Basic properties of apatite cement containing spherical tetracalcium phosphate made with plasma melting method

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Apatite cement (AC) can be injected through syringe and forms apatite mass. Therefore, AC is very useful for a minimally invasive surgical operation aimed for the reconstruction of bone defects. However, injectability of current AC is not satisfactory for its clinical use. In this investigation, therefore, spherical tetracalcium phosphate (s-TTCP) was prepared with plasma melting method and its effect on injectability were evaluated as well as other basic properties of AC. We found much better handling property and injectability when we used s-TTCP as a component of AC (s-AC). For example, cement spread area used as an index of consistency of the s-AC paste was 512 mm^2 whereas that of ordinary AC with irregular TTCP (i-AC) was 158 mm^2 when powder to liquid mixing ratio was 2.5. However, diametral tensile strength of set s-AC (1.4 MPa) was significantly lower than that of set i-AC (10.7 MPa) when the powder to liquid ratio was 4.0. X-ray powder diffraction analysis revealed limited formation of apatite in the case of s-AC. Although there are some drawbacks, we feel the use of spherical particle is very useful to improve the injectability of AC. Therefore, it is important to find suitable method to prepare spherical powder as the component of AC.
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1. Introduction

Many types of apatite cement (AC) were proposed based on the fundamental invention made by Drs. Brown and Chow [1–10]. AC consists of powder and liquid phases and forms apatite (AP: $Ca_{10-x}(HPO_4)x(PO_4)_{6-x}(OH)_{2-x}$) upon mixing based on the dissolution of powder phase and precipitation of AP, the most stable phase thermodynamically, followed by the interlocking of precipitated AP crystals [11]. Animal studies as well as clinical evaluation demonstrated that AC shows excellent tissue response and good osteoconductivity [12–16]. One of the key advantages of AC in contrast to other AP related biomaterials is its setting character. Filling the bone defect with AC results in the reconstruction of bone defect with the apatite having exactly the same shape with the bone defect. Setting character of AC also allows injection of AC paste through syringe. This behavior is very important with respect to the minimally invasive surgical operation [17–19]. For example, injection of AC to the deformed condyle through syringe results in the expansion of the condyle without open surgery.

However, injectability of current AC is unfortunately not satisfactory for its clinical use. Injectability of AC is governed by many factors including the powder to liquid mixing ratio and the use of viscous chemicals [20–23]. However, lower powder to liquid mixing ratio results in the lower mechanical strength and use of viscous chemicals sometimes reduces the tissue response to AC [10]. On the other hand, shape of the powder is expected to have effects on injectability. Powder with spherical shape may contribute good injectability of AC. In fact, it is reported that sphericalization of Portland cement increased its handling property [24]. Many methods were reported on how to prepare spherical powder [24]. Among them, sphericalization method using the surface tension is known to be a good method to prepare ideal spherical powder [24].

The objective of this study was therefore to evaluate the usefulness of AC using spherical tetra-

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calcium phosphate (TTCP) with respect to its inject-ability.

2. Materials and methods

2.1. Preparation of apatite cement

Spherical tetracalcium phosphate (TTCP; $Ca_4(PO_4)_2O$) particles were prepared at Netsuren Co. (Kanagawa, Japan) by plasma melting method using commercially obtained TTCP (Taihei Chemical, Osaka, Japan). In brief, TTCP particle was dropped into reaction vessel filled with Ar where the TTCP was melted by the heat generated by plasma. The irregular form TTCP became spherical due to surface tension and quenched at room temperature during dropping. The irregular form TTCP and spherical form TTCP are denoted as i-TTCP and s-TTCP, respectively, in the following text.

s-TTCP or i-TTCP was mixed with dicalcium phosphate anhydrous (DCPA; $CaHPO_4$, J. T. Baker Chemical Co., NJ) with a medium particle size of 1.2 μm so that the mixing ratio became equimolar using a mixer (SK-M2, Kyoritsuriko, Tokyo, Japan). The mixtures with i-TTCP and s-TTCP are denoted as i-AC and s-AC, respectively.

It is well known that the liquid phase of the AC affects the properties of AC a lot. For example, use of neutral sodium hydrogen phosphate aqueous solution shortens the setting time of AC; this AC is called fast-setting AC (fs-AC) [8, 25]. Use of sodium alginate or other viscous chemicals provide anti-washout ability to the AC; this type of AC is called anti-washout AC (aw-AC) [9, 15, 26, 27]. Although aw-AC shows much better handling property and injectability than conventional AC (c-AC) due to the use of a viscous chemical, sodium alginate, in its liquid phase, we used distilled water which is the liquid phase of c-AC in the present study to focus only on the effect of powder form on injectability and other basic properties of AC.

The powder phase and liquid phase which is distilled water, was mixed with spatula on glass with powder to liquid ratio (P/L ratio) of 1.5 or 4.0

2.2. Consistency evaluation

The consistency of the cement paste was evaluated essentially according to the method set forth in international standard ISO1566 for dental zinc phosphate cement, in which consistency is defined as the diameter of the spread area of the cement paste when a glass plate (140 ± 0.5 g) is placed on 0.5 mL of the paste 3 min after mixing. In the present study, a 2 kg glass plate was placed on $0.2\, cm^3$ of the cement paste and the spread area was measured after 3 min [27–29].

2.3. Injectability evaluation

As an index of injectability of the cement paste, load required to push out the $0.5\, cm^3$ paste from 30 mm length 18 G syringe needle at a rate of $1\, cm^3 \cdot min^{-1}$ was measured using universal testing machine.

2.4. Mechanical strength measurements

The mechanical strength of set AC was evaluated in terms of the diametral tensile strength (DTS). Cement paste was packed in a splitting cylindrical plastic mold (6 mm in diameter × 3 mm in height). Both ends of the mold were then covered by glass plates, clamped, and the paste set by storing in an incubator for 24 h at 37 °C and 100% relative humidity. The diameter and height of each specimen were measured with a micrometer. The samples were crushed with a cross-head speed of $10.0\, mm\, min^{-1}$ using a universal testing machine (AGS-500A, Shimadzu, Kyoto, Japan). The DTS values used were averages of at least eight specimens. The bars in figures denote standard deviation. For statistical analysis, one-way factorial ANOVA and Fisher's PLSD method as a post-hoc test were performed using Stat View 4.02 (Abacus Concepts, Berkeley, CA).

2.5. Powder X-ray diffraction (XRD)

The composition of the set AC was evaluated by means of powder X-ray diffraction (XRD). The XRD patterns of the vacuum-dried samples were recorded with a vertically mounted diffractometer system (Rint 2000, Rigaku, Tokyo, Japan) using Ni filtered CuKα generated at 40 kV and 30 mA. The specimens were scanned from 3 to 60° 2θ (where θ is the Bragg angle) in a continuous mode. JCPDS cards of 9-0432, 25-1137 and 9-0080 is used for as the reference of AP, TTCP and DCPA, respectively.

2.6. Scanning electron microscopy (SEM)

Morphology of the particles before and after spherical-ization using plasma melting method the particle was observed using scanning electron microscope (SEM) (S-700; Hitachi Co., Tokyo, Japan) under an accelerating voltage of 20 kV after gold-coating.

3. Results

Fig. 1 shows the SEM pictures of i-TTCP and s-TTCP made with plasma spray method. As shown, shape of the i-TTCP particles is irregular and has pores on their surface. In contrast, s-TTCP made with plasma spray method is spherical as the name represents. We could find small pores on the surface of some s-TTCP.

Fig. 2 shows the powder XRD patterns of i-TTCP and s-TTCP. Basically, i-TTCP and s-TTCP showed the same patterns typical for tetracalcium phosphate. However, we
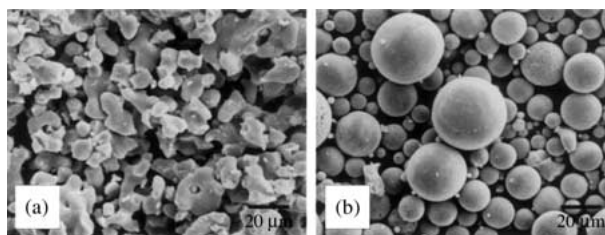

Figure 1 Typical SEM image of the tetracalcium phosphate. (a) i-TTCP; (b) s-TTCP
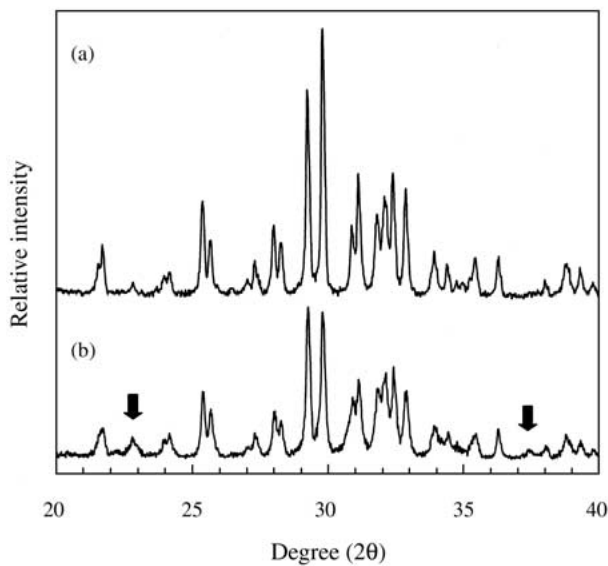
Figure 2 Powder X-ray diffraction patterns of (a) i-TTCP and (b) s-TTCP. Bar in (b) indicate unidentified thermal decomposition product of TTCP formed during the sphericalization of s-TTCP powder using plasma melting method.

T A B L E I Bulk density of several poweders used in this study

Powder	Bulk density $(g\,cm^{-3})$
i-TTCP	0.96 ± 0.05
s-TTCP	1.52 ± 0.06
i-AC	0.61 ± 0.02
s-AC	1.13 ± 0.04

found a small amount of unidentified peaks around $2\theta = 22.8$ and 37.4 in the case of s-TTCP.

Table I summarizes the bulk density of TTCP and AC powders. Bulk density of s-TTCP was approximately 60% higher than that of i-TTCP. Similarly, bulk density of s-AC was approximately 85% higher than that of s-AC.

Fig. 3 shows the cement spread area of the i-AC and s-AC as a function of P/L ratio. In both cases, cement

Figure 4 Injection behavior of s-AC and i-AC prepared with powder to liquid ratio of 3.0, 3.5, and 4.0. Percentage of injected AC though 30 mm 18 G syringe were plotted against the load required to push the injector.

spread area decreased with an increase in the P/L ratio. Cement spread area of s-AC was much larger than that of i-AC regardless of the P/L ratio when compared with the same P/L ratio. For example, cement spread area of s-AC was $512 \pm 67\,mm^2$ whereas that of i-AC was $158 \pm 32\,mm^2$ when the P/L ratio was 2.5. These results indicated that s-AC shows much better handling properties than i-AC.

Fig. 4 shows the injection profile of ACs with *P/L* ratio of 3.0, 3.5 and 4.0. As shown, load required to inject AC increased with the amount of AC injected. We found none of the AC was fully injectable within this experimental condition. Larger load was required to inject i-AC than s-AC at the same P/L ratio.

Fig. 5 summarizes the DTS value of i-AC and s-AC as a function of incubation time. As shown in this figure, DTS value of i-AC was much larger $(p < 0.01)$ than
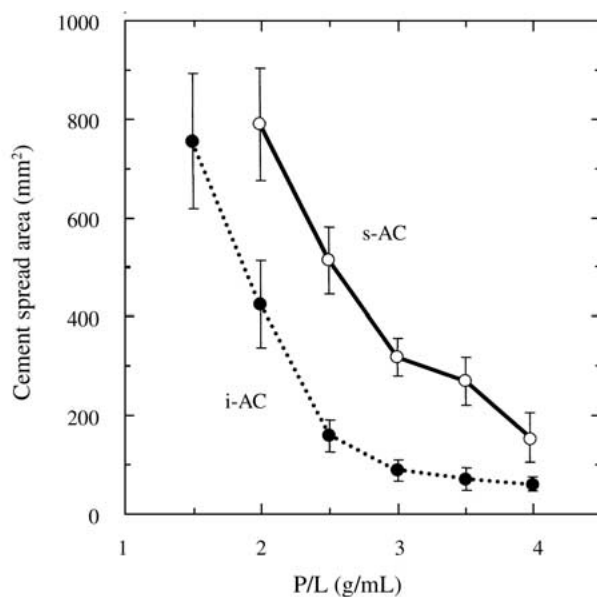

Figure 3 Comparison of the cement spread area as an index of the consistency of cement paste of s-AC and i-AC as a function of powder to liquid mixing ratio. ●, i-AC; ○, s-AC. $n = 3$
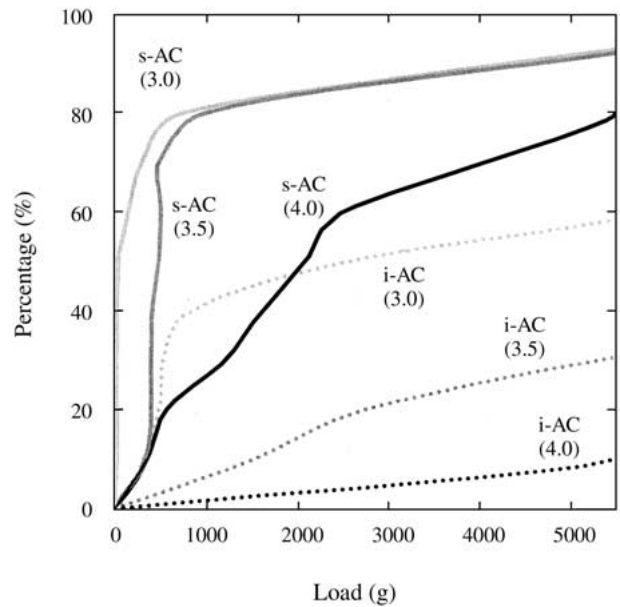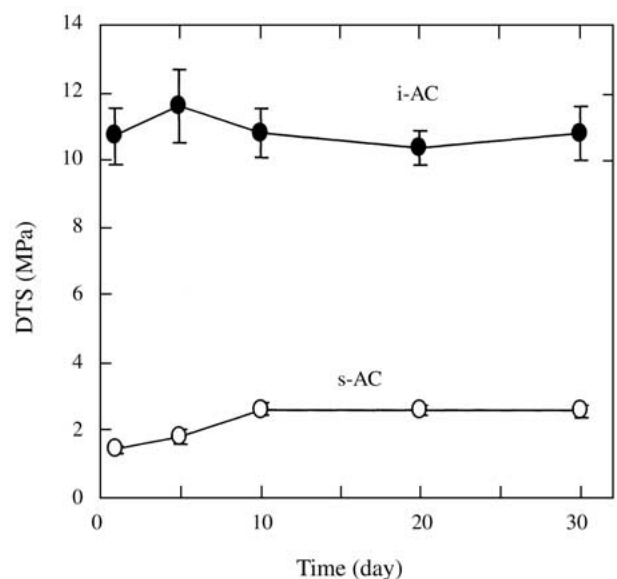

Figure 5 Evaluation of diametral tensile strength (DTS) versus incubation time for s-AC and i-AC. (P/L = 3.5 g/ml; $n = 8$ samples) ●, i-AC; ○, s-AC. Powder to liquid mixing ratio was 3.5. $n = 8$
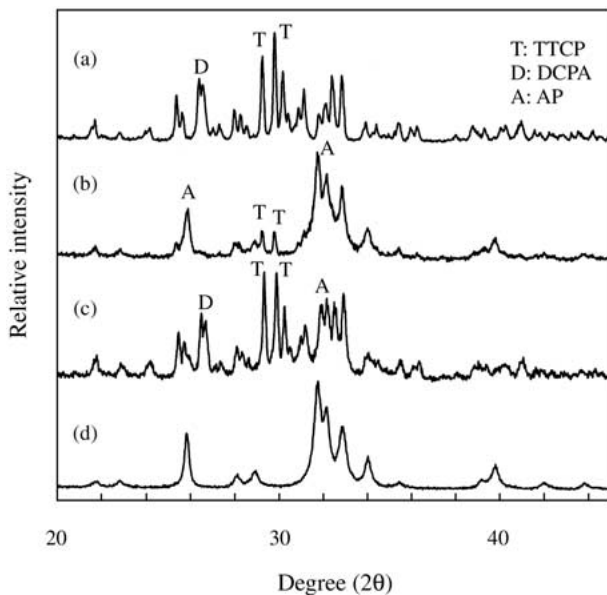
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Figure 6 Powder X-ray diffraction patterns. (a) Powder phase of i-AC, i.e. equimolar mixture of TTCP and DCPA; (b) set i-AC; (c) set s-AC; (d) commercially obtained hydroxyapatite.

s-AC regardless of the incubation time. DTS values of i-AC were the same regardless of the incubation time. In contrast, DTS value of the s-AC increased with incubation time up to 10 days then showed the same DTS value. DTS value of the i-AC was significantly ($p < 0.01$) higher than that of s-AC regardless of the incubation time.

Fig. 6 summarizes the XRD patterns of powder phase of (a) i-AC, (b) set i-AC, and (c) set s-AC. XRD pattern of (d) crystalline apatite is also listed for comparison. Set AC indicates AC where powder phase and liquid phase were mixed with powder to liquid ratio of 3.5 and kept in an incubator at 37 °C and 100% relative humidity for 24 h Set i-AC shows the typical apatitic patterns with small amount of unreacted TTCP. In contrast, we found limited amount of apatite formation in the set s-AC and larger amount of unreacted TTCP and DCPA.

4 Discussion

The results obtained in this study demonstrated clearly the usefulness of spherical powder in the component of apatite cement to improve the handling property and injectability of apatite cement. As shown in Fig. 1, we could prepare ideal spherical shaped TTCP. Spherical powder itself is easy to roll and thus shows good handling properties and injectability when paste is prepared using spherical powder. Also, it should be noted that the paste became fluid with less amount of liquid phase since no captured liquid exist in the case of spherical powder. Figs. 3 and 4 indicate s-AC is very useful to obtain good handling property and injectability. However, Fig. 4 also indicates that perfect injectability cannot be obtained by s-AC, i.e. although the injectability protocol was very severe, none of the ACs were fully injectable. Despite s-AC showed much better injectability than i-AC, the load required to extrude both ACs increased significantly after 80% of cement injection. This behavior is also observed in the case of i-AC. For example, after the injection of approximately 50% of the i-AC, only a limited amount of AC was injected even though further load was applied to the injector. This is explained by a phase separation problem, i.e. liquid phase is preferentially injected out of the syringe due to an effect of filtering through the powder phase. However, as it has been reported, this problem can be avoided by using certain viscous chemicals or additives to the liquid phase [20–23]. Despite this was not the main objective of this research, the authors feel that the use of a viscous chemical is a requirement for the fabrication of injectable AC.

The result obtained in Fig. 4 also showed that lower P/L ratio is preferable with respect to the injectability. This result is easy to accept since more wet paste can go through the syringe. However, lower P/L ratio result in the set AC with low mechanical strength [10]. Therefore, we feel that lower P/L ratio should be avoided as much as possible.

s-AC showed higher bulk density when compared with i-AC as shown in Table I. Since mechanical strength of AC increases with increase in the density of AC, set s-AC should show higher mechanical strength than set i-AC. Unfortunately, we found opposite results. We found much lower ($p < 0.01$) DTS value in the case of set s-AC than set i-AC. This result can be explained by the limited apatite formation. The XRD pattern shown in Fig. 6 shows set i-AC was almost apatite whereas only a small amount of apatite was formed in the case of s-AC. Since only the difference between i-AC and s-AC is TTCP, s-TTCP is the cause of this limited apatite formation and resulting lower mechanical strength. As shown in Fig. 2 comparison of the XRD patterns of i-TTCP and s-TTCP showed the presence of trace amount of thermal decomposition product in s-TTCP. In addition to this XRD detectable thermal decomposition product, some amorphous phase may be formed in the s-TTCP. These products are thought to inhibit the apatite formation in the s-AC.

Longer incubation of s-AC resulted in the slight but significant ($p < 0.01$) increase in the mechanical strength of s-AC as shown in Fig. 6. However, incubation of s-AC longer than 10 days resulted in no increase in mechanical strength.

Although the mechanical strength of s-AC is higher than some of the other proposed ACs, e.g. 0.4 MPa as DTS value for AC consisting of thermally decomposed α-TCP and TTCP [30], its value is too low for the TTCP-DCPA system cement. Fortunately many methods are proposed to prepare spherical powder. Since the result obtained in this study demonstrated the effectiveness of the spherical powder to improve the handling and injectability of AC, further studies to prepare AC with improved injectability without losing mechanical strength await based on this initial study.

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