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(54) **Title:** HOMOGENEOUS PERSISTENT LUMINESCENCE NANOCRYSTALS AND METHODS OF PREPARATION AND APPLICATION THEREOF

(57) **Abstract:** This invention provides a groundbreaking approach to PLNPs and their preparation. In particular, the synthetic methodology disclosed herein fundamentally differs from the traditional solid-state annealing reactions that require extreme and harsh reaction conditions. In one unique aspect of the invention, a simple, one-step mesoporous template method utilizing mesoporous silica nanoparticles (MSNs) is disclosed that affords *in vivo* rechargeable NIR-emitting mesoporous PLNPs with uniform size and morphology. In another unique aspect of the invention, the novel synthetic approach is based on aqueous-phase chemical reactions conducted in mild conditions, resulting in uniform and homogeneous PLNPs with desired size control {e.g., sub-10 nm}.



HOMOGENEOUS PERSISTENT LUMINESCENCE NANOCRYSTALS AND METHODS OF PREPARATION AND APPLICATION THEREOF

Priority Claims and Related Applications

[0001] This application claims the benefit of U.S. Provisional Application No. 62/127,376, filed March 3, 2015, the entire content of which is incorporated herein by reference for all purposes.

Technical Fields of the Invention

[0002] The invention generally relates to materials and methods for persistent luminescence. More particularly, the invention relates to uniform and homogeneous persistent luminescence nanoparticles, and methods of preparation and application thereof.

Background of the Invention

[0003] Persistent luminescence (PL), also called afterglow or long-lasting phosphorescence, is the phenomenon encountered in materials that glow well after the end of an excitation with UV or visible light. PL materials have been used in a wide variety of applications, such as luminous dials and displays, fiber-optic thermometers, and forensic and military identification materials. While the mechanism underlying PL is not fully understood, it is generally agreed that the phenomenon involves energy traps in a material that are filled during excitation. After excitation, the stored energy is gradually released to emitter centers.

[0004] In near infrared (NIR) persistent luminescence, PL phosphors can store excitation energy in energy traps while continuing to emit photons for weeks after excitation ceases. (Pan, et al. **2012** *Nat Mater* 11, 58; Abdukayum, et al. **2013** *J Am Chem Soc* 135, 14125; Liu, et al. **2013** *Sci Rep-Uk* 3, 1554.) Studies have been done to explore advanced biomedical applications for NIR persistent luminescence nanoparticles (PLNPs) that can emit PL in the optical bio-imaging window (~650–1000 nm) for hours, or even days, after cessation of excitation. (Pan, et al. **2012** *Nat Mater* 11, 58; Maldiney, et al. **2014** *Nat Mater* 13, 418.) The temporal separation of excitation and afterglow properties of these persistent phosphors makes them ideal as *in vivo* optical imaging contrast reagents. (Chermont, et al. **2007** *P Natl Acad Sci USA* 104, 9266; Maldiney, et al. **2011** *J Am Chem Soc* 133, 11810; Maldiney, et al. **2011** *Acs Nano* 5, 854.)

Excitation resource and relevant complicated optics that are necessary for traditional fluorescence imaging are no longer needed.

[0005] Until now, persistent luminescence has relied on short-wavelength excitation (e.g., ultraviolet light), which has rather limited tissue-penetration depth. (Clabau, et al. **2005** *Chem Mater* 17, 3904; Lin, et al. **2001** *J Mater Sci Lett* 20, 1505; Rodrigues, et al. **2014** *J Mater Chem C* 2, 1612; Rodrigues, et al. **2012** *J Phys Chem C* 116, 11232.) To address this problem, a NIR-light-stimulated PL mechanism was proposed in $\text{LiGa}_5\text{O}_8:\text{Cr}^{3+}$, to release energy trapped in deeper energy levels of the phosphor. In this case, however, the energy must be pre-charged by UV-light and the photo-stimulated emission continues to weaken after each cycle of photo-stimulation and finally becomes extinguished. (Liu, et al. **2013** *Sci Rep-Uk* 3, 1554; Zhuang, et al. **2013** *J Mater Chem C* 1, 7849.) Very recently, the PL phosphor, $\text{ZnGa}_2\text{O}_4:\text{Cr}^{3+}$ (ZGC), was found to be activatable using tissue-penetrable red light, which means that energy can be recharged and NIR PL imaging is no longer limited by the luminescence-decay life-time of the phosphor. (Maldiney, et al. **2014** *Nat Mater* 13, 418.) Thus ZGC is arguably the optimal rechargeable NIR persistent emitting phosphor reported to date.

[0006] Despite such inspiring progress, production of uniformly structured NIR PL ZGC phosphors remains challenging. Advances in the development of PLNPs for both basic research and commercialization have been hampered by their complicated synthesis methods. To make such NIR-persistent phosphors bulk crystal requires temperatures greater than 750 °C in traditional solid-state annealing reactions. (Pan, et al. **2012** *Nat Mater* 11, 58; Clabau, et al. **2005** *Chem Mater* 17, 3904; Setlur, et al. **2008** *J Appl Phys* 103, 053513.) Moreover, to convert such bulk crystal into nanoparticles that are sufficiently disperse for biological applications, certain tedious physical treatments such as grinding or laser ablation must be utilized. (Abdukayum, et al. **2013** *J Am Chem Soc* 135, 14125; Liu, et al. **2013** *Sci Rep-Uk* 3, 1554; Maldiney, et al. **2014** *Adv Funct Mater* DOI 10.1002/adfm.201401612; Maldiney, et al. **2014** *Nanoscale* 6 (22), 13970-13976; Maldiney, et al. **2012** *Opt Mater Express* 2, 261.) The afforded products are generally highly heterogeneous and suffer from severe agglomeration. In addition, bio-imaging applications generally require that the nanocrystals be biocompatible, which means that the PLNPs need to be comparable in size to the biomolecules they label, so as not to interfere with cellular systems.

[0007] Thus, novel and improved synthetic methodologies, in particular aqueous-phase chemical synthesis for sub-10 nm NIR PLNPs that are uniform and can be homogeneously dispersed in a carrying medium (*e.g.*, aqueous solution), are strongly desired.

Summary of the Invention

[0008] The invention provides novel PLNPs that are uniform and homogeneous, aqueous-phase chemical synthesis of such PLNPs, and their applications in various fields including biomedical, cosmetics, plastics, inks, security, *etc.*

[0009] In one aspect, the invention generally relates to a method for preparing mesoporous persistent luminescence nanoparticles. The method includes providing mesoporous silica nanoparticles having mesopores of defined size and morphology; and reacting precursors to form persistent luminescent nanoparticles templated by the mesopores of the mesoporous silica nanoparticles under conditions sufficient to form mesoporous persistent luminescence nanoparticles.

[0010] In another aspect, the invention generally relates to mesoporous persistent luminescence nanoparticles prepared by a method disclosed herein.

[0011] In yet another aspect, the invention generally relates to a method for preparing persistent luminescence nanoparticles. The method includes conducting a hydrothermal chemical reaction in an aqueous phase under conditions sufficient to form uniform and homogeneous persistent luminescence nanoparticles.

[0012] In yet another aspect, the invention generally relates to persistent luminescence nanoparticles prepared by a method according to a method disclosed herein.

[0013] In yet another aspect, the invention generally relates to rechargeable persistent luminescence nanocomposites comprising doped zinc gallates $\text{ZnGa}_2\text{O}_4:\text{Cr}$ that are substantially uniform and homogeneous with a median particle size of less than about 1,000 nm and having specific surface area from about $50 \text{ m}^2/\text{g}$ to about $600 \text{ m}^2/\text{g}$ wherein the mesoporous persistent luminescence nanocomposites is capable of NIR-emitting in the range from about 650 nm to about 900 nm after multiple emission and recharge cycles (*e.g.*, greater than 5, greater than 10).

Brief Description of the Figures

[0014] **FIG. 1.** Illustration of the synthesis of PL-functionalized MSNs and their *in vivo* imaging application.

[0015] **FIG. 2.** Optimization of the experimental conditions. (A) XRD and (B) photoluminescence spectra (excitation wavelength, 254 nm) of the mZGC samples synthesized at various temperatures, (C) PL excitation and emission spectra (tested using the phosphorescence mode of the fluorimeter), (D) PL decay curve of mZGC synthesized at 600 °C, excited by UV lamp (254nm). Sample mass for above measurements is 100 mg.

[0016] **FIG. 3.** Morphologies and porous structure of MSNs and mZGC. FESEM images of (A) MSNs and (B) mZGC. (C) TEM and (D) HRTEM images of mZGC, (E) N₂ adsorption/desorption isotherms, and (F) pore-size distributions of MSNs and mZGC.

[0017] **FIG. 4.** *In situ* simulated deep-tissue charging properties of mZGC. (A) Optical image of *in situ* excitation, (B) PL spectra covered and uncovered by pork layer, (C) *in vitro* charged and recharged decay curves of mZGC. All spectra were recorded with the ZGC under an 8-mm pork layer.

[0018] **FIG. 5.** Recharged *in vitro* PL imaging of mZGC covered by an 8-mm pork layer.

[0019] **FIG. 6.** *In vivo* recharging of mZGC for PL imaging using a white LED. (A) First charging, (B) 10 min after first charging, (C, E, G, I) second to fifth recharging at time intervals of 10 min, (D, F, H) 10 min after second, third, and forth recharging, (J) background control imaging of a mouse without injection of mZGC.

[0020] **FIG. 7.** Biodistribution of mZGC, 2 h after tail-vein injection.

[0021] **FIG. 8.** Optimization of Cr³⁺ doping concentration (vs. Zn) in mZGC.

[0022] **FIG. 9.** Ibuprofen storage/release properties of mZGC. The ibuprofen storage and release experiment was performed according to a previous report. Briefly, 60 mg of the MSNs/ZnGa₂O₄:Cr³⁺ were immersed into 1 mL of 20 mg/L ibuprofen cyclohexane solution for 24 h. Excess ibuprofen cyclohexane solution was removed by centrifugation and decantation. The cyclohexane in the mesoporous silica was evaporated in a 50 °C air dryer for 1 h. The *in vitro* release of ibuprofen was performed by immersing 60 mg of the drug loaded mZGC in 60 mL of simulated body fluid at 37 °C. 100 µL of the mixed solution was taken to test the released amount of ibuprofen at fixed time intervals; this was centrifuged to determine the released ibuprofen in the supernatant by detecting the absorbance. The initial content of ibuprofen on MSNs and mZGC is 146.3 mg/g and 103.9 mg/g respectively, by elemental analysis.

[0023] **FIG. 10.** Cell toxicity of mZGC. The cytotoxicity was tested using 3-(4,5-dimethyl-2-thiazolyl)-2,5diphenyl-2H-tetrazolium bromide (MTT, sigma, USA) assay using human umbilical vein endothelial cells (HUVE cells, ATCC, CRL-1730).

[0024] **FIG. 11.** Spectrum of the LED torch used in experiments. Wavelengths longer than 600 nm have better biological tissue penetration ability than shorter ones, and result in the charging of mZGC *in vivo*.

[0025] **FIG. 12.** Schematic illustration of the synthesis and imaging applications of ZGC PLNPs.

[0026] **FIG. 13.** (A) TEM and size distribution, (B) HRTEM, of a single crystal, (C) selected area electron diffraction pattern, and (D) X-ray diffraction analysis of ZGC-1.

[0027] **FIG. 14.** The disperse stability of ZGC-1 and ZGC-2 in water (pH=6.5). (A) Dynamic light scattering patterns of ZGC-1 in water before and after storage of 1 month, (B1, B3) bright field and (B2, B4) corresponding luminescence pictures of ZGC-2 in water, (C1, C3) bright field and (C2, C4) corresponding luminescence pictures of ZGC-1 in water. Persistent luminescence intensity is expressed in false color units (1 unit = $2.107 \text{ photons} \cdot \text{s}^{-1} \cdot \text{cm}^{-2} \cdot \text{sr}$) for all images.

[0028] **FIG. 15.** Decay curves of ZGC-1 excited by various wavelength.

[0029] **FIG. 16.** Superior PL properties of ZGC-1. (A) PL spectra and (B) decay curves of ZGC-1 and ZGC-2 excited at 650 nm for 200 s using a xenon lamp as the light source. PL spectra and decay curves were recorded 30 s after the stop of the excitation. Sample mass = 100 mg.

[0030] **FIG. 17.** Analysis of the imaging ability of ZGC-1 and ZGC-2. (A) 50 mg in a black 96-well-plate; (B) *in vivo* activated imaging after subcutaneous injection (50 μL , 2 mg/mL). Persistent luminescence intensity is expressed in false color units (1 unit = $1.10^6 \text{ photons s}^{-1} \cdot \text{cm}^{-2} \cdot \text{sr}$) for all images. ·

[0031] **FIG. 18.** Comparative deep tissue imaging of ZGC-1 and ZGC-2. (A and B) deep tissue *in vivo* imaging before and after *in situ* excitation; (C–H) repeated imaging before and after 2nd, 3rd, and 4th *in situ* excitations, time interval = 30 min. Excitation with a white LED (5000 lumen) light source for 30 s. Persistent luminescence intensity is expressed in false color units (1 unit = $4350 \text{ photons s}^{-1} \cdot \text{cm}^{-2} \cdot \text{sr}$) for all images. ·

[0032] **FIG. 19.** Imaging applications of ZGC PLNPs after ex vivo (A) and in vivo (B) being excited using a white LED light source (5000 lumen).

[0033] **FIG. 20.** Luminescence spectra of PLNPs, mSiO₂/ZnGa₂O₄ doped with diverse dopant ions.

Detailed Description of the Invention

[0034] This invention provides a groundbreaking approach to PLNPs and their preparation. In particular, the synthetic methodology disclosed herein fundamentally differs from the traditional solid-state annealing reactions that require extreme and harsh reaction conditions.

[0035] In one unique aspect of the invention, a simple, one-step mesoporous template method utilizing mesoporous silica nanoparticles (MSNs) is disclosed that affords *in vivo* rechargeable NIR-emitting mesoporous PLNPs with uniform size and morphology.

[0036] For example, uniform sized NIR-emitting mesoporous SiO₂/ZnGa₂O₄:Cr³⁺ persistent luminescence nanocomposites were made by a simple, one-step mesoporous template method. The near-infrared persistent luminescence of the nanocomposites can be recharged for multiple times both *in vitro* under deep tissue simulation (*e.g.*, 8 mm pork slab) and in a live mouse model. This unconventional approach paves the way for the synthesis and wider application of deep tissue rechargeable ZGC persistent luminescence materials in photonics and biophotonics.

[0037] In another unique aspect of the invention, the novel synthetic approach is based on aqueous-phase chemical reactions conducted in mild conditions, resulting in uniform and homogeneous PLNPs with desired size control (*e.g.*, sub-10 nm). The PLNPs can be homogeneously dispersed in a carrying medium (*e.g.*, aqueous solution).

[0038] For example, the aqueous phase synthesis afforded uniformed NIR PL ZGC nanoparticles. The synthesis temperature was significantly decreased from up 1000 °C needed for existing methods to around 250 °C, which greatly simplifies the reaction equipment. The as-synthesized ZGC nanocrystals can be made as small as a size of 8±4 nm with narrow size distribution, which is important for its potential biomedical applications. The ZGC nanocrystals can be easily distributed into water after a simple acid washing post-treatment, which facilitates its surface modification of diverse functional group including biomolecules (*e.g.*, antibody). It has been demonstrated that the PLNPs so prepared provide significantly better PL intensity than those synthesized by the traditional annealing method and can be activated in deep tissue covered by 1 cm pork layer, which enables it for various *in vivo* applications.

[0039] In one aspect, the invention generally relates to a method for preparing mesoporous persistent luminescence nanoparticles. The method includes providing mesoporous silica nanoparticles having mesopores of defined size and morphology; and reacting precursors to form persistent luminescent nanoparticles templated by the mesopores of the mesoporous silica nanoparticles under conditions sufficient to form mesoporous persistent luminescence nanoparticles.

[0040] In certain embodiments of the invention, the specific surface area of the mesoporous silica nanoparticles is from about 100 m²/g to about 2,000 m²/g (*e.g.*, from about 100 m²/g to about 1,000 m²/g, from about 100 m²/g to about 800 m²/g, from about 100 m²/g to about 600 m²/g, from about 100 m²/g to about 400 m²/g, from about 200 m²/g to about 2,000 m²/g, from about 500 m²/g to about 2,000 m²/g, from about 800 m²/g to about 2,000 m²/g, from about 1,000 m²/g to about 2,000 m²/g).

[0041] In certain embodiments of the invention, the mesoporous persistent luminescence nanoparticles are NIR-emitting in the range of about 650 nm to about 900 nm.

[0042] In certain embodiments of the invention, the mesoporous persistent luminescence nanoparticles are rechargeable.

[0043] In certain embodiments of the invention, the mesoporous persistent luminescence nanoparticles are doped zinc gallates ZnGa₂O₄:Cr.

[0044] In certain embodiments of the invention, reacting precursors to form persistent luminescent nanoparticles is conducted at a temperature in a range from about 400 °C to about 700 °C (*e.g.*, from about 400 °C to about 650 °C, from about 400 °C to about 600 °C, from about 400 °C to about 550 °C, from about 400 °C to about 500 °C, from about 450 °C to about 700 °C, from about 500 °C to about 700 °C, from about 550 °C to about 700 °C, from about 600 °C to about 700 °C).

[0045] In certain embodiments of the invention, wherein persistent luminescence is detectable at least 5 hours (*e.g.*, at least 10 hours) after excitation.

[0046] In certain embodiments of the invention, the formed persistent luminescent nanoparticles are substantially uniform and homogeneous with median particle sizes less than about 1,000 nm (*e.g.*, less than about 500 nm, less than about 200 nm, less than about 100 nm, less than about 50 nm, less than about 20 nm, less than about 10 nm).

[0047] In another aspect, the invention generally relates to mesoporous persistent luminescence nanoparticles prepared by a method disclosed herein.

[0048] In yet another aspect, the invention generally relates to a method for preparing persistent luminescence nanoparticles. The method includes conducting a hydrothermal chemical reaction in an aqueous phase under conditions sufficient to form uniform and homogeneous persistent luminescence nanoparticles.

[0049] In certain embodiments of the invention, the hydrothermal chemical reaction is conducted in aqueous phase at a temperature from about 150 °C to about 300 °C (*e.g.*, from about 150 °C to about 280 °C, from about 150 °C to about 250 °C, from about 150 °C to about 230 °C, from about 150 °C to about 200 °C, from about 180 °C to about 300 °C, from about 200 °C to about 300 °C, from about 250 °C to about 300 °C).

[0050] In certain embodiments of the invention, the formed persistent luminescent nanoparticles are substantially uniform in particle size of less than about 10 nm (*e.g.*, less than about 8 nm, less than about 6 nm, less than about 5 nm).

[0051] In certain embodiments of the invention, the persistent luminescence nanoparticles are NIR-emitting in the range from about 650 nm to about 900 nm (*e.g.*, from about 650 nm to about 850 nm, from about 650 nm to about 800 nm, from about 650 nm to about 750 nm, from about 750 nm to about 900 nm, from about 800 nm to about 900 nm).

[0052] In certain embodiments of the invention, the mesoporous persistent luminescence nanoparticles are doped zinc gallates $\text{ZnGa}_2\text{O}_4\text{:Cr}$.

[0053] In certain embodiments of the invention, persistent luminescence is detectable at least 5 hours (*e.g.*, at least 10 hours) after excitation.

[0054] In certain embodiments of the invention, the specific surface area of the mesoporous silica nanoparticles is from about 100 m²/g to about 2,000 m²/g (*e.g.*, from about 100 m²/g to about 1,000 m²/g, from about 100 m²/g to about 800 m²/g, from about 100 m²/g to about 600 m²/g, from about 100 m²/g to about 400 m²/g, from about 200 m²/g to about 2,000 m²/g, from about 500 m²/g to about 2,000 m²/g, from about 800 m²/g to about 2,000 m²/g, from about 1,000 m²/g to about 2,000 m²/g).

[0055] In yet another aspect, the invention generally relates to persistent luminescence nanoparticles prepared by a method according to a method disclosed herein.

[0056] In yet another aspect, the invention generally relates to rechargeable persistent luminescence nanocomposites comprising doped zinc gallates $\text{ZnGa}_2\text{O}_4\text{:Cr}$ that are substantially uniform and homogeneous with a median particle size of less than about 1,000 nm and having specific surface area from about $50 \text{ m}^2/\text{g}$ to about $600 \text{ m}^2/\text{g}$ wherein the mesoporous persistent luminescence nanocomposites is capable of NIR-emitting in the range from about 650 nm to about 900 nm after multiple emission and recharge cycles (*e.g.*, greater than 5, greater than 10).

[0057] In certain embodiments of the invention, the rechargeable persistent luminescence nanocomposites are rechargeable *in vivo* by tissue-penetrable red excitation.

[0058] In certain embodiments of the invention, the specific surface area of the mesoporous silica nanoparticles is from about $50 \text{ m}^2/\text{g}$ to about $600 \text{ m}^2/\text{g}$ (*e.g.*, from about $50 \text{ m}^2/\text{g}$ to about $500 \text{ m}^2/\text{g}$, from about $50 \text{ m}^2/\text{g}$ to about $300 \text{ m}^2/\text{g}$, from about $50 \text{ m}^2/\text{g}$ to about $200 \text{ m}^2/\text{g}$, from about $50 \text{ m}^2/\text{g}$ to about $100 \text{ m}^2/\text{g}$, from about $100 \text{ m}^2/\text{g}$ to about $600 \text{ m}^2/\text{g}$, from about $200 \text{ m}^2/\text{g}$ to about $600 \text{ m}^2/\text{g}$, from about $300 \text{ m}^2/\text{g}$ to about $600 \text{ m}^2/\text{g}$, from about $400 \text{ m}^2/\text{g}$ to about $600 \text{ m}^2/\text{g}$).

[0059] In certain embodiments of the invention, the median particle sizes are less than about 1,000 nm (*e.g.*, less than about 500 nm, less than about 200 nm, less than about 100 nm, less than about 50 nm, less than about 20 nm, less than about 10 nm).

[0060] In certain embodiments of the invention, the persistent luminescence nanocomposites can emit multiple colors (2, 3, 4 or more colors).

[0061] In certain embodiments of the invention, the persistent luminescence nanocomposites are components of an imaging or diagnostic probe or a labeling agent for biomedical assays (*e.g.*, western blot).

[0062] In certain embodiments of the invention, the persistent luminescence nanocomposites are components of a drug delivery vehicle or a therapeutic agent.

[0063] In certain embodiments of the invention, the persistent luminescence nanocomposites are components of a nail polish.

[0064] In certain embodiments of the invention, the persistent luminescence nanocomposites are components of a hair spray.

[0065] In certain embodiments of the invention, the persistent luminescence nanocomposites are components of ink for latent fingerprint, and for art illustration and paints, tracer in dark

conditions such as at night and submarine condition, for constructions, living goods and utility device.

[0066] In certain embodiments of the invention, the rechargeable persistent luminescence nanocomposites are homogenously dispersed in a solution or suspension of water or an organic solvent.

I. ONE-STEP TEMPLATED SYNTHESIS OF NIR-EMITTING MESOPOROUS PLNPS

[0067] Because of their readily controllable synthesis and resulting morphology, super-high specific surface area, huge pore volume, and good biocompatibility, MSNs are widely utilized in biology, drug delivery, and medicinal applications to encase various functional molecules/luminescence contrast reagents. (Yamashita, et al. **2013** *Advanced Drug Delivery Reviews* 65, 139; Liu, et al. **2013** *Angew Chem Int Edit* 52, 4375; Fan, et al. **2014** *Biomaterials* 35, 8992.)

[0068] As first disclosed herein, MSNs can be used to template the synthesis of ZGC NIR persistent phosphors *in situ* with defined size and morphology (mZGC). Since temperatures beyond 750 °C may lead to the collapse of the mesoporous silica nanostructures, the reaction temperature for the phosphor synthesis was explored systematically in this study. It was found that at 600 °C, the as-synthesized mZGC preserves defined size, morphology and mesoporous nanostructure of the MSNs as well as possessing optimal luminescence properties. Further, the performance of mZGC in imaging was measured both *in vitro* and *in vivo* to assess potential applications in biophotonics. It was demonstrated that as-synthesized mZGC can be recharged in a simulated deep-tissue environment (~8 mm pork slab) *in vitro* using red light. Moreover, it was observed that mZGC can be repeatedly activated *in vivo* for persistent luminescence imaging in a live mouse model using white LED as a light source.

Formation of mZGC Using MSNs as Nanoreactors

[0069] The MSNs were impregnated with ZGC nitrate precursor solutions, which entered the nanochannels of MSNs with ease due to the capillarity of the mesopores. The optimal used composition was determined at Zn/Ga/Cr=1/1.997/0.003 by molar ratio (**FIG. 8**). ZGC was formed in the nanochannels of the MSNs after vacuum drying and annealing, as shown in **FIG. 1**.

By measuring the mass of the used silica and the total mass of the as-formed nanocomposites, the content of ZGC in mZGC was calculated to be 10.4% by weight. (**Table 1**). Such a simple, one-step mesoporous template method allowed preparation of NIR-persistent-luminescent mesoporous nanocomposites that combine the unique optical properties of NIR persistent phosphors and the mesoporous attributes of mesoporous silica.

Table 1 Test of the ZGC content in as-synthesized mZGC product

MSNs	mZGC	Increased mass	ZGC content in mZGC (by weight)
400.0 mg	445.7 mg	45.7 mg	10.4%±0.4%
400.0 mg	448.1 mg	48.1 mg	
400.0 mg	445.4 mg	45.4 mg	

Table 2 Mass loss of mZGC after rinsed in PBS for 48 hours

mZGC	Rinsed and dried mZGC	decreased mass	Mass loss by weight
100.0 mg	99.7 mg	-1.7 mg	0.2 %±1.5%
100.0 mg	101.4 mg	+1.4 mg	
100.0 mg	100.1 mg	+0.1 mg	

[0070] X-ray diffraction (XRD) and luminescence measurements were used to confirm the nature of the as-synthesized nanocomposite. The XRD results (**FIG. 2A**) showed that the diffraction characteristics (peaks at 30.4°, 35.78°, 43.50°, 57.48°, 63.12°) of the ZGC crystal already appear when the annealing temperature reaches 500 °C, which then become clear when the temperature increases to 600 °C. This is in agreement with the presence of the spinel phase ZnGa₂O₄ (JCPDS index no. 01-086-0410). However, impurity peaks start to appear at 700 °C, which indicate the formation of metal silicates (arrows in **FIG. 2A**). As a result, it was observed that the PL intensity (**FIG. 2B**; 650–750 nm) initially increases with temperature (from 400–600 °C) and reaches the optimal intensity at 600 °C before decreasing at 700 °C.

[0071] The phosphorescence excitation spectrum of the sample synthesized at 600 °C consists of three ZGC characteristic excitation bands (**FIG. 2C**). These bands, which range from <350 nm, 350–470, and 470–650 nm, can be attributed to the band-to-band transitions of ZnGa_2O_4 , $^4\text{A}_2 \rightarrow ^4\text{T}_1$, and the $^4\text{A}_2 \rightarrow ^4\text{T}_2$ transition of Cr^{3+} , respectively. (Bessiere, et al. **2011** *Opt Express* 19, 10131.) Among these bands, the absorbance at 600–650 nm is responsible for the *in vivo* recharging using deeper-tissue-penetrating red light. (Maldiney, et al. **2014** *Nat Mater* 13, 418.) The PL spectrum (**FIG. 2C**) of the mZGC, which is around 696 nm (inside the NIR imaging window, which ranges from 650–900 nm), is also consistent with the results found in the traditional solid-state reaction. (Bessiere, et al. **2011** *Opt Express* 19, 10131; Zhuang, et al. **2013** *Appl Phys Express* 6, 052602; Zhuang, et al. **2014** *J Mater Chem C* 2, 5502.) The PL of ZGC can be detected even after more than 5 h followed by an excitation for 5 min with a UV light source (254 nm; **FIG. 2D**). Synthesis of zinc gallate based phosphors can usually only occur at temperatures at least 750 °C. (Bessiere, et al. **2011** *Opt Express* 19, 10131; Zhuang, et al. **2013** *Appl Phys Express* 6, 052602; Allix, et al. **2013** *Chem Mater* 25, 1600.)

[0072] The decrease in synthesis temperatures from those generally used implies that the utilization of the pores of MSNs as nanoreactors facilitates the formation of ZGC phosphor. (A. R. West, Solid-State Chemistry and Its Applications (second edition), *John Wiley & Sons, Ltd.*, **2014**, 187.) Homogeneous distribution of these reactants in confined mesoporous nanostructures results in a higher reactivity than would otherwise be the case; such phenomena were observed previously. (Zhan-Jun, et al. **2012** *J Mater Chem* 22, 24713; Chen, et al. **2014** *Adv Mater* 26, 4947; Li, et al. **2014** *Opt Express* 22, 10509; Li, et al. **2014** *Green Chem* 16, 2680.)

Morphology and Porous Structure of mZGC

[0073] This substantial decrease in synthesis temperature is of vital importance to generate mZGC nanocomposites successfully since the nanochannels of MSNs start to collapse when annealed at temperatures higher than 600 °C. (Li, et al. **2013** *Micropor Mesopor Mat* 176, 48.) The field-emission scanning electron microscopy (FESEM) images of the as-synthesized MSNs (**FIG. 3A**) and the corresponding mZGC (**FIG. 3B**) indicate that the MSNs survive calcination at 600 °C. No apparent morphological change could be observed. Furthermore, in the transmission electron microscopy (TEM) and high-resolution-TEM (HRTEM) images (**FIG. 3C, 3D**), tiny dark spots (ZGC nanoparticles) appear homogeneously in the nanochannels of the MSNs.

[0074] N_2 adsorption/desorption was performed to study the influence of the synthesis process on the specific surface area and pore-size distribution of the MSNs. Apparent mesoporous characteristics of plateau regions can be observed both for the MSN templates and for the as-synthesized mZGC (**FIG. 3E**). The specific surface area of the MSNs according to the Brunauer–Emmett–Teller (BET) method decreased from 554.2 m²/g to 214.6 m²/g, while the pore volume decreased from 0.3395 cm³/g to 0.1562 cm³/g, which might arise from the formation of particles in the mesopores. The synthesis of mZGC from the MSNs did not decrease the overall average pore size of the carriers. In fact, a slight increase in average pore size from 2.450 nm to 2.912 nm was observed, as shown in **FIG. 3F**, which may be explained by the ZGC existing as isolated, tiny particles in the nanochannels of the MSNs, and this is also evidenced by the HRTEM image. The mesoporous properties of the as-synthesized mZGC were also verified by the sustained release of a widely used model cargo, ibuprofen (**FIG. 9**). Thus, the PL nano-carrier approach disclosed herein synergized both unique optical properties of ZGC and the cargo storage/release properties of MSNs.

Imaging Capability of mZGC Through Simulated Deep Tissue

[0075] Most of the current PL phosphors can only be excited effectively under blue or even UV light, which can hardly penetrate the deep tissue of animals. (Pan, et al. **2012** *Nat Mater* 11, 58; Chermont, et al. **2007** *P Natl Acad Sci USA* 104, 9266; Clabau, et al. **2005** *Chem Mater* 17, 3904.) The PL excitation band from 600–650 nm is in the transmission window of biological tissue (600–1100 nm) and thus gives us the opportunity to recharge the energy-exhausted mZGC in deep tissue *in situ*. (Maldiney, et al. **2014** *Nat Mater* 13, 418.) Since there is no standard method to accurately evaluate the deep-tissue-imaging ability of PL phosphors, a meat-covering method is proposed for comparison according to our previous report on up-conversion imaging (**FIG. 3A**). (Chen, et al. **2012** *Acs Nano* 6, 8280.) The as-synthesized mZGC sample disk could be excited at 620 nm when covered by 8 mm of pork. After switching off the light used for excitation, the PL spectrum can be recorded using a fluorospectrometer (FluoroMax-3, HORIBA, USA) fitted with a photomultiplier tube (PMT) detector. A similar PL spectrum can be obtained to that of the uncovered sample, which means that the NIR PL of mZGC can efficiently penetrate pork tissue as thick as 8 mm, as shown in **FIG. 4B**. The *in situ* excited PL of mZGC could be reproduced consecutively *in situ* more than five times on any occasion, as shown in **FIG. 4C**.

[0076] With the demonstration that the PL of mZGC can be charged by red light under an 8 mm pork layer, for the first time its applications are studied in deep-tissue imaging using a white light-emitting diode (LED) as the excitation light source (spectrum shown in **FIG. 11**). The rechargeable PL imaging of the mZGC sample was performed five times under a pork layer of 8 mm without any obvious signal weakening, which implies its potential for use in *in vivo* imaging applications (**FIG. 5**). For *in vivo* applications, good biocompatibility is anticipated since the NIR PL phosphor, ZGC, is mainly incorporated within the mesopores of the MSNs, which are well-known to be biocompatible. No apparent cellular toxicity was observed for mZGC under an exposure concentration as high as 50 mg/L (**FIG. 10**).

[0077] To study the *in vivo* chargeability of mZGC, the mZGC saline solution (200 μ L, 5 mg/mL) was injected into a live mouse through the tail vein. After exposure to white LED light, a satisfactory PL imaging picture was obtained, as shown in **FIG. 6**. Moreover, after re-performing the *in situ* excitation, the PL of ZGC was recharged again. No apparent decrease in PL signal was observed after five imaging/recharging cycles (**FIG. 6**). A high signal-to-noise ratio of $\sim 40:1$ was obtained by comparing the PL signal from liver area of mouse with and without injecting mZGC. The biodistribution of the mZGC was studied after euthanasia of the mouse (2 h after injection). Most of the PL signals emanate from the liver and spleen, which is consistent with the *in vivo* imaging results (**FIG. 7**). Given the *in vivo* chargeable PL attributes, the detection of the PL functionalized mesoporous carriers *in vivo* is not limited by the decay of the PL intensity. Thus, the as-synthesized mZGC has great potential to become a new class of mesoporous nanocomposites with NIR PL properties.

[0078] Thus, disclosed herein is an *in vivo* rechargeable NIR-emitting mesoporous $\text{SiO}_2/\text{ZnGa}_2\text{O}_4:\text{Cr}^{3+}$ PL nanocomposites. By using the mesopores of MSNs as a reaction template, the synthesis temperature of the persistent phosphor, $\text{ZnGa}_2\text{O}_4:\text{Cr}^{3+}$ was decreased from higher than 750 $^\circ\text{C}$ in a solid-state reaction to only 600 $^\circ\text{C}$. At this lower temperature, both the unique mesoporous attributes, and the uniform size and morphology of the MSNs were retained in the nanocomposites. For the first time, it was confirmed that mZGC could be repeatedly charged *in situ* under a deep-tissue layer of 8 mm. The deep-tissue chargeable PL properties of mZGC also ensured its repeatable recharged PL imaging in a live mouse model. It is worth noting that this observation is the first direct evidence that persistent luminescence can be recharged *in vivo* for multiple times. This concept of utilizing mesoporous silica as nanoreactor to fabricate ZGC PL

nanoparticles with uniform morphology and preserved porous nanostructure will be significant in directing the synthesis of mesoporous PL systems with diverse PL phosphors and paves a new way to the wide application of deep tissue rechargeable ZGC in photonics and biophotonics.

Experimental

Materials

[0079] Tetraethoxysilane (TEOS), ethanol, diethanolamine (DEA), ammonium hydrate, cetyltrimethylammonium bromide (CTAB), Ga_2O_3 , $\text{Zn}(\text{NO}_3)_2 \cdot 6\text{H}_2\text{O}$, $\text{Cr}(\text{NO}_3)_3 \cdot 9\text{H}_2\text{O}$, and concentrated nitric acid were all of analytical standards, purchased from Sigma-Aldrich and were used as-received. $\text{Ga}(\text{NO}_3)_3$ solution was prepared by dissolving Ga_2O_3 in 1:1 concentrated nitric acid followed by air drying at 105 °C to remove excess nitric acid, and then redissolving in deionized water.

Synthesis of MSNs

[0080] The MSN synthesis was modified from that in a previous report. (Zhan-Jun, et al. **2012** *J Mater Chem* 22, 24713.) Briefly, 7 mL of ethanol, 0.2 g of CTAB, and 50 μL of diethanolamine were dissolved in 25 mL of water under stirring at 60 °C for 30 min to prepare a transparent solution. Then, 2 mL of tetraethoxysilane were added rapidly. The reaction was finished after stirring for another 2 h. Mesoporous silica nanospheres (about 100-nm diameter) were collected by centrifugation and calcination at 550 °C for 2 h to remove CTAB and possible organic residues.

Synthesis of mZGC nanocomposites

[0081] A precursor solution was prepared by dissolving the corresponding nitrates in water/ethanol (1/1, v/v). The final concentration of Zn^{2+} , Ga^{3+} , and Cr^{3+} was controlled to be 0.5 mol/L, 0.9985 mol/L, and 0.0015 mol/L, according to a stoichiometric ratio of Zn/Ga/Cr (1/1.997/0.003, molar ratio), respectively. 200 μL of the precursor solution were mixed with 200 mg mesoporous silica and the mixture was dried in a vacuum oven at 50 °C for 12 h. The samples were then put into a muffle furnace and the temperature was slowly increased by 5 °C/min.

In vitro and in vivo PL imaging of mZGC

[0082] In vitro imaging was performed by putting the powder (100 mg) sample into a black 96-well plate covered with an 8-mm layer of pork tissue. The PL signal from the covered mZGC was recorded after illumination using an LED (5000 lumen) for 15 s. The *in vivo* imaging was conducted by injection of the mZGC dispersion in phosphate-buffered saline (PBS; 5 mg/mL) through the tail vein. The sample was stored in a dark box for 1 day before injection to ensure no pre-activation occurred.

Characterization

[0083] X-ray powder diffraction (XRD) measurements were performed on a diffractometer equipped with Cu K α radiation ($\lambda = 1.5418 \text{ \AA}$) (Panalytical X'pert PRO, The Netherlands). The morphology of the samples was inspected using field-emission scanning electron microscopy (FESEM, HITACHI S-4800, Japan) and transmission electron microscopy (TEM, HITACHI H-7650, Japan) at accelerating voltages of 5 and 100 kv, respectively. High-resolution TEM (HRTEM) images were recorded using a JEM-1200EX II transmission electron microscope. N₂ adsorption/desorption isotherms were obtained on a full-automatic physical and chemical adsorption apparatus (micromeritics, ASAP2020C, USA). Pore size distribution was calculated from the adsorption branch of N₂ adsorption/desorption isotherm and the Brunauer-Emmett-Teller (BET) method. The BET specific surface areas were calculated using the data between 0.05 and 0.35 just before the capillary condensation. The total pore volumes were obtained by the *t*-plot method. Total organic carbon analyzer was used to determine the exact loading level of ibuprofen on mZGC using a CNS elemental analyzer (Elementar Portfolio, Vario MAX, Germany). The spectra and lifetimes of the samples were tested using powdered samples. The photoluminescence spectra and time-decay curves were measured using a fluorospectrophotometer (FluoroMax-3, HORIBA, USA). The absorbance was detected on a UV-vis spectrometer (Thermo, Evolution300, USA). The PL imaging of mZGC was conducted in a Xenogen IVIS imaging system.

II. FACILE SOLUTION-PHASE CHEMICAL SYNTHESIS OF NIR PERSISTENT LUMINESCENCE NANOCRYSTALS

[0084] Hydrothermal synthesis refers to the synthesis by chemical reactions of substances in a sealed heated solution above ambient temperature and pressure. This technique is considered to be one of the widely used technologies to produce nanocrystals (Shi, et al. **2013** *Chem Soc Rev* 42, 5714.)

[0085] Existing fabrication of NIR ZGC PLNPs must undergo solid-state-annealing method at extreme high temperatures and complicated physical methods have to be used to convert as-synthesized large bulk crystals into nanosized particles. As the result, these particles are usually heterogeneous, relative larger, and form agglomerates fast in solution. In addition, a key requirement for bioimaging applications is that the nanocrystals be biocompatible, which means that they need to be comparable in size to the biomolecules they are labeling, so as not to interfere with cellular systems. To date, however, there has been no report of direct aqueous-phase chemical synthesis of NIR PL materials, not to mention sub-10 nm nanoparticles.

[0086] As disclosed herein, water-soluble $\text{ZnGa}_2\text{O}_4\text{Cr}_{0.004}$ PLNPs, with sub-10-nm size and intense PL properties were directly synthesized in aqueous solution by hydrothermal method. An initially attempt was to prepare PLNPs by dissolving $\text{Zn}(\text{NO}_3)_2$, $\text{Ga}(\text{NO}_3)_3$, and $\text{Cr}(\text{NO}_3)_3$ precursor solutions in a sealed Teflon-lined autoclave and underwent a hydrothermal processing at 250°C for 10 h (**FIG. 12**). ZGC PLNPs were generated with a narrow size distribution and an average size of 8 ± 4 nm (denoted as ZGC-1; **FIG. 13A**). The ultra-small size was further confirmed by analysis of the full width at half maximum values of the XRD diffraction peaks and by the Scherrer equation, with an average calculated particle size of 8.7 ± 2.4 nm. As shown in **FIG. 13B**, as-synthesized ZGC-1 has a clear lattice fingerprint of a (311) plane of cubic ZnGa_2O_4 . The selected area's electron diffraction pattern (SAED) result, shown in **FIG. 13C**, indicates that the ZGC nanoparticles have a clear crystal structure and that all of the diffraction circles can be attributed to cubic ZnGa_2O_4 . The formation of pure ZnGa_2O_4 was also confirmed by analysis of its XRD pattern to that of standard data (JCPDS card, no. 01-082-0466; **FIG. 13D**).

[0087] A stable dispersed colloidal solution is usually a premise for the surface modification of nanoparticles. Unfortunately, the re-dispersion of ZGC synthesized by conventional solid state annealing reaction (ZGC-2) is quite difficult due to the severe agglomeration caused by high temperatures. In contrast, the as-synthesized ZGC-1 can be re-dispersed (or “dissolved”) easily in water forming a transparent colloidal solution after a simple acid treatment. Dynamic light

scattering analysis was used to analyze the storing stability of ZGC-1. No significant change was observed in the hydrodynamic size distribution of ZGC-1 colloid even after storing for one month (**FIG. 14A**). In contrast to the as-synthesized ZGC-1, apparent precipitation were observed 30 minutes after milling and ultrasonic treatment of the ZGC dispersion, which was synthesized according to high temperature annealing method (ZGC-2) (**FIG. 14B and 14C**).

[0088] Further systematically studied were the PL properties of ZGC-1 under diverse excitation wavelengths ranging 500–650 nm using the xenon lamp in a fluorimeter as the light source without any corrections made (**FIG. 15**). The results indicated that the PL decay curves of the ZGC-1 can be excited by wavelengths as far as 650 nm. Importantly, during five repeated excitation and decay cycles at 650 nm, it was found that the PL intensity of ZGC-1 was nearly double relative to ZGC-2 30 s after the stop of the excitation (**FIG. 16**).

[0089] A comparison imaging experiment was performed both *in vitro* and *in vivo* using a commercial white LED (5000 lumen, CREE-T6) as light source. By applying the ROI tools of the imaging device, the charging ability of ZGC-1 vs ZGC-2 was compared. The *in vitro* imaging results indicate an increase of PL intensity of ZGC-1 by a factor of 2.7, as shown in **FIG. 17A**. While the *in vivo* results indicate a factor of 2.0 for ZGC-1, as shown in **FIG. 17B**.

Simulated deep-tissue imaging was further performed with live mouse models covered by a pork slab ~1 cm thick. The PL of ZGC-2 could hardly be observed under our simulated deep-tissue environment, whereas ZGC-1 still provided clear, quality images (**FIGs. 18A and 18B**). The PL signal is eliminated completely 30 min after excitation without influencing the next imaging cycle. Once again, it can be reactivated at a desired time to provide PL images (**FIGs. 18C-18H**).

[0090] Based on our observations, ZGC-1 is more suited to deep-tissue imaging applications. The *in vivo* imaging performance of ZGC-H was also performed using a live mouse model (**FIG. 19**). The PL of ZGC-1 could be applied to track its bio-distribution in live mouse. After the *ex vivo* charged PL was exhausted, the PL emission could be recharged *in vivo* by illumination the mouse with a white LED. Although the intensity is relatively weak than that excited *ex vivo*, the total traceable time was not limited by the decay attribute of persistent phosphor. More importantly, it was found that the ZGC-1 PLNPs can be *in vivo* activated through bony structure after being injected inside the spinal tube. The PL imaging result in the live mouse model is consistent with the one performed after open the spinal bone, indicating potential applications in the central neural system or any imaging application through the bone.

Experimental

Materials

[0091] Ga_2O_3 , $\text{Zn}(\text{NO}_3)_2 \cdot 6\text{H}_2\text{O}$, $\text{Cr}(\text{NO}_3)_3 \cdot 9\text{H}_2\text{O}$, ammonium hydroxide, hydrochloride acid, and concentrated nitric acid were all analytical reagents and used as received. $\text{Ga}(\text{NO}_3)_3$ solution was prepared by dissolving Ga_2O_3 in 1:1 concentrated nitric acid followed by air drying at 105 °C to remove the excess amount of nitric acid and re-dissolved in deionized water. 1 mol/L $\text{Zn}(\text{NO}_3)_2$, 2 mol/L $\text{Ga}(\text{NO}_3)_3$, 4 mmol/L $\text{Cr}(\text{NO}_3)_3$ were stored as precursor solution. A little nitric acid was used to prevent hydrolysis of $\text{Ga}(\text{NO}_3)_3$. Polyethyleneimine (PEI) and bovine serum albumin (BSA) were purchased from Sigma-Aldrich and used directly without further purification.

Synthesis of ZGC-1 by hydrothermal process

[0092] A certain amount of $\text{Ga}(\text{NO}_3)_3$ (2 M), $\text{Zn}(\text{NO}_3)_2$ (1 M), $\text{Cr}(\text{NO}_3)_3$ (4 mM) precursor solution were mixed with predetermined molar ratio to form a precursor solution. Ammonium hydroxide (28%, wt) was added quickly under vigorous stirring to reach a pH of 9. The metal hydroxide precursor was sealed into a Teflon-lined autoclave and then performed hydrothermal process at 220 °C for 10 hours. ZGC nanocrystals can be obtained within a wide total concentration molar ratio of precursor, which influenced the product sizes and distributions. In a typical procedure, 2 mmol of $\text{Zn}(\text{NO}_3)_2$, 2 mmol of $\text{Ga}(\text{NO}_3)_3$, 0.004 mmol $\text{Cr}(\text{NO}_3)_3$ were mixed together under vigorous stirring. The total volume was adjusted to 15 mL by adding deionized water. Concentrated ammonium hydroxide (28%) solution (about 1 mL) was added rapidly to adjust the pH to be 9~9.5. White precipitate was formed immediately. After further stirring for half an hour, the mixture were transmitted into a Teflon-lined autoclave (25 mL) and sealed. The autoclave was put in an oven at 220 °C for 10 hour and then cooled to room temperature naturally. The white precipitate could be obtained easily by centrifugation. The product could be dispersed in 0.01 M HCl forming transparent solution. Possible ZnO impurity could be removed by acid washing. ZGC nanocrystals could be obtained by centrifugation after diluted with excess isopropanol. Finally, ZGC nanocrystals were redispersed in deionized (DI) water for further characterization and imaging application. The storing concentration of ZGC is approximately 4 mg/mL and used for further modification.

[0093] For the dispersion in cell culture medium, 200 μ L ZGC-1 storage solution was first diluted with 1 mL water and then added into 5 mL of cell culture medium drop by drop under stirring. A clear solution can be obtained after 10 min ultrasonic dispersion.

[0094] PEI could be adsorbed to the crystal surface of ZGC-1 due to the electrostatic interaction with the negatively charged ZGC-1 nanocrystals, forming PEI modified ZGC (ZGC/PEI). Briefly, 1 mL of ZGC-1 storing solution was added into 10 mL 1 mM of HCl solution, and stirring for 30 min. 1 mL of PEI solution (20%, wt) was then added rapidly under vigorous stirring. ZGC-PEI could be collected by centrifugation after diluted with excess isopropanol. ZGC-PEI was redispersed in DI water under ultrasonic dispersion.

[0095] ZGC-1 was also modified with BSA. 1 mL of ZGC-1 solution (4 mg/mL) was added into 10 mL of BSA solution (1% by weight) drop by drop under stirring followed by further stirring for another 30 min. BSA modified ZGC-1 (ZGC-BSA) was collected by centrifugation and redispersed in 10 mL of DI water by ultrasonic dispersion.

Synthesis of ZGC-2 by hydrothermal assisted solid state annealing method

[0096]

ZGC-2 was synthesized for comparison according to reported hydrothermal assisted annealing method. 1 mmol of $\text{Zn}(\text{NO}_3)_2$, 2 mmol of $\text{Ga}(\text{NO}_3)_3$, 0.004 mmol $\text{Cr}(\text{NO}_3)_3$ were mixed together under vigorous stirring. The total volume was adjusted to 10 mL by adding deionized water. Concentrated ammonium hydroxide (28%) solution (about 1 mL) was added rapidly to adjust the pH to 7.5. The mixture was sealed into a Teflon-lined autoclave at 120 $^\circ\text{C}$ for 24 hour. The resulting precipitate has no PL properties and was sintered at 750 $^\circ\text{C}$ for 5 hours. A small amount of 5 mM NaOH solution was added to the powder product to form a slurry followed by wet grinding for 1 hour with a mortar and pestle. The product was washed by ethanol (90%) and then dried at 60 $^\circ\text{C}$.

Synthesis of ZGC-3 by using hydrothermal post-treatment

[0097]

The as-synthesized ZGC-2 was mixed with ammonium aqueous solution (pH 9~9.5) and sealed in an autoclave, which underwent a hydrothermal procedure at 220 $^\circ\text{C}$ for 10 hours. The as-synthesized ZGC-3 was centrifuged and dried at 80 $^\circ\text{C}$.

Imaging

[0098] A white LED (5000 lumen, CREE-T6) was used for all the imaging experiments as the light source. For the *in vitro* experiment, 50 mg of ZGC sample was put in one well of a black 96-well-plate. The plate was exposed to the LED for 30 s and then put into the IVIS imaging system to detect the PL signal. The *in vivo* imaging was performed after subcutaneous injection of the sample dispersion (50 μ L, 2 mg/mL) and 30 s of *in situ* LED excitation. The *in vivo* deep tissue recharged PL of ZGC was performed by covering the subcutaneous injection site with a 1-cm pork slab. The imaging was performed immediately after 30 s of *in situ* LED excitation.

[0099] Thus, a novel aqueous phase-based synthetic methodology has now been demonstrated for the preparation of uniformed NIR PL ZGC nanoparticles that can be homogeneously dispersed in an aqueous solution or suspension. The synthesis temperature was significantly decreased from up 1000 °C to 250 °C, which greatly simplifies the reaction equipment. The as-synthesized ZGC nanocrystals can be made as small as a size of 8 ± 4 nm with narrow size distribution, which is important for its potential biomedical applications (**FIG. 13**). The ZGC-1 product can be easily dispersed into water after a simple acid washing post-treatment, which will facilitate its surface modification of diverse functional group including biomolecules (*e.g.*, antibody)(**FIG. 14**). The ZGC-1 product can be activated in deep tissue covered by 1 cm pork layer, which will facilitate its applications in animal models (**FIG. 18**). The ZGC-1 has shown significantly superior PL intensity than that synthesized using traditional annealing method (**FIG. 16-18**).

[00100]

the luminescence color of the PLNPs can be further tuned by altering the dopant ions, such as, Gd^{3+} (blue), Bi^{3+} (white), Mn^{2+} (orange) and Cr^{3+} (near-infrared). Due to the uniform morphologies and ultra-small sizes, persistent luminescence nanoparticles disclosed herein can be easily dispersed into various solvents, such as water, ethanol, ethyl acetate.

[00101]

LNPs disclosed herein may be used in biomedical application. The NIR persistent luminescence mZGC may be developed as an *in vivo* traceable drug delivery system. PLNPs can also be used in tumor imaging. As no excitation will be needed during the imaging procedure, high quality imaging result can be obtained by removal of the bio-autofluorescence caused by excitation.

mZGC disclosed herein can not only provide tumor imaging ability but also can carry load of functional molecules such as gene, protein, theronostic reagents.

[00102]

LNPs may be applied in versatile applications in addition to their vast utilities in the healthcare and medical research. For example, they can be dispersed easily into solutions such as nitrocellulose ethyl acetate solution, which is the main content of most commercial nail polish. Also, the PLNPs can be dispersed in polyvinyl acetate/ethanol solution, which is a common solvent used in hair spray. The addition of persistent luminescence nanoparticles in nail polish or hair spray will form colorful afterglow emissions. At night or wherein the light is dark, the afterglow emission can form cool, beautiful nails or hair. Similar applications may also be found in shoe polish, hair spray, art pigment, etc.

[00103]

isible persistent luminescence materials have found application as afterglow fingermark powders used with the visualization of latent fingermarks deposited on multicolored substrate surfaces that can present a contrast problem if developed with regular fingermark powders. Persistent phosphors can provide a strong afterglow effect which aid in the establishment of better fingermark detection. Regard the NIR persistent phosphor, such as our synthesized mZGC, the mZGC disclosed herein can not only image the latent fingermark but also be utilized in information encryption. If mZGC is printed on papers, the NIR persistent luminescence cannot be seen by naked eyes. The NIR luminescence can only be visualized by using special NIR imaging instrument. Thus, NIR persistent luminescence has great potential in the field of information encryption and counterfeit labels.

[00104] In this specification and the appended claims, the singular forms "a," "an," and "the" include plural reference, unless the context clearly dictates otherwise.

[00105] Unless defined otherwise, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art. Although any methods and materials similar or equivalent to those described herein can also be used in the practice or testing of the present disclosure, the preferred methods and materials are now described. Methods recited herein may be carried out in any order that is logically possible, in addition to a particular order disclosed.

Incorporation by Reference

[00106] References and citations to other documents, such as patents, patent applications, patent publications, journals, books, papers, web contents, have been made in this disclosure. All such documents are hereby incorporated herein by reference in their entirety for all purposes. Any material, or portion thereof, that is said to be incorporated by reference herein, but which conflicts with existing definitions, statements, or other disclosure material explicitly set forth herein is only incorporated to the extent that no conflict arises between that incorporated material and the present disclosure material. In the event of a conflict, the conflict is to be resolved in favor of the present disclosure as the preferred disclosure.

Equivalents

[00107] The representative examples are intended to help illustrate the invention, and are not intended to, nor should they be construed to, limit the scope of the invention. Indeed, various modifications of the invention and many further embodiments thereof, in addition to those shown and described herein, will become apparent to those skilled in the art from the full contents of this document, including the examples and the references to the scientific and patent literature included herein. The examples contain important additional information, exemplification and guidance that can be adapted to the practice of this invention in its various embodiments and equivalents thereof.

What is claimed is:

CLAIMS

1. A method for preparing mesoporous persistent luminescence nanoparticles, comprising:
providing mesoporous silica nanoparticles having mesopores of defined size and morphology; and
reacting precursors to form persistent luminescent nanoparticles templated by the mesopores of the mesoporous silica nanoparticles under conditions sufficient to form mesoporous persistent luminescence nanoparticles.
2. The method of claim 1, wherein the specific surface area of the mesoporous silica nanoparticles is from about 100 m²/g to about 1,200 m²/g.
3. The method of claim 1 or 2, wherein the mesoporous persistent luminescence nanoparticles are NIR-emitting in the range of 650 nm and 900 nm.
4. The method of any of claims 1-3, wherein the mesoporous persistent luminescence nanoparticles are rechargeable.
5. The method of any of claims 1-4, wherein the mesoporous persistent luminescence nanoparticles are doped zinc gallates ZnGa₂O₄:Cr.
6. The method of any of claims 1-5, wherein reacting precursors to form persistent luminescent nanoparticles is conducted at a temperature from about 400 °C to about 700 °C.
7. The method of any of claims 1-6, wherein persistent luminescence is detectable at least 5 hours after excitation.
8. The method of any of claims 1-7, wherein the formed persistent luminescent nanoparticles are substantially uniform and homogeneous with particle sizes less than about 1,000 nm.
9. Mesoporous persistent luminescence nanoparticles prepared by a method according to any of claims 1-8.
10. A method for preparing persistent luminescence nanoparticles, comprising conducting a hydrothermal chemical reaction in an aqueous phase under conditions sufficient to form uniform and homogeneous persistent luminescence nanoparticles.
11. The method of claim 10, wherein the hydrothermal chemical reaction is conducted in aqueous phase at a temperature from about 150 °C to about 300 °C.

12. The method of claim 10 or 11, wherein the formed persistent luminescent nanoparticles are substantially uniform with a median particle size of less than about 10 nm.
13. The method of any of claims 10-12, wherein the persistent luminescence nanoparticles are NIR-emitting in the range of 650 nm and 900 nm.
14. The method of any of claims 10-13, wherein the mesoporous persistent luminescence nanoparticles are doped zinc gallates $\text{ZnGa}_2\text{O}_4\text{:Cr}$.
15. The method of any of claims 10-14, wherein persistent luminescence is detectable at least 5 hours after excitation.
16. Persistent luminescence nanoparticles prepared by a method according to any of claims 10-15.
17. Rechargeable persistent luminescence nanocomposites comprising doped zinc gallates $\text{ZnGa}_2\text{O}_4\text{:Cr}$ substantially uniform and homogeneous in particle sizes of less than about 1000 nm and having specific surface area from about $50 \text{ m}^2/\text{g}$ to about $600 \text{ m}^2/\text{g}$ wherein the mesoporous persistent luminescence nanocomposites is capable of NIR-emitting in the range of 650 nm and 900 nm after multiple emission and recharge cycles.
18. The rechargeable persistent luminescence nanocomposites of claim 17, being rechargeable *in vivo* by tissue-penetrable red excitation light.
19. The rechargeable persistent luminescence nanocomposites of claim 17 or 18, capable of emitting multiple colored lights.
20. The rechargeable persistent luminescence nanocomposites of any of claims 17-19, wherein the persistent luminescence nanocomposites are components of an imaging or diagnostic sensing probe or a labeling agent for a biomedical assay.
21. The rechargeable persistent luminescence nanocomposites of any of claims 17-20, wherein the persistent luminescence nanocomposites are components of a drug delivery vehicle.
22. The rechargeable persistent luminescence nanocomposites of any of claims 17-21, wherein the persistent luminescence nanocomposites are components of a therapeutic agent.
23. The rechargeable persistent luminescence nanocomposites of any of claims 17-22, wherein the persistent luminescence nanocomposites are components of a nail polish.

24. The rechargeable persistent luminescence nanocomposites of any of claims 17-23, wherein the persistent luminescence nanocomposites are components of a hair spray.
25. The rechargeable persistent luminescence nanocomposites of any of claims 17-24, wherein the persistent luminescence nanocomposites are components of a latent fingermark in ink.
26. The rechargeable persistent luminescence nanocomposites of any of claims 17-25, being homogenously dispersed in a solution or suspension.

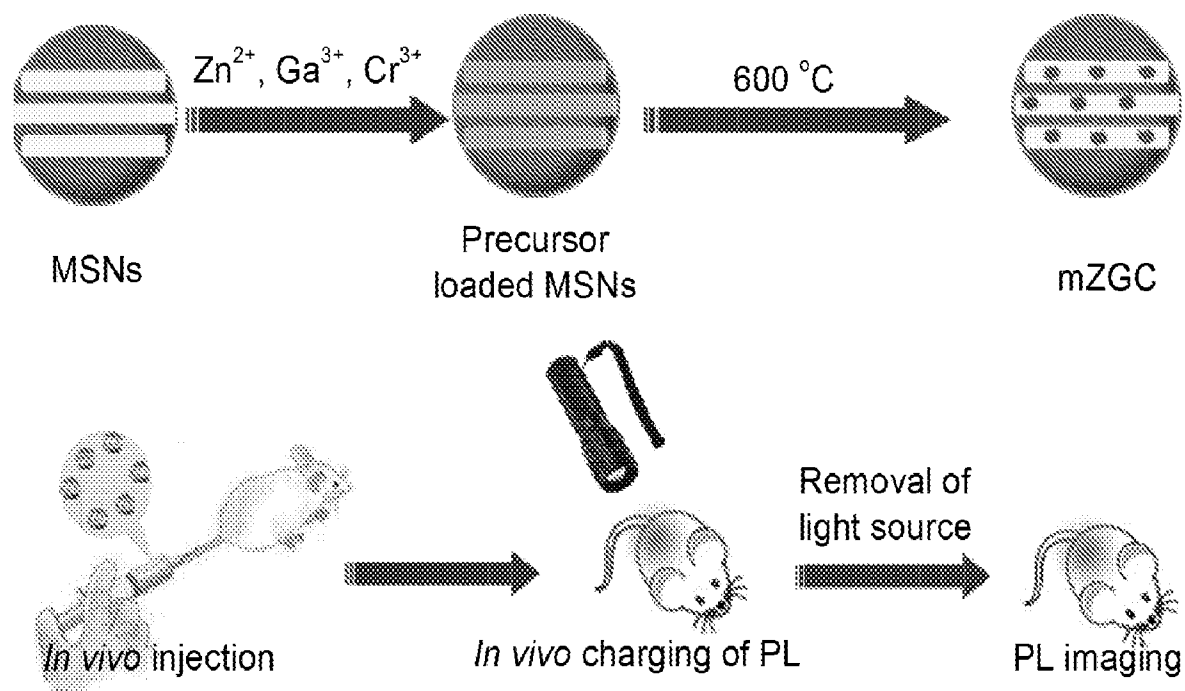


FIG. 1. Illustration of the synthesis of PL-functionalized MSNs and their *in vivo* imaging application.

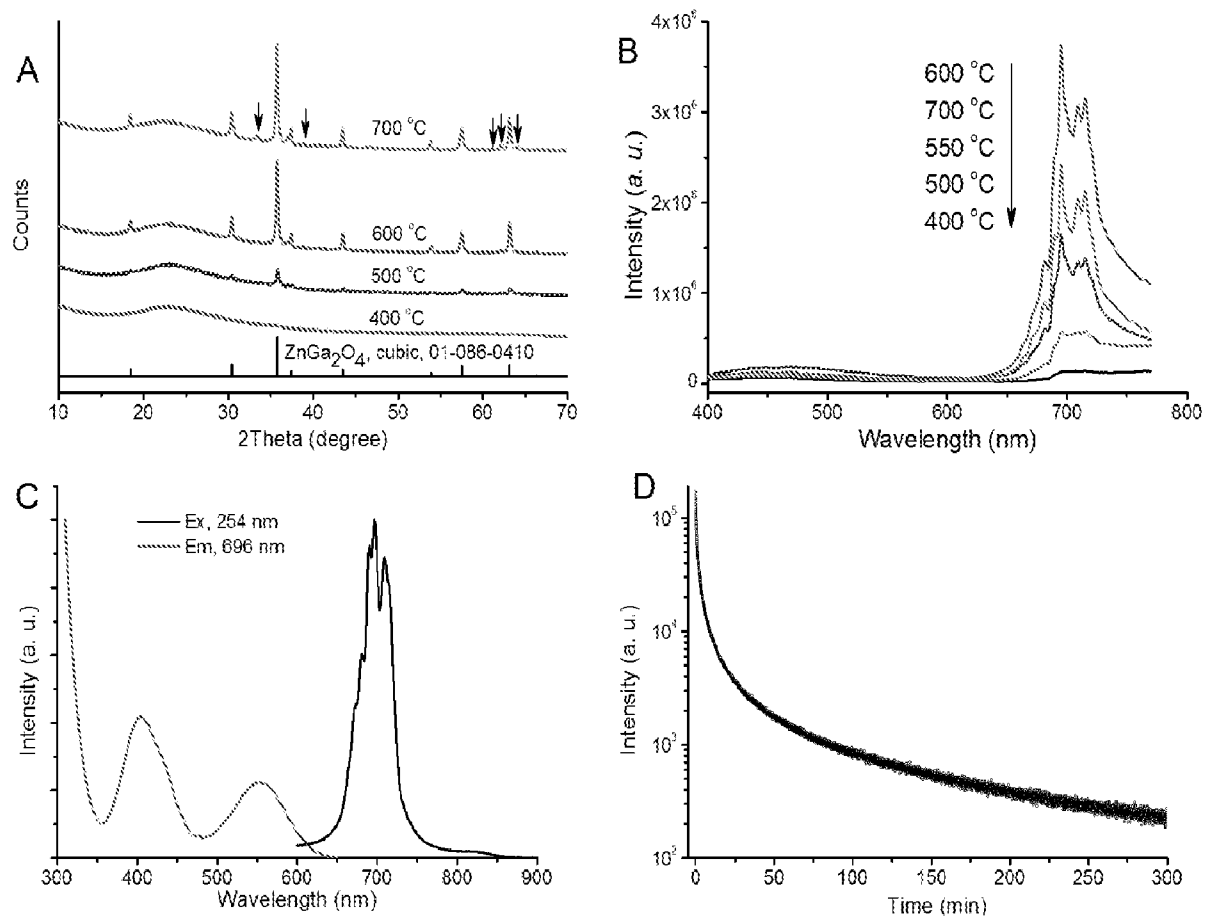


FIG 2. Optimization of the experimental conditions. (A) XRD and (B) photoluminescence spectra (excitation wavelength, 254 nm) of the mZGC samples synthesized at various temperatures, (C) PL excitation and emission spectra (tested using the phosphorescence mode of the fluorimeter), (D) PL decay curve of mZGC synthesized at 600 °C, excited by UV lamp (254nm). Sample mass for above measurements are 100 mg.

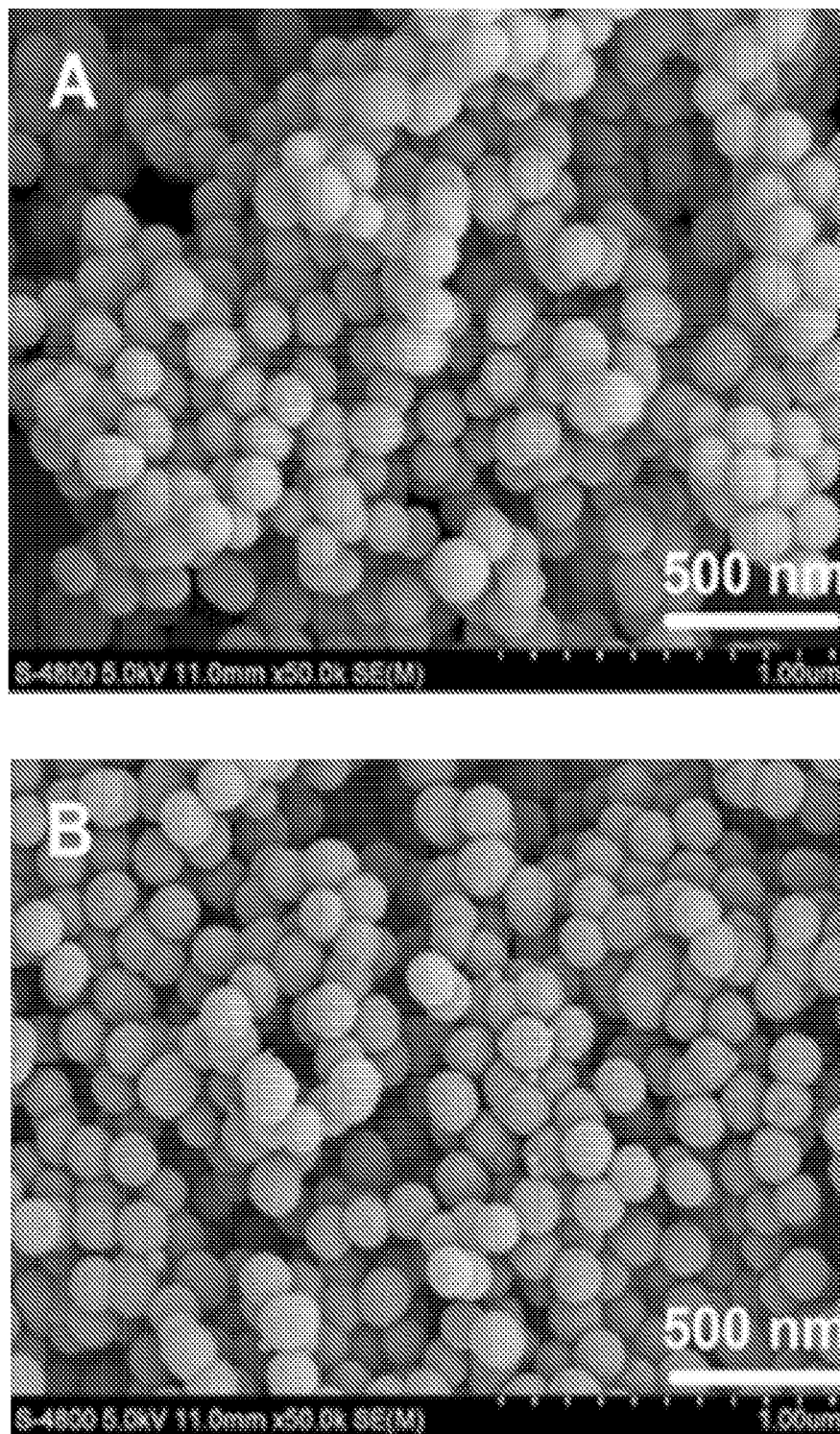


FIG. 3. Morphologies and porous structure of MSNs and mZGC. FESEM images of (A) MSNs and (B) mZGC.

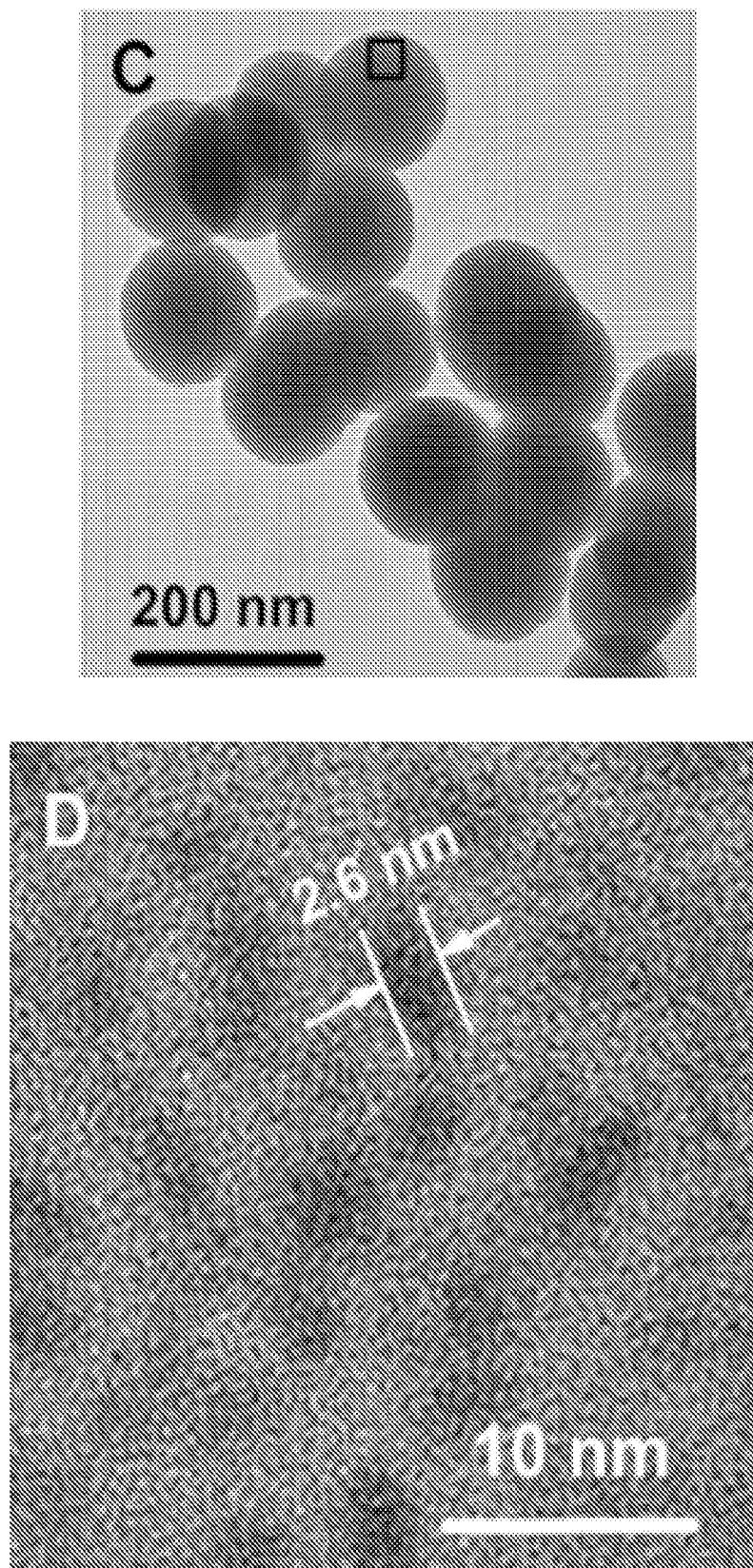


FIG. 3. (Cont'd). (C) TEM and (D) HRTEM images of mZGC, (E) N₂ adsorption/desorption isotherms, and (F) pore-size distributions of MSNs and mZGC.

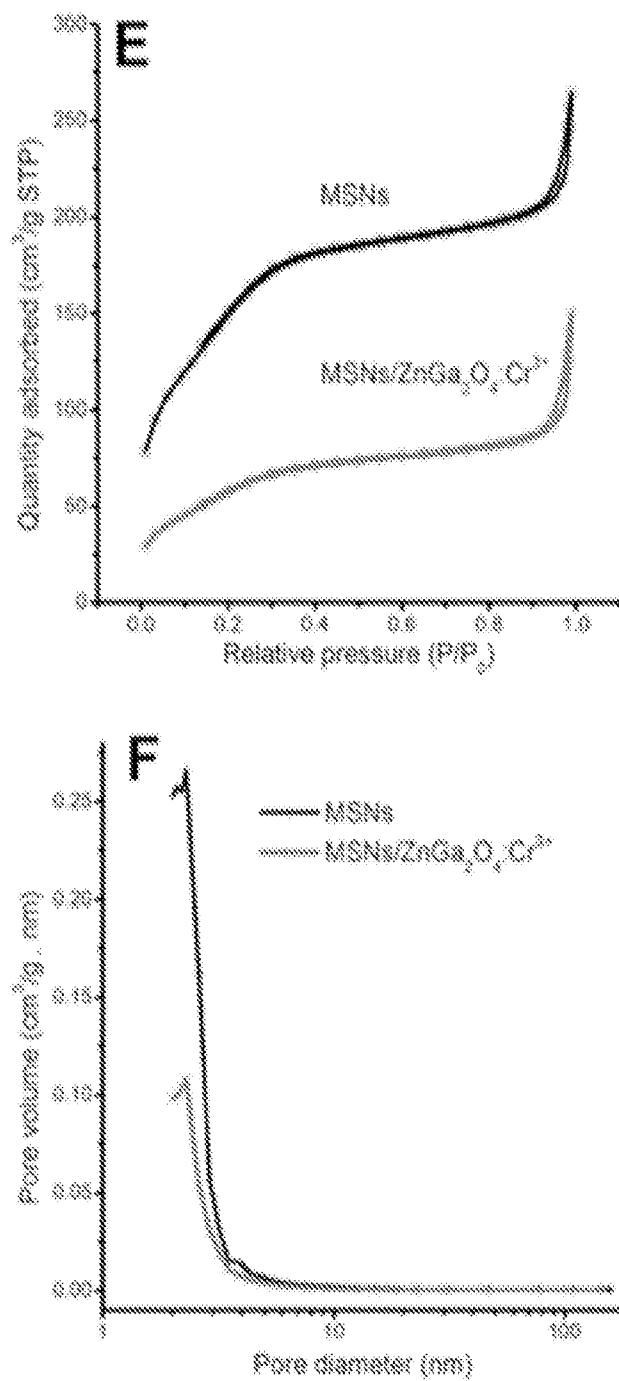
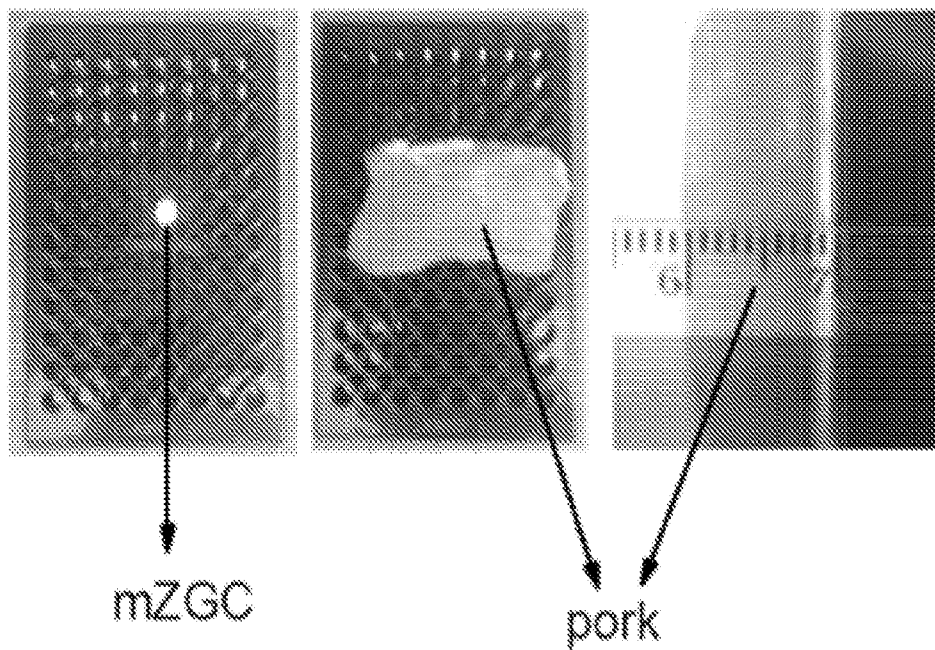


FIG. 3. (Cont'd). (E) N₂ adsorption/desorption isotherms, and (F) pore-size distributions of MSNs and mZGC.

A.



B.

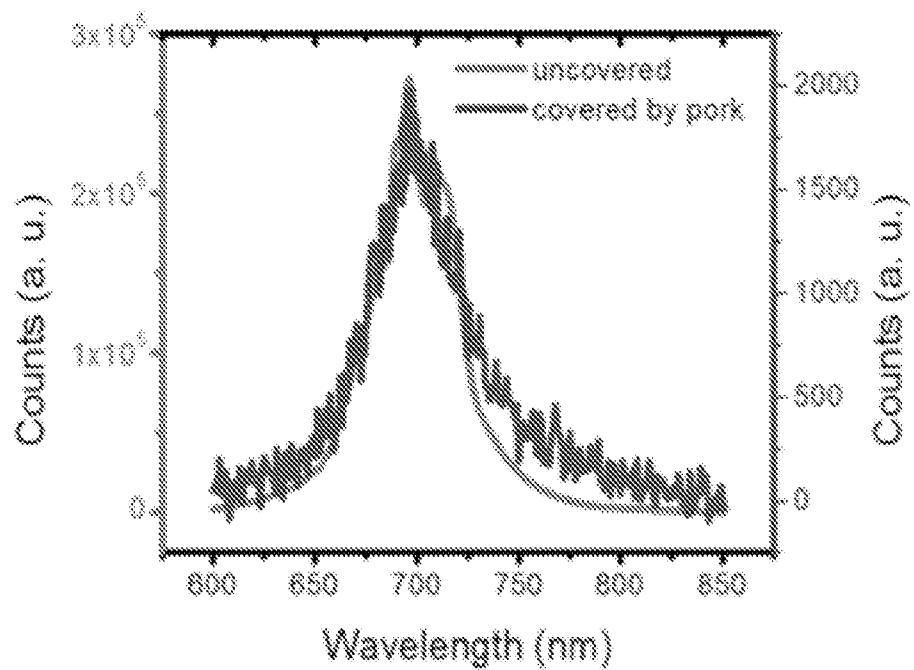


FIG. 4. *In situ* simulated deep-tissue charging properties of mZGC. (A) Optical image of *in situ* excitation, (B) PL spectra covered and uncovered by pork layer.

C.

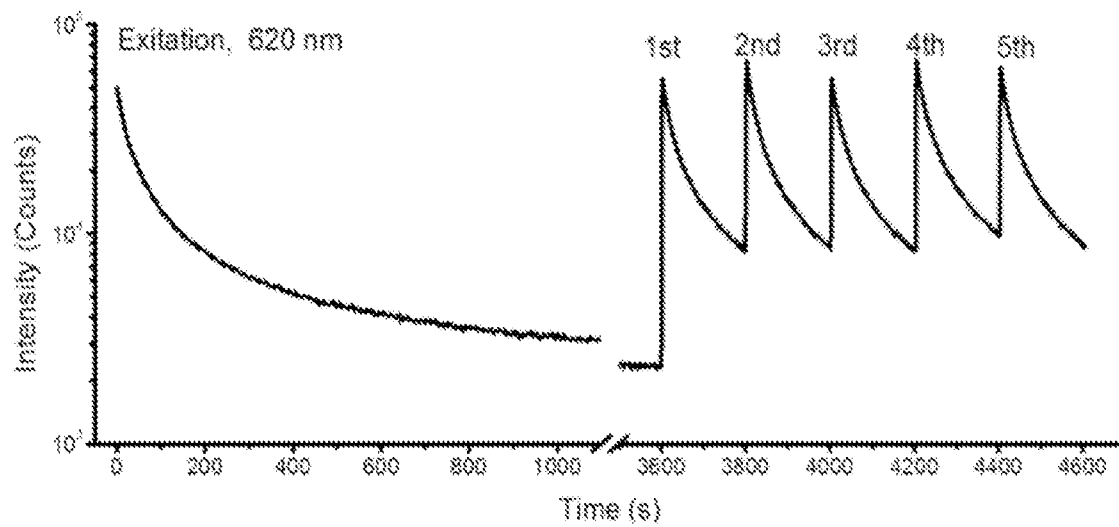


FIG. 4. (Cont'd) (C) *in vitro* charged and recharged decay curves of mZGC. All spectra were recorded with the ZGC under an 8-mm pork layer.

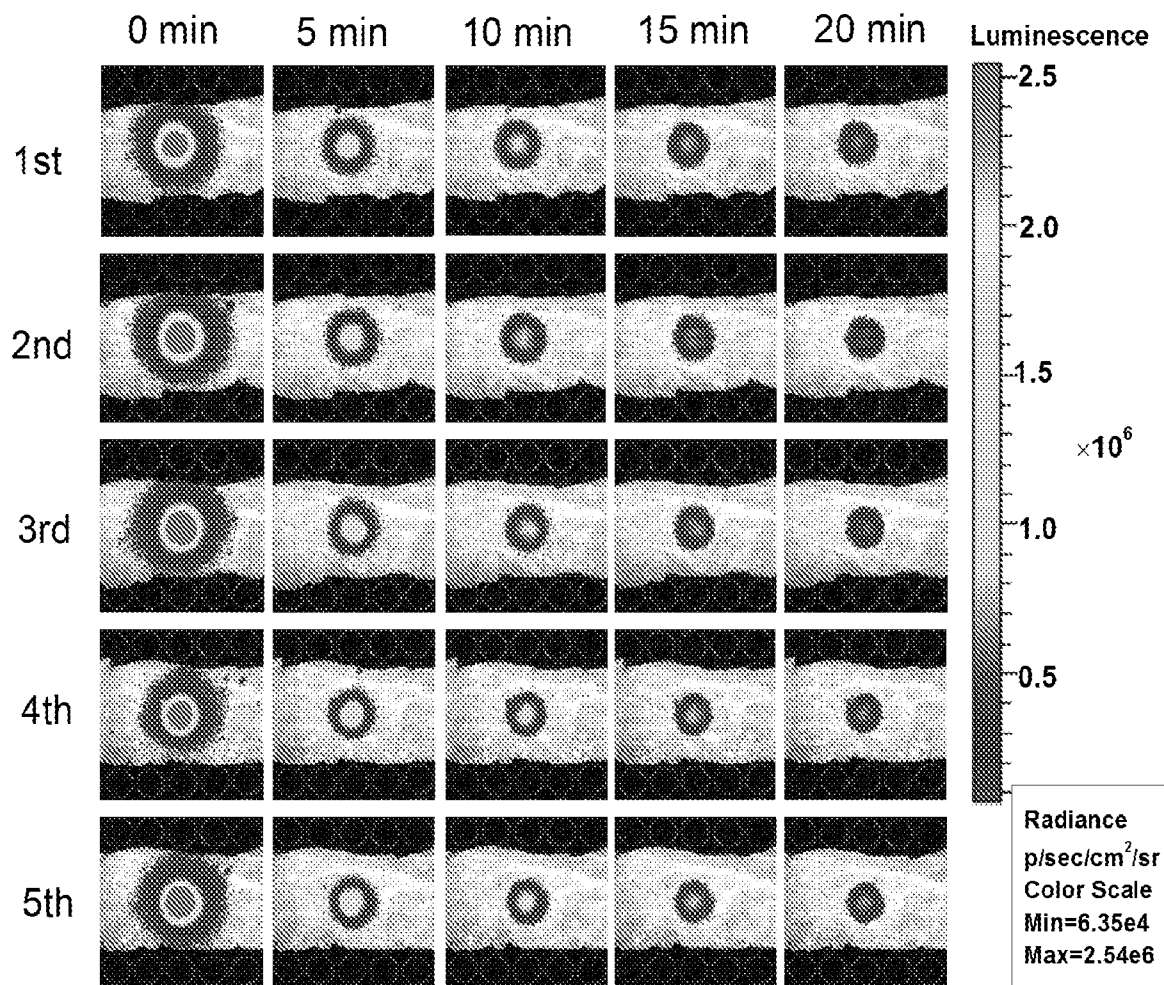


FIG. 5. Recharged *in vitro* PL imaging of mZGC covered by an 8-mm pork layer.

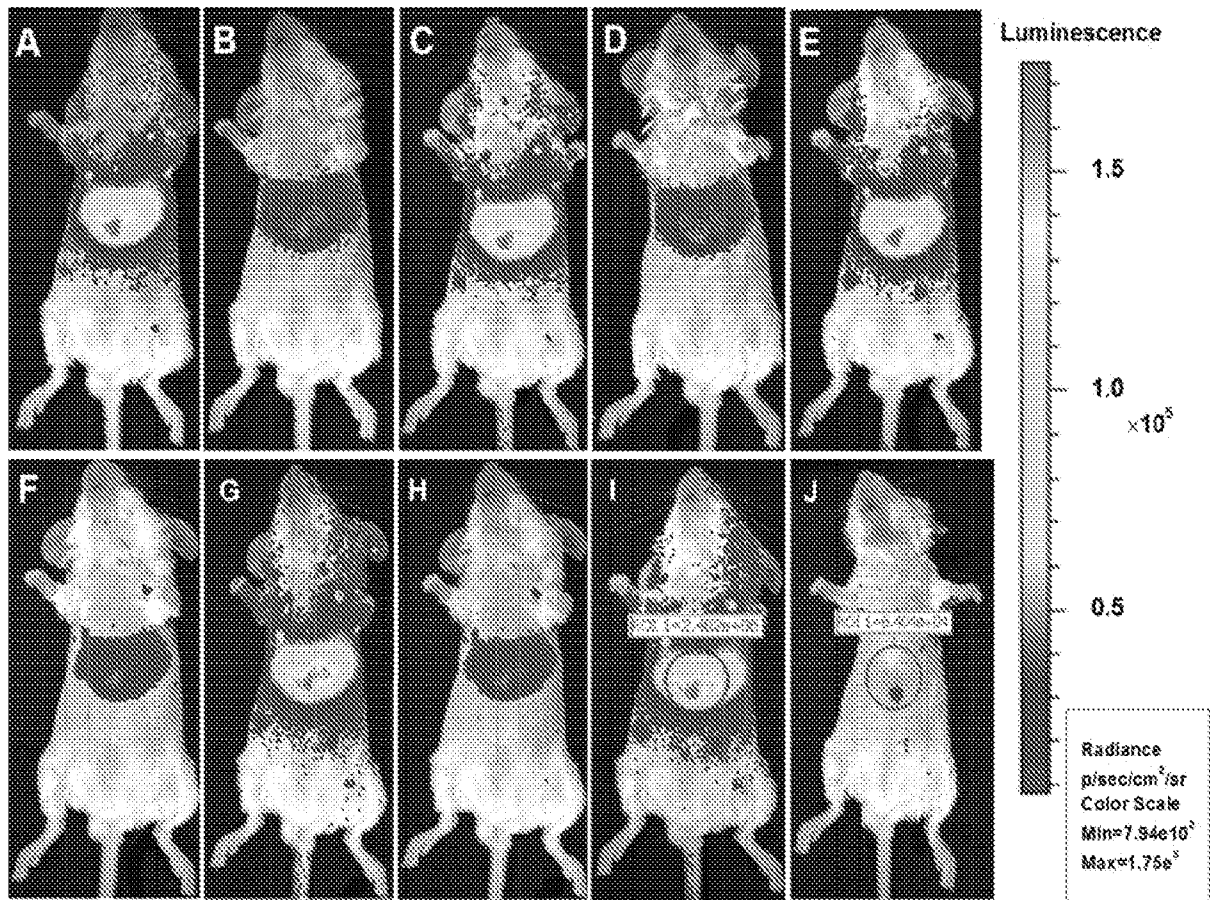


FIG. 6. *In vivo* recharging of mZGC for PL imaging using a white LED. (A) First charging, (B) 10 min after first charging, (C, E, G, I) second to fifth recharging at time intervals of 10 min, (D, F, H) 10 min after second, third, and forth recharging, (J) background control imaging of a mouse without injection of mZGC.

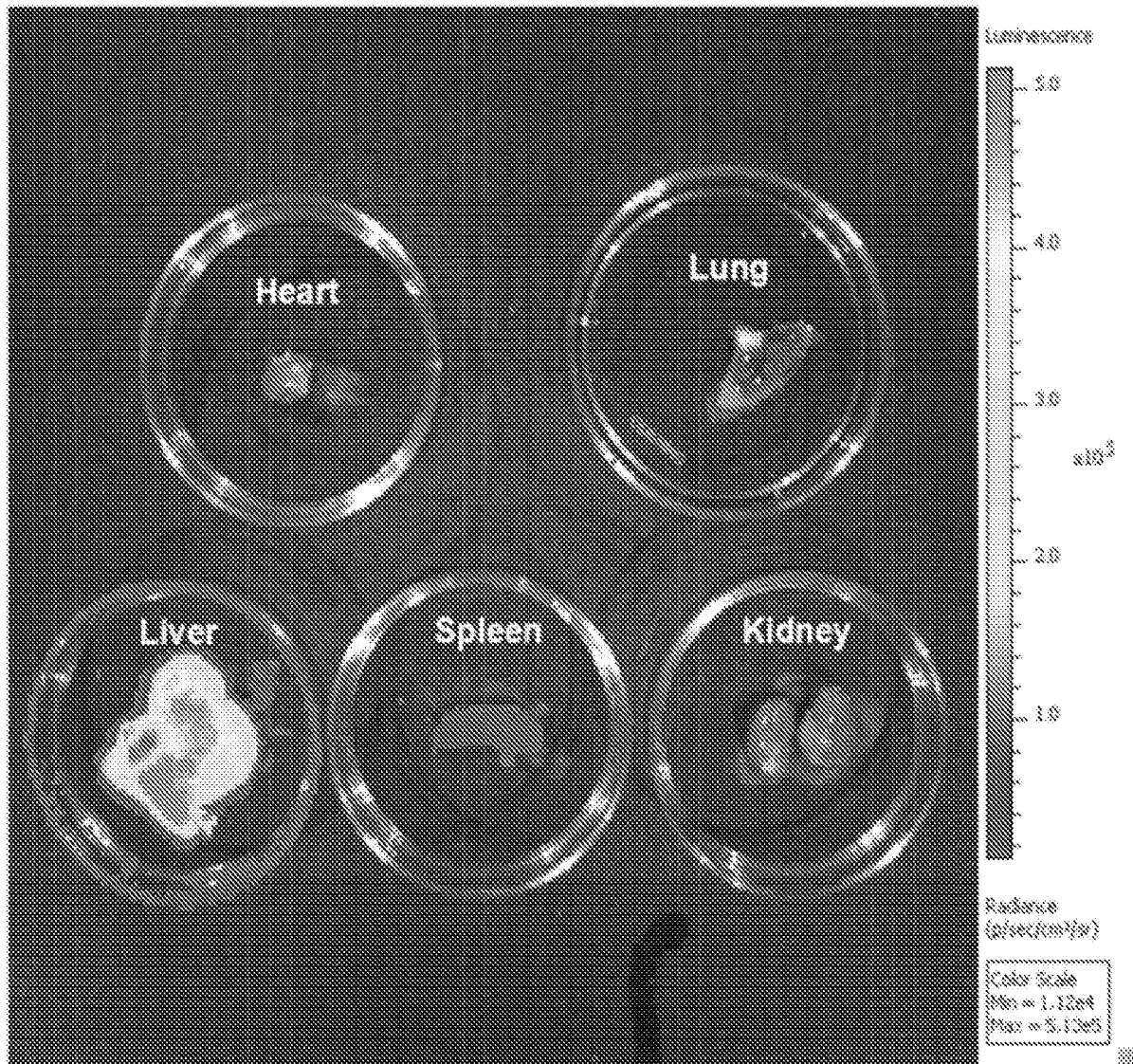


FIG. 7. Biodistribution of mZGC, 2 h after tail-vein injection.

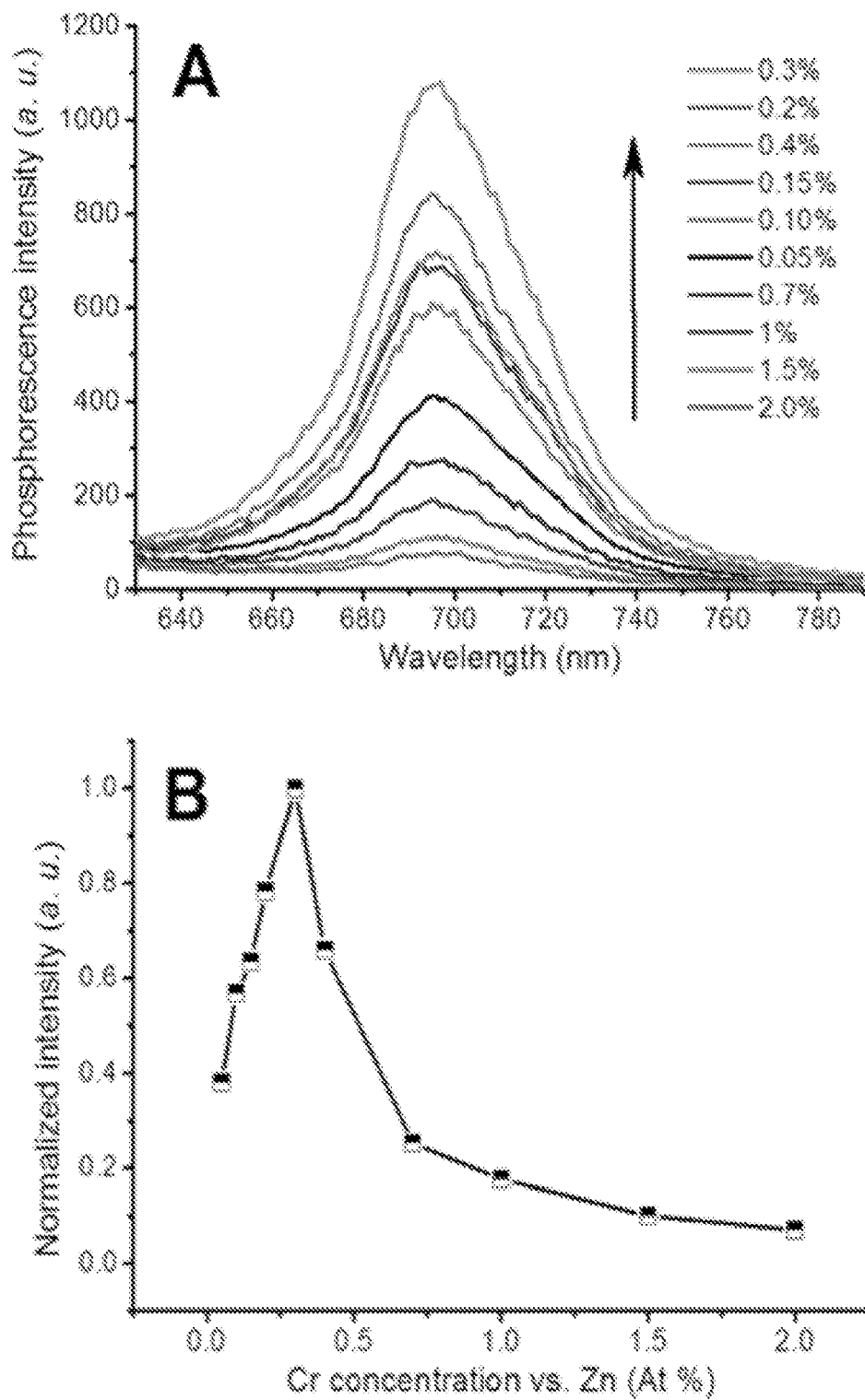


FIG. 8. Optimization of Cr^{3+} doping concentration (vs. Zn) in mZGC.

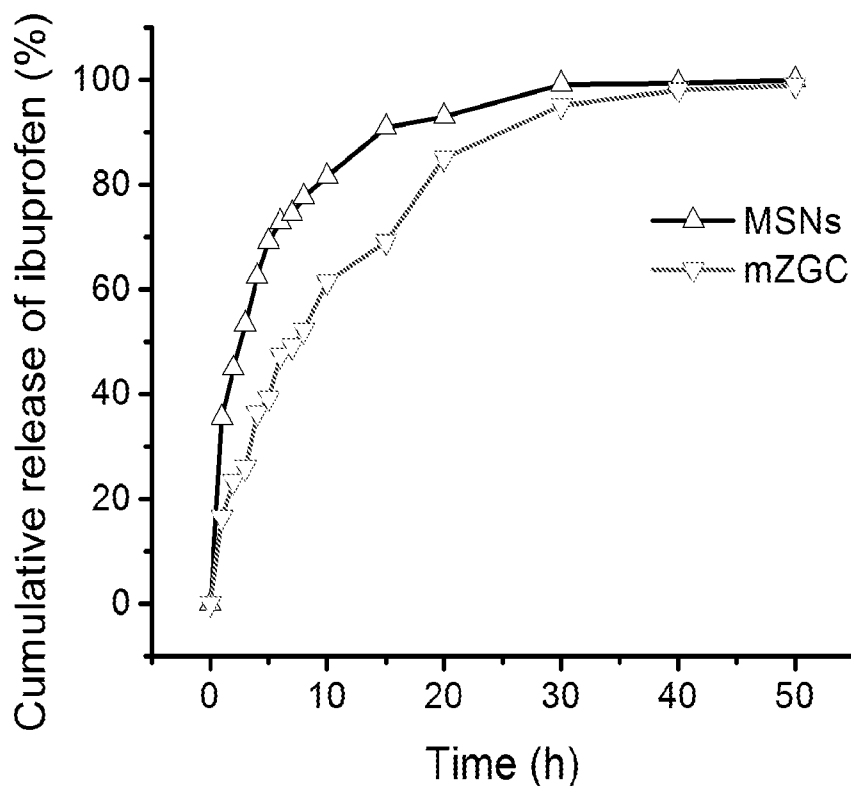


FIG. 9. Ibuprofen storage/release properties of mZGC. The ibuprofen storage and release experiment was performed according to a previous report.^[1] Briefly, 60 mg of the MSNs/ $\text{ZnGa}_2\text{O}_4:\text{Cr}^{3+}$ were immersed into 1 mL of 20 mg/L ibuprofen cyclohexane solution for 24 h. Excess ibuprofen cyclohexane solution was removed by centrifugation and decantation. The cyclohexane in the mesoporous silica was evaporated in a 50 °C air dryer for 1 h. The *in vitro* release of ibuprofen was performed by immersing 60 mg of the drug loaded mZGC in 60 mL of simulated body fluid at 37 °C. 100 μL of the mixed solution was taken to test the released amount of ibuprofen at fixed time intervals; this was centrifuged to determine the released ibuprofen in the supernatant by detecting the absorbance. The initial content of ibuprofen on MSNs and mZGC is 146.3 mg/g and 103.9 mg/g respectively, by elemental analysis.

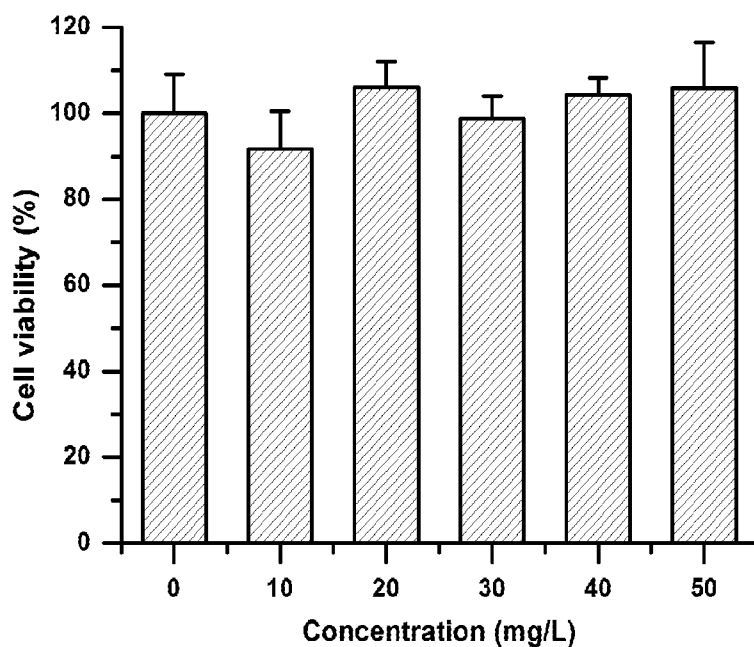


FIG. 10. Cell toxicity of mZGC. The cytotoxicity was tested using 3-(4,5-dimethyl-2-thiazolyl)-2,5diphenyl-2H-tetrazolium bromide (MTT, sigma, USA) assay using human umbilical vein endothelial cells (HUVE cells, ATCC, CRL-1730).

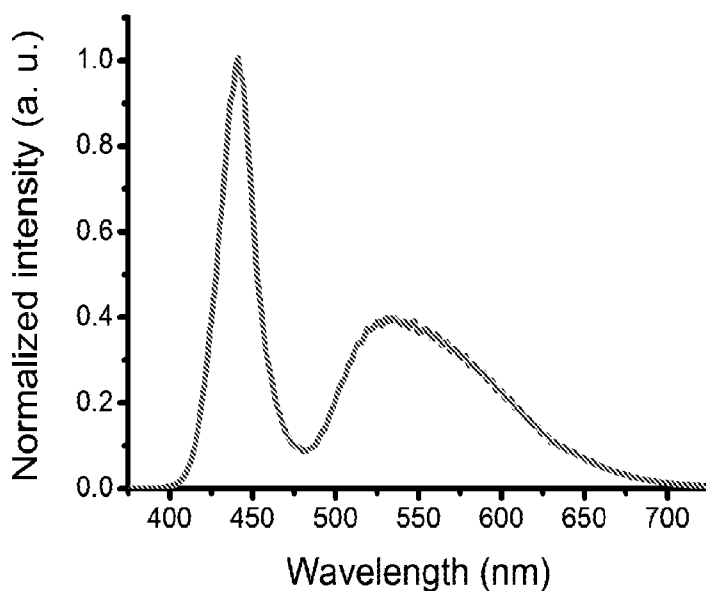


FIG. 11. Spectrum of the LED torch used. Wavelengths longer than 600 nm have better biotissue penetration ability than shorter ones, and result in the charging of mZGC *in vivo*.

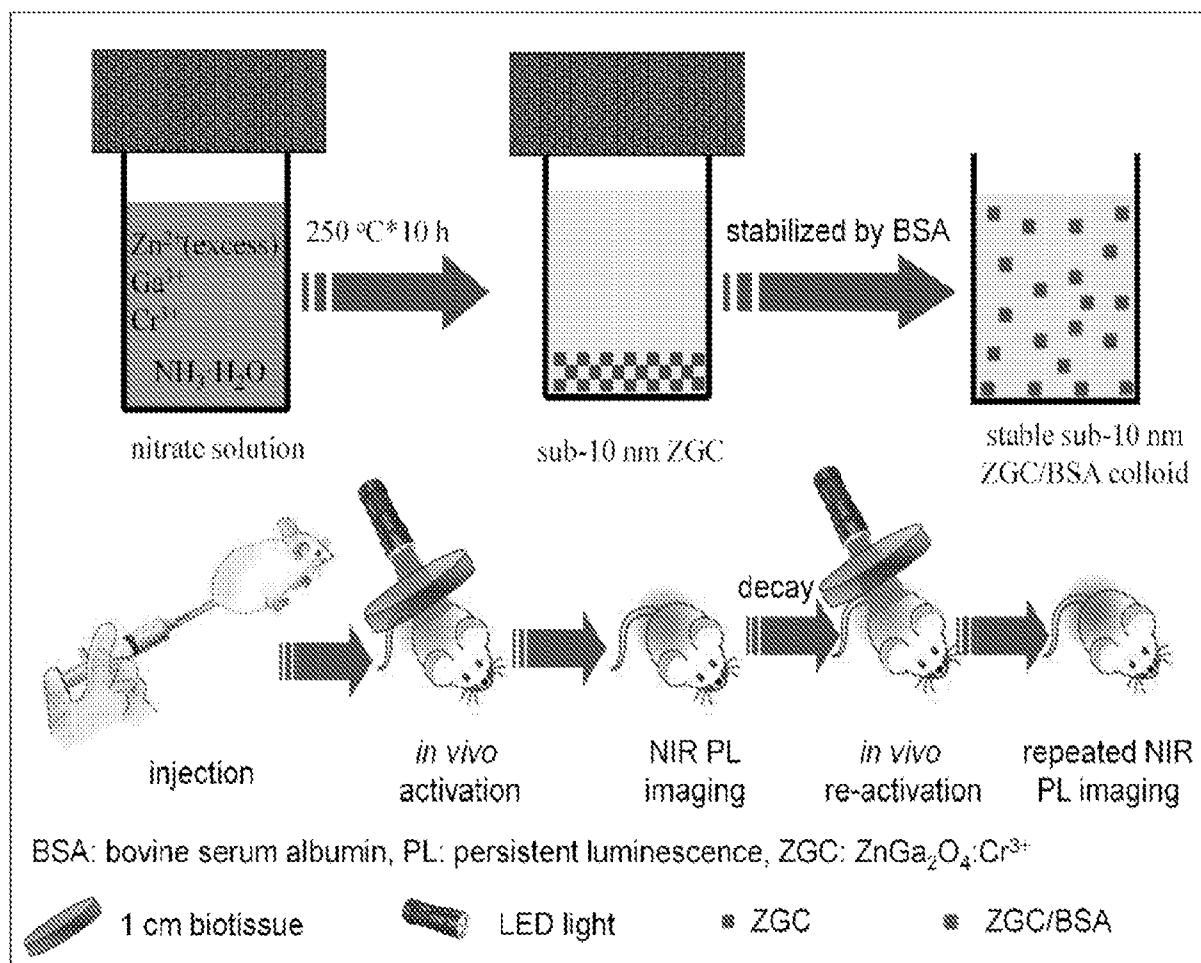


FIG. 12. Schematic illustration of the synthesis and imaging applications of ZGC PLNPs.

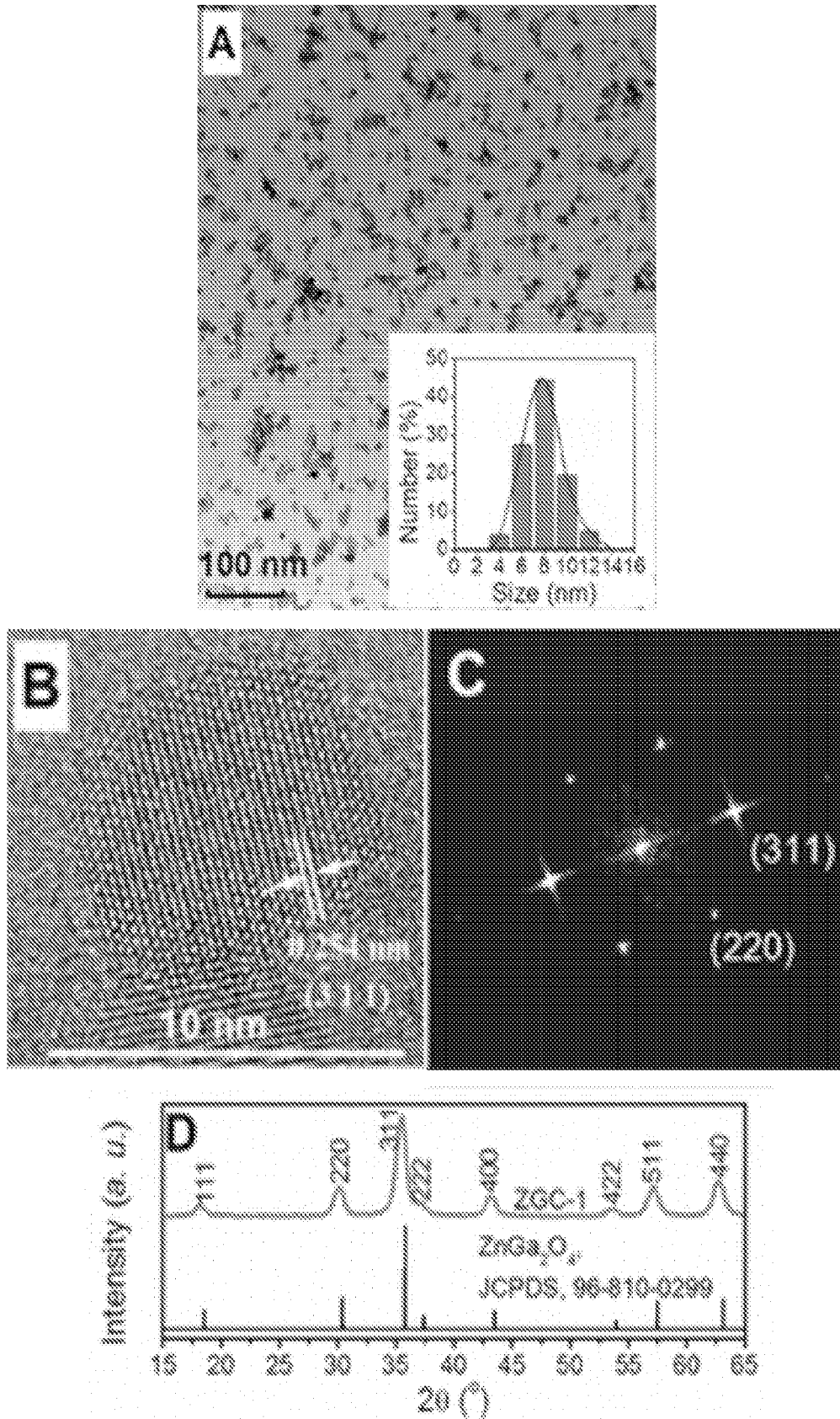


FIG. 13. (A) TEM and size distribution, (B) HRTEM, of a single crystal, (C) selected area electron diffraction pattern, and (D) X-ray diffraction analysis of ZGC-1.

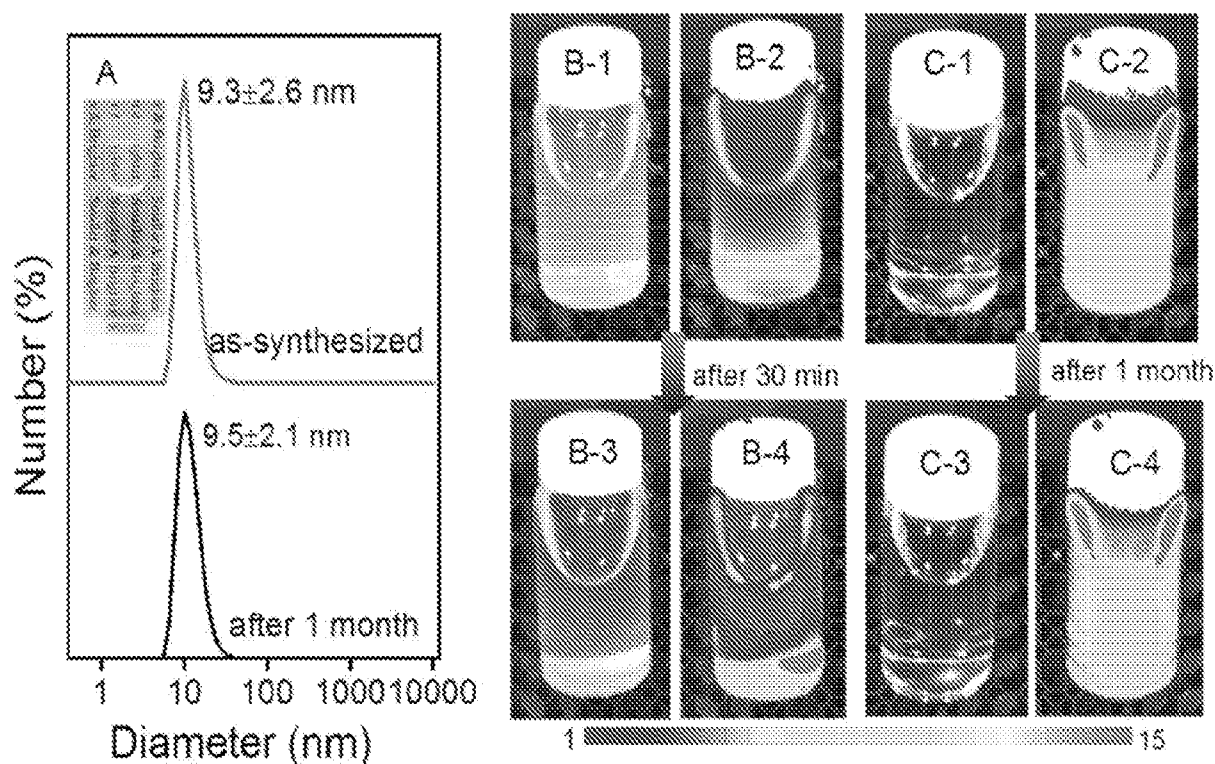


FIG. 14. The disperse stability of ZGC-1 and ZGC-2 in water (pH=6.5). (A) Dynamic light scattering patterns of ZGC-1 in water before and after storage of 1 month, (B1, B3) bright field and (B2, B4) corresponding luminescence pictures of ZGC-2 in water, (C1, C3) bright field and (C2, C4) corresponding luminescence pictures of ZGC-1 in water. Persistent luminescence intensity is expressed in false color units (1 unit = $2.107 \text{ photons} \cdot \text{s}^{-1} \cdot \text{cm}^{-2} \cdot \text{sr}$) for all images.

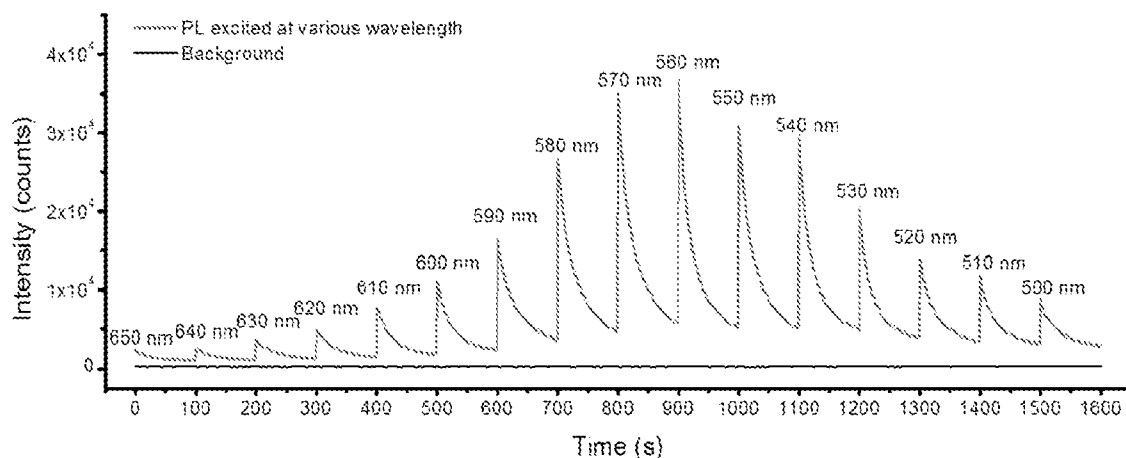


FIG. 15. Decay curves of ZGC-1 excited by various wave length.

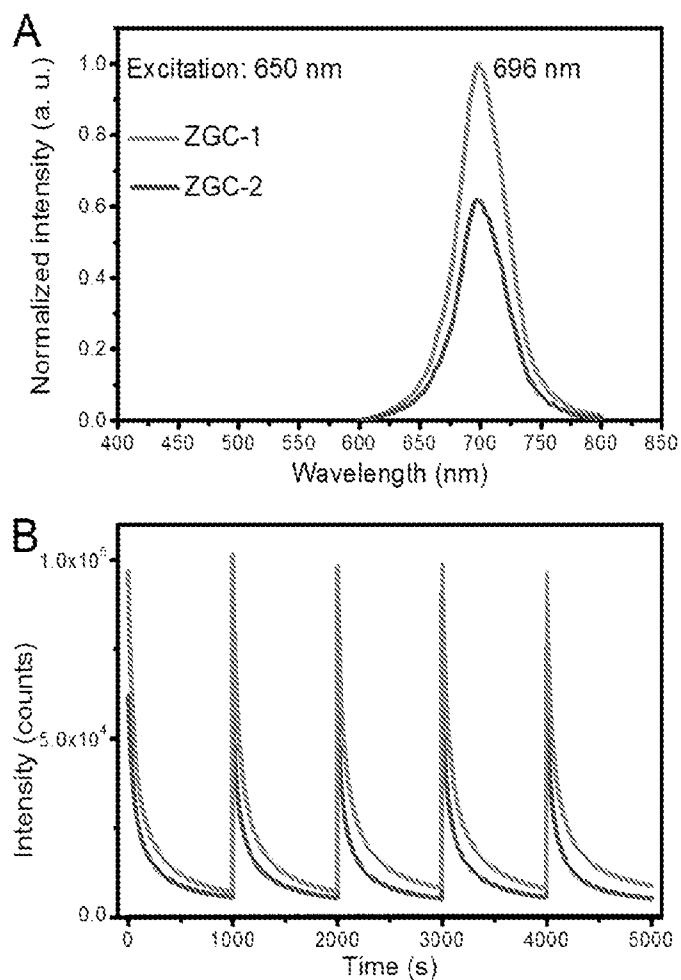


FIG. 16. Superior PL properties of ZGC-1. (A) PL spectra and (B) decay curves of ZGC-1 and ZGC-2 excited at 650 nm for 200 s using a xenon lamp as the light source. PL spectra and decay curves were recorded 30 s after the stop of the excitation. Sample mass = 100 mg.

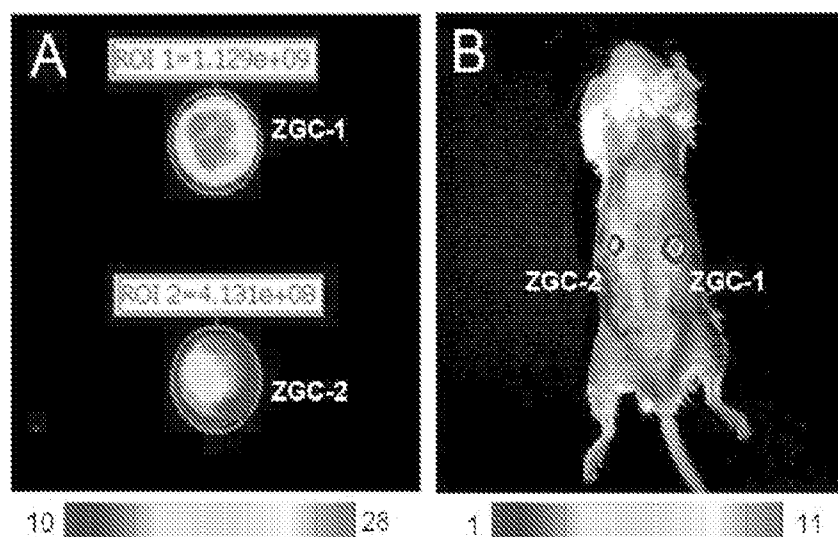


FIG. 17. Analysis of the imaging ability of ZGC-1 and ZGC-2. (A) 50 mg in a black 96-well-plate; (B) *in vivo* activated imaging after subcutaneous injection (50 μ L, 2 mg/mL). Persistent luminescence intensity is expressed in false color units (1 unit = $1 \cdot 10^6$ photons $s^{-1} \cdot cm^{-2} \cdot sr$) for all images.

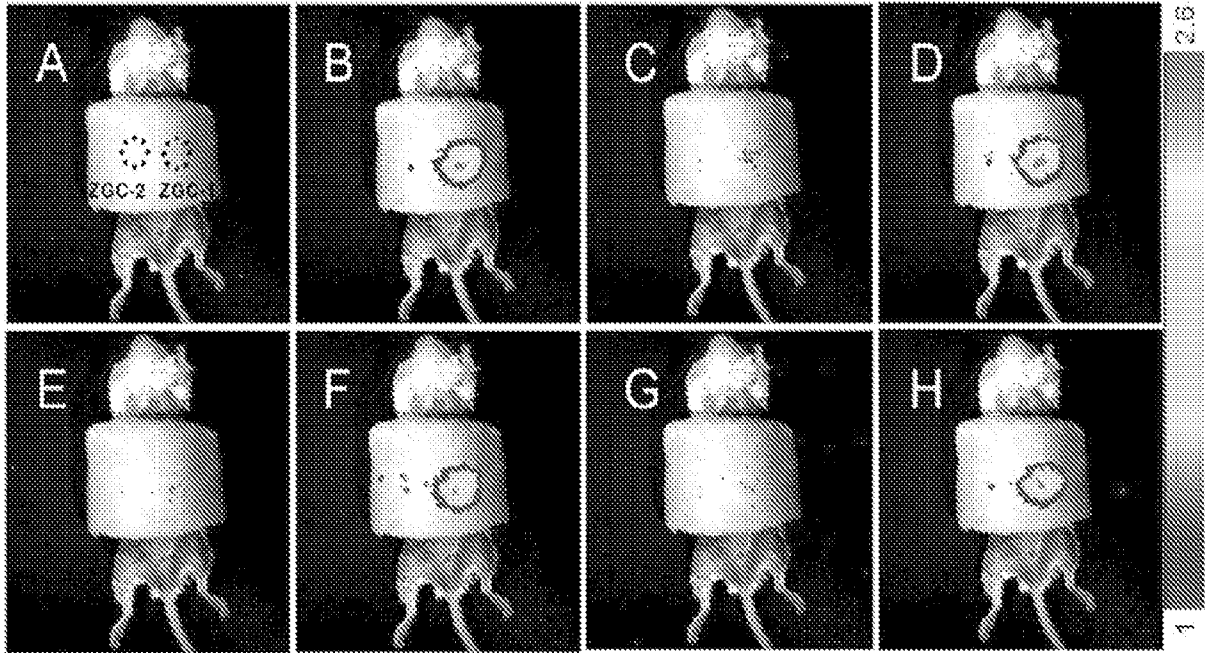


FIG. 18. Comparative deep tissue imaging of ZGC-1 and ZGC-2. (A and B) deep tissue *in vivo* imaging before and after *in situ* excitation; (C–H) repeated imaging before and after 2nd, 3rd, and 4th *in situ* excitations, time interval = 30 min. Excitation with a white LED (5000 lumen) light source for 30 s. Persistent luminescence intensity is expressed in false color units (1 unit = $4350 \text{ photons s}^{-1} \cdot \text{cm}^{-2} \cdot \text{sr}$) for all images. ·

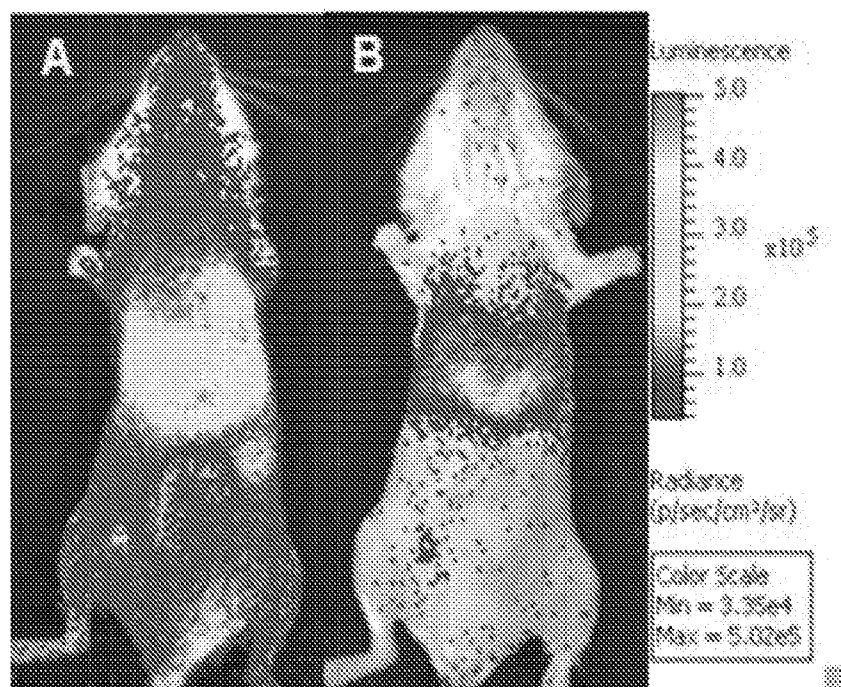


FIG. 19. Imaging applications of ZGC PLNPs after ex vivo (A) and *in vivo* (B) being excited using a white LED light source (5000 lumen).

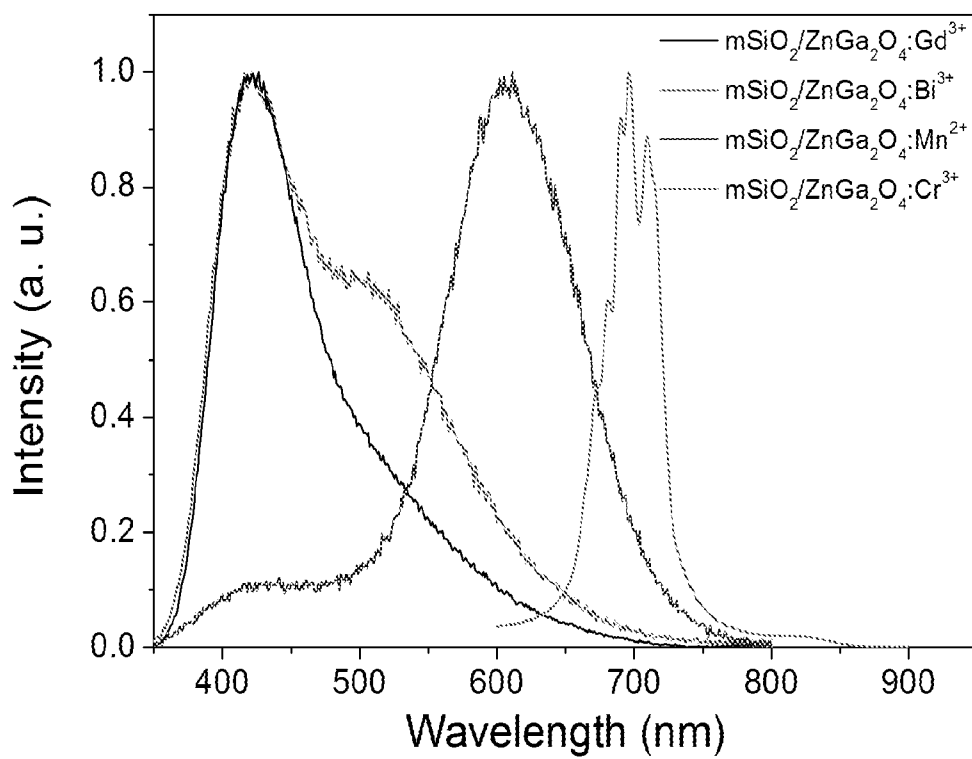


FIG. 20. Luminescence spectra of PLNPs, $\text{mSiO}_2/\text{ZnGa}_2\text{O}_4$ doped with diverse dopant ions.