

Review Paper

Application of calcium phosphate materials in dentistry: A review

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ABSTRACT

Calcium phosphate materials are similar to bone in composition and having bioactive and osteoconductive properties. They have different forms, as cements, composites and coatings, which are used in many dental applications. This article reviews the latest update of their applications in dentistry the studies of improving the mechanical properties of these materials. Notable research is highlighted, regarding application of calcium phosphate into various fields in dentistry and improvement of their mechanical properties. This article deals with most common types of these materials including, calcium phosphate, hydroxyapatite and tricalcium phosphate.

Keywords: Calcium phosphate materials, Dentistry, Hydroxyapatite, Tricalcium phosphate

INTRODUCTION

Calcium phosphate materials are bioactive materials that show a positive interaction with living tissue that includes also differentiation of immature cells towards bone cells. In contrast to bioinert materials, there is chemical bonding to the bone along the interface, thought to be triggered by the adsorption of bone growth-mediating proteins at the biomaterials surface. Hence there will be a biochemically-mediated strong bonding osteogenesis. In addition to compressive forces, to some degree tensile and shear forces can also be transmitted through the interface ("bony ingrowth"). The stable phases of calcium phosphates depend considerably upon temperature and the presence of water, either during processing or in the use environment [1].

Calcium phosphate materials have received a lot of research attention in recent years due to their chemical similarity to calcified tissue (bones, teeth). They are attractive biomedical materials owing to their excellent biocompatibility. The first calcium phosphate materials were used in the 1920s. They were used as bone substitute or bone graft to promote new bone formation [2].



In 1971, Monroe and his colleagues reported a method for the preparation of a calcium phosphate, principally mineral calcium-fluor-apatite, and suggested the possible use of this apatite ceramic as dental and medical implant materials [3]. The first dental application was reported by Nery et al. [4] more than many years later using a synthetic porous material obtained by sintering a “tricalcium phosphate reagent” that was originally described by the authors as “tricalcium phosphate” but later demonstrated to consist of a mixture of hydroxyapatite and tricalcium phosphate [5].

Applications of calcium phosphate include repair of periodontal defects, augmentation of alveolar bone, sinus lifts, tooth replacement and repair of large bone defects caused by tumors [5-13]. They are used in tissue engineering for bone or dentine regeneration [13-17]. Calcium phosphates are also used in the form of injectable cements [18-19] or as coatings on titanium and titanium alloy implants to combine the bioactivity of the calcium phosphates and the strength of the metal [20-21].

The purpose of the present paper is to review the use of calcium phosphate materials in dentistry. Emphasis will be given to the hydroxyapatite and tricalcium phosphate. This review summarizes brief history, dental applications, and methods for improving their properties.

Hydroxyapatite

Hydroxyapatite is the most documented calcium phosphate ceramic, and can be used in bulk form or as a coating. This material can be classified according to its porosity, phase, and processing method. It is widely preferred as the biomaterial of choice in both dentistry and orthopaedics due to its favorable osteoconductive and bioactive properties [22]. Synthetic hydroxyapatite is similar in composition to the mineral component of bone and teeth. Table 1 shows the structural similarities between hydroxyapatite, enamel, dentine and bone [23]. This similarity makes it the most clinically used as biomaterial for medical and dental applications [24].

Table 1. Chemical and structural comparison of teeth, bone and hydroxyapatite (HA)

Composition, wt%	Enamel	Dentine	Bone	HA
Calcium	36.5	35.1	34.8	39.6
Phosphorous	17.1	16.9	15.2	18.5
Ca/P ratio	1.63	1.61	1.71	1.67
Total inorganic (%)	97	70	65	100
Total organic (%)	1.5	20	25	--
Water (%)	1.5	10	10	--

Although hydroxyapatite has favorable bioactive and osteoconductive properties that result in rapid bone formation in a host body and strong biological fixation to bony tissues [25], it possesses low mechanical strength and fracture toughness, which is an obstacle to its applications in load-bearing areas [26]. Typical properties of dense hydroxyapatite are given in Table 2 [27]. Thus, the enhancement of the mechanical properties of hydroxyapatite would extend its scope of applications. Hydroxyapatite is either used as a bioactive coating on implants or reinforced with tough phases such as metal or ceramic phases, in order to achieve the mechanical characteristics needed for biomedical applications.

Table 2. Typical properties of dense hydroxyapatite

Properties	Amount
Theoretical density	3.156 g/cm ³
Hardness	500-800 Vickers, 2000-3500 Knoop
Tensile strength	40-100 MPa
Bend strength	20-80 MPa
Compressive strength	100-900 MPa
Fracture toughness	1 MPam ^{1/2}
Young's modulus	70-120 GPa

Hydroxyapatite has been used successfully in clinical and animal studies for endodontic treatment including pulp capping, repair of mechanical bifurcation perforation, apical barrier formation and repair of periapical defects [28-31]. Jean et al. [28] suggested that the degree of mineralization of reparative dentine formation obtained with tricalcium phosphate-hydroxyapatite was quicker and thicker when compared with that produced by calcium hydroxide. Additionally, hydroxyapatite has been used as filler for reinforcing dental resins [32,33], coating in both orthopaedic and dental implant [34,35], restoration of edentulous atrophic ridges [36], perio-infrabony pockets [37], periodontal defects [38], under and around failing sub-periosteal metal implants [39], ridge augmentation prior to implantology for metal prosthetics [40].

IMPROVING THE PROPERTIES OF HYDROXYAPATITE

Hydroxyapatite composites

Combinations of hydroxyapatite with synthetic polymers or metallic agents are called hydroxyapatite composites. They have been developed and studied in purpose to improve the mechanical properties of porous hydroxyapatite. Many reinforcements including particles, platelets, whiskers, long fibers, partially stabilized zirconia, metal dispersoids, and polymers have been used in hydroxyapatite to improve their reliability [41-44].

Deng et al. [45] added nanocrystalline hydroxyapatite to a polylactide solution to form solvent-cast composite matrices and found a steady increase in tensile modulus as hydroxyapatite loading increased from a low of 1.66 GPa for polymer without hydroxyapatite up to 2.47 GPa for 10.5% hydroxyapatite content. Wang et al. [46] combined polyamide, a bioinert polymer, with both microcrystalline and nanocrystalline hydroxyapatite and compared resulting bending strength and tensile strength. As the ceramic content of each composite increased, so did the bending strength.

For both bending and tensile strength, the addition of nanocrystalline hydroxyapatite increased the properties over those with microcrystalline hydroxyapatite. It was theorized that the smaller crystals of the nanocrystalline hydroxyapatite resulted in higher surface areas and thus greater surface energy, surface activity, and thus bonding between the polymer and the hydroxyapatite.

Abu Bakar et al. [47] examined the effect of varying amounts of hydroxyapatite added to polyetheretherketone as an injection-molded composite by varying hydroxyapatite content between 0-40% by volume. Results indicate that Young's modulus increased from

approximately 3-15 GPa as hydroxyapatite content increased from 0-40% but tensile strength decreased from 80-44 MPa along the same increase in hydroxyapatite content.

Balac et al. [48] attempted to understand the effect of hydroxyapatite particle shape and volume fraction in a polylactide/collagen/hydroxyapatite composite scaffold using finite element analysis and found fewer stress concentrations throughout the matrix with an increased hydroxyapatite volume fraction but a reduced dependence on this as the hydroxyapatite particles were modeled as spherical, suggesting yet another design consideration for the composite scaffold. Hydroxyapatite matrix composites containing 20-30% Fe-Cr alloy long metal fibers showed the highest values of fracture toughness and fracture strength for hydroxyapatite based materials as reported by Suchanek and Yoshimura [49].

Ramires et al. [50] tested titanium oxide and hydroxyapatite composites, formed by sol-gel method, for biocompatibility and cell response. Their results showed that the combination was biocompatible and excellent at promoting cell activity. Volceanov et al. [51] investigated the influence of zirconia addition to a hydroxyapatite matrix on mechanical strengths and the interaction mechanism between zirconia and its polymorphs with calcium phosphates after sintering at 1250°C. Their results highlighted that there were improved mechanical properties for hydroxyapatite matrix composites cured in air at 1250°C. Some authors added small amounts of P-glass into hydroxyapatite ceramic to improve sinterability and mechanical properties of the dense body, as well as biological properties [52-54].

Ferraz et al. [52] fabricated glass-hydroxyapatite composite coatings, using a plasma spraying technique, and experimented it *in vitro* with osteosarcoma cells. Their findings showed favorable cellular responses. Others claimed that the inclusion of phosphate based glasses produced significant improvement in mechanical properties [55, 56].

Hydroxyapatite coatings

Bioactive calcium phosphate ceramics as coatings on bioinert metallic substrate have received worldwide attention in both orthopaedic and dental implant due to their biocompatibility and their ability to bond directly to bone [34, 35, 57]. However, there are several factors that may influence the performance of any hydroxyapatite coating such as: coating thickness, chemical composition, crystallinity, phase purity, cohesive and adhesive strengths, and resorption resistance. Adhesion strength of the coating to the implant surface appears to be a property that needs to be maximized to avoid cracking, shearing off, and chipping of the hydroxyapatite coating during emplacement of the implant. The ideal hydroxyapatite coating would be one with low porosity, strong cohesive strength, good adhesion to the substrate, a high degree of crystallinity and high chemical purity and phase stability [58].

In 1960s, the concept of biological fixation of load-bearing implants using bioactive hydroxyapatite and calcium phosphate coatings was proposed as an alternative to cemented fixation. Since Furlong and Osborn first began clinical trials using the hydroxyapatite-coated implants in 1985 [59], it has been reported that hydroxyapatite coatings can successfully enhance clinical success, and a less than 2% failure rate was reported during a mean follow-up study of 10 years [60,61]. Hydroxyapatite is stable in a body fluid, whereas tricalcium phosphate is rather soluble in the fluid [62]. Many studies have indicated that the dissolution of well-crystallized hydroxyapatite in the human body after implantation is too low to achieve optimum results. On the other hand, the dissolution rate of tricalcium

phosphate ceramics is too fast for bone bonding. To achieve an optimum dissolution rate of bone graft materials, research has focused mainly on biphasic calcium phosphate ceramics composed of hydroxyapatite and tricalcium phosphate [63, 64]. It is generally known that tricalcium phosphate is more soluble than hydroxyapatite at physiologic pH and more susceptible to bioresorption [65]. Partial dissolution of the calcium phosphate macrocrystals followed by an increase in the calcium and phosphate ion concentrations in the local environment is thought to be important for the excellent osteoconductivity and tight chemical bonding of the bioactive ceramics with bone [66]. Although greater, unpredictable solubility of the tricalcium phosphate coating may cause earlier failure of a hydroxyapatite/tricalcium phosphate-coated implant at the bone-implant interface [67], gradual resorption of this coating and replacement with new bone might be desirable to prevent the late complications of calcium phosphate coatings [68].

Since the clinical success of orthopaedic and dental implants depend on the osseointegration at the bone-implant interface; surfaces of bone-contacting devices would be desirable to be compositional, structural and functional analogous to that of human bone. Surface composition containing calcium and phosphate; display good cytocompatibility and enhanced bone contact and greater new bone apposition, particularly calcium. Okamoto et al. [69] reported that a significantly higher number of cells adhered to hydroxyapatite than to uncoated titanium. Wong et al. [70] compared the osseointegration of commercial implants in the trabecular bone of mature miniature pigs for 12 weeks. Their results showed excellent osseointegration of the hydroxyapatite coated implant. Likewise, Cao et al. [71] showed successful osseointegration of hydroxyapatite coatings with surrounding bone tissue when a hydroxyapatite coated implant was placed within living bone. Also, the success or failure of hydroxyapatite coated orthopaedic implants; depends on the control and consequences of cell behaviour post implantation [72]. Thus, the first and essential step for bone tissue-implant interface studies is *in vivo* tests using osteoblast cells due to the important role in which they play in the osteointegration of the implant. They have the ability to synthesise and produce extracellular matrix and to control its mineralization and thus regulate the “ingrowth” of bone to the implant. Rouahi et al. [73] examined the growth of Saos-2 cells on discs of microporous and non-porous hydroxyapatite in comparison to titanium. The surface morphology was found to have an effect on the behaviour of the cells. Richard et al. [72] cultured cells on calcium-deficient hydroxyapatite thin films produced using electrodeposition. Areas of the coating with two different morphologies and compositions were examined and the results were compared to those for cells cultured on cell culture plastic. In this study cell morphology, cell viability, cell proliferation and gene expression were examined over 28 days. The differentiation of osteoblast cells was found to be enhanced on the calcium phosphate coating compared to the titanium plate. Yang et al. [74] reported that cell proliferation and type I

collagen synthesis were higher on porous surfaces than on dense ones. This is related to greater protein absorption and to the increased surface area available for cell attachment. Wang et al. [75] carried out a study to determine the effect of the phase composition of calcium phosphate ceramics on osteoblast behaviour. The compositions studied were pure hydroxyapatite, a 70/30 mixture of hydroxyapatite and tricalcium phosphate and a 35/65 mixture of hydroxyapatite and tricalcium phosphate and pure tricalcium phosphate. In their study, the phase composition of the ceramics did not have a significant affect on the expression of the osteonectin and production of bone sialoprotein and osteocalcin in SaOS-2 cells.

Histologically comparing osseous apposition to hydroxyapatite coated implants and titanium implants has demonstrated mineralization of bone directly on hydroxyapatite surfaces with no fibrous tissue layer formation. However, a predominately fibrous tissue interface was observed on titanium implants, with only minimal areas of direct bone contact [76]. In addition, in an animal study hydroxyapatite-coated implants showed an increased coronal bone growth that was not observed with titanium implants [77]. Maintaining a bony osseous crest is essential clinically because it may prevent peri-implant saucerization and subsequent pocket formation [78, 79]. Other histometric studies in animal models have also exemplified that bone adapts in much less time to hydroxyapatite-coated implants than to titanium implants [80, 81].

Another area of recent advance is the use of drug releasing layers on hydroxyapatite coatings. These layers are designed to supply drugs, for example antibiotics and antiresorptive drugs, locally to the bone surrounding the implant. Drug releasing layers have been produced from numerous different polymeric and ceramic materials. The benefits of these drug release coating layers have been shown by a number of researchers [82, 83]. Ogiso et al. [82] used the antiresorptive drug zoledronate grafted to a hydroxyapatite coated implant. *In vivo* studies in rats showed an increase in mechanical fixation of the implants. Martins et al. [83] found that their collagen-hydroxyapatite composite paste had potential for use in sustained antibiotic release.

Biomimetic process (definition ?)

Some authors reported deposition of long and thin needle-shaped crystals of enamel-like calcium phosphate onto a bioactive glass in a supersaturated calcifying solution containing recombinant porcine amelogenins [84, 85]. It has been realized that nucleation and growth of calcium phosphate crystals *in vivo* are modulated by specific proteins in mineralizing tissues, intrinsically by functional groups in proteins. Other authors reported that some functional groups have the ability to induce bone-like apatite nucleation through a biomimetic way [85, 86]. A self-assembled monolayer (SAM) technique is an effective way to fabricate charged surface terminated with polar head groups [87]. The deposition of bone-like apatite could improve the biological properties for potential restorative

application. Thus, biomimetic strategies developed to design new materials, which are expected to improve biological and mechanical performance for biomaterials [88, 89].

Many researchers used bovine and human serum *in vitro* to analyze protein adsorption on biomaterials [90, 91]. The reactions occurring at the surface of biomaterials in contact with protein containing solutions have also been studied with Dulbecco's Modified Eagle's minimum essential medium supplemented with 10% Nu-Serum [92], which contains growth factors, hormones and vitamins. A step further to simulate *in vitro* the real condition of biomaterials immersed into body fluids is the immersion in cell-containing solutions. Osteoblast cells have often been used to understand the influence of the presence of biomaterials on cells, different tests can be done. Usually cell morphology, adhesion and proliferation are examined. Then, cell activity can be tested by the amount of some specific enzymes produced. For example, osteoblasts which are synthesizing bone matrix produce alkaline phosphatase. Another important protein that can be evaluated is osteocalcin. This is a non-collagenous extracellular matrix protein, and its presence is indicative of the beginning of bone mineralization.

Calcium phosphate cement systems

Calcium phosphate cement was discovered by Brown and Chow in the 1980's. This type of cement can be prepared by mixing a calcium phosphate salt with water or with an aqueous solution to form a paste that reacts at room or body temperature, giving rise to a precipitate containing one or more calcium phosphates, which sets by the intercrossing of the crystals of this precipitate.

This cement consists of two components, one basic and one acid, which react when mixed with water, producing one or more products with an intermediary acidity [93, 94].

In 1982, LeGeros et al. [95] presented preliminary studies on the possibility of developing apatitic calcium phosphate cements, with the rationale that such cements would have the unique combination of the following properties: (i) compatibility with the tooth mineral; (ii) adjustability of composition (with or without F⁻, Mg²⁺, Sr²⁺, etc.); and (iii) esthetics.

Calcium phosphate cements have been evaluated as one of the potential materials for bone tissue engineering. An advantage of calcium phosphate cement is that they can be directly injected into the bone defect and allowed to set *in situ*. Calcium phosphate cements also are biocompatible and resorbable; they can be synthesized with a macroporous structure having micropores that are very crucial for cellular growth and infiltration [96, 97].

In 1987, Brown and Chow developed a novel class of low temperature setting calcium phosphate cements from precursors such as dicalcium phosphate dihydrate, dicalcium phosphate anhydrous, and tetra calcium phosphate [98]. These low temperature apatites, are receiving a great deal of attention due to their ability to set at physiological temperature to form hydroxyapatite that resembles biological apatites without the addition of any additives [99, 100]. This is highly advantageous because acrylic cements currently used for orthopaedic applications require high temperature for setting and use of toxic reagents [99]. Another advantage of calcium phosphate cement is that during the setting reaction only a small amount of heat is released as compared to polymethylmethacrylate cements and also the volume of calcium phosphate cement remains constant during the setting reaction [99].

Upon mixing with water or aqueous solution, the calcium phosphate cement dissolves and precipitates into a less soluble calcium phosphate. During precipitation, the calcium phosphate crystals increase in size and get inter-locked thus providing structural rigidity to the cement. Hydroxyapatite thus formed in aqueous solution is poorly crystalline [99]. When used for *in vivo* applications, a thick paste of calcium phosphate cement can be formed in the presence of water or aqueous solutions which can be injected or sculpted during surgery into the defect site and self hardens to form hydroxyapatite *in situ* [100-102]. Hence these biomaterials do not require shaping and can be prepared at operating room conditions. They provide excellent contact between the bone and the graft. Since most of the current orthopaedic implants are available in hardened form, the moldability and *in situ* hardening of calcium phosphate cement along with its osteocompatibility make it a desirable alternative for current orthopaedic implants. Moreover, since the calcium phosphate cements are fabricated at room or at body temperatures, also they can be used as drug delivery vehicle for antibiotics, anti tumor drugs, anti inflammatory drugs, and growth factors [103, 104].

However currently available calcium phosphate cement systems are far from ideal properties due to the discrepancies in the setting time, mechanical properties, and *in vivo* response of the cements [105]. Also, they are used under development for furcation sealing [106], root surface desensitization and root apex sealing or root canal filling [107,108]. The abilities of self-setting, fair compressive strength and biocompatibility suggest that calcium phosphate appears superior to pure calcium hydroxide, thus this material may have potential for dentine regenerating pulp capping or lining materials [109, 110]. Calcium phosphate cement systems also have been used as bone fillers and to deliver bioactive agents due to its osteoconductivity, osteotransductivity, and suitable mechanical properties [107-114].

Tricalcium phosphate

Tricalcium phosphate exists in many polymorphs (α , β , γ and super- α) [115]. The only two polymorphs phases (α and β) are used as biomaterials. These phases have received much attention [116]. However, despite the extensive research since the early 1970s, there is still lack of clarity concerning this material. The use of resorbable tricalcium phosphate materials is preferred since they will be in the long term replaced by bone.

Clarke et al. [117] reported a method of preparing tricalcium phosphate ceramic and suggested its use as a bone graft material. Levin et al. [118] reported that the first dental application of a tricalcium phosphate ceramic in periodontal defects in dogs. Koenigs et al. [119] used resorbable form of tricalcium phosphate ceramic to induce apical closure. Formation of mineralized tissue occurred within the root canal, but was incomplete. Roberts and Brilliant [120] used tricalcium phosphate ceramic to induce apical closure in human permanent pulpless teeth with large open apices, but found it to be no more effective than calcium hydroxide. Brown and Chow [121] tested a tricalcium phosphate and brushite combination. X-Ray diffraction revealed a conversion to HA in a few minutes with compressive strengths of up to 500 psi. Coviello and Brilliant [122] tested the apical barrier of 101 teeth. They found that no difference in healing between cases treated with tricalcium phosphate or calcium hydroxide. Gruninger et al. [123] tested a combination of tricalcium phosphate, hydroxyapatite and sodium fluoride as a bone implant material. They determined the material to be neither toxic nor mutagenic, and not resorbable. They encouraged the evaluation of these materials as root canal filler. Functionally graded

coatings consisting of fluorine-substituted apatite (FA) and beta-tricalcium phosphate (β -TCP) were also produced by Wong et al. [124]. The coating produced had four layers, the outermost layer containing FA + 50 wt% TCP, the next FA + 40 wt% TCP, + 30 wt% TCP and finally the innermost FA + 20 wt% TCP. The HA component of the coating is expected to enhance early-stage bone ingrowth and bone bonding, whereas the remaining porous FA component aims achieve long-term fixation of an implant. Tricalcium phosphate materials mostly behave as osteoconductive materials, which permits bone growth on their surface or into pores, channels or pipes [125]. Tricalcium phosphate is biocompatible material and useful for inducing hard tissue formation [126,127]. It has been used as capping agent [126], cleft palate [128], apical barrier [122], apexification [129], vertical bone defect [130], and implants coating [65]. Tricalcium phosphate is a resorbable phase calcium phosphate and exhibits some good properties. It has also been shown to support bone growth [1131]. However, it is difficult to sinter, shows poor mechanical strength and low resistance to crack-growth propagation. Further, the rate of resorption of tricalcium phosphate is fast and uncontrolled [132]. Unpredictable solubility of the tricalcium phosphate coating may cause earlier failure of coated implant.

CONCLUSION

The applications of calcium phosphate materials in dentistry still limited and need further investigations to improve their properties and extend their clinical use.

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((((اسم البحث))))

فؤاد الصبري
الكلية

ملخص

تتشابه مركبات فوسفات الكالسيوم في التركيب مع المركبات الأساسية للعظام ، وتمتلك موادها الأساسية خاصية القدرة على تدعيم و التئام الأنسجة العظمية ، كما أن مركبات فوسفات الكالسيوم لها أشكال مختلفة منها المادة الإسمنتية والطلائية المستخدمة كثيرا في تطبيقات طب الأسنان. بين هذا البحث أحر التطبيقات العلمية التي أجريت لتطوير الخواص الميكانيكية والأنواع الشائعة لمركبات فوسفات الكالسيوم مثل كالسيوم فوسفات، هيدروكسي أبتيث وفوسفات الكالسيوم الثلاثية واستخداماتها.

كلمات مفتاحية: مركبات فوسفات الكالسيوم، طب الأسنان، هيدروكسي ابتيث، فوسفات الكالسيوم الثلاثية