

Role of magnesium on the biomimetic deposition of calcium phosphate

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Abstract Biomimetic depositions of calcium phosphate (CaP) are carried out using simulated body fluid (SBF), calcifying solution and newly developed magnesium containing calcifying solution. Calcium phosphate has a rich phase diagram and is well known for its excellent biocompatibility and bioactivity. The most common phase is hydroxyapatite (HAp), an integral component of human bone and tooth, widely used in orthopedic and dental applications. In addition, calcium phosphate nanoparticles show promise for the targeted drug delivery. The doping of calcium phosphate by magnesium, zinc, strontium etc. can change the protein uptake by CaP nanocrystals. This work describes the role of magnesium on the nucleation and growth of CaP on Ti and its oxide substrates. X-ray diffraction studies confirm formation of HAp nanocrystals which closely resemble the structure of bone apatite when grown using SBF and calcifying solution. It has been observed that magnesium plays crucial role in the nucleation and growth of calcium phosphate. A low magnesium level enhances the crystallinity of HAp while higher magnesium content leads to the formation of amorphous calcium phosphate (ACP) phase. Interestingly, the deposition of ACP phase is rapid when magnesium ion concentration in the solution is 40% of calcium plus magnesium ions concentration. Moreover, high magnesium content alters the morphology of CaP films.

1. Introduction

Calcium phosphate (CaP) is a bioceramic and the synthetic forms have the potential for biomedical applications [1–3]. Magnesium is a vital component of bone mineralization. Bone mineralization is a complex phenomenon and so extremely challenging to mimic the process completely [4, 5]. Initially, authors of this work tried to use simulated body fluid (SBF) [6], but found the deposition to be very slow (requires at least 4 weeks). Since the work reported here compares the growth of calcium phosphate, a slightly concentrated SBF (1.5SBF), a calcifying (Ca/P) solution [7], a modified calcifying (m-Ca/P) solution and magnesium containing m-Ca/P solution are used. Even higher concentration of SBF, i.e. 5SBF is a natural choice for faster deposition of CaP. However, the crystallinity of apatite is reduced when grown using 5SBF [8]. Another important aspect is unlike CaP grown using 1.5SBF, Ca/P solution, m-Ca/P solution where deposition takes place uniformly, apatite grown in 5SBF is less uniform and show evidence of poor adhesion with the substrate [8, 9].

In this work, biomimetic deposition of calcium phosphate with a controlled incorporation of magnesium from a low to high level of Mg^{2+} ion concentration in a modified calcifying solution is presented. Understanding the effect of magnesium on the nucleation and growth of calcium phosphate on implant materials is the central theme of this work.

2. Experimental methods

2.1. Preparation of solution

The ion concentrations of human blood plasma and different solutions used in this work are presented in table 1.



Table 1. Ion concentrations (mM) of human blood plasma and different solutions for the deposition of CaP films

Ion	Human blood plasma	SBF	1.5SBF	Calcifying solution Ca/P	Modified Calcifying solution m-Ca/P	m-Ca/P with Mg content
Na ⁺	142.0	142.0	213.0	25.5	76.5	76.5
K ⁺	5.0	5.0	7.5	—	—	—
Mg ²⁺	1.5	1.5	2.25	—	—	5–0.625
Ca ²⁺	2.5	2.5	3.75	2.5	12.5	7.5–11.875
Cl ⁻	103.0	147.8	221.7	5.0	25.0	25.0
HCO ₃ ⁻	27.0	4.2	6.3	18.0	54.0	54.0
HPO ₄ ²⁻	1.0	1.0	1.5	—	—	—
PO ₄ ³⁻	—	—	—	2.5	7.5	7.5
SO ₄ ²⁻	0.5	0.5	0.75	—	—	—

The reagents NaCl, NaHCO₃, KCl, K₂HPO₄·3H₂O, MgCl₂·6H₂O, CaCl₂, Na₂SO₄ and Na₃PO₄·12H₂O, as received from Merck and Sigma Aldrich are used to prepare the solutions in de-ionized water. The solutions are prepared at 37.0±0.5 °C using a heating magnetic stirrer (Arex Digital Pro, Velp Scientifica). The pH of the solutions at each stage is measured by using a pH meter (Cyberscan Ph2100, Eutech Instruments). Finally, pH is adjusted within the range 7.3–7.4 by adding required amount of Tris or Hepes and HCl and maintaining temperature of the solution at 37 °C. The detailed procedure is described elsewhere [8, 9]. The films are grown at a temperature of 37 °C.

2.2. Recording variation of pH with time

The pH variation of different solutions with substrate immersion time is recorded using the Cyberscan Ph2100 pH meter interfaced with the CyberComm Pro Data Acquisition Software.

2.3. Characterization of prepared samples

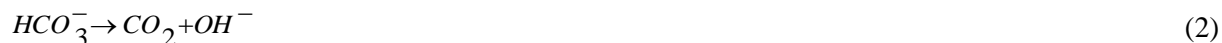
The surface morphology and elemental analyses of the samples are examined using field emission scanning electron microscope (FE-SEM, SIGMA, Zeiss) equipped with energy dispersive x-ray (EDX) spectrometer. XRD patterns are recorded in the powder diffractometer (D8 Advance, Bruker AXS). The Cu K_α line 1.5406 Å is used at a low grazing angle of 1°. The intrinsic broadening due to the diffractometer is corrected by recording the XRD pattern of the standard corundum sample under identical conditions. XRD patterns of the samples are acquired in the 2θ range 20–55° and step size of 0.02° and integration time of 1 s/step.

3. Results and discussion

3.1 pH variation

The pH variation (figure not shown here) of different solutions with Ti substrate immersion time is investigated. The starting pH values are 7.38 and burgeon during first 3 hours. With time, pH gradually increases. The increase in pH of a solution is primarily due to the release of CO₂ gas as [8]





With increase in pH, respective solutions achieve supersaturation which in turn triggers deposition of calcium phosphate. However, the increase in pH of solutions occurs at different pace for different solutions. Since these changes happen at the early stages of deposition of CaP so provides an initial impression that the precipitations of CaP phase might not be the same in all cases.

3.2 Microstructure

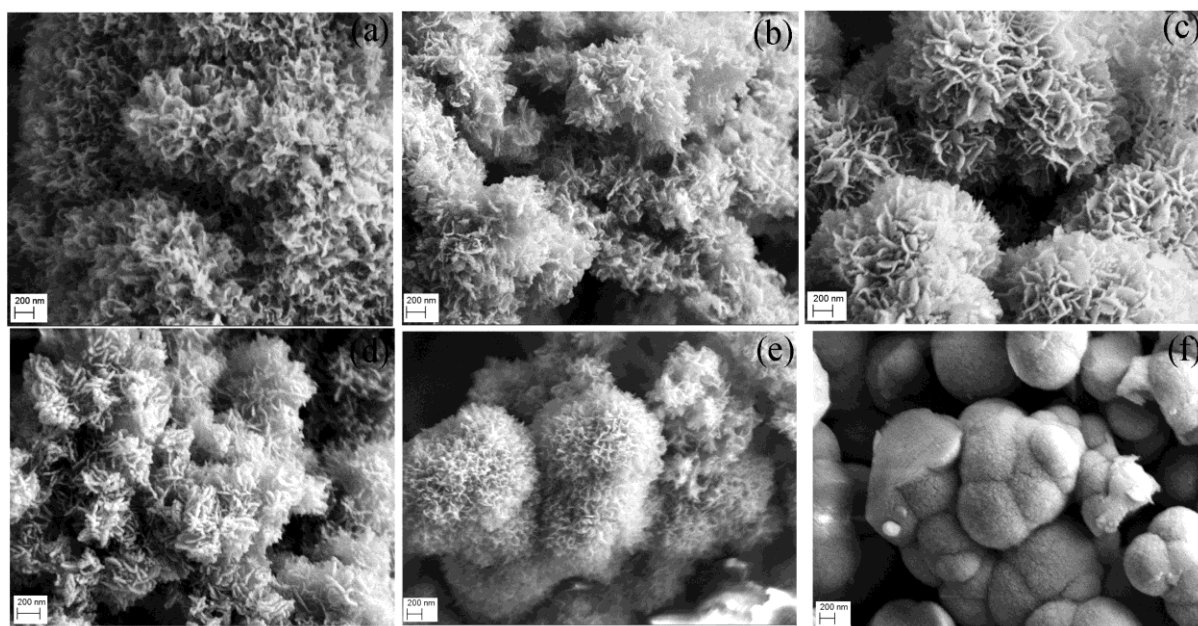


Figure 1. High resolution FE-SEM images of CaP films grown on Ti substrate using (a) 1.5 SBF, (b) 5.0 SBF, (c) calcifying solution, (d) modified calcifying solution, (e) modified calcifying solution with moderate Mg content, (f) modified calcifying solution with high Mg content

The surfaces of CaP structures grown on Ti using various solutions are composed of uniformly distributed micron sized spherical particles. Higher resolution FE-SEM images (Figure 1) show that the surfaces of CaP consist of nano-sized plates arranged to form flowery structures. These plate shaped tiny particles are closely connected to each other and are characteristics of bone apatite [6–8]. Interestingly, such morphology prevails in CaP structure at varying Mg^{2+} concentrations in the m-Ca/P solution from low to moderate level. However, higher magnesium content (40% of calcium plus magnesium ions concentration) alters the morphology drastically to globular pattern signifying that the magnesium content in solution plays a crucial role in the precipitation of CaP structure. EDX results are summarized in table 2. Ca/P atomic percentage ratio deviates from that of the stoichiometric hydroxyapatite (HAp) which is 1.67. However, the calcium deficiency cannot be solely attributed to the magnesium substitution at the Ca site in the HAp lattice. Calcium deficient carbonate apatite is quite common in biomimetic deposition of CaP.

3.3 X-ray diffraction and analysis

Figure 2A shows XRD patterns of biomimetic CaP films grown on Ti substrate using 5 SBF, 1.5SBF, Ca/P, m-Ca/P, and m-Ca/P solutions with Mg source. X-ray line profile fitting for XRD pattern of CaP film with 7.2% Mg to Ca atoms concentration (corresponding to 10% of magnesium to calcium

plus magnesium ions concentration in m-Ca/P solution, i.e. m-Ca/P solution with moderate Mg content) is shown in figure 2B. XRD patterns reveal formation of HAp phase in all cases except for CaP films grown using m-Ca/P solution with high Mg content (corresponding to 40% of magnesium to calcium plus magnesium ions concentration in m-Ca/P solution, i.e. 29.6% Mg to Ca atoms concentration) which is amorphous (table 2).

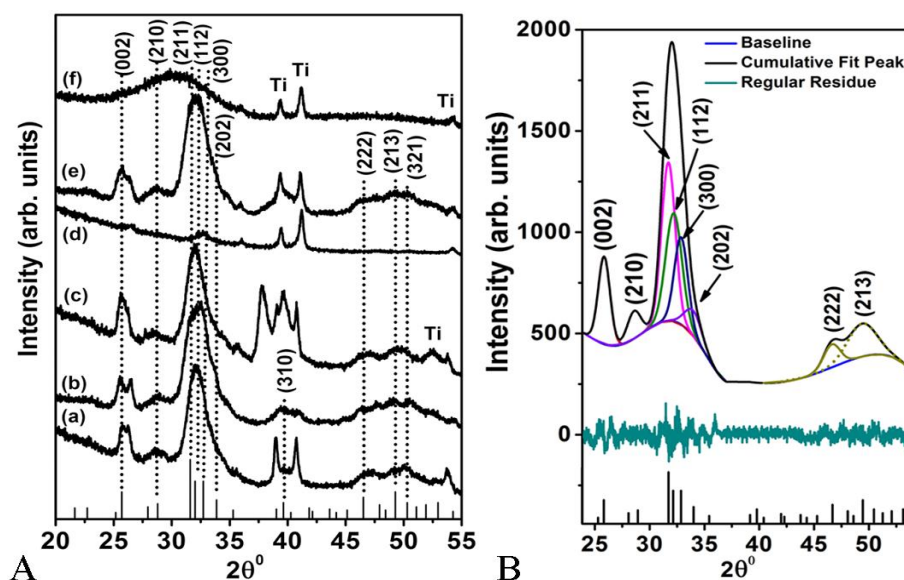


Figure 2. A. XRD patterns of CaP films grown on Ti substrate using (a) 1.5 SBF, (b) 5.0 SBF, (c) Ca/P solution, (d) m-Ca/P solution, (e) m-Ca/P solution with moderate Mg content, (f) m-Ca/P solution with high Mg content, B. Representative X-ray line profile fitting for HAp with moderate Mg content.

Table 2. Mg content, phase, and crystallite size of biomimetic CaP films

Solution	% Mg/Ca	% Mg/(Ca+Mg)	Phase	Ca/P	(Ca+Mg)/P	<i>t</i> (nm)
Ca/P	0	0	Carbonate HAp	1.35	1.35	9
m-Ca/P	0	0	Carbonate HAp	1.49	1.49	3
	4.8	4.6	Carbonate HAp	1.34	1.40	6
m-Ca/P with Mg content	7.2	6.7	Carbonate HAp	1.43	1.54	6
	7.7	7.1	Carbonate HAp	1.55	1.67	8
	29.6	22.9	ACP	1.07	1.38	—

HAp is nanocrystalline with apparent domain size in the range 3–9 nm. The result is consistent with TEM observation (data not presented here). The appearance of the most intense peak at $\sim 32^\circ$ might be ascribed to the collective contributions from (211), (112), (300), and (202) diffraction planes which is obvious from the X-ray line profile fitting as shown in figure 2B. Raman spectra (figure not shown here) further suggest that nanocrystalline HAp has B-type substitution with carbonate ions replacing some of the phosphate ions in the HAp. At the same time, with Mg source in the solution, Mg substitutes for Ca in the HAp lattice. EDX analyses (table 2) suggest 4.8–7.7% substitution of Mg to Ca in the nanosized carbonate apatite. With greater Mg content (29.6% Mg to Ca atoms) the

amorphous calcium phosphate (ACP) phase is formed. In order to understand the effect of Mg substitution, the unit cell parameters are determined from X-ray line profile fittings for various Mg concentrations. The variation is shown in figure 3.

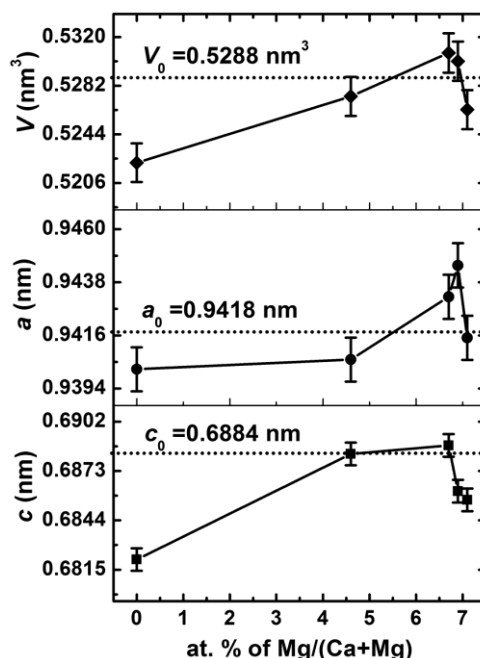


Figure 3. Variations of lattice parameters c , a , and unit cell volume V of CaP as a function of Mg impurity concentration. The dashed lines represent standard values for a , c , and V taken from ICDD file 9-432.

It is observed that the unit cell initially experiences extension along a - and c -directions of the hexagonal HAp with increasing Mg concentration. However, beyond 6.7% of Mg concentration, the unit cell undergoes substantial contraction in the c -direction. However, the lattice contraction along a -direction is not so significant even at 7.1% of Mg concentration. The unit cell volume follows a similar trend. Interestingly, such fluctuations in a , c , and V are observed at different Mg concentrations even with equal apparent domain size (6 nm). The evident lattice distortion is better decided by the impurity concentration. Even if the domain sizes are equal, marked variations in a , c , and V are noticed.

4. Conclusions

Biomimetic approach to CaP nanocrystals reveal that a modified calcifying solution may be suitably used for Mg doping experiments. The growth of HAp nanocrystals on Ti substrate from a supersaturated calcifying solution at physiological temperature and pH of human blood plasma has been demonstrated through XRD, Raman, and electron microscopy studies. The apparent domain size lies in the range 5–8 nm with low to moderate Mg concentration. Interestingly, such extent of magnesium substitution does not inhibit the HAp crystal growth. A controlled Mg incorporation could induce lattice distortion in the carbonate apatite. However, higher Mg level is found to be responsible for the precipitation of ACP films. Moreover, the precipitation of ACP is much faster than HAp growth suggesting that the role of Mg is more varied here and is not just limited to inducing the lattice distortion in the HAp lattice. It is well known that Mg is important for human metabolism and magnesium deficiency in bone can lead to orthopedic problems. Thus the presented method of controlled incorporation of Mg in biocompatible CaP might be a potential way to produce next generation biomaterials for biomedical applications.

Acknowledgments

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