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The Effect of α-TCP Particle Size on Mechanical and Setting Properties of Calcium Phosphate Bone Cements

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Background: Calcium phosphate cements (CPCs) show several advantages over other materials, used for bone repair. For example, they are injectable, easily shapeable and remained localized. Therefore, they fill effectively bone defects with an irregular shape. Furthermore, CPCs are very bone compatible and also osteoconductive.

Objectives: This work aimed to investigate the effect of particle size on the mechanical and setting properties of α-tricalcium phosphate (α-TCP) based cements. The rate of conversion of reactants to nano-hydroxyapatite (nHA) in the medium of human blood plasma is also studied.

Materials and Methods: In this study, we prepared CPCs consisting of α-TCP (61%), dicalcium phosphate (DCP) (26%), calcium carbonate ($CaCO_3$), hydroxyapatite (HA) (3%) as powder phase, in a solution of 3 wt% NaH_2PO_4 as liquid phase. In the next step, three different cements with the same formulation but different α-TCP particle sizes (4 μm, 10 μm, 22 μm) were prepared. Finally, we evaluated the setting time, compressive strength and the rate of conversion of reactants to apatite phase in blood plasma.

Results: Based on the results, the initial setting time decreased from 30 minutes for CPC with α-TCP particle size of 22 μm to 15 minutes for the cement with α-TCP particle size of 4 μm. Also, the cement prepared with the least α-TCP particle size exhibited the maximum compressive strength after setting. The results revealed that reduction of α-TCP particle size, the main component of the CPC, favors conversion of cement constituents to needle-like nano-apatite crystals when soaking in human blood plasma, and this leads to increment of mechanical strength.

Conclusions: In α-TCP based CPCs, reduction of α-TCP particle size favors the conversion of the cement constituents to nano-apatite crystals (when soaking in human blood plasma), which leads to reduction of setting time and increase in mechanical strength of CPCs.

Keywords: Bone Cements; Hydroxyapatite; Particle Size

1. Background

Calcium phosphates based materials are similar in composition to the mineral components of bone tissue, and this property has made them the most promising materials for replacement of damaged bones. Calcium phosphate cements (CPCs) are cementing systems, consisting of powder and liquid phases; their mixing causes a chemical reaction accompanied by setting and subsequent hardening.

CPCs are designed for plastic filling of bone defects, and joining bone fragments. They combine features such as osteoconductivity (ability to maintain vital functions of osteogenic cells), biocompatibility (lack of negative reactions to body) and formability (ability to fill cavities with the complex configuration). CPCs are injectable, which allows them to enter directly into the bone. By controlling the phase composition, it is possible to regulate the rate of bio-degradation (dissolving in the body) of CPCs (1).

α-TCP based calcium phosphate cement is one the most important commercial CPCs available in the market. Z. Kojic et al. investigated the irritative property of α-TCP to rabbit skin, and the results revealed that α-TCP was neither toxic nor irritant, both in solid and paste form (2). Non-toxicity, bioactivity, both in vitro and in vivo, and also being more bioreabsorbable than many other bioceramics (such as α-TCP and HA) has made α-TCP an ideal bone substitute (2-5).

The initial setting time (Si), the final setting time (Sf) and the ultimate compressive strength are important terms in clinical requirements for calcium phosphate bone cements. Si indicates the time when the paste cannot be deformed without damaging the structure of the hardening cement, and Sf indicates the time when the cement can be touched without scratching it. These times (Si and Sf) have clinical significance as the cement paste should be implanted before Si, and the wound can be closed after Sf (6).

Implication for health policy/practice/research/medical education:
Calcium phosphate cements (CPCs) show several advantages over other materials used for bone repair. For example, they are injectable, easily shapeable, and remained localized. Therefore, they fill effectively bone defects with an irregular shape. In this study, the effect of reactants particle size on some key properties of CPCs was investigated, which helps improving the quality of these cements.

However, poor mechanical properties of CPCs have limited their applications to low loaded or unloaded bearing organs (7). This problem is even enhanced by the fact that in vivo resorption of CPC is very slow (8). The CPCs should have a compressive strength of at least 30 MPa (Megapascal). The importance of other mechanical properties such as flexural strength, tensile strength, hardness and elastic modulus depends on the application environment (4).

Thus, the importance of studying parameters affecting CPC properties such as setting time, and compressive strength appears. Initial setting time, final setting time, and compressive strength strongly depend on various parameters, including particle size of reactants, time, concentration of liquid phase, and liquid to powder ratio (6, 9).

The influence of the particle size of the reactants on the setting time and hardening properties has been studied in detail in other hydraulic cements, such as Portland cement (10, 11). In the field of calcium phosphate cements, some authors studied the influence of the particle size of the reactants on the final mechanical properties of some cements, such as dicalcium phosphate (DCP) and tetracalcium phosphate (TTCP) based cements (11) or dicalcium phosphate dihydrate (DCPD) and TTCP based cements (12).

2. Objectives

This work aimed to investigate the effect of particle size on the mechanical and setting time properties of α-TCP based cements. The rate of conversion of reactants to nano-hydroxyapatite (nHA) in the medium of human blood plasma is also studied.

3. Materials and Methods

3.1. Synthesis of α-TCP and nHA

α-TCP and nHA were synthesized and characterized according to the method described by Shahrouzi J et al. (13). The synthesized α-TCP was milled three separate times in a planetary ball-mill to obtain three different particle sizes. The particle size distribution of α-TCP was measured by laser particle size analyzer instrument (Fritsch particle sizer analysette).

3.2. Cement Preparation

The powder phase of the cement was composed of 61% α-TCP, 26% DCP, 10% $CaCO_3$ and 3% nHA (5) which mixed with aqueous solution of 3 wt% $NaH_2PO_4.2H_2O$ (from Merck, Germany) as liquid phase at liquid-to-powder ratio of 0.35 mL/g until a homogenous paste obtained.

3.3. Characterization

3.3.1. Setting Time

Powder phase components were mixed in a mortar for about two minutes and then mixed with liquid phase thoroughly to form a homogenous paste. The initial and final setting times of the cements were measured using the Gillmore needles according to the C266-89 ASTM standard (13, 14).

3.3.2. Mechanical Strength

To prepare specimens for measuring compressive strength (CS) of the cements, the powder phase and liquid phase were mixed to form a paste and then poured into a cylindrical Teflon mold (6 mm in diameter and 12 mm in height). The specimens were removed from the mold after 5 hours and kept in human blood plasma solution for 12 days. A Universal Testing Machine (STM 120, Santam Co.) was used for measuring the compressive strength of specimens at the loading rate of 1 mm/min (13).

3.3.3. Phase Analysis

The phase composition of synthesized α-TCP, nHA, and the cements before and after soaking in the human blood plasma were checked by X-ray diffraction (XRD) using automated Phillips PW3710 diffractometer with Cu-kα radiation operated at 40 kV and 10 mA (13).

3.3.4. Microstructure Observation

The surface morphology of the gold-coated cements was also analyzed by scanning electron microscopy (SEM, Stereoscan S360 Cambridge Ltd.) at the operating voltage of 20 kV and electrical current of 10 mA (13).

3.3.5. Statistical Analysis

All experiments were performed at least three times. Data were processed by Excel Microsoft 2010, and the results were presented as mean ± standard deviation (SD).

4. Results

4.1. Characteristics of Starting Materials

Figure 1 Shows XRD spectrum of synthesized α-TCP, as the major component of starting powders. All observed diffraction peaks are related to α-TCP, and no impurity phases were found.

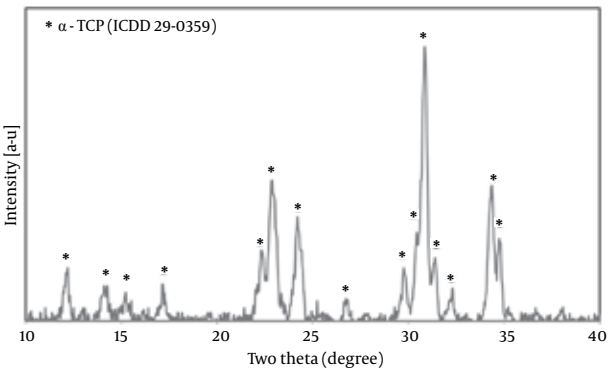

Figure 1. XRD pattern of synthesized α-TCP shows that all observed diffraction peaks relate to α-TCP and no impurity phases are found

The particle-size distribution of the α-TCP after being milled in planetary ball-mill for three different periods of times, obtained by laser diffraction showed that about 90% of the total volume of α-TCP ground for 45 minues was under 18.05 μm diameter and mean size of the α-TCP particles was about 4 μm, while the mean size of the α-TCP particles ground for 30 and 15 minutes were respectively 10 and 15 μm.

The XRD pattern of biomimetically synthesized nHA is illustrated that all major peaks relate to nHA, except two peaks (at 2θ = 29 and 30°) which correspond to remaining TTCP.

The rod-like particles with diameter lower than 50 nm and length-to-diameter ratio higher than 3 can be observed by transmission electron microscopy as shown in Figure 2. Some authors suggested that the HA crystals tend to grow along [0001] direction, resulting in needle-like morphology. Also, nHA had a mean particle size of 1 μm.

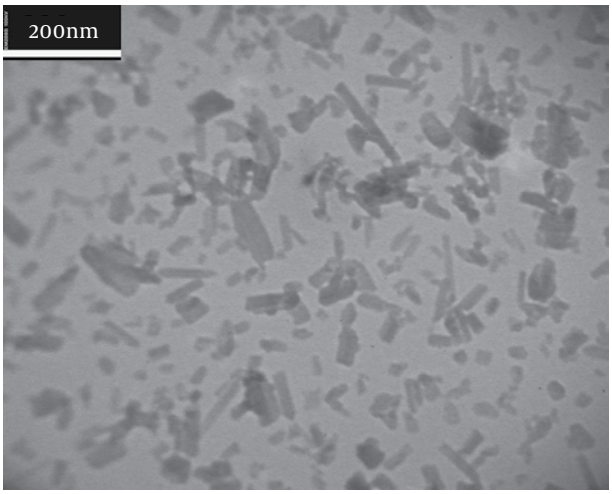

Figure 2. TEM Image From Synthesized Nano-Structured Hydroxyapatite

4.2. Setting Time

Figure 3 shows the initial and final setting times of the cements prepared with three different α-TCP particle sizes. According to results, both initial and final setting times of the cements are in direct relation with α-TCP particle size. The final setting time of the cements prepared α-TCP particle sizes of 4, 12 and 22 μm are respectively 26, 29 and 32 minutes. Hence, the final setting time of the cements increased with increment of α-TCP particle size.

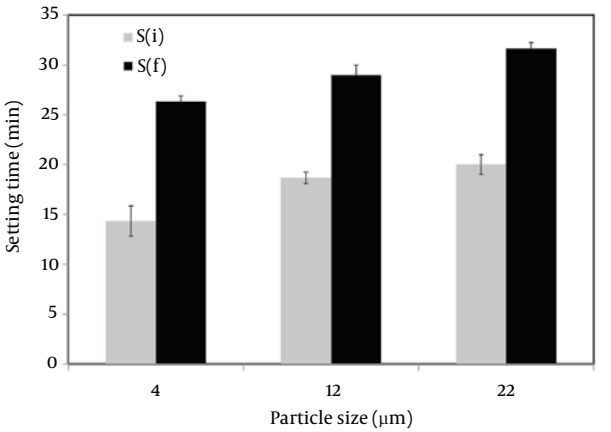

Figure 3. Initial (Si) and final (Sf) Setting time of CPCs prepared at different particle sizes of α-TCP

4.3. Compressive Strength

Figure 4 shows the wet compressive strength (CS) of the cements after soaking in human blood plasma for different periods of time versus α-TCP particle size. The results revealed that the wet compressive strength of the cements decreased with the increment of α-TCP particle size. The wet compressive strength of specimen prepared with α-TCP particle size of 4 μm was more than twice the CPC prepared with α-TCP particle size of 22 μm both after 12 days of soaking in human blood plasma.

The ascendant trend for CS of the specimens was continued by keeping the specimens for more days in human blood plasma. The maximum value for CS of cements related to the specimens prepared with α-TCP particle size of 4 μm and soaked in human blood plasma for 12 days (~27 MPa).

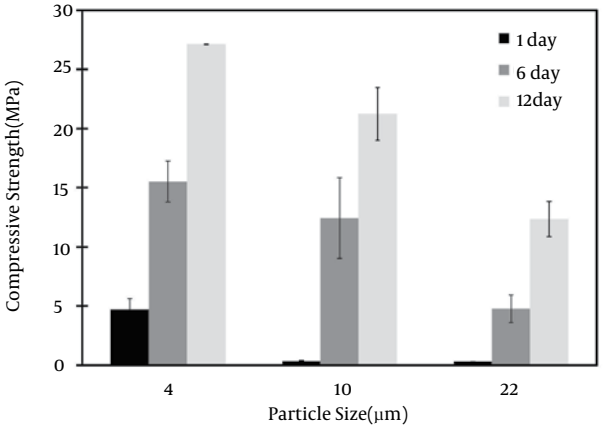

Figure 4. Compressive strength of CPCs prepared with different particle sizes of α-TCP after being soaked in human blood plasma for 1, 6 and 12 days

4.4. Phase Composition of Cements

Figure 5 shows XRD spectrum of reactants of the powder phase and also the cements prepared with three different particle size of α-TCP soaked in human blood plasma for 12 days. As the results reveals, although α-TCP is still the dominant phase and conversion of starting materials to apatite has not completed yet, but the intensity of peaks related to HA has increased as the α-TCP particle size decreases.

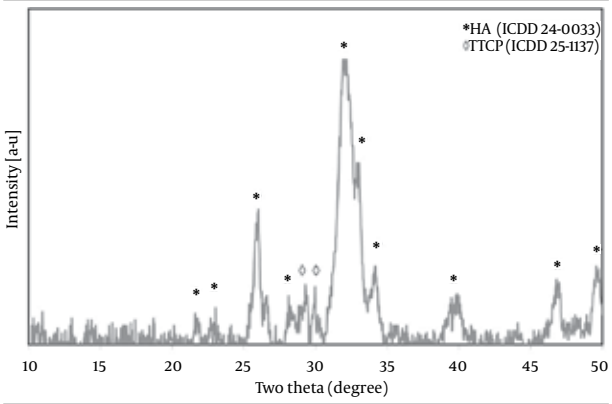

Figure 5. XRD Patterns of the powder phase reactants and cements prepared with three different particle sizes of α-TCP after being soaked in human blood plasma for 12 days

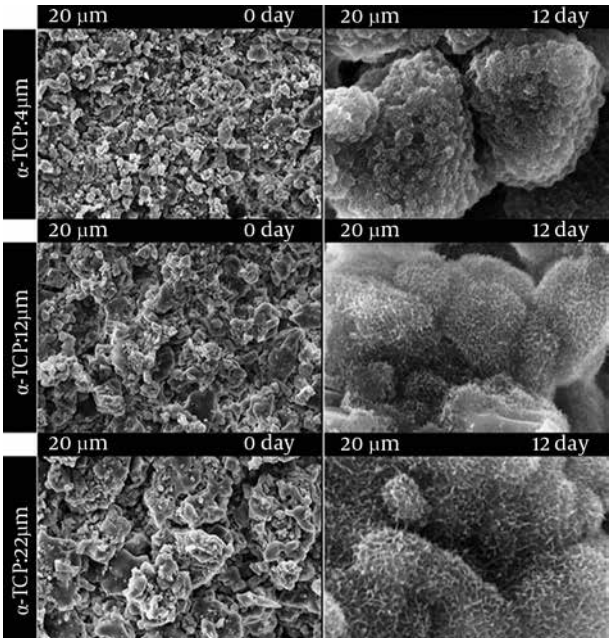

Figure 6. SEM images of cement prepared with three different α-TCP particle sizes before and after being soaked in human blood plasma fir 12 days

In other words, by reduction of α-TCP particle size, the main component of the CPC powder phase, the conversion rate of cement components to nanoapatite when soaking in human blood plasma has increased.

4.5. Morphology of Cements

Figure 6 shows the surface morphology of the cements prepared with three different α-TCP particle sizes before and after soaking in human blood plasma for 12 days. In the microstructure of samples related to before soaking in human blood plasma, polyhedral particles are observed which in the cements prepared with finer α-TCP, these polyhedrons are smaller in size. It also seems that by increasing the α-TCP particle size, the size of pores has increased. In the images related to the cements after soaking in human blood plasma for 12 days, spherical particles consisting of needle-like crystals entangled in each other are observed which relates to HA phase. By increment of α-TCP particle size, the needle like phase has become more delicate and also more in quantity.

5. Discussion

Particle size is one of the most important parameters that affects the hydration and setting process of calcium phosphate cements. As the results of this investigation indicate, change in α-TCP particle size, as the major component of the powder phase, affects some key characteristics of the cements such as its setting time, compressive strength, morphology, and phase composition.

Setting time of the CPCs is associated with the solid network formation, influenced by the rate of the hydration process. The required energy for forming this solid network is provided by the surface energy produced from formation of product material in the liquid phase. Thus, the higher rate of hydration reaction leads to higher supersaturation rate and consequently, reduction in setting time of the cement. Because the reactants with smaller particle size have higher specific area (and therefore, higher surface energy), after mixing with liquid phase, they reach the supersaturation state faster. These facts support our results regarding the setting time.

Although the quantity of hydration product was a main factor affecting the macro-properties of the cement, the compressive strength was also associated with the porous structure and the matches of all particles. Dehydrated particles took part in matches among the grains as the "micro-framework". The smaller the grain of the starting material, the faster the hydration process, and the more hydration product would be formed.

In the presence of proper quantities of framework material, the adhesive strength among the grains would rise, and so would the value of the compressive strength at macroscopy. Otherwise, a fast hydration reaction would produce more defects in the hydration product crystal, which would make it difficult to reach thermodynamic stability. Such metastable particles would reach thermodynamic equilibrium through a dissolution-reprecipitation process, which might destroy the existing structure of setting cement slurry formed at the initial stage and decreases the compressive strength (15).

Physically, it is accepted that in calcium phosphate cements, the cement hardening is due to the entanglement of the crystals in the product phase, therefore, the morphology and size of these crystals should condition the final strength of the cement. As the cement studied in this research is an apatite cement, the entanglement of the Hydroxyapatite crystals defines the strength of the cement. The higher amount of the compressive strength in cements prepared with fine α-TCP is due to the smaller entangled crystals. Because small crystals have more contact points, and their porosity is low (10).

It can be concluded that reduction of α-TCP particle

size as the major component of powder phase leads to increment of compressive strength of the α-TCP calcium phosphate cements and also decreases both their initial and final setting times. Moreover, reduction of α-TCP particle size favors the conversion of the cement constituents to nano-apatite crystals when soaking in human blood.

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Authors' Contribution

Study supervision and drafting of the manuscript: Dr M. Shahrezayee; Acquisition, analysis, and interpretation of data, as well as drafting of the manuscript and statistical analysis: J. Shahrouzi; Study concept and design, statistical analysis, administrative, technical, and material support: Dr S. Hesaraki; and Study supervision, administrative, technical, and material support: Dr A. Zamanian.

Financial disclosure

There was no financial interest.

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