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Multifunctional near infrared-emitting long-persistence luminescent nanoprobes for drug delivery and targeted tumor imaging

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ABSTRACT

In this paper, near infrared-emitting long-persistence luminescent porous $Zn_{1.1}Ga_{1.8}Ge_{0.1}O_4:Cr^{3+}$, Eu^{3+} @SiO_2 nanoprobes have been prepared using mesoporous silica nanospheres both as morphology-controlling templates and as vessels. These nanoprobes possessed an excellent capacity for drug delivery and allowed for real-time monitoring of the delivery routes of the drug carriers *in vivo*. The nanoprobes demonstrated a typical mesoporous structure, a brighter NIR emission (696 nm) and a long afterglow luminescence that persisted for 15 d. Furthermore, after surface modification with folic acid (FA), a tumor-targeting group, these nanoprobes exhibited an excellent ability to target tumors with high sensitivity *in vitro* and *in vivo*. Importantly, these modified nanoprobes could accurately diagnose tumors and allow for long-term tumor monitoring *via in situ* and *in vivo* re-excitation by a red LED lamp. Furthermore, the drug release data demonstrated that the modified nanoprobes could be loaded with a large amount of doxorubicin hydrochloride (DOX) and showed sustained release behavior. Together, the results of this study indicate that these nanoprobes can accurately diagnose tumors, allow for long-term *in vivo* and *in situ* monitoring and release DOX *in situ* to cure tumors.

1. Introduction

Mesoporous silica materials have attracted increasing attention for drug delivery in recent decades due to their unique properties, which include a stable mesoporous structure, high specific surface area, easy surface modification and excellent biocompatibility [1–4]. However, because of the complicated environment *in vivo*, drug carriers entering into biological host systems are difficult to monitor in real time and also may not be able to effectively locate the targeted site (*e.g.*, a tumor), which would decrease the pharmacological function and potentially result in serious adverse reactions and side effects. Thus, it is critical to realize real-time monitoring of the delivery routes of drug carriers *in vivo* [5].

Fluorescent labeling has been proven to be a simple and effective way of monitoring the route of drug carriers *in vivo* [6]. Mesoporous silica materials functionalized to have fluorescent properties have attracted much interest because of their potential application in tracking drug carriers [7,8]. To date, a large number of silica-based fluorescence labeling materials, such as organic dyes, upconverting nanoparticles and semiconductor quantum dots (QDs), have been applied for drug delivery [9–12]. However, there are still some critical limitations for using these fluorescent probes to monitor drug delivery *in vivo*. Due to serious photo-bleaching and poor stability, organic dyes are only suitable for observation over short time periods [13]. Although fluorescent nanoprobes, such as semiconductor nanocrystals and upconverting nanocrystals, can effectively overcome the problems of photo-bleaching and instability associated with traditional organic fluorophores [14–16], these photoluminescent nanoprobes still have some shortcomings for practical applications. For example, low photoluminescent signals and strong auto-fluorescence from biological tissues, can drastically decrease the signal-to-noise ratio (SNR) and can even result in false diagnosis [17,18]. Such intrinsic disadvantages would affect their utility for tracking drug delivery *in vivo*.

Recently, there has been increasing interest in employing long-persistence luminescent nanoparticles (LPLNPs) for *in vivo* imaging. Because the long afterglow of these nanoparticles can last for several hours after they are excited *in vitro*, real-time *in vivo* imaging can be achieved after injection without requiring any external illumination source. Thus, the SNR can be significantly improved by removing the background noise originating from *in situ* excitation [19,20]. Moreover, the afterglow luminescence of near infrared (NIR)-emitting long-persistence luminescent nanoparticles (NLPLNPs) (the afterglow wavelength varies from 650 nm to 900 nm) falls within the tissue transparency window, where light attenuation is largely due to scattering rather than absorption, which is advantageous for long-

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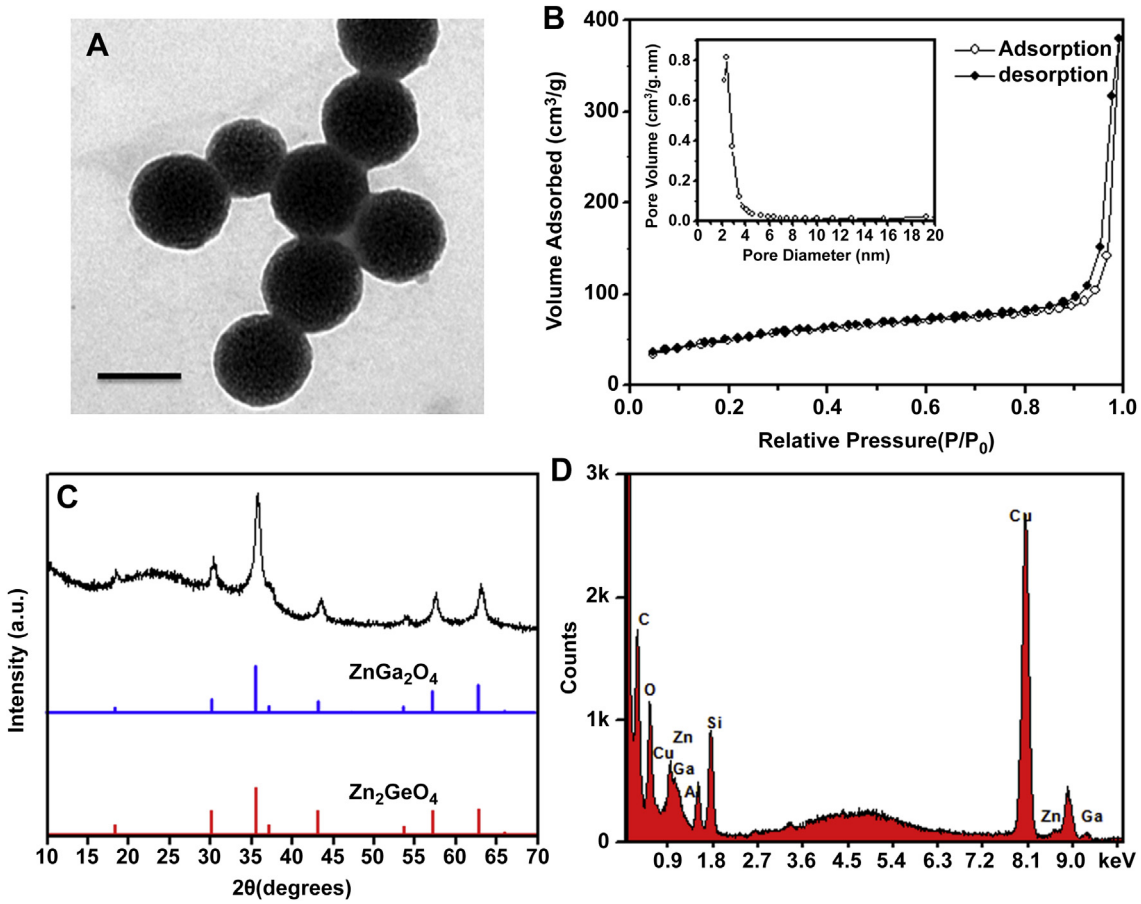

Fig. 1. Characterization of NLPLNPs@MSNs. (A) TEM image of NLPLNPs@MSNs. (B) N_2 adsorption/desorption isotherm and pore size distributions (inset) of NLPLNPs@MSNs. (C) XRD patterns of NLPLNPs@MSNs. (D) EDS of NLPLNPs@MSNs. Scale bar = 50 nm.

term *in vivo* imaging with deep penetration and a high SNR [21]. Scherman and co-workers designed NLPLNPs ($CaMgSi_2O_6$: Eu, Pr, Mn) to realize *in vivo* imaging for more than 1 h [22]. Moreover, Yan et al. have utilized NLPLNPs ($Zn_{2.94}Ga_{1.96}Ge_2O_{10}:Cr^{3+}$, Pr^{3+}) to diagnose tumors and realize long-term monitoring of tumors [23]. More recently, new and non-toxic NLPLNPs ($ZnGa_{1.995}Cr_{0.005}O_4$) with a narrow size distribution were used to realize tumor targeting and efficient cell tagging to track the biological fate of cells *in vivo*, without any acute toxicity, in healthy mice [24]. Although the materials mentioned above have exhibited great advantages for long-term *in vivo* imaging with a high SNR, they were still unsuitable for use as drug carriers because of their lack of a porous structure and the non-uniform particle sizes and morphologies, all of which have hindered the application of NLPLNPs in tracking drug delivery *in vivo*. Therefore, it is necessary to develop novel monodisperse NLPLNPs with a porous structure to realize not only tumor diagnosis but also the drug delivery.

Here, we utilized mesoporous silica nanospheres (MSNs) both as drug carriers and as morphology-controlling templates to design porous NLPLNPs. Furthermore, based on a recent report, the long afterglow performance of $Zn_{1.1}Ga_{1.8}Ge_{0.1}O_4:Cr^{3+}$ was the best among those of all germanium substituted $ZnGa_2O_4$ ($Zn_{1+x}Ga_{2-2x}Ge_xO_4:Cr^{3+}$), including $ZnGa_{1.995}Cr_{0.005}O_4$ and $Zn_{2.94}Ga_{1.96}Ge_2O_{10}:Cr^{3+}$ [25]. Thus, in this paper, we loaded $Zn_{1.1}Ga_{1.8}Ge_{0.1}O_4:Cr^{3+}$, Eu^{3+} into the pores of MSNs to prepare a novel trackable drug carrier ($Zn_{1.1}Ga_{1.8}Ge_{0.1}O_4:Cr^{3+}$, Eu^{3+} @SiO_2) (NLPLNPs@MSNs). Due to the MSN templates, the obtained samples showed a porous structure, spherical morphology, narrow size distribution, lager specific surface area and large pore capacity, indicating excellent drug carrier properties. Moreover, the NLPLNPs@MSNs demonstrated a brighter NIR emission (696 nm), and the long afterglow luminescence persisted for 15 d. In particular, the NLPLNPs@MSNs could be excited by a red LED lamp *in vivo*, indicating the capacity for real-time tracking and imaging continuously *in vivo* for long periods of time. Furthermore, after surface modification with folic acid (FA), a tumor-targeting group, the NLPLNPs@MSNs exhibited excellent tumor targeting ability with high sensitivity both *in vitro* and *in vivo*. All the results indicate that NLPLNPs@MSNs-FA can accurately diagnose tumors, be used for long-term *in vivo* and *in situ* monitoring and release a drug *in situ* to cure tumors.

2. Material and methods

2.1. Materials

$Zn(NO_3)_2{\cdot}6H_2O$, $Ga(NO_3)_3{\cdot}6H_2O$, $Cr(NO_3)_3{\cdot}9H_2O$, $Eu(NO_3)_3{\cdot}6H_2O$, and GeO_2 were purchased from Aladdin (Shanghai, China). Tetraethoxysilane (TEOS), 3-aminopropyl triethoxysilane (APTES), hexadecyl trimethyl ammonium bromide (CTAB), dimethylformamide (DMF), dimethyl sulfoxide (DMSO), ethanol, dichloromethane and diethanolamine were purchased from Shanghai Chemical Reagent Company (Shanghai, China). *N*-(3-Dimethylaminopropyl)-*N*′-ethylcarbodiimide hydrochloride (EDC), *N*-hydroxysuccinimide (NHS), 4-dimethylaminopyridine (DMAP), folic acid (FA), DOX and methyl thiazolyl tetrazolium (MTT) were

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