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Review Article

Prospective of Calcium Phosphate Cements for Bone Regeneration in Relation to Physicochemical, Mechanical and Biological Properties

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Keywords Calcium phosphate; Biocement; Injectability; Cohesion; Fracture toughness; Otseoinductivity; Drug delivery

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; HA: Hydroxyapatite; TCP: Tricalcium Phosphate; DCPA: Dicalcium Phosphate Anhydrous; TTCP: Tetracalcium Phosphate; DCPD: Dicalcium Phosphate Dihydrate; MCPA: Monocalcium phosphate anhydrous; MCPM: Monocalcium Phosphate Monohydrate; ALP: Alkaline Phosphatase; BMSCs: Bone Marrow Stromal Cells

Abstract

Purpose: Calcium phosphate ceramics have been widely used as biomaterials for regeneration of bone tissue because of their ability to induce osteoblastic differentiation in progenitor cells. Calcium Phosphate Cements (CPCs) are prepared from different calcium phosphate powder precursors by mixing them together with aqueous phase followed by setting and hardening into monolithic solid. This article provides an overview on the chemistry, kinetics of setting and handling properties such as 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 discussed. CPCs are currently used for repair of non-load bearing bone defects due to its brittle nature and low flexural strength. Processing strategies to improve mechanical strength, fracture toughness and reliability of CPCs are also highlighted here. Further, a systematic discussion on the effects of 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. Moreover, 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.

Results: There are mainly two types of CPCs such as apatitic and brushitic that differ in their setting and hardening kinetics and in general brushitic CPC exhibits faster setting kinetic and lower mechanical strength. Setting kinetics, injectability and cohesive strength of both types of CPCs can be regulated by varying powder particle size, polymeric additives and viscosity of mixing liquid. The shortcomings in mechanical properties of such type of cements can be addressed by using bimodal size distribution of precursor particles, incorporating dual setting character in the cement or by preparing biodegradable or bioinert fiber reinforced cement composite. Calcium phosphate cement appears to possess excellent biological properties. CPC properties such as surface charge, crystallinity, slower and controlled degradation, micro and macro tomography positively influence several chronological events such as protein adsorption and cell adhesion that ultimately governs osteoblastic differentiation of progenitor cells. Because of its macro and micro porous structure, CPC serves as an excellent candidate 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, by virtue of its biodegradability. Due to its poor mechanical properties, CPCs' clinical applications are currently limited to craniofacial reconstruction. Further research is necessary to exploit its excellent biological properties with concomitant strengthening of mechanical reliability for its widespread clinical applications.

Conclusions: CPCs possesses a huge prospect to serve as next generation bone substitute material and further research is necessary to ameliorate its performance under clinical situations.

Introduction

In the early 1980s, scientists at the American Dental Association LeGeros et al. [1] and Brown and 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). The discovery of self-setting CPC was a significant achievement in the field of bioceramics for bone regeneration, since its 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, [10] Charrière et al. found that hydroxyapatite cements have the potential to be structural biomaterials while brushite cements are suitable as bone fillers. Typical applications of

Table 1: CPC formulations approved by FDA.

Product	Manufacturer	Applications
Alpha- BSM	Etex Corporations Cambridge, MA	Filling bone voids and defects
Bone Source	Striker How medica Osteonics Rutherford, NJ	Craniofacial
Skeletal repair systems (SRS)	Norian Corporation Cupertino, CA	Skeletal distal radius fractures, craniofacial

CPCs are treatment of maxillofacial defects or deformities [11] or the repair of craniofacial defects [12], with the possibility of applying it in moderately load-bearing defects, such as in vertebroplasty or kyphoplasty [13-15].

Food and Drug Administration (FDA), USA first approved CPC for human use in 1996. Since then, a number of CPC products (Table 1) have been approved in different countries for a variety of clinical applications including cranio-maxillofacial, dental and orthopedic trauma. Despite CPCs' high potential and wide acceptance as biomaterials for bone regeneration, some crucial issues still need to be solved to satisfy clinical requirements [16,17]. Specifically, CPCs without any additives suffer from poor injectability [18,19] and are prone to disintegrate upon early contact with blood or biological fluids [20]. CPCs are limited in application to non- or moderate load-bearing musculoskeletal defects [21,22] 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 (Table 1).

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 [23]. 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 [24,25]. 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) [26,27]. 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 [28,29]. Based on differential solubilities of cement raw materials and their setting product and thus controlled by continuous dissolution and subsequent precipitation reaction, setting of CPC takes place as shown in Figure 1. The final phases after cement setting will differ depending on the pH of liquid mixture in which setting reaction occurs. Neutral or basic pH environment will result in stoichiometric or calcium deficient hydroxyapatite as set product, while secondary protonated dicalcium phosphates such as brushite ($CaHPO_4 \bullet 2H_2O$) or monetite ($CaHPO_4$) will be formed at an acidic pH (Figure 1).

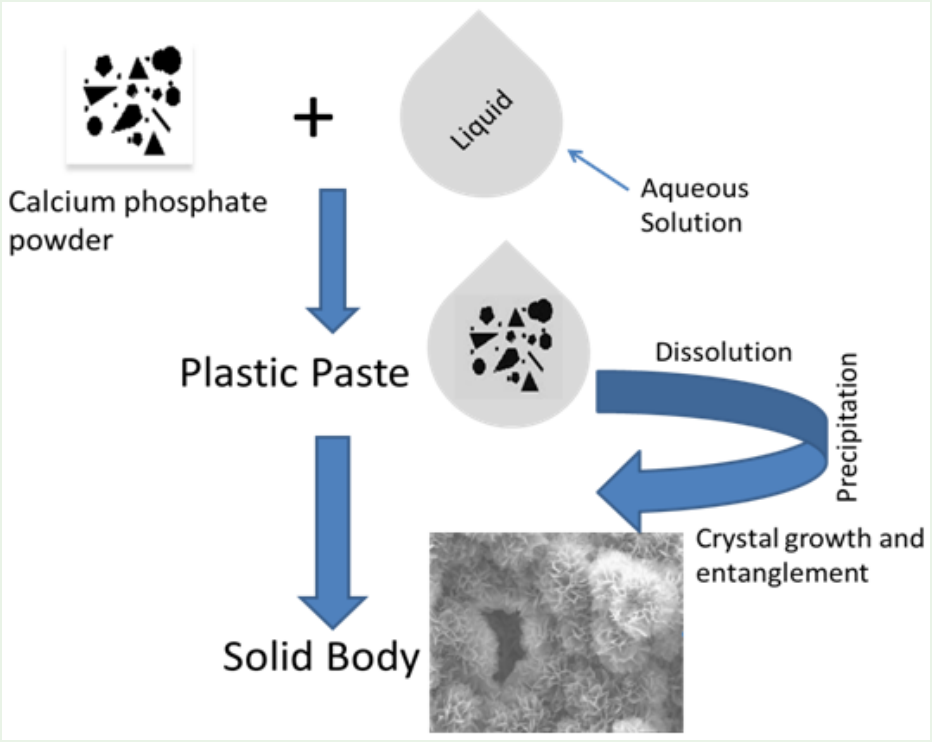

Figure 1: Setting mechanism of calcium phosphate cements.

All reactions between calcium phosphate compounds that occur in an aqueous environment can be characterized as dissolution/re-precipitation reactions. For example, in the a formulation of basic component TTCP and acidic component DCPA based cement paste, dissolution of TTCP and DCPA would lead to a solution composition that is highly supersaturated with respect to HA, resulting in HA precipitation. The driving force for such a reaction is the relative solubilities of the reactants and product for a given solution composition. In the above example, the TTCP+DCPA → HA reaction occurs because both TTCP and DCPA are considerably more soluble than HA. Setting reaction mechanisms in other CPC mixtures that contain other calcium phosphate starting materials are in fact quite similar and may be understood by analyzing the solubility behavior of the compounds involved.

The main constituents of Dicalcium Phosphate (DCP) cements are alkaline calcium sources such as β- TCP, α- TCP, TTCP, CaO etc., an acidic phosphate source such as MCPM or MCPA and water. For example, β-TCP/MCPM cements starts their setting reaction by the dissolution of MCPM, which causes a rapid and pronounced decrease in pH nearly to 2.5 [30,31]. In case the cement contains excess MCPM, pH of the cement mixture will remain low even after the completion of setting reaction. On the contrary, if the cement formulation possesses excess β-TCP, pH of cement paste will settle at 5 after completion of setting reactions [32]. Similar variation in pH is also observed in other brushite cement systems, such as that contains calcium oxide and MCPM [32].

Composition of both solid and liquid phases governs the kinetics of CPC setting. TenHuisen and Brown [30] 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, complexing and adsorbing ability of citrate ions onto α-TCP crystals and apatite nuclei retard both the formation of crystal nuclei and their further growth and entanglement and thus slows down CPC's setting kinetics. Because of similarity in chemical composition of mammalian bones to that an ion-substituted calcium deficient apatite, apatite cements have been more extensively investigated (Table 2).

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 [34-36].

Setting time

The factors that promote faster setting kinetics can potentially reduce the setting time of CPCs. Such factors are (i) smaller particle size i.e. high specific surface of precursors; (ii) low crystallinity; (iii) accelerators in the liquid and solid compositions; (iv) higher setting temperature; and (v) a low liquid-to-powder ratio (L/P ratio) [37]. 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 [38]. Thus it is critical to prepare 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 [39], hydroxypropyl methylcellulose (HPMC) [40,41], hyaluronic acid [42], chitosan [43,44] and modified starch [45], though can prolong setting time and hamper mechanical strength [46], has found to be effective in improving cohesion and anti-washout properties of CPCs.

Injectability

Bohner and Baroud [47] 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 [48,49]. Because of higher zeta potential depending on pH of the slurry and adsorption of charged species onto particle surface, electrical double layer repulsion between the particle surfaces in CPC increases resulting in lesser friction among the particles during ejection from the syringe and thus enhanced injectability of CPC paste (Figure 2).

Table 2: Classification of CPCs and their overall characteristics.

Apatite- forming CPCs		Brushite forming CPCs
Hydrolysis: Hydrolysis of a α -TCP	Acid –base reaction: TTCP+DCPA/DCPD	Acid- base reaction: β- TCP+ MCPM/MCPA
End product: CDHA/ HA		End product: DCPD
		Better solubility under physiological conditions
		Faster reaction and setting
		Lower mechanical strength
		Faster degradation and resorption in vivo
Most commercial products		

Effect of addition of Polycrboxylic Acids into CPC

A number of carboxylic acids, such as glycolic, citric, tartaric, malonic, malic, succinic, and maleic acids readily form calcium complexes as well as relatively insoluble and often amorphous Ca-carboxylate compounds. These acid solutions, when mixed with a powder containing one or more of calcium phosphate compounds, produce fast hardening cement [50-52]. Although in most cases final cement products are apatitic in neutral or alkaline pH, DCPD and DCPA as final product in CPC could also be formed if the molar Ca/P ratio in the cement formulation is lower than about 1.3 or the setting reaction is carried out at pH less than 4.2 [52]. Citric acid reportedly promotes hardening reaction and increases mechanical strength of CPCs by means of a chelating reaction with calcium ions [53,54].

Setting of apatitic cement in neutral or alkaline pH, results in less amount of free calcium ions because majority of calcium ions are involved in complexation reaction with citrate and that drags cements dissolution and hydration equilibrium in forward direction to make it faster. On the other hand, brushite cements generally have lower mechanical strengths than those HA-forming CPCs without use of an organic acid solution as the liquid. At pH less than 6.5, under acidic condition, there exists very few free carboxylate ions as Ca^{+2-} carboxylate complex is insoluble and forms protective coatings on CPC grains that retards further hydration of CPC and hence setting of CPC is delayed as shown in Figure 3. In general, higher proportion of precursor phase in citric acid solution in acidic pH signifies a longer setting time in cement samples. At the same time, a shorter setting time is observed in CPCs using basic solution of citrate ions as indicated by higher proportion of final set product. Gbureck et al. found zeta potential of TTCP and DCPA in water was 18.4 ± 1.9 mV and 15.0 ± 1.8 mV, respectively, whereas that for the same powders in a trisodium citrate solution was 50.1 ± 1.0 mV and 50.6 ± 3.8 mV [55]. As a result, a CPC prepared from TTCP, DCPA and water achieved an injectability of 59%, while under the same conditions with added trisodium citrate solution, the same CPC formulation achieved an injectability of 97.4%. However, improvement on injectability of CPC formulations by increasing zeta potential of the slurry is limited due to its detrimental effects on reducing particle attraction that may negatively influence cohesive properties of cement formulations [56] (Figure 3).

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 [57-60], reinforcement with resorbable as well as strong and tough fibers to the cement matrix [61,62] or using dual-setting cements where a dissolved monomer is simultaneously cross-linked during cement setting [63-65]. 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 [66] and brushite [67,68] forming biocements. Moreover, higher zeta potential on particle surface due to adsorption of ionic moieties such as tartrates or citrates [69] from the cement liquid helps in dispersing agglomerates of finer particles and ensure homogeneous particle distribution and better packing as shown in Figure 4a. As a result, porosity was decreased from 37% to 25% and compressive strength was enhanced from 50 to 79 MPa [66] in a CPC comprising monomodal sized. α-tricalcium

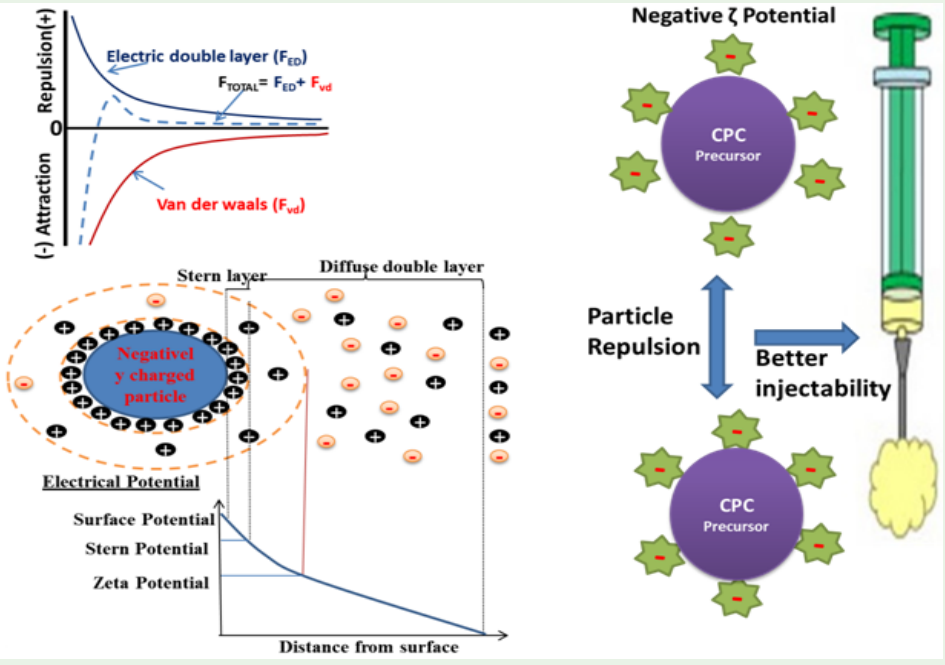

Figure 2: Effect of zeta potential of CPC slurry in particle deagglomeration and improving its injectability.

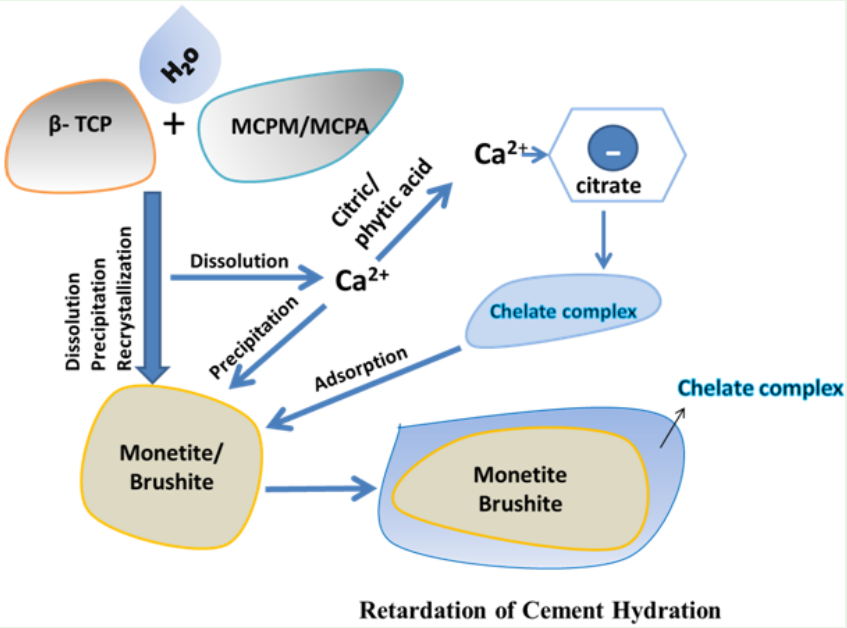

Figure 3: Effect of polycarboxylic acid in retarding setting behavior of CPC.

phosphate with d_{50}~9.8 µm and 13-33 wt% fine sized CaHPO4 filler with d_{50}~1.16 µm in 0.5 M trisodium citrate solution in the cement liquid [66]. Agglomerated structure and more ordered arrangement of particles always results in lesser degree of freedom for particle packing, higher interparticle porosity and hence lesser density as compared to more dispersed and less ordered particle arrangement as evident from Figure 4b.

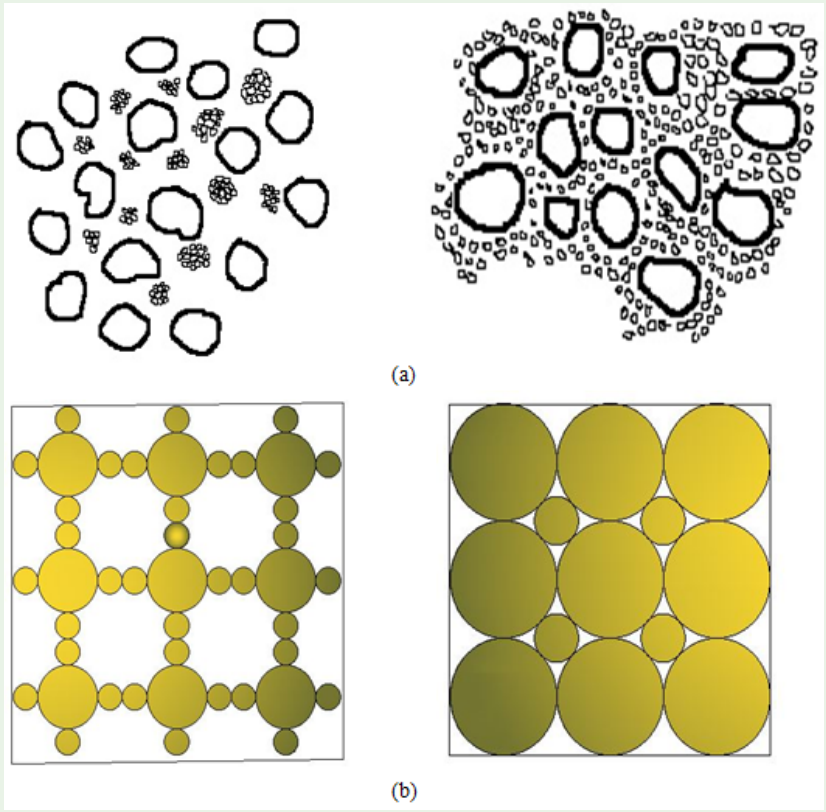

Figure 4: Effect of particle deagglomeration and size distributon on (a) particle packing in CPC formulation (b) pore development in CPC.

Dual setting cements

Reduction in brittleness and an increase in strength of CPC can be obtained by mixing carboxylic acid or organic phosphate biopolymer moieties e.g., polyacrylic acid [70], polymethyl vinyl ether maleic acid [71], poly [bis(carboxylatophenoxy) phosphazene] [72] or poly(vinyl phosphonate) [73] in the cement liquid. Mixing these moieties helps in the formation of intra or inter-chained Ca^{2+} organic anion chelates [72] 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 by this processing approach with practically unaltered rheological properties of the fresh cement paste [74-76]. This strategy of fracture strength enhancement in CPC has been described in Figure 5.

Silica is commonly added to CPCs to enhance bioactivity, cohesion and mechanical strength [77] by incorporation of non-reactive silica fillers in cements [78] or addition of non-reactive calcium phosphate particles to an in situ forming sol-gel processed silica matrix [79]. In addition, Geffers et al. [80] 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 (Figure 5).

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 Fiber Reinforced CPC (FRCPC) has been observed [81-90]. The enhancement in mechanical strength for FRCPC depends on several parameters such as (1) matrix composition and strength, (2) fiber volume fraction, orientation, aspect ratio and tensile modulus as well as (3) the interface properties between matrix and fibers [79]. Apart from an increase in bending strength from 10-15 MPa for pure CPC to a maximum strength of 45 MPa (polyglactin fibers) [87] and 60 MPa (carbon fibers) [89], 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 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 [91] (Figure 6).

In the field of civil engineering, for fiber-added composite cements, there primarily exists three mechanisms of fiber reinforcement namely fiber bridging, crack deflection and frictional sliding (Figure 6). Specifically, in the event of crack initiation in cement matrix, the fibers bridge the crack to hinder its further opening and propagation. By virtue of crack deflection by the fibers, crack travels longer distance to propagate, and thereby consumes more energy in freshly formed surfaces. These two strengthening mechanisms are reported to be in operation to contribute majorly in enhancing the fracture toughness of human bone, which is a hierarchical composite consisting of hard apatite nanoparticles and polymeric collagenous fibers [92,93]. Finally, during the pull out, frictional sliding of

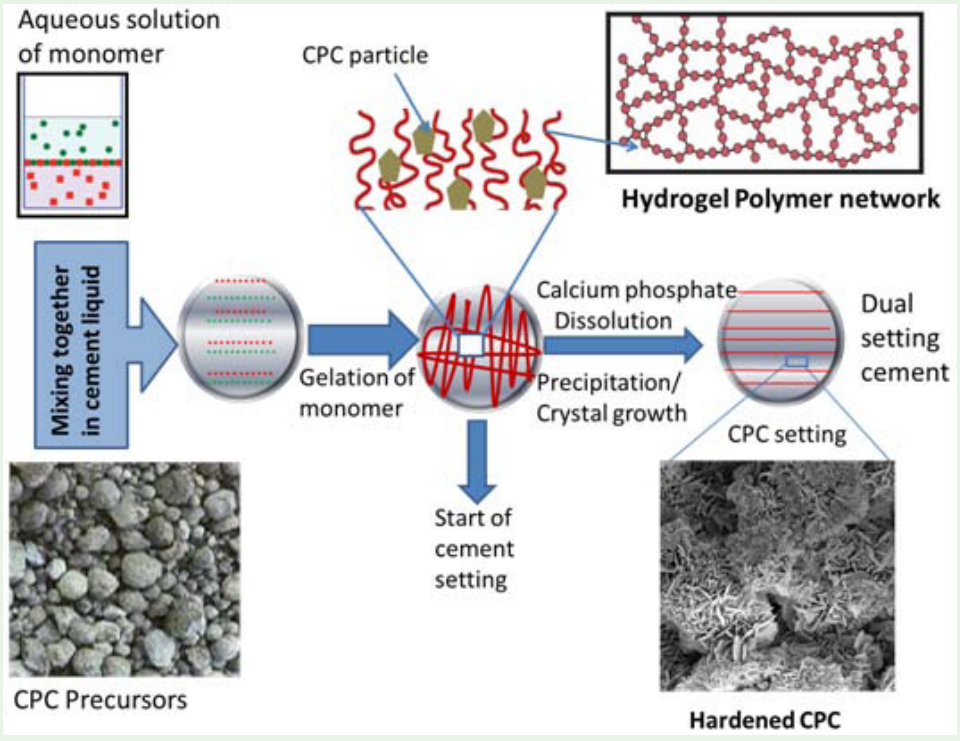

Figure 5: Mechanism of dual setting character in CPC.

fibers against the matrix further consumes the applied energy and contributes to enhancement of fracture resistance of the composite [94]. Due to chemical similarity between CPCs and cements for civil engineering, it is hypothesized that addition of strong and tough fibers in CPC formulations, its fracture toughness can be enhanced with the help of above mentioned toughening mechanisms.

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 [95]. 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 presence of osteogenic supplements, the order of osteoinductive potentials for various calcium phosphates in CPC follows as TCP > BCP> HA > ACP [96]. In presence of osteogenic supplements, the better performance of HA over ACP may be attributed to the higher crystallinity [97] of the former, while the better performance of TCP over HA and BCP under the same condition may stem from the higher solubility of TCP [98]. At the same time, the higher osteoinductive potential of TCP reported by Yuan et al. may be related to higher microporosity relative to HA and BCP, which can facilitate protein adsorption [98].

On the otherhand, osteoinductive potentials exist for CPCs in the absence of osteogenic supplements follows the order: BCP > TCP > HA, which reveals the fact that CPC properties can significantly influence osteoinduction [96]. The osteoinductive capacity of CPCs in vivo seems to be driven by the solubility and resorptive capacity of CPCs. From this standpoint, β-TCP and ACP appear to exhibit higher osteoinductivity and faster bone in-growth than a slowly dissolving CPC such as HA. However, for in vivo stability in longer duration and sustained osteoconduction, HA may be more suitable candidate [96].

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 ERK1/2-pathway. Furthermore, CPC properties such as surface charge and crystallinity influence several chronological events such as protein adsorption and cell adhesion, which 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 [99]. At greater concentrations of phosphate, cell apoptosis took place without significantly affecting cell differentiation. On the otherhand, 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 [100] 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, nanoporosity of CPC may not facilitate the release of adsorbed 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

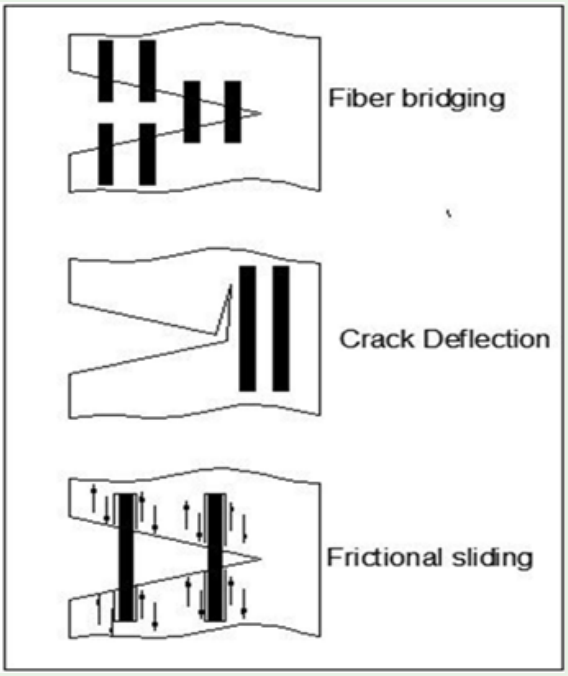

Figure 6: Effect of fibre addition on reinforcement of CPC.

(DL-lactic-co-glycolic acid) (PLGA) micro-spheres containing CPC than the release of the protein from the microspheres alone [101]. 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 [87].

There may be three possible ways by which most of the drug molecules remain entrapped between the entangled CPC crystals as described in Figure 7I. They may remain dissolved within the existing pores between CPC crystals as shown in Figure 7Ia. Another possibility is that the drug molecules may be adsorbed or chemically bound to the surfaces of newly formed CPC crystals depending on the pH of surrounding liquid phase [Figure 7Ib]. And lastly, when the drug concentration reaches above its supersaturating limit in entrapped liquid phase, it may be deposited as solid drug aggregate on the surfaces of CPC crystals as in Figure 7Ic.

The efficacy of calcium phosphate cements as carriers of different types of drug, such as antibiotics, analgesics, anticancer, anti-inflammatory, as well as growth factors has been investigated extensively [102-107]. In general, in apatitic cements, antibiotics tend to increase their setting times and reduce their mechanical strength [108-110]. Though some CPCs are resorbable, in most of the CPCs 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 alter 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 [111]. With the availability of some degree of interconnecting pores in the cement matrix, surrounding physiological fluids can penetrate into adjoining CPC crystals and the drug release can predominantly occur by diffusion through the fluid filling the pores [Figure 7IIa]. On the other hand, when cement resorption is relevant, mainly in brushite cements or carbonate-containing cements, with consequent increase in pore size and porosity in matrix, mobility of the solubilized drug is enhanced and also contact area of matrix with surrounding liquid is increased. As a result, speeding up of drug release [112] is observed [Figure 7IIb].

Otsuka et al. 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 [113,114]. 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 [Figure 7IIc], or to other changes due to protein adsorption or cell activity (Figure 7).

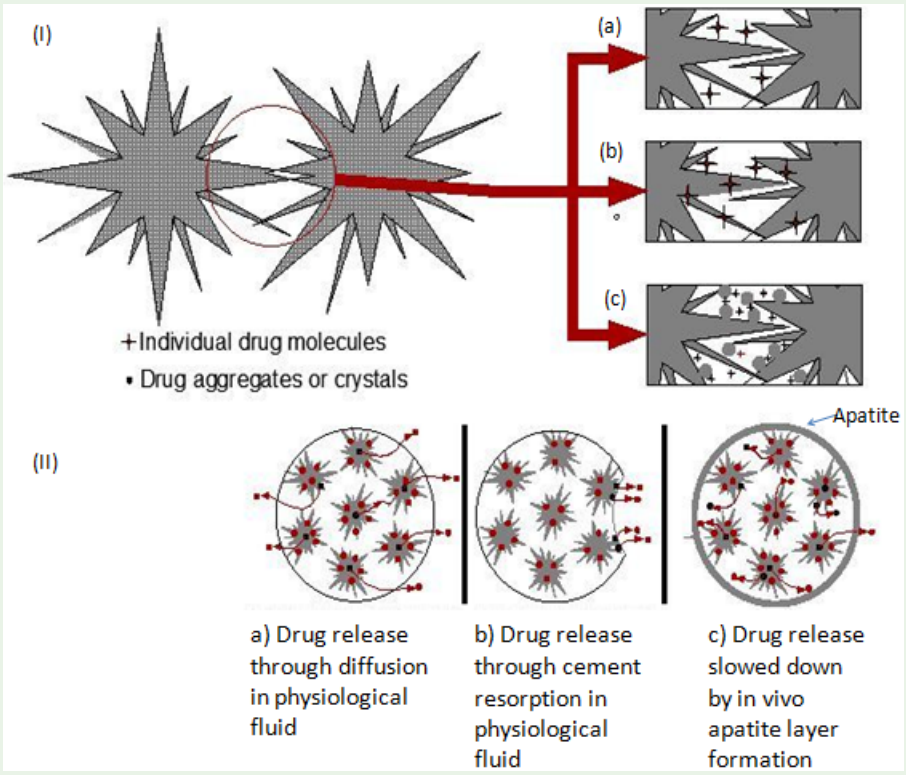

Figure 7: (I) Different ways of drug entrapment in CPC matrix: (a) In liquid within the pores individual molecules are dissolved; (b) Individual drug molecules are adsorbed or chemically bound to the surface of CPC crystals; (c) Drug crystals or aggregates in solid form are physically adsorbed onto CPC surfaces. (II) Drug release mechanism from CPCs can take place in the following manners: (a) When the rate of CPC degradation is lower than drug diffusion, drug release is primarily controlled by diffusion of the drug through the liquid surrounding the cement, (b) When the rate of cement degradation is faster than drug diffusion, drug release rate is controlled by CPC's degradation and (c) in some cases with bioactive cements, an apatite layer can be formed on the surface of the cement after implantation in vivo and this relatively restricts the release of drug to the surrounding tissue.

Table 3: Potential advantages and Challenges behind development of CPCs.

Advantages	Challenges
Controlled biodegradability: Implanted CPC can be replaced by newly formed bone after a period of time. Therefore, complications arises due to long term use of HA based implant can be avoided.	Can be washed out from the applied sites in excess of blood confluence. Setting of CPC is recommended under compression.
Injectability and mouldability: Can be placed at surgical sites without much difficulty or even in a noninvasive way.	Microstructural control: Microstructural development in CPC such as total porosity, pore size and its distribution cannot be controlled easily.
Excellent carrier of biomolecules: It can be used to deliver growth factors, antibiotics, morphogenic proteins at local sites.	Mechanical limitation: Can fail under stress during or after soft tissue formation without going into hard callous or bone formation stage in load bearing sites.
Excellent biocompatibility	Fatigue failure: CPCs are very much susceptible to fatigue failure.
Osteoconduction and osteoinduction: at early stages, biological properties are similar to those of HA	Osteogenic properties: Biological properties, such as osteoinductivity, drug release behavior of CPCs need to be regulated to enhance its performance as bone substitute material.
Excellent material for bone regeneration at non load or moderate load bearing sites.	

Conclusions

The chemistry and kinetics of 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. Fibers degradability and strength retention of FRCPC for long term in vivo use need to be properly addressed and researched. The prospect of more ductile cement hydrogel composites on course of setting of cement needs to be investigated for better biological performance. Studying fatigue properties of CPCs in load-bearing defect models requires proper attention (Table 3). 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 (Table 3).

References

1. LeGeros RZ, Chohayeb A, Shulman A. Apatitic calcium phosphates: possible dental restorative materials. J Dent Res. 1982; 61: 343.
2. Brown WE, Chow LC. A new calcium phosphate setting cement. J Dent Res. 1983; 62: 672.
3. Brown WE, Chow LC. A new calcium phosphate water setting cement. In Cements Research Progress; Brown WE, Ed American Ceramic Society: Westerville. 1986; 352-379.
4. Brown WE, Chow LC. Dental restorative cement pastes. U.S Patent No: 4,518,430. 1985.
5. Gruninger SE, Siew C, Chow LC, O'Young A, Tsao NK, Brown WE. Evaluation of the biocompatibility of a new calcium phosphate setting cement. J Dent Res. 1984; 63: 200.
6. Bohner M, Gbureck U, Barralet JE. Technological issues for the development of more efficient calcium phosphate bone cements: a critical assessment. Biomaterials. 2005; 26: 6423-6429.
7. Ginebra MP, Traykova T, Planell JA. Calcium phosphate cements as bone drug delivery systems: a review. J Control Release 2006; 113: 102-110.
8. Ginebra MP, Canal C, Espanol M, Pastorino D, Montufar EB. Calcium phosphate cements as drug delivery materials. Adv Drug Deliv Rev. 2012; 64: 1090-1110.
9. Khairoun I, Magne D, Gauthier O, Bouler JM, Aguado E, Daculsi G, et al. In vitro characterization and in vivo properties of a carbonated apatite bone cement. J Biomed Mater Res. 2002; 60: 633-642.
10. Charriere E, Terrazzoni S, Pittet C, Mordasini P, Dutoit M, Lemaitre J, et al. Mechanical characterization of brushite and hydroxyapatite cements. Biomaterials. 2001; 22: 2937-2945.
11. Bohner M, Gbureck U, Barralet JE. Technological issues for the development of more efficient calcium phosphate bone cements: a critical assessment. Biomaterials. 2005; 26: 6423-6429.
12. Von Gonten AS, Kelly JR, Antonucci JM. Load-bearing behavior of a simulated craniofacial structure fabricated from a hydroxyapatite cement and bioresorbable fiber-mesh. J Mater Sci Mater Med. 2000; 11: 95-100.
13. Blattert TR, Jestaedt L, Weckbach A. Suitability of a calcium phosphate cement in osteoporotic vertebral body fracture augmentation a controlled, randomized, clinical trial of balloon kyphoplasty comparing calcium phosphate versus polymethylmethacrylate. Spine. 2009; 34: 108-114.
14. Maestretti G, Cremer C, Otten P, Jakob RP. Prospective study of standalone balloon kyphoplasty with calcium phosphate cement augmentation in traumatic fractures. Eur Spine J. 2007; 16: 601-610.
15. Tarsuslugil SM, O'Hara RM, Dunne NJ, Buchanan FJ, Orr JF, Barton DC, et al. Development of calcium phosphate cement for the augmentation of traumatically fractured porcine specimens using vertebroplasty. J Biomech. 2013; 46: 711-715.
16. Bohner M, Gbureck U, Barralet JE. Technological issues for the development of more efficient calcium phosphate bone cements: a critical assessment. Biomaterials. 2005; 26: 6423-6429.
17. Bohner M. Design of ceramic-based cements and putties for bone graft substitution. Eur Cell Mater 2010; 20: 1-12.
18. Bohner M, Baroud G. Injectability of calcium phosphate pastes. Biomaterials. 2005; 26: 1553-1563.
19. Habib M, Baroud G, Gitzhofer F, Bohner M. Mechanisms underlying the limited injectability of hydraulic calcium phosphate paste. Acta Biomater. 2008; 4: 1465-1471.
20. Khairoun I, Driessens F, Boltong MG, Planell JA, Wenz R. Addition of cohesion promoters to calcium phosphate cements. Biomaterials 1999; 20: 393-398.

21. Canal C, Ginebra MP. Fibre-reinforced calcium phosphate cements: a review. J Mech Behav Biomed Mater. 2011; 4: 1658-1671.

22. Kruger R, Groll J. Fiber reinforced calcium phosphate cements - on the way to degradable load bearing bone substitutes? Biomaterials. 2012; 33: 5887-5900.

23. Chen WC, Lin JH, Ju CP. Transmission electron microscopic study on setting mechanism of tetracalcium phosphate/dicalcium phosphate anhydrous-based calcium phosphate cement. J Biomed Mater Res A. 2001; 364: 664-671.

24. Chow LC. Next generation calcium phosphate-based biomaterials. Dent Mater J. 2009; 28:1-10.

25. Fernandez E, Gil FJ, Ginebra MP, Driessens F, Planell JA, Best SM. Calcium phosphate bone cements for clinical applications. Part I. Solution chemistry. J Mater Sci-Mater M. 1999; 10: 169-176.

26. Chow LC. Calcium phosphate cements. Monogr. Oral Sci. 2001; 18: 148-163.

27. Nancollas GH, Zawacki SJ. Calcium phosphate mineralization. Connect. Tissue Res. 1989; 21: 239-244.

28. Bohner M, Gbureck U, Barralet JE. Technological issues for the development of more efficient calcium phosphate bone cements: a critical assessment. Biomaterials. 2005; 26: 6423-6429.

29. Dorozhkin SV. Calcium orthophosphate cements for biomedical application. J Mater Sci. 2008; 43: 3028-3057.

30. Bohner M, Gbureck U. Thermal reactions of brushite cements. J Biomed Mater Res B Appl Biomater. 2008; 84: 375-385.

31. Bohner M, van Landuyt P, Merkle HP, Lemaitre J. Composition effects on the pH of a hydraulic calcium phosphate cement. J Mater Sci Mater Med. 1997; 8: 675-681.

32. Nurit J, Margerit J, Terol A, Boudeville P. pH-metric study of the setting reaction of monocalcium phosphate monohydrate/calcium oxide-based cements. J Mater Sci Mater Med. 2002; 13: 1007-1014.

33. TenHuisen KS, Brown PW. The effects of citric and acetic-acids on the formation of calcium-deficient hydroxyapatite at 38˚C. J Mater Sci-Mater M. 1994; 5: 291-298.

34. Bohner M, Gbureck U, Barralet JE. Technological issues for the development of more efficient calcium phosphate bone cements: a critical assessment. Biomaterials. 2005; 26: 6423-6429.

35. Bohner M, Doebelin N, Baroud G. Theoretical and experimental approach to test the cohesion of calcium phosphate pastes. Eur Cell Mater. 2006; 12: 26-35.

36. Perez RA, Kim H, Ginebra M. Polymeric additives to enhance the functional properties of calcium phosphate cements. J Tissue Eng. 2012; 3: 204-208.

37. Bohner M. Reactivity of calcium phosphate cements. J Mater Chem. 2007; 17: 3980-3986.

38. Dorozhkin SV. Calcium orthophosphate cements for biomedical application. J Mater Sci. 2008; 43: 3028-3057.

39. Ishikawa K, Miyamoto Y, Kon M, Nagayama M, Asaoka K. Non-decay type fast-setting calcium phosphate cement: composite with sodium alginate. Biomaterials. 1995; 16: 527-532.

40. Cherng A, Takagi S, Chow LC. Effects of hydroxypropyl methylcellulose and other gelling agents on the handling properties of calcium phosphate cement. J Biomed Mater Res. 1997; 35: 273-277.

41. Liu W, Zhang J, Weiss P, Tancret F, Bouler JM. The influence of different cellulose ethers on both the handling and mechanical properties of calcium phosphate cements for bone substitution. Acta Biomater. 2013; 9: 5740-5750.

42. Alkhraisat MH, Rueda C, Marino FT, Torres J, Jerez LB, Gbureck U, et al. The effect of hyaluronic acid on brushite cement cohesion. Acta Biomater. 2009; 5: 3150-3156.

43. Liu H, Li H, Cheng W, Yang Y, Zhu M, Zhou C. Novel injectable calcium phosphate/chitosan composites for bone substitute materials. Acta Biomater. 2006; 2: 557-565.

44. Takagi S, Chow LC, Hirayama S, Eichmiller FC. Properties of elastomeric calcium phosphate cement-chitosan composites. Dent Mater. 2003; 19: 797-804.

45. Wang XP, Chen L, Xiang H, Ye JD. Influence of anti-washout agents on the rheological properties and injectability of a calcium phosphate cement. J Biomed Mater Res. 2007; 81: 410-418.

46. Khairoun I, Driessens F, Boltong MG, Planell JA, Wenz R. Addition of cohesion promoters to calcium phosphate cements. Biomaterials 1999; 20: 393-398.

47. Bohner M, Baroud G. Injectability of calcium phosphate pastes. Biomaterials. 2005; 26: 1553-1563.

48. Liu W, Zhang J, Weiss P, Tancret F, Bouler JM. The influence of different cellulose ethers on both the handling and mechanical properties of calcium phosphate cements for bone substitution. Acta Biomater. 2013; 9: 5740-5750.

49. Sarda S, Fernandez E, Nilsson M, Balcells M, Planell JA. Kinetic study of citric acid influence on calcium phosphate bone cements as water-reducing agent. J Biomed Mater Res. 2002; 61: 653-659.

50. Yoshikawa M, Terada Y, Toda T. Setting time and sealing ability of alpha-tricalcium phosphate cement containing titanic oxide. J Osaka Dent Univ. 1998; 32: 67-70.

51. Doi Y, Shimizu Y, Moriwaki Y, Aga M, Iwanaga H, Shibutani T, et al. Development of a new calcium phosphate cement that contains sodium calcium phosphate. Biomaterials. 2001; 22: 847-854.

52. Yokoyama A, Yamamoto S, Kawasaki T, Kohgo T, Nakasu M. Development of calcium phosphate cement using chitosan and citric acid for bone substitute materials. Biomaterials. 2002; 23: 1091-1101.

53. Sarda S, Fernández E, Nilsson M, Balcells M, Planell JA. Kinetic study of citric acid influence on calcium phosphate bone cements as water-reducing agent. J Biomed Mater Res. 2002; 61: 653-659.

54. Yokoyama A, Yamamoto S, Kawasaki T, Kohgo T, Nakasu M. Development of calcium phosphate cement using chitosan and citric acid for bone substitute materials. Biomaterials. 2002; 23: 1091-1101.

55. Gbureck U, Barralet JE, Spatz K, Grover LM, Thull R. Ionic modification of calcium phosphate cement viscosity. Part I: hypodermic injection and strength improvement of apatite cement. Biomaterials. 2004; 25: 2187-2195.

56. Bohner M, Doebelin N, Baroud G. Theoretical and experimental approach to test the cohesion of calcium phosphate pastes. Eur Cell Mater. 2006; 12: 26-35.

57. Moreau JL, Weir MD, Xu HHK. Self-setting collagen-calcium phosphate bone cement: Mechanical and cellular properties. J Biomed Mater Res A. 2009; 91: 605-613.

58. Schneiders W, Reinstorf A, Biewener A, Serra A, Grass R, Kinscher M, et al. In Vivo Effects of modification of hydroxyapatite/collagen composites with and without chondroitin sulphate on bone remodeling in the sheep tibia. J Orthop Res. 2009; 27: 15-21.

59. Tamimi F, Kumarasami B, Doillon C, Gbureck U, le Nihouannen D, Cabarcos EL, et al. Brushite-collagen composites for bone regeneration. Acta Biomater. 2008; 4: 1315-1321.

60. O'Hara RM, Orr JF, Buchanan FJ, Wilcox RK, Barton DC, Dunne NJ. Development of a bovine collagen-apatitic calcium phosphate cement for potential fracture treatment through vertebroplasty. Acta Biomater. 2012; 8: 4043-4052.

61. Canal C, Ginebra MP. Fibre-reinforced calcium phosphate cements: a review. J Mechan Behav Biomed Mater. 2011; 4: 1658-1671.

62. Dos Santos LA, de Oliveira LC, Rigo ECD, Carrodeguas RG, Boschi AO, de Arruda ACF. Fiber reinforced calcium phosphate cement. Artif Org. 2000; 24: 212-216.

63. Dos Santos LA, Carrodeguas RG, Boschi AO, de Arruda ACF. Fiber-enriched double-setting calcium phosphate bone cement. J. Biomed Mater Res A. 2003; 65: 244-250.

64. Dos Santos LA, Carrodeguas RG, Boschi AO, de Arruda ACF. Dual-setting calcium phosphate cement modified with ammonium polyacrylate. Artif Org. 2003; 27: 412-418.

65. Wang J, Liu CS, Liu YF, Zhang S. Double-network interpenetrating bone cement via in situ hybridization protocol. Adv Funct Mater. 2010; 20: 3997-4011.

66. Gbureck U, Spatz K, Thull R, Barralet JE. Rheological enhancement of mechanically activated alpha-tricalcium phosphate cements. J. Biomed Mater Res. B Appl. Biomater. 2005; 73: 1-6.

67. Engstrand J, Persson C, Engqvist H. The effect of composition mechanical properties of brushite cements. J. Mech Behav Biomed Mater. 2014; 29: 81-90.

68. Hofmann MP, Mohammed AR, Perrie Y, Gbureck U, Barralet JE. High-strength resorbable brushite bone cement with controlled drug-releasing capabilities. Acta Biomater. 2009; 5: 43-49.

69. Barralet JE, Tremayne M, Lilley KJ, Gbureck U. Modification of calcium phosphate cement with alpha-hydroxy acids and their salts. Chem Mater. 2005; 17: 1313-1319.

70. Chen WC, Ju CP, Wang JC, Hung CC, Lin JHC. Brittle and ductile adjustable cement derived from calcium phosphate cement/polyacrylic acid composites. Dent Mater. 2008; 24: 1616-1622.

71. Watson KE, Tenhuisen KS, Brown PW. The formation of hydroxyapatite-calcium polyacrylate composites. J Mater Sci Mater Med. 1999; 10: 205-213.

72. Greish YE, Brown PW, Bender JD, Allcock HR, Lakshmi S, Laurencin CT. Hydroxyapatite-polyphosphazane composites prepared at low temperatures. J. Am Ceram Soc. 2007; 90: 2728-2734.

73. Greish YE, Brown PW. Chemically formed HAp-Ca poly (vinyl phosphonate) composites. Biomaterials 2001; 22: 807-816.

74. dos Santos LA, Carrodeguas RG, Boschi AO, de Arruda ACF. Dual-setting calcium phosphate cement modified with ammonium polyacrylate. Artif Organs. 2003; 27: 412-418.

75. Sugawara A, Antonucci JM, Takagi S, Chow LC, Ohashi M. Formation of hydroxyapatite in hydrogels from tetracalcium phosphate/dicalcium phosphate mixtures. J. Nihon Univ Sch Dent. 1989; 31: 372-381.

76. Rigo ECS, dos Santos LA, Vercik LCO, Carrodeguas RG, Boschi AO. Alpha-tricalcium phosphate- and tetracalcium phosphate/dicalcium phosphate-based dual setting cements. Lat. Am. Appl. Res. 2007; 37: 267-274.

77. Zhou H, Luchini TJF, Agarwal AK, Goel VK, Bhaduri SB. Development ofmonetite-nanosilica bone cement: a preliminary study. J Biomed Mater Res B Appl Biomater. 2014; 102: 1620-1626.

78. Van den Vreken NMF, de Canck E, Ide M, Lamote K, van der Voort P, Verbeeck RMH. Calcium phosphate cements modified with pore expanded SBA-15 materials. J Mater Chem. 2012; 22: 14502-14509.

79. Andersson J, Areva S, Spliethoff B, Linden M. Sol-gel synthesis of a multifunctional, hierarchically porous silica/apatite composite. Biomaterials. 2005; 26: 6827-6835.

80. Geffers M, Barralet JE, Groll J, Gbureck U. Dual-setting brushite-silica gel cements. Acta Biomater. 2015; 11: 467-476.

81. Krueger R, Groll J. Fiber reinforced calcium phosphate cements-On the way to degradable load bearing bone substitutes?. Biomaterials. 2012; 33: 5887-5900.

82. Xu HHK, Eichmiller FC, Giuseppetti AA. Reinforcement of a self-setting calcium phosphate cement with different fibers. J Biomed Mater Res. 2000; 52: 107-114.

83. Zhang Y, Xu HHK. Effects of synergistic reinforcement and absorbable fiber strength on hydroxyapatite bone cement. J Biomed Mater Res A. 2005; 75: 832-840.

84. Xu HHK, Quinn JB, Takagi S, Chow LC, Eichmiller FC. Strong and macroporous calcium phosphate cement: Effects of porosity and fiber reinforcement on mechanical properties. J Biomed Mater Res. 2001; 57: 457-466.

85. Xu HHK, Quinn JB. Calcium phosphate cement containing resorbable fibers for short-term reinforcement and macroporosity. Biomaterials. 2002; 23: 193-202.

86. Xu HHK, Simon CG. Self-hardening calcium phosphate cement-mesh composite: Reinforcement, macropores, and cell response. J Biomed Mater Res A. 2004; 69: 267-278.

87. Xu HHK, Quinn JB, Takagi S, Chow LC. Synergistic reinforcement of in situ hardening calcium phosphate composite scaffold for bone tissue engineering. Biomaterials. 2004; 25: 1029-1037.

88. Gorst NJS, Perrie Y, Gbureck U, Hutton AL, Hofmann MP, Grover LM, et al. Effects of fibre reinforcement on the mechanical properties of brushite cement. Acta Biomater. 2006; 2: 95-102.

89. Zhao P, Sun K, Zhao T, Ren X. Effect of CNTs on property of calcium phosphate cement. Key Eng. Mater. 2007; 336: 1606-1608.

90. Muller FA, Gbureck U, Kasuga T, Mizutani Y, Barralet JE, Lohbauer U. Whisker-reinforced calcium phosphate cements. J. Am. Ceram. Soc. 2007; 90: 3694-3697.

91. Maenz S, Kunisch E, Muehlstaedt M, Boehm A, Kopsch V, Bossert J, et al. Enhanced mechanical properties of a novel, injectable, fiber-reinforced brushite cement. J. Mech. Behav. Biomed. Mater. 2014; 39: 328-338.

92. Ritchie RO. The conflicts between strength and toughness. Nat Mater. 2011; 10: 817-822.

93. Launey ME, Ritchie RO. On the fracture toughness of advanced materials. Adv Mater. 2009; 21: 2103-2110.

94. Xu H, Quinn JB, Takagi S, Chow LC, Eichmiller FC. Strong and macroporous calcium phosphate cement: effects of porosity and fiber reinforcement on mechanical properties. J Biomed Mater Res. 2001; 57: 457-466.

95. Albrektsson T, Johansson C. Osteoinduction, osteoconduction and osseointegration. Eur Spine J. 2001; 10: S96-S101.

96. Samavedi S, Whittington AR, Goldstein AS. Calcium phosphate ceramics in bone tissue engineering: A review of properties and their influence on cell behavior. Acta Biomaterialia. 2013; 9: 8037-8045.

97. Hu QH, Tan Z, Liu YK, Tao JH, Cai YR, Zhang M, et al. Effect of crystallinity of calcium phosphate nanoparticles on adhesion, proliferation, and differentiation of bone marrow mesenchymal stem cells. J Mater Chem. 2007; 17: 4690-4698.

98. Yuan HP, Fernandes H, Habibovic P, de Boer J, Barradas AMC, de Ruiter A, et al. Osteoinductive ceramics as a synthetic alternative to autologous bone grafting. Proc Natl Acad Sci U S A. 2010; 107: 13614-13619.

99. Liu YK, Lu QZ, Pei R, Ji HJ, Zhou GS, Zhao XL, et al. The effect of extracellular calcium and inorganic phosphate on the growth and osteogenic differentiation of mesenchymal stem cells in vitro: implication for bone tissue engineering. Biomed Mater. 2009; 4: 025004.

100. Espanol M, Perez RA, Montufar EB, Marichal C, Sacco A, Ginebra MP. Intrinsic porosity of calcium phosphate cements and its significance for drug delivery and tissue engineering applications. Acta Biomaterialia. 2009; 5: 2752-2762.

101. Ruhe PQ, Hedberg EL, Padron NT, Spauwen PH, Jansen JA, Mikos AG. rhBMP-2 release from injectable poly(DL-lactic-co-glycolic acid)/calcium-phosphate cement composites. J Bone Jt Surg. 2003; 85: 75-82.

102. Otsuka M, Matsuda Y, Fox JL, Higuchi WI. Novel skeletal drug delivery system using self-setting calcium phosphate cement 9: effects of the mixing solution volume on anticancer drug-release from homogeneous drug-loaded cement. J Pharm. Sci. 1995; 84: 733-736.

103. Otsuka M, Matsuda Y, Suwa Y, Fox JL, Higuchi WI. A novel skeletal drug-delivery system using self-setting calcium-phosphate cement. 4. Effects of the mixing solution volume on the drug-release rate of heterogeneous aspirin-loaded cement. J Pharm. Sci. 1994; 83: 259-263.

104. Hamanishi C, Kitamoto K, Tanaka S, Otsuka M, Doi Y, Kitahashi T. A self-setting TTCP-DCPD apatite cement for release of vancomycin. J. Biomed Mater Res. 1996; 33: 139-143.

105. Blom EJ, Klein-Nulend J, Klein CPAT, Kurashina K, van Waas MAJ, Burger EH. Transforming growth factor-β1 incorporated during setting in calcium phosphate cement stimulates bone cell differentiation invitro. J Biomed Mater Res. 2000; 50: 67-74.

106. Blom EJ, Klein-Nulend J, Wolke JGC, Kurashina K, van Waas MAJ, Burger EH. Transforming growth factor-beta 1 incorporation in an alpha-tricalcium phosphate/dicalcium phosphate dihydrate/tetracalcium phosphate monoxide cement: release characteristics and physicochemical properties. Biomaterials. 2002; 23: 1261-1268.

107. Blom EJ, Klein-Nulend J, Wolke JGC, van Waas MAJ, Driessens FCM, Burger EH. Transforming growth factor-beta 1 incorporation in a calcium phosphate bone cement: material properties and release characteristics. J Biomed Mater Res. 2002; 59: 265-272.

108. Takechi M, Miyamoto Y, Ishikawa K, Nagayama M, Kon M, Asaoka K, et al. Effects of added antibiotics on the basic properties of anti-washout-type fast-setting calcium phosphate cement. J Biomed Mater Res. 1998; 39: 308-316.

109. Ratier IR, Gibson SM, Best M, Freche JL, Lacout F, Rodriguez. Behaviour of a calcium phosphate bone cement containing tetracycline hydrochloride or tetracycline complexed with calcium ions. Biomaterials. 2001; 22: 897-901.

110. Huang Y, Liu CS, Shao HF, Liu ZJ. Study on the applied properties of tobramycin-loaded calcium phosphate cement. Key Eng Mater. 2000; 192: 853-862.

111. Ginebra MP, Traykova T, Planell JA. Calcium phosphate cements as bone drug delivery systems: a review. Journal of Controlled Release. 2006; 113: 102-110.

112. Otsuka M, Matsuda Y, Wang Z, Fox JL, Higuchi WI. Effect of sodium bicarbonate amount on in vitro indomethacin release from self-setting carbonated-apatite cement. Pharm Res. 1997; 14: 444-449.

113. Otsuka M, Nakahigashi Y, Matsuda Y, Fox JL, Higuchi WI, Sugiyama Y. A novel skeletal drug delivery system using self-setting calcium phosphate cements VIII: the relationship between in vitro and in vivo drug release from indomethacin-containing cement. J. Control. Release. 1997; 43: 115-122.

114. Otsuka M, Nakahigashi Y, Matsuda Y, Fox JL, Higuchi WI. A novel skeletal drug delivery system using self-setting calcium phosphate cement 7: Effect of biological factors on indomethacin release from the cement loaded on bovine bone. J. Pharm. Sci. 1994; 83: 1569-1573.