Chapter 7 Sintering Behavior of RF Plasma Synthesized and Spray Dried Calcium Phosphate Powders Assessed by a Dilatometer

Introduction

Studies on the spark plasma sintered bioceramic have been highlighted the problem of achieving high densities with enhanced bio-mechanical properties and without excessive grain growth. The main effective parameter for spark plasma sintering was sintering temperature. However, for conventional sintering of compacted powders, it had not been clearly illustrated the effects of sintering temperatures, sintering duration and heating rates on the sintering abilities of the used powders that experienced spray drying and/or RF plasma steps. This chapter aimed at establishing baseline conditions that allowed more thorough assessments of the effects of several parameters on the pressureless densification behavior using a dilatometer.

7.1 Processing of samples

High green body density would improve the sintering ability of powders. While, too high pressing pressures leave residual stresses in the green compacts that could led to cracking before sintering. Thus, the RF plasma processed and spray dried green compacts for the dilatometer were prepared by a hydraulic under moderate pressures of 200 MPa and 130 MPa, respectively, for 1 min at room temperature. Compaction was performed without binder or other sintering aids, to minimize the effects of additives. The linear shrinkage of these green bodies, measured from room temperature to preset sintering temperature at a constant heating rate, was studied using a push rod type dilatometer.
7.2 RF plasma processed powders

7.2.1 Results

7.2.1.1 Effects of sintering temperature

To investigate the micro-structural evolution during the densification steps, R15 powder, which was prepared at 15 kW in the RF plasma, was sintered at various temperatures for 2 hrs. Figure 7.1 presented the SEM images of these sintered samples. As shown, all these obtained ceramics were quite porous. The measurement of grain sizes was difficult due to these fine grain sizes and high porosity levels.

At the sintering temperature of 1100 °C, a non-uniform sintering was observed. It was found that the formation and growth of neck occurred between the ultrafine particles. The larger particles remained undensified or with limited densification. When the sintering temperature was increased to 1200 °C, the micron particles began to sinter. To decrease the numbers of boundaries as well as the free surface areas, the fine grains formed from the nano particle were merged into the micron sized ones (shown in Figure 7.1 (b)). The pores were diminished gradually by the driving force of temperature. They existed in the form of continuous open porosity.

Further increasing in sintering temperature to 1300 °C resulted in an apparent rise in densification. But the pores still existed in the form of continuous open porosity. Sintering at 1400 °C led to relatively denser microstructure compared with the samples sintered at low temperature. The linked pores became relatively smaller. They were partially reduced to closed pores. These images indicated that the RF plasma processed particles were difficult to be sintered within 2 hrs through the traditional sintering.

Actually, the sintering temperature for dense bioceramic depended strongly on the
powder properties and powder packing. Different agglomerate properties could result in variable compaction/densification behavior for individual agglomerates as shown in Figure 7.1. Due to the complex relationship between agglomerate packing and the coexistence of inter- and intra-agglomerate pores, the green density of the compact was important to determine the shrinkage behavior. In the compact of spherical particles, before the neck formation started, pores had the shape of the space among the particles with the sharp edges. After the formation of necks between particles as shown in Fig. 7.1 (a-b), the spheroid pores with smooth corners were formed between the particles. Due to the particle size distribution in the powder (finer particles sintered first) and the presence of agglomerates, differential densification resulted in inhomogeneous densification. In macro scale, higher shrinkage in lateral dimension changed the spheroid shape of pores to tubular (elongated). Tubular pores shrank in the length, when the shrinkage increased in thickness by further increase in sintering temperature or holding time.

Also, due to the relatively low sintering activation energy caused by low surface area of the RF plasma processed powders, it was quite difficult to obtain high green density compacts for the solid particles processed through RF plasma. Thus the green compacts with low green densities led to the formation of porous structured ceramic as observed in Figure 7.1.

In addition, as discussed in the previous Chapter 5, there were several calcium phosphate phases in the RF plasma processed powder, which included HA, α-TCP and TTCP. It has also found the presence of oxyhydroxyapatite (Ca_{10}(PO_4)_6(OH)_{2-2x}O_x) or even oxyapatite (Ca_{10}(PO_4)_6O) in this RF plasma processed powder. These phases behaved as composite systems during sintering. The TCP would act as a limiting
agent of the sintering of the HA matrix by standing in the way of diffusion on account for the decrease of the densification ratio of the material.

Besides the temperature effects, the final sintered properties also depended largely on the phase content of the starting powder. Large amounts of secondary calcium phosphate phases such as TCP, TTCP might hinder the sintering process. Figure 7.2 presented the XRD patterns of the ceramic sintered at various temperatures. HA, TTCP and a mixture of both $\alpha$- and $\beta$-TCP were observed in the obtained ceramic samples that were fired below 1200°C. Upon increasing sintering temperature to above 1300 °C, $\alpha$-TCP and TTCP were mainly detected in the sintered samples.

Referring to the maximum peak intensities, the relative concentration was defined as the ratio of the maximum intensity of different CaP phase to that of the maximum peak of HA. Thus, the relative contents of TTCP, HA, and $\alpha/\beta$-TCP in the ceramics could be determined. As presented in Figure 7.2, the relative intensities of the TTCP peaks increased gradually as the sintering temperature increased. At above 1100 °C, the peak intensities of TTCP were obviously predominant. The relative intensities of TCP changed slightly.

The Raman spectra provided additional information on the phase transformation of calcium phosphate at various sintering temperatures (Figure 7.3). Shoulders and main band observed in the $\nu_1$ mode region (at ~960 cm$^{-1}$) of phosphate in the ceramics were assigned to different environments of the phosphate groups. With the increase in sintering temperature, the main $\nu_1$ vibration of phosphate at around 960 cm$^{-1}$ slightly shifted to the higher vibrational positions. While, the shoulder peak at about 950 cm$^{-1}$ shifted toward to the lower vibrational positions. This finding might be caused by the amount of various calcium phosphate phases in the ceramic. The $\nu_2$, $\nu_4$ and $\nu_3$
domains of the ceramic were difficult to assess the phosphate groups due to the low intensities of the peaks. No obvious peaks belong to hydroxyl modes were detected, which indicated the final products were dehydrated calcium phosphates.

Both the XRD patterns and Raman spectra confirmed that the final products sintered using the dilatometer were made of a complicated mixture of different calcium phosphate phases. Though most of these calcium phosphates have been proved to be bioactive, the microstructure observed under SEM indicated many pores existed in the bioceramic samples, which were detrimental to the mechanical properties in the future application. Considering the SEM images presented in Figure 7.1 and XRD results shown in Figure 7.2, the sintering temperature of 1200 ℃ should be an appropriate sintering parameter. However, the sintering duration should be extended till near fully dense ceramics could be produced.
Figure 7.1 SEM images of R15 sintered at various temperatures: (a) 1100 °C, (b) 1200 °C, (c) 1300 °C, and (d) 1400 °C.
Figure 7.2 XRD patterns of sintered of R15 powders in the dilatometer at various temperatures with a heating rate of 5 °C/min.

Figure 7.3 Raman spectra of sintered of R15 powders in the dilatometer at various temperatures at a heating rate of 5 °C/min.
7.2.1.2 Effects of sintering duration and heating rates

For the further investigation on the densification, the green compacts were sintered at 1200 °C for various dwell times. The duration was set for 5, 10 and 20 hrs. Figure 7.4 presented the SEM microstructure of sample sintered for various durations. The effect of holding time to remove the porosity was pronounced. The pores existed in the form of continuous open porosity when the dwell time was less than 5 hrs (shown in Figure 7.1). When the duration increased to 5 hrs, it was found that the inter-agglomerate joining increased. The linked pores were reduced in size to closed pores, which indicated the sintering had reached an intermediate or the second sintering stage. After extending to 10 hrs, the effect of holding time to remove the porosity was not obviously pronounced. For longer duration of 20 hrs, as shown in Figure 7.4 (e-f), few pores were detected in the ceramic. The sintering was able to reach the final stage. The intra-agglomerate pores reduced in size, and eventually were eliminated at this stage. The grain size was only about 2-3 μm. Such a small grain size after sintered for 20 hrs should be attributed to the dense structure of starting powder. Normally, green compact with a dense structure fabricated under very high pressure showed neither rapid densification nor rapid grain growth even at high sintering temperature. However, green compact with a loose structure exhibited a rapid increase in the sintered density and grain size as the sintering temperature increased [168]. As shown in Figure 7.1 (d), the second stage of sintering was reached in even shorter time at higher temperatures (such as 1400 °C). At even higher temperature and longer dwelling time, the remaining closed pores would be eliminated when grain growth became a predominant phenomenon. This behavior could be explained by Lange’s theory [169]. It was suggested [41] that the pores could not remain along the
boundaries until they were eliminated at higher sintering temperature. This pore-boundary separation formed closed pores as shown in Figure 7.1 (d).

Figure 7.4 Microstructure images of R15 sintered at 1200°C under the heating rate of 5 °C/min for:
(a-b) 5 hrs, (c-d) 10 hrs; (e-f) 20hrs.

Figure 7.5 showed the influence of heating rates (5, 10, and 20 °C/min) on the SEM microstructures of the ceramic sintered at 1200 °C for 5 hrs. It was obvious that denser disks were obtained at higher heating rates. The grain size was around 1-2 μm
at the heating rate of 5 °C/min. While, the grain size increased to ~4 and ~5 µm at the rate of 10 °C/min and 20 °C/min, respectively. The slowest heating rate was expected to correspond to more advanced sintering as the samples spent longer time at high temperatures. The density variation of sintered samples of the present composition fabricated using various sintering parameters were given in Table 7.1. The maximum density of 2.86 g/cm³ was obtained at a heating rate of 10 °C/min. Compared with the sample sintered at a heating rate of 10 °C/min, the sample density obtained at a heating rate of 20 °C/min dropped slightly to 2.77 g/cm³. This was attributed to that a low heating rate could increase the degree of sintering resulting in a higher density.

Table 7.1 Densities of the ceramics at various sintering conditions.

<table>
<thead>
<tr>
<th>Sintering Temperature (°C)</th>
<th>Dwell time (hrs)</th>
<th>Heating rate (°C/min)</th>
<th>Density (g/cm³)</th>
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<tbody>
<tr>
<td>1000</td>
<td>2</td>
<td>5</td>
<td>2.51</td>
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<tr>
<td>1100</td>
<td>2</td>
<td>5</td>
<td>2.48</td>
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<tr>
<td>1200</td>
<td>2</td>
<td>5</td>
<td>2.62</td>
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<tr>
<td>1300</td>
<td>2</td>
<td>5</td>
<td>2.53</td>
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<td>5</td>
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<td>1200</td>
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<tr>
<td>1200</td>
<td>20</td>
<td>5</td>
<td>2.96</td>
</tr>
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</table>

Three measurements were averaged for each sample.
Figure 7.5 Influence of the heating rate on the microstructures of the ceramics sintered at 1200 °C for 5 hrs: (a) 10°C/min; (b) 20°C/min.

![Microstructure images showing different heating rates](image)

Figure 7.6 XRD patterns of the ceramics sintered at 1200 °C for 5 hrs at various heating rates.

![XRD patterns showing different heating rates](image)
7.2.1.3 Shrinkage behavior

Figure 7.7 (a) showed the densification characteristics through the dilatometer during heating to 1300 °C. It was observed that the transition from expansion to shrinkage of R15 powders started at around 510 °C. This observation was very important in that the existing structure must display self-consistent behavior on both sides of the transition. Structural features that led to macroscopic expansion at low temperatures should be influential in triggering shrinkage.

The rate of sintering was very slow up to 1080 °C. Above this temperature, shrinkage rate increased significantly showing a maximum around 1300 °C. The shrinkage rate decreased at above 1300 °C. It was due to the contribution of a grain growth mechanism.

In the initial stage of sintering below 1080 °C, the crystallites in each agglomerate were sintered first, and densified at a low rate due to the energetic, sinteractive nano-sized crystallites [170], as shown in Figure 7.1 (a). The low sintering rate observed should contribute to the small amount of nano-particles. Small particles between the hard agglomerates were consumed via grain growth providing additional shrinkage. These nano-scaled features constituted contact points that could inhibit smaller center-to-center distances between relatively large particles. There was little difference, energetically, between the surface smoothing of a nano-sized peak and the surface consumption of a nano-sized particle. Via the elimination of pinning fine particles, surface diffusion could result in densification in real systems containing a poly-disperse range of particle sizes.

Likely neck formation at relatively low temperatures corresponding to the observed
macroscopic change suggested that, taken globally, the formation of small sinter neck overcame the continuing thermal expansion of the particles. The subsequent macroscopic result was a dimensional decrease due to repacking.

Shrinkage accompanying sintering of the agglomerates would produce tensile stress between adjacent agglomerates. This stress, if sufficiently large, could break away the sintered agglomerates from the surrounding matrix, leaving a gap that could not be closed by subsequent sintering. An increased temperature was required to drive the sintering further to produce an increased densification.

As could be seen from Fig 7.7 (b), the densification rate showed a maximum at around 1280 °C. Above 1300 °C, the shrinkage rate decreased. The shrinkage curve was uniform. It did not show obvious steps as a function of temperature at above 1100 °C. The presence of a sharp peak in the shrinkage rate indicated a uniform removal of pores and the development of a mono-modal pore size distribution in the sample, as observed under SEM. Different ranges of pore sizes, e.g. in bimodal pore size distribution, resulted in steps in the shrinkage curve. It was ascribed to the different nature of the size distribution of the pores due to the presence of agglomerates in the powder. Shrinkage in porous body first started in agglomerates since the driving force was higher due to the high pore coordination number inside agglomerates. At high temperatures, inter-agglomerate densification would follow.

The formed linking pores would be gradually diminished by the driving of temperature. If the sintering duration was extended longer than 2 hrs, the pores would be reduced to closed pores. Relatively dense ceramics would then be obtained. While, less favorable agglomerate bonding or more internal agglomerate fractures could result in either large gap along the inter-agglomerate interface or within the
agglomerates themselves as sintering proceeds. Also from the XRD patterns, it indicated that the sintering temperature at 1300 °C was too high, and the decomposition of HA into secondary phases were detected.

Figure 7.7 Densification curves of R15 during heating to 1300 °C:
(a) shrinkage behavior; and (b) densification rate, \( \frac{dL}{L_0} \)/dt.
7.2.2 Discussion

To assess the pressureless sintering behavior of the RF plasma processed powders, the whole sintering process should be investigated in terms of powder characters and sintering conditions.

Densification in pressureless sintering was related to the system itself. The densification theory in pressureless sintering had been a fundamental theme for several decades [171]. Full densification was a prerequisite for the achievement of the intrinsic properties of advanced bioceramic. According to Coble [172], the solid state sintering could be divided into three stages.

The first or initial stage of sintering involved the interface formation and the neck growth between primary particles (the contact area between the particles increase from ideally point zero) to a certain extent. The neck growth would cease when an equilibrium configuration was reached. The initial stage of sintering involved no grain growth and no appreciable shrinkage.

The second or intermediate stage of sintering started when grain growth began. During this stage, grain boundaries formed extensively. While, pores were still connected with each other, and formed a continuous pore network, i.e., pore channel existed, whereas the grain boundaries were isolated. No continuous grain boundary networks were formed. Most densification and microstructure changes took place in this stage. This stage took place normally at a temperature 1/4-3/4 of the melting point in Kelvin degrees [171]. In this study, the sintering reached the intermediate stage at all the sintering temperatures within the duration of 2 hrs.

After sintered for 20 hrs at the temperature of 1200 °C, nearly dense ceramic was
obtained as presented in Figure 7.4. As shown, the pores became isolated. Grain boundaries formed a continuous network, indicating that the third or final stage of sintering started. Isolated pores located at grain boundaries, or linear junctures of three grains or point junctures of four grains, and/or entrapped in grains. At this final stage, density increased slightly but the grains grew rapidly.

The sintering ability of ceramic was closely related to the particle size of the starting powders. It was important to get as small particle size and agglomeration free particles as possible at reasonable cost. As it was well known, the general driving force at the ceramic sintering was the surface free energy of particles. The smaller the particle size, the higher the surface free energy, the easier surface atom diffusion between neighboring particles, and the lower the sintering temperature. The large surface area of nano powders provided a strong driving force for both sintering and grain coarsening. Correspondingly, sintering temperature of these nano particles was lower than that of larger particles. As observed, the nano sized particles in the RF plasma processed powders initially started to sinter at a temperature of 510 °C, which was quite low when compared with micrometer sized powders.

The stability of HA had been discussed in Chapters 5 and 6. It was reported that HA decomposed within the temperature range 1200-1450 °C, which depended strongly on the characteristics of the HA powders [173].

In a moisture-filled environment, TCP would react with TTCP or CaO in the starting powder to revert back to HA coupled with the reduction of the CaO phase. This could be postulated in the following reactions:

\[ 2 \text{Ca}_3(\text{PO}_4)_2 + \text{Ca}_4\text{P}_2\text{O}_9 + \text{H}_2\text{O} \rightarrow \text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2 \]  \[ 7-1 \]

\[ 3 \text{Ca}_3(\text{PO}_4)_2 + \text{CaO} + \text{H}_2\text{O} \rightarrow \text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2 \]  \[ 7-2 \]
However, the present sintering was conducted in the atmosphere of nitrogen. The moisture was too low to promote the hydrolytic reaction to HA but to TTCP and oxyapatite (Ca$\text{_{10}}$(PO$_4$)$_6$O). The transformations could be explained as follows:

$$2 \text{Ca}_3(\text{PO}_4)_2 + \text{Ca}_4\text{P}_2\text{O}_9 \rightarrow \text{Ca}_{10}(\text{PO}_4)_6\text{O} \quad [7-3]$$

$$\text{Ca}_3(\text{PO}_4)_2 + \text{CaO} \rightarrow \text{Ca}_4\text{P}_2\text{O}_9 \quad [7-4]$$

Both the above two chemical reactions might occur at the sintering temperature below 1200 °C, while the reaction of TCP and CaO controlled mainly the sintering. Thus, the reduction of CaO and increase in TTCP were observed in the sintered samples.

At temperature higher than 1200 °C, oxyapatite decomposed in two compounds: $\alpha$-TCP and TTCP according to the reaction:

$$\text{Ca}_{10}(\text{PO}_4)_6\text{O} \rightarrow 2 \text{Ca}_3(\text{PO}_4)_2 + \text{Ca}_4\text{P}_2\text{O}_9 \quad [7-5]$$

Therefore, from the above analysis, the detected apatite structure in the samples sintered below 1200 °C was actually a compound of oxyhydroxyapatite and/or oxyapatite. Further increase in sintering temperature to 1300 °C, the relative density of the sample declined to 2.53 g/cm$^3$. This was attributed to the decomposition of dehydrated apatite into TTCP and $\alpha$-TCP phases. Almost no apatite could be traced at the sintering temperature of 1400 °C.

Considering the densities listed in Table 7.1, it was possible to reduce the sintering temperature by prolonging sintering time. While, it should be prohibited to use the long sintering time because the consequent risk of over firing, excessive grain growth and possible high production cost.

As shown in Figure 7.5, the heating rates also influenced the microstructure of the
present samples. A noticeable difference in the grain size was observed. The grain size decreased, and became more uniform with increasing heating rates. A faster heating rate may produce densification with a smaller grain size due to the activation energy for densification being significantly greater than that for grain growth. Thus, the effect of heating rate on density (Table 7.1) was likely to be due to the differences in the relative rates of grain growth and densification. However, the smallest grain size in all samples was about half a micron. For the traditional sintering, too fast heating rate would damage the heating elements, which should be considered in the industrial production. Also, slow heating was detrimental to rearrangement of particles in the compacts [174].

Kim et al [175] illustrated the effects of sintering rates on the densification. It was reported that the higher sintering rate accompanied higher thermal gradient between the surface and the center of a sample. It was likely that the outmost region of the sample sintered much faster than the core region. When the outside of the sample was well densified, the inside of the sample was not able to densify because the outer skeleton constrained the densification geometrically. Thus, many large pores would be left inside the densified ring. Therefore, the drop in density at heating rate of 20 °C/min was dominantly attributed to the presence of large pores inside the sample.

As proposed by Ryu et al [176], the densification at high heating rate in the solid phase occurred by rearrangement of the primary particles instead of the surface area change mechanism. This mechanism would result in insufficient shrinkage of the sintered sample as well as the decrease in density.

However, due to the increased sintering degree, the decomposition of the starting powder would also be enhanced at the low heating rate of 5 °C/min leading to the
relative low density of 2.73 g/cm$^3$. The high sintering temperature could help to accelerate fully dense ceramic within a short dwell time. However, as shown in Figure 7.2, decomposition occurred predominantly at high sintering temperature, which was detrimental to the final products in the future medical application. The disadvantages of using a high sintering temperature also included high production cost, high maintenance fee, difficulty in quality control and high equipment cost.

### 7.3 Effect of silica to the densification

#### 7.3.1 Results

The effect of silica to the densification was also studied using the dilatometer. Figure 7.8 (a) exhibited the shrinkage curves of HA as a function of sintering time for various silica contents. The densification curves presented a sigmoidal shape. The onset temperature of shrinkage curves depended on silica contents and shifted to lower temperatures with increasing silica. It was found that the onset sintering temperature of spray dried HA began at 841 °C. The total shrinkage (22%) was similar to that of 1SiHA (23%), whose curve also showed a sharp fall. The sintering process of SD HA specimen completed at about 1180 °C. But slight swelling was observed in the isothermal heating stage of 1300 °C. At the final temperature, there were distinct differences in the total shrinkage, with the 1SiHA specimen displaying the greatest shrinkage. For the samples prepared with 1SiHA and 3SiHA powder, they began to shrink at lower temperature (onset temp=600 °C) and stopped to shrink later than that of SD HA. The dilatometry curve of 1SiHA sample approached to a near constant value towards the end of the shrinkage process. On the other hand, for the 3SiHA sample, the shrinkage decreased during the isothermal heating stage at 1300 °C. Sintering seemed to progress at a much slower rate and did not complete within the
holding time, as the dilatometry curve was unable to progress to a constant value before the dynamic cooling takes place.

In order to investigate the shrinkage behavior of the spray dried powders in detail, the sintering shrinkage rate, i.e., the first derivative (dL/dt) with respect to time was calculated. Figure 7.8 (b) showed the curves of the compacts doped with different amount of silica as a function of sintering time. Compacts without silica exhibited a highest shrinkage rate. Compared with that of pure HA compacts, the maximum shrinkage rates of these silica doped samples occurred at higher temperatures which were 930 °C, 1143 °C and 1250 °C for HA, 1SiHA and 3SiHA, respectively. Several peaks were also observed in the shrinkage rate curves. These steps of the densification behavior might be attributed to the different nature of the size distribution of pores.

From the above data, it was evident that the presence of silica increased the onset sintering temperature, but retarded the finishing of densification. The increase in the onset sintering temperature should be attributed to the melting of amorphous silica doped in the HA powders that caused a localized increase of the temperature. The presence of a liquid phase bonded the different powder particles together, and accelerated the sintering. Whereas after the formation of the liquid phase, the silica converted into crystalline silicon dioxide that retarded the mobility of hydroxyl groups in the apatite powders, and thus limited the sintering procedures.
Figure 7.8 Dilatometer results of green compacts with a heating rate of 5 °C/min:
(a) densification behavior; (b) shrinkage rates.

7.3.2 Discussion

Shrinkage was governed by the rate of densification and through reduction in
porosity. It was therefore important to mention any correlation between porosity and shrinkage, and also if additional factors such as secondary phases correlated with changes observed for shrinkage. The differences in the microstructure of SD HA and 3SiHA were shown in Figure 7.9. It was found that grain sizes within the 3SiHA specimen were around 2-3 µm, while the grain sizes of the HA specimen were less than 1 µm. Low grain growth indicated that the mobility of pores was higher than that of grain boundaries. The grain size of the silica-containing ceramic was slightly larger than that of HA compact, which might be due to the enhancement of the sintering rate by providing a path for rapid atomic diffusion in the presence of a liquid phase.

A homogeneous distribution of silica in the grain boundaries was very important because the inhomogeneous presence of small amounts of liquid phases gave rise to the abnormally exaggerated grain growth. The segregation of silica into grain boundaries was found to deteriorate the mechanical properties. When silica was added, an amorphous phase was formed in competition with the crystalline material. The amorphous phase locating at the grain boundaries and triple point of the sintered ceramics would impede the transport of oxygen ions from grain to grain. Figure 7.9 (a) showed clearly the impedance effect.

The effects of silica of the densification of apatite should be differentiated from the traditional sintering and spark plasma sintering. During spark plasma processing, silicate was substituted into the apatite structure by the high energy plasma caused between the particle gaps. The particles were heated from both inside and outside of the particles. While, the dilatometer sintered samples were heated from outside to inside, so the thermal energy for compaction could not be as effectively transmitted to the sample as SPS processing. Thus the effect of silica to the densification of apatite
in the dilatometer was different from those obtained from SPS system. In the dilatometer, the significant particle rearrangement caused by the presence of silica would lead to local grain growth during sintering. The preferential sintering of agglomerates would contribute to the coarsening of grain sizes in the silica doped HA ceramic.

Figure 7.9 Microstructures of 3SiHA (a) and SD HA (b) after sintered in the dilatometer.
7.4 Chapter summary

This chapter discussed the effects of various sintering parameters (temperatures, durations, and heating rates) on the densification behavior of RF plasma processed powders. The ultrafine particles in the RF plasma processed powders sintered first at around 500 °C. The sintering temperature influenced the decomposition of HA into TCP and TTCP. The decomposition was detrimental to the densification of the final products. The influence of the silica to the shrinkage behavior of the spray dried powders was also evaluated. Silica was found to have a different role to the densification in the traditional sintering and spark plasma sintering steps. From the results and discussion, not only the densification steps of these powders were concluded definitely, but also the advantages of spark plasma sintering procedure were indirectly revealed.
Chapter 8 In vitro tests of SPS ceramic

Introduction

In vitro and in vivo studies indicated the biologic apatite deposited on the surface of Ca-containing implants [176, 177]. This apatite layer served as a substrate for subsequent protein adsorption and bone cell attachment. The formation of apatite layers depended on direct interactions of bone matrix and osteoblasts with biomaterials. The proteins (cell receptors) adsorbed to the material surface (cell ligands), resulting in cell attachment on the material surface. The surface chemistry and physical factors such as crystallinity, particle size, porosity, topography and surface energy, would directly influence cell attachment, proliferation and differentiation of the cells [178]. It was reported that osteoblasts appeared to be very sensitive to minor variations in surface composition and topography [176]. The evaluation of cell-material (CaP) interactions was of main importance when developing new materials for biomedical application. The inductive response of apatite was complex, and not fully understood. Moreover, the in vitro biocompatibility of the material component had already been assessed by a cell line in order to test cytotoxicity of the materials [179].

Bioceramic structures made of HA were capable of inducing bone growth. However, the main limitation of HA was its poor mechanical properties, which included low fracture toughness and poor strength. Spheroidized HA powder obtained through RF plasma spraying had been sintered using a SPS system with the aim of enhancing its mechanical properties [180].
In the present study, RF plasma processed CaP powders, spray dried silica doped HA powders and HA powders had been employed as feedstock in the sintering process. The biological properties of these sintered compacts had been evaluated \textit{in vitro} immersion in SBF and cell culture work.

\textbf{8.1 \textit{In vitro} tests through simulated body fluids}

\textbf{8.1.1 Results of SBF}

The starting powder was prepared at the working plate power level of 10 kW in the RF plasma. This powder (R10) was sintered in the SPS system at 1000 °C, 1100 °C, and 1200 °C for 3 min. After polishing, the SPS compacts with a diameter of 12 mm were soaked in 15 ml of SBF, and kept at 37 °C in a water bath for various immersion times.

After immersion, the SPS disks were immediately cleaned with deionized water, and dried in air. The ion concentrations of the solutions were measured through the ICP atomic emission spectrometer. An average of three measurements was taken for each solution sample.

The surface morphology of the samples after immersion in SBF was shown in Figure 8.1. The micrograph (a) in Figure 8.1 corresponded to the sample surface before immersion.
Figure 8.1 Bone-like apatite layers on the surface of SPS samples sintered at 1100 °C for 3 min after immersion different periods in SBF: (a) 0 day; (b) 1 day; (c) 3 days; (d) cross-section view of the apatite layer (c); (e) 2 weeks; (f) 4 weeks; and (g-h) high magnification of apatites.
After 1 day of immersion, randomly occurring spherical structures were seen on the sample surface. After 3 days of immersion, the surface was covered with a layer. This thin layer (~5 µm) showed some cracks. No obvious morphological changes in the layer could be distinguished with an increase in the soaking time. Another common characteristic was the formation of cracks of a tortoiseshell character. The morphology of these cracks was similar to those formed naturally on a mud deposit upon drying.

The high magnification SEM micrograph revealed a layer made of a network of plate-like structures, which were in fact re-precipitated apatite. The re-precipitated apatite layer on bioactive materials, known as hydroxy-carbonated apatite, had been extensively studied [181].

![Thin film XRD patterns of apatite layers on SPS compacts sintered at 1100 °C for 3 min after immersion for various periods in SBF.](image)

Figure 8.2 Thin film XRD patterns of apatite layers on SPS compacts sintered at 1100 °C for 3 min after immersion for various periods in SBF.

The formed apatite layers were examined by thin film XRD method. The results were shown in Figure 8.2. The peaks from the XRD patterns matched the peaks of the
HA structure. Comparatively, the intensity of these peaks gradually decreased with the immersion time, which indicated that a partially amorphous Ca-P layer was being formed. The anomalous high intensity peak corresponding to the $[0002]$ reflection revealed that the apatite layer was growing with a preferential orientation along $[0002]$. With an increase in the immersion time, the orientation was more obvious.

![Graph showing Ca and P concentration over immersion periods in SBF.](image)

Figure 8.3 The curves of Ca and P concentration versus immersion periods in SBF.

Three measurements were averaged for each sample.

The calcium (Ca) and phosphorous (P) concentrations after immersion in the SBF were tested using ICP. Figure 8.3 presented the concentration changes in the SBF
solutions as a function of immersion time. Ca and P concentrations decreased in the solution, which suggested that precipitation of Ca and P was taking place leading to the formation and growth of the Ca-P layer. This decrease was more evident in the first two weeks of immersion (known as induction period). During this induction period, the apatite nuclei were growing to form a continuous layer by consuming higher amounts of Ca and P.

8.1.2 Discussion of SBF

According to Kokubo et al [182], the *in vitro* immersion of bioactive materials in SBF was thought to reproduce *in vivo* surface structure changes in materials such as bioactive glass and glass-ceramic. The grown layer was sometimes called a bone-like apatite. The formation of biological apatite on the surface of implanted synthetic calcium phosphate ceramic had been reported to go through a sequence of chemical reactions [183].

The nucleation of apatite at a given temperature could be increased by the following two ways: increasing the supersaturation or lower the interfacial energy at a given supersaturation. Though SBF was supersaturated to apatite, the interfacial of crystalline HA was not low enough to form nuclei of apatite. However, the samples used in this study had been subjected to RF plasma and SPS, which had resulted in the formation of secondary calcium phosphate phases such as amorphous phase, TCP as discussed in Chapter 5. Whereas due to the small amount level, these secondary phases were not obviously detected in the samples. When these samples were immersed in the SBF, dissolution would occur from these secondary calcium phosphates within 24 hrs. $\text{Ca}^{2+}$ and $\text{PO}_4^{3-}$ were released leading to the higher supersaturation of $\text{Ca}^{2+}$ and $\text{PO}_4^{3-}$ in the SBF. The first nuclei formed on the surface of
the sample, where the relatively high supersaturation could be maintained steadily. The surface roughness due to the scratches was favorable for the nuclei to anchor. The favorable micro-environment for nucleation of bone-like apatite must be that concentrated with calcium and phosphate ions in the immediate vicinity of the surface upon which the apatite anchored. The morphological evolution of the apatite layer grown from the rough surface to the smooth hillocks indicated a poly-nuclear growth process, during which new nuclei started rapidly before the proceeding layer growth was completed by the consumption of calcium and phosphate ions in SBF.

Carbonate ions together with other electrolytes, which were from the SBF solution, would also be incorporated in the new apatite micro-crystals forming on the sample’s surface [183].

Along with the formation of HCA, cracks of tortoiseshell character appeared on the newly-formed layer (shown in Figure 8.1). This was probably due to the release of growth strains induced during its formation. As the apatite nano-crystals grew and increased in size, its lattice parameters would be a little different from those of compacts due to the incorporation of new atoms. This difference would result in strains formed within the new apatite. The roughness of sample surface would also contribute to the presence of growth strains to make the new apatite layer connected together. All these growth strains built up gradually, and increased with immersion time. If the new apatite layer could not release them through plastic deformation, it would crack even peel off to release the growth strains.

As observed, the new formed apatite layer had a preferential orientation along [0002] direction in the apatite structure. This could be attributed to the changes of surface zeta potential. The zeta potential was the electrical voltage difference between
the surface of colloid and the suspension liquid. It was caused by the surface charge. Kokubo et al [184] discussed that the zeta potential of the surface of HA varied with soaking time in the SBF solution. When HA was immersed in the SBF, it initially had a negative zeta potential that increased to a maximum positive value within 3 hrs. Then this value decreased rapidly to a negative value within 6 hrs and finally would gradually converge to a constant negative value. The transport of chemical ions to a solid/liquid interface always happened by ionic diffusion through the Nernst diffusion layer. After being delivered by diffusion to the interface, ions were absorbed onto the surface of apatite. There was an adsorption resistance for ions to be adsorbed onto the surface. In order to overcome the resistance, the ions made a diffusive jump toward the surface to a distance corresponding to their size [185]. As shown in Figure 8.4, due to higher concentration of OH\(^{-}\) (when compared with PO\(_4^{3-}\)), adsorption of protons would occur easily. If OH\(^{-}\) from SBF solution bonded to the sample’s surface in such a way that the O-H bond direction was perpendicular to the sample surface and parallel to that O-H on the (000l) planes of HA (c-axis of HA structure). It would lead to the oriented nucleation. The HA crystals would tend to grow along the [000l] direction, which was the fastest growth direction for HA, resulting in a plate-like morphology.

There were two advantages for such an orientated structure as a bone implant in the medical application. First, the orientation was the same as that of HA in natural bone where the apatite was highly oriented with (000l) planes perpendicular to the collagen axis, that was, the [000l] direction was parallel to the collagen axis. Second, the formed apatite was a precursor in the biomineralization of natural bone to enhance biointergration between the ceramic and surrounding bone tissue.
These SBF *in vitro* results presented the biological properties of SPS ceramic. The ceramic could be used in the following characterization through osteoblastic cell lines to investigate the cellular reactivities on the ceramic surfaces.

![Diagram of calcium phosphate bonded material](image)

Figure 8.4 A schematic showing the formation of preferential apatite along [0001] on the SPS ceramic surface in the SBF.

### 8.2 Cell culture

The previous study showed that a biological apatite layer was formed when SPS sample was soaked in SBF [180]. However, despite its importance in the bioceramic field, there remained a need to correlate the chemical and physical composition of hydroxyapatite with cellular interaction. In this study, the cellular activities of bone cells on these disks were studied. These SPS disks were seeded with human osteoblast-like cells to investigate surface-dependent responses of bone-forming cells. The cell morphology was assessed using SEM. The cell proliferation and differentiation was assessed using biochemical techniques.

#### 8.2.1 Sample characterization

Cell culture tests were used to study cell attachment, proliferation on SPS disks. The cell attachment and cell proliferation were observed under SEM. A colorimetric
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assay (MTT) was also used to quantitatively determine cell proliferation and viability on the ceramic. Using the standard curve, the MTT results were converted to living cell number. Before cell seeding, the surfaces nature that could influence the cellular activity and proliferation were characterized through XRD method, contact angle analysis and roughness tester.

Table 8.1 Powder feedstock used and surface roughness of SPS disks.

<table>
<thead>
<tr>
<th>Powder feedstock</th>
<th>ID of ceramic</th>
<th>Surface roughness (Rₐ; nm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pure HA</td>
<td>H</td>
<td>64.5</td>
</tr>
<tr>
<td>RF plasma processed at 15kW</td>
<td>R15</td>
<td>172</td>
</tr>
<tr>
<td>RF plasma processed at 21kW</td>
<td>R21</td>
<td>198.3</td>
</tr>
</tbody>
</table>

The surfaces of the SPS disks before cell culture were characterized for roughness using Surfpak Roughness Tester. The feedstock used for the SPS were listed in Table 8.1. The roughness results exhibited in Table 8.1 provided evidence of increased surface roughness in R15 and R21 compared with H. Webster et al [210] discussed that the dimensions of nanometer or fine surface features gave rise to larger amounts of interparticulate voids (with fairly homogeneous distribution). The increase in the surface roughness led to increased surface area. The previous result [176] had shown better cell attachment on rougher surfaces.

The dispersive components and polar component of SPS ceramic surface energies were listed in Table 8.2. It was found that the polar components of R15 and R21 were slightly lower than that of sample H. According to wettability criteria [211], the decrease in polar species was not favored for interaction between ceramic and bone cells or proteins.
Whereas, not only the polar components of ceramic surfaces but also the surface topography and chemical composition should be taken into account the influence of the cell response. The increase in the surface roughness led to the increased surface area. An increase in surface area correlated to an increase in osteoblast adhesion and proliferation. Thus, the combined effect of surface energies and surface roughness would contribute to the cell attachment on the ceramic surface.

Table 8.2 Surface energies of different SPS disks.

<table>
<thead>
<tr>
<th>Ceramic</th>
<th>Dispersive component (mJ/m²)</th>
<th>Polar component (mJ/m²)</th>
<th>Total surface energy (mJ/m²)</th>
</tr>
</thead>
<tbody>
<tr>
<td>H</td>
<td>32.23</td>
<td>15.81</td>
<td>48.04</td>
</tr>
<tr>
<td>R15</td>
<td>31.88</td>
<td>15.35</td>
<td>47.23</td>
</tr>
<tr>
<td>R21</td>
<td>33.75</td>
<td>15.17</td>
<td>48.92</td>
</tr>
</tbody>
</table>

Five measurements were averaged for each sample.

8.2.2 Cell culture results and discussions

8.2.2.1 Morphology of osteoblasts

After 2 days culture on the disks, it was found that the cells had attached on the sample surface. The typical osteoblast morphologies under SEM were exhibited in Figure 8.5 after fixing. The majority of the cells had a flattened appearance with a predominantly polygonal morphology. A rough texture was observed due to the presence of numerous blebs on the surface of the cells. The cells were attached to the biomaterials exhibiting filopodia-like with various lengths and lamellipodia-like extensions. Close contact between the cells and the substrate was established through extending filopodia, which initiated cellular anchorage on the substrate. This result
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indicated that a bioactive surface provided preferential sites for cell attachment and in return, recruited proteins and other growth factors to enhance cellular activity.

Osteoblasts were anchorage-dependent cells, and their morphology was determined by the properties of the substrate. The flatten appearances indicated a high affinity to the substrate surface. The more flatten cells would produce more collagen than those less flattened cells. Depending on cell types, filopodia developed from lamelliapodia provided cell a more defined direction to migrate [186].

The initial point of contact between the cell and the substrate has been shown to be a random process that was largely determined by the distribution of adhesion proteins adsorbed onto the surface of the biomaterials [187]. Serum was known to contain the glycoproteins fibronectin and vitronectin, and these were known to adsorb onto the surfaces of biomaterials [188]. Furthermore, exclusion of these proteins from cell culture had shown to cause a decrease in the total number of cellular attachments on biomaterials [189]. Cells used these proteins as anchoring points that enabled them to attach to the substrate through extending filopodia, followed by the ‘zipping-up’ of the cytoplasm between the filopodia to form flattened regions.

Cells interacted with the adsorbed proteins through membrane integral proteins (integrins) localized at the region of focal contacts. Integrins acted as signal transducers linking the extracellular matrix to the intracellular protein, in particular cytoskeleton actin (a protein in muscle) filaments forming stress fibers, which in return regulated cellular morphology and gene expression.
8.2.2.2 Cell proliferation on SPS compacts from RF plasma processed powders

The recruitment of osteoblastic cells played a crucial role in osteogenesis (bone growth) [179] since the bone formation depended mainly on the number of osteoblastic cells rather than the osteoblast activity. The data from MTT assay reflected the ability of cell proliferation and cytotoxicity on the ceramic. A total of

Figure 8.5 SEM images of human osteoblast-like cells on the SPS disks.
five points were averaged for the MTT data. The higher the MTT results, the more the viable cells.

As shown in Figure 8.6, the RF plasma processing samples could effectively increase the cell proliferation rate compared with the sample H. The higher the RF plasma power level, the higher the cell proliferation.

It was known that the physicochemical features of the ceramic surface could affect the reorganization of proteins on ceramic specimen and change the profile of adsorbed proteins. The differences in protein adsorption could result in very different initial cellular behavior. Surface roughness improved surface wetting properties. This could affect directly cell attachment via enhanced formation of focal contacts or through selective adsorption of serum proteins required for cell attachment. The rougher R21 ceramic surface helped adsorb more proteins compared with HA and R15 samples. Thus, it enhanced osteoblast functions and cell proliferation.

It had been demonstrated that osteoblast-like cell behavior such as cell attachment efficiency, spreading as well as cell migration was also dependent on the materials surface chemistry [187] and the concentration of Ca ions in the culturing medium [190].

The instability of TCP and TTCP in the R15 and R21 disks was thought to be a factor that accelerated osteoconductivity, and improved the chemical affinity and connectivity with the bone tissue in vivo [190]. Referred to the peaks intensities in XRD patterns as shown in Figure 8.7, there were larger amount TTCP, α-TCP and CaO in R21 than those in R15. The comparative dissolution behavior [104] of TTCP, HA, β-TCP and α-TCP in increasing order was shown below:
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HA<β-TCP<α-TCP<CaO<TTCP

In the present study, the dissolution of Ca ions from the ceramic surfaces, resulted in an interfacial supersaturated condition with the already present Ca ions in the medium. The supersaturation of Ca ions stimulated consequently the proliferation of osteoblasts [190] when compared with sample H. While, no obvious precipitation of carbonated apatite was observed on the sample surface after the cell culture. This may be ascribed to the more acidic micro-environment produced by osteoblasts, in which the amorphous phase dissolved at an accelerated rate [191].

However, excessively high solubility and reactivity of bioceramic surfaces might result in damage to adherent cells, which might stimulate correspondingly inflammatory responses in surrounding tissues [190]. The high solubility of the TTCP and TCP was found to be the dominant factor. They would decrease the viability of the cells by causing their rupture during initial anchoring phase. The failure of the initial attachment between the surrounding tissues and the implant materials might cause acute inflammation, and thus delay wound repair.

Figure 8.6 MTT results of the SPS disks after cell culture for 2 and 4 days. Cell seeding density: 2x10^4/cm². Four measurements were averaged for each sample.
8.2.2.3 Effects of silica on the cell proliferation and cell differentiation

It had been reported that nucleation of bioactive apatite around silicate-substituted HA was enhanced by increased dissolution of calcium and phosphate ions from the implant [192]. It was also important to emphasize the effect of silica on cell behavior on SPS disks. This study investigated the cell attachment, proliferation, differentiation and cytotoxicity assessment on these silica dope HA \textit{in vitro}. The cells cultured on the sterile plastic Petri dish were used as a positive control group that confirmed the assay conditions were accurate.

As shown in Figure 8.8, MTT results provided the evidence of increased osteoblast viability on the silica doped disks. It was also found that the osteoblast viability on the disks was slightly higher than those cultured in the Petri dish after culturing for 2 days. While after 4 days culture, the results from sample HA and 1wt\% silica doped HA (1SiHA) were lower than that of reference number.
Figure 8.8 Relative osteoblast viability on the SPS disks doped with different amount of silica. Cell seeding density: $2 \times 10^4$/cm$^2$. Five measurements were averaged for each sample.

The secretion of AKP was analyzed and presented in Figure 8.9, it was found that similar amount of AKP was secreted in the medium after culture for 2 days. An increase in cell proliferation was detected with an increase in the dopant level of silica. While, the highest cell differentiation levels were detected in the 1SiHA sample after 6 days culture.

The presence of silica played an integral role during the bone mineralization processes. It was a fundamental constituent of collagen and as being an essential element in the formation in the formation of collagen. Other study had elaborated the role of silicon or silica in osteoblast function [194]. The effects of orthosilicic acid on collagen type I synthesis had demonstrated the enhanced differentiation of osteoblastic cell lines exposed to orthosilicic acid. As discussed in chapter 6, the presence of soluble silicate caused the rapid release of silicate and Ca$^{2+}$ ions leading to rapid bone cell proliferation. Silicon release, when compared with pure HA ceramic, had shown to raise AKP expression in human osteoblast-like cells. However, as
discussed previously, too much release of Ca\(^{2+}\) caused by the presence of TCP would also result in the increased inflammatory effect to the osteoblasts, which would suppress the secretion of AKP, as shown in Figure 8.9.

Figure 8.9 Effect of silica to the relative secretion of AKP on the silica dope HA disks. Cell seeding density: \(2 \times 10^4/\text{cm}^2\). Four measurements were averaged for each sample.

8.3 Chapter summary

In the simulated body environment, these SPS sintered compacts showed excellent bioactivity. A plate-like apatite layer was formed on the surface of compacts. These apatite crystals grew with the (0001) planes. Their c-axes were roughly consistent with
the assembled state of bone. Cell culture results indicated that osteoblasts grew well on the sample surfaces with cells appearing well-flattened and maintaining their polygonal morphology. Furthermore, the results indicated that ceramic surface chemistry contributed mainly to the cellular attachment on the SPS disks. In addition, highest cell growth was observed on the SiHA disks that also displayed relatively high cell differentiation compared with the samples without doping silica. In conclusion, the \textit{in vitro} results demonstrated that the presence of silica improved generally the cell proliferation and differentiation. This behavior could be explained by the surface characteristics affecting the selective adsorption of serum proteins.
Chapter 9 General Discussion

HA and generally bioactive calcium phosphate materials have received considerable attention as materials for implants and bone augmentation procedures, because they chemically bonded directly to bone when implanted, resulting in the formation of a strong bone-implant interface [4, 26, 27, 30]. The plasma sprayed HA and other calcium phosphate materials were the major applications in the load-bearing conditions. The nanometer characteristics, necessary to create an optimal implant, have not yet been fully investigated and used in the industry. The successful industrial application of the RF induction plasma process helped to transfer the technology from laboratory to industrial scale application. The present work was a project aiming at studying the RF induction plasma sprayed HA that had a nanometer characteristic. The results presented in Chapter 5 indicated that the RF plasma spray was capable of developing nano-structured powder particles from starting spray dried HA with attractive properties.

Figure 9.1 presented the HA powder, before and after application of the RF induction plasma spheroidisation processing. The particle size of RF plasma processed powder became smaller compared with the starting powder, which was caused by powder densification via plasma processing. Many ultrafine particles were also detected on the surfaces of the spherical particles. The presence of these ultrafine particles was attributed to the vaporization and condensation of melting part of the surface materials of starting powder.

The RF plasma processing removed preferentially the lower melting point component in the starting HA, with the amount depending upon the heat transfer. The
change in chemistry occurred on the outside of the molten particles where the heating conditions were the most intense and phosphate removal was easiest. Thus, a partial decomposition of the starting HA occurred in this study, which was due to the combined effect of high temperature and the residence time in the plasma. It was found that HA had decomposed into different calcium phosphate phases that contained TTCP, TCP and CaO. The amount of these phases depended on the extent of heat transfer and was dictated by particle size, particle velocity, and location within the flame of the traversing particles. The relatively small particle size (~20 µm) of the starting powder in the dehydroxylated state promoted the decomposition.

![Before and After images](image)

Figure 9.1 HA powder became dense and spherical after treatment by RF induction plasma spray.

Many nano particles were obtained in the RF plasma sprayed powder, as shown in Figure 9.1. These nano particles were examined using TEM. Figure 9.2 presented the diffraction pattern from these particles. The diffuse scattered ring around the bright central spot and distinct diffraction maxima indicated that the predominant phase in the ultrafine particles was amorphous. The presence of amorphous phase was attributed to the rapid cooling rate after plasma processing and it played an important role in the formation of biominerals [109, 130]. This amorphous phase could be converted into apatite structures whereas apatitic structure of HA crystals remained
unchanged even the implantation periods were prolonged. The rate of new bone formation on the apatites converted from amorphous calcium phosphate implants was much greater than that on HA crystals implanted directly into the body.

![Figure 9.2 A TEM morphology (a) and a selected area diffraction pattern of nano particles prepared in RF plasma.](image)

We have investigated the spark plasma sintering ability of the RF plasma spheroidized HA powders. The results showed that the RF plasma sprayed powder could be effectively sintered to high densities within 3 minutes by a SPS system. The highest relative density was around 97% to the theoretical density of stoichiometric HA. Gu et al [148] commended that the short duration required for SPS sintering was presumably due to the cleaning of the particle surface prior to sintering. The high current input also contributed to the activation of the powder particle surface that enhanced diffusion during subsequent densification.

Due to the high surface energy of the nano particles, the ultrafine particles in the RF plasma sprayed powder started to sinter at lower temperature than the large particles. Additional information on sintering ability was provided by a dilatometer sintering system, as discussed in Chapter 7. The results indicated that the nano particles in the RF plasma processed powder began to sinter at around 500 °C whereas the large particles started to densify at around 1100 °C. The different calcium
phosphate phases experienced complicated chemical reactions during sintering at low water pressure level. TCP and CaO reacted together to form TTCP. Also, a chemical reaction between TCP and TTCP occurred to form the oxyapatite whose chemical structure was similar to that of stoichiometric HA. $\alpha$-TCP (monoclinic) transformed to $\beta$-TCP (orthorhombic) after sintering in the temperature range of 1000-1100 °C. $\beta$-TCP changed at approximately 1120 °C to the high temperature phase $\alpha$-TCP. These combined chemical reactions contributed to the different phases in the final ceramic after sintering.

It was also found that the densification of RF plasma sprayed powder started at higher temperature than the spray drying powders. The difference of sintering ability should be attributed to the different characters of starting feedstock used for SPS. Spray dried powder were loose particles whereas the RF plasma spheroidized HA powder were dense particles. The intra-agglomerate pores formed dominantly in the spray dried powder. This type of pore was similar to the particle size, which was readily eliminated at low sintering temperature. Since the low fraction of fine particles in the RF plasma sprayed powders, the inter-agglomerate pores in these denser particles would control predominately the sintering. Therefore, these pores could not be removed immediately, and needed to be sintered at high temperature.

The present investigation on the in vitro behavior of the ceramic prepared using the RF plasma sprayed powder suggested the improved bioactivity by way of facilitating the formation of apatite layer with a preferential orientation along $[0002]$ direction. The high bioactivity of HA ceramic prepared by SPS might be ascribed to result from a $\text{OH}^-$ and/or $\text{Ca}^{2+}$ ion deficiency caused by (i) a localized increase in the temperature at grain boundary of the matrix; (ii) a small presumably electric poling effect by
resulting a weak polarization during the spark plasma sintering treatment.

The osteoblast cells cultured on the various SPS calcium phosphate surface displayed normal morphological features. They had attached and undergone spreading on all the surfaces. The relationship between the osteoblast cells and the ceramic surface was influenced by the surface characters, such as surface topography and surface chemistry. The instability of TCP and TTCP in the ceramic prepared from the RF plasma sprayed powder stimulated the cell proliferation rates compared with the pure HA ceramic. Thus, the adhesion on pure HA ceramic surface was slower than on the ceramic prepared from RF plasma sprayed powder.

The similarity of HA to bone mineral led to the extensive use of HA as a bone grafting material in hard tissue implants. Silicon levels up to 0.5 wt % were observed in the active growth areas of rats, such as the osteoid. The current study was carried out to introduce different amount of silica (0, 1, 3, and 5 wt %) into the starting HA slurry to get homogenous distributed silica. It has been expected that the high energy source, such as RF plasma and spark plasma, was able to promote the silicon substitution possibility into the apatite structure. The results indicated that silicate substitution in apatite was obtained, while not all of the silica reacted with apatite. Around 30 atomic% of the silica has substituted into the apatite structure. To maintain the charge balance, some hydroxyl vacancies must be also introduced in the silicate substituted apatite. The release of hydroxyl group resulted in the formation of pores in the ceramics. The higher the dopant level, the more the presence of pores.

Additionally, it was found that the presence of silica destabilized the thermal stabilities of HA accelerated the decomposition into other calcium phosphate phases. The main secondary calcium phosphate phase was β-TCP. A local increase in
temperature resulted in the formation of some liquid phase of silica, which corresponded to reduce interfacial energy. Therefore, the transformation of apatite into TCP was promoted. This meta-stable $\beta$-TCP would exist near to the pores.

The *in vitro* tests with regard to osteoblast cell proliferation and differentiation were performed to evaluate the biocompatibility of the silica doped HA ceramic. The results from Chapter 8 suggested that rapid bone cell proliferation was obtained, which was due to the rapid release of $\text{SiO}_4^{4-}$ and $\text{Ca}^{2+}$ from the ceramic. This was attributed to that the incorporation of silicate ions into the HA structure increased the number of defect structures. The defect structures were the specific sites that were most favorable to dissolution. Therefore, by increasing the number of defect sites, the solubility of the HA in a biological fluid is increased, as was its rate of cell proliferation.

Finally, to conclude, the most important research contributions made by this study were that it provided evidences in the following three aspects:

1. Confirmation that RF plasma spray could be used to produce nano-structured calcium phosphate powder that had great potentials for industrial applications;

2. Establishment of evidence that spark plasma sintering could be used to produce bioceramic with enhanced bio-mechanical properties;

3. Explanation of the effect of doping silica (~5 wt %) in affecting the densification behavior and biocompatibility of dense calcium phosphate ceramic.
Chapter 10 Conclusions and Recommendations

10.1 Conclusions

The present work was a project aimed at studying and characterizing the nano-structured bioceramic products through a spark plasma sintering system. Generally, the results have demonstrated that the nano-structured calcium phosphate (CaP) powder was synthesized through an inductively coupled radio frequency (RF) plasma system that employed spray dried powder as feedstock. This powder can be densified to high density (98% to theoretical density) using an SPS system. The final bioceramic products were analyzed through mechanical and biological properties to evaluate the potential biomedical applications in the future. Therefore, five conclusions can be drawn from this study:

1. The RF plasma technique showed its capability of spraying nano-structured calcium phosphate powders with suitable phase composition. The RF plasma sprayed powder exhibited different particle size distribution arising from the imperfect evaporation of feedstock in the plasma flame. It was found that the characteristics and residence time of the feedstock in the RF plasma flame determined ultimately the extent of starting HA decomposition.

2. The Rietveld analysis revealed that there was around 75 wt % of amorphous phase in the RF plasma sprayed ultrafine particles. The effect of the working plate power of the RF plasma system on the various decomposition trends was suggested to be due to the combined influence of the plasma flame temperature and flame velocity.
Conclusions

3. The recrystallization behavior of the amorphous phase in the ultrafine powder was determined by DSC to be ~650 °C. After heat treatment at 800 °C in air, β-DCP and HA phases were found to be dominant in the ultrafine powder. The presence of β-DCP was attributed to the direct decomposition of HA feedstock in the RF plasma flame followed by the rapid cooling rate.

4. The spark plasma sintering technique demonstrated its capacity to densify effectively the calcium phosphate powder to high densities with enhanced bio-mechanical properties. The introduction of silica (~ 5 wt %) resulted in the decomposition of HA into other phases after sintering. The Raman spectra, lattice parameters and XPS results proved the possibility of silicate substitution in these SPS ceramic and the RF plasma processed silica doped powder.

5. The in vitro test revealed the excellent bioactivity of the SPS compacts immersed in the simulated body environment. A plate-like apatite layer was precipitated on the surface of compacts during the in vitro ageing. These apatite crystals grow with the (0001) planes assembling the state of natural bone, which could regulate the biomineralization in vitro. In addition, the in vitro cell culture results claimed that osteoblast cells displayed cellular attachment and proliferation on the ceramic surface. In addition to surface roughness, the ceramic surface chemistry affected mainly cellular responses, enhancing cell adhesion and proliferation. Moreover, the in vitro cell culture results also revealed that the introduction of silica into apatite structure enhanced the cell proliferation and differentiation.

10.2 Recommendations

This project has successfully prepared nano-structured biomaterials using a radio
frequency plasma technique. On the basis of information presented in this thesis, the author concentrated on the assessment of the new nano-structured bioceramic using traditional analysis methods. There were several recommendations for the future studies in this field:

1. Future researchers should give careful consideration to the working plate power levels of the RF plasma system. The judicious selection of the working parameters of RF plasma and SPS systems may influence significantly the bio-mechanical properties of the final products.

2. In addition to the currently required characterization, broad assessments should be conducted of the silicate substitution possibilities in these new biomaterials.

3. The further analysis of medium after cell culture need to be carried out, which will provide information on the *in vitro* behaviors of these SPS ceramics.

4. An evaluation of the strengths and weaknesses, short-term and long-term successes and failures through the *in vivo* study would be extremely helpful for the medical application in the human bodies.
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