Prospective of Calcium Phosphate Cements for Bone Regeneration: Physicochemical, Mechanical and Biological Properties

Abstract

Calcium phosphate ceramics (CPCs) have been widely used as biomaterials for the regeneration of bone tissue because of their ability to induce osteoblastic differentiation in progenitor cells. This article provides an overview on the chemistry, kinetics of setting and handling properties (setting time, cohesion and injectability) of CPCs for bone substitution, with an emphasis on their mechanical and biological properties. The processing parameters that can be adjusted to control the setting process, injectibility and cohesive strength are presented. CPCs are used for the repair of non-load bearing bone defects due to the brittle nature and low flexural strength of such cements. Processing strategies to improve mechanical strength, fracture toughness and reliability of CPCs are discussed. A systematic discussion on the effects of the physical (e.g. surface roughness) and chemical properties (e.g. solubility, crystallinity) of CPCs on protein adsorption, cell adhesion and osteoblastic differentiation *in vitro* is presented. Furthermore, the physical and chemical properties of CPCs that govern its efficacy as carrier and candidate biomaterials for controlled release of variety of drugs and bioactive molecules are elaborated. Future research directions to improve the performance of CPCs are highlighted and briefly discussed.

Keywords: Calcium phosphate; Biocement; Injectability; Cohesion; Fracture toughness; Osteoinductivity; Drug delivery

Review Article

Volume 2 Issue 6 - 2017

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Received: April 30, 2017 | **Published:** September 13, 2017

Abbreviations: ACP: Amorphous Calcium Phosphate; BCP: Biphasic Calcium Phosphate; CPC: Calcium Phosphate cement; CNT: Carbon Nanotube; FAK: Focal Adhesion Kinase; FRCPC: Fiber Reinforced Calcium Phosphate; HAP: Hydroxyapatite; TCP: Tricalcium Phosphate; DCPD: Dicalcium Phosphate Dihydrate; ALP: Alkaline Phosphatase

Introduction

In the early 1980s, scientists at the American Dental Association Le Geros et al. [1] and Brown & Chow [2-5] first explored the possibility of generating a monolithic calcium orthophosphate ceramic at ambient or body temperature via a cementation reaction among one or more component of calcium orthophosphate precursors. Presently this type of materials is referred to as calcium phosphate cements (CPC) and the discovery of self-setting CPC was a significant achievement in the field of bioceramics for bone regeneration, since it's self-setting nature opened up the door for minimally invasive surgical techniques as compared to classical surgical methods [6]. The aim of biomimetic CPC is to temporarily play the role of artificial bone with minimal interference on bone functions and properties until a new bone has been grown in its place. Apart from excellent biological behaviour exhibited by CPC, its injectability, hardenability *in vivo* at body temperature [7,8], mouldability and adaptation to the surrounding bone even for irregularly shaped cavities, represent its unique advantage over other bioceramics, which are difficult to machine and shape [9]. After a comprehensive mechanical characterization for both hydroxyapatite and brushite forming CPC, Charrière et al. [10] found that hydroxyapatite cements have the potential to be structural biomaterials while brushite cements are suitable as bone fillers. Typical application of CPCs are treatment of maxillofacial defects or deformities [6] or the repair of craniofacial defects [11], with the possibility of applying it in moderately load-bearing defects, such as in vertebroplasty or kyphoplasty [12-14].

Despite CPCs' high potential and wide acceptance as biomaterials for bone regeneration, some crucial issues still need to be solved to satisfy clinical requirements [6,15]. Specifically, CPCs without any additives suffer from poor injectability [16,17] and are prone to disintegrate upon early contact with blood or biological fluids [18]. CPCs are limited in application to non- or moderate load-bearing musculoskeletal defects [19,20] because they lack enhanced toughness, reduced brittleness and improved reliability. The purpose of this mini review is to study the chemistry, physical, mechanical and biological properties of CPCs with special emphasis on various parameters that can improve their properties for wider clinical applications.

Chemistry of CPC setting

The setting reaction is associated with dissolution of more soluble calcium phosphate phase with the consequent super saturation of calcium and phosphate ions and reprecipitation

of least soluble calcium phosphate phase [21]. Once the ionic concentration reaches a critical value, the nucleation of the new phase occurs surrounding the powder particles that keeps growing and entangles with each other as the dissolution of the reagents continues [22,23]. Apatite is the most stable calcium phosphate at pH >4.2 (37˚C) and brushite is the most stable one at pH<4.2 (37˚C) [24,25]. That is why, although various mixtures of calcium and phosphate sources as precursors for CPC exists, there are in principle only two cement types i.e. apatite with various stoichiometric composition between $Ca_9(PO_4)_5HPO_4OH$ and $Ca_{10}(PO_4)_6(OH)_2$ or brushite ($CaHPO_4 \bullet 2H_2O$, DCPD) in final setting reaction [6,26]. Composition of both solid and liquid phases govern the kinetics of CPC setting. Ten Huisen & Brown [27] found that the kinetics of apatite CPC hardening from α-TCP precursors strongly depends on both the concentration and the type of acid. Due to the increased solubility of the reactant phases at lower pH, acetic acid accelerates the CPC setting reaction. On the other hand, the complexing and adsorbing ability of citrate ions onto α-TCP crystals and apatite nuclei retards both the formation of crystal nuclei and their further growth and entanglement and thus slows down CPC's setting kinetics.

Handling properties of CPC

Besides having excellent biocompatibility and bioactivity, two other main advantages of CPCs are its injectability and self-setting capability *in vivo* at body temperature. However, without any physical, chemical and compositional modification, CPCs normally possess a relatively long setting time, poor injectability and poor cohesion [6,28,29].

Setting time

The factors that promote faster setting kinetics can potentially reduce the setting time of CPCs. Such factors are

A. Smaller particle size i.e. high specific surface of precursors;

B. Low crystallinity;

C. Accelerators in the liquid and solid compositions;

D. Higher setting temperature; and

E. A low liquid-to-powder ratio (L/P ratio) [30].

However, too short a setting time may make CPCs unworkable during total surgical implantation, whereas unexpectedly longer setting time may cause severe inflammatory responses and disintegration of CPC implants [26]. Thus it is critical to prepare a cement with an optimum and suitable setting time, preferably a few minutes.

Cohesion and anti washout ability

The processing steps that generate strong attractive forces between CPC particles or weaken the forces acting between the paste and the surrounding fluids; i.e., osmotic pressure, can be applied to improve cohesion of CPCs. Thus, a smaller particle size and control over the (liquid/ powder) L/P ratio can be strategically used to strengthen particles' interactions, thus improving cohesion. Moreover, enhancing the viscosity of the mixing liquid by dissolving biopolymers such as sodium alginate [31], hydroxypropyl methylcellulose (HPMC) [32,33], hyaluronic acid [34], chitosan [35,36] and modified starch [37], though can prolong setting time and hamper mechanical strength [18], has found to be effective in improving cohesion and anti-washout properties of CPCs.

Injectability

Bohner & Baroud [16] redefined the injectability of CPC paste as the ability to stay homogeneous during injection without any filter-pressing and independent of the injection force. A series of theoretical and experimental studies, found that parameters such as decreasing particle size, using round particles, using deagglomerated particles, using a broad particle size distribution, increasing L/P ratio, adding ions or polymers, decreasing particle interactions and increasing the viscosity of the mixing liquid can be applied to improve injectability of CPCs, but the best strategy is to increase the viscosity of the mixing liquid [33, 38].

Strengthening of CPC

To reduce brittleness of CPC and to improve their mechanical performance for load-bearing applications several research efforts are in progress. These include, but are not limited to, modification of the cement liquid with polymeric additives such as collagen [39-42], reinforcement with resorbable as well as strong and tough fibres to the cement matrix [19,43] or using dual-setting cements where a dissolved monomer is simultaneously cross-linked during cement setting [44-46]. The most significant reinforcement strategies for CPCs are based on either intrinsic porosity reduction or extrinsic material modifications such as through fiber addition, incorporating dual setting character.

Biomodal particle size distribution

With the concept of finer particles filling the interstitial spaces in binary particle size distribution in the cement pastes that is normally occupied by water, the possibility for porosity reduction in CPC has been demonstrated for both hydroxyapatite [47] and brushite [48,49] forming biocements. Moreover, higher zeta potential on particle surface due to adsorption ionic moieties such as tartrates or citrates [50] from the cement liquid helps in dispersing agglomerates of finer particles and ensure homogeneous particle distribution and better packing. As a result, porosity was decreased from 37% to 25% and compressive strength was enhanced from 50 to 79 MPa [47] in a CPC comprising. α-tricalcium phosphate having mono modal size distribution with d50 ~9.8 μm and 13-33 wt% fine sized CaHPO4 filler with d50~ 1.16 μm and using 0.5 M trisodium citrate solution in the cement liquid [47].

Dual setting cements

Reduction in brittleness and an increase strength of CPC can be obtained by mixing carboxylic acid or organic phosphate biopolymer moieties e.g., polyacrylic acid [51], polymethyl vinyl ether maleic acid [52], poly [bis(carboxylatophenoxy) phosphazene] [53] or poly(vinyl phosphonate) [54] in the cement liquid. Mixing these moieties helps in the formation of intra- or inter-chained Ca^{2+} – organic anion chelates [53] with a highly reactive cement component, mostly tetracalcium phosphate, from the cement powder.

An alternative strategy is to use reactive monomer systems dissolved in the cement liquid, that simultaneously react during cement setting by a gelation–polymerisation process to give rise to an interconnecting hydrogel matrix with embedded cement particles, that subsequently sets and hardens by a continuous dissolution–precipitation reaction. Not only the total porosity reduction, but with the possibility of a high polymer loading in the cement, strength and toughness of CPC can be increased in this processing approach with practically unaltered rheological properties of the fresh cement paste [45,55,56].

Silica is commonly added to CPCs to enhance bioactivity, cohesion and mechanical strength [57] by incorporation of non-reactive silica fillers in cements [58] or addition of non-reactive calcium phosphate particles to an in situ forming sol-gel processed silica matrix [59]. In contrast, Geffers et al. [60] applied the concept of dual setting cements using pure inorganic materials with pre-hydrolysing tetraethyl orthosilicate (TEOS) and a brushite forming cement paste under acidic conditions.

Fiber reinforcement

Using different types of biocompatible and degradable (Polyglactin 910, poly (caprolactone), Poly lactide -co- glycolide etc.) as well as non -biodegradable (CNT, aramid etc.) fibers, an increase in mechanical strength of fibre reinforced CPC (FRCPC) has been observed [20,61-69]. The enhancement in mechanical strength for FRCPC depends on several parameters such as

A. Matrix composition and strength,

B. Fibre volume fraction, orientation, aspect ratio and tensile modulus as well as

C. The interface properties between matrix and fibres [59].

Apart from an increase in bending strength from 10-15 MPa for pure CPC to a maximum strength of 45 MPa (polyglactin fibers) [66] and 60 MPa (carbon fibers) [68], the work of fracture for FRCPC usually increased by at least one order of magnitude. While reinforcement with long fibers significantly hampers the workability of CPC pastes and impedes a minimal invasive surgery by injection, CPC pastes filled with short fibers up to a fiber length of 1 mm and a fiber volume of 7.5% have been found to be injectable in surgical sites [70].

Osteoinductive Properties in CPC

Osteoinduction is the property of a material by virtue of which it recruits and induces progenitor or undifferentiated cells to differentiate towards the osteoblastic lineage [71]. Osteoconductivity and osteoinductivity depend strongly on the physical and chemical properties of CPCs. Differences in osteoinductivities for CPC formulation may originate from variable degree of chemical properties such as stoichiometry, crystallinity, solubility etc. and topographical features such as microporosity and roughness. Studies have revealed that while all CPCs stimulate bone cell differentiation in the presence of osteogenic supplements, the order of osteoinductive potentials for various calcium phosphates in CPC follows as TCP > BCP> HAP > ACP [72]. In presence of osteogenic supplements, the better performance of HAP over ACP may be attributed to the higher crystallinity [73] of the former, while the better performance of TCP over HAP and BCP under the same condition may stem from the higher solubility of TCP [74]. At the same time, the higher osteoinductive potential of TCP reported by Yuan et al. [74] may be related to higher microporosity relative to HAP and BCP, which can facilitate protein adsorption.

On the other hand, osteoinductive potentials exist for CPCs in the absence of osteogenic supplements follows the order: BCP > TCP > HAP, which reveals the fact that CPC properties can significantly influence osteoinduction [72]. The osteoinductive capacity of CPCs *in vivo* seems to be driven by the solubility and resorptive capacity of CPCs. From this standpoint, b-TCP and ACP appear to exhibit higher osteoinductivity and faster bone in-growth than a slowly dissolving CPC such as HAP. However, for *in vivo* stability in longer duration and sustained osteoconduction, HAP may be more suitable candidate [72].

For exhibiting osteoinduction, facilitated cell adhesion is highly essential. High crystallinity and low solubility in CPC offers stable surfaces for cell adhesion, primarily because of low ion exchange between the CPC and the aqueous phase, and slow rates of recrystallization from solution. Moreover, cell adhesion seems to be facilitated by the direct adsorption of negatively charged cell-adhesive proteins on positively charged surfaces (e.g. cationic calcium sites on CPCs). Also, surface topography plays a key role in controlling protein adsorption and consequently cell adhesion. It has been observed that surface roughness in CPC can modulate osteoblastic differentiation by controlling the adsorption of cell-adhesive proteins, subsequent phosphorylation of FAK and activation of the ERK 1/2-pathway. Furthermore, CPC properties such as surface charge and crystallinity influences several chronological events such as protein adsorption and cell adhesion, that ultimately govern osteoblastic differentiation.

In particular, change in ionic environment because of ions release and recrystallization from and onto CPCs, can modulate local pH and extracellular ion concentration, and influence cell viability and differentiation. Extracellular concentrations of Ca^{2+} and phosphate ions directly govern cell proliferation and differentiation. Liu et al. discovered that externally supplied 1.8 and 0.09 mM Ca^{2+} and phosphate ions promoted the proliferation and differentiation of rabbit BMSCs respectively [75]. At greater concentrations of phosphate, cell apoptosis took place without significantly affecting cell differentiation. On the other hand, as observed by decreased ALP production and type I collagen/ osteocalcin mRNA expression, greater Ca^{2+} concentrations inhibited cell differentiation but promoted matrix mineralization.

CPC for drug delivery

Because of CPC's capability to incorporate drugs and other bioactive molecules, to retain it in a specific target site, and to deliver it progressively with time in the surrounding tissues, and its injectability and biodegradability, CPCs are potentially very attractive and useful in treatments of different skeletal diseases, such as bone tumours, osteoporosis or osteomyelitis, that otherwise require long and painful therapies. CPCs can be designed with soluble porogen to have larger pores than other mesoporous drug carriers, which would allow them to deliver not only small-molecule drugs, but also macromolecules, such as

growth factors, or peptide and protein-based drugs. Ginebra et al. [76] found that bimodal pore size distribution in set CPC that varied with the processing parameters, affected the adsorption and penetration of BSA differently. They concluded that effective surface area should be calculated considering protein size and pore diameter and protein adsorption and penetration is governed by the pore size in between aggregates, not the intercrystallite voids.

Moreover, the nanoporosity of the CPC may not facilitate the release of the protein, but may further restrict its release because of the high binding affinity of the protein for CPC. A similar trend was observed when release of human recombinant BMP-2 (rhBMP-2) found to be very limited, much slower from rhBMP-2 loaded poly (DL-lactic-co-glycolic acid) (PLGA) micro-spheres loaded CPC than the release of the protein from the microspheres alone [77]. This was explained on the basis of physical entrapment of the microparticles within the nanoapatitic porous cement. Thus development of CPCs with high total porosity does not ensure higher protein loading and controlled release, unless there exists an adequate pore size distribution in the matrix [76].

The efficacy of calcium phosphate cements as carriers of different types of drugs, such as antibiotics, analgesics, anticancer, anti-inflammatory, as well as growth factors has been investigated extensively [78- 83]. In general, in apatitic cements, antibiotics tend to increase their setting times and reduce their mechanical strength [84-86]. Though some CPCs are resorbable, in most of the CPC studied as drug-carriers, the rate of matrix degradation is much slower than the release rate of loaded drug. Thus the drug release is assumed to be mainly controlled by the process of diffusion through the cement matrix and not by the degradation of the same. On the contrary, if pore size and total porosity in the cement alters considerably with time, drug release kinetics no longer follow Higuchi's law with the consequence that drug diffusion through the CPC matrix is not the only mechanism that controls drug release [7]. Otsuka et al. [87,88] found linear drug release kinetics from studied CPCs at the initial stage both *in vivo* and *in vitro*, but release rate of drugs *in vivo* appeared much slower than that *in vitro* during the last stage of the study. The difference in such release behaviour was attributed to bioactive character of the CPC that caused some surface changes in the cement due to the formation of an apatite layer, or to other changes due to protein adsorption or cell activity.

Conclusion

The chemistry and kinetics of the setting, handling properties, mechanical and biological properties of CPCs for bone substitution were reviewed. Many processing parameters, such as powder particle size, composition, additives can be varied to control the setting process, cohesive strength and concomitantly to improve the handling properties of CPCs. Improvement of both cohesion and injectability simultaneously can be achieved by increasing the viscosity of the mixing liquid in cement paste. Incorporation of fibers may result in reinforcement of CPC matrix, but restrict CPC's injectibility for minimal invasive application techniques. Fibres degradability and strength retention of FRCPC for long term *in vivo* use need to be properly addressed and researched. The prospect of during setting, leading to more ductile cement–hydrogel composites need to investigated for better biological performance. Studying fatigue properties of CPCs in load-bearing defect models requires proper attention. There exists strong interrelation between physical, chemical properties of CPCs and its osteoconductive and osteoinductive potential. Investigation on the activation of specific signaling molecules in response to CPCs and inter dependence between various pathways can lead us to understanding the mechanisms of CPC-mediated osteogenesis. Further research efforts are needed towards processing of osteoinductive CPCs that support adhesion, growth and differentiation of stem cells without any necessity of osteogenic supplements and growth factors delivery from CPC matrix. A lot of work is needed to generalize laws that can predict drug release profile of these types of materials to obtain reproducible and predictable drug delivery systems.

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Citation: Dasgupta S, (2017) Prospective of Calcium Phosphate Cements for Bone Regeneration: Physicochemical, Mechanical and Biological Properties. Adv Tissue Eng Regen Med Open Access 2(6): 00049. DOI: 10.15406/atroa.2017.02.00049

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Citation: Dasgupta S, (2017) Prospective of Calcium Phosphate Cements for Bone Regeneration: Physicochemical, Mechanical and Biological Properties. Adv Tissue Eng Regen Med Open Access 2(6): 00049. DOI: 10.15406/atroa.2017.02.00049

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Citation: Dasgupta S, (2017) Prospective of Calcium Phosphate Cements for Bone Regeneration: Physicochemical, Mechanical and Biological Properties. Adv Tissue Eng Regen Med Open Access 2(6): 00049. DOI: 10.15406/atroa.2017.02.00049