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Mechanical properties of three different compositions of calcium phosphate bioceramic following immersion in Ringer's solution and distilled water

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Abstract Dissolution tests were carried out to compare the mechanical properties of calcium phosphate based bioceramics with different compositions, before and after ageing for various time periods in Ringer's solution (pH 7.2) or distilled water (pH 7.2 and 4.0) at 37°C. The results indicate that the sample composition seems to have more of an effect on the mechanical properties than does the storage environment. No obvious decrease in mechanical properties was found after samples had been aged in the various solutions during the different time periods. This indicates that these samples could be of significant clinical interest as their good structural properties were retained.

1 Introduction

Dissolution tests on calcium phosphate bioceramics can be used to assess their potential in vivo bone bioactivity [1]. Generally such tests have been carried out in simulated body fluid (SBF) [1–3], a solution with ion concentrations and pH value similar to those of human blood plasma, or water [4–6]; the majority utilise a standard pH of 7.2–7.4. It has been reported that the dissolution rate of β-tricalcium phosphate based bioceramics (β-TCP) was 3–12 times faster than that of stoichiometric hydroxyapatite (HA) [7]. In vitro studies also revealed that the α-TCP had a higher dissolution rate than β-TCP. Thus the diminishing order of relative solubility has been suggested as α-TCP $>$ β-TCP $\gg$ HA [8, 9]. However, there is limited information on such tests carried out in water or in a more acidic environment corresponding to, for example, localized osteoclast activity in vivo where the pH can be as low as pH 4.0 [5].

The dissolution behaviour of calcium phosphate samples immersion in Hank's solution has been investigated by Ding et al. [10] The results showed porous calcium phosphate gradually lost the strength with increasing immersion time. A weight lost of 6–11% after 90 days immersion was also reported. Porter et al. [11] immersed calcium polyphosphate (CPP) in tris-buffered solution and found a significant decrease in bending stress. The samples sintered at 585°C reduced in strength by over 50% after 1 day of immersion and by over 95% after 5 days of immersion. Some samples could not be tested after 5 days as they had lost structural integrity and failed during handling. The strengths of samples sintered at 600°C were reduced by 25% after 1 day and by 60% after 10 days of immersion. Similar observations were reported by Pilliar et al. [5] after immersing CPP in tris-buffered solution (pH 7.4) or potassium hydrogen phthalate buffered solution (pH 4.0) at 37°C for periods up to 30 days. An initial rapid loss of strength by 1 day followed by a more gradual strength loss was observed for samples after soaking in buffered solutions at both pHs. Samples made from fine powder had slightly lower strengths after immersion in solution at pH 4.0 compared to pH 7.4 but there was no such finding for samples based on coarse powder.

A variety of bone replacement materials have been developed in recent years and, in the past, the majority have been limited to non-load bearing applications [12, 13]. More

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recently, calcium phosphate based ceramics with the potential for load bearing have been developed. These materials combine both good mechanical properties with an interconnected network of pores in the appropriate size range for bone ingrowth [14]. However, little is known about their relative behaviour in vitro. It is of interest therefore to immerse samples in SBF or water for extended time periods to investigate the effect on the mechanical properties of both the type of medium and the pH over different time periods.

2 Materials and methods

2.1 Calcium phosphate samples

Calcium phosphate ceramics with interconnecting porosity were fabricated by a technique of vacuum impregnation of reticulated polymeric foams with ceramic slip as described in an earlier publication using 20 ppi foam templates which resulted in a pore size in the range of 197–254 μm [15]. This range was selected as being the most appropriate for bone ingrowth. Samples with three different ratios of HA/α-TCP/β-TCP were produced as shown in Table 1. The sintering temperature was 1,150°C for samples of COMP3 and 1,280°C for samples of COMP1 and COMP2. The COMP1 sample had a relatively high HA content which would be expected to result in a slower rate of dissolution. The COMP2 sample comprised 37.21% HA, 20.81% α-TCP and 41.98% β-TCP. The compositions of samples of COMP3 were mainly β-TCP which, from the literature, would be expected to result in a faster rate of dissolution than the COMP1 samples. Large samples with dimensions of 45 × 45 × 17 mm were made and then sectioned to smaller bars of 45 × 5 × 4 mm for dissolution and mechanical tests. The surfaces of the samples were ground flat using grade 600 silicon carbide paper. The samples were then cleaned in an ultrasonic bath to remove any dust that may have collected in the sample pores.

2.2 Dissolution testing

Ringer's solution was prepared by dissolving eight Ringer's solution tablets (VWR International Ltd., Leicestershire, England) in one litre distilled water (Fisher Scientific UK). Hydrochloric acid; HCl and Tris-hydroxymethyl-amino-methane; $(CH_2OH)_3CNH_2$ (Sigma-Aldrich Company Ltd., Gillingham, UK) were used to adjust the pH value. Ringer's solution at pH 7.2 and distilled water at pH 7.2 and 4.0 were prepared. Samples with three different compositions were immersed in Ringer's solution or distilled water at the two pHs for periods of up to 26 weeks; the temperature was maintained at 37°C. For each test, seven bars were placed in 945 ml of solution. Post immersion, the samples were removed from the solutions and dried prior to mechanical testing.

Table 1 The phase compositions of the samples before dissolution tests

	HA (%)	α-TCP (%)	β-TCP (%)
COMP1	75.96	2.31	21.73
COMP2	37.21	20.81	41.98
COMP3	3.28	0	96.72

2.3 Weight changes

Changes in weight over the time periods were recorded. The weight changes were calculated by comparing the dry weight (Ws) of the sample after immersion for a predetermined time with the original dry weight (Wo) of the sample as in the equation:

$$\text{Weight change}(\%) = (\text{Ws} - \text{Wo})/\text{Wo} \times 100$$

2.4 Measurement of mechanical properties

The mechanical strengths of the samples were measured to investigate the relationship between the weight and strength changes after various periods of immersion. Four-point bending and compressive tests were carried out using an Instron 1195 testing machine.

2.4.1 Four-point bend testing

Six samples from each group were tested to obtain an average value. The test was carried out using rectangular bars with dimensions of 45 × 5 × 4 mm. The samples were tested with a crosshead speed of 0.5 mm/min and the span was 20 × 40 mm. The four-point bend strengths (σ) were calculated using the equation:

$$\sigma = 3\text{F}(\text{L} - \text{L}_1)/2\text{bd}^2$$

where F is applied force (N); L, the span of the support loaders (mm); L_1, the separation of the loading span (mm); b, sample width (mm); d, sample thickness (mm).

2.4.2 Compressive testing

Compression test samples were made with dimensions of 12 × 5 × 4 mm. Prior to testing, a thin layer of rubber was placed between each end of the sample and the loading plates of the machine to reduce any shear stress concentration on the ends of the samples. The samples were loaded up to fracture with a constant cross-head speed of

2 mm/min. Six samples from each group were tested to obtain an average value for the compression strength.

Compressive strength is calculated by the equation:

$$\sigma = \mathrm{F}/\mathrm{A}$$

where σ is compressive stress (Pa); F, applied force (N); A, the specimen cross-sectional area (mm^2);

2.5 Scanning electron microscopy (SEM)

The surface morphology of samples was examined using scanning electron microscopy (SEM) (Jeol, JSM6480LV) with an accelerating voltage of 10–20 kV. The working distance used was 20 mm. Before observations, the samples were coated with a thin layer of gold to avoid the build up of static electrical charge during the microscopy session.

3 Results and discussion

All the results for the weight changes, four point bend tests and compressive strengths were analysed statistically using the standard student *t*-test.

3.1 Weight changes

3.1.1 Effect of composition

Figure 1a shows the weight changes of samples after immersion in Ringer's solution at pH 7.2. It can be seen that samples of COMP2 have an obvious tendency to increase in weight. There is a small weight gain after 1 week but a more noticeable weight gain by week four which remains at about 2% up to the 26 week time point. In contrast, there were no significant weight changes for samples of COMP1 and COMP3, both of which showed slight weight gains and losses at different time intervals. Similar observations were found for samples after immersion in distilled water at pH 7.2 and 4.0. The weight changes of the samples with three compositions after immersion in distilled water at pH 4.0 are shown in Fig. 1b. A significant increase in weight was observed for samples of COMP2. No obvious weight change was found for the samples of COMP1. A slight decrease was found for the COMP3 samples.

The weight change was more significant for the samples of COMP2 where the weight gain was ~2% after immersion for 26 weeks in Ringer's solution at pH 7.2 and ~1.25% after immersion for 26 weeks in distilled water at both pH 7.2 and 4.0. The weight changes for other two samples were below 0.2% in the three solutions.

The weight increases of samples of COMP2 can be related to the SEM observations as precipitates were seen on the surfaces as seen in Fig. 2a. The weight loss in samples of COMP3 could relate to their apparent higher solubility as seen in Fig. 2b. Overall, the weight changes were small but were significant for COMP2.

3.1.2 Effect of time period

As can be seen in Fig. 1, samples of COMP2 showed a significant increase in weight during the first 4 weeks followed by a tendency to continue to increase slightly in weight up to 26 weeks of immersion time in the various solutions. After immersion in Ringer's solution, COMP1 samples tended to decrease in weight slightly for up to 4 weeks. A weight gain with immersion time was observed for the COMP1 samples for immersion times of over 8 weeks as seen in Fig. 1a. This phenomenon is also related to the SEM observations; samples of COMP1 seem to undergo more rapid dissolution in first few weeks of immersion followed by more obvious deposition behaviour. Although a reduction in weight was found for samples of COMP1 after immersion in distilled water at pH 4.0 as seen in Fig. 1b, this loss decreased with immersion time. Samples of COMP3 had a tendency to slightly decrease in weight after immersion in various solutions. However, no significant relationship was found between weight changes with immersion time for sample of COMP3.

3.1.3 Effect of solution and pH

In general, lower weight gains (~1.5%) for the samples of COMP2 after immersion in distilled water at pH 7.2 were found when compared to ~2% after immersion in Ringer's solution at pH 7.2 as seen in Fig. 1. This was a surprising observation as it was anticipated that the solubility and hence weight change would be greater for samples of COMP3 particularly in the more aggressive environment of pH 4 and in distilled water. For samples of COMP1 the weight changes observed were minor up to 26 weeks. Slight differences were observed in that the samples stored in Ringer's solution showed a very slight increase in weight whilst those stored in distilled water generally showed a slight weight loss. This could be explained in terms of the higher starting ion concentrations in Ringer's solution which could result in a more rapid rate of deposition. Similar weight change behaviour for each sample was found after immersion in solution at different pHs. The results show the pH of solution does not have a noticeable effect on weight changes. In contrast, for COMP3 a slight increase in weight was observed samples stored in both solutions; this was more obvious for samples stored in distilled water at pH4.

The results show that the weight changes are strongly affected by sample composition and to some degree by the soaking solution. Immersion time period also has a slight

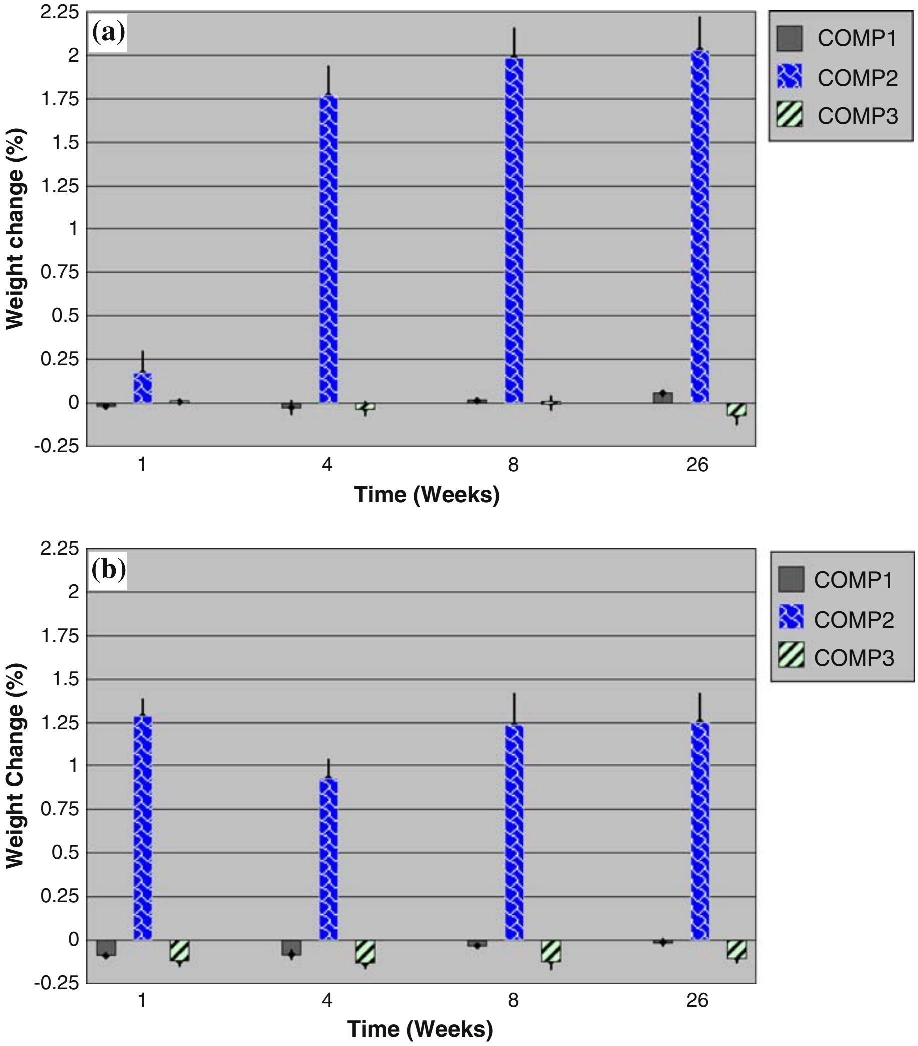

Fig. 1 The weight changes of the samples after immersion in **a** Ringer’s solution at pH 7.2 **b** distilled water at pH 4.0 for up to 26 weeks

effect whereas the effect of varying solution pH on weight changes was not not obvious.

3.2 Measurement of mechanical properties

3.2.1 Four-point bend strength

3.2.1.1 Effect of composition Figure 3 shows changes in the four-point bend strength of samples of the three compositions after immersion in Ringer’s solution at pH 7.2 and distilled water at pH 4.0 for up to 26 weeks. Overall, samples of COMP1, with the highest HA content, have the greatest strengths and the COMP3 samples have significantly lower strengths than other samples irrespective of the period of immersion. The difference between samples with different compositions seems to be more marked with immersion time. Samples of COMP3 with a composition primarily of β-TCP have the lowest strengths which may be due to the lower sintering temperature of 1,150°C compared to 1,280°C for the other samples. A general observation is that there was a slight tendency for the bend strength of COMP1 samples to increase in all storage environments. In contrast to this, COMP3 samples remain the same in Ringer’s solution but reduce in strength in distilled water. There does not appear to be a strong relationship between the bend strengths and weight changes.

3.2.1.2 Effect of time period Samples of COMP1 seem to have a tendency to increase in bend strength with immersion time irrespective of the medium used. Although, slight variations in bend strength with immersion time were observed for samples of COMP2, there are no obvious trends for bend strengths in relation to immersion time. Samples of COMP3 seem to have a slight tendency to decrease in bend strength with immersion time in distilled water. However, there was no obvious trend for bend strengths in relation to immersion time for sample of COMP3 in Ringer’s solution.

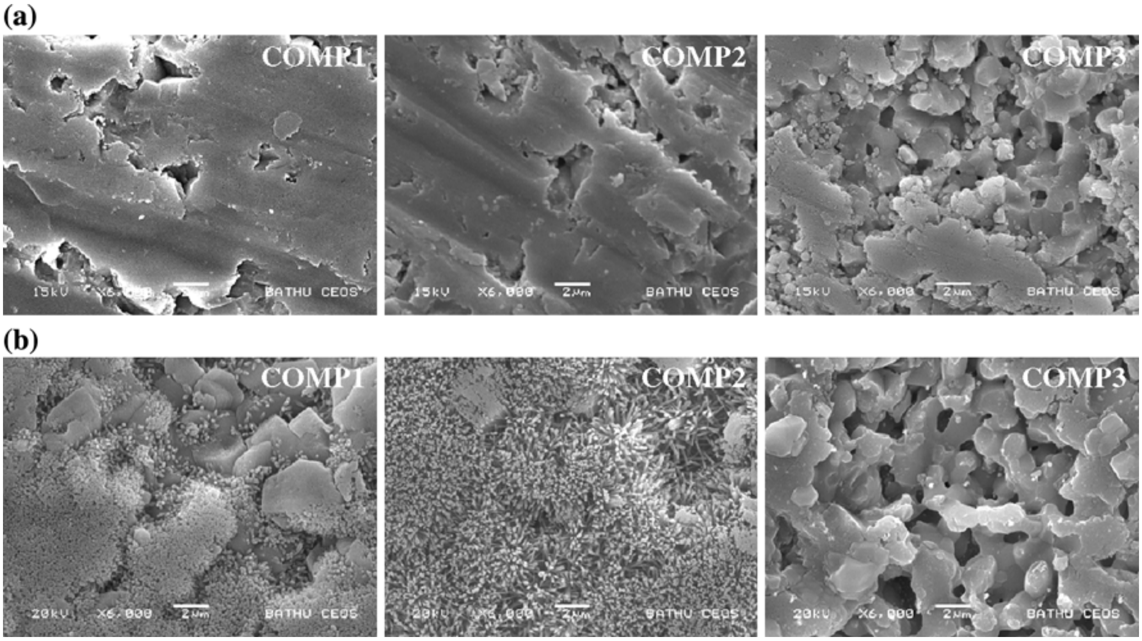

Fig. 2 Micrographs of the surfaces of samples **a** COMP2 **b** COMP3 after immersion in Ringer's solution at pH 7.2 for 26 weeks

After 26 weeks of immersion, a significant increase in bend strength was found for sample of COMP1. In contrast to this, no obvious change was seen for samples of COMP2. A slight decrease was found for samples of COMP3 after immersion in distilled water but there was no obvious change in Ringer's solution.

3.2.1.3 Effect of solution and pH There does not appear to be a strong effect of solution on bend strengths. Similar bend strength values were found for samples of COMP1 and COMP2 immersed in different solutions for 26 weeks. Slightly lower bend strengths were found for samples of COMP3 after immersion for 26 weeks in distilled water than Ringer's solution. No noticeable effect of solution pH on bend strength was found; the samples behaved in a similar manner at both pH 7.2 and pH 4.0.

Generally, the four-point bend strength was found to vary with sample composition irrespective of irrespective of time period or media. No obvious influence of solution and pH on bend strength were found other than a slight influence of solution on the bend strength of samples of COMP3.

3.2.2 Compressive strength

3.2.2.1 Effect of composition The changes in the compressive strength of samples of the three compositions after immersion in Ringer's solution at pH 7.2 and distilled water at pH 4.0 are shown in Fig. 4. The sample composition had a major effect on the properties as the results always appeared in the same relative order for compressive strengths in the various solutions. Significantly lower strengths were found for the samples of COMP3, which may be due to the lower sintering temperature. In contrast to this, COMP1 samples always have the highest strength. The order for long-term compressive strengths in various solutions is seen as sample COMP1 $>$ COMP2 $\gg$ COMP3 which is the same relative order as the bend strengths.

3.2.2.2 Effect of time period There was no obvious tendency for the compressive strengths to change with immersion time, with both increases and decreases observed with immersion time as shown in Fig. 4. Additionally, no obvious decrease in compressive strength was found after samples were aged in the various solutions for up to 26 weeks compared to "as-received".

3.2.2.3 Effect of solution and pH There was no significant effect of solution on the compressive strengths. Similar values of compressive strength were seen for samples with the same composition after immersion in different solutions. No major influence of pH on the compressive strength was found. The sample composition appeared to have a dominant effect. Neither time period nor the storage environment were found to significantly effect the compressive strength.

3.3 Scanning electron microscopy of the microstructure

Micrographs of typical as-received samples are shown in Fig. 5. The angular nature of the porosity can clearly be

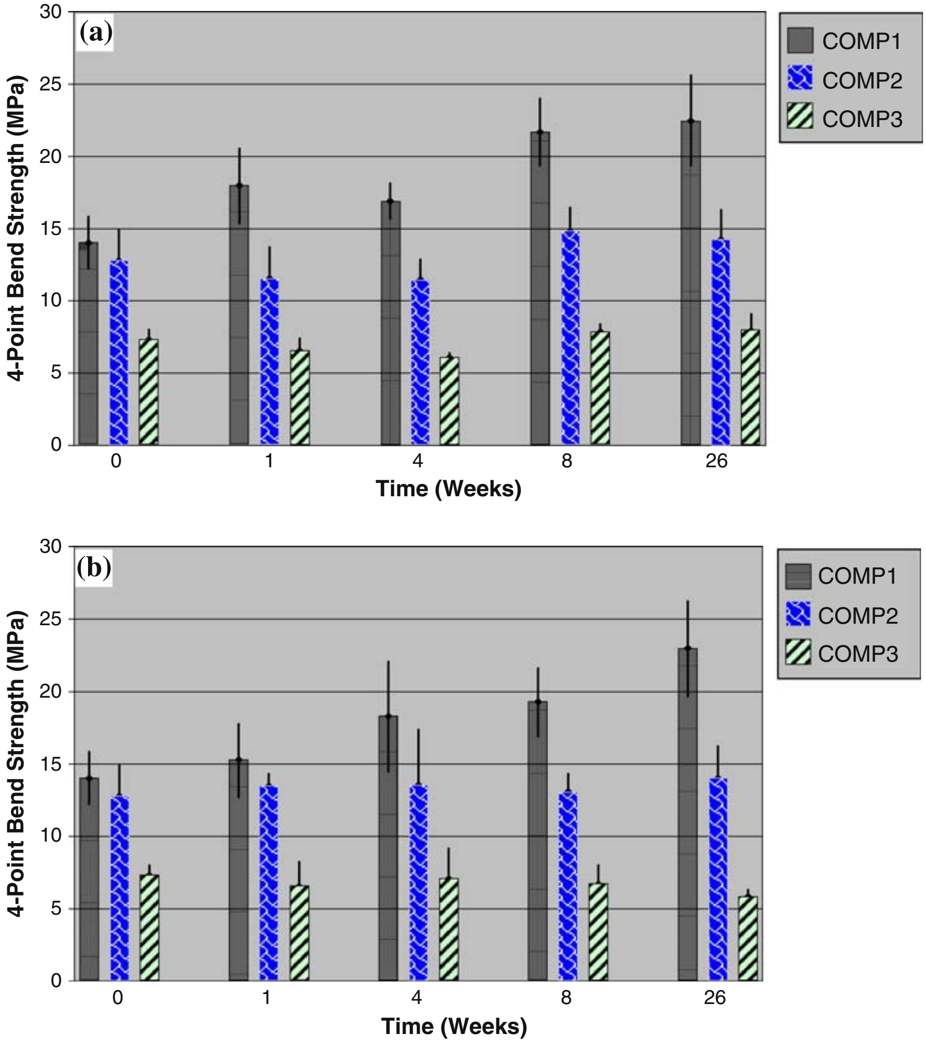

Fig. 3 The changes of 4-point bend strength after immersion in **a** Ringer's solution at pH 7.2 **b** distilled water at pH 4.0 for up to 26 weeks

seen. This may be of relevance when considering the change in mechanical properties in relation to composition and time period. Micrographs of the surfaces of samples with the three compositions, post immersion in the two storage environments, for which results have been presented in this paper, for 26 weeks, are shown in Fig. 6. On the macroscale, noticeable dissolution behaviour can be seen on the edge of pores for the sample of COMP2 in the two different media. Obvious dissolution behaviour can also be seen for the sample of COMP1 after immersion in Ringer's solution but this appears less significant in distilled water. No obvious dissolution behaviour can be seen for the sample of COMP3 in the various media. This is in contrast to the microstructural observations in Fig. 2.

The pores in the as-received samples, Fig. 5, are very angular in appearance with sharp corners which could act as stress raisers in the sample. Following exposure to the storage environment there is evidence of rounding of these sharp edges, particularly in samples of COMP2. It is proposed that the increase in bend strength for COMP2 samples may be related to this observation as the stress concentrating effect is reduced with time. However, given the relative order of solubility for the samples, COMP1 < COMP2 < COMP3, this effect would have been expected to be observed most strongly in samples of COMP3. As can be seen from the SEM observations and the bend strengths this was not the case.

4 Conclusions

Dissolution tests have been carried out on porous calcium phosphate bioceramics with three different compositions in various solutions. There was no significant relationship found between the weight changes and mechanical strengths of the calcium phosphate ceramics immersed in Ringer's solution at pH 7.2 and distilled water at pH 7.2 and 4.0. However, the sample composition seems to have major effect on the properties as the results always appear in the same relative order for weight change in the various

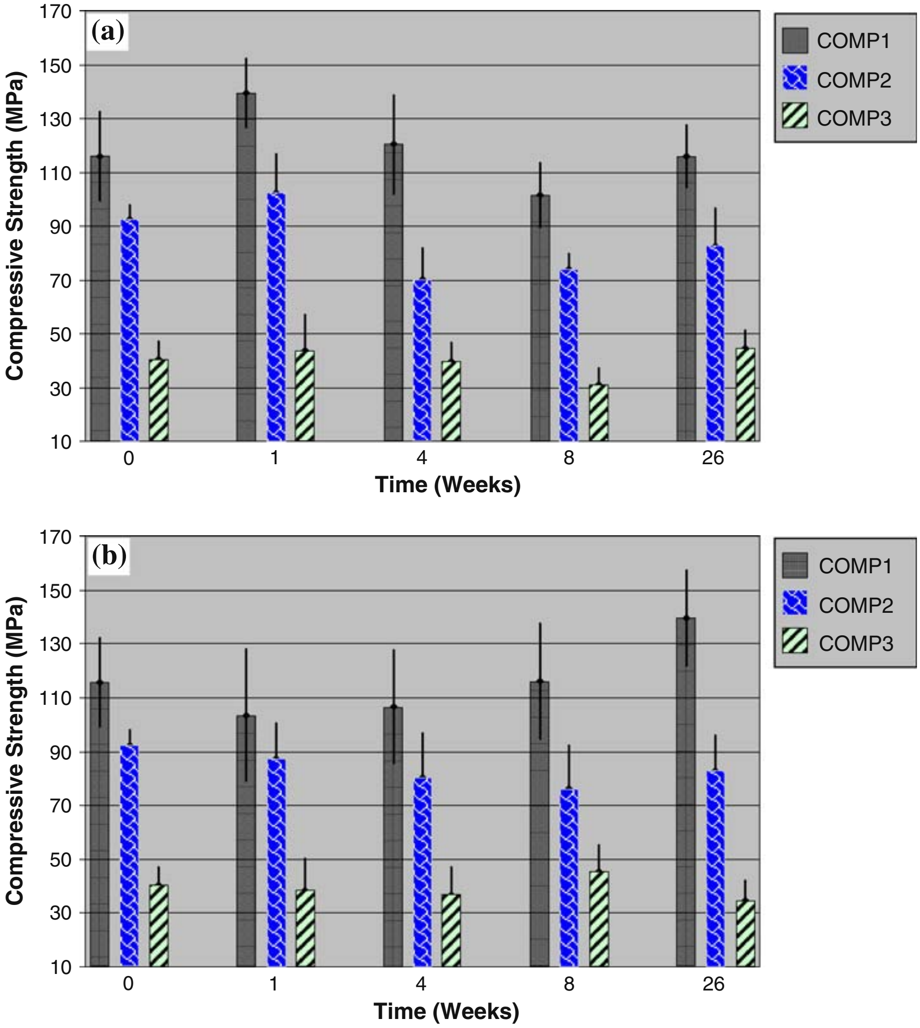

Fig. 4 The changes of compressive strength after immersion in **a** Ringer's solution at pH 7.2 **b** distilled water at pH 4.0 for up to 26 weeks

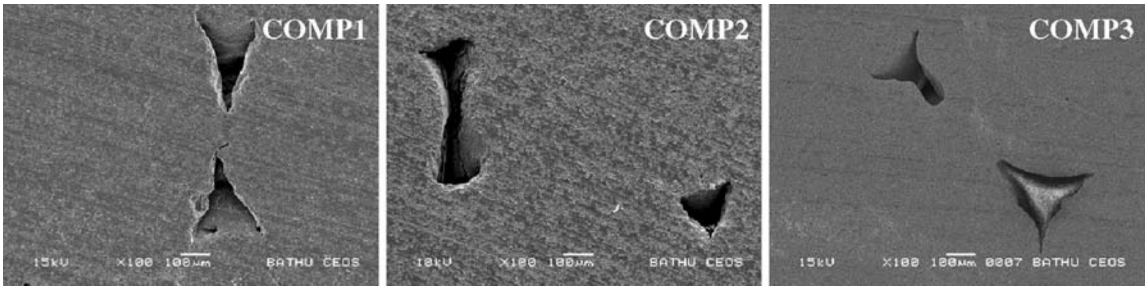

Fig. 5 Micrographs of typical as-received samples with three compositions

solutions. Although the order for weight change is different to that for mechanical strengths, the same relative order for four-point bend and compressive strengths can be seen irrespective of the storage environment. Samples of COMP1, with the greatest HA content, always have the highest mechanical strengths. A significantly lower strength was found for samples of COMP3 which can be attributed to the low sintering temperature and high β-TCP content. The results show these samples could be of significant clinical interest as, contrary to other published work, no obvious decrease in mechanical properties was found after samples had been aged in the various solutions for up to 26 weeks. The information gained from this research will provide a useful platform for further study.

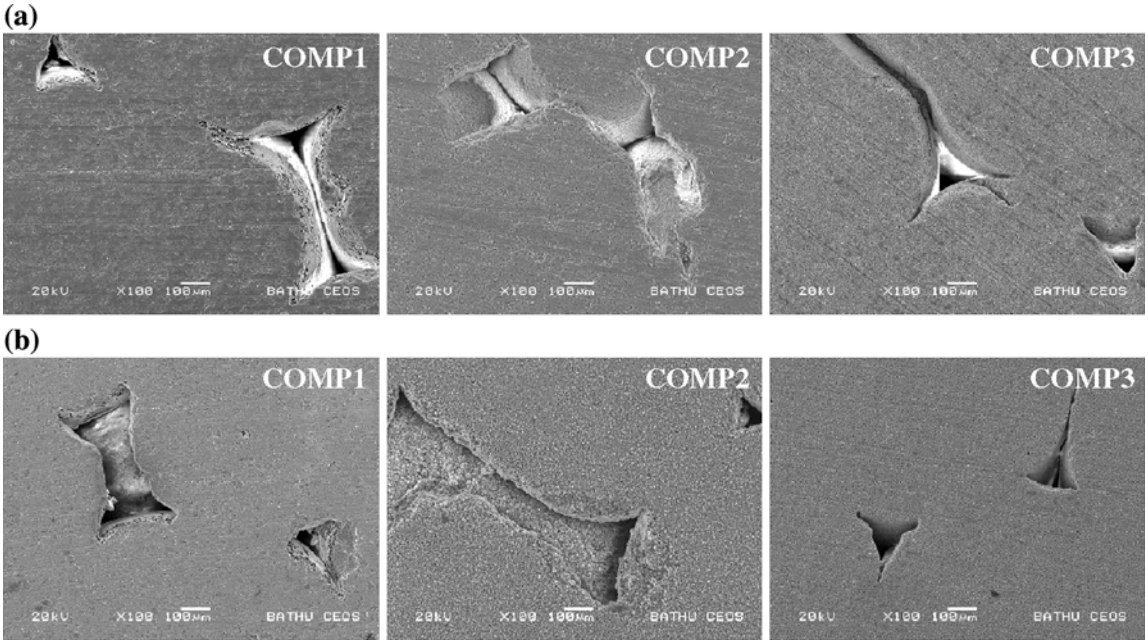

Fig. 6 SEM micrographs of samples with three compositions after immersion in **a** Ringer's solution at pH 7.2 **b** distilled water at pH 4.0 for 26 weeks

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