Persistent Luminescence Nanoparticles | Very Important Paper

Non-Aqueous Sol–Gel Synthesis of Ultra Small Persistent Luminescence Nanoparticles for Near-Infrared In Vivo Imaging


Abstract: Ultra-small ZnGa2O4:Cr3+ nanoparticles (6 nm) that exhibit near-infrared (NIR) persistent luminescence properties are synthesized by using a non-aqueous sol–gel method assisted by microwave irradiation. The nanoparticles are pegylated, leading to highly stable dispersions under physiological conditions. Preliminary in vivo studies show the high potential for these ultra-small ZnGa2O4:Cr3+ nanoparticles to be used as in vivo optical nanotools as they emit without the need for in situ excitation and, thus, avoid the autofluorescence of tissues.

Nanotechnology-based devices and nanomaterials have been demonstrated to be very useful for improving the efficiency and efficacy of medical diagnosis. For molecular imaging, nanoscale materials such as noble metals, semiconductors, and metal oxides, have their own specific advantages[1] when it comes to being used as nanoparticles for each imaging mode, particularly for optical[2] and magnetic resonance[3] imaging. For optical imaging, semiconductor quantum dots (QDs) possess major optical advantages over fluorescent organic molecules, advantages that include high stability against photo-bleaching, intrinsic brightness, and the ability to tune their optical properties through changing their size, shape, and composition.[5] Recently, the synthesis of persistent luminescence nanoparticles (PLNPs) was described, allowing very sensitive optical detection in vivo (Scheme 1) by avoiding the autofluorescence of tissues.[6] Persistent luminescence is an optical phenomenon in which the excitation energy leads to the creation of an electron/hole pair located near doping ions, which are close to physically near structural defects.[7] At the end of the excitation, the carriers are slowly released from the trapping defects by thermal assistance and radiatively recombine to produce a persistent luminescence that can last for several hours.[8] Consequently, compared with conventional fluorescent nanoparticles, PLNPs improve the signal-to-noise ratio by avoiding the autofluorescence of tissues.

However, because of their large hydrodynamic size (>100 nm) PLNPs are quickly taken up and sequestered by the reticuloendothelial system (RES).[7,8b] Hence, it remains a challenge to produce appropriately sized biocompatible luminescent of tissues.[8b] These authors contributed equally to this paper and should be considered as joint first authors.

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Scheme 1. Schematic representation of pegylated USPLNPs used for in vivo imaging without the need for in situ excitation.
cent nanoparticles (between 5 and 50 nm) with long blood circulation times, both to minimize renal filtration and to limit the opsonization and RES clearance, which prevent organ targeting. A few studies present the synthesis of ultra-small persistent luminescence nanoparticles (USPLNP), but these do not have suitable colloidal stability for biomedical applications and/or luminescence within the NIR range.

Herein, we describe a facile one-pot synthesis of ZnGa$_2$O$_4$:Cr$^{3+}$ as NIR USPLNPs with an average diameter of 6 nm by using a non-aqueous sol–gel method assisted by microwave irradiation. Indeed, this type of non-aqueous benzyl alcohol route has been established as a very powerful technique that takes place at moderate temperature and pressure to obtain very small metal oxide nanoparticles with high crystallinity, purity, and reproducibility. Microwave heating offers a reduction of the reaction time (30 min) in comparison to traditional heating in an autoclave (48 h). One special advantage of this technique is that the one-pot process does not need further high-temperature treatment, which is conventionally used for the synthesis of such materials and generally leads to coalescence and larger particles. Moreover, this method allows control of the crystal growth without the need to use additional surfactants or ligands. The nanoparticles are then easily surface functionalized after synthesis, avoiding ligand exchange processes. Polyethylene glycol phosphonate moieties (PO-PEG) are used as surface ligands. The phosphonate group presents high affinity for the metallic surface, whereas the PEG chain improves the biodistribution of the NPs in vivo.

Nanocrystals were obtained by mixing the Zn$^{2+}$ and Ga$^{3+}$ chelates with a stoichiometric ratio (1:2) in benzyl alcohol under microwave irradiation (30 min) in the presence of a small amount of Cr$^{3+}$ (see the experimental methods in the Supporting Information for further details). Owing to their similar ionic radii, Ga$^{3+}$ was substituted by Cr$^{3+}$, which is in a distorted octahedral coordination. Then, the nanocrystals were surface passivated with PO-PEG by simply mixing the USPLNPs and PO-PEG in an acidic medium (see the Supporting Information). Excess coating molecules were eliminated by ultrafiltration. The TEM image (Figure 1 a) shows uniform nanocrystals with a median diameter of 6.1 nm. The standard deviation of 0.2 was deduced by simulating the diameter distribution with a log-normal function (see Figure S1 in the Supporting Information). High-resolution TEM (inset, Figure 1 a) shows a highly crystallized single crystal. The electron diffraction pattern of a typical selected area of USPLNPs shows rings from the (220), (311), (400), (422), (511), (440), and (533) planes of the cubic spinel ZnGa$_2$O$_4$ structure (Figure 1 b). The crystallinity of the ZnGa$_2$O$_4$ nanocrystals was confirmed by infrared spectroscopy (Figure 1 c) with the two typical Zn–O (610 cm$^{-1}$) and Ga–O (465 cm$^{-1}$) vibration bands from ZnGa$_2$O$_4$ in an Fd$\overline{3}m$.
space group. The FTIR spectrum for the free PO-PEG molecules (middle grey curve) is characterized by the strong vibration band due to ethylene oxide at 1100 cm\(^{-1}\) and the characteristic band of an asymmetric phosphonate stretch,\[20\] \(\nu_c(PO_2^-)\), at 1061 cm\(^{-1}\). The PEG band dominates the USPLNP@PO-PEG spectrum (upper black curve), with the addition of the Zn–O and Ga–O vibration bands, which clearly indicates the PO-PEG coating on USPLNP surface.

We also noticed that the characteristic band of the asymmetric phosphonate stretch, \(\nu_c(PO_2^-)\), is broader and slightly blueshifted to 1042 cm\(^{-1}\), suggesting PO-PEG complexation to the USPLNP surface through the phosphonate group.

The stability of nanomaterials is a key parameter for biomedical applications. The USPLNP@PO-PEG formed highly stable dispersions over a broad range of pH (2–10) (Figure 1 e, solid circles) compared with the bare nanoparticles that were only stable in acidic media and agglomerated at physiological pH (Figure 1 e, open circles) owing to an isoelectric point around pH 8 (see Figure S2 in the Supporting Information). At physiological conditions (pH 7.4, [Na\(^+\),Cl\(^-\)] = 300 mmol L\(^{-1}\), \(T = 37^\circ\)C), the as-prepared nanocrystals formed highly stable dispersions that remained stable for more than three days (Figure S3 in the Supporting Information). The hydrodynamic diameter and surface zeta potential of the USPLNP@PO-PEG were found to be 16 nm and \(-3\) mV, respectively (Figure 1 f, g). Considering the size of the mean crystalline core (6 nm in diameter) and a layer of PEG coating,\[21\] this hydrodynamic diameter value suggests very low aggregation of the synthesized materials. The neutral zeta potential confirms the PEG grafting at the outer of the nanoparticle surface. The number of PO-PEG molecules per nanoparticle was determined by thermogravimetric analysis (TGA) and was found to be 90 molecules per nanoparticle (31.5 wt %), corresponding to a surface area of 79 \(\AA^2\) for each PO-PEG ligand molecule (Figure 1 d). This surface area is in good agreement with the results obtained from the surface modification of iron oxide particles with phosphonate moieties.\[22\] Hence, the coating density is estimated to be 1.3 molecules per nm\(^2\), which confirms a high PEG density that should decrease the uptake by reticuloendothelial system organs (mostly the liver and spleen) and improve the long term biodistribution of the NPs.\[11b\]

Undoped ZnGa\(_2\)O\(_4\) shows blue luminescence (Figure S4 in the Supporting Information), which is attributed to self-activated centers of the octahedral Ga–O group in the spinel lattices and the Ga\(^{3+}\) ions combining with UV-generated free electrons produced in oxygen vacancies.\[11b\] With Ce\(^{3+}\) doping, a redshift of luminescence is observed compared with undoped ZnGa\(_2\)O\(_4\) (Figure 2a).\[23\] The excitation spectrum (Figure 2a, left curve) shows that USPLNPs exhibit a strong peak at 254 nm, which is in good agreement with the result from the UV/Vis absorption spectrum (Figure S5 in the Supporting Information). The emission spectrum (Figure 2a, right curve) shows a peak at 695 nm. When excited, Ce\(^{3+}\) emits via the \(2E \rightarrow 4A_2\) transition, which gives rise to a far-red luminescence with a maximum at 695 nm in the zinc gallate host.\[24\] This wavelength range is particularly suitable for in vivo imaging as it corresponds to a transmission maximum for biological tissues.\[25\] The room-temperature persistent luminescence decay of USPLNP is presented in Figure 2b after UV excitation at 254 nm for two minutes before measurement.

At the end of the excitation, long-term persistent luminescence was observed for more than 4 h (Figure 2b) owing to the slow release of the stored energy of excitation.\[26\] The persistent luminescence decayed quickly at the beginning, with a lifetime around 4 min, but then became stable over the period range of 20–240 min after excitation (lifetime equal to 72 min, Figure S6 in the Supporting Information). The long persistent luminescence lasts more than 4 h, which allows the NPs to be used as optical probes for biomedical applications. The suitability of these pegylated USPLNPs for use as in vivo NIR luminescent nanoprobes is illustrated in Figure 3. After excitation for two minutes at 254 nm, the USPLNPs were injected into the caudal vein of a BALB/c mouse (\(n = 3\)) and the luminescence signal was detected without the need of in situ excitation throughout the imaging (see the experimental methods within the Supporting Information for more details).

Figure 3b shows the whole-body distribution of the pegylated USPLNPs: this is the first time that ultra-small (diameter < 10 nm) PLNP have been detected in vivo. The biodistribution was confirmed by ex vivo analysis 6 h after the injection, demonstrating their long term circulation. For ex vivo quantification
tion, a UV source was shined on the different organs for two minutes in order to reactivate the persistent luminescence from the USPLNPs. The USPLNPs were retained in various organs, including the heart, lungs, liver, spleen, and kidneys. The long-term circulation and widespread organ distribution of the USPLNPs offer a variety of potential imaging targets such as those for cancer or cardiovascular diseases. The minimal RES capture may also prevent their long term accumulation and possible toxicity concerns.

A preliminary in vitro toxicity study of USPLNP@PEG nanoparticles has been performed on endothelial cells. Endothelial cells were chosen not only as mimics of the vascular space but also because they are one of the major components of the physiological barrier that carries nanoparticles from the blood to surrounding tissues after intravenous injection. No toxicity was observed. The viability of endothelial normal cells remained above 90% after 24 h incubation of the cells with USPLNP@PEG even at concentrations as high as 1.0 mg mL$^{-1}$ (Figure S7 in the Supporting Information). These results confirm previous studies on the apparent low toxicity of such PLNP materials.

In summary, the ultra-small persistent luminescence nanoparticles (6 nm), USPLNP, were synthesized by using the non-aqueous sol–gel method to give nanoparticles with high crystallinity and purity without the need for any additional ligands. The USPLNPs were pegylated by using phosphonate moieties as a strong chelating agent, leading to highly stable dispersions under physiological conditions. The NIR emission, located at 700 nm, is particularly suitable for in vivo imaging as it corresponds to the region of minimal tissue absorbance. This preliminary in vivo study paves the way for the use of USPLNPs as optical nanotools that emit without the need for continuous in situ excitation and that avoid tissue autofluorescence. The ultra-small nanoparticle size and the PEG passivation reduce the RES clearance, enhance the blood circulation time, and allow widespread organ distribution. The results presented here could address a major issue regarding the difficulty associated with increasing peripheral blood circulation, while limiting clearance by the liver and spleen, a combination that presents new opportunities for functional imaging diagnostics by conjugating such NPs with targeting molecules such as peptides or antibodies.

**Figure 3.** In vivo imaging in BALB/c mice ($n = 3$) injected with a suspension of pegylated USPLNPs: (a) Visible preview; (b) Luminescence acquisition after UV excitation; (c) ex vivo imaging of liver, spleen, kidneys, heart, and lungs and corresponding biodistribution for each organ 6 h after systemic injection. Animal studies were conducted in agreement with the French guidelines for animal care in compliance with procedures approved by the Paris Descartes University ethics committee for animal research (ref. CEEA34.12.12).

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Persistent luminescent nanoparticles (PLNPs) avoid the autofluorescence of tissues that arises from the continuous in situ excitation that conventional fluorescent nanoparticles require. However, because of their large hydrodynamic size (>100 nm) PLNPs are quickly taken up and sequestered by the reticuloendothelial system. In their Communication on page 4 ff., Y. Lalatonne and co-workers describe ultra-small ZnGa$_2$O$_4$:Cr$^{3+}$ nanoparticles (6 nm) that exhibit near-infrared persistent luminescence and high stability under physiological conditions. The PLNPs could be excited prior to injection into mice and the resultant persistent luminescence signal was detected for up to 4 h after excitation, showing retention of the PLNPs in various organs, including the heart, lungs, liver, spleen, and kidneys.