Colloids and Surfaces B: Biointerfaces 146 (2016) 363–374

Contents lists available at ScienceDirect

Colloids and Surfaces B: Biointerfaces

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

Different hydroxyapatite magnetic nanoparticles for medical imaging: Its effects on hemostatic, hemolytic activity and cellular cytotoxicity

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ARTICLE INFO

Article history:
Received 22 February 2016
Received in revised form 20 June 2016
Accepted 24 June 2016
Available online 25 June 2016

Keywords:
Biomaterial
Nanotechnology
Hemocompatibility
Magnetic resonance imaging

ABSTRACT

Magnetic nanoparticles (MNPs) should be highly biocompatible, stable and safely eliminated from the body, and can therefore be successfully used in modern medicine. Synthetic hydroxyapatite (HAP) has well established biocompatible and non-inflammatory properties, as well as a highly stable and flexible structure that allows for an easy incorporation of magnetic ions. This study characterized and compared the *in vitro* cytotoxicity and hemocompatibility of hydroxyapatite MNPs doped with different ions (Gd^{3+}/Fe^{2+}/Fe^{3+}/Co^{2+}). HAP doped with 10% of Gd and Fe(III) presented the highest magnetic moments. Our results showed that Gd doped HAP nanoparticles are non-cytotoxic, hemocompatible, non-hemolytic and non-thrombogenic, in contrast with Fe(III) doped HAP that can be considered thrombogenic. For these reasons we propose that, Gd doped HAP nanoparticles have the most potential for application as a MRI contrast agents. However, use of Fe (III) doped HAP as MRI contrast agents should be further investigated.

1. Introduction

Over the last decade, numerous characteristics of nanoparticles (NPs) such as their size (from 1 up to 100 nm), administration route and biodistribution have been explored and channeled into delivery systems, targeting therapies and medical diagnoses [1–3]. Magnetic based nanoparticles, namely magnetic nanoparticles (MNPs), have several applications in modern medicine, from tumor imaging with magnetic resonance imaging (MRI) modality [4], to cancer hyperthermia therapy and even targeted drug/gene delivery systems [5,6].

Magnetic Resonance Imaging (MRI) is a clinical imaging technique that allows for noninvasive tomographic visualization of anatomic structures with high spatial resolution and soft tissue contrast. Contrast agents of MRI, composed of paramagnetic, superparamagnetic and ferromagnetic materials, can change the image contrast between normal and diseased tissue [6]. It is well accepted that nanosized magnetic contrast agents present distinct advantages over conventional contrast agent magnetic resonance imaging (MRI) modality. Chief among these advantages are their high surface area, their ability to be delivered to a specific cancer site by active targeting, and the possibility of engineering their blood circulation half-life [1,2].

Hydroxyapatite ($Ca_{10}(PO_4)_6(OH)_2$(HAP)) is a bioceramic material with a calcium-to-phosphorus ratio similar to that of natural bone and teeth. There is therefore great clinical interest in its use, since it is biocompatible, bioactive and biodegradable. It is currently used for bone graft substitutes, such as porous granules, block scaffolds and coatings over metallic implants for bone regeneration [7]. Particular physicochemical properties of the apatite structure allow it to form many different compositions, therefore allowing

Abbreviations: MNPs, Magnetic nanoparticles; MRI, Magnetic resonance imaging; HAP, Hydroxyapatite; hDMECs, Human Dermal Microvascular Endothelial Cells; APTT, Activated Partial Thromboplastin Time; PT, Prothrombin Time; TCPS, Tissue Culture Polystyrene.

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http://dx.doi.org/10.1016/j.colsurfb.2016.06.042
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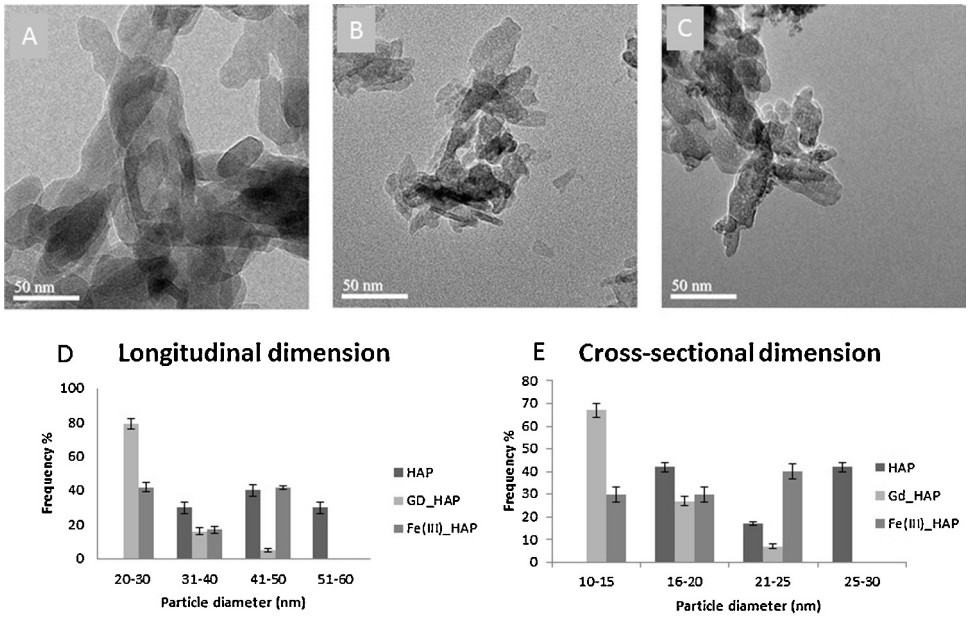

Fig. 1. Bright-field TEM images of (A) non-doped and doped hydroxyapatite (HAP): (B) Gadolinium doped hydroxyapatite (Gd_HAP) and (C) iron (III) doped hydroxyapatite (Fe(III)_HAP). (D) Longitudinal size distribution of HAP,Gd_HAP and Fe(III)_HAP nanoparticles; Cross sectional size distribution of HAP,Gd_HAP and Fe(III)_HAP nanoparticles.

an easy incorporation of ions in the crystal lattice [8]. To date, several methods of preparing HAP nanoparticles have been developed, including sol-gel, biomimetic deposition, electrodeposition, ultrasonic spray freeze-drying, spray dry, combustion synthesis and the wet chemical route [9,10].

The most efficient method of administering magnetic nanoparticles to reach target tissues is intravenously [3,11], yet despite the importance of the intravenous method very few studies have addressed the hemostatic and thrombogenic effects on human blood of nanoparticles containing magnetic metal ions.

Magnetic contrast agents have been widely used in MRI diagnostics. The most clinically used ones are the gadolinium complexes that have seven unpaired electrons and a large magnetic moment [4,5]. Most of the gadolinium based MRI contrast agents available are non-specific for a particular organ. Although, there are organ-specific contrast agents (Gd-based and non Gd-based) designed to specifically accumulate in a given organ or tissue such as liver and lymph nodes or for MR angiography[12]. These contrast agents have an organic ligand with affinity to a specific type of cells. For example hepatobiliary contrast agents bind to a specific receptor site on the hepatocyte cell membrane [13]. On the other hand, superparamagnetic iron oxide (SPIO) particles for parenteral use are coated with various substances (such as albumin, a hydrophilic polymer, starch or dextran) to facilitate uptake by the reticuloendothelial system. SPIO particles are mainly T2-agents while the gadolinium and manganese-based products are mainly T1 agents. T1 and T2 relaxation times affect the signal intensities of tissues being imaged and are therefore crucial for creation of the final images. Signal in MR images can be high or low (bright or dark), depending on the pulse sequence used, and the type of tissue of interest [14,15].

Taking the above issues into account, the main goal of this study was to produce, characterize and compare hydroxyapatite nanoparticles doped with particular magnetic species (Gd, Fe(II), Fe(III) and Co) and evaluate their potential as MRI contrast agents with regard to their hemostatic, cytotoxic and hemolytic properties.

2. Methods

2.1. Hydroxyapatite-based nanoparticles preparation and characterization

The hydroxyapatite-based nanoparticles were prepared by the wet chemical precipitation method [16]. A control of pure hydroxyapatite nanoparticles was prepared using 40 mL of 0.5 M $Ca(OH)_2$ (98% extra pure, Acros Organics), then heated to and maintained at 100 °C, to which 40 mL of 0.3 M H_3PO_4(85 wt% solution in water, Acros Organics) was added at a rate of 500 μL/min. For the magnetic nanoparticles preparation, magnetic dopants such as: gadolinium chloride hexahydrate $Cl_3Gd{\cdot}6H_2O$ (99%, Sigma Aldrich), Cobalt nitrate hexahydrate $Co(NO_3)_2{\cdot}6H_2O$ (98 +%, Acros Organics), Iron nitrate nonahydrate $Fe(NO_3)_3\ 9H_2O$ (98 + %, Acros Organics) and iron chloride tetrahydrate $FeCl_2{\cdot}4H_2O$(99 + %, Acros Organics) were added simultaneously with the phosphate precursor H_3PO_4 and dropwise to the $Ca(OH)_2$ solution at the rate of 500 μL/min. The amount of dopant ions added depended on the required percentage of doping (2.5%, 5% and 10%) with respect to the atomic percentage of Ca^{2+}. All solutions were prepared with deionized water and the (Ca + doping ion)/P molar ratio was maintained at 1.67 in all procedures. Throughout the reaction, pH was maintained at ~7.4 using ammonium hydroxide (25% solution in water, Acros Organics). After the reaction, the mixture was kept in these conditions for 2 h and aged at RT overnight. Prior to the drying step at 60 °C, samples were centrifuged (5 min, ~2070*g*) and washed 3 times with hot deionized water. Finally, all nanoparticles were powdered using mortar and pestle and stored in a desiccator.

2.2. Size and morphology

To determine size and morphology of pure and doped HAP nanoparticles, transmission electron microscopy (TEM) analysis was performed in a JEOL 2010 F in bright-field mode and operated at 200 kV (JOEL, Tokyo, Japan). For the TEM sample preparation the as-received powder was first dispersed in ethanol and sonicated for fifteen minutes to reduce particle agglomeration. After sonication, particles were deposited onto carbon lacey TEM grids for subsequent observation. TEM images were then analyzed using Image