

*Full Length Research Paper*

# A novel injectable and degradable calcium phosphate/calcium sulfate bone cement

Wei Zhou, Youdi Xue, Xubiao Ji, Guoyong Yin, Ning Zhang and Yongxin Ren\*

Department of Orthopedics, The First Affiliated Hospital With Nanjing Medical University, Nanjing, China.

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The aim of this study was to obtain a composite material which not only has suitable workability but also has excellent degradability. A novel injectable and degradable bone cement consisting of a mixture of calcium phosphate ( $\alpha$ -tricalcium phosphate;  $\alpha$ -TCP) and calcium sulfate dihydrate ( $\text{CaSO}_4 \cdot 2\text{H}_2\text{O}$ ; CSD) powders as the solid phase and an aqueous solution containing 2.5 weight percent of  $\text{Na}_2\text{HPO}_4$  as the liquid phase, was prepared. Seven groups with differing CSD content (0, 5, 10, 20, 30, 40 and 100%) were tested. The injectability of  $\alpha$ -TCP increased with increasing proportions of CSD in the mixture; a small addition of CSD powder sharply decreased the setting time of the cement, but further addition caused the setting time of the mixture to increase again. Simultaneously, adding CSD to  $\alpha$ -TCP had little effect on the setting temperature. The compressive strength of the cement decreased with increasing content of CSD except in the 5% group, because addition of a small amount of CSD to the cement induced the appearance of fiber-like crystallization; this was confirmed by scanning electron microscopy (SEM). Furthermore, the bone cement mixture had good degradability in simulated body fluid (SBF) when the content of CSD was increased.

**Key words:** Calcium phosphate, calcium sulfate dehydrate, bone cement, injectability, degradability, bone repair.

## INTRODUCTION

Percutaneous vertebroplasty (PVP) and percutaneous kyphoplasty (PKP) are minimally invasive operations used to treat deformities of the vertebral body caused by osteoporotic compression fractures or vertebral tumors. These operations are reported to be effective in improving the stability of the spine, reducing pain and improving deformities (Hollingworth and Jarvik, 2006). However, the commonly used vertebral body filler, the plastic polymethyl methacrylate (PMMA), is not an ideal material as it is associated with many intraoperative and postoperative complications. Because the setting process can generate heat, and the temperature of polymerization may reach 40 to 120°C *in vitro*, so any contiguous tissue, especially the spinal cord and nerve roots, may be at risk of damage (Park et al., 1999). Absorption of the unpolymerized toxic monomer, methacrylate, may also create complications including hypotension shock and fat embolism when PMMA is applied (Harris et al., 1975;

Phillips et al., 1971). In addition, PMMA is not an osteoconductive or biodegradable material, it fails to integrate and substitute with the bone (Bartucci et al., 1985). Furthermore, foreign body reactions at the bone/PMMA interface will continue over the long term, eventually resulting in the resorption of the bone, and a subsequent decrease in compressive strength (McAfee et al., 1986). In some studies, new vertebral body fractures were found in vertebrae adjacent to the augmented vertebral body at follow-up (Frankel et al., 2007; Grados et al., 2000; Fribourg et al., 2004). In view of the disadvantages of traditional bone cement, it is essential to find a novel biodegradable, nontoxic and injectable vertebral body filler material.

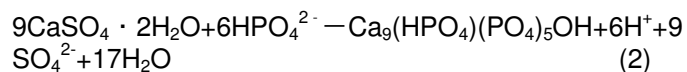
Calcium phosphate ( $\alpha$ -tricalcium phosphate;  $\alpha$ -TCP), which are used for bone substitution and augmentation, were first fabricated by Brown and Chow (1985) and are labeled as self-setting cements. TCP, when compared with other bone substitute materials (especially ceramic bone substitutes), provide excellent biocompatibility and self-setting ability (Costantino et al., 1991; Constantz et al., 1995). The properties of degradability, osteocon-

\*Corresponding author. E-mail: [renyongxin@yahoo.com.cn](mailto:renyongxin@yahoo.com.cn), [zhangning2003@medmail.com.cn](mailto:zhangning2003@medmail.com.cn).

ductivity and constant setting temperature are also advantages over traditional bone cement (Costantino et al., 1991; Constantz et al., 1995). However, when used as bone cements, TCP are too stable to be absorbed *in vitro*, and may take one or two years to achieve complete degradation (Nilsson et al., 2002). Calcium sulfate (CS) bone cements have also been used as bone substitutes for over 100 years, and have proved to be safe and biocompatible (Nilsson et al., 2004; Song et al., 2004). Recently, Woo KM et al. (2009, 2010) found that CS (especially alpha-form CS) strongly promoted gene expression of osteoblast phenotypes including Runx2, alpha1 (I) collagen, osteocalcin, and bone sialoprotein. However, after extensive application, CS bone cements have been criticized for their rapid resorption (Blaha, 1998), which results in complete absorption before the bone tissue has enough time to grow into the defect. In addition, when compared with the strength of vertebral cancellous bone, the mechanical strength of calcium sulfate bone cements was also insufficient.

Many studies have been carried out to improve the characteristics of TCP and CS. Urban et al. (2007) found that the mixture of TCP and CS could degrade much more quickly than TCP, but more slowly than the degradation speed of CS. Nilsson et al. (2002) found that after absorption of the CS in a TCP/CS mixture, the remaining particles of TCP became encapsulated by new bone tissue, and this facilitated the formation of surrounding new bone. Accordingly, this material exhibited excellent osteoconduction. Furthermore, these materials performed better than CS when used for reconstruction of major bone defects. From these studies, we concluded that these two simple materials can interact with each other without losing each of their intrinsic properties when mixed together; meanwhile the overall property of the mixture was improved.

In order to obtain a material which could satisfy the requirements of PVP and PKP surgery, we mixed  $\alpha$ -TCP and CSD powders together with an aqueous solution in this study. Both  $\alpha$ -TCP and CSD reacted with the solution, the product was CDHA ( $\text{Ca}_9(\text{HPO}_4)(\text{PO}_4)_5\text{OH}$ ) (1)(2), which supplied mechanical strength, osteoconductivity and degradability.



From this combination, we desired to obtain biomaterials with excellent degradability and mechanical strength suitable for application in treating deformities of the vertebral body.

## MATERIALS AND METHODS

### Preparation of cement

Alpha-tricalcium phosphate ( $\alpha$ - $\text{Ca}_3(\text{PO}_4)_2$ ), was gratuitously provided

by the Department of Materials Science and Engineering, Tsing Hua university and calcium sulfate dihydrate ( $\text{CaSO}_4 \cdot 2\text{H}_2\text{O}$ , Sigma-Aldrich, C3771) served as the basis for the experiments. CSD was added to the powder phase of  $\alpha$ -TCP at 0, 5, 10, 20, 30, 40, 100% in weight, and all were then combined with the liquid phase which was an aqueous solution containing 2.5 weight % of  $\text{Na}_2\text{HPO}_4$ . The ratio of liquid to powder (L/P) was 0.4 ml/g. The specimens of TCP/CSD composite cements with different calcium sulfate contents were prepared by keeping the paste in beakers which were stored in a constant-temperature oven at 37°C and 100% relative humidity.

### Setting time and setting temperature test

The setting time of bone cements included cohesion time (TC), initial setting time (TI) and final setting time (TF). The standard setting times are: 1)  $3 \text{ min} \leq \text{TI} < 8 \text{ min}$ ; 2)  $\text{TI} - \text{TC} \geq 1 \text{ min}$ ; and 3.  $\text{TF} \leq 15 \text{ min}$ .

The setting time of the samples was measured using Gilmore apparatus. This apparatus includes two needles of different weight. The lighter needle (weight:  $113 \pm 0.5 \text{ g}$  and diameter:  $2.12 \pm 0.05 \text{ mm}$ ), which provided 0.3 MPa static pressure, was used to test the initial time, while the heavier needle (weight:  $453.6 \pm 0.5 \text{ g}$  and diameter:  $1.06 \pm 0.05 \text{ mm}$ ), which provided 5 MPa static pressure, was used to test the final time. The time interval from initiation, when the powders were mixed with the liquid, to the time when the lighter needle loaded on the surface of the sample, did not make any visible impression; under conditions of 100% relative humidity at 37°C was considered the initial time. At the same time, the final time was considered when the heavier needle loaded on the surface of the sample did not make any visible impression. Setting temperature was measured using an infrared thermo-detector while the solid phase was mixed with the aqueous solution for 60 s at 20°C.

### Injectability test

The composite cement powders were mixed with the liquid phase to form a homogeneous mixture under conditions of 100% relative humidity at 37°C. The paste was then transferred into a syringe with a capacity of 5 ml and an opening nozzle diameter of 2.0 mm. After the cement powder had been in contact with the liquid for 90 s, the paste was extruded using a mechanical testing machine (Hualong HWT-200, Hualong, Shanghai) compressed at the tip of the syringe. The extrusion speed was kept constant at 15 mm/min, while the force was recorded. The extrusion procedure ended when the force reached 100 N, and the injectability was taken to mean the amount of the paste which could be extruded from the syringe, expressed as a percentage by weight of the total.

### Compressive strength test

The bone cement samples were molded into cylindrical columns of 6 mm in diameter and 12 mm in length. The samples were incubated in 100% relative humidity at 37°C for 24 h. Compressive strength of each sample was then measured using a computer-controlled Universal Testing Machine. The cross-head speed was 1 mm/min. For each measurement, an average of five replicates was used.

### Degradability test *in vitro*

*In vitro* degradation of the injectable cement was investigated by

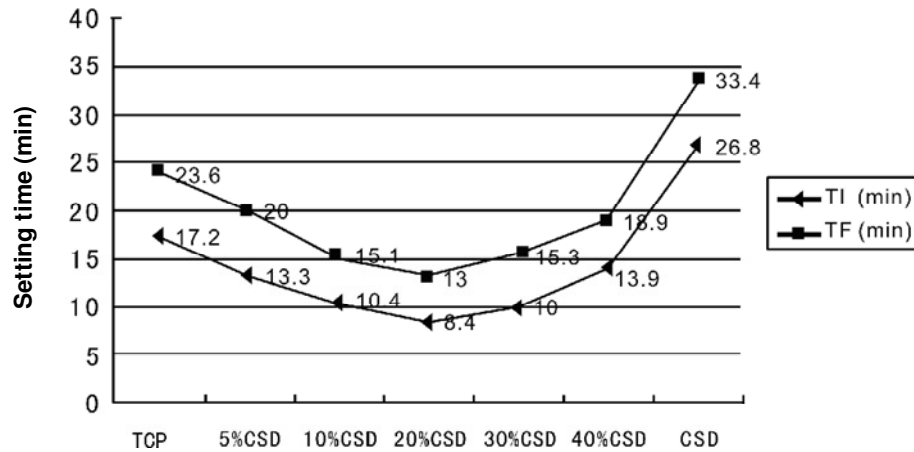


Figure 1. Setting time of bone cement mixtures with differing CSD content.

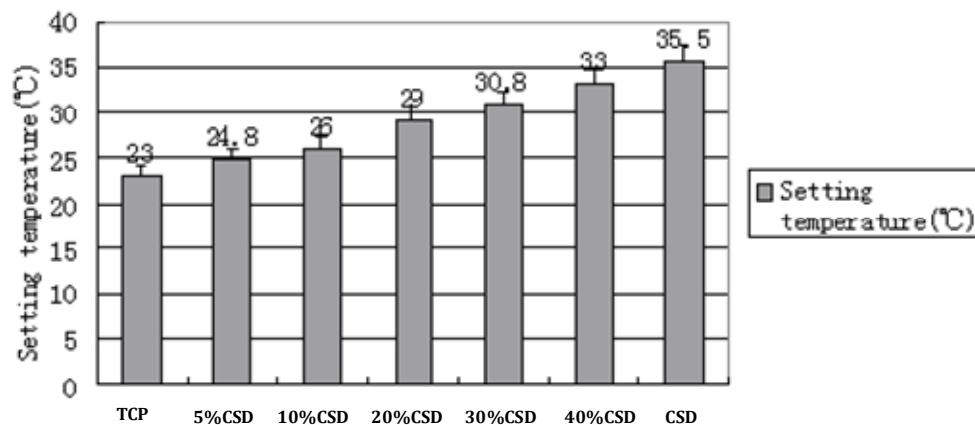


Figure 2. Setting temperature of bone cement mixtures with differing CSD content.

immersing the samples in simulated body fluid (SBF), which was simulated with Tris-HCl solution (temperature of 36.5°C and pH of 7.25). The degradation rate of the cement was characterized by analyzing its weight loss rate. The bone cements were molded into cylindrical columns of 5 mm in diameter and 10 mm in length. 20 samples were prepared of each cement type. After setting for 48 h at 37°C and 100% humidity, samples were dried at 60°C for 24 h. Samples with initial weight ( $W_i$ ) were immersed into the Tris-HCl solution at 37°C. The solutions were exchanged every day. At intervals of three weeks, five samples were removed from the solution, cleaned with deionized water, dried at 60°C for 24 h and their final weight ( $W_f$ ) was recorded. The weight loss rate of each sample was calculated according to the equation: weight loss rate (%) =  $(W_i - W_f) / W_i \times 100\%$ .

## RESULTS

### Setting time and setting temperature test

Figure 1 shows the setting time of cements with differing contents of CSD. A lower content of CSD resulted in a

significant decrease in the setting time. While with a higher CSD content, setting time increased. Figure 2 shows the setting temperature on composites with varying contents of CSD. The addition of CSD had a small effect on the setting temperature.

### Injectability

Figure 3 shows the injectability on composites with varying contents of CSD. Injectability increased with increasing CSD content in the mixtures.

### Compressive strength

The relationship between CSD content and compressive strength of the composite cement is shown in Figure 4. The compressive strength of the hardened composite cement increased with the addition of a small amount of

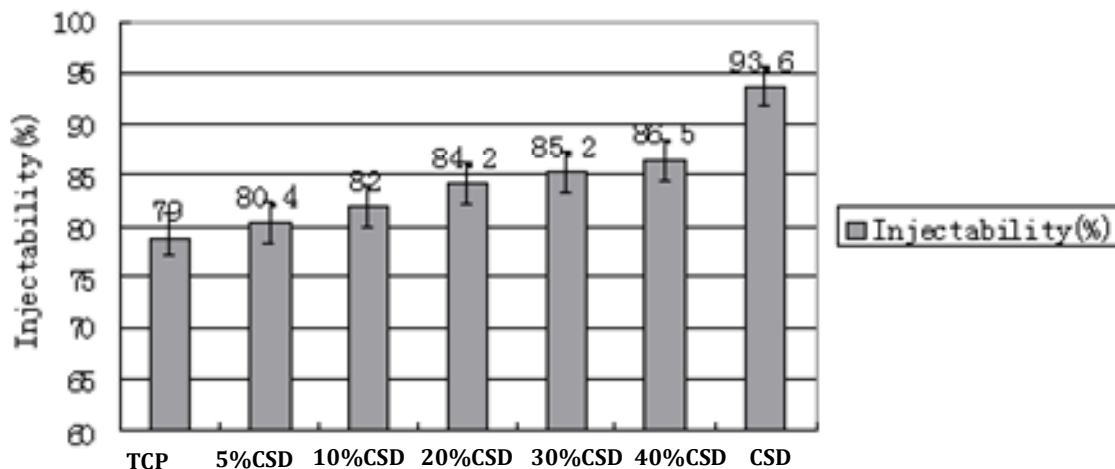


Figure 3. Injectability of bone cement mixtures with differing CSD content.

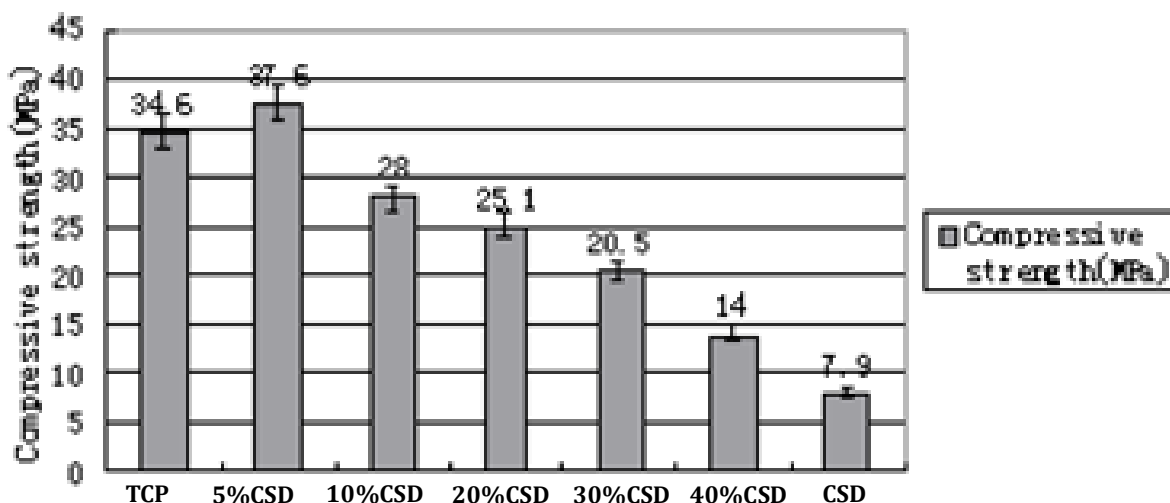


Figure 4. Compressive strength of bone cement mixtures with differing CSD content.

CSD (5%). However, when the CSD content in the composite cement increased from 10 to 40%, a decrease in the compressive strength was observed.

### Degradability

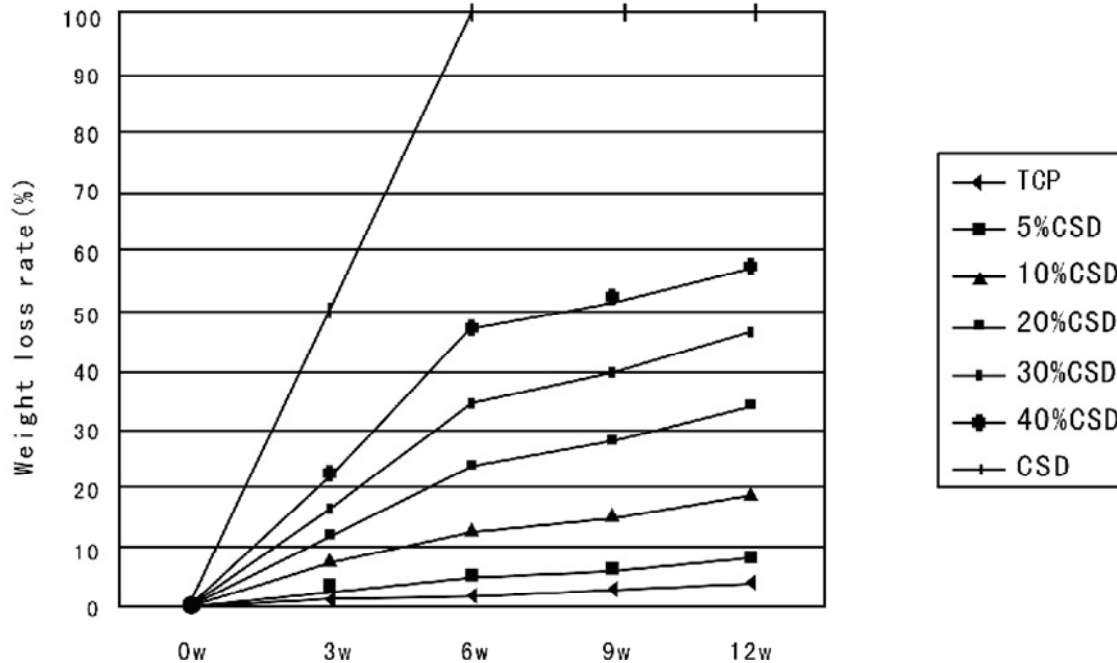
The relationship between the weight loss rate of the composite cement and the immersion time in SBF is shown in Figure 5. The weight of the composite cement decreased over time and this weight loss occurred more rapidly as the CSD content in the composite increased. The results indicate that adding CSD to  $\alpha$ -TCP could increase the degradation of composite cement.

### DISCUSSION

CSD has been proved to be both safe and biocompatible.

However, the absorption of CSD is faster than the formation of new bone *in vivo*, which is deleterious to reconstruction of the bone defect. Calcium phosphate show good biocompatibility and adequate mechanical properties but have slow absorption rates *in vivo*. The injectable and degradable TCP/CSD cement prepared in this study had better properties and is likely to prove superior to other conventional calcium phosphate cement biomaterials, and as such has a huge potential for repair or substitution of bone fractures and defects.

The most important property of an injectable vertebral substitute may be a suitable mechanical strength similar to that of vertebral cancellous bone. The compressive strength of the composite cement varied as the amount of CSD increased, with a small addition (5% CSD) augmenting compressive strength. This may be because a small amount of CSD can influence the hydration of  $\alpha$ -TCP, and thus induce the growth and modify the crystal



**Figure 5.** Degradability of bone cement mixtures with differing CSD content at different time intervals.

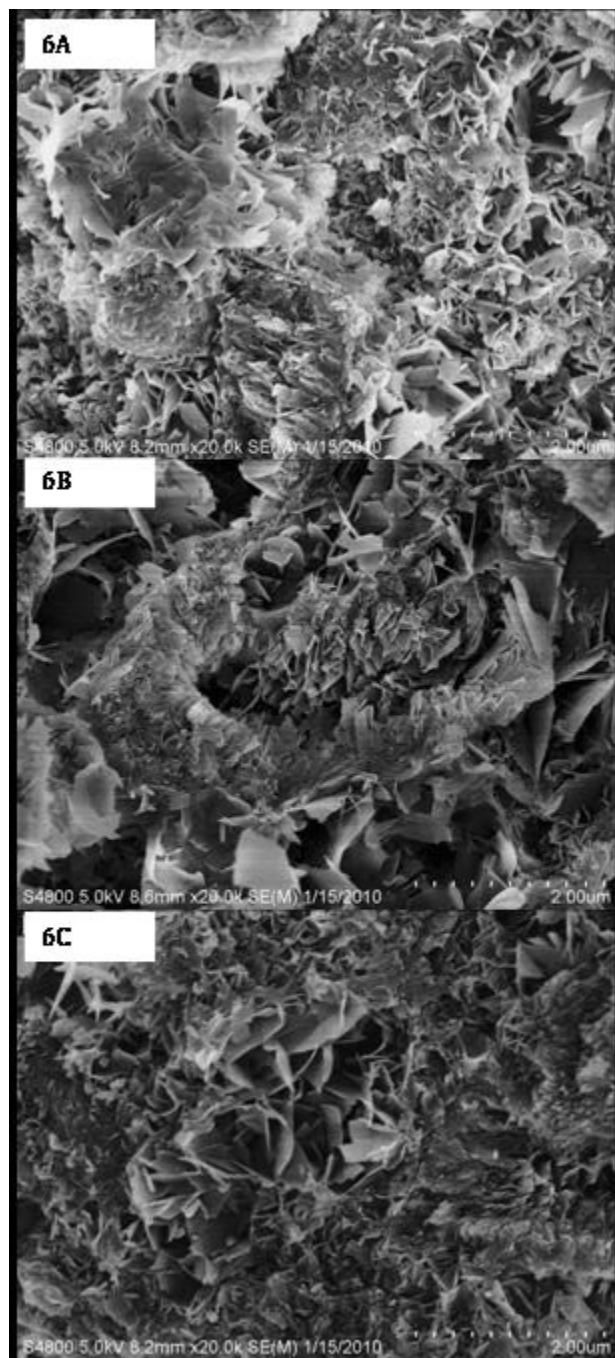
structure of CDHA. Differences in the fracture surfaces between  $\alpha$ -TCP, and  $\alpha$ -TCP with 5 and 10% CSD, after setting for 48 h at 37°C and 100% humidity, are shown in Figure 6.

However, when the amount of CSD exceeded 10%, the compressive strength of the composite cement decreased as the CSD content increased. These results show that increasing CSD content has a marked effect on the compressive strength of the cement. The decrease in compressive strength of the composite cement is probably due to the addition of CSD to  $\alpha$ -TCP damaging the bonds between  $\alpha$ -TCP crystals. Large amount of CSD may affect the workability and induce an incomplete hydration of  $\alpha$ -TCP, resulting in decreased compressive strength of the composite cement.

As with any injectable bone cement, a suitable setting time is very important, because the cement must react slowly enough to provide enough time for the surgeon to perform the implantation, but quickly enough to avoid delaying the operation. For vertebral application, particularly precise control of the cement setting rate is desired. The setting rate should then increase as fast as possible during the hardening phase. Ideally, good mechanical properties should be reached within minutes after initial setting. Khairoun et al. (1997) set a standard for setting time in clinical use: the initial setting time should range from 3 to 8 min, and the final setting time should be less than 15 min. In this study, the addition of CSD had a complex influence on the setting time of the mixed materials. While a significant decrease in the setting time was observed with the addition of a small amount of CSD, addition of a large amount resulted in an

increased setting time. This phenomenon had also been described by Bohner and Schmid (2004). The setting time of cement generally corresponds to the onset of the setting reaction (Fernandez et al., 1996). Therefore, the setting time depends on the initial setting rate of the cement. The setting rate of the cement is not a constant value, but varies with time. A particular cement might have a short setting time, but might require a long time to reach complete conversion of the TCP into CDHA (Bohner and Schmid, 2004).

On the basis of kinetics and solubility considerations, in order for CDHA to crystallize, the solution has to be supersaturated. When  $\alpha$ -TCP is mixed with water, the aqueous solution is initially under saturated towards CDHA and  $\alpha$ -TCP. Therefore,  $\alpha$ -TCP starts to dissolve. When TCP-water mixtures have reached equilibrium, the resulting solution is supersaturated towards CDHA. Therefore, at a certain point in the dissolution process, the mixing solution becomes supersaturated towards CDHA, and CDHA begins precipitating. CDHA precipitation eventually results in the entanglement of CDHA crystals, and hence to the hardening of the mixture. During setting,  $\alpha$ -TCP continuously dissolves and is replaced by CDHA. The setting reaction stops when all  $\alpha$ -TCP has been transformed into CDHA. The setting time decreases in the presence of a small amount of CSD, because at neutral and slightly basic pH values, CSD is more soluble than both  $\alpha$ -TCP and CDHA. Therefore, the presence of CSD crystals in the cement should lead to a rapid increase in the concentration of  $\text{Ca}^{2+}$  in the mixing liquid, and hence to a fast attainment of supersaturation in the mixing liquid (towards CDHA).



**Figure 6.** SEM micrographs of the fracture surfaces of  $\alpha$ -TCP (A),  $\alpha$ -TCP with 5% CSD (B), and  $\alpha$ -TCP with 10% CSD(C) after setting for 48h

As a result, the setting time decreases due to an increase in the initial setting rate. In contrast, a large addition of CSD into the mixing liquid increases the saturation of the mixing liquid towards  $\alpha$ -TCP, hence causing a decrease of the dissolution rate of  $\alpha$ -TCP. The latter effect is expected to decrease the transformation rate of  $\alpha$ -TCP into CDHA. Therefore, an increased amount of CSD should lead to an increase in the setting

time. The results of X-Ray diffraction of  $\alpha$ -TCP and 5% CSD (Figures 7A, B and C) show that the intensity peak of  $\alpha$ -TCP was much higher in a mixture of  $\alpha$ -TCP with 5% CSD than in the pure  $\alpha$ -TCP.

Injectability was also investigated in this study. There are many different ways to measure the injectability of bone cement; in this study, an objective method reported by Khairoun et al. (1998) was used. The injectability of bone cement increased with the increase of CSD content in the TCP mixture. The degradability of TCP has been investigated for many years; some study shows that degradability of some TCP is very slow both *in vivo* and *in vitro*. In order to improve the degradability of TCP, CSD was added to TCP in this study. These composite cements were found to have good degradability when the CSD content in the composite cement was more than 10%. The higher degradation rate of the composite cement in SBF was due to degradation of CSD. CSD on the surface would quickly dissolve and form a number of micropores in the surface layer of the composite cement, which could then increase the area of the cement in contact with the solution. The degradation rate of the composite cements could be improved by increasing the CSD content, indicating that degradability of composite cements could be controlled by adding CSD. Figure 8 shows the different micropore structures seen in SEM micrographs of the fracture surfaces of 5% CSD and 30% CSD after 12 weeks in Tris-HCl solution.

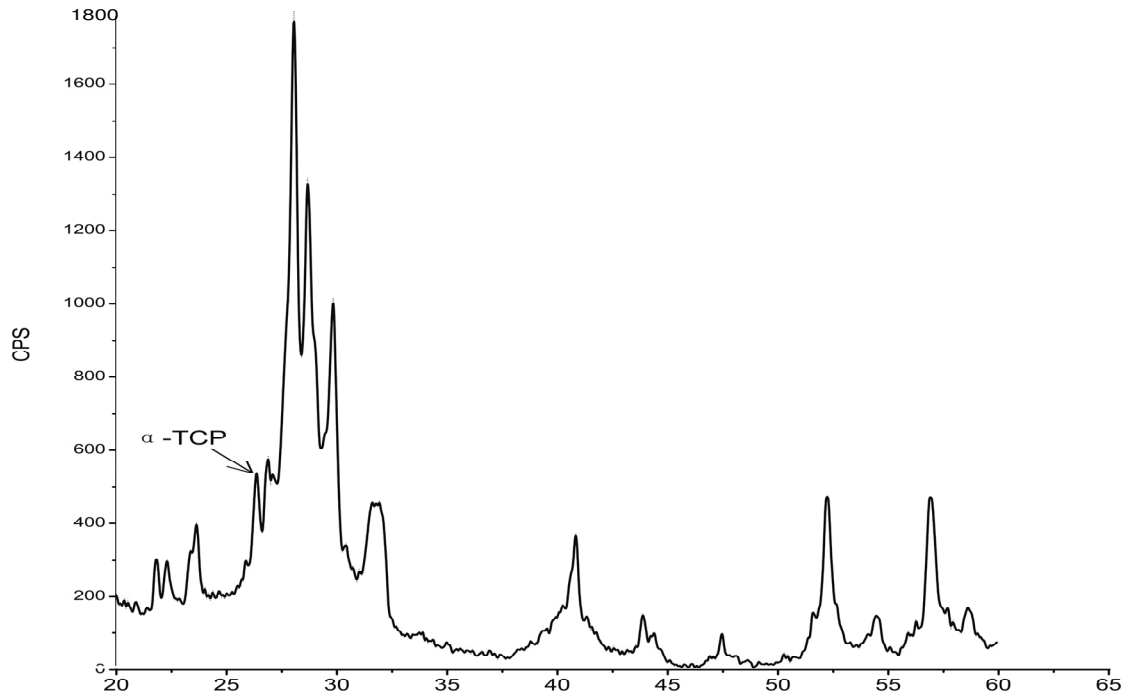
## Conclusion

In this study, in order to investigate the effect of addition of CSD to  $\alpha$ -TCP, mixtures of materials containing differing contents of CSD and  $\alpha$ -TCP were prepared. Following preparation, the materials were mixed with an aqueous solution containing 2.5 weight % of  $\text{Na}_2\text{HPO}_4$ , and characteristics of the materials were then analyzed.

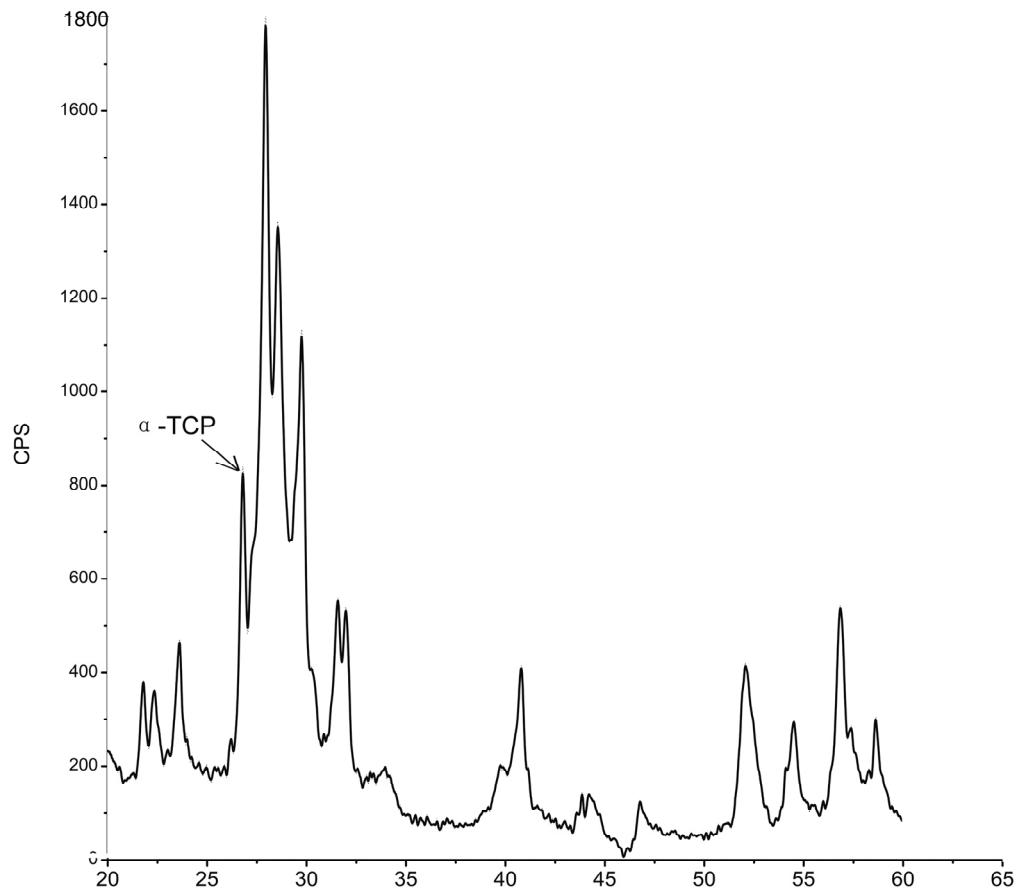
The bone cement containing CSD and  $\alpha$ -TCP provides a new way to prepare degradable and bioactive bone substituted materials. The composite bone cement prepared in this experiment has a reasonable setting time and setting temperature, suitable compressive strength, excellent injectability and degradability. Simultaneously, the bone cement mixture had good degradability and bioactivity when the content of CSD was increased. In view of the advantages of the mixtures as bone substitution, such novel composite cement could be suitable for use in minimally invasive operation of spinal surgery and has promising prospects.

## ACKNOWLEDGEMENTS

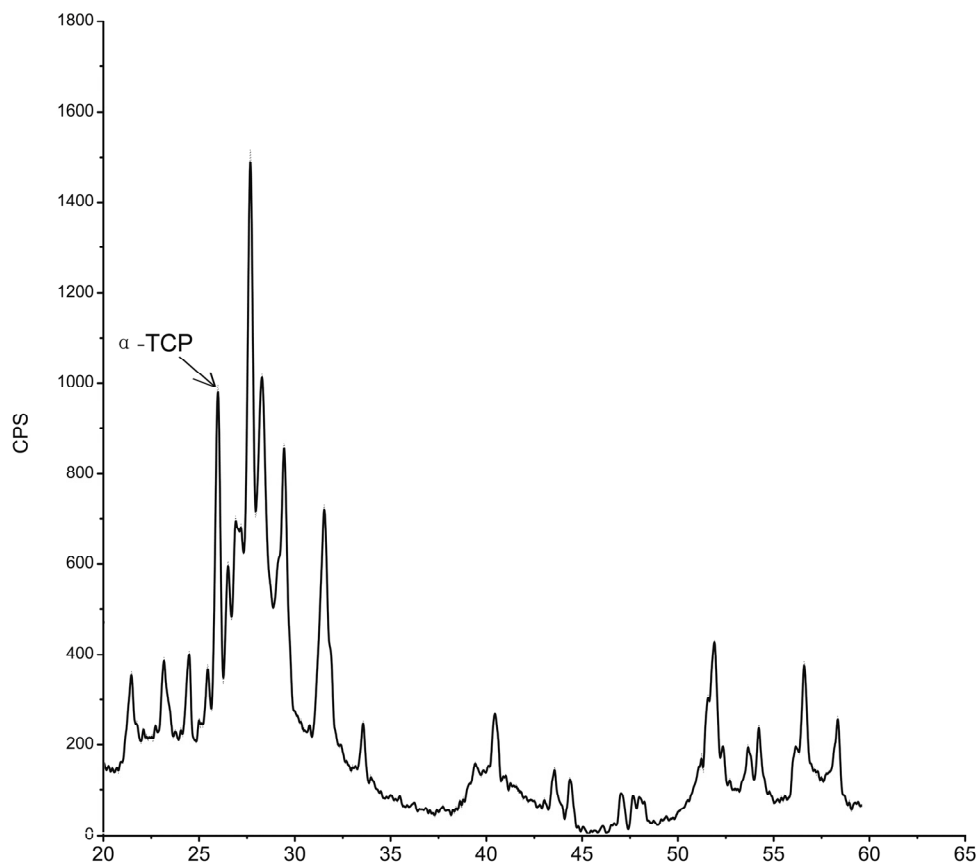
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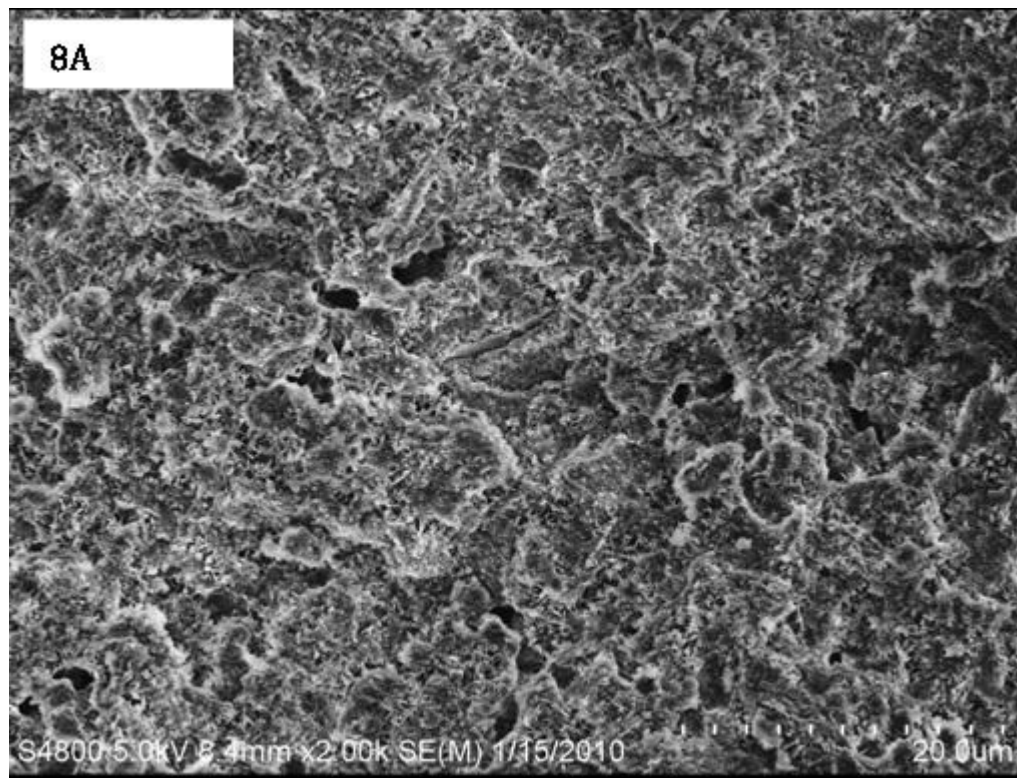
**Figure 7A.** XRD spectra of the cement samples of  $\alpha$ -TCP with 5% CSD.



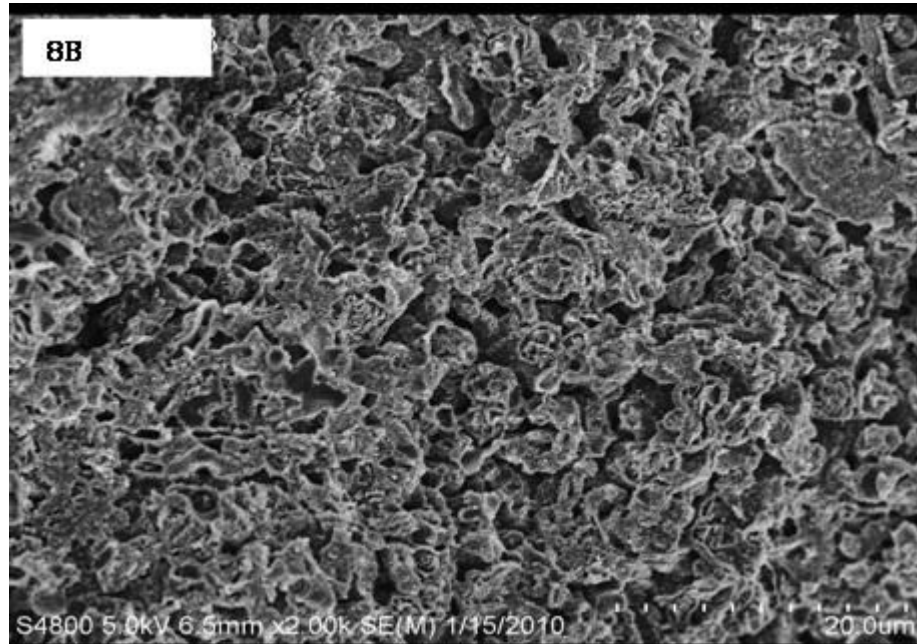
**Figure 7B.** XRD spectra of the cement samples of  $\alpha$ -TCP with 10% CSD.



**Figure 7C.** XRD spectra of the cement samples of  $\alpha$ -TCP after setting for 48h.







**Figure 8.** SEM micrographs of the fracture surfaces of  $\alpha$ -TCP (A) and 30% CSD (B) following dissolution in SBF for 12 weeks.

## REFERENCES

- Bartucci EJ, Gonzalez MH, Cooperman DR, Freedberg HI, Barmada R, Laros GS (1985). The effect of adjunctive polymethylmethacrylate on failures of fixation and function in patients with intertrochanteric fractures and osteoporosis. *J. Bone Joint Surg. (Am)*, 67:1094-1097.
- Bohner M, Schmid H (2004). New hydraulic cements based on  $\alpha$ -tricalcium phosphate-calcium sulfate dehydrate mixtures. *Biomaterials*, 25: 741-749.
- Blaha JD (1998). Calcium sulfate bone-void filler. *Orthopedics*, 21: 1017-1019
- Brown W, Chow L (1985). Dental restorative cement pastes. US Pat, 4518430.
- Costantino PD, Friedman CD, Chow , Pelzer HJ, Sisson GA Sr (1991). Hydroxyapatite Cement: Basic chemistry and histologic properties. *Arch Otolaryngol Head Neck Surg.*, 117: p. 379.
- Constantz BR, Ison IC, Fulmer MT, Poser RD, Smith ST, VanWagoner M, Ross J, Goldstein SA, Jupiter JB and Rosenthal DI (1995) Skeletal repair by in situ formation of the mineral phase of bone. *Science*, 267:1796.
- Fernandez E, Ginebra MP, Boltong MG, Driessens FCM, Ginebra J, DeMayer EAP, Verbeeck RMH, Planell JA (1996) Kinetic study of the setting reaction of a calcium phosphate bone cement. *J. Biomed. Mater Res*, 32: 367-374.
- Frankel BM, Monroe T, Wang C (2007). Percutaneous vertebral augmentation: an elevation in adjacent-level fracture risk in PKP as compared with PVP. *Spine*, 7: 575-582.
- Fribourg D, Tang C, Sra P, Delamarter R, Bae H (2004). Incidence of subsequent vertebral fracture after kyphoplasty. *Spine*, 29: 2270-2276.
- Grados F, Depriester C, Cayrolle G, Hardy N, Deramond H, Fardellone P (2000) Long term observations of vertebral osteoporotic fractures treated by percutaneous vertebroplasty. *Rheumatology*, 39: 1410-1414.
- Harris NH, Miller AJ, Bourne R, Wilson S, Kind P (1975). Proceedings: experimental investigation of fat embolism after the use of acrylic cement in orthopaedic surgery. *J. Bone Joint Surg. (Br)*, 57: 245-246.
- Hollingworth W, Jarvik JG (2006). Evidence on the effectiveness and cost-effectiveness of vertebroplasty: A review of policy makers' responses [J]. *Acad. Radiol.*, 13: 550-555.
- Khairoun I, Boltong MG, Driessens FC, Planell JA (1997). Effect of calcium carbonate on clinical compliance of apatitic calcium phosphate bone cement. *J. Biomed. Mater Res.*, 38: 356-360.
- Khairoun I, Boltong MG, Driessens FC, Planell JA (1998). Some factors controlling the injectability of calcium phosphate bone cements. *J. Mater Sci. Mater Med.*, 9: 425-428.
- McAfee PC, Bohlman HH, Ducker T, Eismont FJ (1986). Failure of stabilization of the spine with polymethylmethacrylate: a retrospective analysis of twenty-four cases. *J. Bone Joint Surg. (Am)*, 68: 1145-1157
- Nilsson M, Fernandez E, Sarda S, Lidgren L, Planell JA (2002). Characterization of novel calcium phosphate/sulphate bone cement. *J. Biomed. Mater. Res.*, 61: 600-607.
- Nilsson M, Wang JS, Wielanek L, Tanner KE, Lidgren L (2004). Biodegradation and Biocompatibility of calcium sulphate-hydroxyapatite bone substitute. *J. Bone Joint Surg. Br.*, 86: 120-125.
- Park CK, Allen MJ, Schoonmarker J, Yuan P, Bai B, Yuan HA (1999). Gelfoam as a barrier to prevent polymethylmethacrylate induced thermal injury of the spinal cord: *in vitro* and *in vivo* studies in pigs. *J. Spinal Disord*, 12: 496-500.
- Phillips H, Cole PV, Lettin AW (1971). Cardiovascular effect of implanted acrylic bone cement. *BMJ*, 3: 460-461
- Song HR, Oh CW, Kyung HS, Park IH, Kim PT, Baek SH, Kim SJ, Lee ST (2004). Injected calcium sulfate for consolidation of distraction osteogenesis in rabbit tibia. *J. Pediatr Orthop. B*, 13: 170-175.
- Urban RM, Turner TM, Hall DJ, Inoue N, Gitelis S (2007). Increased bone formation using calcium sulfate-calcium phosphate composite graft. *Clin. Orthop. Relate Res.*, 459: 110-117.
- Woo KM, Yu B, Jung HM, Lee YK (2009). Comparative evaluation of different crystal-structured calcium sulfates as bone-filling materials. *J. Biomed. Mater Res. B Appl. Biomater*, 91: 545-554.
- Woo KM, Jung HM, Song GA, Lee YK, Baek JH, Ryoo HM, Kim GS, Choung PH (2010). Modulation of the resorption and osteoconductivity of alpha-calcium sulfate by histone deacetylase inhibitors. *Biomaterials*, 31: 29-37.