NaGdF_4$:Yb/$NaNdF_4$:Yb nanoplates (NPs) with controllable surface

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roughness were synthesized and firstly used to evaluate the surface roughness dependent BBB permeability without any assist of specific bio-conjugation. The hexagon-disc, multi-petals and six-petals shaped NPs can be obtained by delicate tuning the reaction kinetics of the shell coating process. Furthermore, the $NaGdF_4$ inner layer and the $NaNdF_4$ out layer endow the NPs with magnetic resonance imaging (MRI) and NIR-II region fluorescence (FL) properties with negligible background autofluorescence and minimal scattering for bioimaging. Benefited from the multi-model bioimaging capabilities, the obtained NPs were used in the evaluation of the surface roughness dependent BBB transportation. The systematic *in-vivo* NIR-II FL imaging and MRI of intracranial glioblastoma results show that the surface bare six-petals NPs with the highest roughness can cross the BBB more efficiently than not only other NPs with less roughness, but also the receptor-conjugated NPs, which lay solid the foundation for the diagnosis and therapy of brain tumor.

2. Material and method

2.1. Materials and characterization

2.1.1. Materials

All solvents used were of analytical grade without further purification. Gadolinium (III) chloride anhydrous ($GdCl_3$, 99.99%), yttrium (III) chloride anhydrous (YCl_3, 99.9%), ytterbium (III) chloride anhydrous ($YbCl_3$, 99.9%), erbium (III) chloride anhydrous ($ErCl_3$, 99.9%), thulium (III) chloride anhydrous ($TmCl_3$, 99.9%), neodymium (III) chloride hexahydrate ($NdCl_3$, 99.9%), sodium trifluoroacetate (Na-TFA, 98%), 1-octadecene (ODE, 90%), oleic acid (OA, 90%), (ethylene glycol)—distearoyl phosphatidylethanolamine, (DSPE-PEG_{2000}-NH_2) were purchased from Avanti Polar Lipids. Rose Bengal (99%) was bought from Sigma Aldrich. CCK-8 was obtained from Shanghai Dongin Chemical Technology Co. Ltd.

2.1.2. Characterization

Transmission electron microscopy (TEM) measurements were carried out on a JEM 2100F microscope (Japan) operated at 200 kV. The samples were first dispersed in ethanol and then collected by using copper grids covered with carbon films for measurements. Energy-dispersive X-ray spectroscopy (EDX) was performed on a JEM 2100F EDX instrument. Scanning electron microscopic (SEM) images were obtained on a Philip XL30 microscope (Germany). The nanoplates were dispersed in cyclohexane and directly dropped on copper net for SEM and TEM. The uperconversion luminescence (UCL) and NIR II FL spectra were characterized on a Hitachi Fluorescence Spectrometer F4500 instrument equipped with a 0—2 W adjustable continuous-wavelength laser (808 nm, Beijing Hi-Tech Optoelectronic Co., China) as the excitation source. X-ray diffraction (XRD) patterns were recorded with a Burker D8 powder X-ray diffractometer (Germany) using Cu Kα radiation (40 Kv, 40 mA). MRI was obtain by Siements 1.5 T Avanto and NIR II FL imaging was carried by InGaAs under 808 nm irradiation.

2.2. Synthesis of core/shell/shell $NaYF_4$:Yb,Er@$NaGdF_4$:Yb@$NaNdF_4$:Yb nanoplates with controllable surface roughness

Lanthanide doped core/shell structured nanoplates was synthesized using the successive layer-by-layer (SLBL) strategy reported by us previously (see Supporting Information for details).[15] The coating of $NaNdF_4$:Yb layer with controllable surface roughness can be synthesized under the guidance of kinetics-mediation strategy. Typically, 5 mL as prepared $NaYF_4$:Yb,Er@$NaGdF_4$:Yb was mixed with 8.0 mL of oleic acid (OA) and 12.0 mL of octadecylene (ODE). The flask was pumped down at 70 °C for 30 min to remove cyclohexane, any residual air. Subsequently, the system was switched to Ar flow and the reaction mixture was further heated to 280 °C at a rate of ~20 °C/min. Then Nd-Yb-OA (1.0 mL) and Na-TFA-OA (0.5 mL, sodium trifluoroacetate dissolved in oleic acid) shell precursors were alternately introduced by dropwise addition at 280 °C and the time interval between each injection was 15 min. There are four groups of Nd-Yb-OA and Na-TFA-OA. The reaction was finished after another 100 min under 280 °C. The shell roughness can be well tuned by changing the concentration of the shell precursors (0.05 M Nd-Yb-OA for the six-petals coating and 0.2 M Nd-Yb-OA for the hexagon-disc coating). The resultant nanoplates were precipitated by addition of ethanol, collected by centrifugation at 6000 rpm for 5 min, washed with ethanol several times, and re-dispersed in chloroform for further use.

2.3. In-vitro BBB transportation evaluation of six-petals, multi-petals and hexagon-disc

2.3.1. Cell culture

BCECs were purchased from Cell bank of Chinese Academy of Sciences which were obtained from human brain. BCECs for cell viability and cellular uptake studies were cultured at 37 °C and with 5% CO_2 in Roswell Park Memorial Institute medium (RPMI) 1640 supplemented with 15% fetal bovineserum (FBS) and 1% penicillin/streptomycin. U87MG for cell viability and cellular uptake studies were cultured at 37 °C and with 5% CO_2 in Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% fetal bovine serum (FBS) and 1% penicillin/streptomycin.

2.3.2. Trans-well experiment

Polycarbonate 24-well transwell membranes of 1.0 μm mean pore size, 0.33 cm^2 surface areas (FALCON Cell Culture Insert, Becton Dickinson Labware, USA) were applied for trans-well experiment. BCECs were seeded at $2*10^5$ cells/filter on 12-well plates coated with fibronectin. The tight junction of BECEs was monitored using an epithelial voltammeter (Millicell-RES, Millipore, USA), and the cells with Transendothelial Electrical Resistance (TEER) values above 200 Ωcm^2 were selected for the transfer experiments. U87MG cell medium was added in the bottom chamber for latter U87MG cellular uptake. Then BCECs was co-incubated with hexagon-disc@Tf (transferrin), six-petals, multi-petals and hexagon-disc (400 μg/mL) for 4 h. After incubation, all the medium were collected from each chamber. Then tight junction of BCECs were washed with phosphate buffer saline (PBS) for removing cell membrane adhered NPs on cell membrane. Then, they were striped from filter by cell scraping. Finally, cells were rinsed twice with PBS and digested by 5% SDS to obtain the intracellular NPs. Finally, cells were rinsed twice with PBS and digested by 5% SDS to obtain the intracellular nanoplates. After being wash three times by PBS, all cellular bare-nanoplates and transferrin modified nanoplate were resuspended in PBS. Nanoparticles distribution was evaluated in the three compartments: free particles in the upper chamber, entrapped particles in the BCECs tiight junction and particles recovered in the bottom chamber after transporting through the BCECs tight junction. Finally, NPs distribution (the Gd^{3+} concentrations from $NaGdF_4$:Yb) was evaluated by Inductively Coupled Plasma Mass Spectrometry (ICP-MS).

2.4. In-vivo BBB transportation evaluation of six-petals, multi-petals and hexagon-disc

2.4.1. NIR-II FL bioimaging

The female balb/c mice with brain tumor were administered with a single dose of six-petals, multi-petals and hexagon-disc (15 mg/kg, n = 4) *via* tail injection. Downconversion luminescence (DCL) imaging was obtained by InGaAs camera under