Role of Amorphous Calcium Phosphate in Dentistry

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Abstract

Objective: Amorphous calcium phosphate (ACP) is a reactive solution capable of releasing calcium and phosphate ions, conversion to apatite and remineralization of mineral tooth structure. This study reviews ACP structure, its properties and its applications in dentistry.

Methods: In this review study, keywords including "amorphous calcium phosphate", "mineralization", "hydroxyapatite", "casein phosphopeptide", "tissue engineering" and "dentistry" were searched in articles published during 1953-2013 in PubMed, Science Direct, Google Scholar, Embase and Medline databases. A total of 134 articles were evaluated.

Results: ACP had high adhesion, adjustable dissociation rate, bone induction and excellent biocompatibility without cell toxicity. ACP alone or in combination with casein phosphopeptide (CPP) is incorporated into toothpastes, chewing gums, mouth rinses, tooth bleaching gels and food products to enhance remineralization and prevent demineralization. Also, it is used as filler in many dental materials namely glass ionomers, composite resins and bonding agents. Implant surface coating with ACP by radio frequency magnetron sputtering improves osseointegration especially at the final stages of healing.

Conclusion: ACP is suitable for tissue regeneration and healing and is a potential remineralizing agent in dentistry.

Key words: Amorphous calcium phosphate, Casein phosphopeptide, Dentistry, Hydroxyapatite, Mineralization, Tissue engineering.

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Introduction:

ACP is a super-saturated solution of solid calcium phosphate particles. Crystalline octa-calcium phosphate (OCP) or apatite products are derived from it (1). It plays a specific role as a precursor to bioapatite and in the transitional phase of biomineralization (2). In the living creatures some amorphous minerals exist that are biologically named biominerals and their formation process is called biomineralization(1). The morphology and structure of non-crystalline ACP includes repeating atomic chains with short domains/amplitude. It has been shown that many minerals are formed as amorphous pre-phases (3). These amorphous minerals in dental and skeletal structures of marine invertebrates are commonly calcium orthophosphate (3). Also, similar compounds of amorphous calcium phosphate exist in mitochondria (1) and sarcoplasmic reticulum (4) of vertebrates, parts of the internal ear of shark fetus, milk of mammals (5, 6) and tooth enamel (7). Despite numerous studies, presence of ACP compounds in hard tissues namely bone and tooth has not been confirmed (8-10). Moreover, ACP in the form of amorphous mineral phases has been found in the biomineralized structures of a group of animals including auto lit shark and also in the form of a carbonated hydroxyapatite

precursor in crab's teeth and in fine bones of sea horses (11-13). Recent studies on the formation of teeth and bone emphasize the need for the presence of transitional amorphous mineral precursors for the mineralization of calcium carbonate and calcium orthophosphate bases in vertebrates and invertebrates (8-10, 12, 14). The role of ACP in formation of nano-structured hydroxy apatite in highly organized structures has been evaluated in some previous studies (9). ACP compared with HA and tricalcium phosphate (TCP) has better osteoinductive properties, biodegradability and bioactivity (15). Use of ACP has become popular in medicine, orthopedics and dentistry attributed to its favorable characteristics such as high adhesion, adjustable degradation rate, osteoinduction and excellent bioactivity without cell toxicity (16, 17). This study reviews the structure, properties and biological application of ACP in dentistry.

Methods:

In this review study, a search in PubMed, Science Direct, Google Scholar, Embase and Medline databases was carries out using "amorphous calcium phosphate", "mineralization", "hydroxy apatite", "casein phosphopeptide", "tissue engineering" and "dentistry" keywords. Studies published during 1953-2013 were searched; 134 were selected and reviewed.

Results:

1. Structural characteristics of ACP: The history of ACP goes back to 1955 when Robinson and Wastone found the newly formed mineral component in immature bones that did not have a crystalline structure (8, 18). ACP was first introduced by Aarons Ponser in mid 1960 (15). Amorphous ACP was formed by incidental combination of 30mM of calcium chloride and 20mM of sodium acid phosphate (19, 20).

Eanes, *et al*. (1965) introduced ACP as a bone constituent (19). ACP content of bone decreases by aging (21). Under *in vivo* conditions ACP shows better osteoinductive properties than HA due to its high reactivity with body fluids. Also, it has a biodegradability superior to that of TCP (22).

Having nanometer-sized particles is among the most important characteristics of ACP. The primary size of these particles ranges from 40-100 nm (18, 23). Synthesized ACP components under electron microscope are in the form of ionic clusters with 9.5 Angstrom diameter with fixed dimensions and chemical composition of $Ca_4(Po_4)_6$ (24, 25)(Figure 1). Water present in ACP (15-20%) usually fills the spaces in between ionic clusters (26).

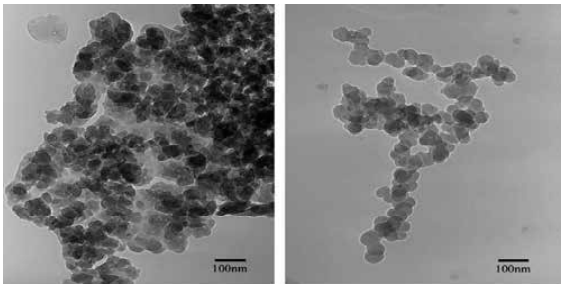

Figure 1- Image of ACP under bright field TEM

Apatite crystals are formed as the result of dissolution of ACP particles in a fixed thermodynamic phase. The results of *in vitro* and in-vivo studies have shown that in different pH values, amorphous calcium phosphate deposit is formed by 1.43/1.5M calcium/phosphate and 1.50-1.76 M of different carbonates (23, 27). Wuthier, *et al*. (1985) reported that ACPcan also deposit with a lower ratio of calcium/phosphate ions in a more acidic pH (28).

In the physiologic pH, ACP is first dissolved and re-deposition of solid phase of OCP occurs through the growth of core and its hydrolysis to a more stable apatite phase (29). OCP may be a mediator in conversion of ACP to phosphate calcium apatite (30). In a lower pH (below 9.25)

OCP and in higher pH values apatite is formed (31). Although the exact mechanism of ACP stability has yet to be clearly known, by presence of sufficient amounts of Mg^{+2}, F^{-}, carbonates, pyrophosphates, diphosphonates, nucleotides or polyphosphoryl metabolites, conversion of synthetic ACP to hydroxyapatite can be prevented (32, 33).

Different ions and proteins are involved in biomineralization of ACP to HA (13, 34). Dentin protein matrix is a biomineralized protein (35) comprised of two main peptides that act as a core for deposition of crystalline calcium phosphate in direct transformation of ACP to HA (14).

2. Application of ACP in dentistry:

As stated earlier, the first studies on synthetic ACP were performed in mid 1960 (16). Recently, application of ACP products in medicine and dentistry has been a subject of attention. This material is also used as filler in ionomer cement (36) for restoration of carious lesions to enhance their remineralization or prevent demineralization (37) in the form of colloidal suspension in tooth pastes (Enamelon TM), Recaldent chewing gums, tooth bleaching gels and mouthwashes (38-40).

A: CPP-ACP products

Casein phosphopeptide contains two common peptides of α_{s1}-casein and β-casein (40). CPP contains multiple rows of phosphoserine among which, 8-9 phosphate groups belong to α_{s1}-casein and 5 groups belong to β-casein (40). Since a specific size is required for coring, phase transportation and deposition, phosphoserine clusters stabilize ACP in CPP-ACP complexes and prevent their growth (41, 42). CPP-ACP nano-complexes due to the small size of particles are capable of penetrating deep into enamel porosities, remineralize enamel crystals and prevent enamel demineralization (43).

GC Tooth Mouse: This product is in the form of a soft, sugar-free, water-based topical crème (39) and is used for remineralization of dentin and enamel for prevention of caries (41, 42,44, 45). It is also used for treatment of tooth hypersensitivity following bleaching, ultrasonic and hand scaling and also as abrasive prophylactic paste (41, 42). Studies have reported that one time use of this product can decrease enamel erosion due to acidic foods and beverages by increasing the calcium content in the saliva and dental plaque and decreasing the critical pH required for dissolution of enamel minerals (39, 40, 45). In-vivo and *in vitro* studies published in 2013 have stated that CPP-ACP is more effective than sodium fluoride mouthwash (46) and fluoridated toothpaste (47) for remineralization of enamel caries. Also, microscopic assessments show that use of CPP-ACP significantly decreases enamel and dentin wear (48). Despite the mentioned advantages, Moezizadeh and Motamedi (2012) demonstrated that application of GC Tooth Mouse to the dentin surface decreases the bond of light cure glass ionomer unless polyacrylic acid is applied before the use of glass ionomer (49).

Xylitol or Sorbitol chewing gums: Recaldent is among these products (40, 49). Recaldent is a very soluble and stable CPP complex containing hydroxide, phosphate and calcium ions that have a more significant anti-caries effect compared to CPP-ACP complex (50).

Toothpastes: Enamelon TM is a commercial product of ACP-containing toothpaste combined with sodium calcium phosphosilicate (39, 40, 51). It is used to remove dentin hypersensitivity at the cervical area (52). CPP-ACP and fluoride have significant effects on decreasing caries (36). Thus, CPP-ACFP as an additive in fluoride toothpastes confers considerable anti-caries effect.

Mouth rinses: CPP-ACP in mouth rinses significantly increases the level of calcium and phosphate ions in supragingival plaque. The results of a study by Rose (2000) showed that the adhesion of Streptococcus mutans bacteria to CPP-ACP compounds is twice their adhesion to

pellicle (53). Thus, adhesion of CPP-ACP to dental plaque provides a large source of calcium in plaque that leads to gradual release of free calcium. A competition between CPP-ACP and calcium for calcium receptors has been reported by Rose (2000). Thus, the attachment between calcium and pellicle and attached bacteria is decreased (54).

Food products: CPP-ACP, with no adverse effect on taste, can be a selective candidate for treatment of demineralization (55). Recent studies have shown that application of CPP-ACP in drinks (56), sweets (57) and milk products (5, 6, 58) can prevent their cariogenic properties (58). One study showed that sweets containing a minimum of 3w% CPP-ACP, by gradual release of calcium phosphate when consumed,can be used as an anti-caries product (40).

Dental bleaching agents: Pastes containing CPP-ACP applied to teeth before or after bleaching (59) or mixed with the bleaching gel in similar ratios (60) have shown to have the ability to prevent hardness reduction and surface roughness of bleached teeth without interfering with the effects of bleaching.

Azarpazhooh and Limeback (2008) reported that due to the insufficiency of clinical trials and their inefficacy, they could not cast a final judgment on the long-term effects of casein derivatives especially CPP-ACP in prevention of caries, dentin sensitivity or xerostomia (40).

B: ACP as filler in resin compounds:

ACP has been used as filler in polymer resins (61). Studies have shown that composites containing ACP have excellent biocompatibility and may be capable of remineralizing the teeth because they release significant amounts of calcium and phosphate into the saliva. These materials are deposited on teeth as apatite minerals and may simulate the role of bone and tooth HA (62, 63). Also, these composites compared to ceramic fillers or silanized glasses are more hydrophilic and by forming weak interfaces show lower durability and mechanical properties. Due to excess water sorption of resins and fillers, a weak bond is formed between the matrix and filler and provides a low quality treatment in the clinical setting (39, 62, 64). Polyethylene hydrophilic oxides present in these composites, despite the ability to form multiple stable hydrogen bonds, affect the affinity of ACP for accumulation and water sorption and also influence the mechanical properties of composites (65).

At present, nano-composites containing amorphous nanocalcium phosphate (NACP) particles have a combination of favorable mechanical properties and acid neutralization enhancing tooth remineralization (66). For the first time in 2011 it was demonstrated that nano-particulate ACP composites had flexural strength and modulus of elasticity equal or higher than those of commercial composites. By increased release of calcium and phosphate similar to composites containing calcium phosphate, they have the remineralizing ability and can be considered as anti-caries restorations (67). They also have strength higher than RMGI and inhibit the growth of S. mutans and decrease the risk of secondary caries (68).

Orthodontic adhesives containing ACP compared to conventional adhesives have lower bond strength but have shown satisfactory clinical results (69, 70).

New bonding systems containing ACP (Aegis Ortho) compared to conventional orthodontic adhesives (Transbond XT) have shown less flexural strength indicating greater material loss at the bracket-adhesive interface compared to adhesive-enamel interface (61).

Bonding agents containing silver nanoparticles (NAg) and NACP decrease acid production and number of microorganisms in dental plaque biofilm without compromising the bond strength. These new bonding agents, due to the remineralizing effects of NACP and antibacterial activity of NAg may be extensively used in bonding systems in the future (71).

C: Application of ACP for bone repair

Many studies have evaluated calcium phosphate containing compounds such as ACP and HA for bone repair. These compounds do not have the side effects of immune system stimulation and inadequate blood supply associated with conventional materials (72, 73). Different ACP compounds due to excellent biocompatibility, superior osteoinduction compared to apatites and higher biodegradability compared to TCP are extensively used (74, 75). New bone formation depends on the degree of ACP absorption and apatites with weak crystalline structure (76). Combination of ACP with polylactic acid is used for reinforcing cell biocompatibility and forming a porous surface for the fabrication of bone and cartilaginous scaffolds (73, 77).

Non-crystalline compounds of carbonated calcium phosphate along with polylactic and glycolic acids are biodegradable and can form a porous bone scaffold and thus are ideal for bone repair (78). Bone-like apatites are also used for optimal bone formation due to ideal surface structure for osteoblasts (78).

D: Dental implants coated with ACP

The most common approach for dental implant surface preparation includes physical changes in the topography or alterations in the chemical composition of surface with presence of non-organic phases (79). Regardless of surface modification, the ultimate goal is to improve primary bone healing and osseointegration allowing implant loading in the soonest time possible (80-82).

Titanium dental implants covered with calcium phosphate have greater osseointegration compared to the uncoated ones. Phase and microstructure of calcium phosphate coats affect the osseointegration (83). Although coating the titanium surface of implants with calcium phosphate leads to primary osseointegration, degradation of the coated layer and implant-coat debonding have also been reported affecting the long-term prognosis (84).

Different techniques such as plasma spray, sputtering, sol-gel technique and electrophoretic deposition have been reported for the application of calcium phosphate to the implant surface. Plasma spray coating is highly popular due to high deposition rate. However, this method creates a weak bond to metal substrate (84). ACP coat applied to implants made of titanium or Ti-6A1-4V alloy using Resonance Frequency (RF) magnetron sputtering forms a very thin 500nm layer with bond strength above 60MPa at room temperature (84).

According to Yokota *et al*. (2012) ACP coated dental implants enhance bone formation compared to uncoated implants. On the other hand, ACP coating is bio-absorbable (83). Bonfante *et al*. (2012) stated that chemical and topographic differences exist between the Ti-6A1-4V implants treated with plasma-sprayed hydroxyapatite (PSHA) and ACP-coated surfaces. When assessed by bone and tissue morphological parameters, both surfaces show osteoconductive properties in root-form implants (85).

A study in 2012 showed that Ti-29Nb-13Ta-4.6 Zr (TNTZ) implants made of a new alloy of beta-titanium, provide an osseointegration similar to that of pure implants. Surface coating of TNTZ implants with ACP by RF magnetron sputtering improves osseointegration especially at the final phases of healing (86).

Discussion:

ACP is found in the skeleton of many living creatures particularly invertebrates. It can also be synthesized using highly advanced techniques (34). ACP can be converted to OCP and apatite depending on the environmental pH (31).

ACP is incorporated into some dental products in the form of CPP-ACP nano-complexes with remineralizing properties. It also prevents demineralization (43). However, long-term studies are not available on its effects on

prevention of tooth hypersensitivity (87, 88).
Composites containing ACP have anti-caries properties but due to hydrophilicity have lower durability and mechanical properties than other composites (62, 64). At present, nanotechnology has greatly helped in enhancing their mechanical properties (67).
Combination of ACP and polylactic acids can be used for the fabrication of bone and cartilaginous scaffolds (73, 77).
Application of ACP due to its bio-absorption and osteoconduction properties for implant surface coating can enhance bone formation and osseointegration (83). Based on the method of application in surface of TNTZ implants, it can improve osseointegration at the final stages of healing (86).

Conclusion:

ACP is a mineral phase formed in mineralized tissues and is involved as an important mediator in apatite formation. ACP is a suitable material for tissue regeneration and repair and is a potential remineralizing agent in dentistry.

This compound alone or in combination with casein derivatives (such as CPP) is a safe and effective material to promote oral health. It can be incorporated into dental hygiene products due to optimal mechanical properties and excellent biocompatibility and is increasingly used in dentistry. ACP compared with TCP and HA has the ability to induce bone scaffold and is more biodegradable. When used for titanium implant surface coating, it can improve osseointegration. Considering the advances in tissue engineering and biomaterial science, application of ACP will be increased in the future.

Conflict of Interest: "None Declared"

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