Materials Science & Engineering C 81 (2017) 422–431

Contents lists available at ScienceDirect

Materials Science & Engineering C

journal homepage: www.elsevier.com/locate/msec

Luminomagnetic Eu^{3+}- and Dy^{3+}-doped hydroxyapatite for multimodal imaging

Annemarie Tesch[a], Christoph Wenisch[a], Karl-Heinz Herrmann[b], Jürgen R. Reichenbach[b], Paul Warncke[c], Dagmar Fischer[c], Frank A. Müller[a],*

[a] Otto Schott Institute of Materials Research (OSIM), Friedrich Schiller University, Loebdergraben 32, 07743 Jena, Germany
[b] Medical Physics Group, Institute of Diagnostic and Interventional Radiology, Jena University Hospital, Philosophenweg 3, 07743 Jena, Germany
[c] Institute of Pharmacy, Friedrich Schiller University, Philosophenweg 14, 07743 Jena, Germany

ARTICLE INFO

Keywords:
Hydroxyapatite
Europium
Luminescence
MRI
Contrast agent

ABSTRACT

Multimodal imaging has recently attracted much attention due to the advantageous combination of different imaging modalities, like photoluminescence (PL) and magnetic resonance imaging (MRI). In the present study, luminescent and magnetic hydroxyapatites (HAp) were prepared via doping with europium (Eu^{3+}) and dysprosium (Dy^{3+}), respectively. Co-doping of Eu^{3+} and Dy^{3+} was used to combine the desired physical properties. Both lanthanide ions were successfully incorporated in the HAp crystal lattice, where they preferentially occupied calcium(I) sites. While Eu-doped HAp (Eu:HAp) exhibits dopant concentration dependent persistent PL properties, Dy-doped HAp (Dy:HAp) shows paramagnetic behavior due to the high magnetic moment of Dy^{3+}. Co-doped HAp (Eu:Dy:HAp) nanoparticles combine both properties in one single crystal. Remarkably, multimodal co-doped HAp features enhanced PL properties due to an energy transfer from Dy^{3+} sensitizer to Eu^{3+} activator ions. Eu:Dy:HAp exhibits strong transverse relaxation effects with a maximum transverse relaxivity of 83.3 L/(mmol·s). Due to their tunable PL, magnetic properties and cytocompatibility Eu:-, Dy:- and Eu:Dy:HAp represent promising biocompatible ceramic materials for luminescence imaging that simultaneously may serve as a contrast agent for MRI in permanent implants or functional coatings.

1. Introduction

The biomineral hydroxyapatite (HAp, $Ca_{10}(PO_4)_6(OH)_2$) is the major inorganic component of mammalian hard tissues, like bone and teeth [1]. Synthetic HAp can easily be synthesized by wet chemical precipitation or sol-gel as well as solid state reaction [2]. This artificial HAp mimics the physicochemical properties of natural HAp and is consequently used as an implant material in biological environments (e.g., bone cement or bioactive coatings on metallic implants) [3,4]. In its biological form, HAp or bone apatite, is highly substituted with ions like Na^+, K^+, Mg^{2+}, HPO_4^{2-} and CO_3^{2-}. Hence, the crystal lattice of HAp exhibits high tolerance towards substitutions and distortions [5]. Therefore, synthetic HAp can also be doped with several metal ions in order to improve properties, like bioactivity, degradation rate, antibacterial characteristics, luminescence and magnetic properties [6–10].

Multimodal imaging represents an approach that has gained much interest in recent years. Several imaging techniques are typically combined in a single diagnostic step. Imaging modalities, like computer tomography, magnetic resonance imaging (MRI) and photoluminescence (PL) tomography, can, for instance, be applied subsequently or in some specific equipment configuration even simultaneously to the same sample. One major advantage of this approach is the more precise diagnosis of complex diseases, like tumors. Here, different imaging modalities can be used to improve the localization of findings in the patient's body or to increase the specificity of the diagnostic findings. Also, multimodal imaging is patient-friendly due to time savings making repeated consultations potentially obsolete. Since different imaging modalities are involved, however, a multimodal contrast agent responding to the different types of radiation or (electro-)magnetic fields associated with the modalities, is required.

One new promising approach for a multimodal contrast agent is the use of doped HAp as a ceramic nanoparticle-sized contrast agent or marker for biomedical luminescence imaging. Single-modal HAp contrast agents have been synthesized by using rare earth elements (REE) as dopants, such as, e.g., europium (Eu^{3+}) or terbium (Tb^{3+}) [7,11–13]. Due to their narrow emission band caused by f-f intraorbital

---

* Corresponding author.
E-mail addresses: Annemarie.Tesch@uni-jena.de (A. Tesch), Christoph.Wenisch@uni-jena.de (C. Wenisch), Karl-Heinz.Herrmann@med.uni-jena.de (K.-H. Herrmann), Juergen.Reichenbach@med.uni-jena.de (J.R. Reichenbach), Paul.Warncke@uni-jena.de (P. Warncke), Dagmar.Fischer@uni-jena.de (D. Fischer), Frank.Mueller@uni-jena.de (F.A. Müller).

http://dx.doi.org/10.1016/j.msec.2017.08.032
Received 11 April 2017; Received in revised form 10 August 2017; Accepted 10 August 2017

electronic transitions and steady photo bleaching, lanthanides are suitable substituents to synthesize luminescent HAp [14]. Eu^{3+} is quite a suitable dopant ion for HAp, as it is easily incorporated in the HAp crystal lattice due to its similar ionic radius. Beyond, Eu^{3+} exhibits excellent optical properties and emits in the visible region showing dominantly red luminescence with a high Stokes shift that facilitates excitation and subsequent detection [15]. Various methods can be used to synthesize luminescent doped HAp, including wet chemical precipitation [16], sol gel synthesis [17] and solid state reactions [18]. The PL properties of Eu-doped calcium phosphates depend on several factors, like dopant concentration, synthesis temperature [19], crystallinity [20] and crystallite size [21].

Another important property that can be utilized for multimodal imaging is the paramagnetic behavior of rare earth elements. Magnetizable materials are employed as contrast agents for MRI. Ions like gadolinium (Gd^{3+}) and dysprosium (Dy^{3+}) have been used for many years to synthesize aqueous paramagnetic contrast agent complexes, which are injected during MRI examination [22,23]. These two ions can also be used to synthesize doped paramagnetic HAp [8,24,25]. A shortening of the transverse magnetization time (T_2) then leads to distinct negative contrast on the corresponding magnetic resonance (MR) images.

In the present study, we demonstrate a new approach towards luminescent and magnetic Eu- and Dy-doped HAp as well as the combination of these desired properties in co-doped Eu:Dy:HAp. For this purpose, a wet chemical precipitation followed by a hydrothermal treatment was used to achieve REE-doped, crystalline and carbonate-free HAp nano-powders. Preserving the beneficial properties of Eu:-, Dy:- and Eu:Dy:HAp nanoparticles, these materials are promising candidates as contrast agent for multimodal imaging in the field of PL tomography and MRI [7,26]. Therefore, as-synthesized samples demonstrate the proof-of-principle for future contrast agent related applications e. g. as injectable luminomagnetic nanoparticles or biocompatible contrastive supplement for calcium phosphate bone cements.

2. Materials and methods

2.1. Synthesis of Eu^{3+}-, Dy^{3+}- and co-doped hydroxyapatite

A hydrothermal co-precipitation method was used to prepare Eu:-, Dy:- and Eu:Dy:HAp carbonate-free HAp with a high degree of crystallinity. Parts of calcium nitrate ($Ca(NO_3)_2{\cdot}4H_2O$, 99%, p.a., Carl Roth GmbH + Co. KG, Germany) were substituted by europium(III) nitrate hexahydrate ($Eu(NO_3)_3{\cdot}6H_2O$, 99.9%, Alfa Aesar GmbH, Germany) and/or dysprosium(III) nitrate pentahydrate ($Dy(NO_3)_3{\cdot}5H_2O$, 99.9%, Alfa Aesar GmbH, Germany) in ratios of 0.5, 1, 1.5, 2, 3, 4, 5 and 10 mol% with respect to charge compensation (denoted as e.g. 0.5Eu:HAp, 0.5Dy:HAp). Mixed calcium-dopant solutions were precipitated with diammonium hydrogen phosphate ($(NH_4)_2HPO_4$, 98%, p.a., Carl Roth GmbH + Co. KG, Germany) under basic conditions. Eu:Dy co-doped HAp samples were synthesized by the precipitation of a calcium solution containing both cations of Eu^{3+} and Dy^{3+} with a total maximum doping concentration of 2 mol%, split in the ratios Eu:Dy of 1:1, 1:3 and 3:1 (denoted as e.g. 0.5Eu:HAp:1.5Dy:HAp). After reaction, the precipitate was transferred into a hydrothermal reactor (4560 Mini Reactor 600 mL, Parr Instruments, USA) and treated at 200 °C and 16.5 bar for 5 h. Finally, the material was washed with deionized water and freeze-dried for 5 h at 0.47 mbar. All salt solutions for the synthesis were prepared by using ultrapure water.

2.2. Characterization

2.2.1. X-ray diffraction

The crystalline phase composition and structure of the doped HAp samples was characterized by X-ray diffraction (XRD, D5000, SIEMENS Diffractometer, Germany) using CuK α radiation ($\lambda = 0.15405$ nm), 40 kV operation voltage and 30 mA operating current. The scanning rate during measurement was 0.02°/s for the angular range of 25–55° 2θ. The degree of crystallinity X_c for REE-doped HAp was determined by using the equation:

$$X_c = \left[1 - \frac{V_{112/300}}{I_{300}}\right] \tag{1}$$

Here, $V_{112/300}$ represents the intensity of the hollow between (112) and (300) reflexes, and I_{300} the intensity of the (300) diffraction peak. Furthermore, mean crystallite sizes D were calculated using Scherrer's equation

$$D = \frac{K{\bullet}\lambda}{FWHM_{002}{\bullet}cos\theta}, \tag{2}$$

with shape factor $K = 0.9$, wavelength λ, $FWHM_{002}$ the full-width-at-half-maximum of the (002) reflex and Θ the angle of this signal.

2.2.2. Fourier transformed infrared spectroscopy

To evaluate the chemical composition, Fourier transformed infrared spectroscopy was performed by attenuated total reflection (ATR-FTIR, Alpha-P, Bruker, Germany). Here, measurements using a diamond crystal were performed with a step size of 1.4 cm^{-1} between 4000 and 400 cm^{-1}. All sample data were normalized to the most intense signal at 1022 cm^{-1}.

2.2.3. Transmission electron microscopy

Transmission electron microscopy (TEM, Zeiss EM 900, Germany) was performed to identify the crystal morphology of doped HAp. For this purpose, an accelerating voltage of 80 kV and a digital reflex camera (EOS 450, Canon, Germany) were used. Statistical crystallite size distributions were analyzed by computation, relying on the length of the predominant crystal direction (c-axis) by analyzing 1000 to 1200 crystals.

2.2.4. Inductively coupled plasma mass and optical spectrometry

To quantify the degree of Eu^{3+} and Dy^{3+} substitution, inductively coupled plasma mass spectrometry (ICP-MS, XSeries II, Thermo Scientific, Germany) was used for lanthanide ion determination and inductively coupled plasma optical emission spectrometry (ICP-OES,725 ES, Varian, Germany) was executed for calcium (Ca) and phosphorous (P) analyses. All powder samples were dissolved in nitric acid.

2.2.5. Photoluminescence properties

The photoluminescence excitation (PLE) and photoluminescence (PL) measurements at $T = 300$ K were performed using a 150 W Xe arc lamp (Tunable PowerArc Illuminator, OBB Corp., USA) as excitation source. To this end, the radiation was guided onto the surface of the samples positioned inside an integrating sphere (IS236A-4, Thorlabs Inc., USA). The emitted radiation was detected by a spectrometer (Maya2000 Pro, Ocean Optics, USA). Furthermore, PL measurements at $T = 5$ K were performed using the third harmonic radiation of a Nd:YAG laser (DPSS 10–100, Innolas, Germany) with an excitation wavelength $\lambda_{ex.}$ of 355 nm. The samples' surfaces were excited at 45° angle relative to the spectrometer (SP-2500i, Princeton Instruments, USA).

2.2.6. Magnetic properties

The magnetic properties of Eu:-, Dy:- and Eu:Dy:HAp were evaluated by using a vibrating sample magnetometer (VSM, MicroMag 3900, Princeton Measurements Corporation, USA). The magnetic field strength H was varied between $-14{,}000$ and $+14{,}000$ Oe during examination of the sample magnetization M. All data were corrected by the sample mass and the mass of the measuring glass vessel.