# A brief review on hydroxyapatite production and use in biomedicine

# *(Uma breve revisão sobre a obtenção de hidroxiapatita e aplicação na biomedicina)*

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**Abstract**

Hydroxyapatite (HAp) is a bioceramic widely studied due to its chemical similarity with the mineral component of bones. Besides, it is biocompatible, bioactive and thermodynamically stable in the body fluid what poses it as an attractive material for a wide range of applications in the biomedical field. Several efforts have been focused on the synthesis of particles of this material aiming to the precise control of size and morphology, porosity and surface area. HAp is widely used as an implant for bone tissue regeneration, as a coating for metallic implants and in a drug-controlled release. In this sense, the objective of this review is to gather information related to HAp, providing readers with information about synthesis methods, material characteristics and their applications.
**Keywords**: hydroxyapatite, synthesis, biomedical applications.

***Resumo***

*A hidroxiapatita (HAp) é uma biocerâmica amplamente estudada devido à sua similaridade química com o componente mineral dos ossos. Além disso, é biocompatível, bioativa e termodinamicamente estável no fluido corporal, o que a coloca como um material atraente para uma ampla gama de aplicações no campo biomédico. Diversos esforços têm sido focados na síntese de partículas deste material visando o controle preciso de tamanho e morfologia, porosidade e área superficial. HAp é amplamente utilizada como implante para a regeneração do tecido ósseo, como revestimento para implantes metálicos e na liberação controlada de drogas. Neste sentido, o objetivo desta revisão é reunir informações relacionadas à HAp, fornecendo aos leitores informações sobre os métodos de síntese, características do material e suas aplicações.*
***Palavras-chave**: hidroxiapatita, síntese, aplicações biomédicas.*

## INTRODUCTION

The knowledge about the structure and chemical composition of bones was gradually acquired over time. It dates back the ancient civilizations that used bones as tools and extended until present time with the understanding of the basic unit that constitutes them [1, 2]. Bone is a hierarchically ordered structure having a uniform arrangement of calcium phosphate (CaP) nanocrystals (with calcium deficiency) in collagen fibrils, which may be settled in a lamellar or layer by layer fashion, depending on the nature of the bone [3]. Bone tissue is a specialized form of connective tissue, metabolically active, composed by an extracellular matrix (ECM) and some cell types, called osteoclasts, osteoblasts and osteocytes. These cells are immersed in the mineralized ECM, composed of organic and inorganic phases, which together give resistance, resilience and hardness to the tissue. Osteoclasts are multinucleated cells that participate in the dynamic and cyclic process of bone resorption and remodeling [4]. They are followed by osteoblasts that deposit the organic part of the matrix and many are trapped in the ECM becoming osteocytes cells. In turn, osteocytes act in the maintenance of bone mineralized matrix. Therefore, the bone has the intrinsic capacity of regeneration and it is the only tissue that is reconstructed without scar formation. However, since bone regeneration depends on the type of defect it is necessary to analyze strategies to improve tissue repair capacity [4].

The inorganic phase of the bone is composed by calcium phosphates (CaP), predominantly in the form of hydroxyapatite [$Ca_{10}(PO_4)_6(OH)_2$, corresponding to 65%-70%], and bone water with 5%-8%. The organic phase is mainly in the form of collagen, a large fibrous protein with repetitive amino acid sequences formed in triple helix structure, equivalent to the remaining portion [5-8]. Collagen is responsible for the elastic resistance of bone, acting as a matrix for the deposition and growth of mineral salts [5, 6, 9, 10]. The bone recovery process can be aided by the use of calcium phosphates such as amorphous calcium

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phosphates (FCA), brushite ($CaHPO_4.2H_2O$), monetite ($CaHPO_4$), tricalcium phosphate [$Ca_3(PO_4)_2$] (TCP), α-TCP and β-TCP, and hydroxyapatite (HAp). The biological and medical interest in these phosphates is due to their biocompatibility, bioactivity, resorbability, and chemical similarity to the mineral component of mammalian bones and hard tissues. However, crystalline HAp has called the attention of researchers because of its thermodynamic stability in the body fluid [6, 7, 11]. The number of routes for the production of HAp particles has increased due to its wide range of applications in the biomedical field, either as an implant material for bone tissue regeneration increasing osteogenesis or as a coating material improving implants bioactivity. Moreover, HAp is seen as a suitable material for the controlled and sustained release of drugs into particular sites, stimulating the growth of osteoblastic cells [12, 13]. This work briefly reviews the state of art related to HAp gathering information about the synthesis methods, material characteristics, use of doping ions, and some applications.

## HYDROXYAPATITE

Living organisms are able to crystallize and deposit various minerals during biomineralization processes, such as CaPs, for example [14]. These, in turn, are produced in vertebrates not only in normal calcifications, occurring for instance in bones and teeth, but also in pathological calcifications as in urinary and dental stones, as well as in atherosclerotic lesions of tissues [15, 16]. Attempts to determine the chemical structure and composition of CaP, formerly called apatites, began in the mid-18$^{th}$ century. However, only in the 19$^{th}$ century, the existence of different phases of CaP was proposed [17]. In Table I, some calcium phosphates are shown; they vary in chemical composition and solubility values. The stability of CaP phases is directly related to both the presence of water in the synthesis stage and the medium where it is applied. At body temperature, which is about 37 °C, only two phases of these phosphates are stable when in contact with body fluids. At pH lower than 4.2, the stable phase is dicalcium phosphate dihydrate ($CaHPO_4.2H_2O$), and at a pH higher than 4.2, the stable phase is HAp [$Ca_{10}(PO_4)_6(OH)_2$]. However, at higher temperatures, other phases may be present, such as calcium β-triphosphate (β-TCP) [$Ca_3(PO_4)_2$] and tetracalcium phosphate [$Ca_4(PO_4)_2O$]. These CaPs, when calcined at a high temperature and in a dry environment, interacting with water or body fluids, degrade and form HAp [39]. HAp is one of the most stable salts at room temperature and it is usually carbonated and calcium deficient with a Ca/P ratio equal to 1.67 [40, 41]. It is a compound of great interest also for catalysis, fertilizer and pharmaceutical industry, protein chromatography applications and water treatment processes [42].

The most commonly found structure of HAp belongs to the hexagonal system with space group $P6_3/m$, showing symmetry perpendicular to three equivalent 'a' axis ($a_1$, $a_2$ and $a_3$), which form angles of 120° to each other. Its unit cell is composed by calcium (Ca) and phosphates and may be represented by $M1_4M2_6(PO_4)_6(OH)_2$, in which M1 and M2 are two different crystallographic positions for 10 calcium atoms. Four Ca atoms are surrounded by nine oxygen (O)

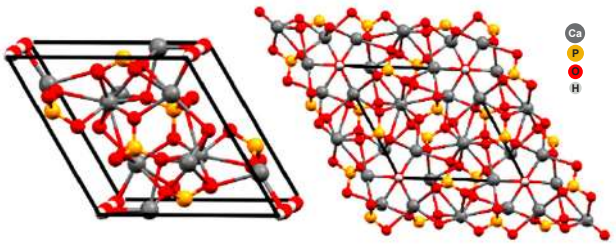


Figure 1: Crystalline structure of the HAp (left) and the projection of the HAp structure in plane 001 (right).
*[Figura 1: Estrutura cristalina do HAp (esquerda) e projeção da estrutura HAp no plano 001 (direita).]*

Table I - Main calcium phosphates.
*[Tabela I - Principais fosfatos de cálcio.]*

| Ca/P | Name | Symbol | Formula | Ref. |
|---|---|---|---|---|
| 0.5 | Monocalcium phosphate monohydrate | MCPM | $Ca(H_2PO_4)_2.H_2O$ | [18, 19] |
| 0.5 | Monocalcium phosphate anhydrous | MCPA | $Ca(H_2PO_4)_2$ | [20] |
| 1.0 | Dicalcium phosphate dihydrate | DCPD | $CaHPO_4.2H_2O$ | [21, 22] |
| 1.0 | Dicalcium phosphate anhydrous | DCPA | $CaHPO_4$ | [23, 24] |
| 1.33 | Octacalcium phosphate | OCP | $Ca_8(HPO_4)_2(PO_4)_4.5H_2O$ | [25, 26] |
| 1.5 | α-Tricalcium phosphate | α-TCP | $\alpha$-$Ca_3(PO_4)_2$ | [27, 28] |
| 1.5 | β-Tricalcium phosphate | β-TCP | $\beta$-$Ca_3(PO_4)_2$ | [29, 30] |
| 1.2-2.2 | Amorphous calcium phosphate | ACP | $Ca_x(PO_4)_y.nH_2O$ | [31, 32] |
| 1.5-1.67 | Hydroxyapatite deficient in calcium | CDHA | $Ca_{10-x}(HPO_4)_x(PO_4)_{6-x}(OH)_{2-x}$ (0<x<1) | [33, 34] |
| 1.67 | Hydroxyapatite | HA or HAp | $Ca_{10}(PO_4)_6(OH)_2$ | [35, 36] |
| 2.0 | Tetracalcium phosphate | TTCP | $Ca_4(PO_4)_2O$ | [37, 38] |

*Note: the solubility values of each CaP can be found in [15].*

atoms of the phosphate groups at the M1 position, belonging to the $PO_4$ tetrahedron. Six other Ca atoms are surrounded by the remaining six O atoms and one of the two OH [3, 43], as shown in Fig. 1. In addition, regardless of its origin, HAp may contain impurities in its structure, such as phosphite ($PO_3^{3-}$), chloride ($Cl^-$), fluoride ($F^-$) and hydroxyl ($OH^-$) ions [17].

HAp has aroused great interest in the field of biomedicine for different applications, such as implants or prostheses in orthopedics, maxillofacial and dentistry, aiming the repair or replacement of hard tissues. This is due to its excellent biocompatibility and bioactivity derived from its chemical analogy to the mineral compounds of human bones and teeth hard tissues, as mentioned before [44]. Moreover, HAp has good mechanical strength, porous structure and osteoconductive, osteoinductive and osteointegrative properties [45]. HAp can be used as an implant material in the form of a solid body with low porosity, granular particles, porous structures and loads. In addition, it can be used as a coating on metal implants improving their biocompatibility [44, 46]. When a HAp-based material is implanted, a free layer of fibrous tissue composed by carbonated apatite is formed on its surface, contributing to the attachment of the implant to the living bone, i.e. improving implant fixation in the surrounding tissues [47]. HAp can promote new tissue growth through the osteoconduction mechanism, without causing systemic or local toxicity, inflammation or similar responses caused by other foreign bodies. The porosity of HAp is one of the most important factors for controlling implant performance, since an open pore structure allows the diffusion of cells responsible for the bone tissue deposition, accounting for a better bio-integration and mechanical stability of the implant. Pores must be interconnected and have dimensions between 100 and 500 μm [48-50]. HAp may also be used as solid scaffolds for tissue reconstitution in tissue engineering to model compounds for biomineralization of human body [9, 10], growth inhibition of many types of cancer cells [51], medicine supply [52], dental enamel repair [53], and injectable bone cement [54]. The mixture of HAp with other phosphates, such as β-tricalcium phosphate (β-TCP) and calcium pyrophosphate, provides greater applicability of this material, since it allows greater cell adhesion and osteogenic characteristics [55]. However, the application of the porous material is restricted to regions where the skeleton is not being mechanically requested, or as a filling of bone cavities because it presents low mechanical resistance. Table II shows the mechanical properties of hydroxyapatite compared to other ceramic materials and some hard tissues of the body. In view of the wide range of HAp applications, several routes have been devised in the last decade aiming the synthesis of HAp. The following discussion presents a description of the routes developed so far.

## SYNTHESIS OF HYDROXYAPATITE

According to the method, reagents and variables adopted, it is possible to obtain materials based on CaP with different phases, which results in materials with diverse characteristics and properties, such as crystalline defects, high surface area, organic material affinity found in physiological medium and so on [46]. HAp can be synthesized by a variety of techniques that can be broadly grouped into six sets of methods: i) dry methods: involve solid state and mechanochemical reactions [63-67]; ii) wet methods: based on low-temperature chemical precipitation [68], co-precipitation [66, 69, 70], sol-gel route [71-76] and hydrolysis [77, 78]; iii) hydrothermal methods: use aqueous solutions of high temperature and high voltage, as hydrothermal [79-81], emulsion and microemulsion [82, 83], and sonochemical [84]; iv) high temperature processes: include combustion [85, 86] and pyrolysis [87, 88]; v) synthesis based on biogenic sources: can be extracted from fish bones [89], shells [90], eggshells [91, 92], bovine bones [93, 94], in the presence of biomolecules [95-97] or biomembranes [98]; and vi) combination of the aforementioned methods. All methods used for synthesizing HAp particles have different processing characteristics and can result in different morphologies, as shown in Table III. Powders' strength and osteointegration are critical characteristics that depend significantly on their microstructure. Therefore, the main challenge in the synthesis of HAp is to control the crystal growth because its microscopic shape, size and size distribution can considerably affect its mechanical properties, processing conditions, surface chemistry, biocompatibility and bioactivity [99-102]. Thus, synthetic procedures that can

Table II - Properties of hydroxyapatite compared to some human tissues.
*[Tabela II - Propriedades da hidroxiapatita comparada a alguns tecidos humanos.]*

| Material | Compressive strength (MPa) | Flexural strength (MPa) | Modulus of elasticity (GPa) | Ref. |
|---|---|---|---|---|
| Hydroxyapatite | 300-600 | 60-115 | 40-120 | [56-58] |
| Stabilized zirconia | 1700-2000 | 421-800 | 195-210 | [59] |
| Alumina | 1000-2800 | 280-420 | 350-400 | [60] |
| Cortical bone | 88-230 | 88-115 | 3-30 | [58, 61, 62] |
| Dentin | 290-380 | 51,7 | 15-20 | [58, 62] |
| Tooth enamel | 250-550 | 10,3 | 10-90 | [61, 62] |

Table III - Methods used to produce HAp.
*[Tabela III - Métodos utilizados para produzir HAp.]*

| Method | Type | Temperature | Morphology | Purity | Ca/P ratio | Particle size | Ref. |
|---|---|---|---|---|---|---|---|
| Dry | Solid-state reaction | ~1000 °C | Miscellaneous | Low | Variable | Micron | [105-109] |
| | Mechanochemical | ~1000 °C | Miscellaneous | Low | Non-stoichiometric | Nano | [90, 110, 111] |
| Wet | Chemical precipitation | 100-1300 °C | Miscellaneous | Variable | Non-stoichiometric | Most nanosized | [46, 112-120] |
| | Hydrolysis | ~900 °C | Miscellaneous | High | Stoichiometric | Variable | [121] |
| | Sol-gel | 500-1300 °C | Miscellaneous | Variable | Stoichiometric | Nano | [46, 71, 122-130] |
| Hydrothermal | Hydrothermal | ~120 °C | Spherical or needle-like | High | Stoichiometric | Nano or micron | [131-138] |
| | Emulsion | ~25 °C | Spherical or needle-like | Variable | Non-stoichiometric | Nano | [83, 139-141] |
| | Sonochemical | 600-1000 °C | Miscellaneous | High | Variable | Nano | [84, 142, 143] |
| High temperature | Combustion | 100-1300 °C | Miscellaneous | High | Variable | Most nanosized | [46, 144-146] |
| | Pyrolysis | ~600 °C | Miscellaneous | Variable | Stoichiometric | Nano or micron | [87, 88] |
| Biogenic sources | | - | Miscellaneous | High | Variable | Variable | [89, 91-97] |
| Combination of methods | | - | Miscellaneous | Variable | Stoichiometric | Most nanosized | [36, 76, 102, 147-149] |

precisely control the geometry of the crystal are of great importance for expanding the potential applications of particles and nanoparticles obtained. Among the traditional methods showed in Table III, the emphasis has been placed on the development of synthesis procedures by precipitation, sol-gel and hydrothermal reaction. Such techniques provide an improvement in the material mechanical properties due to high values of density and homogeneity of the obtained microstructure, even at relatively low sintering temperatures [103]. The preparation of HAp particles with well-defined stoichiometry, high aspect ratio and high crystallinity still has limitations. Conventional mechanical and wet chemical methods are capable of promoting better stoichiometry control of the final product. However, if Ca/P stoichiometry is not adjusted to 1.67 during the precipitation step other phases can be formed, such as β-TCP and CaO [104]. Biomedical applications require precise control of size and morphology of the particles, porosity and surface area, which are fundamental features ensuring the reactivity and interaction with the biological system.

*Dry methods*

Dry methods do not require the use of a solvent. These methods are widely used for mass production of powders with no need for precise control of processing parameters, since they do not strongly influence the characteristics of powders obtained. Solid-state synthesis is a relatively simple and low-cost procedure. In the production of HAp, for example, precursor reagents containing calcium and phosphate are mixed and then calcined at elevated temperatures (approximately 1000 °C) during a defined period of time [105-107, 109]. However, heterogeneous powders results, since secondary phases are present in the final product [107], due to the low diffusion coefficient and the need for long firing times to homogenize the system composition. This is a disadvantage, especially when related to biomedical applications, in which precise control of product characteristics is extremely relevant. The solid-state synthesis of HAp can be performed through two main reactions with water vapor at ~1200 °C [44]:

$$3CaP_2O_7+7CaCO_3+H_2O \rightarrow Ca_{10}(PO_4)_6(OH)_2+ CO_2+7.5O_2 \quad (A)$$

$$3Ca(PO_4)_2+7CaCO_3+H_2O \rightarrow Ca_{10}(PO_4)_6(OH)_2+CO_2+9O_2 \quad (B)$$

Mechanochemical synthesis has been used to improve kinetic performance in the preparation of HAp powder by a dry method. Here, materials containing precursor ions are ground and mechanical energy promotes chemical reactions and structural changes [90, 110, 111]. The method is simple and produces powders with a well-defined structure, enabling the manufacturing of several advanced materials, including HAp [150]. Some of the most relevant reactions involved in the synthesis are [151]:

$$6CaHPO_4.2H_2O+4CaO \rightarrow Ca_{10}(PO_4)_6(OH)_2+4H_2O \quad (C)$$

$$10CaC\,O_3+6(NH_4)H_2PO_4 \rightarrow Ca_{10}(PO_4)_6(OH)_2+8H_2O+10CO_2+6NH_3 \quad (D)$$

$$3Ca_3(PO_4)_2.xH_2O+Ca(OH)_2 \rightarrow Ca_{10}(PO_4)_6(OH)_2+xH_2O \quad (E)$$

$$10Ca(OH)_2+3P_2O_5 \rightarrow Ca_{10}(PO_4)_6(OH)_2+9H_2O \quad (F)$$

*Wet methods*

Wet methods consist of a set of techniques that use reactions in the presence of a solvent medium for the production of powders. These techniques use different temperatures, types of solvents and chemical precursors. Such methods have been conventionally applied to the synthesis of HAp particles with nanometric structure and regular morphology, aiming the process of biomineralization *in vivo* [152, 153]. They also present simple processing, allowing the crystal growth control through its parameters and the attainment of more homogenous materials. However, one disadvantage is still the production of different CaP phases. Chemical precipitation and sol-gel are the most prominent syntheses among wet methods.

*Chemical precipitation*: the synthesis of HAp via chemical precipitation consists of the use of aqueous solutions in which chemical reactions occur between calcium and phosphorus ions, under controlled pH and temperature. The neutralization reaction is the most used, releasing water as a byproduct [12, 112]. The precipitated powder is generally calcined between 400 and 600 °C, or even higher, to obtain a stoichiometric apatite structure [154]. Chemical precipitation is a simple and low-cost method, in addition to producing HAp with characteristics similar to those of bone and dental tissue. However, most synthetic methods lead to the formation of non-stoichiometric products containing various phases resulting from the presence of vacancies and ionic substitutions in the lattice such as carbonates, hydrogen phosphates, potassium, sodium, nitrate and chloride [12]. The morphological properties (shape and size), stoichiometry, specific surface area and degree of crystallinity of the synthesized HAp through precipitation are greatly affected by the synthesis parameters such as temperature [155], time [156], reagent addition rate [155], calcination [156, 157], pH [158], and use of different reagents and their purity. Slow addition of phosphate ions provides lower nucleation rate and higher growth rate of crystals, which leads to larger particles [12, 112]. Moreover, the HAp particle size increases linearly with temperature [156, 157]. Another strategy used to control mineral properties is the biomimetic mineralization, which consists in applying organic macromolecules that act as a model. Such molecules may act as nucleating centers and/or be adsorbed on the crystal surface, varying its properties [151]. HAp obtained by precipitation uses calcium nitrate or calcium chloride salts with ammonium hydrogen phosphate at pH values greater than 4.2, adjusted with concentrated ammonium hydroxide. The reaction temperature can vary between the room temperature and near the boiling point of the water [159, 160]. A general reaction can be shown as follows [161]:

$$10Ca(NO_3)_2+6(NH_4)_2HPO_4+8NH_4OH \rightarrow Ca_{10}(PO_4)_6(OH)_2+20NH_4NO_3+6H_2O \quad (G)$$

Fig. 2 shows the typical steps for production of HAp particles using the chemical precipitation. Briefly, it consists of the dropwise addition of one reagent to another under continuous and slow magnetic stirring, while the molar ratio Ca/P is maintained at 1.67. Finally, the resulting suspension is subjected to atmospheric pressure or immediately washed, filtered, dried, calcined and ground [162]. Rigo et al. [112] produced HAp powder by the precipitation method, with no other phases related to Ca-P system coexisting. The powder obtained was composed of fine particles, forming uniform and dispersed agglomerates. Clinical cases studied have demonstrated that this product is efficient to promote the growth of bone tissue. More recently, Ghosh and Sarkar [46] carried out a study addressing the synthesis of HAp through different chemical routes (precipitation, sol-gel and

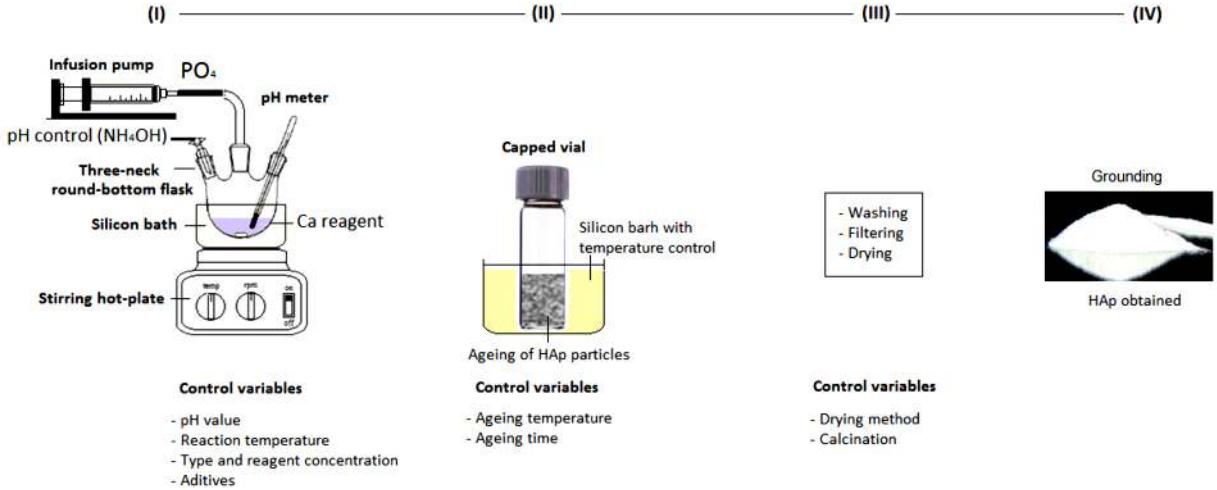


Figure 2: Schematic of chemical precipitation process for production of hydroxyapatite.
*[Figura 2: Esquema do processo de precipitação química para produção de hidroxiapatita.]*

combustion methods) and found that the powder produced by precipitation showed highest density and hardness, besides positive bioactivity. Therefore, precipitation is considered to be the most adequate among the three methods quoted.

*Sol-gel*: the sol-gel process is a wet chemical method well known for its flexibility. It offers different chemistry to fabricate a wide range of structural materials with particle sizes ranging from nanometer to micrometer scale [163-165]. Sol-gel technique involves the conversion of monomers into a sol (a colloidal suspension of solid particles) that acts as the precursor of a 3D network solid phase. Various precursors may be used in the synthesis via sol-gel. In most cases, calcium dioxide or calcium nitrate are reacted with triethyl phosphite or triethyl phosphate in aqueous or organic solution. A general reaction can be shown as follows [151]:

$$10Ca(NO_3)_2+6(C_2H_5O)_3P(O)\rightarrow Ca_{10}(PO_4)_6(OH)_2+\text{by-products} \quad (H)$$

During the process, precursors (usually alkoxides) are mixed, aged, gelled, dried and calcined to remove the organic part (Fig. 3) [35, 164]. The materials here obtained have high purity due to the careful control of process parameters, and are produced with a homogeneous composition at low synthesis temperature, giving rise to ultrafine ceramic powders [71, 86, 122, 166, 167]. The sol-gel technique has been used in conjunction with spinning techniques for the production of ceramic fibers [36, 168-170]. Sol-gel is an effective method for preparation of HAp powders, as it favors mixing in ionic level between calcium and phosphorus, improving chemical and physical homogeneity and resulting in a refined microstructure. This, in turn, favors the reaction and stability within artificial/natural bone interface [124, 147, 154, 171, 172]. Hsieh et al. [71] studied the effect of thermal aging on the molecular structure of HAp precursors for gelation processes of fast and slow drying precursors, subsequently calcined at 600 °C. X-ray diffraction patterns of rapid drying gels revealed an intense peak of CaO, while for slow drying gel the coexistence of major peaks of HAp and a very weak CaO peak indicated the formation of a calcium phosphate complex during aging. Therefore, adequate gel aging tends to complete incorporation of $Ca(NO_3)_2$ into the complex, decreasing CaO formation. In addition, they have found that the CaO derived in the slow drying method can be removed through rinsing with distilled water [71]. Recently, Nazeer et al. [123] have shown that dielectric constant and polarity of the solvent mixture strongly influence the chemical structure and morphological properties of the synthesized calcium phosphate. The water-based composition, which has higher dielectric constant, mainly produced β-calcium pyrophosphate (β-CPP) with a small amount of HAp. Dimethylformamide/water composition produced HAp as the major phase with a small amount of β-CPP; while the tetrahydrofuran/water composition, which has lower dielectric constant, resulted in the formation of pure HAp.

*Hydrothermal methods*

Hydrothermal synthesis can be described as chemical precipitation occurring at elevated temperature and pressure, using organic modifiers to control the morphology and structure of crystals obtained [131, 132]. The corresponding hydrothermal reaction occurs as follows [173]:

$$4Ca(OH)_2+6CaHPO_4.2H_2O\rightarrow Ca_{10}(PO_4)_6(OH)_2+18H_2O \quad (I)$$

Yoshimura et al. [80] produced HAp whiskers under hydrothermal conditions in the presence of disodium ethylenediaminotetraacetic salt ($Na_2EDTA$). The morphology of HAp was significantly affected by the pH, $Na_2EDTA$/Ca ratio and reaction temperature; HAp microspheres with high crystallinity resulted from conditions with high pH, high temperature and moderate retention time. Hoai et al. [138] and Ortiz et al. [135] synthesized HAp nanoparticles by varying the concentrations of hexamethylenetetramine (HMTA) and cetyltrimethylammonium bromide (CTAB), as well as the cooling rate. Rapid cooling produced particles with smaller average sizes, while higher concentrations of additives resulted in the opposite effect. Besides that, Hoai et al. [138] obtained HA nanorods, tested their biomineralization *in vitro* on simulated body fluid and suggested high bioactivity of HAp

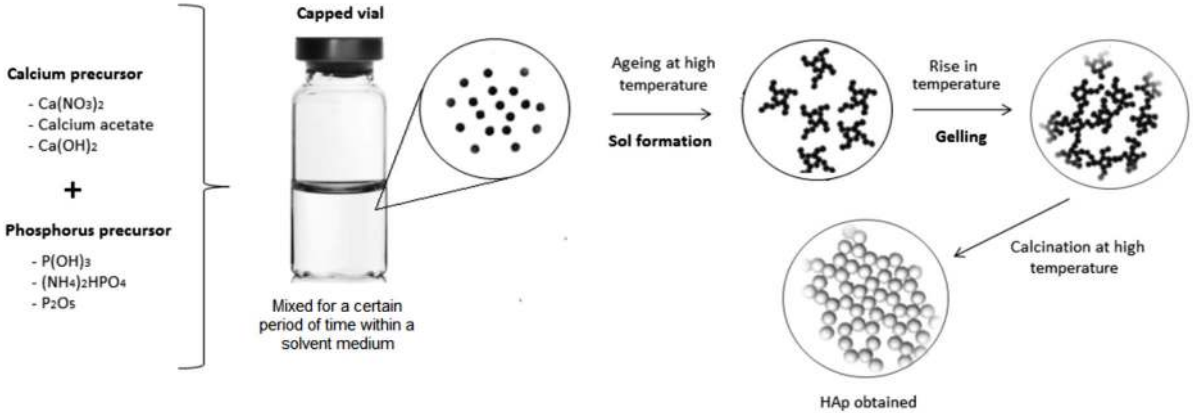


Figure 3: Schematic of the sol-gel process for the production of hydroxyapatite.
*[Figura 3: Esquema do processo sol-gel para produção de hidroxiapatita.]*

prepared by hydrothermal method.

Sonochemical methods allow the synthesis of uniform particles with nanosized products resulting from the chemical reaction that occurs in the presence of ultrasonic radiation [84]. Utara and Klinkaewnarong [84] synthesized nanometric HAp through a sonochemical process (20 kHz) at 25 °C and different irradiation times, followed by calcination at 600 °C. They observed that the smaller diameter (nanorods 8 nm) resulted from an ultrasonic irradiation time of 20 min for a Ca/P ratio of 1.5.

Finally, the synthesis of HAp by emulsion inhibits the formation of agglomerates and makes possible to control the size and morphology of the particles produced [83, 139, 140]. This synthesis can be performed in water-in-oil emulsion [139], oil in water [83], and double emulsion water-oil-water [140] using different surfactants. Recently, Amin et al. [139] produced nanocrystalline HAp by reverse microemulsion technique. They have demonstrated that emulsion reactions control morphology, particle size and minimize the transformation of HAp phases. Ma et al. [83] also sintered spherical nanosized HAp by water-in-oil microemulsion at room temperature in a short time. The particles obtained at 25 °C and a reaction time of 5 h facilitated the formation of spherical HAp with uniformity and regularity.

*High-temperature processes*

High-temperature processes promote decomposition and reactions of HAp precursors leading to the synthesis of materials through short firing cycles. In the combustion synthesis, the process is carried out in a single step, obtaining a final product of high purity as HAp bioceramics. Combustion is a fast, low-energy, exothermic technique in which a self-sustained chemical reaction occurs between an oxidant and a suitable organic fuel such as urea, hydrazine, citric acid, and glycine, in an aqueous medium. The exothermic reaction provides the heat needed to sustain combustion which, once started, does not require any external heat source [144]. However, it is necessary to control processing parameters as fuel/oxidant ratio, furnace initial temperature, fuel nature and amount of initial precursor to keep control of the maximum reaction temperature and consequently the powder final characteristics. Han et al. [85], and more recently Kavitha et al. [144], produced Sr-doped HAp and HAp powders, respectively, through the combustion process. The obtained powders presented high purity, structural and chemical homogeneity and high surface area.

In spray pyrolysis synthesis, particles are formed through the aerosol process that atomizes a solution and heats the droplets to produce solid particles with no addition of fuel. The precursor solution is sprayed in a flame or in a hot zone of an electric oven by an ultrasonic spray generator [87, 88]. Cho et al. [87] synthesized HAp powders via spray-pyrolysis and observed that the reduction of precursor concentration and an increase in the spray pyrolysis temperature caused a decrease in particle sizes.

*Synthesis by biogenic sources*

HAp can be obtained from biogenic sources such as eggshell by means of calcination at high temperature, forming CaO, followed by hydration and reaction with $H_3PO_4$, thus precipitating HAp [91, 92]. Padmanabhan et al. [92] produced a porous HAp/collagen structure in which the obtained HAp was nucleated in the collagen matrix through the chicken eggshell. The synthesized HAp showed chemical interaction with the collagen molecule, resulting in better mechanical properties compared to pure collagen support. Fish scales can be used after the removal of organic components for hydrothermal synthesis of HAp in the reaction with ammonium dihydrogen phosphate. Milovac et al. [89] synthesized HAp coated with polycaprolactone (PCL) through fish scales. Bone-like apatite was formed during *in vitro* mineralization for both pure HAp as in hybrids with PCL polymer. Pal et al. [90] produced HAp through mechanochemical method reacting CaO from mollusk shells and phosphoric acid. *In vitro* biocompatibility studies using osteoblasts (MG63) and fibroblast cells (NIH 3T3) indicated the non-toxic nature of the obtained HAp powder. HAp can be extracted from bovine cortical bone by performing heat treatment at high temperature in order to synthesize HAp powder, followed by milling to obtain particles in the desired size [93, 94].

The synthesis of nanometric particles in the presence of biomolecules derived from various natural materials is another approach to the preparation of HAp using natural sources. Biomolecules, such as amino acids, carotene, papain, carotenoids, and vitamins, are considered as medicinally important materials, and when used in small quantities exert significant control over the synthesis of nanometric HAp, causing a significant change in both size and morphology of the resulting powder [95-97]. Studies have reported the use of natural membranes to produce HAp nanostructures through diffusion-controlled nucleation. For example, the egg membrane, which is composed by interlaced collagen fibers with nanometric pores, can be explored to control the diffusion of phosphate ions and calcium ions during nucleation of HAp, making it possible to obtain HAp with specific morphology [98]. Wang et al. [97] used glutamic acid (Glu) and phosphoserine (Ser-$OPO_3$) as model compounds to modify the synthesis of HAp nanocrystals. Crystals obtained in the presence of amino acids showed plate-like morphology, with Ser-$OPO_3$ being more efficient for nucleation and growth of HAp.

*Combination of methods*

It is possible to combine two or more methods previously described in order to obtain HAp with the desired morphology and properties; for example, the combination of hydrothermal-biochemical, hydrothermal-hydrolysis, microemulsion-hydrothermal methods. It is also possible to modify existing methods for the development of other procedures. One of the most well-known modifications is the synthesis performed through microwave radiation,

used to activate the reaction. Microwave heating generates heat within the samples, reducing reaction time and increasing nucleation rate and growth [95, 98, 174-176]. HAp can be obtained with differentiated structures, such as nanostructured mesoporous microspheres [177], mesoporous lozenges [178], and hollow microspheres [45], using various organic additives, such as amino acids [179, 180], surfactants [160, 180-185], or natural origin substances [186, 187]. The combination of biochemical and hydrothermal procedures by incorporating an aqueous medium into the system can accelerate the kinetic processes that normally limit the rate of reaction in a conventional mechanochemical method. In addition, when comparing to the hydrothermal process, in which a big amount of energy is used to generate high temperatures, high energy consumption in the combined method can be avoided [151]. However, there are also several contributions to the combination of methods previously described with a new procedure, so that they can be considered a hybrid process for the production of nanometric HAp, as it is the case of electrospinning (ES).

HAp has been obtained in form of nanoblasts [182, 183, 188], nanofibers [189-191], nanowires [184], and nanotubes [151, 186]. These structures are said to be 1D and have a size of ≤100 nm in at least one of their dimensions, resulting in high surface activity and an ultrafine structure, similar to the mineral of hard tissues [41]. Consequently, the range of HAp applications is expanded, since nanostructured materials promote better adhesion of osteoblasts and osteointegration of materials, improving reabsorption capacity and bioactivity when compared to micrometric ceramics [14, 102, 103, 151]. Submicron and nanometric fibers have been studied in order to obtain systems with greater flexibility and desired properties for several applications [192]. HAp fibers have been used as another form of production of this material, as well as other biocompatible matrices to prepare composites that offer a solution to the problem of low mechanical resistance of HAp bodies [193-196]. CaP fibers can be obtained by different spinning techniques, starting from synthesis via sol-gel, followed by a direct spinning process as ES, a relatively simple and versatile top-down method for the generation of 1D nanostructures [193, 197]. ES technique uses high voltages as driving-force to form fibers from a precursor solution and deposit them onto a grounded substrate. There are few reports on the production of HAp using the ES technique with different polymeric spinning agents and using as inorganic precursors $Ca(NO_3)_2$, as a calcium precursor, and as phosphorus precursor a phosphate or a phosphite, such as $P_2O_5$, triethyl phosphate, triethyl phosphite and diammonium phosphate. Calcination temperatures in these studies varied between 600 and 800 °C [36, 102, 169, 170, 198-203].

Ramanan and Venkatesh [76] produced HAp fibers prepared via sol-gel using phosphorus pentoxide and calcium acetate as inorganic precursors in 2-butanol solution in distilled water, maintaining the Ca/P ratio at 1.67. Poly(lactic acid) was added as a spinning aid. The fibers obtained were dried and calcined at different temperatures. Their results confirmed the presence of pure HAp phases up to a calcination temperature of 1000 °C. The fibers produced had a uniform diameter of 140 μm and dense microstructure. Wu et al. [170] also produced HAp fibers by electro-winning through a sol-gel system. The authors observed that the surface of the fibers after calcination was rough, because of the polymer complete removal, obtaining fibers from 10 to 30 μm in diameter. In addition, they found that for sintering temperatures of 600 and 700 °C, CaO and β-TCP phases are formed in HAp fibers. More recently, Franco et al. [36] produced nanometric fibers (122±32 nm) of HAp and observed behavior similar to the previous study. After burning at 700 °C, CaO and β-TCP phases began to form due to the decomposition of HAp. This shows the difficulty in obtaining mono-phase fibers of hydroxyapatite with a nanometric scale. Similar results were reported in [102, 148]. Holopainen and Ritala [148] proposed a spinning method called electro-blowing, combining the ES technique with a high-velocity air stream, allowing the production of nanometric fibers (200±70 nm) of hydroxyapatite containing a secondary phase of CaO.

## HYDROXYAPATITE COMPOUNDS

Since HAp is a fragile material, most research is concentrated on the development of coatings and compounds of this material aiming to reduce its structural fragility and increase adhesion strength. To do so, researchers have incorporated inorganic particles that act as multifunctional cross-linkers [204]. Polymer-based hybrids or composites have been produced in order to improve mechanical and biomedical properties in the case of polymeric material [205, 206]. Therefore, HAp can play two roles: reinforces mechanical properties of the polymer matrix and propitiates a bioactive feature to the prosthesis, favoring osteointegration. Polymers used in bone regeneration may be biodegradable or non-degradable from the natural or synthetic origin. However, biodegradable polymers are more attractive, as is seen later. Bonfield et al. [207] used HAp as reinforcement in polymer composites for the first time to analyze the effect of HAp on the mechanical properties of HDPE, for a prospective application in tissue engineering. Their results proved the effectiveness of the reinforcement promoted by HAp in the composite produced, resulting in an appreciable increase in Young's modulus. Among the most commonly used natural polymers in combination with HAp, we have collagen [208-210], chitosan [211-213] and gelatin [214], which promote cell growth due to their similar structure to the extracellular matrix of bone tissue. However, they have an uncontrollable regeneration rate, making them difficult to be used and difficult to handle and process. Polymers of synthetic origin such as polycaprolactone (PCL) [215-218], poly(lactic acid) (PLA) [219-221], and poly(methyl methacrylate) (PMMA) [222] do not reproduce characteristics and behavior of the extracellular matrix, but they have good mechanical properties, besides being easily

mass manufactured, which allows them to be adapted for specific applications.

Bakoš et al. [223] manufactured a HAp/collagen compound aiming to mimic the bone matrix. The composite presented flexural strength of 5.37 kPa which could be applied when better cohesion and stability of the shape are required, confirming the results previously found in [224], while Kozlowska et al. [209] prepared HAp/collagen scaffolds by lyophilization and investigated the biological properties *in vitro*, using mice fibroblast cells, and *in vivo* by implanting them into sub-dermal and peritoneal cavities in rats for up to 30 days. Researchers observed that there was cell viability, besides good biocompatibility. Dalby et al. [225] prepared a HAp/poly(methyl methacrylate) (PMMA) and found its potential to act as substrate for cells resembling human osteoblasts, just like Radha et al. [222], which prepared HAp/PMMA scaffolds by means of thermally induced phase separation (TIPS) and wet-chemical approach, proving their *in vitro* bioactivity and reasonable hemocompatibility. Chaudhuri et al. [226] synthesized composite films of PVA/PVP/HAp, varying the concentration of HAp. Results indicated the biocompatibility of the fibrous meshes containing 8.5 and 5 wt% HAp, using the NIH 3T3 fibroblast lineage. They also showed that the conductivity, the dielectric constant and the hydrophilicity of the components increased significantly by adding a HAp. Within the percolation threshold, the composite with 8.5 wt% HAp exhibited better biocompatibility compared to that of 5 wt% HAp. Haider et al. [227] produced PLA/HAp compounds modified with immobilized insulin, showing increased growth of osteoblasts and accelerated osteogenesis, while Macha et al. [228] have developed controlled release systems of HAp/PLA, loaded with gentamicin (GM). Researchers have reported that HAp particles improve drug stability and availability, as well as control over the release rate. Moreover, *in vitro* studies on human stem cells have shown substantial amounts of cells adhering to the composites. Furthermore, Akindoyo et al. [220] produced PLA/HAp composites by extrusion and injection molding with 10 wt% HAp content. Their results showed that the thermal properties of the composite with HAp were improved along with increases in mechanical properties of about 25%, 20% and 42% in tensile strength, modulus of elasticity and impact strength (Charpy), respectively.

Other materials have also been combined with HAp for production of composites for biomedical applications. Among these we can mention titanium (Ti) [229, 230], titanium dioxide ($TiO_2$) [231, 232], zirconia ($ZrO_2$) [233, 234], and calcium silicate ($CaSiO_3$) [235]. Nie et al. [236] produced porous reduced graphene oxide (RGO)/nHAp scaffolds via self-assembly. They found that carriers with 20% HAp significantly increase proliferation, alkaline phosphatase activity (ALP) and osteogenic gene expression of rat bone mesenchymal stem cells (rBMSCs). *In vivo* tests, performed on rabbits, demonstrated that circular defects with 4 mm diameter were successfully cured after 6 weeks of implantation. In addition, computed tomography (CT) and histological analysis showed improvement in collagen deposition, cell proliferation and bone neoformation in the group treated with 20% nHAp/RGO.

Nanomaterial aggregation is a problem frequently encountered in the production of hybrid materials, which depends on variables such as the type of nanomaterial, surface energy, reagents, the method used for the synthesis of nanoparticles and, especially, hydrophilic-hydrophobic interface between solutions. To minimize this effect, control of the dispersion of nanoparticles can be accomplished by modifying the surface by emulsifying agents in order to retard dissociation of the composite materials and make them specific to the desired application [17]. However, these emulsifying agents must meet certain requirements such as not to compromise biocompatibility, not being cytotoxic or even altering physiological or biological properties of nanoparticles or fillers. Polymeric materials can be used for surface modification and promote, in addition to dispersion, increased factors for cell growth. Hong et al. [237] used ring-opening polymerization to graft poly(lactic acid) (PLLA) into HAp particles, improving PLLA dispersion. The modified compounds promoted increased cell proliferation. Akindoyo et al. [220] used ring-opening polymerization to graft PLLA into HAp particles improving PLLA dispersion. Modified compounds promoted increased cell proliferation.

## DOPED HYDROXYAPATITE

As previously mentioned, natural bone is a composite made of calcium phosphate (CaP), collagen and water, as well as polysaccharides, lipids and proteins in small amounts. CaP is present as crystals of HAp, providing bone stiffness. HAp has its structure composed by hexagonal $Ca^{2+}$ and $PO_4^{3-}$ arrangements on $OH^-$ columns. It is possible to replace calcium by metal ions, and $OH^-$ and $PO_4^{3-}$ groups by some anionic groups such as $Cl^-$, $F^-$, $CO_3^{2-}$ and $VO_4^{3-}$. With the variation of the anionic or cationic type of substitution, HAp structural properties are altered, causing changes in their physical-chemical and biological properties. Such substitutions are accompanied by changes in network parameters and unit cell volume, which are generally related to the size of the cation ionic radius compared to that of $Ca^{2+}$ [238]. Most commonly used cations to replace calcium ions are zinc ($Zn^{2+}$) [239], strontium ($Sr^{2+}$) [240], magnesium ($Mg^{2+}$), and manganese ($Mn^{2+}$). Other materials have been studied, such as silicate ($SiO_4^{4+}$) [241], as well as extra components like silver nanoparticles [242], carbon nanotubes [243-246], graphene and graphene oxide [247, 248], or magnetic nanoparticles ($Fe_3O_4$) [93, 94, 249]. The presence of these dopants in HAp structure significantly contributes to the biostructure and biochemistry, similar to a natural bone [250]. Doping of HAp nanoparticles with Zn or Si ions enhances the biocompatibility of the resulting compound [239]. In addition, bivalent dopant ions present better osteogenic differentiation [251-253] and increased protein absorption [252], while HAp doped with Ag nanoparticles have better antibacterial activity against different types of bacteria [242, 254]. Carbon nanotubes have also been

added to the structure of HAp to improve its mechanical properties, however, at high dosages, this compound may be cytotoxic [246, 255].

Bodhak et al. [256] studied the effect of $Sr^{2+}$ and $Mg^{2+}$ dopants on the structural stability and biological properties of HAp. They found that the presence of these metal ions improved particle stability as well as osteoblast response activities and viable cell density on a negatively charged surface at all periods of culture. Therefore, these ions affect mineral metabolism in the process of bone remodeling and increase apoptosis of osteoclasts, as well as the proliferation of pre-osteoblastic cells. Li et al. [257] doped HAp with $Mn^{2+}$ and $Fe^{3+}$ ions using the wet chemical method and ion exchange mechanism. Both dopants did not cause structure, morphology and size deviations of the crystals, nor toxic effect in osteoblastic cells. Doping of HAp with $Fe^{3+}$ resulted in an increased negative surface charge and, consequently, increased adhesion in osteoblastic cells compared to samples doped with $Mn^{2+}$ and pure HAp. Gautam et al. [174] synthesized HAp composites with $SrCO_3$ and $ZrO_2$ varying the dopant amount in 2, 4, and 6 wt%. Results showed that the presence of dopant aided grain growth during sintering processes, as well as increased wear resistance and specific wear rate. Zhao et al. [51] observed that the choice of dopant ions influences cell behavior; for example, a minimal amount of $Mg^{2+}$ ions in HAp (approximately 1.5 wt%) may lead to significant cytotoxicity in MG63 (osteoblast) cells, but not in rMSCs. In addition, they indicated that doping of HAp with $Mg^{2+}$ nanoparticles could be used to kill cancer cells, eliminating the need to conjugate anticancer drugs to nanoparticles, as well as solving the problem associated with drug toxicity. Begam et al. [258] introduced Zn into HAp block structure through culture also of MG63 cells; they observed that cell viability, adhesion and proliferation rate were better for doped samples, mainly with higher sintering temperature, which can be attributed to the osteoblast activity stimulation caused by Zn.

In this context, the mentioned studies indicate that the use of metal cations incorporated in the structure of HAp promote physical-mechanical and biological benefits when used in tissue engineering. Therefore, the choice of type of chemical compound is directly related to the applicability of the biomaterial, since some chemical components can cause toxicity in the host tissue. However, there are few studies that report biodegradation of these doped materials *in vivo*.

## APPLICATIONS FOR HYDROXYAPATITE

HAp has a high potential for biomedical applications, being quite used in the orthopedic field as material for bone implant and prostheses. Among the most important biomedical applications, HAp can be used in the controlled release of drugs (drug delivery systems), coating of implants, and bone grafts and scaffolds for tissue engineering.

### *Drug delivery system*

There has been a great deal of interest in using HAp as a drug carrier over the last decade. For example, nanorods/nanoparticles have been applied for the controlled release of various proteins and drugs [13, 227, 259]. HAp is mainly used to provide antibiotics to hard tissues [177, 241, 260], and other drugs as anticancer substances [261-263], anti-inflammatory drugs [264-266], anti-osteoporotic substances [267-270], and other molecules such as vitamins, hormones, proteins, and growth factors [260, 271, 272]. For drug release, HAp should have differentiated morphologies that allow the loading of the high capacity of drugs, such as HAp microspheres with a hollow structure, which facilitate the loading of drugs and their controlled release [172]. Ibrahim et al. [273] obtained HAp with high pore volume (1.4 $cm^3/g$) and surface area (284.1 $m^2/g$) by using crude eggshell at room temperature. These features enhanced the incorporation of ibuprofen (model drug; 1.38 g/g HAp), dissolution and controlled release of the drug via carbon dioxide. Dubnika et al. [274] developed a drug delivery system based on Ag-doped HAp and loaded with lidocaine hydrochloride in the presence of chitosan or sodium alginate. Their results suggested that the new hybrid scaffolds have antibacterial activity as well as controlled release of the anesthetic drug for a determined period of time. Composites of HAp/biocompatible polymers such as collagen [275], gelatin [241], chitosan [276, 277], alginate [278, 279], and agarose [260, 280] have been studied for drug release purposes. Uskoković and Desai [276] and Wei et al. [277] performed *in vitro* tests and showed that biopolymer/HAp composites exhibited a larger period of drug release as compared to pure HAp nanoparticles.

Directed (or selective) release systems are also of great interest because they allow release rate and delivery time to be controlled simultaneously, eliminating toxic and collateral effects in healthy tissues. Here, magnetically charged carriers are one of the most efficient methods for selective delivery of drugs to a particular pathological body site [281-285]. In addition, HAp can be used in the treatment of tumors as long-acting support during its gradual release [286-289]. From this perspective, we can see that HAp is considered as a material that has the potential for controlled release systems of pharmaceutical or biological agents, especially for the treatment of bone lesions. However, in view of the need for selectivity and minimization of damage in drug release or tissue treatment, there is a real concern about drug release systems that allow a controlled release of the drug to the target site, as well as the distribution of bioactive factors to different diseases effectively and without causing collateral effects on healthy tissues.

### *Coating of implants*

Most implants used are based on titanium and its alloys due to their excellent mechanical properties, good strength, low density and chemical stability in body fluid. However, they do not have good biocompatibility and osteointegration [290]. Compatibility and bioactivity of the implant material can be improved with the use of biocompatible and bioactive

coatings [291-294]. Among the most used coating materials that meet the above criteria is HAp, which is widely tested because of its chemical stability and osteoconductivity [290]. As mentioned before, HAp adheres firmly to the bone, making implant degradation difficult, as well as increasing osteointegration of implants with surrounding tissues [295, 296]. The coating of HAp should have an adequate thickness for the best implant performance. For example, the thinner the coating, the better the mechanical properties, but in the first few months of implantation, about 10 to 15 μm of the hydroxyapatite surface may dissolve during the bone acquisition process. On the other hand, a coating above 100 to 150 μm can suffer from fatigue failure under tensile load. Hence, the condition required for such application leads to an ideal thickness of approximately 50 μm [56].

Methods used for coating metal implants with HAp include plasma spray [297-301], electrophoretic deposition [302-305], pulsed laser deposition [306-309], sol-gel deposition [310-314], spin-coating [290], sputtering techniques [315-317], ion assisted deposition [296, 318, 319], and many other techniques such as immersion, hot pressing and hot isostatic pressing, thermal spraying and electrostatic spraying [320, 321]. Plasma spraying technique is one of the most economical methods. However, the coatings obtained have limited biomedical applications, as they present structural and chemical properties variation such as poor bond strength between the coating and surface of the metallic implant, not to mention its non-uniform thickness [315, 322, 323]. The sol-gel technique provides chemically homogeneous films with fine grains in the final product, in addition to being simple and inexpensive [313]. Other promising techniques for coating are pulsed laser deposition that produces crystalline and highly adherent films [307] and electrochemical deposition that allows the coating of objects with irregular shapes at low temperatures, promoting the control of coating thickness, crystallinity and phase composition, though presenting low binding strength [304]. The manufacturing technique and its process parameters directly used influence the properties of biocompatible coatings, as well as characteristics of density, phase, chemical composition and material crystallinity [324, 325]. Surface coatings of metal implants with HAp should have a high degree of crystallinity, adequate stoichiometry, porosity and good adhesion to the substrate. Thus, control over the process parameters is fundamental for coating success [172].

LeGeros [326] performed a study that pointed out that osteoblastic cells interact more easily with a HAp-coated surface. In addition, it was observed that the HAp used in the coating shows osteoinductive properties interacting with bone morphogenetic proteins (BMPs). Rigo et al. [327] used HAp coating on the surface of silicon nitride in order to increase their bioactivity and biocompatibility, since pure silicon nitride, despite having good mechanical properties, has limited biomedical applications. Recently Huang et al. [328] produced nanostructured coatings of zinc-doped fluorinated HAp (ZnFHA) deposited on titanium surface by an electrolytic deposition technique. The coatings obtained were dense and free of cracks. In addition, they showed good proliferation of osteoblasts, good corrosion resistance and favorable cytocompatibility for the application as a biomedical implant material. Mihailescu et al. [309] observed that the HAp derived from cattle doped with $MgF_2$ and MgO increases the adhesion of coatings obtained by pulsed laser techniques on Si substrate, as well as its anti-biofilm properties. Other researchers have also added carbon nanotubes [329-331], graphene and graphene oxide [332, 333] in coatings of HAp to improve its mechanical properties. The addition of carbon nanotubes promoted homogeneous and crack-free coatings with higher crystallinity, biocompatibility and bond strength [329, 331]. Moreover, HAp coatings with the addition of graphene oxide and chitosan, produced by electrophoretic deposition, exhibited better biocompatibility and increased suspension stability than HAp deposition, besides decreasing binding with *Staphylococcus aureus* [333].

Bacterial infections are a major problem in the regeneration of bone tissue. Researchers have noticed that after surgical implantation, bacterial cells may adhere to their surface promoting biofilm infection. Therefore, it is essential that the surface material used exhibits not only good biocompatibility but also good antibacterial properties. The antibiotic-loaded HAp can be used as a coating enabling *in situ* delivery of drug to prevent infection or inflammatory reactions after surgical implantation [334]. Addition of inorganic antibacterial factors in HAp coatings has been investigated, with emphasis on silver (Ag) [298, 317, 335]. Guimond-Lischer et al. [298] used vacuum plasma spraying technique to develop HAp-Ag coatings at different concentrations of Ag. The coatings exhibited mechanical and compositional properties that met regulatory requirements, did not exhibit cytotoxicity to primary human bone cells, and exhibited antibacterial activity for *E. coli* and *S. aureus*. As well, magnetite-doped HAp films also have good biocompatibility [336] and destabilizing bacterial adhesion [300].

In summary, such studies prove that the coating of HAp on the surface of implantable materials will help to stimulate bone growth under optimized conditions. However, it is important to emphasize that limitations associated with mechanical properties of this material restrict its applicability. In addition, further studies should focus on improvements in coating deposition methods to obtain implants with good coverage and good properties, as well as promoting the incorporation of biologically active factors, enhancing their therapeutic capabilities, and reducing manufacturing costs.

*Scaffolds for tissue engineering*

Bone grafts are often used to treat bone lesions resulting from infections, tumors or trauma. However, problems related to donor compatibility, immune rejection and pathogen transfer may arise in reconstructive procedures. The use of biocompatible scaffolds has been an alternative

to bone recovery since they act as a support for cell adhesion, viability, proliferation, and differentiation, ensuring neo-tissue growth. Another important criterion is the existence of a highly porous structure with interconnected porous character allowing for cell diffusion, conferring adequate space for bone ingrowth. Macroporous scaffolds allow the formation and mineralization of osteosis by increasing the migration of osteoblasts and osteoprogenitors, while the interconnected micropores promote increased vascularization and diffusion of nutrients during bone reconstruction [337, 338]. Since bone is continuously under request, the mechanical properties of the scaffold should be similar to that of the bone where it is implanted, so that mobilization of the injured site can occur as early as possible [339, 340]. Logeart-Avramoglou et al. [341] do not consider high mechanical resistance as a fundamental priority of the scaffold, since the authors believe that its main function is the stimulation of the growth of the bone tissue in its interior, since the mechanical stability can be reached mostly through appropriate orthopedic devices such as internal and intramedullary pins, or external fasteners. Complementarily, the *in vivo* study in [342] indicated that the compressive strength of HAp scaffolds increases, for example from about 10 to 30 MPa, due to the growth of bone tissue. However, a scaffold must have at least sufficient mechanical strength and fracture toughness to permit its handling, which, according to [343], a mechanical strength between 0.3 and 0.4 MPa is sufficient for proper handling of the scaffold.

In this sense, synthetic materials have been studied to allow treatment and recovery of bone lesions. Such materials, as a temporary extracellular matrix, induce the natural process of tissue regeneration and development. Among these materials, polymer/inorganic composites have been highlighted, a hybrid material that seeks to combine the properties of a polymer matrix with the characteristics of an inorganic phase, such as HAp. The improved properties of hybrid materials result from the combination of compressive strength of inorganic ceramic phase as well as structural toughness and flexibility of polymer, also giving support and protection to the fragile ceramic material [247, 344-347]. Hybrid scaffolds can be made from natural or synthetic polymer matrices. Natural polymers exhibit good cellular affinity, as synthetic ones promote greater mechanical resistance and adjustable rate of degradation. These materials can be found in gel form or not; the former being widely used because of its similarity to extracellular fluids [348]. The natural polymers used are chitosan [239, 349-351], gelatin [241, 248, 352], alginate [271, 346, 353], collagen [354, 355], cellulose [352, 356], and silk fibroin [357, 358]. Among the most used synthetics we have poly(vinyl alcohol) (PVA) [241, 352, 359], poly(methyl methacrylate) (PMMA) [360], polycaprolactone (PCL) [360], polylactic-co-glycolic acid (PLGA) [361], poly(lactic acid)(PLA) [337, 362], etc. HAp makes it possible to increase biocompatibility, osteoconduction, fixation, growth and proliferation of human osteoblastic cells in the composite system [354]. In addition, the content of HAp in the composite scaffolds increases its compressive strength and modulus of elasticity [353]. However, the use of HAp in a composite has some limitations associated with its incorporation into the polymer matrix, such as particle aggregation, especially when using nanoparticles.

The nanostructured hybrid scaffold can be manufactured by *in situ* crystallization of HAp in the polymer matrix by dispersing the HAp nanoparticles in the polymer [349, 350, 353, 354, 360, 363, 364], via HAp synthesis within the polymer matrix itself [365, 366], or even via 3D printing [241, 367, 368]. Juhasz et al. [360] incorporated nanoparticles of HAp and carbonated HAp (nCHA) into the polymer matrix of a poly-2-hydroxyethylmethacrylate/polycaprolactone (PHEMA/PCL) hydrogel with a filler content of 10 wt%. Through these results they observed that the compounds produced exhibited significantly superior cellular activity to human osteoblast-like cells when compared to pure hydrogel samples, indicating the potential of this material for use in bone tissue engineering. Kim et al. [353] have developed scaffolds composed of chitosan/HAp with high strength and controlled pore structures. Researchers observed that increasing HAp content up to 70 wt% increased the compressive strength and elastic modulus of the scaffolds produced, as well as aiding in differentiation and mineralization of MC3T3-E cells. Siqueira et al. [369] produced vertically aligned superhydrophilic carbon/PDLLA nanotubes, then fabricated PDLLA/nanohydroxyapatite (nHAp) scaffolds. Their results showed that nHAp probably acted as a nucleating agent increasing the crystallization rate of PDLLA without structural alteration, presenting no cytotoxic effects and therefore capable of inducing detectable mineralization. In addition, the *in vivo* study showed that PDLLA/nHAp scaffolds mimicked an immature bone and induced bone remodeling. Yang et al. [370] synthesized aliphatic polyurethane (PU) scaffold by a foaming method and incorporated HAp particles into the structure. Simulated body fluid assay demonstrated that the incorporation of 40 wt% HAp particles promoted the biomineralization ability of PU supports, as well as increased *in vitro* proliferation and osteogenic differentiation of sown mesenchymal stem cells. *In vivo* tests performed on nude mice showed that after 8 weeks a considerable amount of vascularized bone tissue with early spinal stromal development was generated in PU/40HAp structures indicating their potential for bone regeneration applications.

Recently, many studies have focused on the development of fibrous scaffolds, particularly those nanostructured, aiming to mimic the fibrous structure of the extracellular matrix (ECM). Fibrous scaffolds present high porosity and surface area and are mainly produced by electrospinning (ES) technique. The nanofibers produced by ES mimic the dimensions of extracellular fibrous ECM and construct a framework that can serve as support and guide for cells in living tissues [102]. However, the ES of biomaterials based on calcium phosphate presents some challenges. The preparation of ceramic fibers involves the removal of polymer components from the newly spun fibers by heat

treatment. However, scaffolds composed only by HAp have fragility characteristics that make it difficult to manipulate and apply them. Thus, the use of hybrid scaffolds, HAp/polymers appears as a promising alternative to extending the applicability of this material and its efficiency in the treatment of tissues. Kim et al. [371] produced nanofibrillar silk fibroin (SF) scaffolds by ES containing up to 20% nHAp which provided high mechanical strength for load applications. However, HAp concentrations above 20% ruptured the polymer chains within the nanofibers which affected the mechanical behavior of the scaffolds, results similar to those found in [372], which indicated that the use of the HAp content above 20% results in the decrease of the flexural modulus of the scaffold, due to the local aggregation of HAp. On the other hand, Ding et al. [373] produced SF nanofibers by electrophoresis containing HAp (10, 20 and 30%) for regeneration of the periosteum and observed that Young's modulus of scaffolds increased with increasing HAp content. In addition, scaffolds containing 30% HAp induced metabolic activity and promoted proliferation of rBMSCs, rather than scaffolds containing 0% HAp. Therefore, SF scaffolds containing 30% HAp are also promising structures to promote bone formation because of their ability to induce mineral deposition and hence cell differentiation.

Another area of scaffold processing that has been calling researcher attention is three-dimensional (3D) printing. Currently, 3D printing techniques have been shown as a promising tool for the production of bone scaffolds for regenerative medical applications [219, 337, 338, 368, 374-377]. This method provides the development of materials with diverse and complex structures through predefined computer designs, as well as making it possible to obtain customized implants or grafts. In this sense, studies have been carried out to develop 3D printing methods of hybrid composites, such as polymer/HAp. Scaffolds printed in 3D were recently produced in PCL/HAp [375, 376] and PLA/HAp [337, 374], and their *in vitro* and/or *in vivo* tests indicated a prospective application for regeneration of bone tissue. In addition to the environment/support for cell proliferation, scaffolds can also act as drug carriers [93, 241, 355]. Therefore, Martínez-Vázquez et al. [241] produced porous 3-D scaffolds composed of gelatin and HAp doped with Si, by rapid prototyping at room temperature, showing good mechanical properties, also allowing the incorporation of vancomycin. The presence of gelatin improved differentiation and MC3T3-E1 osteoblastic cell line gene, while the antibiotic used was gradually released from the scaffold, inhibiting bacterial growth *in vitro*. López-Noriega et al. [355] manufactured collagen/HAp hybrid scaffolds with poly(lactic-co-glycolic acid) (PLGA) microparticles incorporated to the encapsulated pro-osteogenic peptide (PTHrP 107-111). Their results indicated that scaffolds produced good porosity and mechanical properties. In addition, the peptide released from the hybrid showed pro-osteogenic effects on bone cells.

Several studies have investigated the effectiveness of dopant use in the structure of hydroxyapatite, as well as the use of scaffolds of these materials, aiming its application in tissue engineering [258, 378-380]. Dubnika et al. [274] produced HAp-Ag scaffolds to act as a local delivery and delivery system for drugs, functionalized with long-term silver ion release rates. These scaffolds were produced with sodium alginate and chitosan and the rate of *in vitro* release of the drug into simulated body fluid was evaluated, suggesting that the hybrid material developed has antibacterial activity up to one year, as well as controlled administration of the anesthetic drug up to two weeks. Li et al. [381] manufactured lithium (Li) doped HAp scaffolds and evaluated the increase in bone tissue generation seeded with bone marrow mesenchymal stem cells (BMMSCs) preconditioned by hypoxia, indicating that the use of Li as a dopant increased bone density and promoted osteogenic differentiation of BMMSCs, evidenced by the excellent cellular proliferation activity *in vitro*, showing good osteogenesis and angiogenesis potential. Ge et al. [382] produced Src/PLLA doped hybrid HAp scaffolds and performed *in vivo* tests with rabbits. Their results evidenced the effectiveness of the porous structure of this scaffold in the promotion of cell adhesion, proliferation and alkaline phosphatase activity (ALP). Moreover, they showed that the addition of Sr improved bioavailability and bone induction of HAp, also indicating that the dopant has the ability to adsorb proteins and increase the compression modulus. However, scarring of large bone defects is still a challenge for physicians, dentists, and materials engineers. The development of materials that allow the replacement of bone tissue or its regeneration still requires great advances in several mechanisms of healing, regeneration and bone treatment, as well as in the processing of scaffolds. In this context, hybrid scaffolds, consisting of HAp nanoparticles and biodegradable polymers, are materials that are emerging as the most suitable for prospective purposes for bone replacement and regeneration.

## FINAL CONSIDERATIONS

Hydroxyapatite (HAp) is a biocompatible and bioactive material that provides adhesion and cell proliferation of different cell types. It is being used as a carrier and as a loading agent in the controlled release and delivery of drugs, coating on metal materials, and as an orthopedic implant, due to its chemical similarity to the mineral component of mammal bone and hard tissues. Advances related to varieties of methods of synthesis of HAp, as well as knowledge and understanding of cellular reactions involving this material, are being carried out. However, there is still a great demand for the development of efficient, simple and low-cost methods. Furthermore, due to its structural fragility, HAp has limited applications when the bone defect to be repaired is in anatomical regions that are under constant tension. Thus, researchers have been directing studies in obtaining materials that have better mechanical properties. As an alternative, HAp is being used in combination with other materials, increasing its applicability and efficiency in the treatment of tissues, showing a promising methodology and

high potential for the development of scaffolds. However, the development of optimal bone supports, coatings and release systems are still a challenge for the engineering of bone tissue. This makes it necessary to study the development and applicability of this material in different anatomical sites to improve mechanical and biological aspects of HAp-based implants and to optimize their safety and efficiency.

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