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Influence of Pentavalent Dopant Addition to Polarization and Bioactivity of Hydroxyapatite

Jharana Dhal^a, Susmita Bose^b, and Amit Bandyopadhyay^{b,*}

^aDepartment of Physics and Astronomy, Washington State University, Pullman, WA 99164

^bW. M. Keck Biomedical materials Research Lab, School of Mechanical and Materials Engineering, Washington State University, Pullman, WA 99164

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-materials interactions 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.

Keywords

Hydroxyapatite; Polarization; β -TCP; tantalum; contact angle; *in-vitro* study; osteoblast

1. Introduction

Calcium phosphate ceramics are widely used as bone graft substitute because of their excellent bioactivity. Hydroxyapatite (HAp; $\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2$), 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 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].

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*Corresponding author: Prof. Amit Bandyopadhyay. amitband@wsu.edu. Phone (509) 335-4862.

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Basset et al. suggested bone cell could be stimulated electrically [15, 16]. Other research results suggest 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 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 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.) change the surface property of HAp [11, 24–27]. These studies showed that changes in surface properties of HAp improved their structural stability and enhanced biocompatibility. 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 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 doped HAp.

Based on those results, we hypothesize that polarization in conjunction with doping, using higher valent cations can further improve bioactivity of 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 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 trabecular metal in load-bearing implants [29, 30]. Recent study also showed that in the thin film form, Ta^{5+} addition via magnetosputtering to HAp can be accommodated via Ca substitution up to 4.5 atm% without distorting the hexagonal lattice structure of HAp [31]. In our work, doped HAp ceramics was compared with the pure form towards change in activation energy, store charge, and phase transition characteristics. Bioactivity of doped HAp samples was evaluated using human osteoblast cells. *In vitro* study was used to investigate the influences of doping and polarization on bone cell–materials interactions.

2. Experimental Procedures

2.1. Sample preparation and characterization

Three different compositions of HAp were used for this study – pure, 0.25 weight % and 0.5 weight % 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 were ball-milled in an ethanol media at 70 rpm for 24h. Samples were dried in an oven at 80°C for 72h. 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 minutes and then sintered at 1250 °C for 2h 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 density was calculated by normalizing

with the theoretical density of HAp (3.16 gcm^{-3}). Phase analysis was performed with an X-ray diffractometer (Siemens D500 Krytalloflex, Siemens Corporation, NY) using a copper $K\alpha$ radiation with a Ni filter at 30 kV and 35 mA in 2θ ranges between 20 and 60 degrees with a step size of 0.1 degree increment. Phase percentages of different phases in sintered and doped HAp were determined from relative intensity ratio of the corresponding major phases, using Equation 1a and Equation 1b [32, 33].

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

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

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 one hour 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°Cmin^{-1} from room temperature to polarization temperature (T_p) of 400°C and kept at T_p for 1h. Samples were kept under a d.c. electric field (E_p) of 2 kV cm^{-1} for 1h 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 (μCcm^{-1}) and activation energy for dipole relaxation E_{dr} (eV). Arrhenius plots were obtained from TSDC spectrum. Charge storage Q_p and activation energy of dipole relaxation were estimated using Equation 2 and Equation 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 (sec)) at temperature T is expressed by Equation 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 μ m alumina suspension. Polished samples were sonicated for 5 minutes in deionized water and kept in oven at 100 °C for 1h. One μ L 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 1h in air. Contact angles were measured in triplicate with three positions per sample.

2.4. *In vitro* bone cell-materials 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, influence of doping with Ta₂O₅ and combined effect of doping and polarization on bioactivity of osteoblast cells were studied. A 0.25 wt.% Ta₂O₅ doped sample was selected for *in vitro* study due to the presence of high amount of HAp phase (19%) compared to 0.5wt.% sample (9%) as indicated in XRD result. And also these dopants are new in the field of bone implant materials research and the toxicity of using higher amount 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 doped samples without polarization were designated as O-surface. These notations are followed throughout unless specified otherwise.

In vitro bone cell-materials interaction was examined using a 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 2h and seeded on with cells in 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% CO₂. 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 day to measure bone cell proliferation. The MTT reagent 1, 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide, is readily taken up by viable cells and is reduced by the mitochondrial dehydrogenase to blue/purple color formazan product. The intensity of the blue / purple color is an indicator of number of viable cells. Incubated cells were transferred to a new 24-well cell culture plate to prevent contribution from cells grown on the plastic surface of original 24-well plate for cell proliferation study. A 5 mg/ml of MTT solution prepared in phosphate-buffered saline (PBS) was filtered and sterilized. 50 μ L MTT was added to 450 μ L of DMEM culture media enriched with 10% fetal bovine serum. The 500 μ L of diluted MTT solution was added to each specimen in 24-well plates and incubated for 2h at 37 °C to form formazan. 500 μ L of solution made up of 10% Triton X-100, 0.1N HCl and isopropanol were added to dissolve formazan crystals. 100 μ L of dissolved formazan was transferred to 96-well plate in triplicate. The optical density of the solution in each well was measured at a wavelength of 570 nm.

Cell morphology was examined using SEM on samples incubated for 3, 7, and 11 days. Cultured samples were rinsed with 0.1M PBS and fixed overnight at 4°C with 2% paraformaldehyde and 2% glutaraldehyde in 0.1M cacodylate buffer. Fixed samples were treated with 2% Osmiumtetroxide (OsO₄) for 2h at room temperature, dehydrated three times in an ethanol series (30%, 50%, 70%, 95% and 100%) and dried using hexamethyldisilane (HMDS) drying procedure. Dried samples were mounted on aluminum stubs and coated with gold to observe under FESEM (FEI Inc., Hillsboro, OR).

2.5. Statistical Analysis

Each analysis was done in triplicates. Statistical analysis was done on results from MTT assay and contact angle measurements using Student's t-test in which a p-value less than 0.05 was considered significant.

3. Results

3.1. Phase analysis

Figure 1 shows the XRD spectra of doped HAp, pure HAp and Ta₂O₅. The XRD results indicate the presence of β -TCP and small fraction of HAp in all doped samples. However α -TCP was also found in 0.5% doped specimens. The characteristics peaks of HAp, β -TCP and α -TCP in all samples match well with JCPDS file nos. 09-0432 (HAp), 09-0169 (β -TCP) and 09-0348 (α -TCP). From phase analysis using the peak height of 100% peaks, samples doped with 0.25 wt.% Ta₂O₅ showed 19% HAp and 81% β -TCP, whereas samples doped with 0.5% Ta₂O₅ showed 9% HAp, 58% β -TCP and 33% α -TCP. No peaks associated with tantalum, Ta₂O₅, and/or tantalum compounds were found. Relative densities of all samples are reported in Table 1.

3.2. Thermal depolarization

The TSDC of all samples increased as a function of temperature from 200 °C, and reached a maximum current density before started to decrease (Figure 2). Figure 3 shows the curves for activation energy obtained from Arrhenius equation using TSDC data. The maximum current density varied among all samples. However, the highest maximum current density was found for pure HAp. Maximum current density, $J_{\max}$, of 0.5% doped sample was higher than 0.25 wt.% doped sample. The peaks corresponding to doped HAp samples are broader than pure HAp. The stored charge (Q_p) and activation energy of depolarization of all samples are presented in Table 1. The stored charges (Q_p) of all samples followed the similar trend as that of $J_{\max}$.

The stored charge (Q_p) of Ta₂O₅ doped samples is much less than the stored charge of similar concentration of WO₃ doped HAp samples we found in our previous research [34]. We also found that the WO₃ doped samples stored less charge compared to pure HAp.

3.3. Contact angle and wettability measurement

Contact angles of pure HAp were higher than polarized or unpolarized samples doped with 0.25 wt.% Ta₂O₅ (Figure 4). The major phase of doped samples β -TCP had higher contact angle than HAp. The average contact angle of pure HAp surface was 70 degrees, β -TCP surface was 76 degrees, O-Surface was 64 degrees, P-Surface was 52 degrees, and N-Surface was 51 degrees. These results indicate that doped samples are more hydrophilic than pure HAp and β -TCP samples. Similarly, polarized surfaces are more hydrophilic than unpolarized surfaces.

3.4. *In vitro* cell-material interactions

The MTT colorimetric assay indicates that O-, N-, and P-surface samples show higher cell proliferation ($p < 0.05$) than pure HAp for 3, 7, and 11 days samples (Figure 5). Cell proliferation was significantly ($p < 0.05$) higher on N- and P-surfaces compared to O-surface. This result indicates that polarization of samples increase the proliferation of bone cells. Cell proliferation increased for all samples with increasing incubation duration. After 3 day incubation, proliferation on P-surface is not significantly different ($p > 0.05$) from N-surface. This trend was similar even after 7 days of incubation, though cell population increased in all samples. However, after 11 days of incubation, N-Surface had higher

($p < 0.05$) cell population than P-Surface. We have also seen in WO_3 doped HAp in our previous research that the doped samples showed better cell proliferation than pure HAp samples for all day points of study [34]. Proliferation of cells further increased on polarized surface [34].

FESEM images of hFOB cells after different incubation periods attached onto pure HAp, O-surface, N-surface and P-surface samples are presented in Figures 6–8. After 3 days of incubations, pure HAp and O-surface were not fully covered with the cell, though cells on O-surface appears to be flat and more connected than cells on pure HAp (Figure 6). The P- and N-surfaces have higher surface coverage by cells than HAp and O-surface. These cells are well connected to each other with spreading of filopodia. Crystal and apatite like structures visible along with bone cells on polarized surface indicates the start of extra cellular matrix mineralization by day 3 (Figure 6c and 6d).

After 7 days of incubation, N- and P-surfaces show whisker and apatite formation (Figure 7c and 7d) beneath the cell layer. Cells used these whiskers as anchoring site to spread over the N- and P-surfaces. These cells were interconnected and spread over each other. After 7 days of incubation, surface coverage was increased on O-surface. O-surface had more connected cells that were extending more no of filopodias compared to pure HAp surface (Figure 7b). O-surface showed multiple layers of cells compared to a single layer of cell on the pure HAp sample surface (Figure 7a and 7b).

After 11 days of incubation, entire P- and N-surfaces were covered with confluent layers of cells (Figure 8c and 8d). HAp and O-surfaces were not completely covered with cells (Figure 8a and 8b). Cells on O-surface extended large amount of filopodia and interconnected with other cells. In contrast, no crystallization on pure HAp was observed even after 11 days of incubation.

4. Discussion

4.1. Structural consideration of depolarization in Ta_2O_5 doped samples

XRD results show that after sintering doped samples contain high fraction of TCP phase. This result indicates that doping HAp with tantalum and sintering at 1250°C makes HAp phase more unstable. Increasing tantalum dopant concentration from 0.25 wt.% to 0.5 wt.% also forms higher amount of α -TCP phase. This is confirmed by the appearance of strong α -TCP peaks in 0.5% Ta_2O_5 doped sample. Considering the smaller size of Ta^{5+} ($0.68 \text{ \AA}$) compared to the size of Ca^{2+} ($0.99 \text{ \AA}$), it can be hypothesize that Ta^{5+} substitutes either Ca^{2+} and/or takes the interstitial position in HAp, β -TCP and α -TCP phases. Charge imbalance created by doping with Ta^{5+} can be compensated by Ca^{2+} vacancies, which in turn favors the formation of TCP than HAp due to its lower Ca:P ratio. Moreover, α -TCP is the stable phase between 1125°C and 1430°C . Based on our results, tantalum doping not only favors TCP phase formation, it also helps to stabilize TCP phase at room temperature. Absence of any representing peaks for tantalum or tantalum compounds suggests that one of the mechanism of Ta_2O_5 incorporation inside doped sample could be substitution of Ta^{5+} in place of Ca^{2+} inside the lattice structure of HAp and TCP phases in the doped samples. Ta^{5+} substitution of Ca^{2+} in HAp was proved by Ligot et al. through XPS measurement of tantalum doped HAp thin film [31]. Another possible way of incorporation of Ta^{5+} in HAp structure is by substituting for P^{5+} cation as TaO_4^{3-} anion. The end product of this substitution will be TCP and $\text{Ca}_3(\text{TaO}_4)_2$.

TSDC results show that doped HAp stored less charge than pure HAp (Table 1). However no direct correlation can be found between the decrease in stored charge and increase in the fraction of Ta_2O_5 in HAp. We determined the activation energies of depolarization from

Arrhenius plot to understand polarization mechanism and shown in Table 1. In this study, the activation energy (E_{dr}) for depolarization of pure HAp was 0.74 eV and that of doped samples were in the range of 0.63–0.66 eV. A previous study reported activation energy of 0.66 ± 0.1 eV for H^+ migration along the OH^- chain in pure HAp [38]. This result shows that polarization is dominated by H^+ ion migration in HAp phase of doped samples.

If H^+ ion migration in HAp phase is the only reason for polarization in all samples, then the amount of charge stored should be proportional to amount of HAp phase present in doped samples. The fraction of HAp phase decreased with an increase in dopant concentration. However stored charge (Q_p) didn't decrease consistently with the increase in dopant concentration. Increasing the dopant concentration from 0.25% to 0.5 wt.% increased the stored charge. This result indicates H^+ migration is not the only mechanism during polarization. In addition to polarization in HAp phase, other phases are also contributing to the polarization and charge storage.

The β -TCP phase can also contribute to polarization as β -TCP present in larger fraction in doped samples. In a previous study, polarization of β -TCP was attributed to migration of either Ca^{2+} or O^{2-} ions [39]. To verify the contribution of β -TCP on polarization, we estimated the activation energy of pure β -TCP using TSDC curve obtained on β -TCP sample polarized in similar condition as described in this article (Polarization temperature = $400^\circ C$, polarizing field = $2kV/cm$, time = 1h, heating rate for TSDC (β) = $5^\circ C/min$) [34] and compared that with the overall activation energy of doped samples. We found that the activation energy of β -TCP as 1.1 ± 0.15 eV, which was nearly twice as that of the activation energies of doped samples [34]. These results suggest that polarization mechanism in pure HAp and β -TCP phases could not completely explain polarization in doped samples. The activation energies of doped samples were not similar to the activation of energies of the major phases in those samples. For example, activation energy of 0.5wt.% doped sample was similar to that of pure HAp even though doped sample consisted of 9% HAp, 58% β -TCP and 33% α -TCP. The activation energy in β -TCP phase and HAp phase could not clarify the higher charge storage and low activation energy of 0.5% doped sample compared to pure β -TCP phase. We hypothesize that doping can influence the activation energy and change the mechanism of polarization. The substitution of Ta^{5+} in place of Ca^{2+} in both substitutional and interstitial positions in HAp, β -TCP, and α -TCP phase can influence the ionic movement further in the doped samples, and lower the activation energy required for polarization. Moreover, it is common to form glassy phase at the grain boundary in ceramics due to dopant addition, which is difficult to identify at such low dopant concentration. At this point, the exact mechanism of ionic movement and polarization in doped samples are not fully understood and need further investigation.

4.2. Wettability and bioactivity of doped HAp

Contact angle measurements indicate that O-surface samples are more hydrophilic than pure HAp and hence have better wettability and shown in Figure 4. Contact angle on solid surface depends upon surface roughness, grain size and chemistry of the surface [14]. Considering the fact that all these samples were subjected to a similar polishing, we can assume that there was no significant difference in surface roughness among the samples. Our higher contact angle value of β -TCP is supported by reference 40 [40]. Higher contact angle value β -TCP phase is not the reason for the decrease in contact angle of doped samples. It is further supported by the report that in case of HAp and β -TCP biphasic ceramics, the contact angle increases with increasing β -TCP concentration [14]. The improved wettability on the O-surface is attributed to the surface chemistry or change in microstructure due to doping. During doping, Ta^{5+} could substitute Ca^{2+} in HAp and β -TCP structure resulting a more electropositive surface for doped samples than pure HAp. Though Ta^{5+} substitution for Ca^{2+} has not been confirmed in our samples, it has been shown by

Ligot et al. [31] who used XPS to characterize tantalum doped HAp thin film. Higher surface charge means higher surface energy. And higher surface energy leads to better wettability [41]. Wettability change due to higher surface charge can also be explained in terms of interaction of polar water with surface charges. This wettability change with higher surface charge is further confirmed by lower contact angle value on P- and N-surfaces as shown in Figure 4 and compared to O-surface and HAp. The contact angle for N-surface is lower than the P-surface, which is due to favorable orientation of water molecules on N- than P-surface. If Ta^{5+} substitutes P^{5+} in the form of TaO_4^{3-} ion, the decrease in contact angle of unpolarized doped samples could be due to the change in microstructure as well. The change in microstructure can be a result of different size of TaO_4^{3-} ion compared to PO_4^{3-} ion.

Results of cell morphology study indicate that O-surface has better cell coverage and proliferation than pure HAp. Cell coverage and proliferation further enhanced on N- and P-surfaces. Hence current results show that cell adhesion was enhanced on more hydrophilic surface. Previously it had also been reported that hydrophilic surface supports better cell adhesion and spreading [42]. Enhanced osteoblast adhesion and proliferation on N-surface were also observed in other studies [6, 22]. Possible explanation for improved cell adhesion on doped unpolarized and polarized surfaces compared to pure HAp is due to change in surface chemistry and surface energy. Change in surface chemistry and surface energy can influence interaction of certain proteins such as vitronectin or collagen, and enhance osteoblast cell adhesion [43].

These results of cell morphology and cell adhesion were supported by the MTT results shown in Figure 5. Adhesion is required for subsequent function of anchor dependent cells such as osteoblasts [11]. Better adhesion can explain enhancement of osteoblast proliferation. MTT results after 3 and 7 days of incubation show that proliferation on P- and N-surfaces are almost similar ($p > 0.05$). After 11 days of incubation, proliferation on N-surface is significantly ($p < 0.05$) higher than that of P-surface. After 3, 7, and 11 days of incubation, cell proliferation followed the order: N-surface > P-surface > O-surface > pure HAp. The difference in bioactivity of N-surface and P-surface is due to their charge polarity. This difference in charge polarity can lead to preferential adsorption of adhesive proteins. Hence, the difference in protein absorption leads to difference in bioactivity of N-surface and P-surface. We hypothesized that polarization in conjunction with doping using higher valent cations can further improve bioactivity of doped HAp ceramics. Our results show that while doping has a strong influence in phase stability of HAp ceramics, however bone cell materials interactions are influenced primarily by the surface chemistry and wettability. Further research is needed to understand the *in vivo* response of these materials with and without surface charge.

5. Conclusions

Doping with tantalum influenced phase stability, surface chemistry and wettability of HAp ceramics. Addition of Ta stabilized the TCP phases during sintering at 1250 °C in which higher tantalum concentration increased the amount of α -TCP phase. It is found that tantalum doping decreased the contact angle and increased the wettability of HAp. Increase in wettability of doped samples was attributed to the change in surface chemistry due to doping. Polarization also enhanced wettability. Doping with pentavalent tantalum increased the bioactivity of HAp.

Polarization further enhanced bone cell adhesion and proliferation. This increase in bioactivity was attributed to the increase in wettability of doped and polarized samples. The stored charge of HAp was higher than doped HAp samples. However, there is no direct

correlation between the drop in stored charge with the increase in weight percentage of tantalum doping.

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Highlights

- Tantalum doping make HAp unstable during sintering at 1250 °C and forms TCP
- Tantalum doping reduces charge storage ability of HAp ceramics
- Even with lower charge storage ability tantalum doping improves wettability
- Tantalum doping enhances bioactivity of calcium phosphate based sintered compacts

Figure 1. X-ray diffraction results of HAp doped with 0.25wt.% and 0.5wt.% of Ta_2O_5 . Results of pure HAp and Ta_2O_5 are also included for comparison.

Figure 2.

TSDC curves of HAp doped with 0.25 wt.% and 0.5wt.% of Ta_2O_5 . Samples were polarized by 2kV/cm at 400 $^{\circ}\text{C}$ for 1h.

Figure 3.

Activation energy curves for pure HAp and HAp doped with 0.25wt.% and 0.5wt.% Ta₂O₅. Samples were polarized at 400 °C for 1h at 2kV/cm field.

Figure 4.

Contact angles of deionized water on pure β -TCP, pure HAp, O-, P- and N-surfaces (O-, P- and N-Surface named for unpolarized, positive side and negative side of 0.25wt.% doped sample surface respectively). Statistical analysis shows significant differences (* for $p < 0.05$, $n=3$).

Figure 5.

MTT results of pure and doped HAp (unpolarized, N- and P-surfaces) incubated for 3, 7 and 11 days. Statistical analysis shows significant difference (* $p < 0.05$, $n=3$).

Figure 6. Cell morphology on pure and doped HAp (unpolarized, N- and P-surface) incubated for 3 days.

Figure 7. Cell morphology of pure and doped HAp (unpolarized, N- and P-surfaces) incubated for 7 days.

Figure 8. Cell morphology of pure and doped HAp (unpolarized, N- and P-surfaces) incubated for 11 days.

Table 1

Activation energy and charge stored in samples prepared at different conditions^a.

Sample Information	Apparent Density ^b (%)	Charge stored ($\mu\text{C}/\text{cm}^2$)	Polarizing field (kV/cm)	Activation Energy (eV)
HAp	93.10 ± 1.12	629.6	2	0.78
HAp + 0.25 wt% Ta ₂ O ₅	93.20 ± 2.20	69.78	2	0.66
HAp + 0.5 wt% Ta ₂ O ₅	94.30 ± 1.10	87.4	2	0.63

^a All samples are sintered at 1250 °C for 2h and polarized at 400 °C for 1h

^b Apparent density is calculated with reference to the theoretical density of HAp, i.e., 3.16g cm^{-3} . The data is reported as average with one standard deviation calculated over 3 samples.