JOURNAL OF MATERIALS SCIENCE: MATERIALS IN MEDICINE **16** (2005) 399–404

Dissolution and mineralization of sintered and thermally sprayed hydroxy-fluoroapatites

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Hydroxyapatites are commonly used as bone cement, coatings on implants for dental and orthopaedic applications, but also as middle ear implants. These applications all require a different tissue healing response that can be attained by different manufacturing processes or by chemically modifying the composition. During implantation apatites undergo a process of dissolution and mineralization. The degree of dissolution is dependent upon the manufacturing process & is higher for thermally sprayed implant materials. This allows them to integrate to the natural bone. This study tests the dissolution and mineralization of fluoride containing hydroxyapatites through immersion in simulated body fluid. It shows that mineralization occurs more readily in hydroxyapatites than fluorapatites because of their higher dissolution rate. Mineralization was detected most readily by image analysis using scanning electron microscopy than by weight changes using a microbalance. Microscopy allowed small heterogeneous precipitates to be observed during the initial stages of mineralization.

1. Introduction

Hydroxyapatite (HA) is a calcium phosphate similar in composition and structure to natural bone [1]. Consequently it is often used as a coating for orthopaedic implants. During implantation, HA undergoes a process of dissolution and mineralization that allows integration of the bone and implant [2]. Mineralization of HA is thought to be heterogeneous occurring at discontinuities in the lattice where lattice vibrations would facilitate the ordering reaction required for formation of HA crystals [3].

Natural bone accumulates fluoride ions from the blood forming a fluoride containing HA [1]. Synthetically, a fluorapatite (FA) can be produced with a range of fluoride concentrations and has potential relevance for orthopaedic applications. Fluoride ions within the lattice replace hydroxide ions creating a tighter lattice structure. This promotes increased stability and lowers the solubility of FA [4–6]. Fluoride is also thought to directly stimulate bone growth by suppressing the maturation of osteoclasts, inhibiting phagocyte cell activity and minimizing proliferation of fibroblasts [7]. The addition of fluoride ions to a HA bone coating could further improve the bond between the bone and implant. This study compares the dissolution and mineralization behaviour of HA and FA.

Most previous studies on the solubility of HA have observed mineralization using scanning electron microscopy (SEM) [8–10] or by measuring the concentration of ions in the test solution [9]. In this study weight measurements were recorded with a microbalance to detect the stages of dissolution and mineralization. These results were confirmed using scanning electron microscopy (SEM) and atomic force microscopy (AFM) image analysis.

Processing of hydroxyapatite influences grain size, grain orientation, porosity, residual stress, surface topography and crystal perfection (defect concentration). These aspects can change the solubility and possibly the mineralization of the resulting materials. This study will assess the changes in dissolution and mineralization of hydroxyapatite and fluorapatite manufactured with sintering and thermal spray processing approaches.

2. Method

2.1. Preparation of pellets

2.1.1. Sintered pellets

Hydroxyapatite and fluorapatite were prepared by adding 0.6 M diammonium hydrogen phosphate to 1 M calcium nitrate and then maturing the solution, at a pH of approximately 9 as reported previously [11]. Fluoride was combined with the ammonium phosphate for fluorapatite synthesis and added dropwise whilst measurements were taken using a fluoride selective electrode. The powder was then washed with water and

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calcined at 900 °C for one hour. From this powder, 0.7 g was measured for pellet production. Pellets were compacted using uniaxial and cold isostatic pressing. The pellets were then sintered in a tube furnace for one hour at 1200 °C in an air atmosphere purged through boiling water.

2.1.2. Thermal spraying

Powder with a particle size of 20 to 60 μm was injected into a Metco 5P flame spray torch and coated onto a 12 mm diameter titanium coupon mounted in a vacuum chuck. Acetylene and oxygen were used as combustion gases and a stand-off distance of 10 cm was selected. The heat in the process was sufficiently high to produce a ⟨001⟩ type preferred orientation during the coating formation of FA. This process was chosen to avoid decomposition phases (such as calcium oxide, tricalcium phosphate and tetracalcium phosphate) and the amorphous phase that results from rapid cooling.

2.1.3. Polishing

Pellets were ground using a Struers autopolisher to a thickness less than one millimetre after sintering to present a smooth surface with a roughness less than 0.2 μm [12]. This was done using silicon carbide grinding paper at 320, 800, 1200 and 2400 grit. Following the grinding process, polishing occurred on a Dur cloth with 1 μm diamond suspension and a finally on a Chem cloth with 0.05 μm gamma alumina. After polishing was completed the pellets were dried for a period of 45 min.

Prior to drying the thermally sprayed coatings were ultrasonically cleaned in ethanol for four minutes.

2.2. Density measurements

Density measurements were obtained for the sintered pellets prior to polishing on a Mettler Toledo AX machine.

Density measurements for the coatings were obtained by recording the combined density of the coating and substrate. The coating was dissolved in 35 vol% hydrochloric acid, then washed in deionized water, dried in the oven and cooled. Density and weight of the substrate was remeasured. Coating density was determined from this data according to equation 1, where c is the coating and s is the substrate.

$$\rho_{(c)} = \frac{\text{weight}_{(cs)} - \text{weight}_{(s)}}{\text{volume}_c} \tag{1}$$

2.3. pH dissolution tests

Dissolution tests were completed on a PHM 250 Ion Analyzer. Prepared solutions containing 400, 40, 4 and 0.4 ppm calcium were used to calibrate the machine prior to testing. Tests were conducted at a pH of 7.3, 6 and 5. The solutions at pH 5 and 6 were made from potassium acetate buffered with hydrochloric acid whereas the pH 7.3 solution was made using a tris[hydroxymethyl]aminomethane buffer, all at a concentration of 0.1 M.

Pellets were dried for two hours at 100 °C before dissolution and weighed using a Mettler Toledo MX5 microbalance. Pellets were placed separately into the buffered solution. The calcium level was measured for one hour whilst the solution was stirred at 300 rpm. Following dissolution, the pellets were cleaned with ethanol and dried for one hour at 100 °C before reweighing.

TABLE I Comparison of ionic concentrations in simulated body fluid and blood plasma

Ion	SBF	Blood Plasma
Na^+	142.0	142.0
K^+	5.0	5.0
Mg^{2+}	1.5	1.5
Ca^{2+}	2.5	2.5
Cl^-	147.8	103.0
HCO_3^{2-}	4.2	27.0
HPO_4^{2-}	1.0	1.0
SO_4^{2-}	0.5	0.5

2.4. Immersion tests in simulated body fluid

Simulated body fluid with an ionic concentration (Table I) similar to blood was prepared in a plastic container to minimize contamination. The solution was prepared at 37 °C and buffered using hydrochloric acid to a pH of 7.4, then filtered.

HA and FA pellets were dried for two hours at 100 °C before testing. They were then weighed on the microbalance and placed separately into a container of 50 mL SBF. The containers were placed in an environmental shaker at 37 °C rotating at a speed of 100 rpm. Pellets remained in the shaker for a period between one and six days. At the correct time period pellets were removed from the solution and washed in ethanol before drying for one hour at 100 °C and reweighing.

2.5. Image analysis

2.5.1. Atomic force microscopy

A JSTM 4200A Scanning probe microscope (Jeol, Japan) was used to image the surface of sintered pellets. Atomic force microscopy (AFM) allows the topography of the surface to be displayed by measuring the interatomic forces acting on a cantilever. During this experiment the non contact analysis was used. Pellets were placed under the laser and a 1.6 V reference voltage was applied.

2.5.2. Scanning electron microscopy

Scanning electron microscopy (SEM) was used to assess the changes to the material surfaces after immersion in SBF. This was achieved with a Hitachi 5570 SEM (Tokyo, Japan) at 10 kV and a working distance of 10 mm.

3. Results and discussion

3.1. Density measurements

Density measurements of the sintered pellets and thermal sprayed coatings were obtained and found to be greater than 90% (Fig. 1). Sintered pellets showed a density greater than 96% at all concentrations and had a

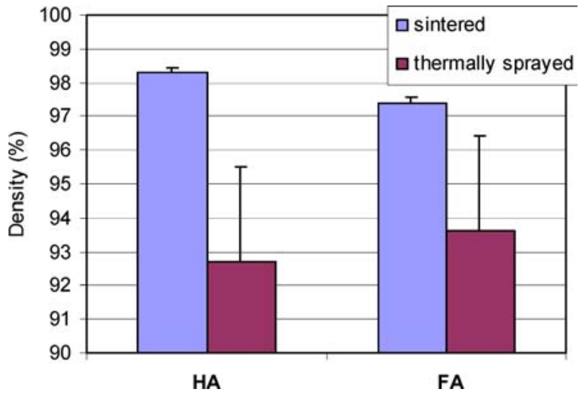

Figure 1 Density Measurements of sintered and thermally sprayed coating of HA and FA.

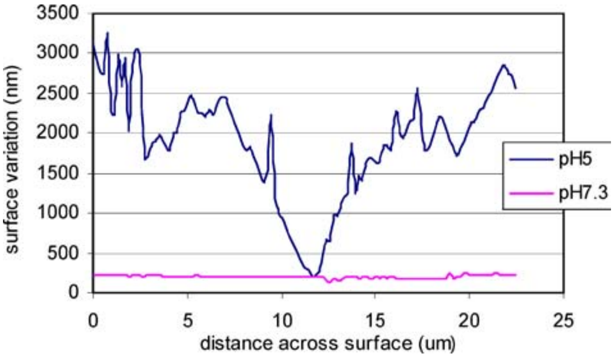

Figure 3 Peak to peak roughness of HA after an hour long dissolution at pH7.3 and pH5.

lower variability than thermally sprayed pellets. Whilst sintered pellets are not used clinically, except for middle ear implants, they provide a reproducible sample for modelling the dissolution and mineralization behaviour of HA-FA.

The lower density of the thermally sprayed coatings are a results of the rapid solidification during production. Coatings are cooled between about 3000 °C and room temperature very quickly. This does not allow time for the pores to be removed from the material. Furthermore, the rapid cooling in the solid phase leads to the retention of a high concentration of defects. Finally, the low fracture roughness of apatite and difference in thermal contraction between titanium and apatite may lead to cracking. The solid phase alone thus has a lower density than material produced from the sintering process.

A lower density material could degrade at a faster rate due to the ability of pores to provide energetically favourable sites for dissolution. Analysis of the dissolution results within this experiment showed no impact of density on the overall dissolution behaviour following weight measurements.

3.2. Dissolution

Dissolution behaviour was observed at a variety of pH levels. Following implantation, particles released from the implant are phagocytosed by macrophages and osteoclasts. During this process the pH can decrease to 4.5 [13, 14]. The more acidic nature of the solution increases the rate of dissolution (Fig. 2). As the pH decreased form 7.3 to 5 the amount of dissolution increased. Interaction between the hydrogen ions, within the solution, and the phosphate ions, within the sample, increase the rate of ionic release [3, 15].

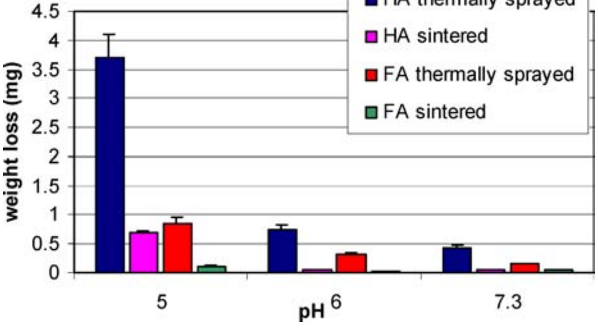

Figure 2 Dissolution behaviour at various pH levels for sintered and thermally sprayed HA and FA following an hour long dissolution.

The trend observed with weight loss measurements was confirmed using AFM. This technique allows topography measurements to be graphed by obtaining a surface measurement for each point on a 25 μm diagonal (Fig. 3). A lower pH shows a higher peak-to-peak roughness on sintered pellets. Following dissolution the surface height variation increased from 100 nm, in a 7.3 pH solution, to 3000 nm, in a pH 5 solution.

Greater dissolution was observed in thermally sprayed coatings due to their lower density and increased residual stress (Fig. 2). Coatings have a smaller grain size, submicron, and a rougher surface than the sintered pellets increasing the overall surface area of the coatings. This increases the sites where dissolution can occur. During thermally spraying the rapid cooling introduces thermal stresses that are relieved during dissolution.

3.3. Mineralization

Dissolution was observed to occur preferentially in thermally sprayed coatings in a mildly acidic medium. This situation occurs clinically following implantation and could result in the formation of a gap between the bone and the implant, preventing integration. The behaviour of the material and the possibility for mineralization will determine whether or not integration will occur.

Mineralization was tested by immersing the samples in simulated body fluid (SBF) for a period between one

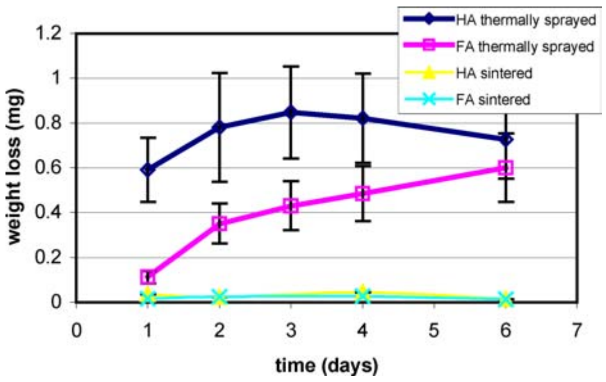

Figure 4 Weight loss in sintered pellets and thermally sprayed coatings following dissolution in SBF over six days.

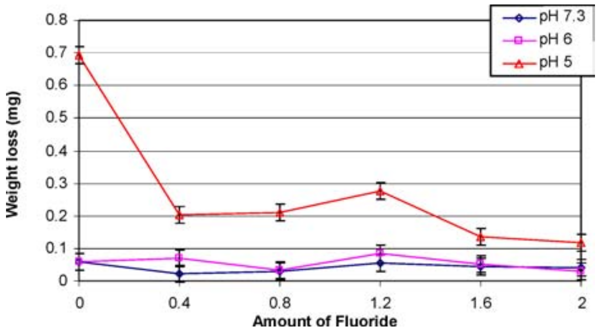

Figure 5 Dissolution in pH medium.

and six days. This solution aimed to simulate the ionic conditions within the body. As observed previously, thermally sprayed coatings underwent a larger dissolution than sintered pellets in a SBF (Fig. 4). Thermally sprayed coatings lost 0.85 mg over three days whilst sintered pellets lost 0.025 mg during a four day dissolution.

Whilst weight loss was observed over the first three days, it was followed by an increase in weight in all samples except the thermally sprayed FA coating between three and six days. Fluorapatite was expected to increase the extent of mineralization due to the higher bond strength of FA. The tighter FA lattice makes formation of apatite energetically favourable. However it is possible that the preferred crystallization direction of the FA in the coating inhibited mineralization.

The addition of fluoride to HA should increase the stability of the implant by providing strong bone-implant interactions. Stability is achieved by optimal bone growth through the energetically favourable mineralization of FA. Fluoride would also improve the bone growth in a cellular medium through the prevention of osteoclasts causing bone resorption and the stimulation of osteoblasts leading to bone formation [7, 16]. Future tests on FA dissolution and mineralization within a cellular environment would confirm the increased bone growth when fluoride is added to HA.

Fluoride compositions of 20, 40, 60 and 80% were tested for dissolution. In most cases the behaviour of the compositions varied with fluoride content. However the 60% sample (indicated by 1.2) followed a different trend (Fig. 5). Previous studies on HA samples

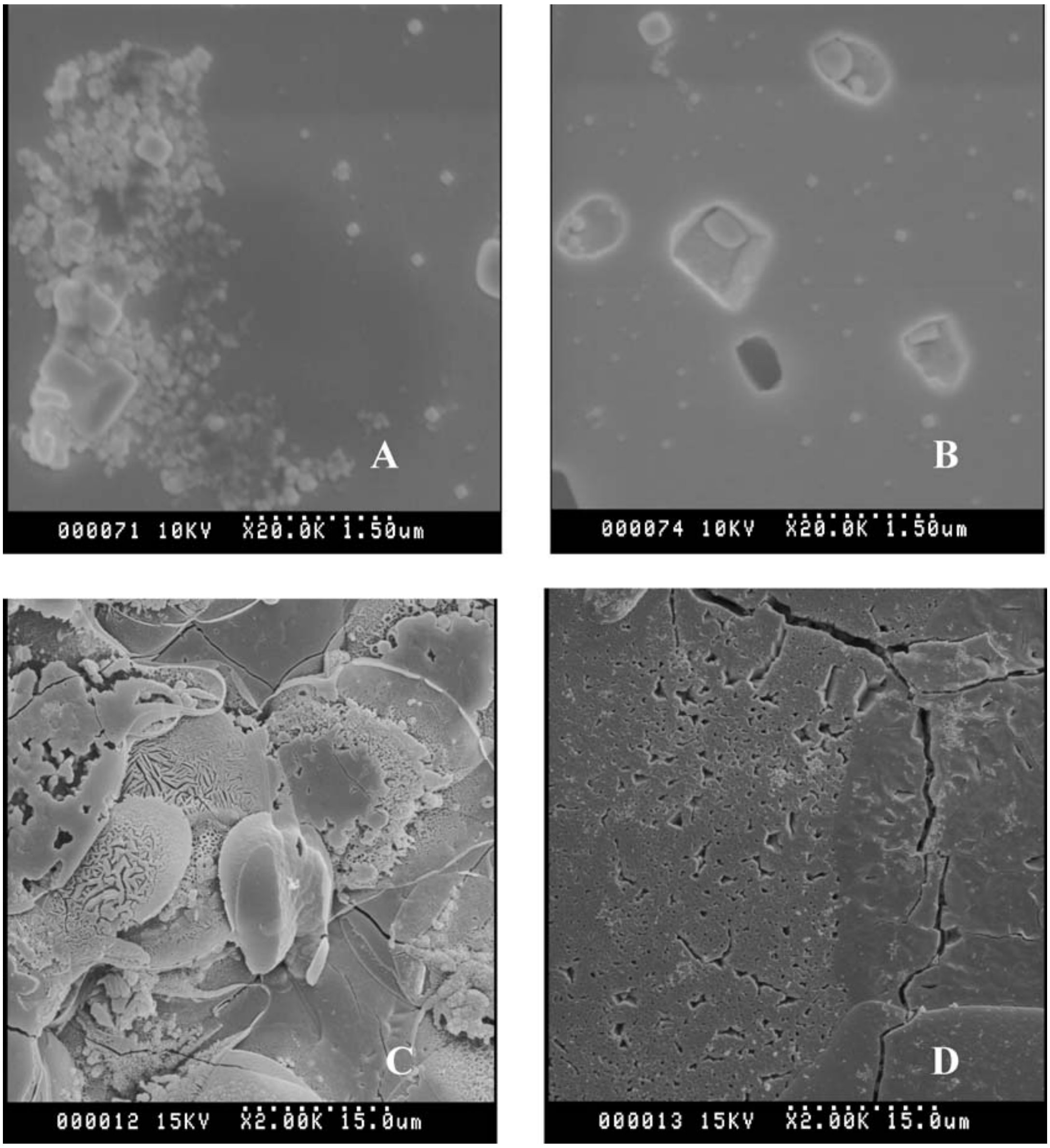

Figure 6 Changes in the topography of sintered (A) HA, (B) FA and thermal sprayed coatings of (C) HA and (D) FA.

containing 60% fluoride showed unusual density and mechanical property behaviour [17]. From this study, the 60% HA-FA sample had the lowest density of the sintered samples at 96%. It also showed the second highest dissolution losing 0.276 mg during an hour long dissolution. This behaviour could optimize the properties of HA and fluoride. The higher dissolution that was observed would increase the surrounding calcium ion concentration stimulating mineralization. Whilst the presence of fluoride would promote bone formation and prevent bone loss.

Results from the weight loss measurements showed considerable variation. To confirm the presence of mineralization at six days samples were observed using image analysis. This confirmed the presence of mineralization in all six days samples, including the FA coating (Fig. 6). Whilst weight measurements showed no mineralization on the FA coating, image analysis allowed observation of mineralization at specific sites. These sites, including pores, were areas where mineralization was energetically favourable.

Since mineralization of FA coatings only occurred at certain sites, dissolution behaviour was the dominant behaviour. This could be seen in the weight measurements. The weight measurements show that the loss of ions from the surface was far greater than the precipitation onto the surface, increasing the weight loss despite the presence of mineralization. Whilst weight loss provides a quick and easy method for determining the dominant mechanism occurring at the surface it is unable to account for small weight changes in mineralization. Similarly the presence of mineralization on an image does not confirm that dissolution is not occurring as both mechanisms occur simultaneously.

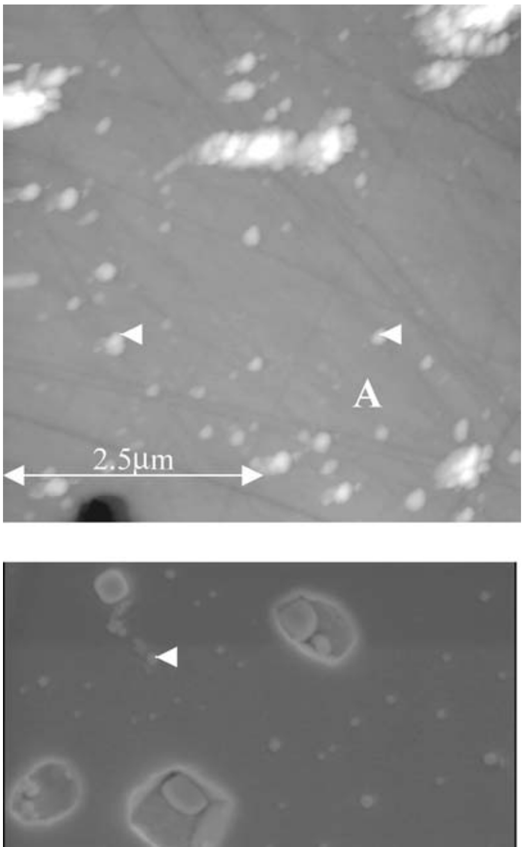

Figure 7 Precipitation (arrowheads) of sintered FA at six days with (A) AFM and (B) SEM.

Mineralization of sintered pellets was observed using both AFM and SEM (Fig. 7). Both results show a 1.5 μm precipitate confirming that either technique can be applied to image the mineralized material. However, AFM is only suitable for flat surfaces, similar to those present in polished sintered pellets. Even at high dissolution rates, for example the pH5 sample, the AFM struggled to obtain topography data. An advantage of using AFM is the ability to complete tests *in situ.* Tests by Leonelli *et al.* (2002) have measured the dissolution of apatites in real time allowing analysis of dissolution as it occurs [18].

Whilst the AFM allowed close examination and roughness profiles for smooth surfaces, the thermally sprayed coatings could only be imaged using SEM. The SEM observations enabled easier analysis of the whole pellet. During AFM analysis the cantilever can only view a small area at one time and movement of the pellet was time consuming. Movement of the pellet within the SEM chamber is easier allowing easy analysis of the entire pellet. Since the mineralization is heterogeneous it is necessary to look at different regions within the pellet. Otherwise it could be assumed that no mineralization had occurred when in fact the area hadn't been analysed yet.

4. Conclusions

Observation of dissolution and mineralization behaviour within ionic medium provides additional insight into changes to HA-FA materials. Improved mineralization was observed in the HA sample, however further tests on FA and 60%HA-FA within a cellular medium could lead to an improved osteoblast differentiation and a prevention of osteoclast formation.

The use of different techniques to measure the extent of mineralization highlighted the advantages of SEM analysis, especially for thermally sprayed samples. Obtaining images of the entire sample allowed the small precipitates to be observed. This confirmed the presence of mineralization despite the continued increase in weight loss.

The manufacturing process significantly influences the properties of hydroxyapatites, imparting a higher resorption for thermally sprayed hydroxyapatites compared to sintered hydroxyapatite. The dissolution was 5 times higher for thermal sprayed hydroxyapatite when measured with an ion selective electrode or 20 times higher when measured with a microbalance.

Acknowledgements

The authors would like to thank Monash University and The Australian Research Council (grants F10017027 and A10017174) for equipment and staff support.

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Received 1 July
and accepted 1 November 2004