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Development of novel upconversion luminescent nanoparticle of Ytterbium/Thulium–doped beta tricalcium phosphate

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ABSTRACT

A novel class of upconversion luminescent nanocrystals of Yb/Tm:calcium deficient hydroxyapatite were synthesized by co-precipitation method in aqueous solution (pH adjusted to 6) and specially heat-treated with microwave radiation at different temperatures (from 900 °C to 1000 °C) and times (2–10 min) to produce small nanocrystals of Yb/Tm:β-tricalcium phosphate (β-TCP). As a result, we report for the first time, a single-phase Yb/Tm-doped β-TCP nanocrystals with a mean crystallite size of 55.3 nm. This material has an efficient visible luminescence from the $^1G_4$ (blue emission) and $^3F_2$ (red emission) and near infrared emission from the $^3H_4$ excited states of Tm^{3+} induced by the $Yb^{3+} \rightarrow Tm^{3+}$ energy transfer under laser excitation (Yb^{3+}) at 972 nm. This β-TCP activated by Yb^{3+} and Tm^{3+} ions constitutes a new nano-fluoroprobe that can be used as optical contrast agents, affording high resolution and sensitivity for visible-near infrared applications.

1. Introduction

Many efforts have been made to conciliate both therapy and diagnoses strategies in just an all-in-one particle, leading to advantages over single approaches [1]. Optical imaging approaches are promising high-resolution modalities for the early cancer detection and treatment, since it is a sensitive, non-invasive and real-time monitoring technique [2]. Despite all benefits, optical technique however relies upon the use of sensitive and stable probes [2].

Ongoing discoveries of potential probe molecules for photodiagnosis and phototherapy are focused on the development of imaging optical sensitivity contrast agents in the near-infrared spectrum (NIR, typically in the 700–1300 nm spectral range). At the NIR spectrum window, biological tissues show very low absorption and auto fluorescence [3]. As a result, the sensitivity for NIR imaging contrast agents is greatly enhanced, affording penetration depths of an order of magnitude greater than that of visible light and low phototoxicity to normal cells and tissues [3]. Fluorescence imaging can be achieved by endogenous (porphyrins, collagens, proteins, etc) or exogenous (optical contrast agents, such polymethines, Cy5.5 and Cy7 [3,4]) molecules. However, fluorescence imaging at depths greater than a few millimeters requires NIR probes and dyes [5].

With the oncoming of the nanomedicine and nanotechnology in the past few decades, this new field has exerted great impact on the development of novel nanoparticle-based probes for in vivo cancer molecular imaging. These probes can target tumor cells either through the EPR effect (enhanced permeability and retention) or by a specific binding between the tumor cell receptors and the biomolecules-functionalized nanoparticles [3]. NIR organic dye-containing nanoparticles have overcome some of the limitations of the conventional NIR organic dyes, such as poor photostability, rapid photobleaching, low quantum yield, low detection sensitivity, high citotoxity, etc. Most of the fluorescent nanoparticles that have been successfully applied for bioimaging applications is typically a core-shell structure, where the core is composed by a fluorescent dye, protected (by the shell) from the photobleaching [3].

A new approach to deliver light into deeper tissues for PDD (photodynamic diagnosis) and PDT (photodynamic therapy) is using NIR-excitable upconversion nanoparticles (UCNPs) as an energy donor. The generation of upconversion (UC) fluorescence is a multiphoton process. UCNPs are usually lanthanide-doped nanocrystals, which emit high-energy photons under the excitation by the NIR light, and they have proven to be valuable and desirable tools for NIRF (near infrared fluorescence) techniques [6–8].

Appropriate loading of photosensitizers (PS) molecules (including silica encapsulation, non-covalently physical adsorption, and covalent conjugation via chemical linkages) to UCNPs is important to allow effective resonance energy transfer from donors to acceptors in NIR-induced PDT [9]. Recent studies have

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demonstrated a significant reduction in tumor growth of the group treated with UCNP loaded PS (mesoporous silica coated Yb: Er:$NaYF_4$, $NaGdY_4$- PEGylation loaded with Ce6 molecules) compared to control group, showing encouraging therapeutic effect upon either local injection or systemic administration of UCNP-PS nanocomplexes [9].

In addition to the application of UCNPs as energy donors, they could also be used to trigger release or ‘uncaging’ of functional biomolecules. Photo-responsive drug release systems have received significant interests, since they can remotely control the releasing of therapeutic drugs at the targeting site [9]. A specific class of UCNPs, usually containing Yb^{3+} and Tm^{3+} (for example Yb:Tm:$NaYF_4$) [9,10] is able to convert the NIR laser excitation into different wavelengths from UV to NIR, and could be a great promising in the designing of NIR-light photoreactions of compounds anchored to their surfaces [9]. Recently it was reported the using of NIR-to-UV UCNPs for photo-controllable gene expression [11], where the authors have used the NIR light treatment to uncaging the pDNA and siRNA by the energy transferred from the UCNPs, photo inducing controlled gene expression and specific gene silencing.

xAs summarized, these nanoprobes play an important role in early detection, screening, image-guided therapy, photodiagnosis, phototherapy, gene therapy for controlled and specific gene delivery/knockdown [9], but brighter UCNPs need to be synthesized with higher quantum yield (UNCPs quantum yields is mostly less than 1%, limiting their using in PDD and PDT [9]) and appropriate loading of PS molecules onto the UCNPs (to allow effective resonance energy transfer from donors to acceptors), by designing special UCNP structures.

In this context, nanosized calcium phosphate (CP) particles are particularly attractive because of the following advantages: 1) it is regarded as safe by the FDA (Federal Drug Administration) [12], since it is the main inorganic constituent of bone and teeth; 2) high biocompatibility and biodegradability; 3) the CP structures can be loaded with multiple therapeutics agents to offer synergistic effects, as photosensitizers, drugs or genes; 4) CP can be coated with biocompatible polymers that render them water-soluble in order to manage the blood circulation time; 5) the surface can be appropriately modified with specific biomolecules (e.g. antibodies, peptides, folates, etc) to provide targeting and selectivity; 6) The luminescent properties of lanthanide-doped CP make them ideal for their using as optical nanoprobes in vivo and in vitro biological applications [10].

Nevertheless, Yb^{3+}/Tm^{3+} doped hydroxyapatite (HA) nanopowder is a weak upconversion luminescent material in the visible due to the intrinsic luminescence quenching of $^1G_4$, $^3F_2$ and $^3H_4$ excited levels of Tm^{3+} due to the optical coupling to the high frequency lattice phonons produced by the constituent OH^- free radicals (3500–2500 cm^{-1}) [13,14]. Recently, it was reported one efficient annealing process that transforms calcium deficient HA (CDHA) nanopowder into β-TCP that activated by Eu^{3+} ions exhibits an intense red luminescent emission twenty-fold higher than those obtained for europium-doped HA structure [15].

In this paper, nanocrystals of Yb/Tm:CDHA were synthesized by co-precipitation method in aqueous solution (pH adjusted to 6) and specially heat-treated with microwave at different temperatures (from 900 °C to 1000 °C) and time to produce small nanocrystals of Yb/Tm:β-TCP with less than 200 nm mean diameter of agglomerated particles and efficient UC luminescence from the $^1G_4$ (blue emission), from the $^3F_2$ (red emission) and from the $^3H_4$ (NIR emission) excited states of Tm^{3+}. This β-TCP activated by Yb^{3+} and Tm^{3+} ions constitutes a special nanophosphor with a vast biological application scope. The multiple processes of energy transfer that occurs when this material is excited around 972 nm were inspected and the UC time constants were determined. The luminescence efficiencies of $^1G_4$ and $^3F_2$ excited states (Tm^{3+}) were measured and the best treatment temperature and time under microwave heating in order to obtain a nanoparticle with an intense emission were established.

2. Experimental procedure

The nanopowders were obtained by the co-precipitation method, wherein the phosphoric acid (Synth – Brazil) diluted in deionized water (0.3 M) was slowly added drop by drop (10 mL min^{-1} rate) into a suspension of Calcium Hydroxide (Synth- Brazil), Ytterbium oxide (5.5%, Sigma Aldrich) and Thulium oxide (0.5%, Sigma Aldrich) (0.5 M). The pH values of the solutions were adjusted to 6.0 by adding HNO_3. After the precipitation, the solutions were aged at room temperature for 24 h. The precipitates were filtered and washed several times with deionized water to remove free ions. Finally, they were dried at 60 °C for 24 h.

The dried powders were heated at $T=900$ °C and 950 °C for 2 or 3 min, 1000 °C for 10 min using an adapted microwave oven, and at 1000 °C for 3 h using a conventional furnace. Samples were characterized by X-ray diffraction (XRD) using a Multiflex Rigaku diffractometer using the Cu kα ($\lambda=0.1542$ nm) radiation. The crystallite size was determined by Scherrer's formula and the (002) X-ray diffraction peak was used. The nanopowders were dispersed in absolute isopropanol and ultrasounds treated to avoid particles agglomeration, and then were dropped on a copper grid to be observed by transmission electronic microscopy (JEM 2010 - JEOL). To Field Emission Gun scanning electron microscopy (FEG JSM 6701F – JEOL) analysis the samples were deposited in a carbon-cement. Granulometry measurements were performed by dynamic light scattering (DLS), in a ZetaPALS-Zeta Potential Analyzer, BrookHaven Corp.

In the luminescence lifetime measurements, the samples were excited by pulsed laser radiation generated by a tunable OPO-IR pumped (Rainbow from OPOTEK, USA) by the second harmonic of a Q-switched Nd:YAG (yttrium aluminum garnet) laser (Brilliant B from Quantel, France). Laser pulse widths of 4 ns at 972 nm were used to directly excite the $^4F_{5/2}$ excited state of Yb^{3+}. Luminescence signals were analyzed by the 0.25 m Kratos monochromator, detected by the EMI S-20 PMT (response time of 10 ns) or using a charge coupled device (CCD) spectrometer coupled to the sample holder containing the nanopowder via optical fiber. Luminescence lifetime was measured using a digital oscilloscope of 100 MS s^{-1} model TDS 410 from TEKTRONIX interfaced to a microcomputer.

3. Experimental results

The as-synthesized Yb(5.5%):Tm(0.5%):CDHA nanocrystals have shown the hexagonal phase of HA ($Ca_{10}(PO_4)_6(OH)_2$) corresponding to the JCPDS file no. 9-432, with a diffraction profile broadening, as seen in Fig. 1.

The samples were calcined at temperatures higher than 900 °C in order to assess the effect of temperature versus time changes on the phase composition and formation of pure β-TCP crystal structure. It is well seen the evolution of deficient hydroxyapatite transformation into β-TCP pattern in Fig. 1. After thermal treatment at 900 °C for 2 min, the diffractogram peaks were indexed according to the standard pattern of HA and β-TCP (JCPDS 09-0169), because this short time (2 min) was not enough for the completely transformation of the powder into the rhomboidal phase. Nevertheless, after 3 min of adapted microwave heating (at 900 °C), and for all the others treatments (samples treated at 950 °C and 1000 °C), the calcined powders showed well characterized peaks of pure β-TCP diffraction pattern (Fig. 1). From the Rietveld refinement it was found out the parameter values as $a=10.4300$ Å and $c=37.3369$ Å.

The morphologies of the powders, before and after thermal treatment, are shown in Fig. 2. Despite the broadening X-Ray