JOURNAL OF MATERIALS SCIENCE: MATERIALS IN MEDICINE **8** (1997) 803–807

Effect of crystal seeding on the hydration of calcium phosphate cement

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In this paper, the effect of crystal seeding on the hydration of calcium phosphate cement (CPC) has been carefully investigated. The setting time of the CPC slurry not containing any crystal seeds was 150 min, while the setting time for the specimen containing 5 wt % low crystallinity hydroxyapatite used as a crystal seed was 7 min. This improvement in the setting time was due to HAP serving as a substrate for heterogeneous nucleation which accelerated nucleation. In addition, the compressive strength of the specimen containing the crystal seeding was deduced and we report values different from those previously reported in the literature. The calorimetric curve indicated that crystal seeding could reduce the induction period. A.c. impedance spectroscopy revealed that at the beginning of hydration, the rate of reaction increased and also that the mean diameter and porosity decreased as the seed content increased. At the end of the hydration reaction the situation was changed with the mean diameter and porosity in the sample without any seeds being a minimum, which indicated that the compressive strength was a maximum. This result could be explained by the dissolution and reprecipitation of small hydration products produced by the high rate of reaction produced by the introduction of the crystal seeds.

1. Introduction

A calcium phosphate cement (CPC) composed of equal-molar amounts of anhydrous dicalcium phosphate (DCPA) and tetracalcium phosphate (TECP) has been recently developed [1, 2]. This CPC could be used in the repair of bone defects or the restoration of anatomical structures in orthopaedic or reconstructive surgery due to its combination of a high biocompatibility and fast self-setting under ambient conditions. The final product of hydration was hydroxyapatite [3, 4] (HAP) which was similar in composition to the mineral component of mammalian hard tissue. The hydration process of CPC was gentle but complex, involving the dissolution of particles, precipitation of product from solution, and reaction and diffusion on the particle surface. It is affected by many factors such as phase composition, particle size, additives etc. A few studies concerning the effect of various parameters on the hydration of CPC have been performed [5], but any effect of crystal seeding on the hydration reaction is currently unclear. Fukase *et al.* [3] and Chow [5] have suggested that the presence of a crystal seed could reduce the setting time and increase the compressive strength, however we will show in the current paper that this is not the case. Moreover, none of the papers currently published in the literature have considered the effect of crystal seeding on the hydration reaction.

The purpose of this work is to identify the influences of a seed crystal on the CPC hydration reaction and the physico-chemical properties of the final products. In order to clarify the mechanism by which the seed crystals influence the hydration, an isothermal conduction calorimeter was used to investigate the reaction rate and a.c. impedance spectroscopy was used to probe the continuous changes in the microstructure.

2. Materials and methods

All the calcium phosphates used in this experiment were synthesized in our laboratory and were single phase materials as determined using X-ray diffraction. The HAP and β-tricalcium phosphate (β-TCP) were prepared with a particle size of 10–20 nm by a precipitation method [6]. Highly crystallized HAP was obtained by heating the low crystallinity HAP at 850 °C for 2 h. The DCPA was prepared by dehydrating $CaHPO_4 \cdot 2H_2O$ by heating samples at 120 °C for about 6 h. The TECP was prepared by heating an equimolar mixture of DCPA and $CaCO_3$ at 1500 °C for 24 h, which was subsequently ground to obtain a suitable particle size. The DCPA was ground in anhydrous ethanol followed by drying at 80 °C. The specific surface areas (SSA) of the TECP and DCPA were 0.732 and 6.90 $m^2\,g^{-1}$, respectively (measured using a BET instrument ASAP2400, Micrometritics Co.).

Selected paper from the 18th European Conference on Biomaterials, Göteborg, Sweden.

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The solid phase consisted of an equimolar mixture of TECP and DCPA. In some samples, hydroxyapatite or β-TCP were introduced as seed crystals to accelerate the rate of the setting reaction. No other additives were used, since nothing else was expected to produce any significant effect on the composition of the cement.

To prepare a slurry specimen, 0.6 g of CPC powder was mixed with 0.15 ml of water so that the powder-to-liquid ratio (P/L) was 4.0 (wt/vol), which is the optimum value determined in a previous study. The solid and liquid were stirred using a stainless steel spatula to form a paste in 1 min.

2.1. Setting time measurement

The slurry was loaded into a glass tube with a diameter of 6 mm and a height of 12 mm. The top and bottom surface of the tube were then tightly covered with two sheets of plastic film held by a "C"-clamp and stored at 37 °C in a 100% humidity box for hydration. The samples were tested at various time intervals using a vicat needle and the time taken until the needle could only penetrate less than 1 mm into the sample was taken as the setting time.

2.2. Compressive strength measurement

The paste was loaded into a stainless steel mould with a diameter of 6 mm and a height of 12 mm with periodic packing by means of a 5.6 mm diameter stainless steel rod. The force applied to the rod during packing was 19.6 N corresponding to a pressure of 8.0×10^5 Pa which is the same conditions as those used by Fukase *et al.* [3]. The specimen was removed and placed in a glass tube (8 mm diameter × 20 mm height) which was then sealed with plastic film and stored at 37 °C in a 100% humidity box for 48 h, at which point the hydration reaction should be completed [4]. The compressive strength of the hardened specimens was then measured at a loading rate of $1\ \mathrm{mm\,min^{-1}}$ using a universal testing machine (AG-2000A, Shimadzu Autograph, Shimadzu Co. Ltd, Japan). Five specimens were measured at each crystal seed content.

2.3. Isothermal conduction calorimetry

The hydration rate was determined by isothermal conduction calorimetry. In this method, 5 g of CPC powder were placed in the calorimetric cell and 5 ml of the water was poured into a solution cell. After 24 h pre-equilibrium the rates of heat evolution produced by the mixing of the liquid with the solids and subsequent hydration reaction were measured.

2.4. Impedance measurement

The fresh CPC paste was placed in a 4.2 × 1.0 × 1.0 cm cell in which 1 mm thick stainless steel electrodes were attached at each end to make the distance exactly 4 cm. Then the sample contained in the cell was incubated in a 100% relative humidity environment. Impedance data on hydration at different conditions were collected using a potentiostat/galvanostat (Model M273) and a lock-in amplifier (Model 5210, EG&G Princeton Applied Research Co., USA). The measurements were made logarithmically in frequency over the range of 100 kHz to 0.1Hz with 5 readings per decade.

3. Results and discussion

3.1. Effect of crystal seeding on hydration rate

The setting time of the CPC slurry could reflect the hydration rate. Table I indicates the effects of using different materials as the crystal seed on the reaction rate. The content of the seeding materials was always 5%.

Table I indicates that low crystallinity HAP is very effective as a crystal seed, but that β-TCP had little effect on the hydration reaction. Thus low crystallinity HAP was chosen as the crystal seeding material and its influence on the hydration process was studied.

Fig. 1 shows the effect of seed content on setting time. It can be seen from Fig. 1 that adding a small amount of seeding crystals significantly lowers the setting time. The higher the seeding crystal content, the shorter the setting time, although the extent of the lowering of the setting time became smaller as the seed content increased. These results can be explained if the crystal seeds act as substrates for heterogeneous nucleation. Although DCPA and TECP can also act as substrates for heterogeneous nucleation, the ability to promote nucleation was limited by their intrinisic properties.

TABLE I The effect of different materials on the hydration rate

Material	Setting time (min)
No seed	150
β-tricalcium phosphate	140
Highly crystalline hydroxyapatite	17
Low crystallinity hydroxyapatite	7

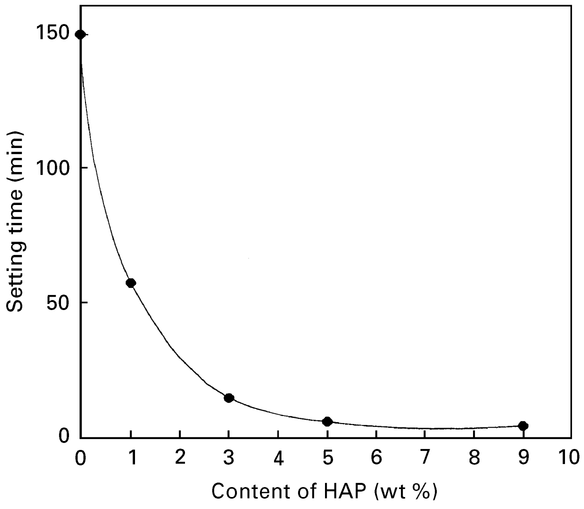

Figure 1 Relationship between the crystal seed content and setting time.

The rate of nucleation in a unit area of substrate can be expressed as:

$$I^{h} = K^{h} \exp\left(-\frac{\Delta G_{k}^{h}}{RT}\right) \tag{1}$$

where I^{h} is the rate of nucleation in a unit area of substrate, K^{h} is a constant irrespective of substrate, R is the universal gas constant, T is temperature and ΔG_{k}^{h} is the potential barrier for heterogeneous nucleation which can be expressed as

$$\Delta G_{k}^{h} = \Delta G_{k} \frac{(2 + \cos\theta)(1 - \cos\theta)^{2}}{4} \tag{2}$$

where ΔG_{k} is the potential barrier for homogeneous nucleation, θ is the contact angle between the crystal nuclei and the substrate.

When the substrate is identical to the nucleating crystal, $\theta = 0$ and consequently $\Delta G_{k}^{h} = 0$ (the potential barrier to nucleation does not exist), however when significant differences exist between the substrate and the nucleating crystal i.e., $\theta = 180^{\circ}, \Delta G_{k}^{h} = \Delta G_{k}$, they cannot interact and so no acceleration of the nucleation, can occur. Using this argument we can suggest that β-TCP was unable to reduce the setting time since it was structurally different from the hydration product, whereas HAP, which is compatable with the CPC product, could decrease the potential barrier for nucleation and therefore accelerate nucleation and growth of the product. The rate of nucleation depends on the area of the substrate in that the larger the substrate area the quicker is the rate of nucleation. Because an increase in the HAP content only raises the substrate area linearly, the extent of the reduction in the setting time is comparatively small. After the point where the surface area of the crystal seed is sufficient for crystal growth, any further increase in the seed content does not result in an increase in the growth rate of the product and the setting time does not further decrease.

The rate of heat evolution during hydration, which reflects the reaction rate of the hydration, was obtained by microcalorimetry (Fig. 2). All the measured curves were similar in that there was an initial period of rapid heat liberation after mixing. This initial peak was associated with wetting, initial dissolution and the establishment of critical degrees of supersaturation. Following the initial peak there was a main reaction peak. The peaks were separated by an induction period. Compared with the behaviour of the sample without any seed, the main reaction peak for the seeded samples shifted to a shorter time and began at a time when the initial peak was still occurring. This indicated that crystal seeding could greatly reduce the induction period.

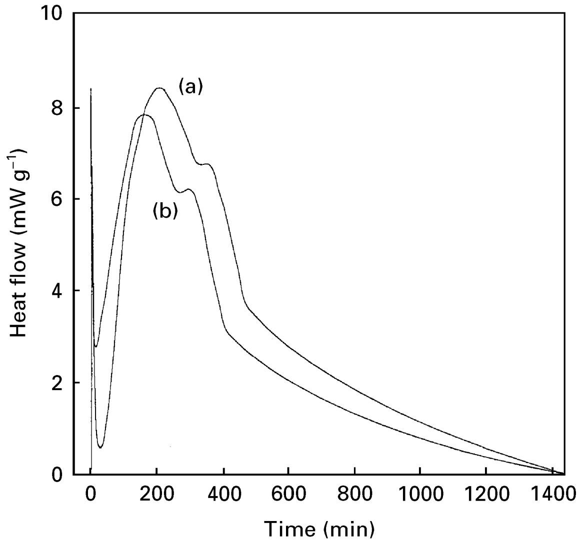

Figure 2 Calorimetric curves showing the rate of heat liberated at a temperature of 37 °C for (a) no seeding and (b) 3 wt% HAP.

3.2. Effect of crystal seeding on the compressive strength

The effect of seeding on the compressive strength was investigated and the results (Fig. 3) showed that the compressive strength significantly decreased when crystal seeds were present as compared to the values for the sample without seeds. It should be noted that the effect was comparatively small as the seed content increased. In the system without crystal seeds, the low rate of hydration resulted in a number of thick particle products that appeared after a while. The greater the aspect ratio of the aciculate products, the more easily they tangled, and the higher the strength of the hardened paste. The presence of crystal seeds greatly accelerated the nucleation. In a system of constant supersaturation, the acceleration of the nucleation means that the growth is limited, which resulted in the formation of smaller sized and lower aspect ratio aciculate products. Thus, the measured strength values decreased in seeded samples. It can be concluded from these observations that the effect of the seed content on the rate of nucleation is much smaller than the actual seeding of the sample. Therefore the measured strength values gradually decreased with an increase in the seed content. These results are checked by microstructural examination using a.c. impedance spectroscopy as will be discussed in the next section. It should be noted that our results are different from

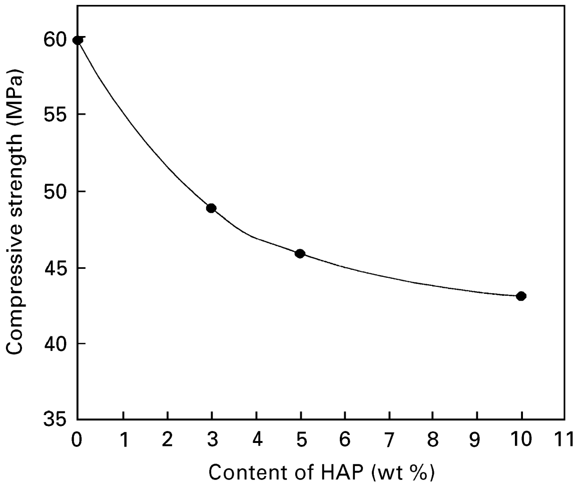

Figure 3 Influence of crystal seed content on the compressive strength of CPC.

these of Fukase *et al.* [3] and Chow [5] who have suggested that an increase in the crystal seed content would improve the strength, but did not provide any detailed data or explanations to support their viewpoint.

3.3. A.c. impedance spectroscopy at various crystal seed contents

A.c. impedance spectroscopy has been shown to be an effective probe to monitor the continuous changes in the microstructure of calcium phosphate cement during the hydration process [8]. Based on the principle of the double layer charge in the electrical field a value for R_2, which is the intercept on the real axis of the a.c. impedance spectrum, is in inverse proportion to Pr_0 (porosity × mean pore size).

Figs 4 and 5 show the impedance spectra obtained for the hydration of CPC at different crystal seed contents at various intervals and they provide values for R_2. It could be observed that the value of Pr_0 for the CPC paste containing seed crystals was less than that in the sample without seeds during the initial stage of hydration, with the difference being most noticeable in the sample containing 7 wt % of seeding crystals. This is because the crystal seeds accelerated the hydration reaction and the faster reaction caused more hydration products to form concurrently. This resulted in a strengthening of the connections between the grains and hence decreased the porosity. After 3 days of hydration, the maximum value of Pr_0 was observed in the sample with 7 wt % of seeds and the minimum value for Pr_0 was observed in the sample without any seeds. This observation can be explained by the seeding increasing the amount of nucleation and thus producing many metastable small particles. During hydration, the samples underwent a dissolution–reprecipitation process which destroyed the original microstructure. The greater the rate of hydration, the more obvious is this effect. Therefore the order of samples in the spectra changed after 24 h and 72 h.

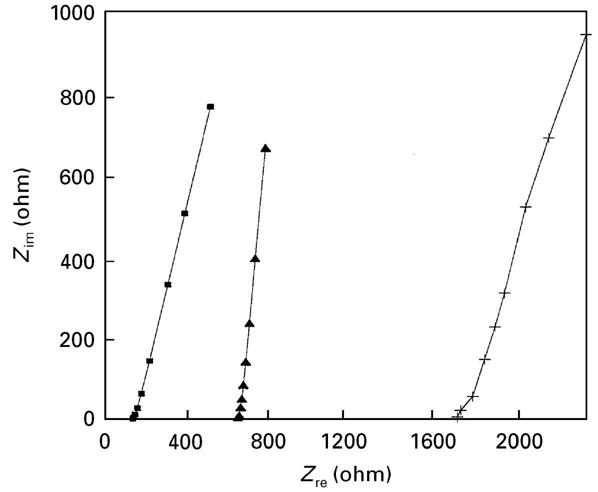

Figure 4 Impedance spectra of hydrating CPC after 3 h at seed contents of: (■) 0 wt %, (▲) 3 wt % and (+) 7 wt %.

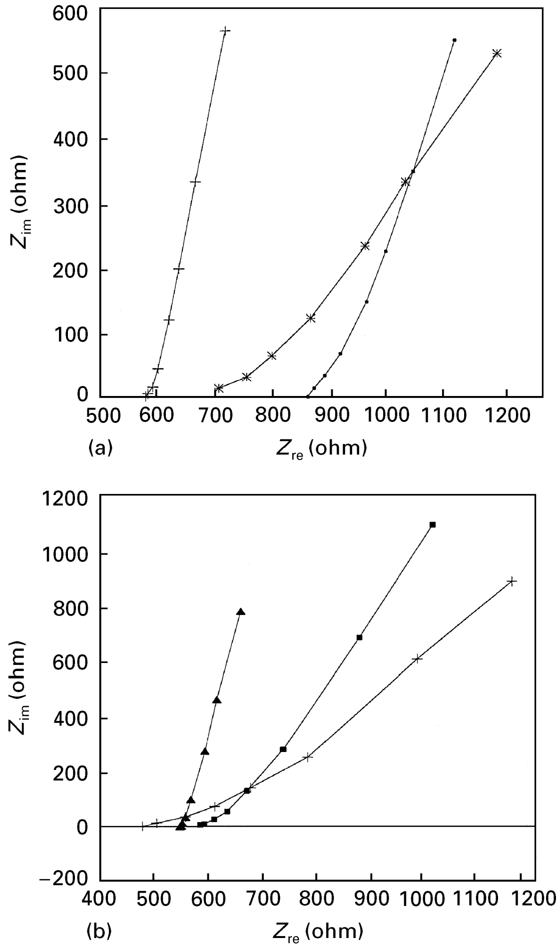

Figure 5 Impedance spectra of CPC samples after (a) 24 h and (b) 72 h. Key: (a) seeding contents of: (●) 0 wt %, (+) 3 wt % and (∗) 7 wt % and (b) seeding contents of: (■) 0 wt %, (▲) 3 wt % and (+) 7 wt %.

In Fig. 5, it is clear that not only is the order of the impedance spectra of the samples altered, but also there is a noticeable deviation from straight line behaviour. The reasons for this deviation should be investigated in future work.

4. Conclusion

The presence of seed crystals of hydroxyapatite in CPC increases the rate of hydration since it acts so as to lower the potential energy barrier to nucleation and also reduces the induction period, with the setting time decreasing with an increase in the seed content. However the seeding of CPC results in a lowering of its compressive strength, a point which was verified by a.c. impedance spectroscopy which is an effective probe to monitor the continuous changes in the microstructure of calcium phosphate cement during hydration.

Acknowledgement

The support of the National Natural Science Foundation of China is gratefully acknowledged.

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Received 5 May
and accepted 22 August 1997