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Influence of pentavalent dopant addition to polarization and bioactivity of hydroxyapatite

Jharana Dhal [a], Susmita Bose [b], Amit Bandyopadhyay [b,*]

[a] Department of Physics and Astronomy, Washington State University, Pullman, WA 99164, United States
[b] W. M. Keck Biomedical materials Research Lab, School of Mechanical and Materials Engineering, Washington State University, Pullman, WA 99164, United States

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ABSTRACT

Influence of pentavalent tantalum doping in bulk hydroxyapatite (HAp) ceramics has been investigated for polarizability and bioactivity. Phase analysis from X-ray diffraction measurement indicates that increasing dopant concentration decreased the amount of HAp phase and increased β-TCP and/or α-TCP phases during sintering at 1250 °C in a muffle furnace. Results from thermally stimulated depolarization current (TSDC) measurements showed that doping hindered charge storage ability in HAp ceramics, and doped samples stored fewer charge compared to pure HAp. However, doping enhanced wettability of HAp samples, which was improved further due to polarization. In vitro human osteoblast cell–material interaction study revealed an increase in bioactivity due to dopant addition and polarization compared to pure HAp. This increase in bioactivity was attributed to the increase in wettability due to surface charge and dopant addition.

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1. Introduction

Calcium phosphate ceramics are widely used as bone graft substitutes because of their excellent bioactivity. Hydroxyapatite (HAp; $Ca_{10}(PO_4)_6(OH)_2$), is a calcium phosphate based ceramic widely used in applications such as bone graft materials because of its structural and compositional similarities to bone [1,2]. HAp physiochemically bonds with natural bone and helps in stabilization of implant [3,4]. However, osteogenic capacity of synthetic HAp compared to natural bone is not ideal, which causes slower tissue in-growth in vivo [5]. Several methods have been proposed to manipulate surface properties of HAp to improve osseointegration that is critical, particularly for older patients with bone disorders like osteoporosis. Some of those methods include inducing electrical charge on the surface through polarization of HAp [6–10], doping with metal cations such as Mg^{2+}, Sr^{2+}, Zn^{2+}, Bi^{3+}, La^{3+}[8,11–13], and mixing of HAp with other calcium phosphate ceramics [14].

Basset et al. suggested that bone cell could be stimulated electrically [15,16]. Other research results suggest that negative surface functional groups could provide preferential sites for nucleation of an amorphous calcium phosphate layer [17,18], which can lead to the formation of the apatite layer, an important biological phenomenon for bone bonding [19]. Polarization has been used as an important tool to manipulate the surface charge of bioceramics [20–22]. Surface charges, depending upon the polarity, were reported to accelerate or decelerate in vitro growth of bone-like apatite crystals on polarized HAp in simulated body fluid (SBF) [6,21], cell adhesion [22], and osteobonding [23]. Polarity could also change the wettability of the HAp surface [6]. Polarity and wettability together can influence the adsorption of bone adhesive proteins and consequently influence adhesion and proliferation of bone cells on the HAp surface [6].

Numerous recent studies reported that doping with divalent ions (Mg^{2+}, Zn^{2+}, Sr^{2+} etc.) or trivalent metal ions (Bi^{3+}, La^{3+}, Y^{3+} etc.) changes the surface properties of HAp [11,24–27]. These studies showed that changes in surface properties of HAp improved their structural stability and enhanced their biocompatibility. In our previous research HAp was doped with Mg, Zn, and Sr in single, binary, and ternary combinations to match that of the bone chemistry [8]. A combined doping of 1% Mg and 1% Sr by weight increased the charge storing ability of doped HAp. The binary doped samples also showed higher bioactivity than pure HAp [28]. Webster et al. [27] compared the effects of doping by several trivalent (Bi^{3+}, Y^{3+}, La^{3+}, and In^{3+}) and divalent cations (Mg^{2+}, Zn^{2+}) and found that bone cell adhered and differentiated earlier on HAp doped with trivalent cations compared to divalent cations. Results of these reported studies indicate that an increase in valence of doped cations can improve the surface property and biocompatibility of the doped HAp.

Based on those results, we hypothesize that polarization in conjunction with doping, using higher valent cations can further improve bioactivity of the doped HAp ceramics. There exists a knowledge gap on simultaneous effects of higher valent cations and surface charge on bioactivity of HAp ceramics. To validate our hypothesis and fill

* Corresponding author. Tel.: +1 509 335 4862.
E-mail address: amitband@wsu.edu (A. Bandyopadhyay).

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the knowledge gap, we have investigated the combined effect of polarization and doping with pentavalent tantalum towards the bioactivity of bulk HAp ceramics. We have chosen tantalum because it is a bioactive metal and has been widely used as a trabecular metal in load-bearing implants [29,30]. Recent study also showed that in the thin film form, Ta^{5+} addition via magneto-sputtering to HAp can be accommodated via Ca substitution up to 4.5 at.% without distorting the hexagonal lattice structure of HAp [31]. In our work, the doped HAp ceramics were compared with the pure form towards change in activation energy, stored charge, and phase transition characteristics. Bioactivity of the doped HAp samples were evaluated using human osteoblast cells. *In vitro* study was used to investigate the influences of doping and polarization on bone cell–material interactions.

2. Experimental procedures

2.1. Sample preparation and characterization

Three different compositions of HAp were used for this study—pure, 0.25 wt.% and 0.5 wt.% of Ta_2O_5 (99.95%, Alfa Aesar) with high purity HAp powders (99.9% purity, Berkeley Advanced Biomaterials, CA). To minimize the formation of agglomerates and to increase the homogeneity of the powder, a mixture of HAp and Ta_2O_5 powders was ball-milled in an ethanol medium at 70 rpm for 24 h. Samples were dried in an oven at 80 °C for 72 h. Disc samples of 12 mm diameter and 1.3 mm thickness were processed by pressing dried powders uniaxially. Disk samples were pressed cold isostatically at 344.74 MPa for 5 min and then sintered at 1250 °C for 2 h in air. Final samples were disk shaped with 10.4 mm in diameter and 1.2 mm in thickness. Archimedes' method was used to measure the apparent density of each composition. The relative densities were calculated by normalizing with the theoretical density of HAp (3.16 g cm^{-3}). Phase analysis was performed with an X-ray diffractometer (Siemens D500 Kristalloflex, Siemens Corporation, NY) using a copper Kα radiation with a Ni filter at 30 kV and 35 mA in 2θ ranges between 20 and 60° with a step size of 0.1 degree increment. Phase percentages of different phases in the sintered and doped HAp were determined from relative intensity ratio of the corresponding major phases, using Eqs. (1a) and (1b) [32,33].

$$\textit{Percent of phase to be determined} = \textit{Relative Intensity ratio of the phase} \times 100 \quad (1a)$$

$$\textit{Relative Intensity ratio} = \frac{\textit{Intensity of the major peak of the phase}}{\sum \textit{Intensity of major peaks of all phases}} \quad (1b)$$

2.2. Electro-thermal polarization and TSDC measurement

Thermally stimulated depolarization current (TSDC) technique was used to estimate stored charge (Q_p) and activation energy of depolarization (E_{dr}) [34,35]. Both sides of the disc samples were coated with silver paint and kept in an oven for 1 h at 200 °C. Silver coated samples were sandwiched between a pair of platinum plates connected to a picoammeter (Model 6487, Keithley Instruments Inc., OH) by silver wires. To polarize, samples were heated under a controlled heating rate of 5 °C min^{-1} from room temperature to polarization temperature (T_p) of 400 °C and kept at T_p for 1 h. Samples were kept under a d.c. electric field (E_p) of 2 kV $cm^{-1}$for 1 h at T_p and were cooled down from T_p to room temperature, while keeping the polarizing field (E_p) still on during the cooling down process. The polarized samples were heated at a rate of 5 °C min^{-1} from room temperature to 550 °C, and TSDC was measured using the same picoammeter.

TSDC curve was used to estimate polarized charge Q_p (μC cm^{-1}) and activation energy for dipole relaxation E_{dr} (eV). Arrhenius plots were obtained from TSDC spectrum. Charge stored Q_p and activation energy of dipole relaxation were estimated using Eqs. (2) and 3, respectively [36].

$$Q_p = \frac{1}{\beta}\int J(T)dT \quad (2)$$

$$\ln(\tau) = \frac{E_{dr}}{kT} + \ln(\tau_0) \quad (3)$$

where $J(T)$ is the depolarization current density at temperature T, β is the heating rate during TSDC measurement and "k" is the Boltzmann constant. The dipole relaxation time (τ (s)) at temperature T is expressed by Eq. (4) [10,36].

$$\tau = \frac{1}{\beta(T)J(T)}\int J(T)dT = \frac{Q_p(T)}{J(T)} \quad (4)$$

2.3. Contact angle measurement

Contact angles of deionized water on β-TCP, HAp and 0.25 wt.% doped sample surfaces (polarized and unpolarized samples) were measured using sessile drop method. We have included β-TCP results, as XRD results indicate that all doped samples have β-TCP as a major phase. Each sample was polished on a sand paper followed by cloth polishing using 1 μm alumina suspension. Polished samples were sonicated for 5 min in deionized water and kept in an oven at 100 °C for 1 h. One μl deionized water was dropped on the sample surface and contact angle was measured using a set-up equipped with a CCD camera (VCA Optima, AST Products, MA). To take measurements on polarized samples, polished samples were polarized between platinum electrodes at 400 °C for 1 h in air. Contact angles were measured in triplicate with three positions per sample.

2.4. In vitro bone cell–material interaction

In vitro studies were conducted on pure and doped HAp, both polarized and unpolarized, following detailed methods outlined elsewhere [6]. Pure and unpolarized HAp was selected as control. In this study, the influence of doping with Ta_2O_5 and combined effect of doping and polarization on bioactivity of osteoblast cells were studied. A 0.25 wt.% Ta_2O_5 doped sample was selected for in vitro study due to the presence of high amount of HAp phase (19%) compared to 0.5 wt.% sample (9%) as indicated in XRD result. And also these dopants are new in the field of bone implant material research and the toxicity of using a higher amount of doping is unknown. We started from low dopant concentration to find any change due to doping. Polished samples for *in vitro* study were polarized between a pair of platinum electrodes on both sides. Among polarized Ta-HAp samples, the negatively polarized surfaces were denoted as the N-surface and the positively polarized surfaces were denoted as P-surfaces. The surfaces of the doped samples without polarization were designated as O-surface. These notations are followed throughout unless specified otherwise.

In vitro bone cell–material interaction was examined using human fetal osteoblast cells (hFOB) derived from bone tissue [37]. The hFOB cells were cultured in a culture plate for 10 days. Samples were sterilized by autoclaving at 121 °C for 2 h and seeded on with cells in a 24-well plate. Pure HAp samples were used as control. One ml of DMEM media enriched with 10% fetal bovine serum was poured into each well and incubated at 37 °C in air with 5% CO_2. We changed cell culture media every alternate day. Incubated samples were used for cell proliferation and cell morphology studies.

MTT assay (Sigma Inc., St Louis, MO) was conducted on samples incubated for 3, 7 and 11 days to measure bone cell proliferation.

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