Chapter

Calcium Phosphate Cements in Tissue Engineering

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Abstract

Calcium phosphate cements (CPCs) consist of a combination of calcium phosphates and a liquid phase, allowing it to fit into the body where it was inserted. Several chemical compositions have been synthesized, promoting specific characteristics to the cements for applications such as bone augmentation and reinforcement and metal implant fixation. The hardening reaction mechanism is at low temperatures and makes it capable of incorporating different drugs and other biological molecules. In addition to the abovementioned advantages, CPCs have excellent bioactivity and osteoconductivity and the ability to form a bone bond. Its function as osteoconductor can be improved by insertion of growth factors. In addition, it is possible to functionalize it with silver ions and use it as a coating of implants, conferring antibacterial properties. In this chapter the physical, mechanical, chemical, and biological properties and the possibility of using these cements as drug carriers or biomolecules will be discussed.

Keywords: calcium phosphates, bone cements, tissue regeneration, drug delivery, osseointegration, antibacterial properties

1. Calcium phosphate cements

Calcium phosphate cements (CPCs) were proposed by Brown and Chow [1] and LeGeros et al. [2] in the 1980s. In 1990, the first CPC was used commercially in the treatment of maxillofacial defects and fractures [3–5].

CPCs consist of a combination of one or more calcium orthophosphate powders in which a liquid phase, usually water or an aqueous solution, is added, allowing it to be set and hardened at the site of the body where it was implanted. This type of cement hardens through a dissolution reaction and a precipitation process, distinguishing itself from other cements that harden through a polymerization reaction. Over time, new compositions have been synthesized promoting specific characteristics to the cements for various applications such as bone augmentation and strengthening [6–14], fixation of metal implants [15, 16], and vertebral fractures [17–19].

The CPCs have the essential advantage of hardening in vivo through a low-temperature reaction. After mixing, the material becomes moldable, and its noninvasive injection represents an important advantage over conventional calcium phosphate ceramics. The fact that this type of cement does not present an exothermic reaction also makes it able to incorporate different drugs and other biological molecules, allowing its application in treatments by drug delivery [20]. In addition to the aforementioned advantages, CPCs have excellent bioactivity and osteoconductivity and an excellent ability to form a bone bond. Its rate of resorption is also

a factor to take into account, since, after modifying its structure, it is possible to modify that rate. Calcium phosphate cements also have disadvantages, namely, their low mechanical performance, which limits their application in bearing situations. Its intrinsic porosity also leads to this material presenting less strength compared to calcium phosphate ceramics [21, 22].

The cements tend to dissolve in order to achieve a stable and less soluble phase, the dissolution being controlled by the pH of the medium. The cements based on hydroxyapatite or brushite are the only final reaction products because they are the most stable at pH > 4.2 and pH < 4.2, respectively, even though there are a large number of formulations. In addition, and because these materials are intended to be used as bone substitutes, it is important to take into account that the values of the compressive strength of the cortical bone vary between 90 and 209 MPa, [23, 24] and the spongy bone varies between 1.5 and 45 MPa. [25]. As reference values, the compressive strength of apatite cements usually ranges from 20 to 50 MPa [26–32]. Brushite CPCs are generally weaker than apatite CPCs being around 25 MPa [33].

In summary, the main characteristics of this type of cement are presented in **Table 1**.

1.1 Physical and mechanical properties

The mechanical properties are the main properties to take into account when developing a biomaterial to apply surgically. The microstructural characteristics (porosity, quantity, size, morphology, and distribution of the crystals formed) of a biomaterial are the determining factor to define the mechanical properties. These characteristics are controlled by the synthesis process and its intrinsic parameters. In addition, all factors, such as chemical composition of cement, relative proportions of reactants, powder or liquid additives acting as accelerators or retarders, particle size, liquid-powder ratio (L/P ratio) (**Figure 1**), applied pressure during synthesis, and aging conditions, will affect its mechanical properties [36].

Unlike bioceramics, which require sintering at high temperatures, CPCs are formed through a dissolution-precipitation process at room or body temperature. During this process, a crystalline matrix is formed in which with the passage of time and matrix it becomes increasingly dense until reaching the maximum mechanical properties [36].

Normally, microporosity ranges between 30 and 55% and is dependent on the L/P ratio; the higher this ratio is, the greater the microporosity [37].

Calcium phosphate properties	
Material type	Ceramic
Liquid phase	Water or aqueous solutions
Powder component	Calcium phosphate powders
Setting reaction mechanism	Dissolutions and precipitation reaction
Reaction products	Calcium phosphates, usually hydroxyapatite or brushite (37°C)
Stability	Resorbable (low or high resorption rate depending on composition and microstructure)
Bioactivity	Bioactive
Applications	Bone regeneration; non-load-bearing applications

Table 1.
The nature and properties of calcium phosphate bone cements (adapted from [34]).

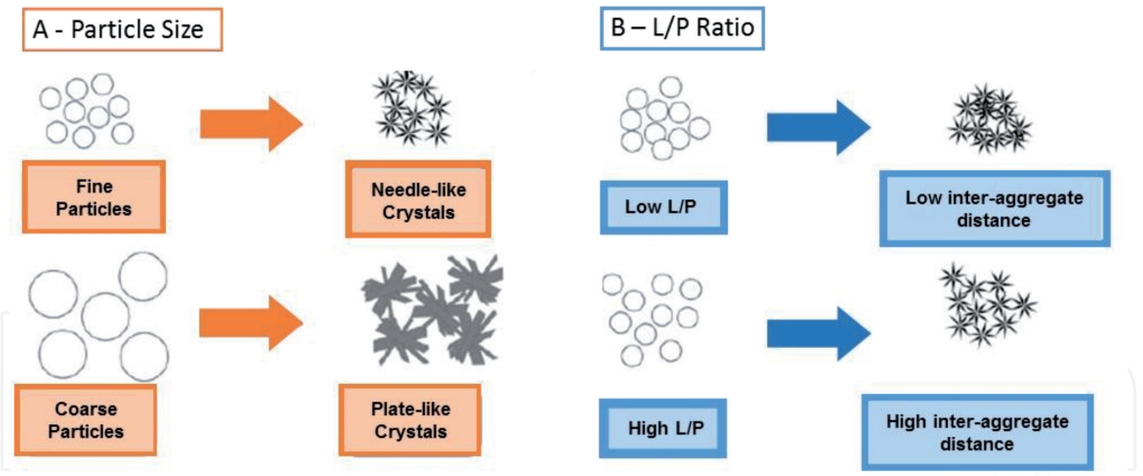

Figure 1.
Microstructure and porosity of CPCs according to particle size and L/P ratio. (A) When the particle size is small, there is an increase in the specific surface area, resulting in the degree of supersaturation. This phenomenon favors the nucleation of crystals and leads to the formation of large quantities of small needle-like crystals. When the particles are larger, the formation of larger plate crystals occurs. Small pores are formed in the cement where small particles are used. (B) Porosity and pore size distribution also vary with the L/P ratio. When the L/P ratio is low, the space between the particles in the mixture decreases, leading to a more compact structure of crystal agglomerates. In contrast, when the L/P ratio increases, the total porosity of the cement increases, and the larger pores are formed due to the increased separation between the aggregates (adapted from [35]).

Although porosity is a disadvantage to be applied in load situations, porosity is sought to improve the resorbability of the material and the extent of bioactivity, increasing the surface area available for reaction. The presence of a certain level of porosity makes this material a good carrier for controlled drug systems [34].

It is possible to promote the creation of macropores in CPCs through two techniques. The leaching of porogen after the adjustment creates macropores; however, it is necessary to add large amounts of porogen, which may compromise the injection process [37–47]. The formation of the pores can also be achieved by the formation of gas foam prior to fixation, but the release of the gas after the introduction of the implant may be harmful to the organism [46, 48–52]. In order to overcome the drawbacks of the techniques mentioned above for obtaining macroporous structures of CPCs, Ginebra et al. proposed the use of self-adjusting injectable macroporous foams composed of a protein-based foaming agent and CPC paste [52].

Regardless of the composition of CPC (apatite or brushite), the strength decreases globally with increased porosity, which is a common occurrence in several materials mainly in porous materials used in bone replacement [36]. In addition to the pore fraction effect, pore size also significantly affects the strength of CPCs. Bai et al. [53] found that the compressive strength is inversely proportional to the size of the macropores through a study in materials with equivalent total porosity but with different sizes of macropores. Through Griffith's classical theory [36], which relates strength to the critical size of the fault, macropores can be considered as failures, thus reducing strength. In addition to the quantity and size of the pores, the characteristics of the crystals (quantity, size, morphology, and distribution) also influence the strength of these cements. The growth of the crystals depends on the kinetics of the dissolution-precipitation reaction of the cement, being controlled by many factors. The smaller the particle size of the starting materials, the faster the material will be converted to apatite, and the crystals formed will not have time to grow. The small size of these crystals will lead to a more dense and crystalline organization, increasing the strength of the cement [54].

One of the conditions that influence the kinetics of apatite formation is aging. The transformation of the initial reactants into apatite becomes faster at higher

temperatures, thus making the structure more homogeneous and denser, making it more resistant. In contrast, high temperatures also cause the development of precipitated apatite crystals more rapidly, resulting in larger crystals, which will negatively influence the resistance [55].

The fixation kinetics may be influenced by the presence of accelerating or retarding substances which are added to the mixture and is an important factor in determining the strength of the structure.

Bermudez et al. [56] and Yang et al. [57] found that by adding certain amounts of apatite, the hardening time of the CPCs is lower, and the compressive strength increases considerably. α-Hydroxylic acids (citric acid or glycolic acid) and their salts (sodium citrate) are also used as retarders. The addition of the same allows to mix and to process more easily, reducing the L/P ratio associated with a decrease of the porosity, improving the strength [58–64]. However, it is necessary to take into account an optimum concentration of these additives since the excess may lead to the opposite effect and decrease the force [63]. In summary, the mechanical properties of the CPCs, and in particular the resistance, depend strongly on the microstructure, which is related to the synthesis process, chemical composition, powder or liquid additives acting as accelerators or retarders, particle size, L/P ratio, and aging conditions. In addition, it has been found that crystalline structures have, with smaller crystals, become more compact and homogeneous and appear to give better mechanical properties than those with larger crystals.

Strength has been the main property to be studied when evaluating mechanical performance; however, the CPCs applied in bone defects are also subject to cyclic loading, and the resistance of CPC to fractures cannot be evaluated by strength alone. In order to adequately evaluate the ability to resist fractures, it is also necessary to take into account fracture toughness that describes the strength of a material containing cracks or notches to resist crack propagation [65, 66].

The toughness of a material depends on its nano-/microstructure and on the possibility of promoting the hardening activation mechanism [67, 68]. Without the activation of significant hardening mechanisms, the fracture toughness of CPCs is very low. Due to the low values of toughness, CPCs are very sensitive to defects and failures. The reliability, that is, the likelihood of failure of brittle materials, is also an important factor when one thinks of applying the cement to load-bearing sites. As previously mentioned, it is possible to improve the hardening mechanism by decreasing porosity, as it is the most damaging factor in mechanical performance. To overcome this problem, it is necessary to decrease the volumetric fraction of the pores in order to achieve a more dense matrix by compacting the cement paste prior to hydration.

Studies have shown that compaction pressure would significantly increase tensile strength [69]. However, when the compaction pressure is above 100 MPa, only a slight decrease in porosity is achieved, and the diametral tensile strength is not substantially improved.

In addition, the use of this method to promote the hardening has the same function of decreasing the L/P ratio, which would influence the workability and injectability of cement pastes, which may exclude the application of this cement in minimally invasive surgery.

In order to overcome this disadvantage, researchers added certain amounts of citric acid to the cement liquid to evaluate its effect on the fixability and fixation properties of apatite cements and found that this addition effectively improves the mechanical properties of the cement. According to this prominent effect of citric acid on strength improvement, Barralet et al. [61] and Gbureck et al. [60] added sodium citrate and compacted the resulting cement slurry, obtaining compressive strengths near the resistance of the cortical bone, that this composition can be used

in load-bearing locations. This resistance can also be achieved by other factors, especially in the use of citric acid but without applying external pressure, varying the particle size and distribution of powdered reagents [70].

1.2 Chemical and biological properties

The main chemical reaction occurring in the setting mechanism is similar in all these systems of cements and can be understood by analyzing the behavior of the solubility of the existing compounds in the composition [71–73]. During the fixation reaction, the two mechanisms present are dissolution and reprecipitation [23]. The dissolution is activated by the release of the calcium and phosphate ions from the starting materials, leading to supersaturation in the solution. After the ionic concentration reaches a critical value, the nucleation of the new phase occurs, usually around the powder particles. This new phase develops in line with the dissolution of the reactants [74].

In the dissolution/reprecipitation mechanism, the formation of the precipitates depends on the relative stability of the various calcium phosphate salts in the system. The existence of a precipitate that grows in the form of the crystal agglomerates determines the force that a cement can acquire [74]. The solubility phase diagram predicts this reaction, describing the evolution of the solubility of a compound through the logarithm of the total concentration of calcium (or phosphate) as a function of pH [71–73].

The less stable phase of calcium phosphate tends to dissolve to form a more stable and less soluble phase.

As mentioned above, apatite is the most stable calcium phosphate (less soluble at a pH above 4.2 at room temperature), and brushite is most stable at a pH below 4.2 [71].

In these reactions the amount of water consumed is nonexistent, or almost nil, being necessary only for the reagents to become viable and to allow homogeneity in the solution. For this reason, water becomes one of the main contributions to the development of porosity in cement, and, therefore, CPCs are intrinsically porous materials.

In addition to this material, in situ, the body temperature allows its molding after mixing, to be injectable and therefore to be used as a carrier for biological drugs or molecules [75–77].

In the industry, the biological responses of materials have been increasingly a property to be taken into account, focusing on improving cell and tissue CPC interactions, as well as their applications in bone tissue engineering [78–82]. The improvement of these interactions is one important factor for the application of biomaterials and their commercialization for clinical applications.

Studies evaluating the in vivo behavior of CPCs show high levels of biocompatibility and osteoconductivity, stimulating tissue regeneration [5, 82–92]. Most apatite cements are reabsorbed by cell-mediated mechanisms. The function of the osteoclastic cells in this process is to degrade the materials layer by layer, starting from the surface which is in contact with the bone to the nucleus. The biodegradability of apatite CPCs is slow but higher than that of the synthesized hydroxyapatite. As noted above, the rate of degradation of apatite cements is controlled by precipitated hydroxyapatite (PHA) crystallinity, specific surface area, and matrix porosity.

The cements based on brushite have a higher reabsorption rate than apatites due to their superior stability in the biological environment [89–91]. However, there is a possibility in vivo of brushite cements to be transformed into PHA and thus reduce their total degradation rate. In order to retard this reaction or to avoid magnesium salts have been added [92].

At a biological level, the mechanism of dissolution is mediated by the action of physiological solutions or by cell-mediated processes (phagocytosis) [93].

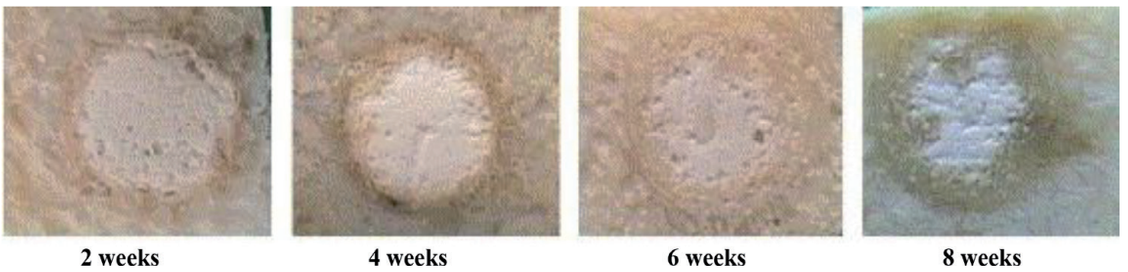

Figure 2.
Image of the drill hole with progressive resorption of the calcium phosphate cement matrix, during 8 weeks [81].

Bone replacement depends on the age, sex, and general metabolic health of the host and the site and volume where it is applied, porosity, crystallinity, chemical composition, particle size, and L/P ratio of the cement. Considering these factors, it can take from 3 to 36 months for the cement to be completely reabsorbed and replaced with bone. However, further studies are needed to confirm the total resorption of the material in order to be applied clinically [94]. Studies have revealed bone development around calcium phosphate cements, demonstrating osteoconductive and osteoinductive characteristics in several cases. It has been shown that within 2 weeks, spicules of living bone with normal bone marrow and gaps in osteocytes can be identified in the cement. After 8 weeks, the cement is almost completely surrounded by new bone. At this stage, no cement reabsorption is typically observed [94]. **Figure 2** shows a progressive resorption of the calcium phosphate cement matrix, with tricalcium phosphate (TCP) granules embedded in a matrix of dicalcium phosphate dihydrate (DCPD) and parallel new bone formation, in a drill hole. After 2 weeks almost the entire surface of the cement was in direct contact with the margins of the bone defect. After 4 weeks, occasional granules of β-TCP and the newly formed bone islets are visible. This area expanded after 6 weeks, involving a progressive reabsorption of the cement matrix and parallel neoformed formation [81].

2. Principal calcium phosphate cements

2.1 Apatite cements

The importance given in the use of apatites in bone replacement is due to the fact that this mineral is the base of the main inorganic part of hard tissues. In fact, nonstoichiometric or calcium-deficient hydroxyapatite (CDHA) is the main mineral phase characteristic of human bones [94]. The CPCs consist of a network of calcium phosphate crystals, with chemical composition and crystal size that can be modified to approximate the biological hydroxyapatite that exists in the living bone [95, 96].

In this regard, it is necessary to clarify that even though the stoichiometric hydroxyapatite has a fixed composition, the apatite structure may exist in a variety of compositions. CDHA comprises in its composition the possibility of varying amounts of calcium, where it is possible to present a completely deficient structure based on this base element. The composition may be expressed as $Ca10-x(HPO4)(PO4)6-x(OH)2-x$, where x ranges from 0 to 1, 0 for stoichiometric hydroxyapatite and 1 for hydroxyapatite totally deficient in calcium. Biological apatite is deficient in calcium containing various ionic substitutions such as Na^+, K^+, Mg^{2+}, F^-, and Cl^- [34].

Apatite cements may form PHA or CDHA through a precipitation reaction. The synthesis of these cements allows the incorporation of different ions in their composition, depending on the initial compounds. The formation of hydroxyapatite that occurs in the cement is compared to the process of formation of new bone

and is also seen as a biomimetic process, because it occurs at body temperature and physiological environment. This may explain the fact that the hydroxyapatite formed in the reactions of calcium phosphate cements is much more similar to biological apatites than the ceramic hydroxyapatite resulting from high-temperature sintering processes [34].

The CPCs lead to the formation of PHA or CDHA and can be divided into three systems (single compound, two compounds, more than two compounds), taking into account the number and type of calcium phosphates used in the synthesis [97].

Monocomponent CPCs are those having a single calcium phosphate reagent that hydrolyzes to form PHA or CDHA. Taking into account that at pH 4.2 the hydroxyapatite is less soluble, any other calcium phosphate present will dissolve, and the PHA will tend to precipitate. However, when the formation of PHA occurs, from the hydrolysis of calcium phosphate, the reaction mechanism becomes very slow, due to a decrease in the level of supersaturation, as the reaction proceeds [13]. In this in which only one compound is present, no release of any acid or no base occurs due to the Ca and P ratio being maintained [34, 35].

The second type of cement that may exist is composed of two calcium phosphates, one acid and the other basic, where they adjust after an acid–base reaction. The most commonly used compound is usually tetracalcium phosphate (TTCP), as it is the only calcium phosphate with a Ca/P ratio higher than PHA. Therefore, TTCP can be combined with one or more calcium phosphates with lower Ca/P ratios to obtain PHA or CDHA, avoiding the formation of acids or bases as final products. The combinations that have been more studied seek to produce cements that adjust to the body temperature in a range of pH around the neutral [34].

The third possible system consists of more than two compounds, including calcium phosphates and other salts. For example, a cement proposed by Norian Corporation [5] is used where calcium phosphates with a Ca/P ratio lower than PHA and CaCO3 are added as an additional source of calcium. The initial configuration process involves the formation of DCPD, later forming dahllite, a carbonated hydroxyapatite similar to the bone mineral [5].

Apatitic CPCs appear as a viscous, easily moldable material; however, their injection is difficult. **Figure 3** shows the microstructure of an apatitic cement after setting. The setting time can also be reduced by means of additives such as with the introduction of PHA particles. These changes in the composition may lead to an

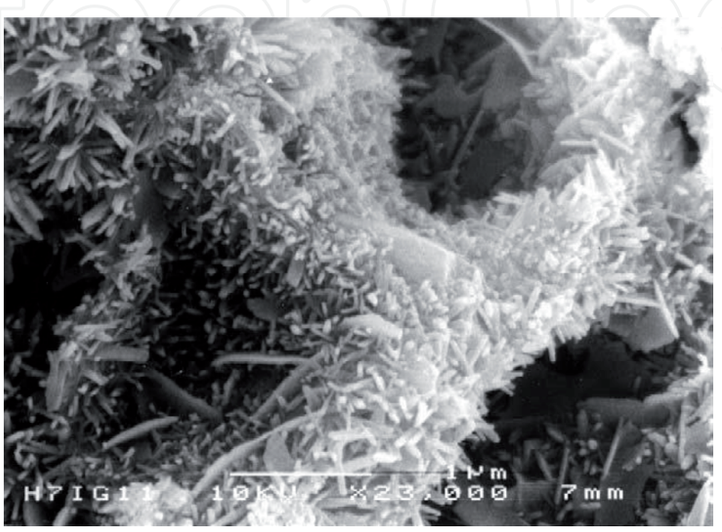

Figure 3.
Microstructure of an apatitic calcium phosphate cement after setting, showing the micro–/nanosize pore structure formed by the entanglement of the precipitated crystals [71].

adjustment time in the range of about 15 minutes. When hardening of the cement paste occurs too fast, the hardened cement must be milled to render it viscous again. Subsequently, the paste hardens due to the precipitation of PHA.

After implantation, the mechanical properties can be altered. Investigations indicate that the mechanical properties of apatite CPC tend to increase, unlike brushite cement, which initially decrease and increase when the bone develops [98, 99].

2.2 Brushite cements

Brushite (DCPD) is an acidic calcium phosphate that has been found in some physiological sites, for example, in bones [100]. Unlike hydroxyapatite, brushite is metastable under physiological conditions [101] and for this reason reabsorbed much faster than CPC apatite; however, there are studies that conclude that DCPD in vivo tends to convert to PHA [26]. Some CPCs were designed to provide brushite as the final product.

Several combinations of compounds have been proposed for the formation of brushite cements; most are β-tricalcium phosphate (β-TCP) and an acid component, namely, monocalcium phosphate monohydrate (MCPM) or phosphoric acid [102–104].

The reaction leading to the formation of brushite CPCs is an acid–base reaction. The brushite paste is acidic during sedimentation because brushite can only precipitate at a pH value below ~6 [105]. The pH of the cement paste tends to change slowly toward equilibrium pH [106]. If the slurry contains an excess of basic phase, the pH tends to equilibrate by crossing the solubility of the base phase with that of the DCPD. The time of stabilization of brushite CPC depends greatly on the solubility of the basic phase: the higher the solubility of the basic phase, the faster the defined time. For example, hydroxyapatite (HA) + MCPM blends have an adjustment time of several minutes. The β-TCP + MCPM mixtures have an adjustment time of 30–60 seconds [107, 108]. However, compounds that inhibit the development of DCPD crystals can be added, increasing the settling time of the β-TCP + MCPM mixtures [109]. The brushite CPC can initially be very liquid and still be defined within a short period of time, unlike the apatite CPC. The brushite CPC is slightly weaker (tensile strength of 10 MPa [110] and compressive strength of 60 MPa) than the apatite CPC (tensile strength of 16 MPa [111] and compressive strength of 83 MPa [112]). The mechanical properties of apatite CPC increase [98], whereas those of brushite CPC decrease [99] This latter phenomenon is attributed to the greater solubility of DCPD in relation to that of PHA [113]. After a few weeks of implantation, the mechanical properties of brushite CPC are promoted by bone growth [99]. Although brushite CPC exhibits biocompatible properties, inflammatory reactions have been reported with the excessive addition of brushite CPC [114]. Investigations indicate that these reactions are due to the transformation of DCPD into PHA [115]. This reaction releases large amounts of acid. The transformation of DCPD into PHA can be avoided with the addition of magnesium ions to the cement [116]. Unlike apatite CPC, brushite CPC cannot be reabsorbed exclusively by osteoclastic activity but also by simple dissolution. Therefore, brushite CPCs degrade at a faster rate than the apatite CPC.

Although brushite demonstrates a higher solubility rate than the other calcium phosphate phases, it is a precursor of the most stable HA phase [117–120]. For this reason, DCPD coatings as an initial step to obtaining HA have been widely used. The synthesis of HA through precipitation mechanisms results in compacted crystals but with sizes difficult to control. Using DCPD as a precursor becomes favorable since it is possible to modify the crystal size of the DCPD through homogeneous precipitation and can be converted directly into HA [119]. In environments with a pH > 6–7, brushite becomes unstable and becomes the most favorable HA phase [121, 122].

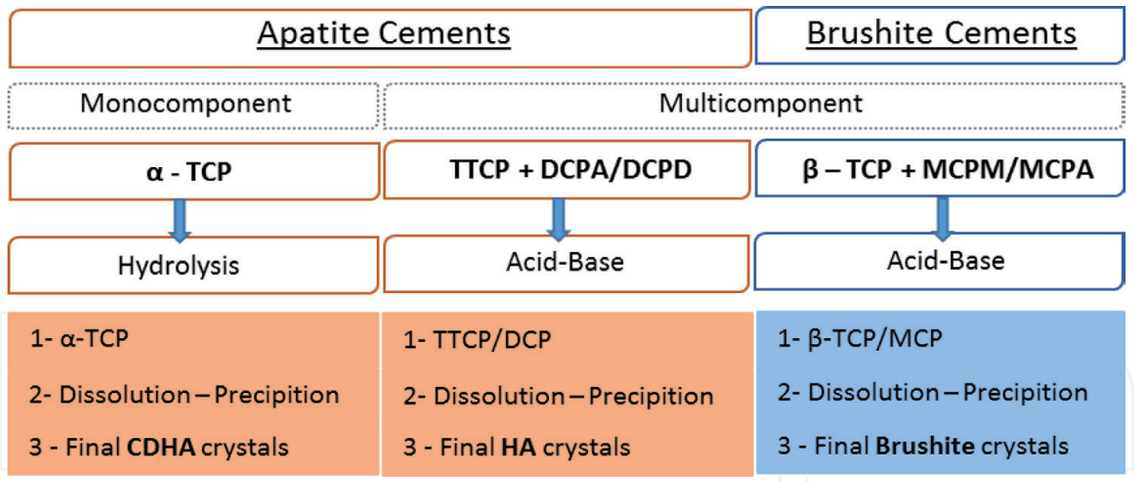

Figure 4.
Classification of calcium phosphate cements, with examples of the most common formulations. From top to bottom, the cements are classified by the type of end product (apatite or brushite), a number of components in the solid phase (single or multiple), type of setting reaction (hydrolysis or acid–base reaction), setting mechanism, and microstructure evolution during setting (adapted from [35]).

The fact that DCPD is able to be more soluble leads to its use in metal implants as a means of increasing the amount of calcium and phosphate ions available in the surrounding tissue of the implant to promote increased osseointegration [118].

The biocompatibility of DCPD as a coating has been demonstrated in several cell lines as, for example, in pre-osteoblastic macrophages [123, 124] and fibroblastic cells [125]. The biocompatibility of DCPD has also been demonstrated when used at a cranial defect site in sheep [126], and the formation of new bone was observed in the absence of inflammation [81]. A clinical study in humans in 2010 effectively used a brushite cement for the repair and increase of pterionic craniotomies, with no inflammation occurring [127].

Figure 4 summarizes how the CPCs are classified by type and number of initial reagents as well as their final product.

3. Calcium phosphate cement applications

3.1 Drug delivery

The main requirements for a substrate to have potential as a drug carrier are to have the ability to incorporate it, to retain it at a specific site, and to distribute it progressively over time in surrounding tissues. In addition, it is beneficial that the material is injectable and biodegradable [77].

Calcium phosphate cements, in addition to allowing hardening at room or body temperature, allow the insertion of various components due to their intrinsic porosity. It is possible to incorporate drugs, biologically active molecules, or even cells without their functions being altered by the effect of temperature or even losing their activity during the procedure (**Figure 5**). This change in the CPCs offers new properties in addition to the osteoconductive characteristic, namely, to increase its capacity for bone regeneration or to support in disorders or pathologies, such as bone tumors or osteoporosis [35].

It is necessary to take into account that the performance of the drug delivery depends on the structural characteristics such as the specific surface area, permeability, matrix degradation rate, drug solubility, or the interaction itself between the matrix and the inserted drug.

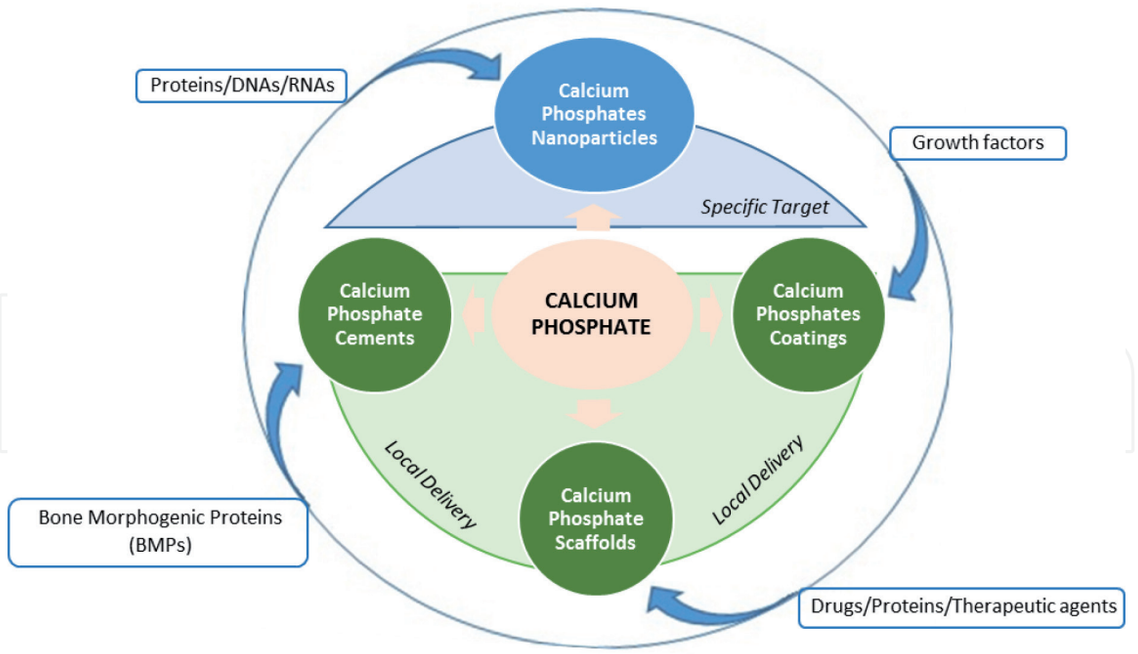

Figure 5.
Scheme about the possible applications of calcium phosphates in the biomolecules and drug delivery (adapted from [128]).

The interaction between the drug and the cement matrix is defined by the drug insertion procedure. Usually, the drugs are added as a powder to the solid phase or dissolved in the liquid part. The method that allows better homogenization of the drug in the matrix is in the liquid phase [35].

Another method for inserting the drug into the matrix is by impregnating solid beads or granules from the cement with a drug solution. This method continues to present benefits compared to other conventional methods even with its impaired injection process. The advantages associated with this method are due to the fact that the consolidation of the material (dissolution-precipitation reaction) leads to the production of hydrated matrices with large specific surface areas that allow the loading of drugs and their release mechanism to be favorable [35].

The two drug encapsulation processes differ greatly in the structural properties of the matrix. In one method the drug is incorporated in the initial phase together with the cement reactants while the matrix is still evolving. Thus, the hardening can last for hours or even days until the suspension of particles evolves into a network of interlaced crystals. In the other case, if the drug is added to the preconceived cement, the matrix structure will always be stable throughout the release process, in addition to possible degradation, and therefore the results cannot be extrapolated to the previous situation. This highlights the need for the studies to be carried out, taking into account the actual conditions of application. The alternative method is to incorporate the drug into polymeric microspheres prior to mixing in the cement. This procedure has two advantages over the other methods presented. It is possible to modify the release kinetics of the drug, and the degradation of the microspheres generates an array capable of being more easily reabsorbed and remodeled [35].

The incorporation of drugs can influence the entire mechanism of action of the cement and compromise its purpose, changing the fit kinetics, rheological characteristics, and microstructural development. For example, molecules that interact with calcium or phosphate ions promote a coprecipitation during the fit or form complexes with Ca^{2+}, promoting a delay in precipitation and modifying viscosity, set time, and cement properties [72, 129, 130].

In addition to the influence that the drug has on the cement matrix, it is also necessary to take into account the influence of the cement on the stability of the drug or bioactive molecule. Due to the dissolution-precipitation process, there is a change in the surrounding pH as well as the change in ionic concentrations, which may influence the functionality of the drug and its release.

Thus, it is beneficial to study the release of drugs introduced from already synthesized cements.

Recently, studies have been developed related to the incorporation of antibiotics, as a preventive method of infections resulting from surgeries or as treatment of bone infections. In addition to antibiotics, studies with anti-inflammatories, antitumor drugs, or hormones have also been disclosed. In another aspect, the incorporation of factors that stimulate bone regeneration, such as bone morphogenetic proteins (BMP) or transforming growth factors-β (TGF-β), has been studied [77, 131].

3.2 Growth factor addition

Growth factors are a large group of proteins that interact at the cellular level [35]. The major families of these proteins are the transforming growth factor-beta superfamily responsible for promoting bone regeneration. The BMPs are part of the TGF-β superfamily and have been widely used in bone regeneration. It is known to play a role as an activating agent in the various biological phenomena responsible for bone formation and therefore can accelerate bone growth. These BMPs stand out from the other growth factors of the large TGF-βSF group because they are osteoinductive. That is, the BMPs act at the level of cell differentiation transforming the pluripotential cells into bone-forming cells, aiding in bone formation outside the bone tissue [77].

These proteins have been produced at an industrial level with a high level of purity; however, it is necessary that their administration is controlled and with adequate therapeutic levels as well as adapted to the tissue targets. In fact, it is known that the injection of such substances alone cannot induce the formation and regeneration of tissues since the protein diffuses very rapidly from the site of implantation. Thus, the CPCs present themselves as good substrates and carriers for these bioactive molecules, also improving their function as osteoconduction [77].

Studies have shown that the superfamily of growth factors stimulates osteoblast proliferation and collagen synthesis in vitro [132] and may increase the size of the cortical bone when applied near the periosteum in vivo [133].

This improvement in bone growth, when applied to cement with these molecules, is due to the adsorption of large doses of rhTGF-β_1 on the surface of the material [134, 135].

Despite this accumulation of the growth factor to the surface, there is a homogenous distribution throughout the cement mass, increasing the time of the release of the growth factors while the degradation of the matrix occurs.

Blom et al. showed that the addition of a human recombinant TGF-β_1 (rhTGF-β_1) to a CPC in the adjustment phase stimulated the differentiation of pre-osteoblastic cells using primary mouse bone cells in vitro [136].

In contrast to the kinetics of drug release, the release of these factors becomes much slower [137].

It has been determined that in the first days, the release rate of the components is higher because the initial release is only of the material present in the surface layer that is in contact with the medium. This increase in the rhTGF-β_1 release was confirmed when the area in contact with the medium occurred by fragmentation. The same phenomenon was observed when BMP-2 human recombinant microspheres were introduced into the CPC. The release of the factor was quite limited due to

the possible physical entrapment of the microparticles inside the porous cement. According to the authors, the nanoporosity of CPC not only did not facilitate the release of the protein but could also limit it because of the high binding affinity of the protein by CPC [138].

Haddad et al. [139] investigated the action of implantation of cement loaded with BMP-2 in the bone repair of a critical-sized calvarial vault defect in rabbits. Compared with control, an increase in bone formation was observed at 45% after 12 weeks of implantation.

Other investigations by Seeherman et al. [140, 141] also demonstrated the efficiency of these combined systems (BMP-2/cement). For example, these composites accelerated the filling of a bone defect by 40% after approximately 4 months of implantation, compared to cement without the protein. This study was done in a primate fibula osteotomy [140].

The composite used in the abovementioned study was also used in rabbit bone defects. After 4 weeks of implantation, an acceleration of reabsorption was observed as well as filling of the defect compared to the base cement. This acceleration led to the complete filling of the defect with new bone 8 weeks after the implant.

3.3 Ion addition

To avoid infections resulting from orthopedic surgery, which usually lead to bone loss or subsequent removal of the implant, alternatives such as antibiotic delivery have been used on the site [142–144]. This transport is usually done using poly(methyl methacrylate) (PMMA) or by encapsulating the drug in the CPC matrix. PMMA beads have the drawback that they are not resorbable and require further surgery to remove them and place new antibiotic-loaded spheres if the goal is to prolong the treatment [145–148]. Faced with this drawback, CPCs have been widely studied as degradable materials capable of carrying antibiotics [77, 145, 148–155]. However, there is a risk of creating bacterial resistance due to low doses of release [156–158]. Thus, the use of surface functionalization of biomaterials as well as the coating of implant surfaces with silver ions has been recurring, conferring antibacterial properties [159–163].

The antimicrobial properties of Ag^{+} ions have been investigated and studied in the field of biomedical engineering [164]. It has been found that bacteria hardly gain resistance to silver-based products and low concentrations are required to have a bactericidal effect [165].

Therefore, both metallic silver and ionic silver were incorporated in several biomaterials, HA [166–169], and bioactive glasses [164, 170–172]. In both structures, silver has relatively low toxicity to human cells [173–176].

Ewald et al. evaluated the antimicrobial properties of silver-loaded bone cements as well as their osteoconductive and resorbable properties. The reagents used were both α- and β-tricalcium phosphates combined with slightly acidic compounds to form HA or brushite cement. This study revealed that it is possible to synthesize cements with antimicrobial activity with effects comparable to antibiotic treatments. **Figure 6** shows the inhibition of both *S. aureus* and *S. epidermidis* cultured on the surfaces of the silver-doped cements. Ag-brushite exhibits more antibacterial properties than Ag–HA. In addition, in the case of brushite cements, silver ions allow the cements to increase in compressive strength by approximately 30% [177].

Several studies have been carried out using silver-doped calcium phosphate cements, and the results have been satisfactory, demonstrating inhibitory effect against certain bacteria [178].

In addition to silver, other ions have been incorporated into materials composed of calcium phosphate. Doping with Co^{2+} showed proangiogenic effects [179, 180].

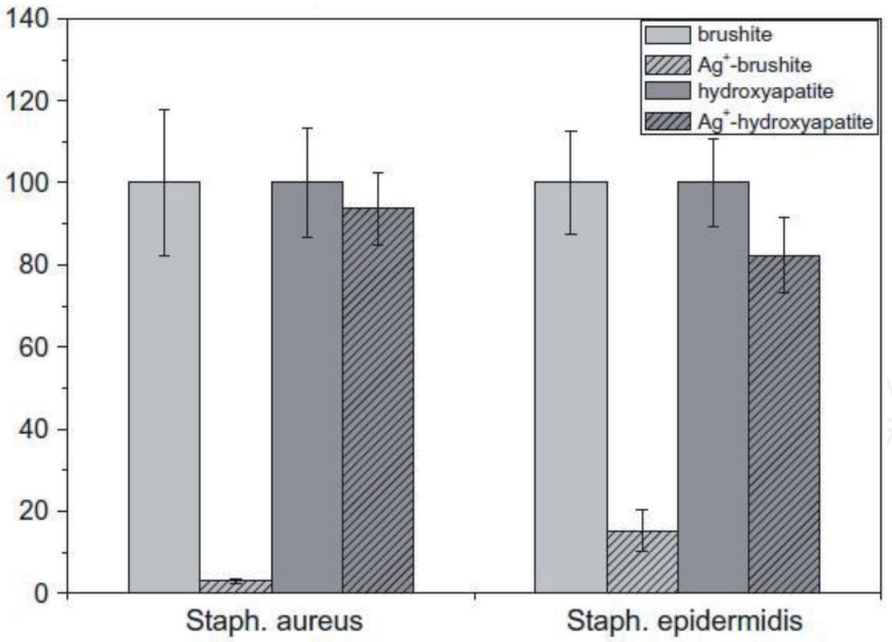

Figure 6.
Bacterial activity of S. aureus *and* S. epidermidis *on cement surfaces (n = 4) determined by the WST-1-test in LB medium [177].*

Modification with Cu^{2+} increased the rate of vascularization [181]. The influence of the introduction of Co^{2+}, Cu^{2+}, and Cr^{3+} on calcium phosphate cement was investigated, evaluating the material properties, proliferation, and osteogenic differentiation of human mesenchymal stem cells in vitro [182]. The Cr^{3+} and Cu^{2+} ions, in this case with less evidence, had positive effects on osteogenic proliferation and differentiation.

Despite all the positive results of this incorporation, the examinations that evaluate the osteogenic capacity in vitro are not enough to estimate the clinical performance of these materials in the bone graft. Since the balance between bone neoformation and material resorption is crucial for successful remodeling, in vitro analysis of osteoclast-mediated degradation of materials is a logical next step in evaluating material remodeling in vivo [183].

However, it is necessary to take into account the dose that is used in the doping process, as investigators have concluded that doses in certain amounts of Cu^{2+} and Co^{2+} can cause cytotoxic reactions to osteoclasts and progenitors of osteoclasts during the initial release of Cu^{2+} and Co^{2+}, respectively. Cements at lower doses are beneficial to bone regeneration since Cu^{2+} at 18 μM completely inhibits reabsorption (but not the formation of osteoclasts), which may be beneficial for patients with osteoporosis and imbalance between bone formation and resorption. The addition of Cr^{3+} to brushite cements increases osteoclastic reabsorption and increases the viability of osteoprogenitor cells compared to cement without the addition of ions [182]. Therefore, Cr^{3+}-doped brushite cements are suggested as a promising new material for application in bone regeneration.

3.4 3D printing of CPCs

The 3D printing technique, or additive manufacture (AM), is based on the addition of layers of powder material producing solid materials with adjustable porosity, through a digital geometric model. This method has been the subject of much research in the medical field such as in the synthesis of the customized scaffold [184]. In addition to the economical and fast production that 3D printing allows, it also allows

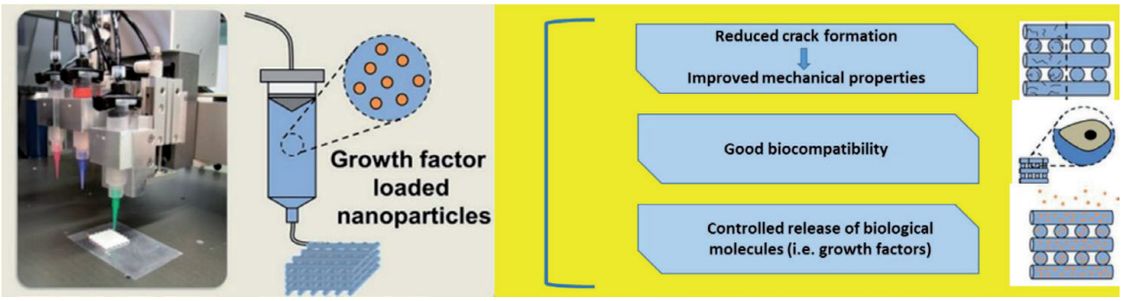

Figure 7.
Scheme of the 3D printing system of the calcium phosphate bone cement scaffolds and its advantages due to the possibility of improving the settings.

the manufacture of pieces with high geometric complexity. All these advantages have made 3D printing very useful in the field of biomedical and tissue engineering due to the ability to replicate the complicated architecture as well as the cellular heterogeneity present in tissues and organs. The bone presents itself as an exquisite structure due to the existence of a complex compound of minerals and an organic matrix. Taking into account this organization, there are several size scales, and it is possible to easily reproduce this structure through 3D printing. In addition, 3D printing is suitable for producing structures derived from medical images, such as CT scans [185, 186].

Recently, the manufacture of the customized scaffolds of the calcium phosphate cements by 3D printing has been described. During printing, highly viscous or pasty materials are dispensed through an adjustable dosing nozzle, resulting in the deposition of CPC layers on a platform with a liquid or air as a plotting medium. After the stabilization of the scaffolds, the water adjustment reaction begins. These stabilization and hardening conditions allow the integration of biological molecules at specific sites [187].

In this way, it is possible to create scaffolds, based on calcium phosphates, more complex with specificities that can promote higher bioactivity or introduce drugs or other therapeutic components, taking into account the patients' needs (**Figure 7**).

In addition, this technique allows the combination of components (i.e., CPC and alginate) to produce structures more resistant to compression and with improved toughness compared to pure CPC supports [188].

4. Conclusions and future perspective

Due to its bioactivity, biocompatibility, osteoconductivity, and osteoinductivity, calcium phosphate cements present an advantageous option in the field of bone tissue engineering, taking into account all the needs that this application demands. In addition, it may be used as scaffolds and transport medium for various biological molecules such as stem cells, drugs, or growth factors. It is also worth mentioning the possibility of producing structures of these cements through 3D printing technology, where it is possible to manufacture intrinsically complex biomimetic structures due to the degree of precision of this technique.

The possibility of building calcium phosphate cements, involving the incorporation of several types of cells, growth factors, molecules, or bioactive glasses, allows favorable results in the vascularization of bone tissues and, consequently, in bone regeneration. This feature, particularly appreciated in large bone regenerations, will allow a considerable increase in the use of these structures in clinical applications. However, more research is needed to consolidate and understand all the information associated with the fundamental mechanisms that promote the development of tissue engineering and regenerative medicine.

The success associated with biomaterials has been underestimated maybe because they have been used clinically for more than 40 years. However, there is still a huge diversity of calcium phosphate-based materials that have not been fully investigated.

Acronyms

AM	manufacture additive
BMP	bone morphogenetic proteins
CDHA	calcium-deficient hydroxyapatite
CPCs	calcium phosphate cements
DCPA	dicalcium phosphate anhydrous
DCPD	dicalcium phosphate dihydrate
HA	hydroxyapatite
L/P ratio	liquid-powder ratio
MCPM	monocalcium phosphate monohydrate
PHA	precipitated hydroxyapatite
PMMA	poly(methyl methacrylate)
TCP	tricalcium phosphate
TGF-β	transforming growth factors-beta
TTCP	tetracalcium phosphate
rhTGF-β_1	human recombinant TGF-β_1

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