Journal of Dental Sciences (2012) 7, 316—323

Available online at www.sciencedirect.com

SciVerse ScienceDirect

journal homepage: www.e-jds.com

REVIEW ARTICLE

First detection, characterization, and application of amorphous calcium phosphate in dentistry

Jie Zhao [a], Yu Liu [a], Wei-bin Sun [a*], Xuebin Yang [b]

[a] *Stomatological Hospital, Nanjing University Medical School, 30 Zhongyang Road, Nanjing, China*
[b] *Biomaterials and Tissue Engineering Group, Leeds Dental Institute, University of Leeds, United Kingdom*

Final revision received 22 February 2012; accepted 10 March 2012
Available online 20 November 2012

KEYWORDS
amorphous calcium phosphate;
apatite;
biomineralization

Abstract *Background/purpose:* This literature review provides an overview of the first detection, structure, chemical composition, morphology characterization, phase transformation, and clinical application of amorphous calcium phosphate (ACP) to dentistry.
Materials and methods: ACP is the essential mineral phase formed in mineralized tissue and the first product to be used as artificial hydroxyapatite. ACP is unique among the calcium phosphates in that it lacks the long-range, periodic atomic scale order of crystalline calcium phosphates. Its X-ray diffraction patterns are broad and diffuse with a maxima at 25° 2θ, and no other different features compared with well-crystallized hydroxyapatite. Under electron microscopy, its morphologic form appears as small spheroidal particles of a few tenths of a nanometer in scale. In aqueous media ACP is easily transformed into crystalline phases such as octacalcium phosphate and apatite, due to the growth of the microcrystal.
Results: ACP has better osteoconductivity and biodegradability than tricalcium phosphate and hydroxyapatite *in vivo*. Moreover, it can increase alkaline phosphatase activity of mesoblasts, enhance cell proliferation activity, and promote cell adhesion. The unique role of ACP in the formation of mineralized tissues makes it a potentially useful candidate for use in materials for tissue repair and regeneration. The same properties may make ACP suitable as a potential remineralizing agent for dental applications.
Conclusion: Recently developed bioactive ACP-filled composites are potentially effective anti-demineralizing/remineralizing agents for the preservation and repair of teeth.

Introduction

Over the last decade, calcium phosphates have been of special interest to dentistry, the orthopedic industry, and

* Corresponding author. Stomatological Hospital, Nanjing University Medical School, 30 Zhongyang Road, Nanjing 210008, China.
E-mail address: wbsun@nju.edu.cn (W.-b. Sun).

1991-7902/$36
http://dx.doi.org/10.1016/j.jds.2012.09.001

medicine because of their excellent performance. This appears logical due to their similarity to the mineral phases of most hard tissues of human bones and teeth. Calcium phosphates of biological significance are summarized in Table 1.[1–5] Amorphous calcium phosphate (ACP) is the initial solid phase that precipitates from a highly supersaturated calcium phosphate solution, converting readily to stable crystalline phases such as octacalcium phosphate (OCP) or apatite products.

ACP is unique among the calcium phosphates. Its morphologic form, structural models, and X-ray diffraction patterns are typical of noncrystalline substances with short-range periodic regularity. ACP has been shown to have better *in vivo* osteoconductivity than hydroxyapatite (HAP) and better biodegradability than tricalcium phosphate (TCP). In addition, it has no cytotoxicity and good bioactivity. These excellent biocharacteristics explain why ACP has potential for wide application in oral biology, dentistry, orthopedic biomechanics, materials, and medicine. This review provides an account of the first detection, structure, composition, and morphologic characterization of ACP, as well as its phase transformation and biomedical applications, especially in dentistry.

First detection of ACP

Generally, it is believed that ACP was first described by Aaron S. Posner[6] in the mid 1960s. He obtained an amorphous precipitate by accident when mixing high concentrations (−30 mM) of calcium chloride and sodium acid phosphate (−20 mM) in buffer. X-ray diffraction revealed the pattern of this rapidly precipitated phase as showing only two very broad and diffuse peaks, with maxima at 25° 2θ with no features, and it was clearly not apatite. This diffraction pattern is typical for substances that lack long-range periodic regularity. Immediately after being mixed, the spontaneously formed precipitate was a noncrystalline, or amorphous, calcium phosphate with calcium-to-phosphorus molar ratio (Ca/P) of 1.50, while after several hours, upon aging, it could convert to poorly crystalline apatite. Afterward, this solid in contact with the precipitating solution converts slowly to crystalline apatite (Ca/P = 1.67) through an autocatalytic mechanism.

Actually, another report appeared in *Nature* in 1965, in which Eanes[7] identified ACP as a bone component. It seemed plausible to Posner that such an amorphous material called ACP was present in bone, and along with the apatite, might account for the broad diffraction pattern of bone mineral and for its variable composition. Posner and his staff[8,9] also described an age-dependent change in the ACP content of bone, with the proportion of ACP decreasing with age. In 1975, ACP was identified as the mineral in the hepatopancreas of the blue crab.[10] X-ray diffraction revealed that the mineralized cytoplasmic structure isolated from the hepatopancreas of the blue crab is very similar in short-range atomic structure to synthetic amorphous calcium phosphate.

Structural studies

After detection of amorphous calcium phosphate, further experiments focused on its structure. It was proposed that synthetic amorphous calcium phosphate particles, which appear as 300−1000 Å spheres in the electron microscope (Fig. 1),[6] the exact size depending on preparation conditions, consist of a random assembly of ion clusters 9.5 Å in diameter, dimensions consistent with the chemical composition $Ca_9(PO_4)_6$. The 15%−20% of water found in synthetic amorphous calcium phosphate was shown to be mostly in the interstices between, and not within, the individual $Ca_9(PO_4)_6$ clusters (Fig. 2).[6,11,12,13] Aggregated particles readily dissolve and crystallize to form apatite, a thermodynamically stable phase, because the binding effect of water is not strong. The typical radial distribution

Table 1 Summary of biologically significant calcium phosphates.[1–5]

Compound	Acronym	Formula	Ca/P molar ratio
Monocalcium phosphate, monohydrate	MCPM	Ca(H2PO4)2·H2O	0.5
Monocalcium phosphate, anhydrous	MCPA or MCP	Ca(H2PO4)2	0.5
Dicalcium phosphate dihydrate, mineral brushite	DCPD	CaHPO4·2H2O	1.0
Dicalcium phosphate anhydrous, mineral monetite	DCPA or DCP	$CaHPO_4$	1.0
Octacalcium phosphate	OCP	Ca8(HPO4)2(PO4)4·5H2O	1.33
α-tricalcium phosphate	α-TCP	α-$Ca_3(PO_4)_2$	1.5
β-tricalcium phosphate	β-TCP	β-$Ca_3(PO_4)_2$	1.5
Amorphous calcium phosphate	ACP	CaxHy(PO4)z·nH2O, n = 3−4.5; 15%−20% H2O	1.2−2.2
Calcium-deficient hydroxyapatite	CDHA or Ca-def HA	Ca10-x(HPO4)x(PO4)6-x(OH)2-x ($0 < x < 1$)	1.5−1.67
Hydroxyapatite	HA, Hap, or OHAp	$Ca_{10}(PO_4)_6(OH)_2$	1.67
Fluorapatite	FA or FAp	$Ca_{10}(PO_4)_6F_2$	1.67
Oxyapatite	OA, OAp, or OXA	$Ca_{10}(PO_4)_6O$	1.67
Tetracalcium phosphate, mineral hilgenstockite	TTCP or TetCP	$Ca_4(PO_4)_2O$	2.0

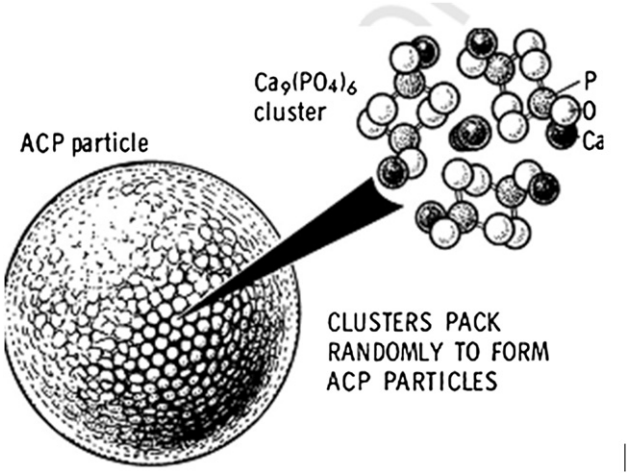

Figure 1 Structural model of ACP.[6] ACP = amorphous calcium phosphate.

functions of noncrystalline ACP cluster structures, calculated from X-ray diffraction patterns, is only two very broad and diffuse peaks showing the rapid drop-off of atomic periodicity. Short-range order exists in these amorphous structures but no long-range order, such as that found in crystalline hydroxyapatite. Infrared analysis showed a similar lack of crystalline order about the PO_4 anions in the ACP structure.[14]

It is now generally agreed that both *in vitro* and *in vivo* precipitation reactions at sufficiently high supersaturation and pH result in the initial formation of amorphous calcium phosphate with a molar calcium/phosphate ratio of about 1.5, in the range of 1.34—1.50, with different pH and 1.50—1.67 when adding different amount of carbonates.[15] However, Wuthier et al[16] reported that ACP, with a Ca/PO_4 molar ratio as low as 1.15, precipitated at more acidic preparative pHs, e.g., 6.9.

More importantly, it has actually been proven that ACP particles are a nanometer particle. Primary practical sizes of ACP are about 20—300 nm. The morphology of ACP solids appears to include a curvilinear aspect when viewed by TEM, rather than the faceted, angular shape of crystalline calcium phosphates.[17] However, this curvilinear appearance has only been clearly established for dried ACP.[12] The initial flocculates collected immediately after precipitation of highly hydrated ACP have a low-contrast disk-shaped appearance. High-contrast spherical particles begin to appear as ACP suspensions age, and become the dominant shape with time.[18]

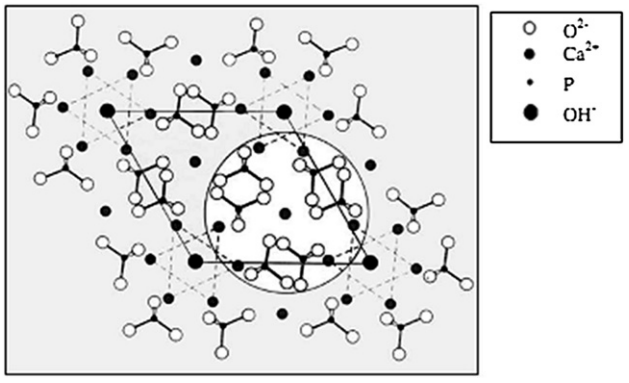

Figure 2 The relationship between ACP and HA. The circle shows the amorphous cluster corresponding to $Ca_9(PO_4)_6$ cluster.[6,11,12] ACP = amorphous calcium phosphate; HA = hydroxyapatite.

Its disordered structure means ACP has high reactivity with body fluid, causing substantial dissolubility and fast apatite reprecipitation. Accordingly, ACP has been proven to have better *in vivo* osteoconductivity than hydroxyapatite and better biodegradability than tricalcium phosphate.[12] The ACP precipitate, with little long-range order, is a highly unstable phase and hydrolyzes almost instantaneously into more stable phases. In the presence of other ions or under *in vivo* conditions, ACP may persist for appreciable periods due to kinetic stabilization.[19] Although the exact mechanism of stabilization of amorphous calcium phosphate is not understood, the presence of Mg^{2+}, F^-, carbonate, pyrophosphate, diphosphonate, polyphosphorylated metabolites, or nucleotides, in sufficient quantity will prevent the transformation of synthetic amorphous calcium phosphate to hydroxyapatite.[1,20]

ACP as precursor in biomineralization and preparation

It has been stated that ACP likely plays a special role as a precursor to bioapatite and as a transient phase in biomineralization. In solutions, ACP is converted readily to stable crystalline phases such as octacalcium phosphate or apatite products. One biomineralization strategy that has received significant attention in recent years is mineralization via transient precursor phases.[21] Transient amorphous mineral phases have been detected in biomineral systems in different phyla of the animal kingdom.[22,23] ACP has been previously reported in the otoliths of blue sharks and also shown to form as a precursor phase of carbonated hydroxyapatite in chiton teeth.[24] The presence of an abundant ACP phase has also been demonstrated in newly formed zebrafish fin bony rays.[25] The disordered phase is a precursor of crystalline carbonated hydroxyapatite. The initially extracted amorphous mineral particles transform into a crystalline mineral phase with time, and the proportion of crystalline mineral increases during bone maturation. The transient ACP phase may conceivably be deposited directly inside the gap regions of collagen fibrils, but it may also be delivered as extrafibrillar particles.[25] This may be consistent with a study showing that collagen mineralization via a transient ACP precursor phase *in vitro* can produce aligned intrafibrillar carbonated apatite crystals.[26]

Several animal studies carried out in different systems *in vivo* also have reported the presence of transient precursor calcium phosphate phases in the deposition of carbonated hydroxyapatite. Beniash, for example, performed a comprehensive analysis of the mineral phases in the early secretory enamel of mouse mandibular incisors using four methods of physical characterization. That study proposed that the outer, younger, early secretory enamel

contains a transient disordered ACP phase, which transforms with time into the final apatite crystalline mineral.[22]

A variety of proteins and ions have been proposed as involved in the biomineralization of ACP to HAP.[27,28] Dentin matrix protein 1 (DMP1) is one of these. A report by He showed that two peptide motifs identified in DMP1 [motif-A (ESQES) and motif-B (QESQSEQDS)] enhanced *in vitro* HAP formation when immobilized on a glass substrate.[29] Similarly, another experiment found that the synthesized artificial protein composed of these peptide motifs of DMP1 facilitated reorganization of the internal structure of amorphous particles into ordered crystalline states, i.e., the direct transformation of ACP to HAP, thereby acting as a nucleus for precipitation of crystalline calcium phosphate.[30]

Studies on the preparation of hydroxyapatite [$Ca_{10}(PO_4)_6$-$(OH)_2$], the synthetic prototype of bone mineral, showed that the initial solid phase that precipitates from a calcium phosphate solution depends on the degree of its supersaturation.[15] A noncrystalline ACP precursor approximating $Ca_9(PO_4)_6$ in composition appears under conditions of high supersaturation.[6,21] This precursor ACP, unless stabilized in some way, transforms to the thermodynamically more stable calcium phosphate phases, or leads to an autocatalytic solution-mediate crystallization process.

On the other hand, the first solid to form in low supersaturated solutions is hydroxyapatite, with Ca/P ratio of 1.67 obtained without precursor phases, so ACP is considered as a "mandatory precursor to apatite," and may be used in dilute solutions to form apatite without going through this precursor.[21] The pH value also affects the initial solid phase in the precipitation of calcium and phosphate ions. OCP is the crystalline phase that initially forms when the reaction pH is less than 9.25, whereas apatite preferentially forms at higher pHs.[31] As we know, at neutral pH and moderate supersaturation, ACP is often the first deposit to form *in vitro*.[32] Transformation mechanisms of ACP to apatite at physiological pH have been described as follows: first ACP dissolution, then a transient OCP solid phase reprecipitation through nucleation growth, finally hydrolysis of the transient OCP phase into the thermodynamically more stable apatite by a topotactic reaction, which usually takes more than 10 hours.[32]

Based on the analysis of measured precipitate induction times and the structure of the developing solid phase, Feenstra[33] proposed OCP might be an intermediate stage in the conversion of ACP to apatite calcium phosphate. Since OCP or apatite crystals were generally found in association with the ACP spherules, it is possible that ACP acts as a template for the growth of these crystal phases. Their formation, however, appears to take place by consuming ions largely supplied from the surrounding solution, rather than from direct hydrolysis of the solid amorphous material. Transformation experiments of ACP at pH 10 showed that transformation of ACP to poorly crystalline HAP might proceed without change in the local calcium environment, but with the development of longer-range order in the structure.

However, by contrast to the results obtained at pH 10, under physiologic conditions the picture is quite different. Tung used a titration method to study the conversion of high-concentration ACP slurry to an apatite. A typical conversion kinetics clearly indicated two processes: the first process consumes acid with the conversion of ACP to an OCP-like intermediary, and the second process consumes base with the conversion of the OCP-like intermediate to apatite or, possibly, direct conversion of ACP to apatite. Now, it is proposed that a stoichiometric HAP is formed when there is no OCP-like intermediate phase, and a non-stoichiometric apatite product is formed when an OCP-like intermediate phase is involved.[34]

Application to oral science

ACP has been widely applied in dental or oral science due to its excellent bioactivity, high cell adhesion, adjustable biodegradation rate and good osteoconduction.[15,35–37] As we mentioned above, the first quantitative studies on synthetic ACP were done in the mid 1960s.[6] From then on, increasing attention has been attracted in the development and the application of the ACP-containing products, especially in the dental and orthopedic industry. It is also used as filler in ionomer cements to fill carious lesions or as a colloidal suspension in toothpastes, chewing gums or mouthwashes to promote remineralization of carious lesions and/or to prevent tooth demineralization.[13]

Carrier in dental prophylaxis

Casein phosphopeptides (CPPs) contain the cluster sequence of -Ser (P)-Ser (P)-Ser (P)-Glu-Glu from casein.[38,39] Through these multiple phosphoseryl residues, CPP have a remarkable ability to stabilize clusters of amorphous calcium phosphate into a state-forming CPP-ACP complex, preventing their growth to the critical size required for nucleation, phase transformation and precipitation. In the United States, up to now, this product is primarily used in abrasive prophylaxis pastes, and secondarily for treatment of tooth sensitivity, especially after in-office bleaching procedures, ultrasonic scaling, hand scaling or root planing. However, its use for remineralizing dentin and enamel and preventing dental caries is an off-label application. Outside the United States, the products are marketed as GC Tooth Mousse.[40–42]

Moreover, the results from a clinical trial of a mouthwash used thrice daily containing CPP-ACP showed that the calcium and inorganic phosphate content of supragingival plaque increased after use of the mouthwash over a three-day period.[43] Rose measured the affinity and capacity of *Streptococcus mutans* for CPP-ACP, demonstrating that CPP-ACP binds with about twice the affinity for calcium of the bacterial cells, up to a value of 0.16 g/g wet weight cells. Hence, CPP-ACP binds well to plaque, providing a large calcium reservoir within plaque and slowing diffusion of free calcium.[44] Additional evidence also reported by Rose indicates that CPP-ACP would compete with calcium for plaque Ca binding sites. This will reduce the amount of calcium bridging between the pellicle and adhering cells and between cells themselves.[45] This is likely to restrict mineral loss during a cariogenic episode and provide a potential source of calcium for the inhibition of demineralization and assist in subsequent remineralization.[45,46]

A human *in situ* caries model has been used by Reynolds to study the ability of the 1.0% CPP, 60-mM $CaCl_2$ and 36-mM sodium phosphate, pH 7.0 solution to prevent enamel demineralization. Two exposures of CPP-ACP solution per day to one side of the enamel slabs produced 51 $\pm$ 19% reduction in enamel mineral loss compared to the control side. Plaque exposed to CPP-ACP had 2.5 times more Ca and phosphorus than control plaque.[47] Reynolds also conducted an experiment using an *in vitro* model system to study the effects of CPP-ACP solutions on remineralization of artificial lesions in human third molars. After a 10-day remineralization period, all solutions deposited mineral into the bodies of the lesions, with the 1.0% CPP-calcium phosphate (pH 7.0) solution replacing 63.9 $\pm$ 20.1% of mineral lost at an average rate of 3.9 $\pm$ 0.8 $\times$ 10^{-8} mol hydroxyapatite/m^2/s. The remineralizing capacity was greater for the solutions with higher levels of CPP-stabilized free calcium and phosphate ions.[48]

The CPP-ACP and fluoride were shown to have additive effects in reducing caries incidence,[49,50] so CPP-ACFP may be added into current fluoride-containing toothpastes as an additive to improve efficacy. Furthermore, recent studies indicate that CPP-ACP can be incorporated into confectionery and drinks without adverse organoleptic effects.[51] CPP-ACP is a natural derivative of milk, and therefore could have an important role as a food additive for the control of dental caries.[52] However, in 2008 Azarpazhooh[39] systemically reviewed 98 articles on the clinical efficacy of casein derivatives and concluded that there is insufficient evidence in clinical trials (in quantity, quality or both) to make a recommendation regarding the long-term effectiveness of casein derivatives, specifically CPP-ACP, as preventing caries *in vivo* and treating dentin hypersensitivity or dry mouth.

Filler in polymeric composites

ACP, a postulated precursor in the formation of biological hydroxyapatite, has been evaluated as a filler phase in bioactive polymeric composites.[53] During the last decade, Skrtic[2,54–56] has been developing unique biologically active restorative materials containing ACP as filler encapsulated in a polymer binder, which may stimulate the repair of tooth structure because of releasing significant amounts of calcium and phosphate ions in a sustained manner. In addition to excellent biocompatibility, the ACP composites release calcium and phosphate ions into saliva milieus, especially in the oral environment caused by bacterial plaque or acidic foods. Then these ions can be deposited into tooth structures as apatite mineral, which is similar to the hydroxyapatite found naturally in teeth and bone.[57,58]

However, it has been reported that orthodontic ACP-containing adhesive appears to lower bond strength. Dunn[59] conducted an *in vitro* study to compare it with the conventional resin-based orthodontic adhesive. Foster also compared the shear bond strength (SBS) of orthodontic brackets using ACP-containing adhesive to a conventional adhesive and a resin-modified glass ionomer. In these experiments, ACP-containing adhesive was demonstrated to possess low, but satisfactory, bond strength needed to function as an orthodontic adhesive.[60] When comparing four new ACP-containing bonding systems, including Aegis Ortho, with a conventional bracket bonding system (Transbond XT), it was found that the traditional bonding systems achieved greater bond strength than the newer ones. However, Aegis Ortho had bond strength sufficient to be useful for orthodontics at 24-hour postcure time, but the bracket might drift because of the low viscosity of the material during laboratory bonding.[61] The authors also found that Aegis Ortho had lower flexural strength, which would explain the material failure at the adhesive-bracket interface rather than at the enamel adhesive interface.[61]

Compared with the more commonly used silanated glass or ceramic filler, the more hydrophilic and biodegradable ACP-filled composites exhibit inferior mechanical properties, durability, and water sorption characteristics.[62] The uncontrolled aggregation of ACP particulates along with poor interfacial interaction plays a key role in adversely affecting their mechanical properties.[63] Their clinical applicability may be compromised by relatively poor filler/matrix interfacial adhesion and also by the excessive water sorption that occurs in both the resin and filler phases of these composites.[49,54,64,65]

In addition, it is possible to improve the remineralizing potential of ACP composites by introducing Si or Zr elements during the low-temperature synthesis of the filler. Si- and Zr- ACPs enhanced the duration of mineral ion release through their ability to slow down the intra-composite ACP to HAP conversion.[2] Antonucci[66] also stated the possible role on nonionic and anionic surfactants and poly (ethylene oxide; PEO) introduced during the preparation of ACP on the particle size distribution and compositional properties of ACP fillers. The hydrophilic PEO is widely used in water compatible polymer systems because of its proven ability to undergo multiple hydrogen bonding interactions and stabilize cations through multiple chelation. Incorporating PEO in ACP fillers would also be expected to affect not only the tendency of ACPs to form aggregates but also the water content of the ACP.[67] These properties would finally affect both ion release kinetics and the mechanical stability of composites. According to this study, surfactants introduced during the precipitation of ACP stabilized the amorphous solid phase against conversion to apatite. The particle size of ACP was moderately reduced because of the introduction of the anionic surfactant. Addition of PEO resulted in more pronounced ACP agglomeration but no changes in ACP water content. Both surfactants and PEO lead to no improvement in the dry biaxial flexure strength of composites compared with the control Zr-ACP composites. However, their strength after prolonged exposure to aqueous milieus was reduced drastically in contrast to the controls.[66]

Scaffold in bone tissue engineering

Various compounds from the calcium phosphate family have been extensively investigated as hard tissue repair materials due to their excellent biocompatibility.[68] It has been shown that the rate of new bone formation coincides more closely with the resorption rate of poorly crystalline apatites and ACP.[69] Additionally, ACP shows better

osteoconductivity *in vivo* than apatite, and its biodegradability is higher than that of tricalcium phosphate.[31]

Clinically, ACP is widely accepted for autografts and allografts to repair fractures and other bone defects. Recently, materials with ACP, hydroxyapatite and other calcium phosphate family members have been extensively investigated for alternative bone repair, due to the limitations of traditional materials, including potential immunogenicity, insufficient supply, and so on.[3,4] ACP and even ACP/biopolymer composites have received intense attention because of their applications as a bone tissue—engineering scaffold. The good properties of these materials, such as biocompatibility and osteoconductibility, and their ready conversion to bone apatite formation *in vivo* make them a seemingly perfect class emerging materials for bone substitution and reparation.

Studies have shown that bone-like apatite shows appropriate surface characteristics for osteoblast cells to adhere and grow, and, as a result, facilitates bone formation, and regeneration. Ambrosio synthesized an amorphous carbonated calcium phosphate ceramic encapsulated within bioresorbable PLAGA microspheres. A bioresorbable, highly porous, three-dimensional scaffold may be produced after sintering the composite microspheres together. These noncrystalline and carbonated materials may be ideal for tissue ingrowth and potentially suitable for bone repair applications.[70]

ACP also has been incorporated into porous poly (L-lactic acid; PLLA) to create a desired pore wall surface within bone tissue engineering scaffolds.[71] After being soaked in PBS, the ACP aggregates in the composite experienced a fast and *in situ* transformation into bone-like apatite. The cell culture results also evidenced that the ACP/PLLA composite had enhanced cytocompatibility. It has been demonstrated that ACP/PLLA material, which can experience morphologic variations in its microstructure, is also a suitable candidate to serve as scaffold for cartilage tissue engineering.

Moreover, another experiment has been done to explore the feasibility of restoring periodontal defects with dental follicle cells (DFCs)-β-TCP complex. It has been proposed that DFCs combined with β-TCP bioceramics could offer a novel therapeutic strategy for restoring periodontal defects.[72]

In summary, ACP is usually formed in a meta-stable phase when calcium ions and phosphate ions in aqueous solution react to precipitate. The X-ray diffraction pattern, structure, morphology and infrared analysis results show ACP solids have typical noncrystalline character with short-range order, instead of long-range periodic regularity. ACP can act as an important intermediate product for apatite formation *in vitro* and *in vivo*. It converted to apatite in water, and a variety of proteins and ions can increase the stability of ACP. ACP is becoming increasingly significant in oral biology, dentistry, orthopedic biomechanics materials and medicine because of its excellent biocharacteristics. We believe that ACP has prospects for wider application due to the fast development of tissue engineering technique and applied material science.

Acknowledgments

This work was supported as a project of Worldwide Universities Network Development Fund (No. 201001168), Natural Scientific Fund of Jiangsu (No. 2010118), and Natural Scientific Fund of China (No. 51142013).

References

1. Johnsson MS, Nancollas GH. The role of brushite and octacalcium phosphate in apatite formation. *Crit Rev Oral Biol Med* 1992;3:61—82.
2. Skrtic D, Antonucci JM, Eanes ED. Amorphous calcium phosphate-based bioactive polymeric composites for mineralized tissue regeneration. *J Res Natl Inst Stand Technol* 2003;108:167—82.
3. Dorozhkin SV. Calcium orthophosphates. *J Mater Sci* 2007;42:1061—95.
4. Dorozhkin SV. Calcium orthophosphates in nature, biology and medicine. *Materials* 2009;2:399—498.
5. Dorozhkin SV. Medical application of calcium orthophosphate. *Bioceramics* 2011;1:1—51.
6. Boskey AL. Amorphous calcium phosphate: the contention of bone. *J Dent Res* 1997;76:1433—6.
7. Eanes ED, Gillessen IH, Posner AS. Intermediate states in the precipitation of hydroxyapatite. *Nature* 1965;208:365—7.
8. Betts F, Blumenthal NC, Posner AS, Becker GL, Lehninger AL. Atomic structure of intracellular amorphous calcium phosphate deposits. *Proc Natl Acad Sci* 1975;72:2088—90.
9. Posner AS, Betts F, Blumenthal NC. Formation and structure of synthetic and bone hydroxyapatite. *Progr Cryst Growth Char* 1980;3:49—64.
10. Blumenthal NC, Betts F, Posner AS. Stabilization of amorphous calcium phosphate by Mg and ATP. *Calcif Tissue Res* 1977;23:245—50.
11. Termine JD, Eanes ED. Comparative chemistry of amorphous and apatitic calcium phosphate preparations. *Calcif Tissue Res* 1972;10:171—97.
12. Dorozhkin SV. Amorphous calcium (ortho) phosphates. *Acta Biomaterialia* 2010;6:4457—75.
13. Combes C, Rey C. Amorphous calcium phosphates: synthesis, properties and uses in biomaterials. *Acta Biomaterialia* 2010;6:3362—78.
14. Eanes ED, Termine JD, Nylen MU. An electron microscopic study of the formation of amorphous calcium phosphate and its transformation to crystalline apatite. *Calcif Tissue Res* 1973;12:143—58.
15. Feenstra TP, De Bruyn PL. Formation of calcium phosphates in moderately supersaturated solutions. *J Phys Chem* 1979;83:475—9.
16. Wuthier RE, Rice GS, Wallace JE, Weaver RL, LeGeros RZ, Eanes ED. kinetic stabilization In vitro precipitation of calcium phosphate under intracellular conditions: formation of brushite from an amorphous precursor in the absence of ATP. *Calcif Tissue Int* 1985;37:401—10.
17. Sun W, Zhang F, Guo J, Wu J, Wu W. Effects of amorphous calcium phosphate on periodontal ligament cell adhesion and proliferation in vitro. *J Med Biol Eng* 2008;28:31—7.
18. Harries JE, Hukins DW, Holt C, Hasnain SS. Conversion of amorphous calcium phosphate into hydroxyapatite. *J Cryst Growth* 1987;84:563—70.
19. Boskey AL, Posner AS. Magnesium stabilization of amorphous calcium phosphate: a kinetic study. *Mater Res Bull* 1974;9:907—16.
20. Posner AS, Betts F, Blumenthal NC. Role of ATP and Mg in the stabilization of biological and synthetic amorphous calcium phosphates. *Calcif Tissue Res* 1977;22:208—12.
21. Wang L, Nancollas GH. Dynamics of biomineralization and biodemineralization. *Met Ions Life Sci* 2010;4:413—56.
22. Beniash E, Metzler RA, Lam RS, Gilbert PU. Transient amorphous calcium phosphate in forming enamel. *J Struct Biol* 2009;166:133—43.

23. Lowenstam HA. Minerals formed by organisms. *Science* 1981; 211:1126—31.
24. Lowenstam HA, Weiner S. Transformation of amorphous calcium phosphate to crystalline dahillite in the radular teeth of chitons. *Science* 1985;227:51—3.
25. Mahamid J, Sharir A, Addadi L, Weiner S. Amorphous calcium phosphate is a major component of the forming fin bones of zebrafish: indications for an amorphous precursor phase. *Proc Natl Acad Sci USA* 2008;105:12748—53.
26. Landis WJ, Glimcher MJ. Electron diffraction and electron probe microanalysis of the mineral phase of bone tissue prepared by anhydrous techniques. *J Ultrastruct Res* 1978;63:188—223.
27. Liu S, Weng W, Li Z, et al. Effect of PEG amount in amorphous calcium phosphate on its crystallized products. *J Mater Sci Mater Med* 2009;20:359—63.
28. Margolis HC, Beniash E, Fowler CE. Role of macromolecular assembly of enamel matrix proteins in enamel formation. *J Dent Res* 2006;85:775—93.
29. He G, Dahl T, Veis A, George A. Nucleation of apatite crystals in vitro by self-assembled dentin matrix protein 1. *Nat Mater* 2003:552—8.
30. Tsuji T, Onuma K, Yamamoto A, Iijima M, Shiba K. Direct transformation from amorphous to crystalline calcium phosphate facilitated by motif-programmed artificial proteins. *Proc Natl Acad Sci USA* 2008;105:16866—70.
31. Tadic D, Peters F, Epple M. Continuous synthesis of amorphous carbonated apatites. *Biomaterials* 2002;23:2553—9.
32. Nagano M, Nakamura T, Kokubo T, Tanahashi M, Ogawa M. Differences of bone bonding ability and degradation behaviour in vivo between amorphous calcium phosphate and highly crystalline hydroxyapatite coating. *Biomaterials* 1996;17:1771—7.
33. Feenstra TP, de Bruyn PL. The Ostwald rule of stages in precipitation from highly supersaturated solutions: a model and its application to the formation of the nonstoichiometric amorphous calcium phosphate precursor phase. *J Coll Interf Sci* 1981;84:66—72.
34. Tung MS, Brown WE. An intermediate state in hydrolysis of amorphous calcium phosphate. *Calcif Tissue Int* 1983;35:783—90.
35. Simon CG, Antonucci JM, Liu DW. In vitro cytotoxicity of amorphous calcium phosphate composites. *J Bioact Compat Polym* 2005;20:279—95.
36. Balasundaram G, Sato M, Webster TJ. Using hydroxyapatite nanoparticles and decreased crystallinity to promote osteoblast adhesion similar to functionalizing with RGD. *Biomaterials* 2006;27:2798—805.
37. Whited BM, Skrtic D, Love BJ, Goldstein AS. Osteoblast response to zirconia-hybridized pyrophosphate-stabilized amorphous calcium phosphate. *J Biomed Mater ResA* 2006;76:596—604.
38. Huq NL, Cross KJ, Reynolds EC. Molecular modelling of the multiphosphorylated casein phosphopeptide alphaS1-casein(59-79) based on NMR constraints. *J Dairy Res* 2004;71:28—32.
39. Azarpazhooh A, Limeback H. Clinical efficacy of casein derivatives: a systematic review of the literature. *J Am Dent Assoc* 2008;139:915—24.
40. Iijima Y, Cai F, Shen P, Walker G, Reynolds C, Reynolds EC. Acid resistance of enamel subsurface lesions remineralized by a sugar-free chewing gum containing casein phosphopeptide-amorphous calcium phosphate. *Caries Res* 2004;38:551—6.
41. Shen P, Cai F, Nowicki A, Vincent J, Reynolds EC. Remineralization of enamel subsurface lesions by sugar-free chewing gum containing casein phosphopeptide-amorphous calcium phosphate. *J Dent Res* 2001;80:2066—70.
42. Ranjitkar S, Kaidonis JA, Richards LC, Townsend GC. The effect of CPP—ACP on enamel wear under severe erosive conditions. *Arch Oral Biol* 2009;54:527—32.
43. Reynolds EC. Anticariogenic complexes of amorphous calcium phosphate stabilized by casein phosphopeptides: a review. *Spec Care Dentist* 1998;18:8—16.
44. Rose RK. Binding characteristics of Streptococcus mutans for calcium and casein phosphopeptide. *Caries Res* 2000;34: 427—31.
45. Rose RK. Effects of an anticariogenic casein phosphopeptide on calcium diffusion in streptococcal model dental plaques. *Arch Oral Biol* 2000;45:569—75.
46. Reynolds EC, Black CL, Cai F. Advances in enamel remineralization: anticariogenic casein phosphopeptide-amorphous calcium phosphate. *J Clin Dent* 1999;10:86—8.
47. Reynolds EC. Anticariogenic phosphopeptides. U.S. Patent. 1991, 5, 015, 628.
48. Reynolds EC. Remineralization of enamel subsurface lesions by casein phosphopeptide-stabilized calcium phosphate solutions. *J Dent Res* 1997;76:1587—95.
49. Reynolds EC. Calcium phosphate-based remineralization systems: scientific evidence? *Aus Dent J* 2008;53:268—73.
50. Reynolds EC, Cai F, Cochrane NJ, et al. Fluoride and casein phosphopeptide—amorphous calcium phosphate. *J Dent Res* 2008;87:344—8.
51. Ramalingam L, Messer LB, Reynolds EC. Adding casein phosphopeptide-amorphous calcium phosphate to sports drinks to eliminate in vitro erosion. *Pediatr Dent* 2005;27:61—7.
52. Reynolds EC. Dairy products and dental health. *Proc Nutr Soc* 1995;19:95—102.
53. Dorozhkin SV, Epple M. Biological and medical significance of calcium phosphates. *Angew Chem Int Ed Engl* 2002;41: 3130—46.
54. Skrtic D, Antonucci JM, Eanes ED, Eichmiller FC, Schumacher GE. Physicochemical evaluation of bioactive polymeric composites based on hybrid amorphous calcium phosphates. *J Biomed Mater Res* 2000;53:381—91.
55. Skrtic D, Antonucci JM, Eanes ED, Eidelman N. Dental composites based on hybrid and surface-modified amorphous calcium phosphates. *Biomaterials* 2004;25:1141—50.
56. Skrtic D, Antonucci JM, McDonough WG, Liu DW. Effect of chemical structure and composition of the resin phase on mechanical strength and vinyl conversion of amorphous calcium phosphate-based composites. *J Biomed Mater ResA* 2004;68:763—72.
57. Olszta MJ, Cheng X, Jee XX. Bone structure and formation: A new perspective. *Mat Sci Eng* 2007;58:77—116.
58. Llena C, Forner L, Baca P. Anticariogenicity of casein phosphopeptide—amorphous calcium phosphate: A review of the literature. *J Contemp Dent Pract* 2009;10:1—9.
59. Dunn WJ. Shear bond strength of an amorphous calcium-phosphate-containing orthodontic resin cement. *Am J Orthod Dentofacial Orthop* 2007;131:243—7.
60. Foster JA, Berzins DW, Bradley TG. Bond strength of an amorphous calcium phosphate-containing orthodontic adhesive. *Angle Orthod* 2008;78:339—44.
61. Minick GT, Oesterle LJ, Newman SM, Shellhart WC. Bracket bond strengths of new adhesive systems. *Am J Orthod Dentofacial Orthop* 2009;135:771—6.
62. O'Donnell JN, Antonucci JM, Skrtic D. Amorphous calcium phosphate composites with improved mechanical properties. *J Bioact Compat Polym* 2006;21:169—84.
63. Schumacher GE, Antonucci JM, O'Donnell JN, Skrtic D. The use of amorphous calcium phosphate composites as bioactive basing materials: their effect on the strength of the composite/adhesive/dentin bond. *J Am Dent Assoc* 2007;138:1476—84.
64. Skrtic D, Antonucci JM, Eanes ED. Effect of the monomer and filler system on the remineralizing potential of bioactive dental composites based on amorphous calcium phosphate. *Polym Adv Technol* 2001;12:369—79.
65. Antonucci JM, Skrtic D. matrix resin effects on selected physicochemical properties of amorphous calcium phosphate composites. *J Bioact Compat Polym* 2005;20:29—49.
66. Antonucci JM, Liu DW, Skrtic D. Amorphous calcium phosphate based composites: effect of surfactants and poly(ethylene

oxide) on filler and composite properties. *J Dispers Sci Technol* 2007;28:819—24.
67. Eanes ED. Amorphous calcium phosphate: thermodynamic and kinetic considerations. In: Amjad Z, ed. *Calcium Phosphates in Biological and Industrial Systems*. Boston: Kluwer Academic Publ; 1998. p. 21—39.
68. Dorozhkin SV. Bioceramics of calcium orthophosphates. *Biomaterials* 2010;31:1465—85.
69. Klein CP, Driessen AA, Groot K, Hooff A. Biodegradation behavior of various calcium phosphate materials in bone tissue. *J Biomed Mater Res* 1983;17:769—84.
70. Ambrosio AM, Sahota JS, Khan Y, Laurencin CT. A novel amorphous calcium phosphate polymer ceramic for bone repair: I. Synthesis and characterization. *J Biomed Mater Res* 2001;58: 295—301.
71. Gao Y, Weng W, Cheng K, et al. Preparation, characterization and cytocompatibility of porous ACP/PLLA composites. *J Biomed Mater ResA* 2006;79:193—200.
72. Zuolin J, Hong Q, Jiali T. Dental follicle cells combined with beta-tricalcium phosphate ceramic: a novel available therapeutic strategy to restore periodontal defects. *Med Hypotheses* 2010;75:669—70.