Chemical Properties of Calcium Phosphate

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Calcium phosphate (CaP)-based biomaterials have superior properties and have been widely used for bone defect repair, due to their similarities to the inorganic components of human bones. The biological performance of CaPs, as a determining factor for their applications, are dependent on their physicochemical properties. Hydroxyapatite (HAP) as the most thermally stable crystalline phase of CaP is mostly used in the form of ceramics or composites scaffolds with polymers. Nanostructured CaPs with large surface areas are suitable for drug/gene delivery systems. CaP is not only a family of natural minerals, but also includes biominerals in humans, which are the main inorganic component of hard tissue (bone and teeth).

calcium phosphate bone regeneration hydroxyapatite

1. Introduction

Bone as a mineralized tissue has an irreplaceable role in supporting and protecting the body of human beings. Defects of bone caused by trauma, aging, inflammation, infection, and tumors seriously affect people's health and normal life [1]. A critical bone defect, which refers to a defect greater than 2 cm in length or greater than 50 percent of the circumference of the defect, cannot completely regenerate by self-growth and requires the use of biomaterials to guide its repair [2]. Millions of bone grafting operations are performed every year in the world for the treatment of critical bone defects, resulting in a huge economic burden to the families of patients and the whole of society [3]. Therefore, the development of high-performance biomaterials for bone repair is of great scientific significance and clinical application value.

At present, the most commonly used methods for treating bone defects include bone transplantation, membrane-guided regeneration, Ilizarov technology, and bone tissue engineering [4][5][6][7][8]. However, these methods are insufficient in meeting the requirements for clinical treatment of bone defects. Autologous bone grafting, the clinical gold standard for treating bone defects, is usually limited by the quantity of available tissue and the risk of secondary surgery and infection, blood loss and operation time [3]. Allografts and xenografts are important alternative options of autografts for orthopaedic applications in terms of osteogenic, osteoinductive, and osteoconductive properties, and there are two main categories, including cellular bone matrices (CBM) and peptide enhanced xeno-hybrid bone grafts developed as commercial products for clinical use. Cellular bone matrices have four necessary and beneficial elements for bone growth and healing: osteoinduction, osteoconduction, osteogenic activity and angiogenic activity [9]. However, CBM have several challenges with respect to intrinsic biological characteristics, such as viable cell sources, donor age at the time of graft harvest, and cell survival after

transplantation, which may cause differences in expected outcomes due to different batches of the same product. Xeno-hybrid bone matrices are appealing, innovative, osteoconductive and osteoinductive bone substitutes to autografts. The compatibility of xeno-hybrid bone matrices in favoring cellular attachment, osseointegration, bone remodeling and satisfactory mechanical performance has been attested by numerous clinical studies [9]. However, further independent clinical studies are required to confirm these promising results and to promote their application. It is worth mentioning that although there are potential risks of infection when using allografts, the allografts are procured, processed, and distributed only by Tissue Banks, which operate under strict guidelines and sterile conditions in Class A environments, which helps to minimize the abovementioned issues [10]. The technology of tissue engineering represents an emerging strategy for repair of bone defect [11]. However, it is still a big challenge to construct functional bone tissue in vitro, due to the proliferation and differentiation of seeding cells, bioactivity of growth factors and physicochemical and biological properties of scaffolds [12][13]. Recently, in-situ tissue engineering has been proposed for autologous tissue regeneration, which is based on tissue-specific scaffolds, by regulating the microenvironment and in vivo recruiting stem and progenitor cells [14][15]. Therefore, the preparation of functional scaffolds with ideal biocompatibility, bioactivity and biodegradability is the critical factor that limits the rapid development of in-situ tissue engineering for bone defect repair.

Synthetic bone scaffolds have been increasingly applied in the field of bone repair. Compared with the autologous bone grafts, although there are some poor properties of osteinductive and osteogenic activities, synthetic bone scaffolds with abundant sources provide a wide variety of choices in structure, chemical/mechanical properties and biological functions to meet specific requirements in bone regeneration [15]. Considering the limitations above-mentioned, artificial bone substitutes have attracted tremendous attention and have been rapidly developed. Among the varied biomaterials used in bone repair, calcium phosphate (CaP)-based biomaterials occupy a particular position as a result of their resemblance to the chemical components and structures of natural bone tissue. CaP is not a specific material but represents a big family of materials that are compounds formed by the reaction of calcium ions and phosphate ions. The apatite reported by Werner in 1788 was the earliest discovered member of CaP [16]. By 1926, Jong revealed the relationship between apatite and the inorganic minerals of bone [17]. Therefore, CaP-based biomaterials (CaPs) were proposed for use as therapeutic agents for bone regeneration [18]. In 1971, Monroe was the first to report the use of CaP ceramics, which are white translucent polycrystalline ceramics that contain hydroxyapatite (HAP) [19]. Since that time, CaP ceramics have been developed greatly for the application of bone repair [20][21]. A CaP bone cement (CPC) was created by the hydrolysis of TCP and was used for the first time in the early 1920s as a treatment for bone repair [18]. Since then, CPC has been prepared with many different chemical formulas and application as described by Cama [22]. Mineralized collagen with orderly, organized collagen and HAP is the basic unit of natural bone tissues and is involved in building complex biomineralized systems with hierarchical structures [23]. Hence, researchers in the field of biomaterials are interested in biomimicking mineralized collagen for developing bone substitute materials by utilizing the biomimetics strategy [24][25]. In 2003, biomimetic mineralized collagen nanofibrils were designed and prepared by Cui et al., which are similar in both composition and structure to natural bone [26]. As of now, various methods have been developed for preparing mineralized collagen, and these products have excellent bioabsorbability and osteoconductive properties, leading to their potential in promoting bone regeneration [27].

2. Chemical Properties of Calcium Phosphate

2.1. Species of Calcium Phosphate

CaP is not only a family of natural minerals, but also includes biominerals in humans, which are the main inorganic component of hard tissue (bone and teeth) [28]. In the past decades, a wide variety of CaP-based biomaterials have been used in bone regeneration studies and clinical applications. As is well known, CaP biomaterials promote cell adhesion and growth, which then induces the formation of new bone minerals via their interaction with extracellular matrix proteins [29]. In the application of bone regeneration, the bioactivity of CaPs is critical and usually varied depending on their species [30]. The bioactive features of CaPs is related with to the degradation properties of CaP [31]. Due to different Ca/P ratios, the different species of CaP biomaterials result in variations in in vitro and in vivo calcium and phosphate ion release. Consequently, the pH of the local microenvironment of bone is affected by the released calcium and phosphate ions, which then influence the viability of osteoblasts and osteoclasts [32][33]. Moreover, the increased concentration of calcium and phosphate ions can promote the formation of bone minerals, as well as affect the expression of osteogenic differentiation-related genes (e.g., Col-I, ALP, OPN, OCN, RunX2 and BMPs) of bone cells [28][34].

Calcium exists wildly in natural bone minerals and is a key ion in forming the bone matrix [35]. Ca^{2+} is also capable of forming and maturing bone tissue by calcification. Ca^{2+} influence the bone cell maturation and bone tissue regeneration by regulating related cellular signaling pathways [36][37]. For instance, Ca^{2+} activating ERK1/2 causes pathway activation of osteoblastic-related bone formation [38]. In addition, an increased life span of osteoblasts has been observed with the activation of the PI3K/Akt signal axis by Ca^{2+} [39]. Meanwhile, phosphate ions degraded from CaP are present in large quantities in the human body, and can be utilized in various physiological systems, including construction of proteins, nucleic acids, and adenosine triphosphate [40]. Approximately 80% of phosphate ions in the body occur with calcium ions in the form of CaP in bone, which affects the development of bone tissue [41]. It is well known that the differentiation and growth of osteoblasts are regulated by phosphate ions by IGF-1, ERK1/2, BMP, and other pathways [42][43].

The osteoconductivity and osteoinductivity of CaP materials are closely related to their physical and chemical characteristics, such as, solubility, stability, and mechanical strength [28], are determined by the species of the CaP materials, Therefore, the selection of one kind of CaP biomaterial from their family according its characteristics is important in preparing biomaterials for the use of bone regeneration. A large number of CaP biomaterials have been used in bone regeneration and other biological research, including tricalcium phosphate (TCP), hydroxyapatite (HAP), amorphous calcium phosphate (ACP), octacalcium phosphate (OCP), dicalcium phosphate anhydrous (DCPA), dibasic calcium phosphate dihydrate (DCPD), and tetracalcium phosphate (TTCP) [44]. Basic information concerning these CaP biomaterials are displayed in the **Table 1** [45]. The crystal phases of calcium phosphate were discovered before the 20th century (amorphous phases were discovered in the 1950s) and accurately characterized during the 20th century (OCP was defined in 1957 [46]). After that, no new crystal phases (non-doped, non-substituted, only Ca, P, O, H) have been reported. Dicalcium phosphate monohydrate ($CaHPO_4 \cdot H_2O$, DCPM) was obtained by controlling the transformation of a special amorphous calcium phosphate

($CaHPO_4 \cdot xH_2O$, ACHP) in a water-deficient environment (water/methanol mixed solvent, or in humid air) by Lu et al. in 2020 [47]. The discovery of DCPM brought a new member to the calcium phosphate family. However, applications of DCPM in bone repair, and even in biomedicine, has not yet been carried out.

Table 1. The basic information of usual calcium phosphate materials.

Name	Formula	Ca/P	Solubility at 25 °C (g/L)
HAP	$Ca_{10}(PO_4)_6(OH)_2$	1.67	~0.0003
α-TCP	α-$Ca_3(PO_4)_2$	1.5	~0.0025
β-TCP	β-$Ca_3(PO_4)_2$	1.5	~0.0005
ACP	$Ca_xH_y(PO_4)_z \cdot nH_2O$, n = 3–4.5, 15–20% H_2O	1.2–2.0	/
OCP	$Ca_8(HPO_4)_2(PO_4)_4 \cdot 5H_2O$	1.33	~0.0081
DCPA	$CaHPO_4$	1.0	~0.048
DCPD	$CaHPO_4 \cdot 2H_2O$	1.0	~0.088
TTCP	$Ca_4(PO_4)_2O$	2.0	~0.0007

HAP: hydroxyapatite, α-TCP: α-tricalcium phosphate, β-TCP: β-tricalcium phosphate, ACP: amorphous phosphate calcium. The solubility date at 25 °C of ACP cannot be measured precisely. However, the comparative solubility in acidic buffer is ACP >> α-TCP >> β-TCP >> HAP.

2.2. Hydroxyapatite

HAP is the most abundant crystal phase of biominerals in human bones, and accounts for ~70% of the dry weight of bone tissue [48]. Among all CaP materials, HAP is only inferior to fluorapatite (FAP) in terms of stability and insolubility. The chemical formula of HAP is $Ca_5(PO_4)_3(OH)$. However, HAP is usually referred to as $Ca_{10}(PO_4)_6(OH)_2$ to indicate the hexagonal unit cell of HAP [49]. There are two approaches for the formation of HAP, including the natural formation process and artificial synthesis. The hexagonal crystal structure of naturally formed HAP usually has defects, which can be filled by vacancies or other ions [45]. However, structural defects in synthesized HAP may depend on synthesis procedures or conditions. Monoclinic and hexagonal crystals are the two crystal phases of synthesized HAP; the monoclinic crystal phase can change to the hexagonal crystal phase when the temperature is higher than 250 °C. The hexagonal crystal structure of HAP is the predominant phase found in the biological environment as a result of its high stability [50]. HAP is considered the most stable phase of CaP and the final mineral phase in bone, whereas the other CaP phases (e.g., ACP and OCP) in bone are precursors, or sub-precursors, that transform into HAP under in vivo or aqueous environments with high pH [28][45]. The phase transformation of several CaPs usually occurs under different conditions [51]. The equilibrium of phase transformation between various CaP phases is related to temperature and the ratio of CaO and P_2O_5. The physicochemical and biological properties of HAP significantly change with the Ca/P ratios and the replacement of ions or vacancies in HAP crystal structure. For example, the mechanical properties of HAP are enhanced with increasing Ca/P ratio, and reach a maximum when the stoichiometric ratio is 1.67. Interestingly, once the Ca/P ratio exceeds 1.67, the strength of HAP decrease [52]. The defects of HAP crystal structure can be replaced with F^-, Cl^-,

CO_3^{2-}, Mg^{2+}, Sr^{2+}, and other ions. As a result of Mg^{2+} replacement, the size and density of HAP nanostructure particles, which contribute to the specific mechanical properties of bones, may be altered [53]. Furthermore, the crystallization of HAP is inhibited by Mg^{2+}, and results in the formation of fewer large crystals and a greater number of apatite nuclei. The significance of this is that nanocrystalline bone apatites are necessary for the proper bone formation–resorption turnover that occurs via bone cells [54]. The replacement of F^- ions can increase stability, while Mg^{2+} enhances biological activity, compared with pure HAP [54]. Several studies have shown that Mg^{2+} has the ability to influence bone metabolism, regulate the activity of osteoblasts and osteoclasts, and to stimulate new bone growth [55][56]. Therefore, artificial Mg substituted HAP in different forms has been carried out, and has displayed advanced bioactivity [57][58]. Furthermore, Mg-based CaP materials can result in neuralization and the synthesis and release of CGRP to promote osteogenesis [59][60][61].

HAP has been used clinically in bone regeneration since the 1980s, as implants and coatings of other implants [49][62]. HAP has good biocompatibility, bioactivity, and osteoconductive properties. In the presence of Ca^{2+} and PO_4^{3-} ions, the surface of HAP can act as a nucleation site for the initiation of biomineralization [63]. Therefore, HAP is used widely for dental surgery, long bone defects, bone nonunion, vertebral fusion operation and maxillofacial repair [64]. The biocompatibility, osseointegration, and bioactivity of metal implants are improved by coating their surfaces with HAP, which enhances the bone contact area and cell adhesion properties of the implants [65]. Moreover, HAP can promote the biomineralization of macromolecule-based scaffolds. HAP nanoparticles penetrate into the demineralized collagen scaffold and serve as mineralization seeds that promote the occurrence of remineralization of the collagen matrix [66].

HAP has high chemical stability, but has weakness in mechanical properties. Stress along the *Z*-axis direction of HAP crystals creates brittleness [67]. It is worth mentioning that wear resistance, the friction coefficient and hardness of dense HAP are similar to natural those of mineralized tissues [68]. The fatigue resistance of dense HAP is superior to porous HAP [52]. As a result, HAP is not used as a load-bearing implant due to its poor mechanical properties, but is usually implanted with granules and porous scaffolding [69]. It is still a huge challenge to improve the mechanical properties of HAP. Metal oxides including zirconia, alumina and titania are common reinforcing agents [70]. However, the biocompatibility and biodegradation properties of HAP-based biomaterials are compromised by the addition of these reinforcing agents, which are bioinert or none-biodegradable [71]. Constructing composites with a polymer is an effective way to improve the mechanical properties of HAP. Natural polymers such as chitosan, hyaluronic acid, silk fibroin and gelatin are common components for fabricating hybrid scaffolds [72]. For instance, the hydroxy propyl methyl cellulose of chitosan has been crosslinked to fabricate chitosan/HAP sponge-like scaffolds which have excellent compressive strength, elasticity and degradability [73]. Considering the importance of mechanical properties for bone repair, particularly in load-bearing bones, further research is necessary to improve the mechanical properties of HAP [74][75].

The biological performance of artificial bone implants is extremely important. HAP is considered to have good biocompatibility and bioactivity in osteoconductivity, but has poor osteoinductivity [63]. Therefore, it is usual to combine HAP with other materials to improve its osteoinductivity. Beta-tricalcium phosphate (β-TCP), another common kind of calcium phosphate used in bone regeneration, has better osteoinductivity than HAP. This biphasic

calcium phosphate (BCP) material has been synthesized by combining HAP and β-TCP to take advantage of the properties of both and obtain better bioactivity for bone regeneration [76]. The BCP material possesses superior bioactivity, biodegradability, osteoinductivity, and mechanical properties than HAP or β-TCP alone, and has greater ability to stimulate osteogenic differentiation of BMSCs [77]. Hence, bone grafts and dental materials are commonly prepared with BCP material [78]. Zhu et al. constructed BCP bioceramics with micro-whiskers and a nanoparticle hybrid structure which may be applied in research on load-bearing bone tissue regeneration to provide mechanical support [79].

HAP is an advanced material for preparing bone grafts owing to its similarity to natural minerals and excellent biocompatibility and osteoconductivity. However, the preparation performance regulation of hydroxyapatite materials for bone regeneration remains a long-term and challenging endeavor. First of all, basic research on hydroxyapatite in bone tissue remains largely unexplored. For example, there is a lack of understanding regarding the factors involved in the formation of hydroxyapatite, including precursors and crystal growth regulatory factors, in the process of bone tissue biomineralization. In addition, the interaction between hydroxyapatite and collagen molecules, particularly how the regular hydroxyapatite-collagen complex is formed, needs to be further studied. On the other hand, there are still many technical and scientific problems associated with the synthesis of hydroxyapatite and the preparation of scaffolds. For instance, the mechanism of hydroxyapatite crystal growth needs to be further explored to control the scale, and a controllable fabrication strategy for ordered biomimetic structures must be developed to fabricate scaffolds with good mechanical properties and controllable porous structures.

2.3. Tricalcium Phosphate

TCP, as one of the most studied calcium phosphate materials, contains two crystalline phases (α-TCP and β-TCP). There are several phases of CaP materials that have similar compositions to TCP, and the term TCP here is used for the phase with a chemical composition of $Ca_3(PO_4)_2$ and a Ca/P ratio of 1.5. Pure crystalline α-TCP cannot be precipitated in aqueous solutions since it is very poorly soluble, unlike β-TCP [80][81]. There are three approaches for synthesizing β-TCP, including solid-state reaction, thermal conversion, and precipitation. Usually, crystalline β-TCP is prepared at a high temperature of ~800 °C such as by thermal decomposition of calcium deficient hydroxyapatite (CDHA), and the other is the solid-state interaction between acidic CaP (i.e., DCPA) and alkaline (i.e., CaO) [45]. As well, it has been shown that β-TCP precipitates in organic solutions, such as ethylene glycol, methanol, tetrahydrofurane, and ethyl propionate [82][83][84]. Tang et al. synthesized β-TCP at a relatively low temperature at about 150 °C in organic solvents (e.g., ethylene glycol) [85]. Moreover, β-TCP transforms into the α-TCP at higher temperatures (above 1125 °C), so α-TCP may be considered as the high-temperature phase of β-TCP [86].

TCP has excellent stability and can be stored in a dry environment at room temperature for a long period of time. β-TCP is more stable than α-TCP according to a density functional study [87]. α-TCP has superior reactivity and specific energy in an aqueous solution than β-TCP, and is capable of being hydrolyzed to CDHA [45]. In clinical applications, β-TCP has higher osteoconductivity and osteoinductivity than HAP and is primarily used in bone

cements and bioceramics [88][89], while α-TCP is normally used in cements, since it is subject to a phase conversion to HAP upon water contact [90][91]. It should be noted that the rate of resorption of pure α-TCP is higher than new bone formation, which leads an imbalance between the process of bone formation and implant degradation [86]. Therefore, α-TCP is usually used as a component in CaP cements with other CaP materials [45]. In contrast, β-TCP has a relatively lower resorption rate than α-TCP, and has good prospects for application in bone regeneration [68]. The nano-porous structure of β-TCP allows for excellent biomineralization and cell adhesion; these properties can stimulate osteoblast and BMSCs proliferation [92]. In addition, compared to HAP, β-TCP has better biodegradability and resorption rate, which can increase the biocompatibility of the implants for bone regeneration [28].

2.4. Amorphous Calcium Phosphates

Amorphous calcium phosphates (ACPs) are a special phase of CaP with various chemical compositions. ACPs have long-range order but short-range disorder regarding their crystal properties [45]. Initially, ACPs were discovered during the preparation of HAP in vitro; therefore, ACPs were considered as precursors of HAP [93]. A study in 1972 found that ACPs were the first phase to form and were transformed into octacalcium phosphate (OCP) during the synthesis of HAP in vitro; the final phase conversion occurred from OCP to HAP [94]. Glimcher et al. believed that ACPs may be the precursor stage of bone formation due to the presence of uniform intra-collagen mineralized particles found in collagen mineralization in vitro through ACPs [95]. ACPs are classified into two groups based on their preparation temperature, namely low-temperature ACPs and high-temperature ACPs [96]. Low-temperature ACPs usually occur as precursors during the precipitation process of other CaP compounds. Since the surface energy of ACPs is lower than that of OCPs and HAPs, ACPs are thought to form at the onset of precipitation [96].

The chemical composition of ACPs depends on pH value and the concentration of calcium and phosphate ions in aqueous solution. The recrystallization of ACPs occurs with increased concentration of Ca^{2+} and PO_4^{3-}. In addition, ACPs may recrystallize slowly or transform into CaP materials with a higher crystalline degree, such as CDHA, in a reaction system with a continuous and mild stirring rate, especially when at higher reaction temperatures [45]. In studies concerning the influence of pH, researchers have discovered that the Ca/P ratio of ACPs increases from 1.18 to 1.53 as the pH value of the system changes from 6.6 to 11.7 [97][98]. Up to now, the atomic distribution in ACPs is still not entirely clear, which is an important research topic in studies of biominerals [96]. Freshly precipitated ACPs usually display spherical-like structures with diameters between 20 and 200 nm as seen by electron microscopy [45]. Some researchers believe that the basic structural unit of ACPs is thought to be a spherical cluster structure with a diameter of 0.95 nm. The ACP chemical formula is $Ca_9(PO_4)_6$ [96][99].

ACPs constitute the initial phase of HAP and are essential components in the process of bone regeneration and bone mineralization due to their particular physical properties and structure [100]. ACPs possess superior biological properties, such as osteoconductivity and biodegradability, leading to a variety of applications including CaP bone cements, biological tissue engineering scaffolds, bone repair biomaterials, and dental implants [101][102]. In addition, the nano-sized clusters in the ACPs have characteristics of large specific surface areas and pH-responsive

degradation, which makes them ideal drug delivery carriers for studies relating to drug loading and controlled release [103].

The preparation of ACPs is regulated by small molecules such as ATP, which can effectively inhibit the phase transformation of ACP [104]. As a result of an ATP-assisted preparation strategy, the product is an ACP composite nanoparticle containing ATP and ADP molecules. Furthermore, the compound has good biocompatibility and osteogenic activity, and can up-regulate the expression of osteogenic genes in BMSCs. An injectable hydrogel prepared by combining ACP compounds with fibrinogen displayed excellent promoting effects in in vivo bone regeneration [105].

An ALP-catalyzed hydrolysis reaction was used to generate EACP nanominerals in an alkalescent aqueous solution similar to mitochondrial surroundings [104]. The mechanism of EACP promoting bone healing was demonstrated in that the ADP/AMP biomolecules and Ca^{2+} ions released from EACP can increase the activation level of AMPK and promote autophagy and osteogenic differentiation in hBMSCs. Additionally, a number of theories suggest that ACP plays an important role in the biomineralization process as a precursor of apatite formation [106][107][108]. There is also evidence that the biomineralization route involves the formation of the mineral phase within matrix vesicles that are associated with small crystals of calcium phosphate mineral [109], which are usually an amorphous phase involved in the formation of these vesicles [110]. Meanwhile, disordered collagen fibrils may contribute to the stabilization of ACP, resulting in both amorphous and crystalline bone mineral [111]. At present, the collagen fibrils as the temptation for bone mineral growth are attracting a great deal of interest in biomineralization research [112]. However, the specific mechanisms by which ACP promotes bone regeneration are highly controversial and require further investigation.

2.5. Application of Other CaP Phases

Tetracalcium phosphate (TTCP) is the most basic CaP phase, which is in a metastable state and gradually hydrolyzed to HAP and $Ca(OH)_2$ in a humid environment or aqueous solution [86]. TTCP often occurs as a by-product of HAP plasma coating, which is a mixture of α-TCP, TTCP and CaO from the high temperature phase [113]. The chemical synthesis process of TTCP can only be carried out in dry air or vacuum environment. It is synthesized by a solid-phase reaction at over 1300 °C. In the presence of water vapor, TTCP is decomposed into HAP [86]. There are three types of TTCP bone cement: single component, multi component, and polymer. In biological applications, TTCP is usually used as a component for preparing self-curing bone cements, biological composites or root canal sealants [114][115][116]. By combining TTCP with DCPA or DCPD (Ca/P = 2.0), bone cements with the stoichiometry composition of HAP can be produced [113]. A set cement with the best mechanical properties was obtained using an equimolar mixture TTCP and DCPA with a particle size (diameter) ratio of approximately 10: 1 [113]. However, another study found that cement with a diameter ratio (TTCP:DCPA) of 20:1 had the highest compressive strength [117].

TTCP bone cements show advanced biological performance. Qin et al. fabricated three-dimensional porous TTCP scaffolds via selective laser sintering technology (SLS) [118]. After immersion in SBF for one day, nanoapatite was

produced on the surface of TTCP scaffolds. The scaffold surface was completely covered with apatite after three days, indicating good biological activity. Furthermore, the biocompatibility of TTCP scaffolds was evaluated by cell culture, which confirmed their high biocompatibility. An evaluation of the histological effects of the TTCP cement applied to the pulp of rat upper incisors demonstrated great advantages over calcium hydroxide ($Ca(OH)_2$) cement [119]. Tsai et al. investigated a single component TTCP cement (containing $(NH_4)_2HPO_4$ as the liquid) in rabbit femurs for 24 weeks in vivo [120]. Following implantation, histological examination of the recovered implants demonstrated good cement-bone host bonding, with cement resorption, new blood vessels, osteoocytes, and osteoblast-like cells identified. At the end of 24 weeks, only a small amount of residual bone cement was found, and the cortical bone was almost completely remodeled.

Octacalcium phosphate (OCP), as a precursor to HAP crystal formation, along with ACP and DCPD, play an important role in bone formation and biomineralization [121][122]. A very similar structure exists between OCP and HAP but OCP is more unstable than HAP and is hydrolyzed to HAP [121]. The mechanism of hydrolysis of OCP is still not completely clear. Two hypotheses, dissolution-reprecipitation mechanism [123] and ion diffusion-crystallization conversion [124], are proposed to explain the hydrolysis of OCP. Eliminating the HPO_4^{2-} from the OCP water layer has been confirmed as a necessary step for phase transformation, and is believed to be the rate-determining factor of the conversion [125]. The transformation of OCP was observed under in vitro and in vivo conditions. Upon being placed in water with a starting pH of 7.2, the mixture of OCP and HAP was examined after 1 h, and at 12 h the structural transition was completed [126]. The OCP was completely hydrolyzed to CDHA within 6 h in deionized water [127]. pH also affects the transformation rate. For example, Suzuki et al. found that OCP hydrolysis was postponed at pH 11 compared with pH 7.4 [128]. Interestingly, the hydrolysis of OCP into HAP is very slow in in vivo conditions. Implanted OCP in a rat calvarial defect hydrolyzed very slowly after 21 days [129]. In SBF at a temperature of 36.5 °C and a pH of 7.25, the hydrolysis of OCP to HAP took place to a small extent over the course of 28 days [130]. In addition, OCP may be non-stoichiometric, and its structure may be calcium-deficient (Ca/P = 1.26) or calcium-excessive (Ca/P = 1.48) [131].

OCP has good osteoinductivity and is widely used in bone repair research, including the coating of metal grafts, the use of CaP bone cement, and the construction of composite bone repair scaffolds [132][133][134]. OCP/Col composite scaffolds constructed from OCP particles and collagen have a higher osteoconductivity than OCP alone, and the osteoconductivity is positively correlated with the dose of OCP [135][136]. By providing a nuclear structure, OCP acts as an initial deposition site for bone, and its conversion to HAP plays a significant role in bone formation, which may explain its beneficial effects on bone growth [129][137][138]. By implanting the precursors of HAP, such as OCP, ACP, and DCPA, along with HAP particles in the subperiosteal region of the mouse calvaria, bone tissue appeared with OCP in approximately one week. At about 3 weeks, bone tissue appeared in ACP and DCPA, and was later found in HAP particles (5 weeks), which further indicates that OCP has good ability to promote bone formation [137]. Moreover, osteoblasts that can initiate bone formation were found on the surface of OCP particles in the OCP group. Ultrastructural SEM examination confirmed that osteoblasts were directly attached to OCP to form bone matrix, and that filaments were formed around OCP. There were many similarities in the composition of the granulated and granular complexes around with OCP to the bone nodules formed during intramembranous osteogenesis [139].

The application of OCP in bone graft biomaterials and bone regeneration has a promising future due to its good osteoconductivity and osteoinductivity. It is of paramount importance to explore and understand the biological mechanism of good osteoinductivity of OCP, as well as the influence of Ca/P stoichiometry and microstructure on its intrinsic biological activity [140][141].

Dicalcium phosphate anhydrous (DCPA) and dibasic calcium phosphate dihydrate (DCPD) are acidic CaP materials. DCPA is an anhydrous crystalline form of DCPD. Since there are no hydrated molecules, the solubility of DCPA is lower than DCPD. Both can be precipitated from an aqueous solution at 100 °C. The difference between DCPA and DCPD is that DCPA does not form in vivo, as confirmed by no DCPA being formed in normal or pathological calcification nodus [45]. DCPA is often mixed with other calcium phosphate materials to prepare bone cement, and it is also used to provide calcium and phosphorus in foods and toothpastes [86][142][143].

DCPD is the dihydrate crystalline state of DCPA [86]. By adjusting pH in the range of 3–4 at room temperature, DCPD can be produced by the neutralizing reaction of Ca $(OH)_2$ and H_3PO_4. Metathesis reactions using calcium-containing phosphates in a slightly acidic environment can also synthesize DCPD [142]. Due to its biocompatibility, biodegradability, and osteoconductivity, DCPD is often used as a component of bone cements and toothpaste to promote bone and tooth mineralization [86][144]. It is worth noting that DCPD was found to be converted to calcium deficient hydroxyapatite (CDHA) in vivo [145]. This conversion process released many acidic substances when excessive DCPD was implanted in vivo, causing a severe inflammatory response [146].

Dicalcium phosphate monohydrate (DCPM) as a crystal phase has a Ca/P ratio of 1:1, and is a new metastable CaP with structural water without DCPD and DCPA [47]. DCPM is formed using ethanol and water mixtures that maintain a low level of hydration and inhibit the formation of DCPD. X-ray powder diffraction (XRPD), was used to determine the crystal structure of DCPM, conformed with a = 8.0063(4) Å, b = 6.7954(5) Å, c = 7.7904(5) Å, α = γ = 90°, β = 91.548(4) Å. In addition, after immersion in water for only one hour, this new crystalline form of calcium phosphate monohydrate transforms into hydroxyapatite, which is the stable form of calcium phosphate found in human bones. This represents a two-fold increase in speed as compared to the dicalcium phosphate dihydrate (DCPD) phase, which is usually used in bone cements today. Furthermore, DCPM can be stabilized by organic molecules such as citrate salts, which are abundant in the human body, and can adsorb a large quantity of small molecules. Consequently, DCPM is an interesting option to encapsulate and release drugs to enhance bone healing and remineralization. However, there is still much work to be done on the features and applications of DCPM in bone repair and biomedicine.

And some commercial products of CaPs were displayed in the **Table 2**.

Table 2. The commercial products of CaPs.

CaP Materials	Product Name	Producer
HAP	Actifuse	ApaTech, UK

CaP Materials	Product Name	Producer
β-TCP	ApaPore	ApaTech, UK
	Bonetite	Pentax, Japan
	Bone Source	Stryker orthopaedics, NJ, USA
	Bioroc	Depuy-Bioland, France
	Cerapatite	Ceraver, France
	Ostim	Heraeus Kulzer, Germany
	Synatite	SBM, France
	adbone® TCP	Medbone, Portugal
	Biosorb	SBM S.A., France
	Cerasorb	Curasan, Germany
	Conduit	DePuy Spine, USA
	Osferion	Olympus Terumo Biomaterials, Japan
	SynthoGraft	Synthograft, MA, USA
	Vitoss	Orthovita, PA, USA
HAP + β-TCP	BCP	Medtronic, MN, USA
	Graftys BCP	Graftys, France
	MBCP	Biomatlante, France
	OsSatura BCP	Integra Orthobiologics, CA, USA
HAP + α-TCP	Skelite	Millennium Biologix, ON, Canada
CDHA	Osteogen	Impladent, NY, USA
ACP + DCPD	Biobon (α-BSM)	Etex, MA, USA
DCPD + β-TCP	ChronOS	DePuy Synthes, PA, USA
TTCP + DCPA + saline	BoneSource HAC	Stryker Instruments, MI, USA
α-TCP + TTCP + $CaHPO_4$ + HAP	BIOPEX	Taisho Pharmaceutical, Japan
HAP + collagen	Healos Fx	DePuy Spine, USA

CaP Materials	Product Name	Producer
HAP + PLLA	SuperFIXSORB30	Takiron, Japan
HAP + Polyethylene	HAPEX	Gyrus, TN, USA
β-TCP + PMMA	Cal-CEMEX	Tecres Spa, Italy

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