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REVIEW

Calcium orthophosphates

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Abstract The present overview is intended to point the readers' attention to the important subject of calcium orthophosphates. This type of materials is of the special significance for the human beings because they represent the inorganic part of major normal (bones, teeth and antlers) and pathological (i.e. those appearing due to various diseases) calcified tissues of mammals. For example, atherosclerosis results in blood vessel blockage caused by a solid composite of cholesterol with calcium orthophosphates. Dental caries and osteoporosis mean a partial decalcification of teeth and bones respectively that results in replacement of a less soluble and harder biological apatite by more soluble and softer calcium hydrogenphosphates. Therefore, the processes of both normal and pathological calcifications are just an in vivo crystallization of calcium orthophosphates. Similarly, dental caries and osteoporosis might be considered as in vivo dissolution of calcium orthophosphates. Conversely, due to a great chemical similarity with the biological calcified tissues, many calcium orthophosphates possess remarkable biocompatibility and bioactivity. Materials scientists extensively use this property to construct artificial bone grafts that are either entirely made of or only surface-coated by the biologically relevant calcium orthophosphates. For example, self-setting hydraulic cements made of calcium orthophosphates are helpful in bone repair, while titanium substitutes covered by a surface layer of calcium orthophosphates are used for hip joint endoprostheses and tooth substitutes. Porous scaffolds made of calcium orthophosphates are very promising tools for tissue engineering applications. In addition, calcium orthophosphates of a technical grade are very popular mineral fertilizers. There is a great significance of calcium orthophosphates for the humankind and, in this paper, an overview on the current knowledge on this subject is provided. To assist and guide the readers, a great number of references to the related publications detalizing various specific aspects of the matter has been collected.

Introduction

Calcium orthophosphates are the chemical compounds of a special interest in many interdisciplinary fields of science, including geology, chemistry, biology and medicine. According to the references, the initial attempts to establish their chemical composition were performed by Berzelius in the middle of the 19th century [1]. Approximately 70 years afterward the idea on existence of different crystal phases of calcium orthophosphates was introduced [2]; mixtures of calcium orthophosphates had been called apatites[1] until then.

By definition, all calcium orthophosphates consist of three major chemical elements: calcium (oxidation

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[1] As a mineral species, apatite was first recognized by the father of German geology Abraham Gottlob Werner (1750–1817) in 1786 and named by him from the Greek απατώ (apato)—''to mislead'' or ''to deceive'', because it had previously been mistaken for other minerals, such as beryl, tourmaline, chrysolite, amethyst, fluorite, etc.

state +2), phosphorus (oxidation state +5) and oxygen (oxidation state –2), as a part of orthophosphate anions. These three chemical elements are present in abundance on the surface of our planet: oxygen is the most widespread chemical element of the earth's surface (47 mass%), calcium occupies the fifth place (3.3–3.4 mass%) and phosphorus (0.08–0.12 mass %) is among the first twenty of the chemical elements most widespread on our planet [3]. In addition, the chemical composition of many calcium orthophosphates includes hydrogen, either as an acidic orthophosphate anion (for example, HPO_4^{2-} or $H_2PO_4^-$), and/or as incorporated water (for example, $CaHPO_4 \cdot 2H_2O$). Diverse combinations of oxides of calcium and phosphorus (both in the presence of water and without it) provide a large variety of calcium phosphates, which are distinguished by the type of the phosphate anion: ortho- (PO_4^{3-}), meta- (PO_3^-), pyro- ($P_2O_7^{4-}$), and poly- ($(PO_3)_n^{n-}$). In the case of multi-charged anions (orthophosphates and pyrophosphates), calcium phosphates are also differentiated by the number of hydrogen ions attached to the anion. Examples include mono- ($Ca(H_2PO_4)_2$), di- ($CaHPO_4$), tri- ($Ca_3(PO_4)_2$), and tetra- ($Ca_2P_2O_7$) calcium phosphates [4–6]. However, only calcium orthophosphates will be considered and discussed in this paper.

The atomic arrangement of calcium orthophosphates is built up around a network of orthophosphate (PO_4) groups, which gives stability to the entire structure. The vast majority of calcium orthophosphates are sparingly soluble in water; however, all of them are easily soluble in acids but insoluble in alkaline solutions. All chemically pure calcium orthophosphates are crystals of white color and moderate hardness. However, natural minerals of calcium orthophosphates are always colored due to impurities, the most widespread of which are ions of iron and rare earth elements. Biologically formed calcium orthophosphates are the major component of all mammalian calcified tissues, while natural calcium orthophosphates are the major raw material for phosphorus-containing fertilizers [7–10].

Geological and biological occurrence

Calcium orthophosphates are abundant in both nature and living organisms. Geologically, natural calcium orthophosphates are found in different regions mostly as deposits of apatites (mainly as natural fluorapatite (FA), chemical formula $Ca_{10}(PO_4)_6F_2$) or phosphorites [8, 11]. The chemical composition of the latter cannot be described so easily. As phosphorites belong to sedimentary deposits, their general appearance and the chemical composition vary a lot. It is a common practice to consider francolite (or carbonate-hydroxyfluorapatite regarded as its synonym) as the basic phosphorite mineral [11–15]. A cryptocrystalline (almost amorphous) variety of francolite (partly of a biological origin) is called collophane (synonyms: collophanit, collophanita, collophanite, grodnolite, kollophan) [16]. It occurs in natural phosphorites predominantly as fossil bones and phosphatized microbial pseudomorphs: phosphatic crusts of chasmolithic biofilms (or microstromatolites) and globular clusters with intra-particular porosities [17]. Natural phosphorites (therefore, francolite and collophane as well) occur in various forms, such as nodules, crystals, or masses. The world deposits of natural calcium orthophosphates are estimated to exceed 150 billion tons; from which approximately 85% belong to phosphorites and the remaining 15% belong to apatites [18].

Natural calcium orthophosphates occur in most geological environments usually as accessory minerals (<5%). Concentrations sufficient for economic use (>15%) are also available. The largest world deposits of natural apatites are located in Russia (the Khibiny and Kovdor massifs, Kola peninsula), Brazil and Zambia, while the largest world deposits of natural phosphorites are located in Morocco, Russia, Kazakhstan, USA (Florida, Tennessee), China and Australia, as well as in the oceans [7–11, 18]. Most of natural calcium orthophosphates occur as small polycrystals. Larger crystals usually have the crystal structure of apatites (hexagonal system, space group $P6_3/m$). Giant crystals including "a solid but irregular mass of green crystalline apatite, 15 feet long and 9 feet wide" and a single euhedral crystal from the Aetna mine measuring 2.1×1.2 m with an estimated weight of 6 tons were found [12, 13]. None of them is a pure compound; they always contain admixtures of other elements. For example, ions of calcium might be partially replaced by Sr, Ba, Mg, Mn, K, Na, Fe; ions of orthophosphate may be partly replaced by AsO_4^{3-}, CO_3^{2-}, and VO_4^{3-}; ions of hydroxide, chloride, bromide, carbonate and oxide may to a certain extent substitute fluoride in the crystal lattice of natural apatites [14]. In principle, the crystal structure of apatites can incorporate half the periodic chart in its atomic arrangement. These substitutions are usually in trace concentrations, but large concentrations and even complete solid solutions exist for certain substituents (e.g., F^- and OH^-). To make things even more complicated, some ions in the crystal structure may be missing, leaving the crystallographic defects, which leads to formation of non-stoichiometric compounds. Figure 1 shows examples of

polycrystalline and single-crystalline samples of natural FA.

Manufacturing of elementary phosphorus (white and red), phosphoric acids, various phosphorus-containing chemicals and, especially, agricultural fertilizers (namely, superphosphate, ammonium orthophosphates) is the major industrial application of natural calcium orthophosphates. This consumes up to 85% of the world production of natural calcium orthophosphates. The total capacity of industrial plants in the world exceeds 25 million tons (as P_2O_5) of phosphate fertilizers per year with the annual increase of 2–3% [8].

In biological systems, calcium orthophosphates occur as the principal inorganic constituent of normal (bones, teeth, fish enameloid, deer antlers and some species of shells) and pathological (dental and urinary calculus and stones, atherosclerotic lesions) calcifications [4, 19–22]. Except for small portions of the inner ear, all hard tissue of the human body is formed of calcium orthophosphates. Structurally, they occur mainly in the form of poorly crystallized non-stoichiometric Na-, Mg-, and carbonate-containing hydroxyapatite (often called biological apatite[2] or dahllite[3]). The main constituents of human bones are calcium orthophosphates (~50–60 wt%), collagen[4] (~30–40 wt%), and water (up to 10 wt%) [26–28]. Detailed information on the chemical composition of the most important human normal calcified tissues is comprised in Table 1. One should note that the values mentioned in Table 1 are approximate; the main constituents can vary by a percent or more [29].

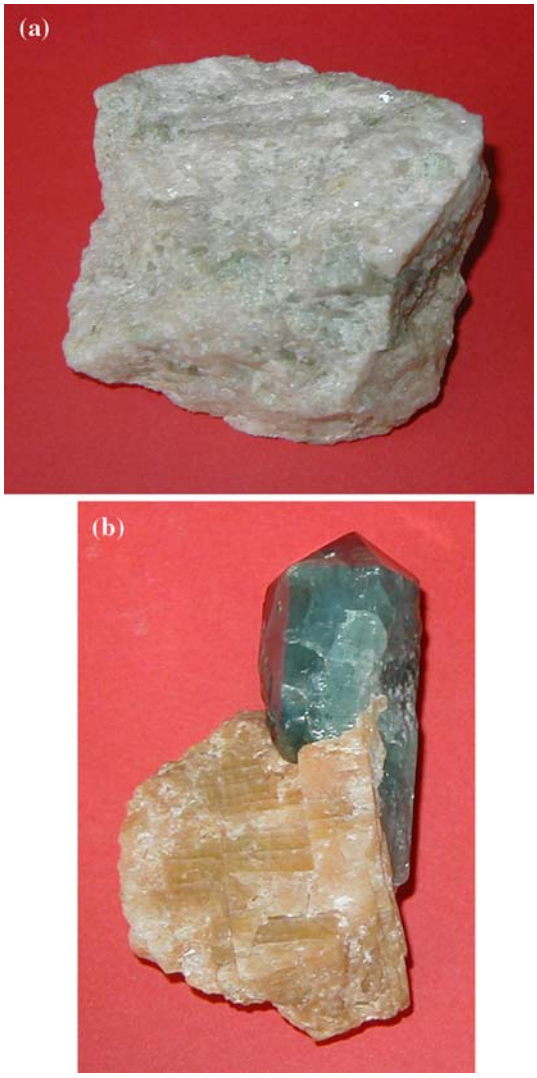

Fig. 1 Polycrystalline (**a**) and single-crystalline (**b**) FA of geological origin. The single crystal has a grey-green color due to incorporated transition metals

The members of calcium orthophosphate family

In the ternary system $Ca(OH)_2$–H_3PO_4–H_2O there are eleven[5] known non-ion-substituted calcium orthophosphates with the Ca/P molar ratio within 0.5 and 2.0 (Table 2). The most important parameters are the molar Ca/P ratio, basicity/acidity and solubility. These parameters strongly correlate with the solution pH. The lower the Ca/P molar ratio is, the more acidic and water-soluble the calcium orthophosphate is [4–6]. Due to the tripotic equilibrium that exists within orthophosphate-containing solutions, variations in pH alter the relative concentrations of the 4 polymorphs of orthophosphoric acid (Fig. 2) and thus both the chemical composition and the amount of the calcium orthophosphates that forms by direct precipitation [32]. A brief description of all calcium orthophosphates

[2] Occasionally "biological apatite" is called "bioapatite" [23–25].

[3] There are reports that dahllite belongs to the francolite group. Natural dahllite might be a rock forming mineral. For example, it is found in some phosphorite concretions of Podolia. In addition, it is found in both massive and accretionary crustal phosphorites [15].

[4] Collagens are fibrous, insoluble proteins found in the connective tissues, including skin, bone, ligaments and cartilage.

[5] In literature occasionally one might find brief notes on the 12th calcium orthophosphate, namely oxyapatite ($Ca_{10}(PO_4)_6O$). A mixture of oxyapatite and HA might be prepared by dehydration of HA at temperatures exceeding ~900°C (e.g., during plasma-spray of HA) only in the absence of water vapor [5, 6, 30]. It also might be crystallized in glass-ceramics [31]. Oxyapatite is very reactive and transforms to HA in contact with water vapor [30]. Oxyapatite is still very poorly known; however, the following data on the solubility constant (K_s ~ 10–69 at 25°C) and crystal structure (hexagonal, $P6_3/62$) are available [5].

Table 1 Comparative composition and structural parameters of inorganic phases of adult human calcified tissues [4, 28]

Composition, wt%	Enamel	Dentin	Cementum	Bone	HA
Calcium[a]	36.5	35.1	[c]	34.8	39.6
Phosphorus (as P)[a]	17.7	16.9	[c]	15.2	18.5
Ca/P (molar ratio)[a]	1.63	1.61	[c]	1.71	1.67
Sodium[a]	0.5	0.6	[c]	0.9	–
Magnesium[a]	0.44	1.23	[c]	0.72	–
Potassium[a]	0.08	0.05	[c]	0.03	–
Carbonate (as CO_3^{2-})[b]	3.5	5.6	[c]	7.4	–
Fluoride[a]	0.01	0.06	[c]	0.03	–
Chloride[a]	0.30	0.01	[c]	0.13	–
Pyrophosphate (as $P_2O_7^{4-}$)[b]	0.022	0.10	[c]	0.07	–
Total inorganic[b]	97	70	50	65	100
Total organic[b]	1.5	20	35	25	–
Water[b]	1.5	10	15	10	–
Crystallographic properties: Lattice parameters (±0.003 Å)					
a-axis, Å	9.441	9.421	[c]	9.41	9.430
c-axis, Å	6.880	6.887	[c]	6.89	6.891
Crystallinity index, (HA = 100)	70–75	33–37	[c]	33–37	100
Typical crystal sizes (nm) [205, 231, 233]	100 μm ×50 × 50	35 × 25 × 4	[c]	50 × 25 × 4	200–600
Ignition products (800°C)	*β*-TCP + HA	*β*-TCP + HA	*β*-TCP + HA	HA + CaO	HA
Elastic modulus (GPa) [607]	80	15	[c]	0.34–13.8	10
Tensile strength (Mpa)	10	100	[c]	150	100

Due to the considerable variation found in biological samples, typical values are given in these cases

[a] Ashed samples

[b] Unashed samples

[c] Numerical values were not found in the literature but they should be similar to those for dentin

is given below. Table 3 comprises their crystallographic data [33].

Monocalcium phosphate monohydrate (MCPM, $Ca(H_2PO_4)_2 \cdot H_2O$; the chemically correct name is calcium dihydrogen orthophosphate monohydrate) is both the most acidic and water-soluble compound. It precipitates from highly acidic solutions that are normally used in industry of phosphorus-containing fertilizer production (''triple superphosphate'') [8]. At temperatures above 100°C, it releases a molecule of water and transforms into MCPA. Due to high acidity and solubility, MCPM is never found in biological calcifications. Moreover, pure MCPM is not biocompartible[6] with bone [34]. However, in medicine MCPM is used as a component of several self-hardening calcium orthophosphate cements [35–39]. In addition, MCPM is used as a nutrient, acidulant and mineral supplement for dry baking powders, food, feed and some beverages [40]. Coupled with $NaHCO_3$, MCPM is used as a leavening agent for both dry baking powders and bakery dough. MCPM might be added to salt-curing preserves, pickled and marinated foods. According to the European classification of food additives MCPM is marked as E341 additive. Occasionally, MCPM is added to tooth pastes. In addition, MCPM might be added to ceramics and glasses, while agriculture is the main consumer of a technical grade MCPM, where it is used as a fertilizer [8, 40].

Monocalcium phosphate anhydrous (MCPA, $Ca(H_2PO_4)_2$; the chemically correct name is calcium dihydrogen orthophosphate anhydrous) is the anhydrous form of MCPM. It crystallizes under the same conditions as MCPM but at temperatures above 100°C (e.g., from highly concentrated mother liquors in the fertilizer production). Like MCPM, MCPA never appears in calcified tissues and is not biocompartible due to its acidity. There is no current application of MCPA in medicine. Due to the similarity with MCPM, in many cases MCPA might be used instead of MCPM [8, 40]; however, highly hydroscopic properties of MCPA reduces its commercial application.

Dicalcium phosphate dihydrate (DCPD, $CaHPO_4 \cdot 2H_2O$; the chemically correct name is calcium hydrogen orthophosphate dihydrate; the mineral brushite[7]) can be easily crystallized from aqueous solutions. It transforms into DCPA at temperatures above 80°C. DCPD is of biological importance because it is often found in

[6] Biocompatibility is the ability of a material to perform with an appropriate host response in a specific application [41].

[7] To honor Prof. George Jarvis Brush (1831–1912), an American mineralogist, Yale University, New Haven, Connecticut, USA.

Table 2 Properties of the biologically relevant calcium orthophosphates [223, 224]

Ca/P molar ratio	Compound	Formula	Solubility at 25°C, –log(K_s)	Solubility at 37 °C, –log(K_s)	pH stability range in aqueous solutions at 25°C
0.5	Monocalcium phosphate monohydrate (MCPM)	$Ca(H_2PO_4)_2 \cdot H_2O$	1.14	Data not found	0.0–2.0
0.5	Monocalcium phosphate anhydrous (MCPA)	$Ca(H_2PO_4)_2$	1.14	Data not found	[c]
1.0	Dicalcium phosphate dihydrate (DCPD), mineral brushite	$CaHPO_4 \cdot 2H_2O$	6.59	6.63	2.0–6.0
1.0	Dicalcium phosphate anhydrous (DCPA), mineral monetite	$CaHPO_4$	6.90	7.02	[c]
1.33	Octacalcium phosphate (OCP)	$Ca_8(HPO_4)_2(PO_4)_4 \cdot 5H_2O$	96.6	95.9	5.5–7.0
1.5	α-Tricalcium phosphate (α-TCP)	α-$Ca_3(PO_4)_2$	25.5	25.5	[a]
1.5	β-Tricalcium phosphate (β-TCP)	β-$Ca_3(PO_4)_2$	28.9	29.5	[a]
1.2–2.2	Amorphous calcium phosphate (ACP)	$Ca_xH_y(PO_4)_z \cdot nH_2O$, n = 3–4.5; 15–20% H_2O	[b]	[b]	~5–12[d]
1.5–1.67	Calcium-deficient hydroxyapatite (CDHA)	$Ca_{10-x}(HPO_4)_x(PO_4)_{6-x}(OH)_{2-x}$ $(0 < x < 1)$	~85.1	~85.1	6.5–9.5
1.67	Hydroxyapatite (HA or OHAp)	$Ca_{10}(PO_4)_6(OH)_2$	116.8	117.2	9.5–12
1.67	Fluorapatite (FA or FAp)	$Ca_{10}(PO_4)_6F_2$	120.0	119.2	7–12
2.0	Tetracalcium phosphate (TTCP or TetCP), mineral hilgenstockite	$Ca_4(PO_4)_2O$	38–44	37–42	[a]

The solubility is given as the logarithm of the ion product of the given formulae (excluding hydrate water) with concentrations in mol/l

[a] These compounds cannot be precipitated from aqueous solutions

[b] Cannot be measured precisely. However, the following values were found: 25.7 ± 0.1 (pH = 7.40), 29.9 ± 0.1 (pH = 6.00), 32.7 ± 0.1 (pH = 5.28) [84]. The comparative extent of dissolution in acidic buffer is: ACP >> α-TCP >> β-TCP > CDHA >> HA > FA [28]

[c] Stable at temperatures above 100°C

[d] Always metastable

pathological calcifications (dental calculi, crystalluria, chondrocalcinosis [4, 19, 20] and urinary stones [21]). In addition, it is found in some carious lesions [43]. It has been proposed as an intermediate in both bone mineralization and dissolution of enamel in acids (dental erosion) [4, 19–21]. In medicine, DCPD is used in calcium phosphate cements [38, 42–48] and as an intermediate for tooth remineralization [49]. DCPD is

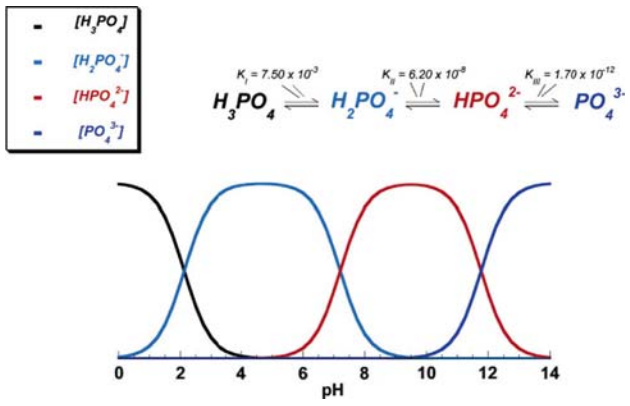

Fig. 2 pH variation of ionic concentrations in tripotic equilibrium for phosphoric acid solutions. Reprinted from [32] with permission

added to toothpaste both for caries protection (in this case, it is coupled with F-containing compounds such as NaF and/or Na_2PO_3F [50–53]) and as a gentle polishing agent. Other applications are: a flame retardant [54], a slow release fertilizer, glass production, as well as calcium supplement in food, feed and cereals [40]. In food industry, it serves as a texturizer, bakery improver and water retention additive. In diary industry, DCPD is used as a mineral supplement. If added to food products, DCPD should be marked as E341 according to the European classification of food additives. In addition, plate-like crystals of DCPD might be used as a non-toxic, anticorrosive and passivating pigment for some ground coat paints.

Dicalcium phosphate anhydrous (DCPA, $CaHPO_4$; the chemically correct name is calcium hydrogen orthophosphate anhydrous; the mineral monetite[8]) is the anhydrous form of DCPD. Like DCPD, DCPA can be crystallized from aqueous solutions but at 100°C. Unlike DCPD, DCPA occurs in neither normal nor pathological calcifications. It is used in calcium phos-

[8] For Moneta (now Monito) Island, which contains a notable occurrence.

Table 3 Crystallographic data of calcium orthophosphates [5, 3, 11]

Compound	Space group	Unit cell parameters	Z^a	Density, g cm^{-3}
MCPM	Triclinic $P\bar{1}$	a = 5.6261(5), b = 11.889(2), c = 6.4731(8) Å, α = 98.633(6)°, β = 118.262(6)°, γ = 83.344(6)°	2	2.23
MCPA	Triclinic $P\bar{1}$	a = 7.5577(5), b = 8.2531(6), c = 5.5504(3) Å, α = 109.87(1)°, β = 93.68(1)°, γ = 109.15(1)°	2	2.58
DCPD	Monoclinic Ia	a = 5.812(2), b = 15.180(3), c = 6.239(2) Å, β = 116.42(3)°	4	2.32
DCPA	Triclinic $P\bar{1}$	a = 6.910(1), b = 6.627(2), c = 6.998(2) Å, α = 96.34(2)°, β = 103.82(2)°, γ = 88.33(2)°	4	2.89
OCP	Triclinic $P\bar{1}$	a = 19.692(4), b = 9.523(2), c = 6.835(2) Å, α = 90.15(2)°, β = 92.54(2)°, γ = 108.65(1)°	1	2.61
α-TCP	Monoclinic $P2_1/a$	a = 12.887(2), b = 27.280(4), c = 15.219(2) Å, β = 126.20(1)°	24	2.86
β-TCP	Rhombohedral $R3Ch$	$a = b$ = 10.4183(5), c = 37.3464(23) Å, γ = 120°	21[b]	3.08
HA	Monoclinic $P2_1/b$ or hexagonal $P6_3/m$	a = 9.84214(8), $b = 2a$, c = 6.8814(7) Å, γ = 120° (monoclinic) $a = b$ = 9.4302(5), c = 6.8911(2) Å, γ = 120° (hexagonal)	4 2	3.16
FA	Hexagonal $P6_3/m$	$a = b$ = 9.367, c = 6.884°Å, γ = 120°	2	3.20
TTCP	Monoclinic $P2_1$	a = 7.023(1), b = 11.986(4), c = 9.473(2) Å, β = 90.90(1)°	4	3.05

[a] Number of formula units per unit cell

[b] Per the hexagonal unit cell

phate cements [47, 55–60]. Other applications include using as a polishing agent, a source of calcium and phosphate in nutritional supplements (e.g., in prepared breakfast cereals, enriched flour, and noodle products), a tabletting aid and a toothpaste component [40]. In addition, it is used as a dough conditioner in food industry.

Octacalcium phosphate (OCP, $Ca_8(HPO_4)_2(PO_4)_4 \cdot 5H_2O$) is often found as an unstable transient intermediate during the precipitation of the thermodynamically more stable calcium orthophosphates (e.g., CDHA) in aqueous solutions. Structurally OCP consists of apatitic layers (with atomic arrangements of calcium and orthophosphate ions similar to those of HA) separated by hydrated layers (water molecules) [4–6, 61]. OCP is of a great biological importance because it is one of the stable components of human dental and urinary calculi [62–64]. OCP was first proposed by Brown [65] to participate as the initial phase in enamel mineral formation, and bone formation through subsequent precipitation and stepwise hydrolysis of OCP was proposed [66]. It plays an important role in in vivo formation of apatitic biominerals. A ''central OCP inclusion'' (also known as ''central dark line'') is seen by transmission electron microscopy in many biological apatites and in some synthetically precipitated HA [67–70]. Although OCP has not been observed in vascular calcifications, it has been strongly suggested as a precursor phase to biological apatite found in natural and prosthetic heart valves [71, 72]. In surgery, OCP is used for implantation into bone defects [73–76]. For the comprehensive information on OCP, the readers are referred to a monograph [64].

β-Tricalcium phosphate (β-TCP, β-$Ca_3(PO_4)_2$; the chemically correct name is calcium orthophosphate tribasic beta) cannot be precipitated from aqueous solutions. It is a high temperature phase of calcium orthophosphates, which only can be prepared by thermal decomposition, e.g. of CDHA, at temperatures above 800°C. Apart from the chemical preparation routes, ion-substituted β-TCP can be prepared by calcining of bones: such type of β-TCP is occasionally called ''bone ash''.

At temperatures above 1125°C, it transforms into the high-temperature phase α-TCP. Being the stable phase at room temperature, β-TCP is less soluble in water than α-TCP (Table 2). Pure β-TCP never occurs in biological calcifications. Only the Mg-substituted form called whitlockite[9] (β-TCMP, β-tricalcium magnesium phosphate, β-$(Ca,Mg)_3(PO_4)_2$) is found in dental calculi and urinary stones [4, 19–21, 80], dentinal caries, salivary stones, arthritic cartilage, as well as in some soft-tissue deposits [4, 19–21]. In biomedicine, β-TCP is used in calcium phosphate bone cements [35, 36, 81–84]. In combination with HA, β-TCP forms

[9] In 1941, to honor Mr. Herbert Percy Whitlock (1868–1948), an American mineralogist, the curator of the American Museum of Natural History, New York City, New York, USA, the term whitlockite was coined as a synonym for β-TCP identified by its X-ray diffraction pattern in phosphate rocks [77]. Therefore, strictly speaking, β-TCMP should be called as a ''magnesium whitlokite''. An iron-containing whitlockite with chemical formula $Ca_9(Mg, Fe^{2+})(PO_4)_6(PO_3,OH)$ exists in nature: is a relatively rare natural mineral but is found in granitic pegmatite and has also been found in meteorites. It can form small, but distinct and well-formed crystals [78, 79].

the biphasic calcium phosphate (BCP) [85–93]. Both β-TCP [94] and BCP [85–93] are widely used as a bone substitution bioceramics. Pure β-TCP is added to some brands of toothpaste as a gentle polishing agent. Multivitamin complexes with calcium orthophosphate are widely available in the market and β-TCP is used as the calcium phosphate there. In addition, it serves as a texturizer, bakery improver and anti-clumping agent for dry powdered food (flour, milk powder, dried cream, cocoa powder). In addition, β-TCP is added as a dietary or mineral supplement to food and feed, where it is marked as E341 according to the European classification of food additives. Occasionally, it might be used as inert filler in pelleted drugs. Other applications comprise porcelains, pottery, enamel, using as a component for mordants and ackey, as well as a polymer stabilizer [40]. β-TCP of a technical grade (as either calcined natural phosphorites or bone dust) is used as a slow release fertilizer for acidic soils [8].

α-Tricalcium phosphate (α-TCP, α-$Ca_3(PO_4)_2$; the chemically correct name is calcium orthophosphate tribasic alpha) is usually prepared from β-TCP at heating above 1125°C and it might be considered as a high temperature phase of β-TCP. However, at the turn of the millennium, it was discovered that if calcium orthophosphates were doped with a certain amount of silica (approx. 1 mol of SiO_2 per 1 mol of HA), α-TCP would become stable at lower temperatures of 800–1,000°C. Such type of α-TCP is called ''silicon stabilized α-TCP'' [95–99].

Although α-TCP and β-TCP have exactly the same chemical composition, they differ by the crystal structure (Table 3) and solubility (Table 2). In addition, β-TCP is more stable than the α-phase [100]. Therefore, of them, α-TCP is more reactive in aqueous systems, has a higher specific energy and it can be hydrolyzed to a mixture of other calcium phosphates. It never occurs in biological calcifications but in medicine chemically pure α-TCP is occasionally used in calcium phosphate cements [38, 45, 47, 48, 57–60, 101]. However, the silicon stabilized α-TCP (more precisely as a biphasic composite with HA) has been commercialized as a starting material to produce bioresorbable porous ceramic scaffolds to be used as artificial bone grafts [94–99]. Theoretical insights into bone grafting properties of the silicon-stabilized α-TCP might be found in [102]. Surface and adsorption properties of α-TCP are available in [103]. α-TCP of a technical grade might be used as a fertilizer [40].

Amorphous calcium phosphate (ACP, $Ca_xH_y(PO_4)_z \cdot nH_2O$, n = 3–4.5; 15–20% H_2O) is often encountered as a transient phase during the formation of calcium orthophosphates in aqueous systems. Usually, ACP is the first phase precipitated from a supersaturated solution prepared by rapid mixing of solutions containing ions of calcium and orthophosphate [5, 104–109]. ACP is thought to be formed at the beginning of the precipitation due to a lower surface energy than that of OCP and HA [105]. The amorphization level of ACP increases with the concentration increasing of calcium- and orthophosphate-containing solutions, as well as at a high solution pH and a low crystallization temperature. A continuous gentle agitation of as precipitated ACP in the mother solution, especially at elevated temperatures, results in a slow recrystallization and formation of better crystalline compounds, such as CDHA [4, 5]. The chemical composition of ACP strongly depends on the solution pH and the concentrations of mixing solutions. For example, ACP with Ca/P ratios in the range of 1.18 (precipitated at solution pH = 6.6) to 1.53 (precipitated at solution pH = 11.7) [5, 110] and even to 2.5 [4, 19, 20] have been described. The presence of ions of pyrophosphate, carbonate and/or magnesium in solution during the crystallization promotes formation of ACP and slows down its further transformation into more crystalline calcium orthophosphates, while the presence of fluoride has the opposite effect [4–6, 28, 111].

The structure of ACP is still uncertain. Infra red spectra of ACP show broad featureless phosphate absorption bands and ACP is X-ray amorphous. Electron microscopy of ACP usually shows spherical particles with diameters in the range of 20–200 nm without distinct morphology. However, there is the possibility that ACP has an apatitic structure but with a crystal size so small, that it is X-ray amorphous. This is supported by X-ray absorption spectroscopic data (EXAFS) on biogenic and synthetic samples [112–115]. On the other hand, it was proposed that the basic structural unit of ACP is a 9.5 Å diameter, roughly spherical cluster of ions with the composition $Ca_9(PO_4)_6$ [5, 110]. These clusters were found experimentally as first nuclei during the crystallization of HA, and a model was developed to describe the crystallization of HA as a step-wise assembly of these units [116] (see HA). Biologically, ACP (often containing Na, Mg, carbonate, and pyrophosphate) is found in soft-tissue pathological calcifications (e.g., heart valve calcifications of uremic patients) [4, 19–21]. In medicine, pure ACP is used in calcium phosphate cements [45–47] and as a filling material in dentistry. Bioactive composites of ACP with polymers have properties suitable for use in dentistry [117–120] and surgery [121–124]. Due to a reasonable solubility and physiological pH of aqueous solutions, ACP appeared

to be consumable by some microorganisms and for this reason it might be added as a mineral supplement to culture media. Non-biomedical applications of ACP comprise its using as a component for mordants and ackey. In food industry, ACP is used for syrup clearing. Occasionally, it might be used as inert filler in pelleted drugs. In addition, ACP is used in glass and pottery production and as a raw material for production of some organic phosphates. A good review on ACP is available [125].

Calcium-deficient hydroxyapatite (CDHA, $Ca_{10-x}(HPO_4)_x(PO_4)_{6-x}(OH)_{2-x}$ $(0 < x < 1)$) can be easily prepared by simultaneous addition of calcium- and orthophosphate-containing solutions into boiling water followed by boiling the suspension for several hours. During this time, initially precipitated ACP is restructured and transformed into CDHA[10]. Therefore, there are many similarities in the structure, properties and application between the precipitated in alkaline solutions (pH > 8) ACP and CDHA. CDHA crystals are poorly crystalline and of submicron dimensions. It has a very large specific surface area, typically 25–100 m^2/g. On heating above 700°C, dry CDHA with Ca/P = 1.5 will convert to β-TCP and that with 1.5 < Ca/P < 1.67 will convert into a mixture of HA and β-TCP (the above-mentioned BCP) [85–93]. A reasonable solid-state mechanism of a high-temperature transformation of CDHA into BCP has been described [129, 130].

The variability in Ca/P molar ratio of CDHA has been explained through different models: surface adsorption, lattice substitution and intercrystalline mixtures of HA and OCP [131]. Due to the lack of stoichiometry, CDHA usually contains other ions. The extent depends on the counter-ions of the chemicals used for preparation (e.g., Na^+, Cl^-). Direct determinations of the structures of CDHA are still missing and the unit cell parameters are uncertain. However, the following lattice parameters have been reported for formate (HCO_2^-) containing CDHA with Ca/P = 1.596 (molar): a = 9.4729(20) and c = 6.8855(9) Å. Ca^{2+} ions were lost exclusively from Ca2 sites, while the PO_4 tetrahedron volume and P–O bonds were 4.4% and 1.4% smaller, respectively, than those in HA [132].

As a first approximation, CDHA may be considered as HA with some ions missing [133]. According to the chemical formula of CDHA (Table 2), there are vacancies of Ca^{2+} (mainly on Ca2 sites [132, 134, 135]) and OH^- ions in crystal structure of this compound. However, nothing is known about the vacancies of orthophosphate ions: in CDHA, a portion of PO_4^{3-} ions is either protonated (as HPO_4^{2-}) or substituted by other ions (e.g., CO_3^{2-}) [136].

Unsubstituted CDHA (i.e. containing ions of Ca^{2+}, PO_4^{3-}, HPO_4^{2-} and OH^- only) does not exist in biological systems. The ion-substituted CDHA: Na^+, K^+, Mg^{2+}, Sr^{2+} for Ca^{2+}; CO_3^{2-} for PO_4^{3-} or HPO_4^{2-}; F^-, Cl^-, CO_3^{2-} for OH^-, plus some water forms biological apatite—the main inorganic part of animal and human normal and pathological calcifications [4, 19]. Therefore, CDHA is a very promising compound for industrial manufacturing of artificial bone substitutes. Non-biomedical applications of CDHA are similar to those of ACP.

Hydroxyapatite (HA (or OHAp),[11] $Ca_5(PO_4)_3(OH)$, but is usually written as $Ca_{10}(PO_4)_6(OH)_2$ to denote that the crystal unit cell comprises two molecules) is the second most stable and least soluble calcium orthophosphate after FA (see below). Chemically pure HA crystallizes in the monoclinic space group $P2_1/b$. However, at temperatures above 250°C, there is a monoclinic to hexagonal phase transition in HA [5, 110] (space group $P6_3/m$) [137, 138]. The detailed description of the HA structure was first reported in 1964 [139], and its interpretation in terms of aggregation of $Ca_9(PO_4)_6$ clusters, the so-called Posner's clusters, has been widely used since publication of the article by Posner and Betts [140]. Some impurities, like partial substitution of hydroxide by fluoride or chloride, stabilize the hexagonal structure of HA at ambient temperature. For this reason, the very rare single crystals of natural HA always exhibit a hexagonal space group. The crystal structure of HA is well described elsewhere [141, 142], the detailed analysis of the electronic structure, bonding, charge transfer, and optical properties are also available [143], while the readers interested in Posner's clusters are referred to other papers [144–147].

Several techniques might be utilized for HA preparation; they can be divided into solid-state reactions and wet methods, which include precipitation, hydrothermal and hydrolysis of other calcium orthophosphates. HA can be prepared in aqueous solutions by mixing exactly stoichiometric quantities of Ca- and PO_4–containing solutions at pH > 9, followed by boiling for several days in CO_2–free atmosphere (the ageing or maturation stage), filtration, drying and, usually, sintering at about 1,000°C [148]. As the first precipitates are rich in non-apatitic environments (see

[10] In some research papers, CDHA is defined as "precipitated HA" [126–128].

[11] It is worth noting that the chemically correct name would be *hydroxylapatite* (perhaps, *hydroxidapatite* would be even better because it relates to calcium hydroxide) while by the medical and material communities it is usually called as *hydroxyapatite*.

ACP and CDHA), the ageing stage appears to be very important: the Ca/P molar ratio of 1.67 was found to attain in as little as 5 h after the completion of the reaction at 90°C [149]. The surface of freshly precipitated HA is composed of a structured hydrated layer containing easily exchangeable mobile ionic species [150]. Usually unsintered HA is poorly crystalline and often non-stoichiometric, resembling the aforementioned CDHA. However, also HA prepared from an aqueous solution can be highly crystalline [151]. Microcrystalline samples of HA can also be prepared by solid-state reaction of other calcium phosphates (e.g. MCPM, DCPA, DCPD, OCP) with CaO, $Ca(OH)_2$, or $CaCO_3$ at temperatures above 1,200°C in an atmosphere of equal volumes of water and nitrogen. HA can be prepared by hydrothermal synthesis [5, 110, 152]. A water-free synthesis can be performed in ethanol from $Ca(OEt)_2$ (Et = ethyl) and H_3PO_4 [153, 154]. In addition, HA might be prepared by mechanochemical synthesis of a dry mixture of CaO and DCPD [155] or from coral skeletal carbonate by hydrothermal exchange [156, 157]. Lower sized particles of HA might be prepared by a pyrosol technique, where an aerosol, containing calcium and orthophosphate ions in the adequate ratio, is transported to a furnace where the pyrolisis takes place [158]. Electrochemical synthesis of nanosized HA has also been described [159]. Two-dimensional nanocrystalline HA might be also synthesized [160]. Space-grown and terrestrial HA crystals were found to differ in size: the former appeared to be at least 1–1.5 orders of magnitude bigger in length [161]. The detailed information on HA synthesis is available elsewhere [162–166]. In addition, there are reviews on HA solubility, crystal growth and intermediate phases of HA crystallization [167], as well as on HA dissolution [168]. The electronic and crystallographic structure of apatites might be found in another paper [169].

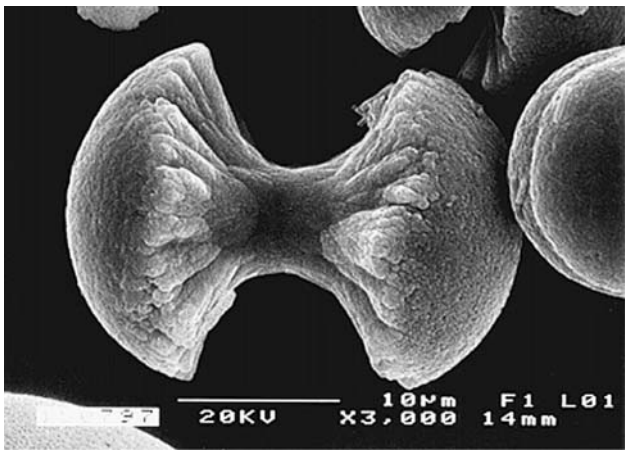

Fig. 3 A biomimetically grown aggregate of FA that was crystallized in a gelatin matrix. Its shape can be explained and simulated by a fractal growth mechanism. Scale bar: 10 µm (taken from. [193] with permission)

Pure HA never occurs in biological systems. However, due to the chemical similarities to bone and teeth mineral (Table 1), HA is widely used as a coating on orthopedic (e.g., hip joint prosthesis) and dental implants [170–176]. A calcium phosphate cement with HA has been developed [42, 44]. Due to the great similarity to biological apatite, HA is used in liquid chromatography of proteins and other biological compounds [177–182] and for drug delivery purposes [183, 184]. Also, HA is added to some brands of toothpaste as a gentle polishing agent instead of calcium carbonate. To conclude this topic, one should mention on other reviews devoted to HA and its biomedical application [185–191].

Fluorapatite (FA (or FAp), $Ca_5(PO_4)_3F$, but is usually written as $Ca_{10}(PO_4)_6F_2$ to denote that the crystal unit cell comprises two molecules) is the hardest (5 according to the Mohs' scale of mineral hardness), most stable and least soluble compound among all calcium orthophosphates (Table 2). Due to these properties, FA is the only calcium orthophosphate that naturally forms large deposits suitable for the commercial use [7–10] (see also Fig. 1). Preparation techniques of the chemically pure FA are similar to the aforementioned ones for HA but the synthesis must be performed in presence of the necessary amount of F^- ions (usually, NaF or NH_4F is added). Under some special crystallization conditions, FA might form unusual dumbbell-like fractal morphology that finally closed to spheres (Fig. 3) [192–197]. A hierarchical structure for FA was proposed [198]. The crystal structure of FA is well described elsewhere [142], while the detailed analysis of the electronic structure, bonding, charge transfer, and optical properties is also available [143]. In addition, there are reviews on FA solubility [167] and the dissolution mechanism [168].

FA easily forms solid solutions with HA with any desired F/OH molar ratio. Such compounds are called fluorhydroxyapatites (FHA) or hydroxyfluorapatites (HFA) and described with a chemical formula $Ca_{10}(PO_4)_6(OH)_{2-x}F_x$, where $0 < x < 2$. If the F/OH ratio is either uncertain or not important, the chemical formula of FHA and HFA is often written as $Ca_{10}(PO_4)_6(F,OH)_2$. The lattice parameters, crystal structure, solubility and other properties of FHA and HFA lay in between of those for the chemically pure FA and HA [199–203].

Similar to pure HA, pure FA never occurs in biological systems. Obviously, a lack of the necessary amount of toxic fluorides (the acute toxic dose of fluoride is ~5 mg/kg of body weight) in living organisms is the main reason of this fact (pure FA contains 3.7% mass. F). Enameloid of shark teeth [28, 204–206] and some exoskeletons of mollusks [207] seem to be the only exclusions because they contain substantial amounts of FA. Among all normal calcified tissues of humans, the highest concentration of fluorides is found in bones and the lowest—in dental enamel[12] (Table 1). However, even in bones, the total amount of fluorides is not enough to form FA; it is generally considered that the inorganic part of bones consists of ion-substituted CDHA. Due to the lowest solubility, good chemical stability and toxicity of high amounts of fluorides, chemically pure FA is rarely used as a bone substituting material [214]. However, due to the ability to form FHA and HFA, occasionally minor amounts of fluorides are intentionally added to calcium orthophosphate biomaterials [215–219]. The effect of fluoride contents in FHA on osteoblast behavior has been described [220].

Tetracalcium phosphate (TTCP (or TetCP), $Ca_4(PO_4)_2O$; the mineral hilgenstockite) is the most basic calcium orthophosphate. However, its solubility in water is higher than that of HA (Table 2). TTCP cannot be precipitated from aqueous solutions. It can be prepared only by a solid-state reaction above 1,300°C, e.g., by heating homogenized equimolar quantities of DCPA and $CaCO_3$ in dry air, or in a flow of dry nitrogen [5, 110, 221]. This reaction should be carried out in a dry atmosphere, in vacuum or with rapid cooling (to prevent uptake of water and formation of HA). TTCP is not very stable in aqueous solutions: it hydrolyses to HA and calcium hydroxide [5, 110]. Consequently, TTCP is never found in biological calcifications. In medicine, TTCP is widely used for preparation of various self-setting calcium phosphate cements [39, 42, 44, 45, 55, 57, 222–224]; however, to the best of my knowledge, there is no commercial bone-substituting product consisting solely of TTCP.

To conclude this part, one should briefly mention on chlorapatite and various ion-substituted calcium orthophosphates. Usually, they are of non-stoichiometric nature and there are too many of them to be discussed in one review; therefore, the readers are referred to books and monographs [4–7, 9, 14, 28, 57, 110, 187, 191]. It is interesting to note, that chemical elements not found in natural bones can be intentionally incorporated into calcium orthophosphate biomaterials to get special properties. For example, addition of Ag [225] and Cu [226] has been used for imparting antimicrobial effect, while radioactive Sm-153 and Re-186 have been incorporated into HA microspheres and injected into knee joints to treat rheumatoid joint synovitis [227].

[12] The amount of fluorides on the very surface of dental enamel might be increased by using fluoride-containing toothpastes and mouthwashes [208–211]. Fluoride-containing toothpastes and mouthwashes are widely used in practice due to the well-known anti-cariogenic effect of fluorides that is related to the solubility decreasing [212, 213].

Biological hard tissues of calcium orthophosphates

Biological mineralization (biomineralization) is the process of in vivo formation of inorganic minerals [205, 206]. As shown in Table 1 and discussed above, in the body of mammals the vast majority of both normal and pathological calcifications consist of ion-substituted calcium orthophosphates, mainly of apatitic structure. The impurities in biological apatite of bones and teeth introduce significant stresses into the crystal structure, which make it less stable and more reactive. Among all substituting ions, the presence of 4–8% of carbonates instead of orthophosphate anions (so called, B-type substitution [4–6]) and of 0.5–1.5% of magnesium is of the special importance because it leads to large lattice strain and significantly increases the solubility [228]. High concentrations of magnesium and carbonates in bone or dentin compared with enamel (Table 1) may explain a higher solubility and a lower crystallinity (smaller crystal size) of bone or dentin compared with enamel. In addition, the crystals of biological apatite are always very small which also increases its solubility when compared with that for the chemically pure HA and even CDHA. Small dimensions and a low crystallinity are two distinct features of biological apatite, which, combined with their non-stoichiometric composition, inner crystalline disorder and presence of other ions in the crystal lattice, allow explaining their special behavior [229]. The major properties of biological apatite are summarized in Fig. 4.

The calcium orthophosphate nature of bones was first determined in 1926 [230]. Nowadays, according to Weiner and Wagner: ''the term bone refers to a family of materials, all of which are built up of mineralized collagen fibrils'' [231, 232]. For mammals, this family of materials includes dentin—the material that constitutes the inner layers of teeth, cementum—the thin layer that binds the roots of teeth to the jaw, deer antlers and some other materials [231, 233]. It is worth noting, that bones and teeth contain almost 99% of the

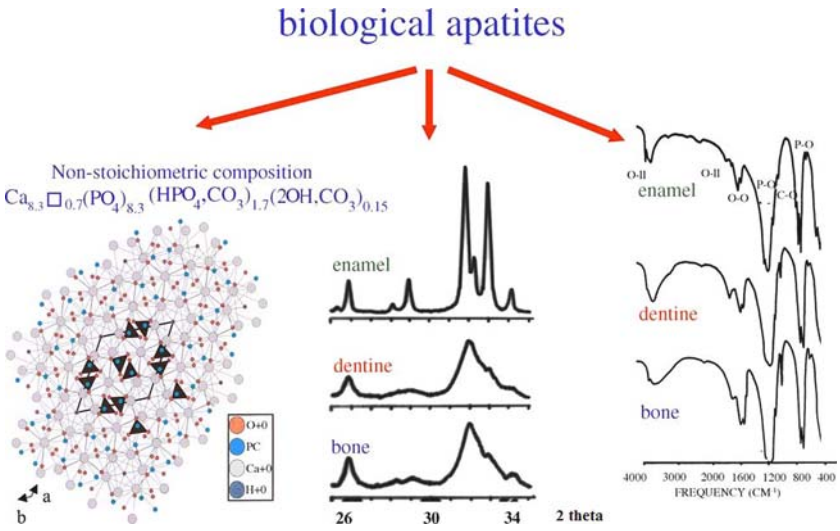

Fig. 4 Crystal structure of biological apatite. Powder X-ray diffraction patterns and infrared spectra of enamel, dentine and bone. Reprinted from [229] with permission

total body calcium and about 85% of the total body phosphorus that amounts to a combined mass of approximately 2 kg in an average person [234, 235]. In addition, it is important to recognize that calcium orthophosphates of bones are by no means inert; they play an important role in the metabolic functions of the body. The recent data on the physico-chemical and crystallographic study of biological apatite have been reviewed elsewhere [236]. Besides, there is a comprehensive review on the application of surface science methods to study the properties of dental materials and related biomaterials [237].

Bone

Bone, also called osseous tissue (Latin: *os*), is a type of hard endoskeletal connective tissue found in many vertebrate animals. All bones of a single animal are, collectively, known as the skeleton. True bones are present in bony fish (osteichthyes) and all tetrapods. Bones support body structures, protect internal organs and in conjunction with muscles facilitate movement. In addition, bones are also involved with cell formation, calcium metabolism and act for mineral storage. From the material point of view, bone is a complicated composite containing both inorganic (Table 1) and biological compounds (chiefly, collagen) [238–242]. The inorganic to biological ratio is approximately 75–25% by weight and 65–35% by volume. It is interesting to note, that bone exhibits several physical properties such as piezoelectricity [243] and pyroelectricity [244].

The stability of the mineral composition of bones has a very long history: calcium orthophosphates were found in dinosaur fossils [245–247]. Bones of modern animals is a relatively hard and lightweight porous composite material, formed mostly of biological apatite (i.e., CDHA with ionic substitutions). It has relatively high compressive strength but poor tensile strength. While bone is essentially brittle, it has a degree of significant elasticity contributed by its organic components. Usually bone is composed of a relatively dense outer layer (cortical or compact bone) covering an internal mesh-like structure of cancellous (other terms: spongy, trabecular) bone, the density of which may vary at different points (Fig. 5). Cortical bone makes up a large portion of skeletal mass; but, due to its high density, it has a low surface area. Cancellous bone has an open meshwork or honeycomb-like structure. It has a relatively high surface area but forms a smaller portion of the skeleton [4, 19, 20, 26–28, 205, 231, 238–242, 248–251]. Bone is a porous material with the pore sizes range from 1 μm to 100 μm in normal cortical bone and 200 μm to 400 μm in trabecular bone. 55–70% of the pores in trabecular bone are interconnected [252].

Bone can be either woven or lamellar. The fibers of woven bone are randomly aligned and as the result have a low strength. In contrast, lamellar bone has parallel fibers and is much stronger. Woven bone is put down rapidly during growth or repair but as growth continues, it is often replaced by lamellar bone. In addition, bones might be long, short, flat and irregular. Long bones are tubular in structure (e.g., the tibia). The central shaft of a long bone is called the diaphysis, and has a medullar cavity filled with bone marrow (Fig. 5). Surrounding the medullar cavity is a thin layer of cancellous bone that also contains marrow. The extremities of the bone are called the epiphyses and are mostly cancellous bone covered by a relatively thin layer of compact bone. Short bones (e.g., finger bones)

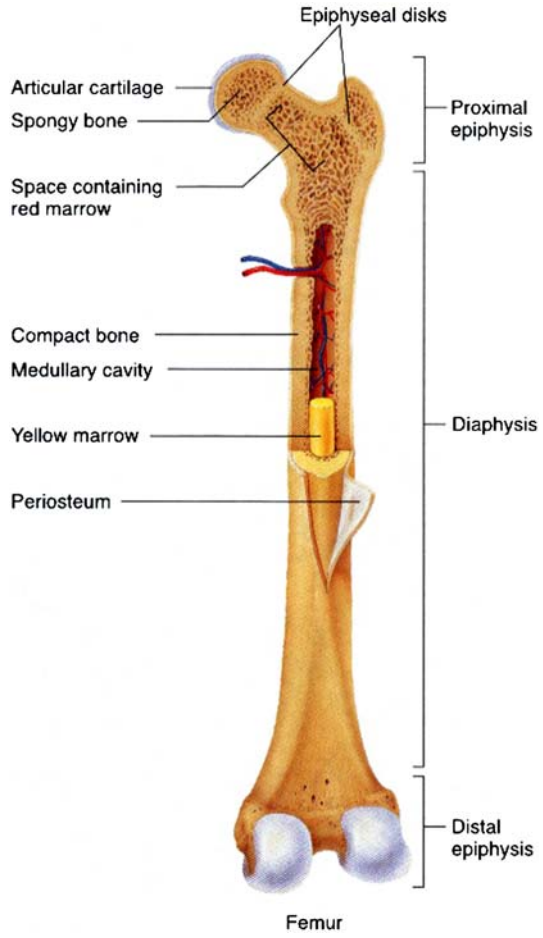

Fig. 5 General structure of a mammalian bone. A very good graphical sketch of the structure of a mammalian bone is available in [229]

have a similar structure to long bones, except that they have no medullar cavity. Flat bones (e.g., the skull and ribs) consist of two layers of compact bone with a zone of cancellous bone sandwiched between them. Irregular bones (e.g., vertebrae) do not conform to any of the previous forms. All bones contain living cells embedded in a mineralized organic matrix that makes up the main bone material. The structure of bone is most easily understood by differentiating between 7 levels of organization because bone exhibits a strongly hierarchical structure [187, 205, 231, 238–251].

Nanoscopically, the constituting building blocks of bone are mineralized collagen fibrils of 80–100 nm thickness and a length of a few to tens of microns. These are composites of biological apatite and molecules of type I collagen [231, 253]. The crystals of biological apatite in bone are always platelet-like (elongated along the crystallographic *c*-axis) and very thin [254, 255], with remarkably uniform thicknesses (determined in transmission electron microscopy) of 2–4 nm (just a few unit cells thick—see Table 1).[13] They are inserted in a parallel way into the collagen fibrils, while the latter are formed by self-assembly of collagen triple helices [231, 256, 257]. The lowest level of hierarchical organization of bone has successfully been simulated by HA precipitation on peptide-amphiphile nanofibers [258]. Unfortunately, the interface between collagen and crystals of biological apatite is still poorly understood; for the available details, the readers are referred to a review devoted to the structure and mechanical quality of the collagen/mineral nano-composite of bones [253]. There is still no clear idea why the crystals of biological apatite are platelet-shaped even though dahllite has hexagonal crystal symmetry [205, 231, 238–251]. One possible reason is that they grow via an OCP transition phase, which crystals are plate-shaped [231].

The processes of bone formation (ossification) and growth are very complicated ones and it is difficult to describe them without making a deep invasion into biology. Briefly, it is considered that bones appear and grow as the result of calcification (or biomineralization) of connective tissues, mainly cartilage. The ossified tissue is invaginated with blood vessels, which bring ions of calcium and orthophosphate to be deposited in the ossifying tissue. Thus, in vivo formation of hard tissues always occurs by mineral reinforcement of the previously formed network of soft tissues.

Cartilage is composed of cells (chondrocytes and their precursor forms known as chondroblasts), fibers (collagen and elastic fibers) and extracellular matrix (proteoglycans, which are a special class of heavily glycosylated glycoproteins) [259–261]. The initial stage involves the synthesis and extracellular assembly of the collagen matrix framework of fibrils. At the second stage, the chondrocytes calcify the matrix before undergoing the programmed cell death (apoptosis). At this point, blood vessels penetrate this calcified matrix, bringing in osteoblasts, which use the calcified cartilage matrix as a template to build bone, thus completing ossification [259–261].

During ossification, the crystals of biological apatite grow with a specific crystalline orientation—the *c*-axes of the crystals are roughly parallel to the long axes of the collagen fibrils within which they are deposited [231, 233–240]. The same is true for dentin and enamel [262, 263] (see below), as well as for more primitive living organisms. For example, in the shell of the fossil mollusk *Lingula unguis* that consists of biological

[13] Due to the nanoscopic dimensions, biological apatite is occasionally called ''nano-apatite'' [231].

apatite, the crystal *c*-axes are oriented parallel to the *β*-chitin fibrils [264, 265]. Therefore, the orientation of biological apatite crystals parallel to the long axes of the organic framework could be a general feature of calcium orthophosphate biomineralization.

Unlike other mineralized tissues, bone continuously undergoes a remodeling process, as it is resorbed by specialized cells called osteoclasts and formed by another type of cells called osteoblasts (so called ''bone lining cells'') in a delicate equilibrium [266, 267]. The purpose of remodeling is the release of calcium and the repair of micro-damaged bones from everyday stress. Osteoblasts contain alkaline phosphatase—this is a weak base. It acts as a local pH increaser, which results in precipitation of biological apatite. In addition, there is one more type of the cells called osteocytes that originate from osteoblasts, which have migrated into, become trapped and surrounded by bone matrix, which they themselves produce [238–241]. The interested readers are suggested to read a review on the interaction between biomaterials and osteoclasts [268].

If osteoblasts can be described as bone forming cells, the osteoclasts can be described as bone destroying cells because osteoclasts mature and migrate to discrete bone surfaces [266, 267]. Upon arrival, active enzymes, such as acid phosphatase, are secreted against the mineral substrate that causes dissolution. This process, called bone resorption, allows stored calcium to be released into systemic circulation, and is an important process in regulating calcium balance [266, 267]. The iteration of remodeling events at the cellular level is influential on shaping and sculpting the skeleton both during growth and afterwards. That is why mature bone consists of a very complex mesh of bone patches, each of which has both a slightly different structure and a different age [205, 231, 233–251].

Still there is no general agreement on the chemical mechanism of bone formation. It is clear that the inorganic part of bone consists of biological apatite, i.e. CDHA with ionic substitutions but without the detectable amounts of hydroxide [269–271]. However, various in vitro experiments on precipitation of CDHA and HA revealed that none of these compounds is directly precipitated from supersaturated aqueous solutions containing calcium and orthophosphate ions: some intermediate phases (precursors) are always involved [4, 19, 20, 67–72, 104–108]. Depending on the solution pH and crystallization conditions, three calcium orthophosphates (DCPD, ACP and OCP) are discussed as possible precursors of CDHA and HA precipitation in vitro. For this reason, the same calcium orthophosphates are suggested as the precursors of biological apatite formation in vivo.

The transient nature of the precursor phase of bone, if it exists at all, makes it very difficult to detect, especially in vivo. However, in 1966 Brown proposed that OCP is the initial precipitate that then acts as a template upon which biological apatite nucleates [272]. This idea was extended in his further investigations [273–276]. The principal support for this concept derived from the following: (i) the close structural similarity of OCP and HA [277]; (ii) formation of interlayered single crystals of OCP and HA (pseudomorphs of OCP); (iii) the easier precipitation of OCP compared with HA; (iv) the apparent plate- or lath-like habit of biological apatites that does not conform to hexagonal symmetry, but looks like a pseudomorph of triclinic OCP; (v) the presence of HPO_4^{2-} in bone mineral, particularly in newly formed bones [236]. Some evidences supporting this idea were found using high-resolution transmission electron microscopy: computer-simulated lattice images of the ''central dark line'' in mineralized tissues revealed that it consisted of OCP [67–69]. Additional evidences of OCP to HA transformation, including a mechanistic model for central dark line formation, were obtained very recently [278].

Simultaneously with Brown, the research group led by Posner proposed that ACP is the initially precipitated phase of bone formation in vivo [279–281]. This conclusion was drawn from the following facts: (i) when calcium orthophosphates are prepared by rapid precipitation from aqueous solutions containing ions of calcium and orthophosphate at pH > 8.5, the initial solid phase is amorphous; (ii) mature bone mineral is composed of a mixture of ion-substituted ACP and poorly crystallized ion-substituted CDHA; (iii) early bone mineral has a lower crystallinity than mature bone and the observed improvement in crystallinity with the age of the bone mineral is a result of a progressive reduction in the ACP content [236, 279–287]. However, there are thermodynamic data proving that the transition of freshly precipitated ACP into CDHA involves intermediate formation of OCP [288, 289].

The nucleation mechanism of bone minerals is not well established, mainly because of the difficulty involved in the nanostructural analyses of bone minerals [290]. Only indirect evidences for the in vivo bone mineral maturation are available. For example, X-ray diffraction patterns of bones from animals of different age show that the reflections become sharper with age increasing [291]. This effect is more pronounced in the crystallographic *a*-axis [(310) reflections] as compared

to the *c*-axis [(002) reflections] [292, 293]. In addition, other changes like an increase of Ca^{2+} content and a decrease of HPO_4^{2-} occur in bone mineral with age [294–296]. Both the crystal sizes and carbonate content were found to increase during aging in rats and cows [296, 297]. From a chemical point of view, these changes indicate to a slow transformation of poorly crystallized non-apatitic calcium orthophosphates into a better-crystallized ion-substituted CDHA [201].

A debate relates to the question whether bone formation is an active or a passive process. An ''active process'' means the assembly of calcium orthophosphate nanocrystals due to activity of the suitable cell (osteoblasts), i.e. within a matrix vesicle. These structures have been discovered by transmission electron microscopy for bone and teeth formation [298, 299]. The ''passive process'' does not require involvement of cells and means mineralization from supersaturated solutions with respect to precipitation of biological apatite. In the latter case, thermodynamically, the mineralization might occur at any suitable nucleus. The collagen fibrils have a specific structure with a 67 nm periodicity and 35–40 nm gaps or holes between the ends of the collagen molecules where bone mineral is incorporated in the mineralized fibril [205, 231, 232]. Such a nucleation within these holes would lead to discrete crystals with a size related to the nucleating cavity in the collagen fibril. It was proposed that a temporary absence of the specific inhibitors might regulate the process of bone formation [300–302].

Finally, let us briefly mention about the practical application of bones. Cut and polished bones from a variety of animals are sometimes used as material for jewelry and other crafts. Ground cattle bone is occasionally used as fertilizer. In the Stone Age, bone was used to manufacture art, weapons, needles, catchers, amulets, pendants, headdresses, etc.

Teeth

Teeth (singular: tooth) are dense structures found in the jaws of many vertebrates. They have various structures to allow them to fulfill their different purposes. The primary function of teeth is to tear, smell and chew food, while for carnivores it is a weapon. Therefore, teeth have to withstand a range of physical and chemical processes, including compressive forces (up to ~700 N), abrasion and chemical attack due to acidic foods or products of bacterial metabolism [237]. The roots of teeth are covered by gums. From the surface teeth are covered by enamel of up to ~2 mm thick at the cutting edges of the teeth, which helps to prevent cavities on the teeth. The biggest teeth of some gigantic animals (elephants, hippopotamuses, walruses, mammoths, narwhals, etc.) are known as tusks or ivory.

Similar to various types of bones, there are various types of teeth. The shape of the teeth is related to the animal's diet, as well as its evolutionary descent. For example, plants are hard to digest, so herbivores have many molars for chewing. Carnivores need canines to kill and tear and since meat is easy to digest, they can swallow without the need for molars to chew the food well. Thus, the following types of teeth are known: molars (used for grinding up food), carnassials (used for slicing food), premolars (small molars), canines (used for tearing apart food) and incisors (used for cutting food). While humans only have two sets of teeth, some animals have many more: for example, sharks grow a new set of teeth every 2 weeks. Some other animals grow just one set during the life, while teeth of rodents grow and wear away continually through the animal gnawing, maintaining constant length [303, 304].

The structure of teeth appears to be even more complicated than that of bone (Fig. 6). Unlike bone, teeth consist of at least two different materials: enamel that is a hard outer layer consisting of calcium

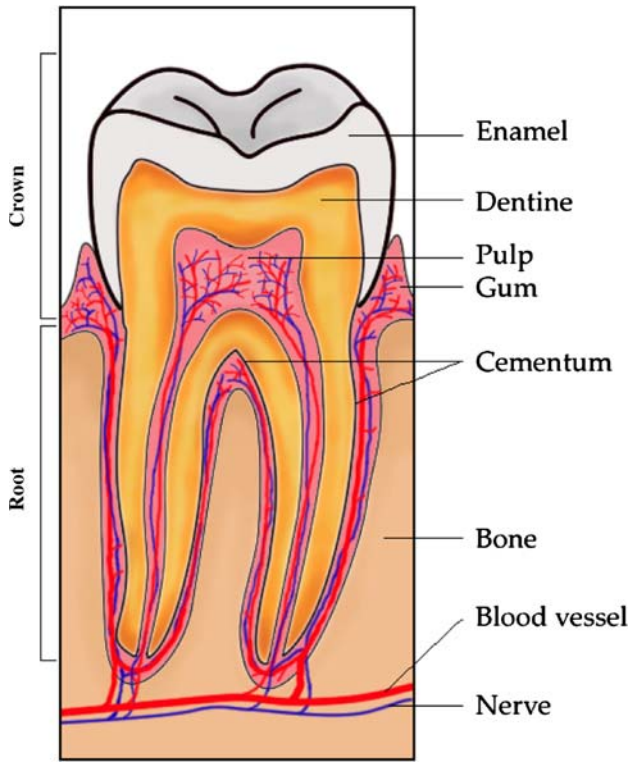

Fig. 6 A schematic picture of a tooth

orthophosphates and dentin, which is a bone-like inner layer, the bulk of the tooth. In addition, there is a thin layer around the tooth roots called cementum—it covers the anatomic root of the tooth. Cementum is a bone-like material similar to dentin, which connects the teeth to the jaw. Finally, there is the core called pulp (commonly called ''the nerve'')—it is a remnant of the embryologic organ for tooth development and contains nerves and blood vessels (Fig. 6) [303, 304]. Both dentin and cementum are mineralized connective tissues with an organic matrix of collagenous proteins, while the inorganic component of them consists of biological apatite. As shown in Table 1, dentin, cementum and bone are quite similar and for general purposes of material scientists they can be regarded as being essentially the same material[14] [5, 110, 205, 231, 233–256, 294, 295]. Thus, most statements made in the previous chapter for bone are also valid for dentin and cementum; however, both dentin and cementum lack vascularization.

Dental enamel is the outermost layer of teeth. It is white and translucent and the true color shows mainly at the cutting edges of the teeth. Enamel is highly mineralized and acellular, so it is not a living tissue. Nevertheless, it is sufficiently porous for diffusion and chemical reactions to occur within its structure, particularly acidic dissolution (dental caries) and remineralization from saliva (possible healing of caries lesions). Enamel is the hardest substance in the body and forms a solid, tough and wear-resistant surface for malaxation. In the mature state, it contains up to 98% of inorganic phase (Table 1). The crystals of biological apatite of enamel are much larger as evidenced by higher crystallinity (reflecting greater crystal size and perfection) demonstrated in their X-ray diffraction patterns, than those of bone and dentin [305]. The organic phase of enamel does not contain collagen. Instead, enamel has two unique classes of proteins called amelogenins and enamelins. While the role of these proteins is not fully understood yet, it is believed that both classes of proteins aid in the enamel development by serving as a framework support [303, 304]. The large amount of minerals in enamel accounts not only for its strength but also for its brittleness. Dentin, which is less mineralized and less brittle, compensates for enamel and is necessary as a support [303, 304]. Shark enameloid is an intermediate form bridging enamel and dentin. It has enamel-like crystals of fluoridated biological apatite associated with collagen fibrils [28, 205]. Due to the presence of fluorides, biological apatite of shark enameloid shows both higher crystal sizes and more regular hexagonal symmetry if compared to non-fluoridated biological apatite of bones and teeth [28].

[14] Strictly speaking, there are some differences. For example, the hardness of live dentin is less than that of enamel but is greater than that of bone or cementum. When pulp of the tooth dies or is removed by a dentist, the properties of dentin change: it becomes brittle, liable to fracture and looses a reparative capability.

Fig. 7 Scanning electron micrograph of the forming enamel of a continuously growing rat incisor showing ordered rods of calcium orthophosphates. Scale bar: 10 μm (taken from [205] with permission)

The basic unit of enamel is called an enamel rod (formerly called an enamel prism), which is a tightly packed mass of biological apatite in an organized pattern. Each rod traverses uninterrupted through the thickness of enamel. They number 5 to 12 million rods per crown. The rods increase in diameter (4 up to 8 μm) as they flare outward from the dentin-enamel junction (DEJ). Needle-like enamel rods might be tens of microns long (up to 100 μm) but sometimes only 50 nm wide and 30 nm thick (Fig. 7) [303–312]. They are quite different from the much smaller crystals of dentin and bone (Table 1), but all of them consist of biological apatite [194, 313–315]. In cross section, an enamel rod is best compared to a keyhole, with the top, or head, oriented toward the crown of the tooth, and the bottom, or tail, oriented toward the root of the tooth. Enamel is a selectively permeable membrane, allowing water and certain ions to pass via osmosis.

The arrangement of the crystals of biological apatite within each enamel rod is highly complex. Enamel crystals in the head of the enamel rod are oriented parallel to the long axis of the rod. When found in the tail of the enamel rod, the crystals' orientation diverges slightly from the long axis [303–305]. The arrangement of the enamel rods is understood more clearly than their internal structure. Enamel rods are found in rows along the tooth (Fig. 7), and within each row, the long

axis of the enamel rod is generally perpendicular to the underlying dentin [303–310]. Recent AFM study indicated that CDHA crystals in enamel exhibited regular subdomains or subunits with distinct chemical properties related to topographical features and gave rise to patterned behavior in terms of the crystal surface itself and the manner in which it responded to low pH [316].

The development of teeth appears to be even more complicated when compared with the afore-described process of bone formation. It is a very complex biological process, by which teeth are formed from embryonic cells, grow and erupt into the mouth. For human teeth enamel, dentin and cementum must all be developed during the appropriate stages of fetal development. Primary (baby) teeth start to form between the sixth and eighth weeks in utero, and permanent teeth begin to form in the twentieth week in utero [303, 304].

As teeth consist of at least two materials with different properties (enamel and dentin), the tooth bud (sometimes called ''the tooth germ''—that is an aggregation of cells that eventually forms a tooth) is organized into three parts: the enamel organ, the dental papilla and the dental follicle. The enamel organ is composed of at least four other groups of cells (for the biological details see [303, 304]). Altogether, these groups of cells give rise to ameloblasts, which secret enamel matrix proteins. The protein gel adjacent to ameloblasts is supersaturated with calcium orthophosphates, which leads to the precipitation of biological apatite. Similarly, the dental papilla contains cells that develop into odontoblasts, which are dentin-forming cells. The dental follicle gives rise to three important entities: cementoblasts, osteoblasts, and fibroblasts. Cementoblasts form the cementum of a tooth. Osteoblasts give rise to the alveolar bone around the roots of teeth (see bone formation above). Fibroblasts develop the periodontal ligaments that connect teeth to the alveolar bone through cementum [303, 304].

The first detectable crystals in enamel formation are flat thin ribbons [308–310], that were reported to be OCP [248, 317–319], β-$(Ca,Mg)_3(PO_4)_2$ [318] or DCPD [269, 271]. During maturation of enamel, the mineral content increases from initially 45 wt.% up to 98–99 wt.% [269, 303, 304]. The enamel crystal rods widen and thicken by additional growth [269, 271, 320, 321] with a simultaneous increase of the Ca/P molar ratio [320, 321] and a decrease in carbonate content [322–324], finally resulting in the most highly mineralized and hardest substance produced by vertebrates.

The crystal faces expressed in enamel are always the (100) face and at the ends presumably (001) [325, 326], which are the ones usually found in HA. The centers of enamel crystals contain a linear structure known as the ''central dark line'' (this line was also observed in bone and dentin), which consists of OCP [67–70]. As described above for bone, X-ray diffraction shows that the crystals of younger dentin are less crystalline than those of more mature dentin [294]. Therefore, maturation of dentin also means a slow transformation of biological calcium orthophosphates from ion-substituted ACP to a better-crystallized ion-substituted CDHA.

The development of individual enamel and dentin crystals was studied by high-resolution transmission electron microscopy [327–329]. Both processes appear to be roughly comparable and were described in a four-step process. The first two steps include the initial nucleation and formation of nanometer-sized particles of biological apatite. They are followed by ribbon-like crystal formation, which until recently was considered as the first step of biological crystal formation [327–329]. These complicated processes, starting with the heterogeneous nucleation of inorganic calcium orthophosphates on an organic extracellular matrix, are controlled in both tissues by the organic matrix and are under cellular control [330]. To complicate the process even further, regular and discrete domains of various charges or charge densities on the surface of CDHA crystals derived from the maturation stage of enamel development were recently discovered by a combination of atomic and chemical force microscopy [331]. Binding of organic molecules (e.g. amelogenin [331]) at physiological solution pH appears to occur on the charged surface domains of CDHA.

The DEJ[15] is the interface between the dentin and enamel. It is the remnant of the onset of enamel formation because enamel grows outwards from this junction [304, 305, 332–334]. The major steps of enamel crystal growth at the junction have been described above but the mechanism of the junction formation is still debatable. Some authors claim that the enamel crystals grow epitaxially on the pre-existing dentin crystals because of a high continuity between enamel and dentin crystals [335–337]. Others have shown that enamel crystals are formed at a given distance from the dentin surface [317–319, 338] and could either reach dentin crystals by a subsequent growth [339] or remain distant [338, 340].

Enamel is formed only during amelogenesis in the jaw. At some point before the tooth erupts into the mouth the ameloblasts are broken down. Consequently, enamel, unlike bones, has no way to regener-

[15] In addition, there is a cementum-enamel junction (CEJ), which is quite similar to DEJ.

ate itself using the process of ''active mineralization'' (see above the debate on bone formation); there is no biological process that repairs degraded or damaged enamel [303, 304]. In addition, certain bacteria in the mouth feed on the remains of foods, especially sugars. They produce lactic acid, which dissolves the biological apatite of the enamel in a process known as enamel demineralization that takes place below the critical pH of about 5.5. Similar process called enamel erosion occurs when a person consumes acid (citric, lactic, phosphoric, etc.) containing soft drinks [306, 341–344]. Evidences exist that there is a preferential loss of carbonates and Mg during the acid dissolution of mineral in dental caries [345]. Luckily, saliva gradually neutralizes the acids that cause the pH of the tooth surface to rise above the critical pH. This might cause partial enamel remineralization, the return of the dissolved calcium orthophosphates to the enamel. Until recently, it was generally agreed, that if there was sufficient time between the intake of foods (2–3 h) and the damage was very limited, the teeth could repair themselves by ''passive mineralization'' [346]. In addition to this, there are data on increased remineralization of tooth enamel by milk containing added casein phosphopeptide-ACP nanocomplexes [347].

Very recently, by using atomic force microscopy nanoindentation technique it was discovered that the previously demineralized samples of enamel further exposed to remineralizing solutions did show a crystalline layer of calcium orthophosphates formed on the enamel surface. Unfortunately, the re-precipitated deposits of calcium orthophosphates always consisted of loosely packed crystals and did not protect the underlying enamel from a subsequent acid attack. Furthermore, these surface deposits have been completely removed by either a toothbrush or a short exposure to an erosive acidic solution [306, 348–350]. In this context, it should be emphasized that the term remineralization, which is often misused in the literature, should imply the process of mineral growth that goes hand in hand with a strengthening effect of the weakened enamel surface. Since no strengthening of an exposure to remineralizing solutions was observed, it might be considered that no ''passive mineralization'' was found (in spite of the real evidence of the re-precipitated surface deposits of calcium orthophosphates) [306, 349, 350]. Thus, the enamel self-repairing ability by the passive remineralization appears to be doubtful, while the active remineralization is impossible.

A content of fluoride added to either toothpaste or mouthwash lowers the solubility of calcium orthophosphates (by formation of FHA on the surface) and therefore improves the acid-resistance of dental enamel [194, 208–213]. In addition, fluorides also reduce the production of acids by bacteria in the mouth by reducing their ability to metabolize sugars.

Finally, let us briefly mention on the practical application of teeth. Due to the relatively small dimensions of normal teeth, only tusks and ivory of giant animals are used. For example, both the Greek and Roman civilizations used large quantities of ivory to make high value works of art, precious religious objects and decorative boxes for costly objects. Ivory was often used to form the whites of the eyes of statues. Prior to the introduction of plastics, it was used for billiard balls, piano keys, buttons and ornamental items. The examples of modern carved ivory objects are small statuary, netsukes, jewelry, flatware handles and furniture inlays.

Antlers

Deer antlers are unique biological structures since their growth rate is without parallel in vertebrates and because they are the only bony appendages in mammals capable of complete regeneration [351, 352]. The antlers are not true horns; they are a simple extension of bone, so they have a matrix of biological apatite similar to that of mammalian bones. Antlers are the large and complex horn-like appendages of deer consisting of bony outgrowths from the head with no covering of keratin as is found in true horns. Similar to bones, antlers contain pores and can withstand applied stresses of over 300 MPa [353], which is even higher than that of bones (Table 1). Each antler grows from an attachment point on the skull called a pedicle. While an antler is growing it is covered with highly vascular skin called velvet, which supplies oxygen and nutrients to the growing bone. Once the antler has achieved its proper size, the velvet starts to dry out, cracks, and breaks off, while the antler's bone dies. Fully developed antlers consist only of dead bone [354–362]. Antlers are shed after mating season and regrown each year. However, people seldom come across the antlers in the woods. Rabbits and rodents such as mice and chipmunks eat antlers (and bones of wild animals after they die) for calcium. Rodents and rabbits also gnaw bones and antlers to sharpen their incisors.

Due to an extremely high growth rate combined with a very fast biomineralization, deer antlers might be a well-suited animal model for studying the disturbances of bone formation induced by additives (e.g., by excess of fluoride) [357]. Moreover, since antlers are periodically replaced, analysis of naturally cast antlers offers the opportunity for a continuous, noninvasive

monitoring of environmental pollution by these additives [356, 357].

Pathological calcification of calcium orthophosphates

In the body of mammals, osteoblasts and odontoblasts fix ions of calcium and orthophosphate and then precipitate biological apatite onto an organic matrix. This is the process of physiological biomineralization that is restricted to the specific sites in skeletal tissues, including growth plate cartilage, bones and teeth [28, 205]. Normally, mammals are supposed to die with calcium orthophosphates located in bones and teeth (and antlers for deer) only and nowhere else, because under normal conditions soft tissues are not mineralized. Unfortunately, owing to ageing, various diseases and under certain pathological conditions blood vessels and some internal organs are calcified as well. This process is called pathological calcification or ectopic mineralization and leads to morbidity and mortality [28, 205].

To the best of my knowledge, the first paper on a negative influence of unwanted depositions of calcium orthophosphates in the body was published in 1946 [363]. Such depositions always lead to various diseases, for instance: soft tissue calcification (in damaged joints, dysfunctional areas in the brain, diseased organs, scleroderma, prostate stones) [364–367], kidney and urinary stones [4, 368, 369], dental pulp stones and dental calculus [62, 63], salivary stones, gall stones, pineal gland calcification, atherosclerotic arteries and veins [370–372], cardiac skeleton, damaged cardiac valves, calcification on artificial heart valves [373–377], cataracts, malacoplakia, calcified menisci [378, 379], dermatomyositis [380]. In addition, there is metastatic calcification of nonosseous viable tissue occurring throughout the body, but it primarily affects the interstitial tissue of the blood vessels, kidney, lungs, and gastric mucosa [381]. Metastatic calcification is defined as the deposition of calcium orthophosphates in previously normal tissue due to abnormal biochemistry with disturbances in the calcium or phosphorus metabolism [382]. Common causes of metastatic calcification include hyperparathyroidism, chronic renal disease, massive bone destruction in widespread bone metastases, and increased intestinal calcium absorption. All these cases are examples of calcinosis, which might be described as formation of calcium orthophosphate deposits in any soft tissue. In dentistry, calculus or tartar refers to hardened plaque on the teeth, formed by the presence of saliva, debris, and minerals. Its rough surface provides an ideal medium for bacterial growth, threatening the health of the gums and absorbing unaesthetic stains far more easily than natural teeth [4].

Table 4 Occurrence of calcium phosphates in biological systems (human) [20]

Calcium phosphate	Occurrence
Biological apatite	Enamel, dentin, bone, dental calculi, stones, urinary stones, soft-tissue deposits
OCP	Dental and urinary calculi
DCPD	Dental calculi, crystalluria, chrondrocalcinosis, in some carious lesions
β-$(Ca,Mg)_3(PO_4)_2$	Dental calculi, salivary stones, arthritic cartilage, soft-tissue deposits
$Ca_2P_2O_7 \cdot 2H_2O$	Pseudo-gout deposits in synovium fluids
ACP	Heart calcifications in uremic patients

Contrary to the mineral phases of normal calcifications (bone, dentine, enamel, cementum, antlers), which consist of only one type of calcium orthophosphate (namely, biological apatite), the mineral phases of abnormal and/or pathological calcifications are found to occur as single or mixed phases of other types of calcium orthophosphates (ACP, DCPD, OCP, β-tricalcium magnesium phosphate) and/or other phosphatic and non-phosphatic compounds (e.g., magnesium orthophosphates, calcium pyrophosphates, calcium oxalates, etc.) in addition to or in place of biological apatite (Table 4) [4, 6, 20, 28, 86, 383–387]. In some cases, the chemical composition of an unwanted inorganic phase might depend on the age of the pathological calcification and its location. For example, DCPD is more frequently found in young (3 months or younger) calculus, biological apatite is present in all ages of calculus, while β-$(Ca,Mg)_3(PO_4)_2$ occurs more frequently in sub-gingival calculus. In mature calculus, the relative abundance of OCP, β-$(Ca,Mg)_3(PO_4)_2$ and biological apatite also differ between the inner and outer layers [20]. It is interesting to note that the mineral phases of animal calculus (e.g., from dog) was found to consist of calcium carbonate and biological apatite, while human calculi do not contain calcium carbonate [20, 388].

Recent findings suggest that the mechanisms and factors regulating physiological biomineralization may be identical or similar to those regulating ectopic mineralization: both are initiated by matrix vesicles, i.e., membrane-enclosed particles released from the plasma membrane of mineralization-competent cells [389]. In addition, other regulators (activators and inhibitors) of physiological mineralization have been identified and characterized, and evidence indicates that the same factors also contribute to the regulation

of ectopic mineralization [389–391]. Besides, the biological fluids (e.g., serum, saliva, synovial fluids) are normally supersaturated with respect to biological apatite [4, 20, 205]; therefore, in principle, calcification is thermodynamically feasible in any part of the body. However, normally it is not the case. Therefore, in the healthy body, the appropriate inhibitory mechanisms must be at work to prevent a superfluous calcification of soft tissues. These inhibition mechanisms are a hot research topic in molecular medicine but this subject is beyond the scope of current review. The interested readers are forwarded, for example, to a very interesting review on molecular recognition at the protein-HA interface [392].

To conclude this part, it is worth reminding that calcium orthophosphates of biological origin are sparingly soluble in aqueous solutions. Removing them from the places of unwanted deposition would be the equivalent of demineralizing bone; that is a challenge. Therefore, most therapeutic approaches are directed at preventing the progression of pathological calcifications. Among them, the chelation therapy might be of some interest to materials researchers because it deals with chemical processes [393, 394].

Calcium orthophosphates as biomaterials and bioceramics

Biomaterials are synthetic or natural materials used to replace parts of a living system or to function in intimate contact with living tissue [41]. They are intended to interface with biological systems to evaluate, treat, augment or replace any tissue, organ or function of the body. *Biomaterials* are different from *biological materials* because the former are the materials that are accepted by living tissues and, therefore, might be used for tissue replacements, while the latter are the materials being produced by biological systems (wood, cotton, bones, etc.). In addition, there are *biomimetic materials*, which are not made by living organisms but have the composition, structure and properties similar to those of biological materials. Bioceramics might be defined as biomaterials having the ceramic origin.

The use of calcium orthophosphates as biomaterials and bioceramics is based upon their similarity with the mineral phase of bone and teeth. Archaeological findings exhibited in museums showed that materials used to replace missing human teeth have included ox teeth, shells, coral, ivory (elephant tusk), wood, human teeth from corpses, and metals (gold or silver) [395]. However, according to the literature, the first attempt to use calcium orthophosphates (it was TCP) as an artificial material to repair surgically created defects in rabbits was performed in 1920 [396]. Unfortunately, up to now, all attempts to synthesize bone replacement materials for clinical applications featuring physiological tolerance, biocompatibility and long term stability have had only relative success; it comes to show the superiority and complexity of the natural structures [229].

Generally, the body might treat artificial implants as bioinert, biotolerant, bioactive or bioresorbable materials [41, 397, 398]. Bioinert (e.g., zirconia, alumina, carbon and titanium) and biotolerant (e.g., polymethyl methacrylate (PMMA), titanium and Co–Cr alloy) materials will evoke a physiological response to form a fibrous capsule, thus, isolating the material from the body. Calcium orthophosphates (both non-substituted and ion-substituted) fall into the categories of bioactive and bioresorbable materials. A bioactive material will dissolve slightly, but promote the formation of a layer of biological apatite before interfacing directly with the tissue at the atomic level, that result in the formation of a direct chemical bond with bone. Such an implant will provide good stabilization for materials that are subject to mechanical loading. A bioresorbable material will dissolve and allow a newly formed tissue to grow into any surface irregularities but may not necessarily interface directly with the material [187, 399–403]. Bioceramics made of dense HA would be a good example of a bioactive material, while porous scaffolds made of BCP (i.e., β-TCP + HA [85–93], α-TCP + HA [95–99]) or bone grafts made of CDHA and/or ACP [123] appear to be the examples of bioresorbable materials. Unfortunately, any calcium orthophosphate bioceramics possesses poor mechanical properties that do not allow them to be used in load-bearing areas. Due to this reason, the medical application of calcium orthophosphates is currently focused on the production of non-load-bearing implants, such as pieces for middle ear surgery, filling of bone defects in oral or orthopedic surgery, as well as coating of dental implants and metallic prosthesis [229]. The mechanical properties of calcium orthophosphate biomaterials and bioceramics were reviewed elsewhere [170, 404]. In addition, there is a good review on the recent developments in processing and surface modification of HA [405].

Biomaterials and bioceramics of calcium orthophosphates are available in various physical forms: particles, blocks (dense or porous), injectable compositions, self-setting cements, coatings on metal implants, composites with polymers, etc. [406]. A porous surface provides mechanical fixation in addition to providing sites on the surface that allow chemical bonding between biomaterials and bone. In a porous form, HA ceramics can be colonized by bone tissue [407,

408]. Therefore, macroporosity (pore size > 100 μm) in solid biomaterials is intentionally introduced by addition of porogens, which are either volatile or soluble substances (e.g., naphthalene, sucrose, $NaHCO_3$, gelatin, PMMA microbeads) [187, 409–412]. Sintering particles, preferably spheres of equal size, is another way to generate porous three-dimensional (3D) bioceramics of calcium orthophosphates. A wetting solution such as polyvinyl alcohol is usually used to aid compaction, which is achieved by cold isostatic pressing the particles into cylinders at approximately 200 MPa [413]. As hardly any effect of macropore size (150, 260, 510 and 1220 μm) was observed on the *in vivo* response [414], there is no need to create bioceramics with very big pores. Microporosity (pore size < 10 μm) results from the sintering process, while dimensions of the pores depend on temperature and sintering time. Creation of the desired porosity is a rather complicated engineering task and the interested readers are referred to special literature [123, 415–421].

The sintering stage appears to be of great importance to produce bioceramics with the required properties. Several processes occur during sintering of calcium orthophosphates. First, moisture, carbonates and all volatile chemicals remaining from the synthesis stage, such as ammonia, nitrates and any organic compounds, are removed as gaseous products. Second, the removal of these gases facilitates the production of dense materials during sintering. Third, these chemical changes are accompanied by a concurrent increase in crystal size and a decrease in the specific surface area. Fourth, there is the chemical decomposition of all acidic orthophosphates and their transformation into other phosphates (e.g., $2HPO_4^{2-} \rightarrow P_2O_7^{4-} + H_2O$). Further details on the sintering process of calcium orthophosphates are available elsewhere [4, 5, 148, 170, 187, 403].

Studies showed that increasing the specific surface area and pore volume of biomaterials for tissue repair might greatly accelerate the kinetic process of biological apatite deposition and therefore enhance the bone-forming bioactivity.[16] More importantly, precise control over porosity, pore size, and internal pore architecture of biomaterials on different length scales is essential for the understanding of the structure–bioactivity relationship and the rational design of better bone-forming biomaterials [422, 423].

Calcium orthophosphates have been clinically applied in many areas of dentistry and orthopedics. Bulk material, available in dense and porous forms, is used for alveolar ridge augmentation, immediate tooth replacement and maxillofacial reconstruction [4, 19, 424]. Further applications include increment of the hearing ossicles, spine fusion and repair of bone defects [425, 426]. In order to permit growth of new bone into a bone defect, the defect should be filled with a suitable bioresorbable material. Otherwise, ingrowth of fibrous tissue might prevent bone formation within the defect. Today, a large number of different calcium orthophosphate bioceramics for the treatment of bone defects is available on the market. As an example, the readers are referred to a thorough physicochemical characterization of 14 calcium phosphate-based bone substitution materials in comparison to natural bone [427]. The commercial and trade-names of most important types of calcium orthophosphate bioceramics might be found in following references [427, 428].

Chemically, calcium orthophosphate bioceramics is based on HA, β-TCP and/or BCP (i.e., a composite of HA and α- or β-TCP) [4, 19, 85–93, 95–100, 102, 103, 424, 429]. General requirements for the ideal bone grafts are as follows: pores of some 100 μm size, a biodegradation rate comparable to the formation of bone tissue (i.e., between a few months and about 2 years) and the sufficient mechanical stability. When compared to α- and β-TCP, HA is a more stable phase under the physiological conditions, as it has a lower solubility and a slower resorption kinetics [4, 19, 424]. As implants made of calcined HA are present in bone defects many years after implantation, bioceramics made of β-TCP, α-TCP or BCP [28, 87, 89, 90, 95–100, 102, 103, 430] is more preferable for medical purposes. According to both observed and measured bone formation parameters, calcium orthophosphates were ranked in order of increasing magnitude as follows: low sintering temperature BCP (rough and smooth) ≈ medium sintering temperature BCP ≈ TCP > calcined low sintering temperature HA > non-calcined low sintering temperature HA > high sintering temperature BCP (rough and smooth) > high sintering temperature HA (calcined and non-calcined) [430]. Figure 8 shows an example of the commercially available calcium orthophosphate bone grafts.

Another bone healing concept was introduced with hydraulic bone cements based on calcium orthophosphates that harden inside bone defects [55, 101, 187, 431–442]. Two major types of cements are possible. The first one is a dry mixture of two different calcium orthophosphates (a basic one and an acidic one) and the setting reaction occurs according to an acid-base reaction. The second type of calcium orthophosphate cements is when the initial and final calcium ortho-

[16] Bioactivity is defined as the property of the material to develop a direct, adherent and strong bonding with bone [175, 176].

Fig. 8 Examples of calcium orthophosphate-based bone substitution materials

phosphates have the same Ca/P molar ratio. Typical examples are ACP with Ca/P molar ratio within 1.50–1.67 and α-TCP: they form CDHA upon contact with an aqueous solution [430, 431]. Mixing with an aqueous solution induces chemical interactions that cause the cement setting. Upon mixing, initial calcium orthophosphate(s) are dissolved and precipitated into less soluble calcium orthophosphates. During the precipitation reaction, new crystals grow and become entangled, thus providing a mechanical rigidity to the cement. Setting of these cements occurs mostly within the first 6 h, yielding an 80% conversion to the final products and a compressive strength of 40–60 MPa. The rate of hardening is influenced by a powder to liquid ratio and addition of other chemicals [55, 101, 431–433, 438, 440–442]. Despite a large number of formulations, the calcium orthophosphate cements can only have three different end products: CDHA, DCPD and ACP [430, 431].

The first animal study on a hydraulic calcium orthophosphate cement was performed in 1991: the cement consisting of TTCP and DCPA was investigated histologically by implanting disks made of this cement within the heads of nine cats [443]. These cements are biocompartible, bioactive and bioresorbable. The structure and composition of the hardened cements is close to that of bone mineral; therefore, the material of these cements can easily be used by bone remodeling cells for reconstruction of damaged parts of bones [101, 187, 430, 431]. The biomechanical evaluation of calcium orthophosphate cements for use in vertebroplasty might be found elsewhere [444]. Unfortunately, the cements possess a low mechanical strength; this property might be improved by reinforcement with polymers [445]. A good adaptation to the defect geometry is the major advantage of bone cements, when compared to implantation of bulk ceramics and scaffolds [101, 170, 222–224, 430, 431, 441, 442].

Injectable bone substitutes (IBS) made of calcium orthophosphates and an aqueous solution of a hydrophilic biodegradable polymer are also known [446–452]. They look like pastes of high viscosity but possessing enough fluidity to be injected into bone defects by a standard syringe with a needle. Creation of the required level of viscosity to prevent IBS from segregation and phase separation during the shelf life is the major task of the polymer in IBS, while calcium orthophosphates is the building material for bone healing. In terms of application, IBS more or less similar to the aforementioned hydraulic bone cements but, unlike the cements, IBS do not possess the self-setting abilities since no chemical reactions occur between the components [453]. Further details on IBS are available elsewhere [446–452]. Very recently, injectable and macroporous calcium orthophosphate cement scaffold, combining the advantages of IBS and hydraulic bone cements, has been developed [454]. The future development of both IBS and hydraulic bone cements might be seen in introduction of living cells into their composition [455, 456].

Calcium orthophosphate coatings on metals are often applied in medicine [457]. Metallic implants are encountered in endoprostheses (total hip joint replacements) and artificial teeth sockets. The requirement for a sufficient mechanical stability necessitates the use of a metallic body for such devices. As metals usually do not undergo bone bonding, i.e. do not form a mechanically stable link between the implant and bone tissue, ways have been sought to improve the mechanical contact at the interface [373, 458, 459]. The major way is to coat the metal with calcium orthophosphate ceramics that generally exhibit bone-bonding ability between the metal and bone [460]. The list of different coating techniques is comprised in Table 5, while the main advantages and drawbacks of each coating technique, as well as the important properties of the deposed calcium orthophosphates, are discussed in details elsewhere [172, 187, 457, 461–463]. HA coating as a system of fixation of hip implants was found to work well in the short to medium term (8 years [464] and 17 years [465]). Long-term results are awaited with great interest. The biomedical aspects of osteoconductive[17] coatings for total joint arthroplasty have also been reviewed [466].

[17] Osteoconductivity is the ability to provide a scaffold or template for the formation of new bone on its surface by attachment, migration, proliferation, and differentiation of bone-forming cells [406].

Table 5 Different techniques to deposit bioresorbable coatings of calcium orthophosphates on metal implants [172, 461]

Technique	Thickness	Advantages	Disadvantages
Thermal spraying	30–200 μm	High deposition rates; low cost	Line of sight technique; high temperatures induce decomposition; rapid cooling produces amorphous coatings
Sputter coating	0.5–3 μm	Uniform coating thickness on flat substrates; dense coating	Line of sight technique; expensive; time consuming; produces amorphous coatings
Pulsed laser deposition	0.05–5 μm	Coating by crystalline and amorphous phases; dense and porous coating	Line of sight technique
Dynamic mixing method	0.05–1.3 μm	High adhesive strength	Line of sight technique; expensive; produces amorphous coatings
Dip coating	0.05–0.5 mm	Inexpensive; coatings applied quickly; can coat complex substrates	Requires high sintering temperatures; thermal expansion mismatch
Sol–gel	<1 μm	Can coat complex shapes; low processing temperatures; relatively cheap as coatings are very thin	Some processes require controlled atmosphere processing; expensive raw materials
Electrophoretic deposition	0.1–2.0 mm	Uniform coating thickness; rapid deposition rates; can coat complex substrates	Difficult to produce crack-free coatings; requires high sintering temperatures
Biomimetic coating	<30 μm	Low processing temperatures; can form bonelike apatite; can coat complex shapes; can incorporate bone growth stimulating factors	Time consuming; requires replenishment and a pH constancy of simulated body fluid
Hot isostatic pressing	0.2–2.0 μm	Produces dense coatings	Cannot coat complex substrates; high temperature required; thermal expansion mismatch; elastic property differences; expensive; removal/ interaction of encapsulation material

Now the bioactivity mechanism should be discussed. Strange enough but careful seeking in the literature resulted in only one publication [172], where the bioactivity mechanism of calcium orthophosphates had been briefly described. Therefore, one should better rely on the bioactivity mechanism of other biomaterials, particularly of bioactive glasses—the concept introduced by Prof. Larry Hench [175, 176]. The mechanism of bonding of bioactive glasses to living tissue involves a sequence of 11 successive reaction steps. The initial 5 steps occurred on the surface of biomaterials are ''chemistry'' only, while the remaining 6 steps belong to ''biology'' because the latter include colonization by osteoblasts, followed by proliferation and differentiation of the cells to form a new bone that had a mechanically strong bond to the implant surface (Fig. 9). Therefore, there is an opinion that in the case of bioactive glasses the border between ''dead'' and ''alive'' is located between stages 5 and 6.

According to Hench, all bioactive materials ''form a bone-like apatite layer on their surfaces in the living body, and bond to bone through this apatite layer. The formation of bone-like apatite on artificial material is induced by functional groups, such as Si–OH (in the case of biological glasses), Ti–OH, Zr–OH, Nb–OH, Ta–OH, –COOH, and $–H_2PO_4$ (in the case of other materials). These groups have specific structures revealing negatively charge, and induce apatite formation via formations of an amorphous calcium compound, e.g., calcium silicate, calcium titanate and ACP'' [175, 176]. For want of anything better, the

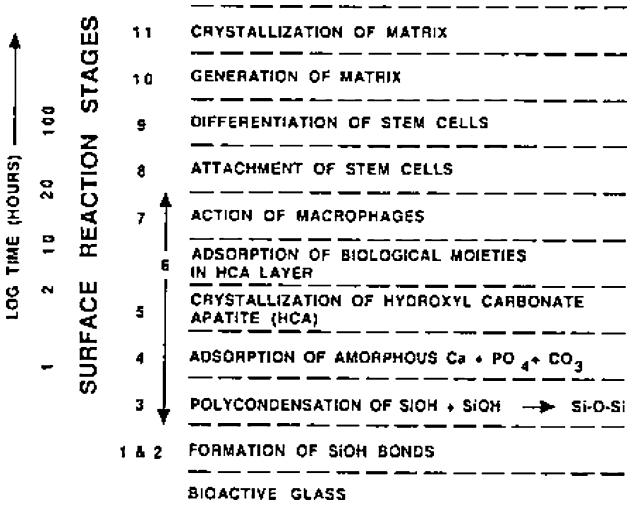

Fig. 9 The sequence of interfacial reactions involved in forming a bond between tissue and bioactive glasses. The border between ''dead'' and ''alive'' occurs approximately at stage 6. Reprinted from [176] with permission

bioactivity mechanism of calcium orthophosphates should also be described by Fig. 9 with omitting of several initial stages, as it was actually made for HA in [172], where 3 initial chemical stages of the Hench's mechanism were replaced by partial dissolution of HA.

To conclude this part, one should briefly mention on a large variety of bone substituting composites made of calcium orthophosphates and organic compounds (usually, polymers, preferably, biodegradable ones). This approach appeared due to the poor mechanical properties (namely: low elasticity, high brittleness, low tensile strength, low fracture toughness and poor impact resistance) of bone substitutes made of calcium orthophosphates only [170, 175, 176]. In addition, it is worth reminding that all biologically formed calcified tissues (bones, teeth, antlers, shells, etc.) appear to be very complicated composites of organic and inorganic phases [4, 19, 20, 28, 162, 205, 231, 232]. In such composites, the mineral component provides the strength whereas the organic component contributes to the ductility. This combination of strength and ductility leads to an energy absorption prior to failure [250]. There is a range of suitable calcium orthophosphates (ACP, OCP, α-TCP, β-TCP, CDHA, HA, TTCP and occasionally FA) and an even greater choice of biocompatible polymers those can be divided into two major groups: synthetic polymers (e.g., polyesters, polymethylmethacrylate, poly-ε-caprolactone) and polymers of biological origin (e.g., collagen, gelatin, chitosan, alginate, modified starch, cellulose esters). Different ways were realized to bring these two components together into a potential implant, like simple mechanical mixing or co-precipitation. It is also possible to introduce porosity into such composites that is advantageous for most applications as bone substitution material. Such composites might possess the unique properties; for example, there is a recent report on shape memory properties of poly(d,l-lactide)/HA composites [467]. The topic of the composite materials made of calcium orthophosphates and organic/biological compounds was first introduced in 1981 by Prof. Bonfield et al. [468]. Nowadays, the synthesis of organic–inorganic hybrids is a strong and very promising subject of research; therefore, the readers are referred to other reviews [187, 469–479].

[18] The term "biomimetics" ("the mimicry of life") was coined by an American inventor, engineer, and biophysicist Otto Herbert Schmitt (1913–1998) in the 1950s.

Biomimetic crystallization of calcium orthophosphates

In general, biomimetics[18] (also known as bionics, biognosis and/or biomimicry) might be defined as application of the methods and systems found in nature to the study, design and construction of new engineering systems, materials, chemical compounds and modern technology. The concept is very old (e.g., the Chinese wanted to make artificial silk 3000 years ago; Daedalus' wings was one of the early design failure) but the implementation is gathering momentum only recently. In spite of the tremendous achievements of modern science and technology, the nature's ability to assemble inorganic compounds into hard tissues (shells, spicules, teeth, bone, antlers, skeletons, *etc.*) is still not achievable by the synthetic procedures. This is not surprising—designs found in nature are the result of millions of years of competition for survival. The models that failed are fossils; those that survived are the success [480]. In the frames of this review, biomimetics is considered as mimicking natural manufacturing methods to generate artificial calcified tissues (grafts, implants, prostheses) those might be used as temporary or permanent replacements of the missing, lost, injured or damaged ones.

As this is mainly the subject of crystallization of calcium orthophosphates, the matter of choosing the correct experimental conditions and well-mimicking solutions is of the primary importance. The easiest way to perform the crystallization would be mixing of aqueous solutions containing the ions of calcium and orthophosphate [4–6]. Unfortunately, such type of crystallization provides precipitates with the properties (chemical composition, Ca/P ratio, crystallinity level, particle size distribution, etc.) far different from those of biological apatite. This can be explained by the following paramount differences between the in vivo and in vitro crystallization conditions [481]:

(i) In vitro crystallization normally occurs at permanently depleting concentrations of calcium and orthophosphate, while the concentrations of all ions and molecules are kept strictly constant during biological mineralization (the same is valid for the solution pH);
(ii) Chemical crystallization is a fast process (time scale of minutes to days), while the biological process is a slow one (time scale of weeks to years);
(iii) Many inorganic, organic, biological and polymeric compounds are present in biological liquids (blood plasma, serum, saliva). Each of these compounds might act as an inhibitor,

nucleator or even as a template for the growth of biological apatite [229]. In addition, each of them somehow influences the crystallization kinetics and might be either incorporated into or co-precipitated with calcium orthophosphates.

The first and the second differences might be overcome by using the appropriate crystallization techniques. The details are available elsewhere [481] but, briefly, the first problem might be overcome by either a continuous flow of a supersaturated solution [482, 483] or using a constant-composition (CC) technique [72, 484, 485]. The second difference might be surpassed by a restrained diffusion of calcium and orthophosphate ions from the opposite directions in, for example, a double-diffusion (DD) crystallization device or in viscous gels [192–194,196,197]. The CC and DD techniques might be combined into a single constant-composition double-diffusion (CCDD) device which currently seems to be the most advanced experimental tool to perform the biomimetic crystallization [481, 486–489]. However, in no case the CCDD device should be considered as the final construction; it still has much room for further improvement, e.g. by upgrading the design of the crystallization chamber [490]. Other constructions, e.g. to study calcification of biological heart valve prostheses [491], are also possible.

The third major difference between the in vivo and in vitro crystallization conditions might be overcome by using the appropriate crystallization solutions [481]. The best way would be to perform experiments using natural liquids (blood serum, saliva, lymph, etc.), but this is not easy due to variability of the chemical and biochemical composition of natural liquids and problems with their storage. As stated before, using supersaturated aqueous solutions containing only the ions of calcium and orthophosphate appears to be unable to mimic the crystallization of biological apatite; therefore, more advanced solutions have been elaborated. To the best of my knowledge, Hanks' balanced salt solution (HBSS) [492] was the first successful simulating medium, containing the ions of calcium and orthophosphate together with other inorganic ions and glucose. HBSS is commercially available and still used in biomimetic experiments [493, 494]; its chemical composition might be taken, e.g., from [495, 496]. Other physiological solutions include α-modified Eagle's medium (α-MEM) which contains numerous organic (e.g., alanine, aspartic acid, glycine, biotin, vitamin C, folic acid, riboflavin) and inorganic (e.g., $CaCl_2$, KCl, NaCl, NaH_2PO_4) compounds [497, 498] and phosphate buffered saline (PBS) that contains only inorganic components (e.g., $CaCl_2$, $MgCl_2$, KCl, KH_2PO_4, NaCl, NaH_2PO_4) [499, 500]. All of them are commercially available.

However, the most popular biomimetic solution is a protein-free acellular simulated body fluid (SBF). It was introduced by Kokubo et al. [501] and occasionally named as Kokubo's SBF. It is a metastable aqueous solution with pH ~ 7.40, supersaturated with respect to the precipitation of HA, containing only inorganic ions in concentrations nearly equal to those in human blood plasma. However, the standard SBF, firstly, contains the tris/HCl buffer, and, second, the concentration of hydrogencarbonate (4.2 mM) is only a fraction of that in blood plasma (27 mM) [501]. The problem of a low concentration of hydrogencarbonate ions has been overcome by first introducing a ''synthetic body fluid'' [502] and later a revised SBF (rSBF) [503, 504]. Due to the chemical similarity with human blood plasma, rSBF currently seems to be the best simulating solution. However, it contains Hepes buffer, loses CO_2 in open vessels and does not contain any organic and/or biological molecules [503, 504]. Other types of SBF are also available; the interested readers are referred to a very recent leading opinion co-authored by the SBF inventor [505], where the entire history and the preparation technique of SBF are well described. The application of SBF for the surface mineralization of various materials in vitro has been reviewed in [506], while the theoretical analysis of calcium orthophosphate precipitation (the driving force and the nucleation rate based on the classical crystallization theory) in SBF is also available [507].

Further attempts to improve the biomimetic properties of SBF and rSBF have been performed. Efforts were made to replace artificial buffers (tris/HCl, Hepes) with simultaneous increasing the concentration of hydrogencarbonates for SBF [508, 509] or avoiding losses of CO_2 from open vessels for rSBF [481, 486–489] by means of permanent bubbling of gaseous CO_2 through the solutions. Addition of the most important organic and biological compounds like glucose [488] and albumin [486] is another direction to improve biomimetic properties of SBFs; further improvements of biomimetic solutions are to be made in future. Occasionally, condensed solutions of SBF (e.g., 1.5-fold, 2-fold [510, 511], 5-fold [512, 513] and even 10-fold [514]) are used to accelerate the precipitation; however, whenever possible this should be avoided because the application of condensed solutions of SBF leads to changes in the chemical composition of the precipitates; namely, the concentration of carbonates increases, while the concentration of orthophosphates decreases [515].

It is very difficult to mimic exactly the calcification process that occurs in bones. A step further would be to perform the precipitation from the simulating solutions on templates of biomineralization proteins for the control of crystal organization and properties. For example, there are successful attempts to crystallize calcium orthophosphates on collagen in order to obtain bone-like composites [516–523]. Although the ultrastructure of bone has not been realized yet, such collagen/calcium orthophosphate composites are currently under investigation for clinical use. Other popular biomimetic matrixes to perform calcium orthophosphate crystallization comprise gelatin [192–194, 196,197, 524–526], chitosan [524, 527, 528], organic polyelectrolytes [529–532], titanium and its alloys [533–540], polymers [541], cellulose [542], self-assembled monolayers [543] and some other materials.

Calcium orthophosphates in tissue engineering

All present day orthopedic implants lack three of the most critical characteristics of living tissues: (i) the ability to self-repair; (ii) the ability to maintain a blood supply; and (iii) the ability to modify their structure and properties in response to environmental factors such as mechanical load [420]. Needless to mention, that bones not only possess all aforementioned properties but, in addition, they are self-generating, hierarchical, multifunctional, nonlinear, composite and biodegradable; therefore, good artificial substitutes must possess similar properties [229].

Tissue engineering is an interdisciplinary field that exploits a combination of living cells, engineering materials and suitable biochemical factors in a variety of ways to improve, replace, restore, maintain or enhance living tissues and whole organs [544]. This field of science[19] started more than a decade ago [546] and nowadays is at full research potential due to the following key advantages: (i) the solutions it provides are long-term, much safer than other options and cost-effective as well; (ii) the need for a donor tissue is minimal, which eliminates the immunosuppression problems; (iii) the presence of residual foreign material is eliminated as well. As 2 of 3 major components (namely, living cells and biochemical factors) of tissue engineering appear to be far beyond the scope of this review, here the topic of tissue engineering is narrowed down to the engineering materials only.

[19] In 2003, the NSF published a report titled: "*The emergence of tissue engineering as a research field*", which provides a thorough description of the history of this field [545].

Cells are generally implanted or seeded into an artificial structure, usually referred to as a scaffold, capable of supporting 3D tissue formation. The scaffolds are temporary matrices for bone growth and provide a specific environment and architecture for tissue development. They serve at least one of the following purposes: (i) allow cell attachment and migration; (ii) deliver and retain cells and biochemical factors; (iii) enable diffusion of vital cell nutrients and expressed products; (iv) exert certain mechanical and biological influences to modify the behavior of the cell phase [547]. To achieve the goal of tissue reconstruction, the scaffolds must meet some specific requirements. A reasonable surface roughness is necessary to facilitate cell seeding and fixation [548–550]. A high porosity and an adequate pore size are very important to provide diffusion throughout the whole structure of both cells and nutrients [549, 551–554]. Biodegradability is very essential since scaffolds need to be resorbed by the surrounding tissues without the necessity of a surgical removal. The resorption rate has to coincide as much as possible with the rate of tissue formation [555]: this means that while cells are fabricating their own natural matrix structure around themselves, the scaffold is able to provide structural integrity within the body and eventually it will break down leaving the newly formed tissue that will take over the mechanical load. Injectability is also an important factor for clinical use [430, 431, 446–451].

In the case of bone grafts, the aim of tissue engineering is to provide an artificially prepared porous scaffold made of calcium orthophosphates that provides the physical and chemical cues to guide cell seeding, differentiation and assembly into 3D tissues of a newly formed bone [556–559]. To meet these needs, much attention is devoted to further modification of calcium orthophosphates. From the chemical point of view, the modifications include synthesis of novel ion-substituted calcium orthophosphates [560–564], while from the material point of view the major research topics include nanocrystalline structures [188, 565–575], organic-inorganic hybrids [187, 469–477, 575–577], fibers [578–583], microspheres [576, 584, 585], 3D scaffolds made of ACP [123], HA [586–588] and BCP [589], structures with graded porosity [590] and hierarchically organized [591]. The influence of the porosity of HA ceramics on in vitro and in vivo bone formation studied by cultured rat bone marrow stromal cells has been studied [592]. The feasible production of ceramic scaffolds with tailored structure and properties opens up a spectacular future for calcium orthophosphates.

There are three principal therapeutic strategies for treating diseased or injured tissues in patients: (i) implantation of freshly isolated or cultured cells; (ii) implantation of tissues assembled *in vitro* from cells and scaffolds; and (iii) in situ tissue regeneration. For cellular implantation, individual cells or small cellular aggregates from the patient or a donor are either injected into the damaged tissue directly or are combined with a degradable scaffold in vitro and then implanted. For tissue implantation, a complete 3D tissue is grown in vitro using patient or donor cells and a bioresorbable scaffold, and then is implanted into the patients to replace diseased or damaged tissues. For in situ regeneration, a scaffold implanted directly into the injured tissue stimulates the body's own cells to promote local tissue repair [544, 593]. In any case, simply trapping cells at a particular point on a surface is not enough: the cells must be encouraged to differentiate, which is impossible without the presence of suitable biochemical factors [594]. All previously mentioned clearly indicates that for the purposes of tissue engineering, calcium orthophosphates play only an auxiliary role; namely, they act as a suitable material to manufacture an appropriate 3D template, substrate or scaffold to be colonized by living cells before the successive implantation. However, the scaffolds themselves might be prepared from not only pure calcium orthophosphates but also organic–inorganic composites [595–598]. The *in vitro* evaluation of potential calcium orthophosphate scaffolds for tissue engineering has been described elsewhere [599], while the data on mechanical properties and porosity of calcium orthophosphates for use in tissue engineering are also available [600, 601]. The effect of a HA-based biomaterial on gene expression in osteoblast-like cells was reported [602], while the influence of adsorbed serum proteins, RGD and proteoglycan-binding peptides on the adhesion of mesenchymal stem cells to HA be also studied [603]. To conclude, the excellent biocompatibility of calcium orthophosphates and their high affinity for proteins and cells makes them very functional for hard tissue regeneration [157, 604–607].

Summary and outlook

In 1998, Prof. Larry Hench published a forecast for the future of biomaterials development [608], where he noted that bioactive materials (calcium orthophosphates, bioactive glasses and glass ceramics) had already improved prostheses lifetime but, unfortunately, any type of prosthesis had mechanical limitations. As the solution, he proposed that biomaterial researchers would need to focus on tissue regeneration instead of tissue replacement. A working hypothesis was announced: ''Long-term survivability of prosthesis will be increased by the use of biomaterials that enhance the regeneration of natural tissues'' [608]. One path to follow is the regeneration of bone using calcium orthophosphate scaffolds that mimic the structure of biological apatite, bond to bone and in some cases activate the genes within bone cells to stimulate new bone growth [420, 544, 593]. Thus, almost 10 years ago Prof. Hench predicted a rapid development of tissue engineering field, where calcium orthophosphates play an auxiliary role. The history shows that tissue engineering, indeed, is a very rapidly developed field of science and research.

However, what can be said about calcium orthophosphates themselves? The major questions on chemistry, crystallization, ion-substitution, crystallography, thermodynamics and phase relationships for the chemically pure compounds have been answered in the XXth century. However, the same topics for ACP, CDHA and the calcium orthophosphates of biological origin, including the control of their morphology and interaction of calcium orthophosphates with various bio- and organic compounds are not well investigated yet. Small amounts of bone-like apatite might be easily prepared by crystallization from SBF and rSBF but what can be said about larger quantities? A standard way of the concentration increasing causes chemical changes in the precipitates [515]. After a necessary technology is developed, one will have to think on scaffold preparation from this material, keeping in mind that any thermal treatment would destroy this material. The existence of oxyapatite remains to be questionable as well. The bioactivity mechanism of calcium orthophosphates requires improving.

Nowadays, the synthesis of organic–inorganic hybrids, perhaps, is the strongest subject of research. For example, even composites of HA with carbon nanotubes already exist [609–611]! In addition, a great attention is paid to manufacturing of calcium orthophosphate cements, multiphase[20] mixtures mimicking as closely as possible the mineral component of biological apatite and the production of calcium orthophosphate substrates for cells and biochemical factors to be used in tissue engineering. The study of nanostructured and nanocrystalline materials made of

[20] For multiphase compositions of various calcium orthophosphates, the problem of accurate phase quantification often arises. The problem is usually solved by the Rietveld refinement and the readers are referred to a very recent paper on this subject [612].

calcium orthophosphates, similar to the complex hierarchical structures of hard tissues (bone and teeth), is also a very attractive field [229]. A work along the ecological ways of synthesis of calcium orthophosphates might be of a great importance as well [613]. A deeper study of the fascinating growth rate of deer antlers might provide new and unexpected approaches to the bone-healing concept, as well as this will be important for further development of both biomimetics and biomineralization. The key future biomedical applications of calcium orthophosphates include (but not limited to) drug delivery systems, carriers of bioactive peptides, growth factors and/or living bone cells [157, 614–616].

To conclude this review, in spite of a rather long history of calcium orthophosphate research, there are still many gaps in our knowledge to be investigated in future.

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