The effects of excess calcium on the handling and mechanical properties of hydrothermal derived calcium phosphate bone cement

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Abstract. The objective of this study is to determine the effects of excess calcium on the handling and mechanical properties of hydrothermal derived calcium phosphate cement (CPC) for bone filling applications. Hydroxyapatite powder was synthesized via hydrothermal method using calcium oxide, CaO and ammonium dihydrogen phosphate, NH₄H₂PO₄ as the calcium and phosphorus precursors respectively. The effects of calcium excess were evaluated by varying the CaO content at 0, 5 and 15 mole %. The precursors were then refluxed in distilled water at 90-100°C and dried overnight until the calcium phosphate powder was formed. CPC was then produced by mixing the synthesized powder with distilled water at the powder-to-liquid (P/L) ratio of 1.5. The result from the morphological properties of CPC shows the increase in agglomeration and particles size with 5 mole % of calcium excess but decreased with 15 mole % of calcium excess in CPC. This result was in agreement with the compressive strength result where the CPC increased its strength with 5 mole % of calcium excess but reduced with 15 mole % of calcium excess. The excess in calcium precursor also significantly improved the setting time but reduced the injectability of CPC.

1. Introduction

Calcium phosphate has been studied as bone repair materials over the decades. The use of calcium phosphate biomaterials, especially hydroxyapatite (HA) has gained popularity because attributed to the chemical similarity with the mineral components of natural bone and teeth [1]. Besides that, it also shows excellent bioactivity, biocompatibility and osteoconductivity for bone implant substitutes [2].

There are wide range of powder-processing techniques to synthesis calcium phosphate biomaterials with the aim to produce distinct particles morphology of HA [3]. Numerous synthesis techniques of HA powder have been developed such as solid state reaction, hydrothermal, hydrolysis, sol-gel and wet-chemical precipitation [4]-[6]. These preparative routes could affect the level of crystallinity, stoichiometry and morphology of synthesis HA. Among these methods, the hydrothermal method is one of the preferable technique due to the high yield of cement production, controlled morphology and stoichiometric Ca/P ratio [7].
For bone filling applications, calcium phosphate cement (CPC) is more attractive compared to HA powder because the ability to mould and shape to fill intricate bone cavities or narrow dental defect sites [8]. CPC element composed of ceramic compounds of calcium and phosphate that may be hardened upon contact with water or aqueous solution. There are two types of CPC: apatite transforming cement like HA, carbonated HA or calcium deficient HA (CDHA), and brushite transforming cement like dicalcium phosphate dihydrate (DCPD) [9]. The apatite cement shows better mechanical properties but lower degradability than the brushite cement [10].

The main advantage of CPC is the handling properties which can be injected and shaped perfectly in situ according to the defect dimension. Investigation on the effects of excess calcium precursor on the injectability, setting time, microstructure and compressive strength of calcium phosphate cement by employing low temperature hydrothermal technique is presented in this work.

2. Experimental procedures

2.1 Synthesis of powder and cement preparation.

The calcium phosphate cement (CPC) was produced by mixing the hydrothermal derived hydroxyapatite (HA) powder with distilled water. To synthesize HA powder, calcium oxide granules, CaO (Bendosen Laboratory Chemicals) and ammonium dihydrogen phosphate, NH₄H₂PO₄ (Friendmann Schmidt Chemical) were used as the precursors and distilled water was used as the solvent. The synthesis route of HA powder via hydrothermal method was reported elsewhere [11]. The chemical reaction to synthesize HA powder is as follows:

\[ 5\text{CaO} + 3\text{NH}_4\text{H}_2\text{PO}_4 \rightarrow \text{Ca}_5(\text{PO}_4)_3\text{OH} + 3\text{NH}_4\text{OH} + \text{H}_2\text{O} \]  

(1)

Based on equation (1), the stoichiometric composition of hydroxyapatite (HA) produced is referred to 1.67 Ca/P ratio. Three synthesized powder were prepared by varying the CaO content: 0, 5 and 15 mole %. The synthesized HA was then dried overnight at 85°C in an oven until the dry powder was produced and then crushed. CPC was produced by mixing the powder and liquid phases homogenously for 3 min at the (P/L) ratio of 1.5. The as-synthesized powder was used as the powder phase, while distilled water was used as the liquid phase. Three different types of cements were prepared based on the variation of calcium excess labelled as CPC-0, CPC-5 and CPC-15. The samples were then fabricated by filling the cement into a teflon (PTFE) mould of 10 mm diameter and 15 mm height. After hardening, the cements were demoulded and left dried in room temperature overnight before characterization. Figure 1 shows the flowchart of the method to produce CPC with calcium excess.

Figure 1. Flowchart of the methods to produce CPC-0, CPC-5 and CPC-15.

2.2 Physical and mechanical characterization

The microstructure analysis of the CPC samples with variation of calcium excess was performed by FESEM (JEOL, JSM 6700F) with a deposition current of 60mA and sputter coating using gold for
50s. Different acceleration condition and voltage were applied to the sample to observe the microstructure of samples.

2.3 Injectability test
The injectability test was done through extrusion of 5 ml non-needle plastic syringe with 2.68 mm inner diameter of canula filled with paste as the working sample. The injectability results of CPC with 0, 5 and 15 mole % of calcium excess were evaluated by observing the extrusion paste of the samples. The syringe was filled with mixture of as-synthesized powder and distilled water at P/L ratio of 1.5 before extruded using a Lloyd Universal Testing Machine, LR 10 K+ model. The crosshead speed and maximum load was constant for all samples at 50mm/min and 300 N, respectively. The injectability results was measured by recording the evolution of the extrusion force and extruding time of the syringes.

2.4 Setting time test
To identify the setting time, Gillmore needles method was used to measure the CPC with variation of calcium excess of 0, 5 and 15 mole %. The Gillmore method was done by following the ASTM C266-08 designation method [12]. After homogenously mixed, the CPC paste was moulded into a PTFE (teflon) mould. The initial and final setting times were determined by holding the light and heavy needles in vertical position and applied to the cement surface until no visible indentation formed.

2.5 Mechanical test
The compression test was done using the Lloyd LR 10 K+ Universal Testing Machine on 10 mm diameter x 15 mm length specimens at 1 mm/min crosshead rate for all samples of CPC.

3. Results and Discussion
The variation of calcium excess has shown some effects on the handling and mechanical properties of hydrothermal derived CPC. Figure 2 shows the extruded paste after injectability test was done on three samples (CPC-0, CPC-5 and CPC-15) with 0, 5 and 15 mole % of calcium excess in CPC, respectively. The P/L ratio and mixing time of all samples were constant at 1.5 and 3 min. CPC-0 and CPC-5 from Figure 2(a) and 2(b) exhibited excellent injectability because the whole paste extruded from the syringe. However, extrusion paste from CPC-0 is continuous compared to CPC-5 due to the reduction of water content of CPC-5 after more calcium content appeared in the paste. The CPC-5 paste started to increase its viscosity because of lack in water content which gives discontinuous form of paste. Figure 2(c) shows sample from CPC-15 where some paste has hardened and cannot be extruded out of the syringe after injected. Overall, increase in calcium excess of CPC can reduce the injectability performance of cement.

![Figure 2](image_url)

**Figure 2.** Extruded paste of samples after injected from the syringe (a) CPC-0, (b) CPC-5 and (c) CPC-15.
Figure 3 presents the initial and final setting times of CPC with the effects of calcium excess. The calcium excess has significantly shortened both the initial and final setting times of CPC. The initial setting time has reduced to 40, 30 and 15 min with 0, 5 and 15 mole % of calcium excess, respectively while the final setting time was 450, 360 and 210 min for CPC-0, CPC-5 and CPC-15, respectively. It can be concluded that the higher calcium excess can increase rate of self-hardening of CPC. This result is in agreement with injectability result where increase in calcium excess can harden the cement faster and reduced injectability.

The results of microstructure analysis using FESEM was shown in figure 4. The particles were in spherical and flakes shapes which represent HA powder. The powder also formed agglomerated particles of a needle like morphology. The particles size increased as the amount of calcium excess increased to 5 mole % but decreased with 15 mole % of calcium excess. Between three samples, CPC-5 shows the highest amount of agglomerated particles where all particles interconnected each other. This is in agreement with compressive strength results shown in figure 5. The highest compressive strength achieved by CPC-5 with 1.682 MPa compared to the compressive strength of CPC-0 and CPC-15 with 1.638 MPa and 1.508 MPa, respectively. This results indicated that, the increase in agglomerated particles may increase the strength of the cement. The agglomeration of particles also the main reason of poor injectability performance of CPC [13]. The mixture with higher calcium excess can be sticky on each other due to the inter-particles attractive force which may harden the cement faster [14].

![Setting Time](image1.png)  ![Compressive Strength](image2.png)

**Figure 3.** Effect of calcium excess on the setting time of CPC.

**Figure 4.** Effect of calcium excess on the compressive strength of CPC.

![Microstructure](image3.png)

**Figure 5.** Microstructure of CPC with different amount of calcium excess (a) CPC-0 (b) CPC-5 and (c) CPC-15.

4. Conclusion

The effects of excess calcium on the injectability, setting time, morphology and compressive strength of hydrothermal derived CPC was successfully studied. The individual particles size and
agglomeration increased with 5 mole% of calcium excess but decreased after 15 mole% of calcium excess in CPC. Increased the calcium content up to 5 mole% also increased the compressive strength in range of 1.638 to 1.682 MPa but decreased to 1.508 MPa with 15 mole% of calcium excess. The results also indicated that, both the initial and final setting times of all samples were improved as the calcium content increased. The initial setting time was 40, 30 and 15 min while the final setting time was 450, 360 and 210 min, respectively for CPC-0, CPC-5 and CPC-15. However, increase in calcium content of CPC could reduce the injectability performance of CPC due to lack of water content of CPC and increase in the rate of self-setting of cement. Overall, injectability is proportional to the hardenability of cement in term of setting.

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