Journal of the European Ceramic Society xxx (xxxx) xxx–xxx

Contents lists available at ScienceDirect

Journal of the European Ceramic Society

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

Original Article

Comparative study of surface properties of Mg-substituted hydroxyapatite bioceramic microspheres

Liga Stipniece[a,*], Valentina Stepanova[a], Inga Narkevica[a], Kristine Salma-Ancane[a], Adrian R. Boyd[b]

[a] Rudolfs Cimdins Riga Biomaterials Innovations and Development Centre of RTU, Institute of General Chemical Engineering, Faculty of Materials Science and Applied Chemistry, Riga Technical University, Pulka 3, Riga, LV-1007, Latvia
[b] Nanotechnology and Integrated Bioengineering Centre (NIBEC), School of Engineering, Ulster University, Shore Road, Newtownabbey, Co. Antrim, BT37 0QB, Northern Ireland, UK

ARTICLE INFO

Keywords:
Hydroxyapatite
Bioceramic
Microspheres
Mg substitution
Protein adsorption

ABSTRACT

Mg-substituted hydroxyapatite (HAp) bioceramic microspheres were prepared by spray drying and subsequent processing at 1173, 1273 and 1373 K. Influence of various Mg substitution levels (up to 0.84 ± 0.10 wt%) on physicochemical properties of the HAp bioceramic microspheres was evaluated. Obtained results were used for the elucidation of the compositional and structural characteristics of the microspheres in conjunction with adsorption of protein, namely, bovine serum albumin (BSA). The primary difference among the microspheres processed at various temperature was the presence or absence of the micropores (< 2 nm in diameter) and mesopores (between 2 and 50 nm). Presence of the micro- and mesopores resulted in higher specific surface area (SSA), enhanced solubility, *i.e.*, ion release, and, accordingly, increase in the amount of BSA adsorbed on the microspheres. Furthermore, the BSA adsorption capacity of the microspheres decreased with increasing Mg content despite of higher SSA.

1. Introduction

The continuously increasing demand to improve life quality concerns an innovative use of specifically designed biomaterials for the repair and reconstruction of diseased or damaged bones. Nowadays the focus has shifted towards bone replacement and repair materials that can mimic living tissues (biomimetic) and assist in the healing process (*i.e.*, be replaced by natural bone): thus, they are bioactive [1]. The most commonly used biomaterials for such applications include calcium phosphates (CaP) [2]. The use of CaP, particularly hydroxyapatite (HAp, $Ca_{10}(PO_4)_6(OH)_2$), for the repair or regeneration of diseased or damaged bone tissues is based generally on the chemical and structural similarity to a mineral component of bone, namely, biological HAp [1,2].

Biological HAp contains various ionic substitutions resulting in a multi-element substituted material [3]. Consequentially, due to their essential functions in many important biological processes, including bone formation, remodelling and metabolism, various biologically active mineral elements have become important bone graft additives for enhanced bone regeneration and repair [4]. Therefore, the most recent studies in the field of bone grafting materials, particularly HAp, have been focused on control of chemical composition in order to optimise bioresorbability and bioactivity, as well as to impart specific properties [5]. As such, a wide range of elements, including, Mg [6,7], Sr [7,8], Zn [8], Ag [9] *etc.* have been strategically incorporated intostructure of synthetic HAp. It has been reported that through several types of substitutions in HAp lattice various properties such as crystal structure (crystallite size and crystallinity), phase and thermal stability and solubility, and adsorption (surface reactivity) can be customized for specific application [3,10].

Among other cationic substitutions for Ca in HAp lattice, in the present study Mg was chosen as it is essential trace element in human bone [11]. Above all, having the optimum amount of Mg in relation to Ca is critical for preventing and reversing osteoporosis [12]. Mg is known to promote cellular adhesion onto the substrate, and is also known to enhance osteoblast-like cell proliferation and differentiation, calcification and angiogenic functions [13]. Thus, incorporation of Mg into the synthetic HAp is important for a number of reasons, including, enhance of bioactivity [14]. In this study relatively low levels, namely close to bone-like amounts (up to 1 wt%) of Mg substitution were chosen due to the potential cytotoxicity of high local concentrations of Mg at site of implantation [15].

---

[*] Corresponding author.
E-mail addresses: liga.stipniece@rtu.lv, ligastipniece@inbox.lv (L. Stipniece).

http://dx.doi.org/10.1016/j.jeurceramsoc.2017.09.026
Received 7 July 2017; Received in revised form 14 September 2017; Accepted 17 September 2017

Please cite this article as: Stipniece, L., Journal of the European Ceramic Society (2017), http://dx.doi.org/10.1016/j.jeurceramsoc.2017.09.026

Porous HAp biomaterials have found to stimulate bone formation and bone bonding, which is related to the specific interactions, *e.g.* ionic exchanges, of their surface with the extracellular fluids [16,17]. Among others, HAp in the form of microspheres has been widely investigated for their potential as local drug- or protein- delivery systems. Furthermore, the microspheres with porous structure provide a high surface area not only for protein adsorption but also cell attachment [18]. The microspheres having capacity to entrap wide range of active ingredients such as drugs and proteins, are used as the topical carrier systems. The microspheres loaded with active ingredients can be incorporated into formulations such as gels, bone cements *etc*. One of the most widely applied techniques to prepare microspheres is spray drying due to its simplicity, the particle size can be controlled in a single step and can be scaled up to ton quantities [19]. The wide use of HAp microspheres is typical 10, 20, 40, and 80 μm in size [20]. However, there are many advantages of reducing size of the microspheres. Mainly, reduced size of the individual particles leads to higher surface area of the compound and, thus, to higher bonding capacity of biomolecules, including proteins [21].

Various proteins have been therapeutically used for the treatment of metabolic disorders and to aid the healing and regrowth of tissues/organs. Thus, a substantial amount of research is focused on effectively delivering these proteins locally to the defective/diseased tissues or organs where they may accelerate or aid the healing process [22]. Moreover, it has been reported that when the bioceramic bone grafts are implanted into a living body, proteins from the surrounding body fluids are spontaneously adsorbed onto their surfaces, and then cellular attachment, proliferation and migration occurs. Summarizing the above-mentioned and considering the widely reported ion-protein interactions, it can be anticipated that HAp have the appropriate properties for protein delivery applications in bone therapeutics [23].

HAp contains two types of binding sites for proteins–positively charged Ca^{2+} and negatively charged PO_4^{3-}. These sites are distributed regularly throughout the crystal structure of the matrix [20]. Considering the abovementioned effect of substituting elements on the HAp properties, substituting Ca, $[PO_2]$ and/or [OH] in the lattice, should result in significant effects for interaction with proteins. Nevertheless, the effect of Mg as well as the ensuing surface properties of the HAp bioceramic microspheres on the loading profile of the proteins has not been emphasized to the best of available literature. Thus, the objective of this study is to elucidate the connection between the related factors, such as specific surface area, pore size distribution, and solubility (*i.e.*, ion release) with the adsorption capacity of proteins (*i.e.*, bovine serum albumin (BSA) as a model protein) on Mg-substituted HAp bioceramic microspheres.

2. Materials and methods

2.1. Sample preparation

Microspheres were prepared by a spray drying method using a table-top spray dryer (Mini Spray Dryer B-290, Büchi, Switzerland). The 0.03 M slurries for spray drying were obtained by precipitating Mg-susbtituted HAp nanoparticles with a (Ca + Mg)/P molar ratio of 1.67, as described in detail elsewhere [6]. The conventional aqueous precipitation method based on an acid-base reaction involving CaO (puriss.; Fluka, Switzerland) and H_3PO_4 (puriss.; 75%, Sigma-Aldrich, Germany) was modified for Mg precursor, namely MgO (reagent-grade; ES/Scharlau, Spain), addition into the synthesis media. Various concentrations of Mg in the products were provided by changing amount of MgO added into synthesis media. The MgO content in the synthesis media varies from 0 wt% to 3 wt% in respect to a Ca source, namely CaO. After aging for ~20 h, the precipitated slurry underwent spray drying by exploiting conditions, which are summarized in Table 1.

The obtained (as-dried) microspheres were sintered at 1173 K for 1 h, 1273 K for 1 h or 1373 K for 1 h in a muffle furnace using a heating/cooling rate of 5 K/min.

Table 1
Spray drying conditions.

Diameter of nozzle, mm	Inlet temperature, K	Outlet temperature, K	Feed flow rate, L/h	Atomization gas (air) flow, L/h
1.5	493	353 ± 5	0.3	246–357

2.2. Physicochemical characterization

Chemical constitutions of the as-dried and sintered microspheres were determined by energy dispersive X-ray spectrometry (EDX; Mira/LMU/Inca Energy 350, Tescan, Czech Republic).

Molecular composition of the sintered microspheres was determined by Fourier transformation infrared spectroscopy (FT-IR; Varian 800, Scimitar Series, USA) using GladiATR™ (Pike Technologies, USA) single reflection attenuated total reflectance (ATR) accessory utilizing a monolithic diamond crystal. Spectra were recorded in the range of 400–4000 cm^{-1} with spectral resolution of 4 cm^{-1} and 50 spectra were co-added. Prior every measurement a background spectrum was taken and deducted from the sample spectrum.

Phase composition of the sintered microspheres was analyzed by X-ray diffractometer system (XRD; X'Pert PRO, PANalytical, Netherlands) operating at 40 KV and 30 mA using a Cu $K\alpha_1$ radiation (λ = 1.5406 Å). Scans were recorded over the range of 2θ from 10° to 70° with an angular step interval of 0.0334°. For the phase identification the International Centre for Diffraction Data (ICDD) was used (card #01-072-1243 for HAp, card #00-009-0169 for β-tricalcium phosphate (β-TCP)).

Sintering behaviour of the as-dried microspheres was investigated by high-temperature optical dilatometer (EM201/HT163, Hesse Instruments, Germany) using following methodology: the powders weighing ~20 mg were compressed into a cylindrically shaped pellets and heated to 773 K at heating rate 80 K/min, and further to 1623 K at heating rate 15 K/min.

Microstructural features of the sintered microspheres were studied by field emission scanning electron microscopy (FE-SEM; Mira/LMU, Tescan, Czech Republic) at acceleration voltage of 30 kV and distance of 10 mm. Samples were sputter coated with thin (15 nm) gold layer. Average diameter of the microspheres was determined using VEGA TC software, measuring at least 100 objects in FE-SEM micrographs for each batch.

The *Brunauer–Emmett–Teller* (BET) method was used to determine specific surface area (SSA) of the as-dried and sintered microspheres by measuring the amount of physically adsorbed N_2 gas at 76 K (Quadrasorb-SI Kr, Quantachrome, USA). The samples were degassed at 373 K for 2 h prior the analyses. The pore size distribution in a range of 0–30 nm were derived from the adsorption branches of the isotherms using density functional theory (DFT).

2.3. Ion release

Solubility, namely cumulative release of Ca^{2+} was determined by placing the Mg-substituted HAp microspheres (200 mg) in tris(hydroxymethyl)aminomethane (TRIS; ACS reagent, ≥99.8%, Sigma Aldrich, USA) and hydrochloric acid (HCl; 37%, Sigma Aldrich, USA) buffer pH 7.4 ± 0.2 (50 mL) at 310 K for 168 h in a table-top environmental shaker-incubator (ES-20, Biosan, Latvia) providing mild, orbital shaking at 70 RPM. TRIS–HCl solution was prepared according to the EN ISO 10993-14:2001. Every 24 h the microspheres were sediment by centrifugation at 3000 RPM for 1 min, "reacted" TRIS–HCl was removed and the microspheres were re-suspended in fresh TRIS–HCl up to