Acta Biomaterialia 9 (2013) 8422–8432

Contents lists available at SciVerse ScienceDirect

Acta Biomaterialia

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

A microwave-assisted solution combustion synthesis to produce europium-doped calcium phosphate nanowhiskers for bioimaging applications

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ARTICLE INFO

Article history:
Received 17 December 2012
Received in revised form 22 May 2013
Accepted 30 May 2013
Available online 11 June 2013

Keywords:
Europium
Calcium phosphate
Fluorescence
Inorganic nanoparticles
Microwave-assisted solution synthesis

ABSTRACT

Biocompatible nanoparticles possessing fluorescent properties offer attractive possibilities for multifunctional bioimaging and/or drug and gene delivery applications. Many of the limitations with current imaging systems center on the properties of the optical probes in relation to equipment technical capabilities. Here we introduce a novel high aspect ratio and highly crystalline europium-doped calcium phosphate nanowhisker produced using a simple microwave-assisted solution combustion synthesis method for use as a multifunctional bioimaging probe. X-ray diffraction confirmed the material phase as europium-doped hydroxyapatite. Fluorescence emission and excitation spectra and their corresponding peaks were identified using spectrofluorimetry and validated with fluorescence, confocal and multiphoton microscopy. The nanowhiskers were found to exhibit red and far red wavelength fluorescence under ultraviolet excitation with an optimal peak emission of 696 nm achieved with a 350 nm excitation. Relatively narrow emission bands were observed, which may permit their use in multicolor imaging applications. Confocal and multiphoton microscopy confirmed that the nanoparticles provide sufficient intensity to be utilized in imaging applications.

1. Introduction

For many biological specimens and systems, optical-based techniques such as fluorescence, luminescence, multiphoton (MP) and confocal microscopy have become extremely useful techniques due to the advent of imaging agents such as fluorescent proteins, the luciferase system and a variety of fluorophores [1]. Imaging agents can be utilized to selectively study biological events and processes such as protein production, cellular trafficking, anatomical/cellular location, etc. Organic dyes, such as fluorescein isothiocyanate (FITC), tetramethylrhodamine isothiocyanate (TRITC) and 4′,6-diamidino-2-phenylindole (DAPI), are the most commonly used imaging agents [2,3]. Organic dyes often have relatively broad emission and excitation spectra and due to their small size are easily internalized by cells, which has permitted their ubiquitous usage in conventional fluorescence microscopy. In traditional fluorescence microscopy, filters are typically 40–50 nm wide and are centered on the peak excitation and emission wavelengths of the common fluorophores. This standardization has greatly minimized equipment costs for traditional fluorescence imaging. However, organic dyes can suffer from photobleaching and their excitation/emission profiles often overlap with one another or autofluorescence from cells, tissues or other environmental components. Therefore, the translation of these dyes to other imaging applications, such as confocal or MP microscopy, may be viewed as less than ideal. These techniques offer improved spatial resolution and are capable of discerning multiple, narrow excitation and emission profiles with suitable imaging agents.

Recently, inorganic nanoparticles, such as quantum dots, silica nanoparticles and calcium phosphates (CaPs), have received considerable attention as alternatives, owing to their resistance to photobleaching, high/enhanced fluorescence yield, narrow emission/excitation peaks and high structural and dispersion stability in vitro and in vivo [4–6]. The optical properties in nanoparticles are dependent on both the particle size and particle morphology as well as the underlying chemical composition. While the size of the nanoparticle may limit internalization in some applications, inorganic nanoparticles have shown promise for bioimaging and

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http://dx.doi.org/10.1016/j.actbio.2013.05.033

gene and drug delivery, which may permit their use in multifunctional biomedical applications [7–10].

Quantum dots (QDs) are currently the most commonly used inorganic nanoparticle for fluorescent imaging [11]. QDs are a type of semiconductor crystal that can be fabricated in the low nanorange (i.e. ~10–50 nm) and have been shown to be extremely efficient in generating high fluorescence with narrow emission spectra [11,12]. This allows for multiple QDs to be used with a single excitation source and for improved multicolor imaging in microscopes with tunable detectors, such as confocal or MP systems [13]. The toxicity effects associated with their use is still largely unknown and may limit their applicability to in vivo studies [14]. Silica nanoparticles are regarded as biocompatible and offer improved stability to surface conjugated organic dyes [7]. The silica matrix is optically transparent and has a highly tunable surface, meaning targeting agents can be used to localize the nanoparticles to specific cellular and intracellular locations [2]. Rapid and recent advances in nanomaterial synthesis techniques have brought fluorescent silica nanoparticle intensities to a level comparable to QDs [15].

CaPs have also been used as fluorescent agents using several different techniques. They can be surface conjugated with fluorophores [16] or encapsulate fluorophores in a micelle type precipitation [17]. CaP materials are also highly adaptable as they allow substitution ions into their lattice structure with relative ease. CaPs doped with lanthanide series elements have shown fluorescence capabilities [18–20]. Differences in crystal structure, lattice substitution and environmental changes affect both the intensity and location of emission and excitation peaks [18,20–22]. Thus, it is possible to discern events such as drug release profiles by simply measuring changes in excitation and emission spectra [23]. The use of the lanthanide series element europium especially has been under recent investigation [18,21–26].

CaPs, such as hydroxyapatite (HA) and tricalcium phosphate, are widely used in biomedical applications such as implant materials, tissue engineering scaffold components and as drug or gene carriers owing to their high biocompatibility [7]. Adding a fluorescent capability to these already diverse materials further extends their functionality. Here, we demonstrate the versatility afforded by a microwave-assisted solution combustion synthesis (MASCS) method in producing a highly crystalline, high aspect ratio (aspect ratio = length/diameter) CaP nanoparticle (i.e. nanowhisker) doped with europium for use as a multifunctional bioimaging agent. The main benefits of using this synthesis method is that doped non-agglomerated, highly crystalline nanowhiskers of varying CaP phases can be produced without necessitating the use of toxic templating agents, specialized equipment, large energy costs or long heating times. The majority of prior studies have produced amorphous and spherical fluorescent CaP nanoparticles [18,19,21,27–30] with a handful of works reporting synthesis of europium-doped CaP nanorods [24,31]. The high aspect ratio, crystalline europium-doped hydroxyapatite nanowhiskers synthesized here utilize a greatly simplified manufacturing process and can fill a specific niche not previously offered by other bioimaging agents. We have chosen europium here based on the availability of data in the literature, but other lanthanide series elements could just as easily be used in this synthesis method to generate fluorescent agents with different excitation and emission profiles.

2. Materials and methods

2.1. Synthesis of europium-doped HA (Eu:HA) using MASCS

Europium-doped (Eu:HA) nanowhiskers (and undoped HA nanowhiskers) were synthesized using a modified microwave-assisted solution combustion synthesis method following the procedure previously described [32]. All the chemical reagents were purchased from Fisher Scientific (Fisher Chemicals, Fisher Scientific, Fair Lawn, NJ).

In short, sodium nitrate, calcium nitrate, potassium phosphate monobasic, nitric acid and urea were sequentially added to 10 ml of deionized water under constant magnetic stirring in a 20 ml Pyrex beaker. For the synthesis of Eu:HA nanowhiskers, 5 mol.% $Eu(NO_3)_3$ was added after the addition of sodium nitrate using the HA formulation. All solutions obtained were clear and contained no precipitates prior to microwaving. The beaker was positioned on the center of a $10 \times 10 \times 1$ cm^3 alumina fiber board and covered with a 250 ml inverted Pyrex beaker. The assembly was then placed in a household microwave oven (Emerson

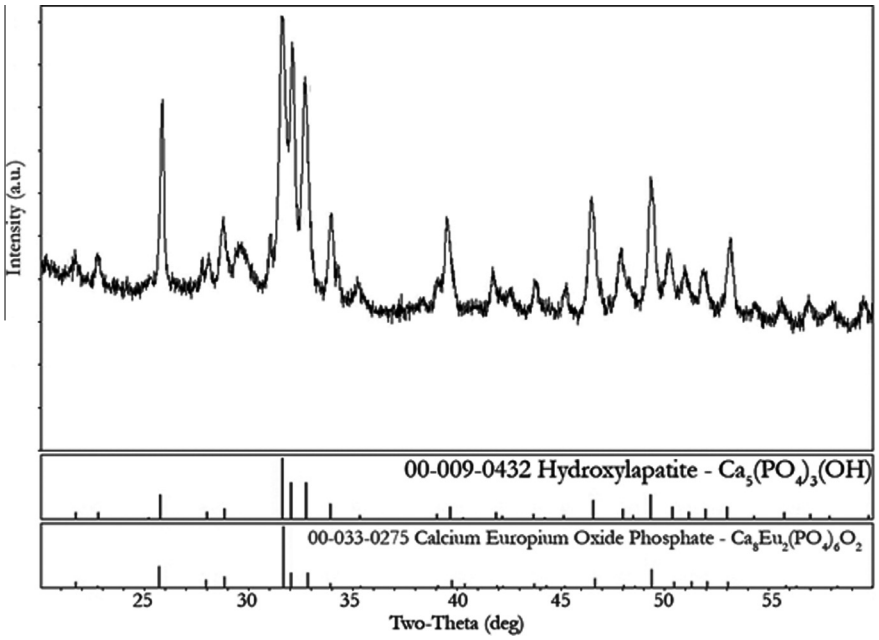

Fig. 1. XRD spectrum of europium-doped hydroxyapatite nanowhiskers.