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Upconverting and persistent luminescent nanocarriers for accurately imaging-guided photothermal therapy

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ABSTRACT

The fluorescence-guided photothermal therapy (FPTT) has great potential in cancer treatment. However, the conventional FPTT has to be stimulated by external light, which tends to increase background noise and leads to the inaccurate infrared light irradiation for PTT. In this study, upconverting and persistent luminescent nanocarriers (UPLNs) loaded mesoporous silica nanoparticles (UPLNs@$mSiO_2$) were first designed to solve the problem mentioned above. The UPLNs cores can effectively reduce the short-lived autofluorescence interference by exerting the delay time between signal acquisition and pulsed excitation light. For testing the luminescence properties, the indotcyanine green (ICG) as photothermal agent was encapsulated into the UPLNs@$mSiO_2$. The experimental results showed that the UPLNs@$mSiO_2$ nanoparticles could significantly reduce the short-lived autofluorescence interference and improve signal-to-noise ratio during FPTT. Our data suggest that UPLNs@$mSiO_2$ may be a promising tool for improving the accuracy of PTT in vivo.

1. Introduction

Photothermal therapy (PTT) as a minimally invasive method of treatment has attracted widespread interest. It can employ photo-absorbing agents to generate heat from the extra optical light, resulting in the 'burning' of cancer cells [1–3]. Cancer cells can be shot because of heat-inducted proteins denaturation and disruption of cell membranes. The key factor of the PTT is whether the extra optical light can precisely irradiate photo-absorbing agents in vivo. To efficiently track the photothermal agents such as indotcyanine green (ICG) in vivo, imaging-guided PTT is a promising method to realize the precise guidance in cancer treatments [4–6]. Among some new kinds of luminescent nanoprobes, the persistent luminescent nanocarriers (PLNs) have attracted enormous attention in recent years, owing to improving the signal-to-noise ratio by avoiding the autofluorescence of tissues, which is very helpful to increase the accuracy of imaging during PTT [7–9].

However, PLNs still suffer from two main limitations during the application in bioimaging filed. 1) Normally PLNs are synthesized through solid-state-annealing way. As a result, these nanoparticles were usually heterogeneous without any hydrophilic groups on the surface. So the aggregated PLNs are easy to be taken up and sequestered by the reticuloendothelial system (RES) in vivo. 2) Usually, PLNs can be excited by ultraviolet (UV) light. Due to the rather limited tissue penetration depth of UV light, these PLNs had to be pre-charged in vitro and cannot be recharged in vivo. This limitation leads to the short imaging time of PLNs. These two challenges severely influence the applications of PLNs in bioimaging.

To improve the size distribution and hydrophilicity of PLNs, various kinds of nanocarriers are used to load the PLNs [10–12]. Among these nanocarriers, mesoporous silica nanoparticles (MSNs) may be a good choice for the surface modification of PLNs. The ordered MSNs have acquired considerable attentions as drug delivery systems because of their stable structure, adjustable pore size, high specific surface area, good biocompatibility, well-defined surface properties [13–15]. This convenient mesoporous silica template method is based on the use of mesoporous silica nanoparticles as hard templates to control the morphologies of the PLNs. The diameter and morphologies of the PLNs could be easily regulated by adjusting the MSNs.

To prolong the imaging time of PLNs during PTT, several methods have been carried out. UV light is attenuated quickly in human tissues

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and causes damage to normal cells [16]. Additionally, protein and nucleic acid are very susceptible to losing their activity. These two challenges severely limit their applications [17]. Near-infrared (NIR) light can be a possible candidate for minimally invasive diagnosis of cancer. In addition, compared with UV light, NIR light can penetrate into tissues up to 10 cm and cause minimal damage to the surrounding tissues and organs [18,19]. Specific probes that can emit light in the NIR range would enhance imaging sensitivity at significant depths of tissue. But how to translate NIR light to visible light for light-triggered release is an urgent and unresolved issue. The upconverting from NIR light to visible light can be realized by upconverting rare earth element Thulium (Tm) and Ytterbium (Yb) which can transfer two or more low-energy pump photons from the NIR to a higher-energy output photon with a shorter wavelength such as UV or visible light [20]. So Tm and Yb can be doped into the PLNs matrix for upconverting imaging which can be a good complementary of persistent luminescent imaging [21–23].

In this paper, the upconverting PLNs and photo-absorbing agents ICG coloaded $mSiO_2$ (UPLNs and ICG coloaded $mSiO_2$) nanoparticles were developed for upconverting and persistent luminescent imaging-guided PTT. This kind of nanodelivery system can simultaneously improve hydrophilicity and imaging time of UPLNs materials [24–26]. The UPLNs and ICG coloaded $mSiO_2$ nanoparticle includes three main parts: 1) The upconverting PLNs, Ca,Ti,Pr,Er,Tm were chosen for upconverting and persistent luminescent imaging, which could be excited by extra NIR light and emit upconverting luminescence, which can be used for imaging in vivo after the persistent luminescence signal became weak; 2) Indocyanine green (ICG), a water soluble anionic tricarbocyanine dye is the only NIR agent approved by FDA which has been widely used clinically for PTT [17]; 3) The MSNs worked as a three dimension hard template for loading and protecting both upconverting PLNs and ICG, which could effectively avoid aggregation.

2. Materials and methods

2.1. Materials

All reagents were used as received without further purification. Indocyanine green, Calcium nitrate tetrahydrate ($Ca(NO_3)_2{\cdot}4H_2O$, ≥ 99.0%), praseodymium(III) nitrate hexahydrate ($Pr(NO_3)_3{\cdot}6H_2O$, ≥ 99.0%), titanium(IV) butoxide ($Ti(OC_4H_9)_4$, 97%), ytterbium (III) chloride hexahydrate ($YbCl_3{\cdot}6H_2O$, 99.99%), Thulium(III) chloride hexahydrate ($TmCl_3$ $6H_2O$), Tetraethylorthosilicate (TEOS, ≥99%), cetyltrime thylammonium bromide (CTAB, ≥99%), ammonium nitrate (NH_4NO_3, ≥99.0%) and nitric acid were all purchased from Sigma-Aldrich (USA); Silane-mPEG (5 k) were obtained from Creative PEG Works (USA).

2.2. Synthesis of persistent luminescent nanocarriers (PLNs)

$CaTiO_3$: 0.1%Pr^{3+} PLNs were synthesized by sol-gel method [27]. Generally, $Ca(NO_3)_2{\cdot}4H_2O$, $Pr(NO_3)_3{\cdot}6H_2O$ and $Ti(OC_4H_9)_4$ were used as the starting materials. First, titanyl nitrate was synthesized by the reaction of $Ti(OC_4H_9)_4$ with nitric acid. Next, $Ca(NO_3)_2{\cdot}4H_2O$ and $Pr(NO_3)3{\cdot}6H_2O$ were dissolved in the titanyl nitrate solution. Then, the mixture solution was stirred for about 2 h at 70 °C. After that the white precursor was collected and heated in a muffle furnace at a temperature of 600–900 °C for 3 h. Finally, $CaTiO_3$: 0.1% Pr^{3+} PLNs were grinded by ball grinder and purified by centrifugation.

2.3. Synthesis of upconverting and persistent luminescent nanocarriers (UPLNs)

($CaTiO_3$: 0.1%Pr^{3+}): Yb^{3+}, Tm^{3+} UPLNs were also synthesized by sol-gel method [27]. $YbCl_3{\cdot}6H_2O$, $TmCl_3{\cdot}6H_2O$ and synthesized $CaTiO_3$: 0.1%Pr^{3+} PLNs were used as the starting materials. First $YbCl_3{\cdot}6H_2O$, $TmCl_3{\cdot}6H_2O$ and PLNs were dissolved in the ethanol. Then, the mixture solution was stirred for about 2 h at 50 °C. Then the white precursor was heated in a muffle furnace at a temperature of 600–900 °C for 3 h. Finally, UPLNs were grinded by ball grinder and purified by centrifugation.

2.4. Synthesis of upconverting and persistent luminescent nanocarriers (UPLNs) loaded mesoporous silica nanoparticles (UPLNs@$mSiO_2$)

UPLNs@$mSiO_2$ nanoparticles were synthesized by CTAB template method [20,28]. Firstly, 1 ml of UPLNs water solution (25 mg/ml) was added into 30 ml of CTAB water solution (8 mg/ml) and stirred overnight. Next, the UPLNs–CTAB solution was added to the mixture of 10 ml of water, 5 ml of ethanol and 200 ml of NaOH solution (2 M) in a round bottom flask with a stirrer. After the temperature was kept stable at 60 °C, 300 ml of TEOS was added into the mixture and the reaction lasted for 3 h. Next, the reaction solution was centrifuged and washed with ethanol 5 times at 10000 rpm. The product was suspended into 30 ml ethanol. Then, in order to eliminate the CTAB template, 30 ml of product was added into a round bottom flask including NH_4NO_3 and topped up with EtOH to 50 ml. After the reaction which lasted for 3 h at 60 °C, the solution was centrifuged and washed with ethanol 5 times at 10000 rpm. Finally, the product was resuspended into 30 ml water for further use.

2.5. Synthesis of ICG and UPLNs coloaded $mSiO_2$ nanoparticles ((UPLNs + ICG)@$mSiO_2$)

Briefly, ICG (5–20 mg) was added into 10 ml of UPLNs@$mSiO_2$ water solution. After stirring for 24 h at room temperature, the solution was centrifuged and washed with water 5 times at 8000 rpm. Finally, the product was resuspended into 10 ml water for further use.

2.6. Characterization

The shape and morphology of the nanoparticles were visualized by transmission electron microscopy (TEM). TEM observation of the nanoparticles was performed at a voltage of 200 kv with a 2010F in bright-field mode. Dilute suspensions of samples in water were deposited on the grid and then air dried; the particle size was measured by dynamic light scattering (DLS). The spectra and lifetimes of the samples were checked by using powdered samples. The photoluminescence spectra and time-decay curves were calculated by using a fluorospectrophotometer. The absorbance was examined on an UV–vis spectrometer. The imaging of the sample was analyzed in a Xenogen IVIS imaging system, according to references [19,29].

2.7. Cytotoxicity assay of different samples

The cytotoxicity of samples against human breast cancer cell line MDA-MB-231 was evaluated in vitro using MTT method8 [30]. The cells at a density of 1×10^4 cells per well were seeded in 96-well culture plates in RPMI 1640 medium containing 10% FBS for 24 h. Then, 100 μl of medium containing the desired amount of samples was added. The cells were incubated for another 24 h at 37 °C. Then, 20 μl of MTT solution was added to each well and incubation was continued for another 4 h. The culture medium was removed, and 200 μl of DMSO was added into each well to solubilize the formazan. The absorbance of each well was measured at 570 nm in a microplate reader (Model 680, Biorad). The survival percentage was then calculated as compared to that of untreated cells. Experiments were carried out in triplicate.

2.8. Persistent luminescent imaging and photothermal therapy efficiency of different samples in vitro

In vitro persistent luminescent imaging was performed by putting the samples into the black 96-well plates. The signal from the covered sample was recorded after illumination by UV light for 60 s. The in