

Fertilizers

Hydroxyapatite



Brushite



DCPD

Bone

Implants



Teeth

β -TCP

Tricalcium phosphate

Biological and Medical Significance of Calcium Phosphates

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Dedicated to Professor Sir John Meurig Thomas on the occasion of his 70th birthday

The inorganic part of hard tissues (bones and teeth) of mammals consists of calcium phosphate, mainly of apatitic structure. Similarly, most undesired calcifications (i.e. those appearing as a result of various diseases) of mammals also contain calcium phosphate. For example, atherosclerosis results in blood-vessel blockage caused by a solid composite of cholesterol with calcium phosphate. Dental caries result in a replacement of less soluble and hard apatite by more soluble and softer calcium hydrogenphosphates. Osteoporosis is a demineralization of bone. Therefore, from a chemical point of view, processes of normal (bone and

teeth formation and growth) and pathological (atherosclerosis and dental calculus) calcifications are just an in vivo crystallization of calcium phosphate. Similarly, dental caries and osteoporosis can be considered to be in vivo dissolution of calcium phosphates. On the other hand, because of the chemical similarity with biological calcified tissues, all calcium phosphates are remarkably biocompatible. This property is widely used in medicine for biomaterials that are either entirely made of or coated with calcium phosphate. For example, self-setting bone cements made of calcium phosphates are helpful in bone repair and titanium

substitutes covered with a surface layer of calcium phosphates are used for hip-joint endoprostheses and tooth substitutes, to facilitate the growth of bone and thereby raise the mechanical stability. Calcium phosphates have a great biological and medical significance and in this review we give an overview of the current knowledge in this subject.

Keywords: bioinorganic chemistry • biomaterials • biomimetic synthesis • biomineralization • materials science

1. Introduction

Calcium phosphates are the most important inorganic constituents of biological hard tissues. In the form of carbonated hydroxyapatite (HA), they are present in bone, teeth, and tendons to give these organs stability, hardness, and function. Calcium phosphate crystals are also found in “dead” nature as mineral deposits of considerable size, having grown over many years under sometimes extreme conditions of pressure and temperature. In contrast, biologically formed calcium phosphates are often nanocrystals that are precipitated under mild conditions (ambient pressure, near room temperature).

The biological formation of minerals by living organisms is commonly called “biomineralization”.^[1–9] Today more than 60 minerals are known that are used by organisms, for

example, for protection (shell), as tools (teeth), as gravity sensors (octocornia or statoliths), or as a skeleton. In terms of absolute quantity, calcium phosphates are minor compared to calcium carbonate (CaCO_3) and silicon dioxide (as silicic acid $\text{SiO}_2 \cdot n\text{H}_2\text{O}$), which both occur in huge amounts in marine single-cell organisms. Another very important class of biominerals are iron oxides that occur, for example, in snail teeth or magnetotactic bacteria.^[1] The presence of calcium phosphates in vertebrates (such as humans) makes them particularly important in biomedicine, as many diseases result from irregularities in the skeletal system (i.e. in bone) or the dental system (in teeth). It must also be stressed that, although the presence of calcium phosphate in these hard tissues is crucial for survival, there are occasions on which calcium phosphate minerals crystallize in an irregular way in undesired regions. These phenomena are called pathological crystallization or ectopic mineralization, of which atherosclerosis, stone formation, or dental calculus are prominent examples.

Herein, we give an overview of the occurrence, formation, and significance of calcium phosphate minerals in living organisms, with a special emphasis on current biomedical questions.

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2. Geological and Biological Occurrence

Calcium and phosphorus are widely distributed elements on our planet. The surface layer of the Earth contains about 3.4 wt % of calcium and 0.10 wt % of phosphorus.^[10] Combinations of oxides of these two elements with or without incorporation of water give different calcium phosphates. Unless doped with a colored transition-metal ion (often the case in nature), all calcium phosphates are white solids. Most calcium phosphates are only sparingly soluble in water, and some can be considered to be insoluble, but all dissolve in acids. Ortho- (PO_4^{3-}), pyro- ($\text{P}_2\text{O}_7^{4-}$), and poly- ($(\text{PO}_3)_n^{n-}$) phosphates can be structurally distinguished. Although calcium pyrophosphates occur in some pathological calcifications, only calcium orthophosphates will be considered here. They are the major component of all human calcified tissues, and natural calcium orthophosphates are the source for phosphorus-containing fertilizers.^[11–14]

Geologically, natural calcium orthophosphates are found in different regions to fluoroapatite deposits, $\text{Ca}_{10}(\text{PO}_4)_6\text{F}_2$, or phosphorites. Most geological environments contain calcium phosphates, usually as accessory minerals (<5 %). In some sedimentary rocks (phosphorites) and rarely in igneous segregations (fluoroapatite), the concentration is high enough to permit an economic use. The largest world deposits of natural phosphate rock are located in Morocco, Russia, Kazakhstan, and the USA (Florida, Tennessee).^[11–14] Most natural calcium phosphates occur as small polycrystals. Larger crystals usually have the crystal structure of apatites (hexagonal system, space group $P6_3/m$, or monoclinic system, space group $P2_1/b$). None of these crystals are pure compounds; they are always admixtures of other elements. For

example, calcium ions may be partially replaced by Sr, Ba, Mg, K, Na, Fe; phosphate ions may be replaced by AsO_4^{3-} , CO_3^{2-} , and VO_4^{3-} ; hydroxide, chloride, bromide, carbonate, and oxide ions may substitute fluoride ions in the crystal lattice. Moreover, some ions in the crystal structure may be missing, which leaves crystallographic defects. This leads to the formation of nonstoichiometric compounds. Figure 1 shows polycrystalline and single-crystalline calcium phosphate minerals.

The major industrial application of calcium phosphate minerals is in the production of agricultural fertilizers. Natural calcium phosphates that are used for fertilizer production can be of geological or of biological origin for example, guano

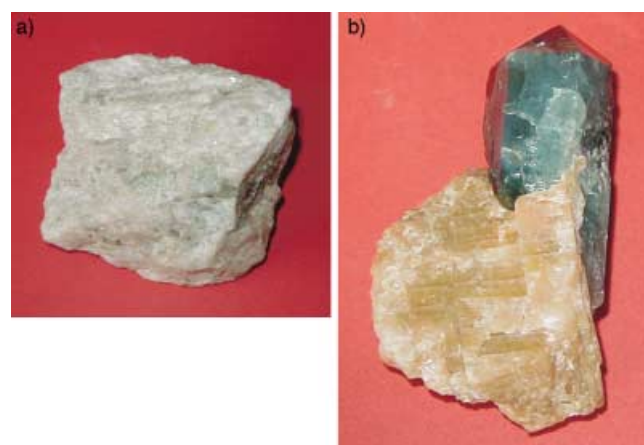


Figure 1. Polycrystalline (a) and single-crystalline (b) fluorapatite (chemical formula: $\text{Ca}_{10}(\text{PO}_4)_6\text{F}_2$) of geological origin. The single crystal has a grey–green color caused by incorporated transition metals.

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(mineralized excrements of birds, accumulated over thousands of years, e.g. in the South Sea at Nauru, Banaba, and Makatea). On the 21 km² island of Nauru, about 2 million tons of fertilizers are mined every year, which is leading to severe ecological problems. The total capacity of industrial plants in the world exceeds 25 million tons of phosphate fertilizers per year (as P₂O₅).^[12]

In biological systems, calcium orthophosphates occur as the principal inorganic constituent of normal (bones, teeth, fish enameloid, and some species of shells) and pathological (dental and urinary calculus and stones, atherosclerotic lesions) calcifications.^[15–18] Structurally, they occur mainly in the form of poorly crystallized nonstoichiometric sodium-, magnesium-, and carbonate-containing HA (often called “biological apatite” or dahllite). The main constituents of human bones are calcium orthophosphates (≈50–60 wt %), collagen (≈30–40 wt %), and water (≈10 wt %). In microscopic studies of the interface between implanted calcium phosphate biomaterials and the host bone, poorly crystallized nonstoichiometric carbonated apatite similar to that of bone apatite was found.^[19–21] Detailed information on the chemical composition of the most important human normal calcified tissues is given in Table 1. Figure 2 shows a picture of a calcined bone, that is, only the calcium phosphate skeleton, after burning off all organic components.

As a variety of stoichiometric calcium phosphates is known, abbreviations have traditionally been introduced to distinguish between the different compounds. Important parameters are the molar Ca/P ratio and the solubility. Table 2 presents the known calcium phosphate phases. For the chemically pure compounds, the Ca/P ratio can be between 0.5–2.0. In general, the lower this ratio, the more acidic and soluble in water the calcium phosphate is (see ref. [22] for the apparent solubility of these phases as a function of pH value and calcium concentration). A brief description of all calcium orthophosphates is given below. Table 3 contains their crystallographic data.

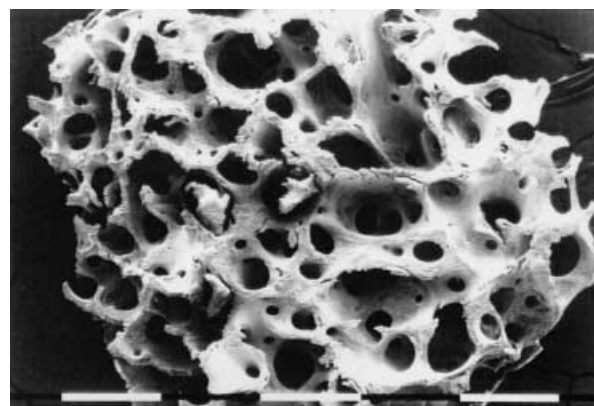


Figure 2. Calcined porous bone (*spongiosa*) showing the high porosity and the interconnecting network of pores (magnification: 20.4 ×).

MCPM (monocalcium phosphate monohydrate, Ca(H₂PO₄)₂·H₂O) is the most acidic and water-soluble calcium phosphate compound. It precipitates from highly acidic solutions that are normally used in the industrial production of phosphorus-containing fertilizer (“triple superphosphate”).^[12] At temperatures above 100 °C, it transforms into MCPA (monocalcium phosphate anhydrate, Ca(H₂PO₄)₂). Because of its comparatively high acidity and solubility, MCPM is never found in biological calcifications. However, MCPM is used in some calcium phosphate cements in medicine.^[23–27] Other applications are as antacids, acidulents, and mineral supplements for baking powders, foods, and beverages.^[28]

MCPA is the anhydrous form of MCPM. It crystallizes under similar conditions as MCPM but at temperatures above 100 °C (e.g. from highly concentrated mother liquors in fertilizer production). Like MCPM, MCPA never appears in calcified tissues, and there is no current application in medicine; it is mainly used as a fertilizer.^[12, 28]

DCPD (dicalcium phosphate dihydrate, CaHPO₄·2H₂O; the mineral brushite) can be easily crystallized from aqueous

Table 1. Comparative composition and structural parameters of inorganic phases of adult-human calcified tissues.^{[a][15, 21]}

Composition	Enamel	Dentin	Bone	Hydroxyapatite (HA)
calcium [wt %] ^[b]	36.5	35.1	34.8	39.6
phosphorus (as P) [wt %] ^[b]	17.7	16.9	15.2	18.5
Ca/P (molar ratio) ^[b]	1.63	1.61	1.71	1.67
sodium [wt %] ^[b]	0.5	0.6	0.9	–
magnesium [wt %] ^[b]	0.44	1.23	0.72	–
potassium [wt %] ^[b]	0.08	0.05	0.03	–
carbonate (as CO ₃ ²⁻) [wt %] ^[c]	3.5	5.6	7.4	–
fluoride [wt %] ^[b]	0.01	0.06	0.03	–
chloride [wt %] ^[b]	0.30	0.01	0.13	–
pyrophosphate, (as P ₂ O ₇ ⁴⁻) [wt %] ^[c]	0.022	0.10	0.07	–
total inorganic [wt %] ^[c]	97	70	65	100
total organic [wt %] ^[c]	1.5	20	25	–
water [wt %] ^[c]	1.5	10	10	–
<i>a</i> axis [Å] ^[d]	9.441	9.421	9.41	9.430
<i>c</i> axis [Å] ^[d]	6.880	6.887	6.89	6.891
crystallinity index, (HA = 100)	70–75	33–37	33–37	100
typical crystal sizes [nm] ^[1, 105, 107]	100 × 50 × 50 μm	35 × 25 × 4	50 × 25 × 4	200–600
ignition products (800 °C)	β-TCP + HA	β-TCP + HA	HA + CaO	HA
elasticity modulus (GPa) ^[261]	80	15	0.34–13.8	10
compressive strength (MPa)	10	100	150	100

[a] Because of the considerable variation found in biological samples, typical values are given in these cases. [b] Ashed samples. [c] Unashed samples. [d] Lattice parameters: ± 0.003 Å.

Table 2. Properties of the biologically relevant calcium orthophosphates.^{[a][103, 104]}

Ca/P ratio	Compound	Formula	Solubility at 25 °C, $-\log(K_{sp})$	Solubility at 37 °C, $-\log(K_{sp})$	pH stability range in aqueous solution at 25 °C
0.5	monocalcium phosphate monohydrate (MCPM)	$\text{Ca}(\text{H}_2\text{PO}_4)_2 \cdot \text{H}_2\text{O}$	1.14	no data	0.0–2.0
0.5	monocalcium phosphate anhydrate (MCPA)	$\text{Ca}(\text{H}_2\text{PO}_4)_2$	1.14	no data	[d]
1.0	dicalcium phosphate dihydrate (DCPD, “brushite”)	$\text{CaHPO}_4 \cdot 2\text{H}_2\text{O}$	6.59	6.63	2.0–6.0
1.0	dicalcium phosphate anhydrate (DCPA, “monetite”)	CaHPO_4	6.90	7.02	[d]
1.33	octacalcium phosphate (OCP)	$\text{Ca}_8(\text{HPO}_4)_2(\text{PO}_4)_4 \cdot 5\text{H}_2\text{O}$	96.6	95.9	5.5–7.0
1.5	α -tricalcium phosphate (α -TCP)	$\alpha\text{-Ca}_3(\text{PO}_4)_2$	25.5	25.5	[b]
1.5	β -tricalcium phosphate (β -TCP)	$\beta\text{-Ca}_3(\text{PO}_4)_2$	28.9	29.5	[b]
1.2–2.2	amorphous calcium phosphate (ACP)	$\text{Ca}_x(\text{PO}_4)_y \cdot n\text{H}_2\text{O}$	[c]	[c]	[c]
1.5–1.67	calcium-deficient hydroxyapatite (CDHA)	$\text{Ca}_{10-x}(\text{HPO}_4)_x(\text{PO}_4)_{6-x}(\text{OH})_{2-x}$ ($0 < x < 1$)	≈ 85.1	≈ 85.1	6.5–9.5
1.67	hydroxyapatite (HA)	$\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2$	116.8	117.2	9.5–12
2.0	tetracalcium phosphate (TTCP)	$\text{Ca}_4(\text{PO}_4)_2\text{O}$	38–44	37–42	[b]

[a] The solubility is given as the logarithm of the ion product of the given formulae (excluding hydrate water) with concentrations in mol L⁻¹. [b] These compounds cannot be precipitated from aqueous solutions. [c] Cannot be measured precisely. However, the following values were reported: 25.7 ± 0.1 (pH 7.40), 29.9 ± 0.1 (pH 6.00), 32.7 ± 0.1 (pH 5.28).^[78] [d] Stable at temperatures above 100 °C. [e] Always metastable. The composition of a precipitate depends on the solution pH value and composition.

Table 3. Crystallographic data of calcium phosphates.^[72, 73]

Compound	Space group	Unit cell parameters ^[a]	Z ^[b]	Density [g cm ⁻³]
MCPM	triclinic $P\bar{1}$	$a = 5.6261(5)$, $b = 11.889(2)$, $c = 6.4731(8)$ $\alpha = 98.633(6)$, $\beta = 118.262(6)$, $\gamma = 83.344(6)$	2	2.23
MCPA	triclinic $P\bar{1}$	$a = 7.5577(5)$, $b = 8.2531(6)$, $c = 5.5504(3)$ $\alpha = 109.87(1)$, $\beta = 93.68(1)$, $\gamma = 109.15(1)$	2	2.58
DCPD	monoclinic Ia	$a = 5.812(2)$, $b = 15.180(3)$, $c = 6.239(2)$ $\beta = 116.42(3)$	4	2.32
DCPA	triclinic $P\bar{1}$	$a = 6.910(1)$, $b = 6.627(2)$, $c = 6.998(2)$ $\alpha = 96.34(2)$, $\beta = 103.82(2)$, $\gamma = 88.33(2)$	4	2.89
OCP	triclinic $P\bar{1}$	$a = 19.692(4)$, $b = 9.523(2)$, $c = 6.835(2)$ $\alpha = 90.15(2)$, $\beta = 92.54(2)$, $\gamma = 108.65(1)$	1	2.61
α -TCP	monoclinic $P2_1/a$	$a = 12.887(2)$, $b = 27.280(4)$, $c = 15.219(2)$ $\beta = 126.20(1)$	24	2.86
β -TCP	rhombohedral $R3cH$	$a = b = 10.439(1)$, $c = 37.375(6)$ $\gamma = 120$	21 ^[c]	3.07
HA	monoclinic $P2_1/b$	$a = 9.84214(8)$, $b = 2a$, $c = 6.8814(7)$ $\gamma = 120$ (monoclinic)	4	3.16
	or hexagonal $P6_3/m$	$a = b = 9.4302(5)$, $c = 6.8911(2)$ $\gamma = 120$ (hexagonal)	2	
TTCP	monoclinic $P2_1$	$a = 7.023(1)$, $b = 11.986(4)$, $c = 9.473(2)$ $\beta = 90.90(1)$	4	3.05

[a] a , b , c are given in Å and α , β , γ in °. [b] Number of formula units per unit cell. [c] Per hexagonal unit cell.

solutions. DCPD transforms into dicalcium phosphate anhydrate at temperatures above 80 °C. DCPD is of biological importance because it is often found in pathological calcifications (dental calculi, crystalluria, chondrocalcinosis,^[15–17] and urinary stones^[18]). DCPD has been proposed as an intermediate in both bone mineralization and dissolution of enamel in acids (dental caries).^[15–18] In surgery, DCPD is used in calcium phosphate cements^[27, 29–34] and, in dentistry, in toothpaste together with fluoride-containing compounds (e.g. NaF) for protection against caries.^[35–38] Other applications are in fertilizers,^[12] glass production, calcium supplements in foods, and mineral supplements in cereals.^[28]

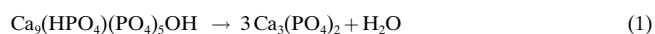
DCPA (dicalcium phosphate anhydrate, CaHPO_4 ; the mineral monetite) is the anhydrous form of DCPD. DCPA, like DCPD, can be crystallized from aqueous solutions but at 100 °C. Unlike DCPD, DCPA occurs in neither normal nor pathological calcifications. It is used in calcium phosphate cements,^[33, 39–44] and other applications are as polishing agents,

sources of calcium and phosphate in nutritional supplements, tableting aids, and toothpaste components.^[28]

OCP (octacalcium phosphate, $\text{Ca}_8(\text{HPO}_4)_2(\text{PO}_4)_4 \cdot 5\text{H}_2\text{O}$) is often found as an intermediate phase during the precipitation of the thermodynamically more stable calcium phosphates (e.g. HA, calcium-deficient HA (CDHA)) from aqueous solutions. OCP consists of apatitic layers (with atomic arrangements of calcium and phosphate ions similar to those of HA) separated by hydrated layers (water molecules). OCP is of great biological importance because it is one of the stable components of human dental and urinary calculi.^[45–47] It plays an important role in the 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 (see below for a detailed discussion).^[48–51] Although OCP has not been observed in vascular calcifications, it has been strongly suggested as the precursor phase to

biological apatites found in natural and prosthetic heart valves.^[52, 53]

β -TCP (β -tricalcium phosphate) is the “true calcium orthophosphate” of the stoichiometric composition $\text{Ca}_3(\text{PO}_4)_2$. It cannot be precipitated from solution, but may only be prepared by calcination, e.g. of CDHA (see below), at temperatures above 800 °C [Eq. (1)]:



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 magnesium-containing form called “whitlockite” (chemical formula: β -(Ca,Mg) $_3(\text{PO}_4)_2$) is found in dental calculi and urinary stones,^[15–18, 54] dental caries, salivary stones, arthritic cartilage, as well as in some soft-tissue deposits.^[15–18] In biomedicine, β -TCP is used in calcium phosphate bone cements.^[23, 24, 55–58] In combination with HA, β -TCP is used as a “biphasic calcium phosphate” (“BCP”)^[59–65] as a bone-substitution ceramic. Other applications include fertilizers,^[12] polishing and dental powders, porcelains, pottery, enamel, and animal food supplements.^[28]

α -TCP (α -tricalcium phosphate, α - $\text{Ca}_3(\text{PO}_4)_2$) is a metastable phase at room temperature, prepared from β -TCP at above 1125 °C. α -TCP is more reactive in aqueous systems than β -TCP and can be hydrolyzed to a mixture of other calcium phosphates. It never occurs in biological calcifications and has a limited application in medicine in calcium phosphate cements.^[26, 31, 33, 34, 41–44, 66] α -TCP is also used as a fertilizer.^[28]

ACP (amorphous calcium phosphate) is often encountered as a transient phase during the formation of calcium phosphates in aqueous systems. Usually, ACP is the first phase that is precipitated from a supersaturated solution prepared by rapid mixing of solutions containing of calcium cations and phosphate anions.^[67–71] The chemical composition of ACP strongly depends on the solution pH value and the concentrations of calcium and phosphate ions in the mother liquor. For example, ACP phases with Ca/P ratios in the range of 1.18:1 (precipitated at solution pH 6.6) to 1.53:1 (precipitated at solution pH 11.7)^[72, 73] and even up to 2.5:1^[15–17] have been described.

The structure of ACP is still uncertain. IR spectra of ACP show broad, featureless phosphate absorption bands. The compounds are amorphous, according to X-ray diffraction experiments. Electron microscopy of ACP usually reveals spherical particles with typical diameters of 20–200 nm. However, it is likely that ACP has an apatitic short-range structure, but with a crystal size so small that it appears amorphous in X-ray diffraction experiments (no coherent X-ray scattering). This is supported by X-ray absorption spectroscopic data (EXAFS; extended X-ray absorption fine structure) on biogenic and synthetic samples.^[74–77] 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 $\text{Ca}_9(\text{PO}_4)_6$.^[72, 73] These clusters were found experimentally as seed nuclei during the crystallization of

HA, and a model was developed to describe the crystallization of HA as a stepwise assembly of these units.^[78] Biologically, ACP (often containing magnesium, carbonate, and pyrophosphate) is found in soft-tissue pathological calcifications (e.g. heart-valve calcifications of uremic patients).^[15–18] In medicine, ACP is sometimes used in calcium phosphate cements.^[31–33] Bioactive composites of ACP with polymers have properties suitable for use in dentistry^[79–82] and surgery.^[83–86]

CDHA (calcium-deficient hydroxyapatite) can be easily prepared by the simultaneous addition of calcium- and phosphate-containing solutions into boiling water, followed by boiling the suspension for several hours. During this time, initially precipitated OCP or ACP (this depends on the solution pH value) are transformed into CDHA. On heating above 700 °C, dry CDHA with Ca/P = 1.5:1 will convert into β -TCP and that with 1.5:1 < Ca/P < 1.67:1 will convert into a mixture of HA and β -TCP (the above-mentioned biphasic calcium phosphate, BCP).^[59–65]

Because of its nonstoichiometric character, CDHA always contains other ions. The extent depends on the counterions of the chemicals used for preparation (e.g. Na^+ , Cl^-). There have been no direct determinations of the structures of CDHA and the unit cell parameters are uncertain. As a first approximation, CDHA may be considered as HA with some ions missing.^[87] According to the chemical formula of CDHA (Table 2), there are vacant calcium ion sites (mainly Ca2 sites,^[88, 89] see HA below) and hydroxide ion sites in the crystal structure of this compound. However, little is known about the vacancies of phosphate ions: in CDHA, part of the phosphate ions is either protonated or substituted by other ions (e.g. carbonate).

Unsubstituted CDHA (i.e. containing calcium, phosphate, hydrogenphosphate, and hydroxide ions only) does not exist in biological systems; it occurs only with ionic substitutions: Na^+ , K^+ , Mg^{2+} , Sr^{2+} for Ca^{2+} ; carbonate for phosphate; fluoride, chloride, and carbonate for hydroxide, and some water, form the so-called “biological apatite” or dahllite—the main inorganic component of animal and human normal and pathological calcifications.^[15, 16] Therefore, CDHA is a very promising compound for the manufacture of artificial bone substitutes.

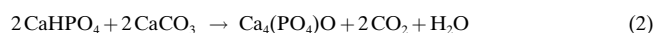
HA (hydroxyapatite, $\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2$) is the most stable and least soluble of all calcium orthophosphates (Table 2). Pure HA crystallizes in the monoclinic space group $P2_1/b$. However, at temperatures above 250 °C, there is a monoclinic to hexagonal phase transition in HA^[72, 73] (space group $P6_3/m$).^[90, 91] Some impurities, like partial substitution of hydroxide by fluoride or chloride ions, 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.

HA can be prepared in aqueous solutions by mixing exactly stoichiometric quantities of calcium- and phosphate-containing solutions at pH > 9, followed by boiling for several days under a CO_2 -free atmosphere, filtration, and drying. Microcrystalline samples of HA can also be prepared by solid-state reactions of other calcium phosphates (e.g. MCPM, DCPA, DCPD, OCP) with CaO, $\text{Ca}(\text{OH})_2$, or CaCO_3 at temperatures

above 1200 °C, in an atmosphere of equal volumes of water and nitrogen. Single crystals of HA can be prepared by hydrothermal synthesis.^[72, 73] A water-free synthesis can be performed in ethanol from Ca(OEt)₂ and H₃PO₄.^[92, 93]

Pure HA never occurs in biological systems. However, because of the chemical similarities to bone and teeth mineral (Table 1), HA is widely used as a coating for orthopedic (e.g. hip-joint prosthesis) and dental implants (reviewed in refs. [94, 95]), and a calcium phosphate cement with HA has also been developed.^[29] Because of the great similarity to bone mineral, HA is also used in liquid chromatography of proteins and other biological compounds.^[96–101]

TTCP (tetracalcium phosphate Ca₄(PO₄)₂O) 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, and thus can only be prepared by a solid-state reaction above 1300 °C, for example, by heating homogenized, equimolar quantities of DCPA and CaCO₃ in dry air, or in a stream of dry nitrogen [Eq. (2)].^[72, 73]



TTCP is not very stable in aqueous solutions; it slowly hydrolyses to HA and calcium hydroxide.^[72, 73] Consequently, TTCP is never found in biological calcifications. In medicine, TTCP is widely used for the preparation of various self-setting calcium phosphate cements.^[27, 29–31, 39, 41, 102–104]

3. Biomineralization and Biological Hard Tissues

Biological mineralization (biomineralization) is the process of in vivo formation of inorganic minerals. As shown in Table 1 and discussed above, in the human body all normal and most pathological calcifications consist of calcium phosphates. Other minerals such as calcium carbonate (found in mollusk shells, algae, fish, ascidians, and plants), calcium oxalate (present in plants), CaSO₄ (jellyfish), SrSO₄ (single-celled sea organisms of the genus *acantharia*), and BaSO₄ (algae), silicon dioxide (marine algae and plants), and iron oxide (in bacteria, limpets, chitons, or mollusk teeth) are also found in biological systems,^[1, 4, 5] but that is another story. Only the chemical and structural peculiarities of calcified tissues consisting of calcium phosphates will be discussed here.

According to Weiner and Wagner, “the term bone refers to a family of materials, all of which are built up of mineralized collagen fibrils”.^[105, 106] This family of materials also includes dentin (the material that constitutes the interior of a tooth), cementum (the thin layer between the root of a tooth and the jaw), and mineralized tendons.^[105, 107] Let us start with the “real” bones.

3.1. Bone

Bone is the major calcification present in a human body.^[1] It serves as structural (mechanical) support for the body

and as the major reservoir of calcium and phosphate ions necessary for a wide variety of metabolic functions. From the chemical point of view, bone is a composite material (Table 1) of calcium phosphate and collagen. The physiological fluids present in bone act as plasticizers. Porosity is an important property of bone, as it allows the body fluids and cells to access the various regions of the osseous tissue while also influencing the mechanical anisotropy.^[1, 5, 15–17, 19–21, 105, 108–112]

Usually bone is composed of a relatively dense outer layer (*Corticalis*; the cortical or compact bone) surrounding a less dense, porous tissue (*Spongiosa*; cancellous bone), which is filled with a gel-like tissue known as bone marrow (Figure 3). Bone is a highly complex material that exhibits a strongly hierarchical structure on different length scales (see refs. [1, 5, 105, 108–112] for detailed discussions).



Figure 3. A noncalcined cancellous bone (femoral head) showing the transition from a more compact outer layer (*corticalis*) to a more porous interior (*spongiosa*).

Microscopically, the constituent building blocks of bone are mineralized collagen fibrils of 80–100 nm thickness and a length of a few to tens of microns (Figure 4). These fibrils are composites of biological apatite (i.e. CDHA with ionic substitutions) and molecules of type I collagen. The crystals of biological apatite in bone are always plateletlike (elongated along the crystallographic *c* axis) and very thin; 2–4 nm (in other words, just a few unit cells thick!—see Table 1). The crystals insert themselves in a parallel fashion into the collagen fibrils, while the latter are formed by self-assembly of collagen triple helices.^[105] Recently, this lowest level of hierarchical organization of bone has been successfully simulated by HA precipitation on amphiphilic peptide nanofibers.^[113] However, the interface between collagen and crystals of biological apatite is still poorly understood. It is not known why the crystals of biological apatite are platelet-shaped.^[1, 5, 105, 108–112]

In general, a sequence of temporal events can be recognized during bone formation. The first stage involves the synthesis and extracellular assembly of the collagen I matrix framework of fibrils, followed by its mineralization. The

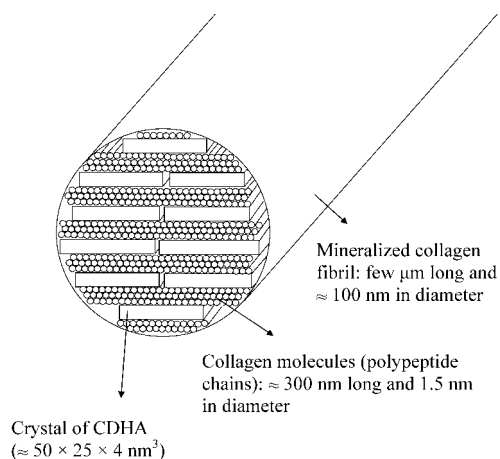


Figure 4. Schematic drawing of the mineralized collagen fibrils that are the basic constituents of bone. Platelet-shaped nanocrystals of CDHA are incorporated in a parallel way between collagen molecules, with the crystallographic *c* axis parallel to the fiber axis.

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.^[5, 105, 107] The same is true for dentin and enamel,^[114, 115] as well as for more primitive living organisms. For example, in the shell of the mollusk *Lingula unguis* which consists of CDHA, the crystal *c* axes are oriented parallel to the β -chitin fibrils.^[116] Therefore, the orientation of CDHA crystals parallel to the long axes of an organic framework could be a general feature of the calcium phosphate biomineralization process.

Unlike other mineralized tissues, bone continuously undergoes a so-called “remodeling” process as it is resorbed by specialized cells called osteoclasts and formed by another type of cells called osteoblasts in a delicate equilibrium. Osteoporosis is the condition in which bone resorption dominates, and in osteopetrosis, the reverse process is dominant. That is why mature bone consists of a very complex assembly of bone “patches”, each of which has a slightly different structure and a different age.^[1, 5, 105, 107–112]

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, that is, CDHA in which some ions have been replaced but (surprisingly!) without detectable amounts of hydroxide ions.^[117–119] However, various in vitro experiments on the precipitation of CDHA and HA revealed that none of these compounds directly precipitates from supersaturated aqueous solutions containing calcium and phosphate ions: some intermediate phases (so-called “precursors”) are always involved.^[15–17, 48–53, 67–71] Three compounds (DCPD, ACP, and OCP) are possible precursors to CDHA and HA precipitation in vitro. Therefore, the same compounds are suggested as the precursors to in vivo bone formation. Evidently, the precursor phase of bone is of a transient nature, which complicates its detection, especially in vivo. In 1966, Brown et al. suggested that OCP is the original precipitate on which biological apatite nucleates in the following step.^[120] This idea was extended in

their further investigations.^[121–124] By use of high-resolution transmission electron microscopy, this hypothesis was supported: computer-simulated lattice images of the “central dark line” in mineralized tissues revealed that it consisted of OCP.^[48–50]

Simultaneously with Brown, the research group led by Posner proposed that ACP is the initially precipitated phase of bone formation in vivo.^[125–127] This conclusion was drawn from the following facts:

- When calcium orthophosphates are prepared by rapid precipitation from aqueous solutions containing calcium cations and phosphate anions at $\text{pH} > 8.5$ in vitro, the initial solid phase that appears is amorphous.
- Mature bone mineral is a mixture of ACP and poorly crystallized CDHA.
- Early bone mineral has a lower crystallinity than mature bone,^[125–133] which suggests that after being formed the crystals of bone mineral undergo some transformations during maturation.

For obvious reasons, there is only indirect evidence for the in vivo crystal growth of bone mineral. Studies of animal bones of different ages showed that the X-ray diffraction peaks become sharper with increasing age, that is, the crystallinity and/or the domain size increase. This change occurs anisotropically, that is, it is more pronounced in the crystallographic *a* axis [(310) reflections] than the *c* axis [(002) reflections].^[134, 135] In addition to this, other changes, such as an increase of calcium content and a decrease of HPO_4^{2-} occur in bone mineral with age.^[136, 137] Both crystal size and carbonate content increase during aging in rats and cows.^[137] From a chemical point of view, these changes indicate a slow transformation of a poorly crystallized CDHA into a better crystallized HA.

There is a current debate on the question of whether bone formation is an active or a passive process. As an “active process”, one describes the assembly of calcium phosphate nanocrystals within a spatially confined compartment of an osteoblast, that is, within a matrix vesicle. These structures have been found by transmission electron microscopy for bone and tooth formation.^[138–140] The term “passive process” comes from the observation that blood serum is supersaturated with respect to calcium phosphate precipitation,^[141] therefore mineralization should occur spontaneously at a suitable nucleus (i.e. on a collagen fibril). The collagen fibrils have a specific structure with a periodicity of 67 nm and 35–40 nm gaps or holes between the ends of the collagen molecules, where bone mineral is incorporated in the mineralized fibril. 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 the temporary absence of specific inhibitors leads to precipitation and thereby regulates this physicochemical bone formation.^[142–144] The question of whether cells do actively form and deposit bone mineral or whether a systemic regulation of inhibitors controls bone formation is still open.^[145] The truth probably lies somewhere in between, that is, calcium phosphate nanocrystals are formed within cells from a supersaturated medium and excreted near the collagen fibers where they are finally deposited.

3.2. Teeth

Teeth are the second major normal calcification present in mammals.^[1] The structure of teeth is even more complicated than that of bone (Figure 5). For example, unlike bone, teeth consist of at least two different biominerals: enamel (outside) and dentin (interior). As shown in Table 1, dentin and bone have many similarities, and in most aspects they can be regarded as being essentially the same material.^[1, 72, 73, 105, 107–112, 136] Therefore, most statements made above for bone are also valid for dentin.

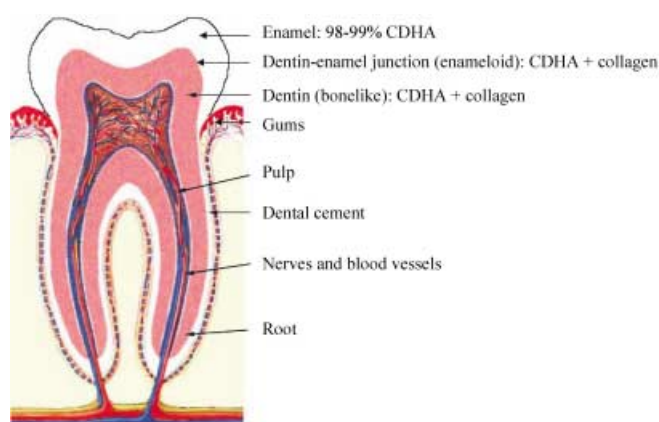


Figure 5. Schematic picture of a tooth and its local chemical composition.

Tooth enamel contains crystals of biological apatite that are much larger than those of bone and dentin (Table 1). In addition, its organic phase does not contain collagen. At the interface between enamel and dentin, there is an “enameloid” phase; a hard tissue that contains enamel-like crystals of biological apatite and collagen fibrils.^[1]

Enamel and enameloid consist of biological apatite crystals that are remarkably different from the other mineralized tissues in humans and vertebrates. In enamel, needlelike crystal rods are tens of microns long (up to 100 μm) but sometimes only 50 nm wide,^[146–150] which is much larger than the mineral crystals of dentin and bone (Table 1), but nevertheless consist of carbonated CDHA.^[151–153] On the surface, there is also some fluoride content in place of hydroxide ions^[154] although the overall content of fluoride ions in enamel is small (about 0.01 wt %;^[16] see also Table 1). Note that fluoroapatite is not found in enamel.^[1]

The enamel crystals are generally organized into parallel arrays under strict biological control. This structure can be deduced from the observation that, at every stage, the parallel arrays are well-ordered and that the crystal rods all have a remarkably uniform cross section (Figure 6).^[146–148] The first detectable crystals in enamel formation are flat, thin ribbons,^[146–148] that were reported to be OCP,^[109, 155–157] β -(Ca,Mg)₃(PO₄)₂,^[156] or DCPD.^[117, 119] During maturation of the enamel, the mineral content increases from initially 45 wt % to 98–99 wt %,^[117] accompanied by widening and thickening of the crystal rods.^[117, 119, 158, 159] Simultaneously, the



Figure 6. Scanning electron micrograph of the forming enamel on a continuously growing rat incisor, which shows ordered rods of calcium phosphates. Scale bar: 10 μm (taken from ref. [1] with permission).

Ca/P ratio increases^[158, 159] and the carbonate content decreases,^[160–162] which finally results in the most highly mineralized and hardest skeletal tissue.

Enamel crystals show the (100) face at the sides and presumably the (001) face at the ends,^[163, 164] as usual for HA. A “central dark line” is observed by TEM in the centers of enamel crystals (also observed in bone and dentin), which consists of OCP.^[48–51] As described above for bone, X-ray diffraction shows that the crystals of “younger” dentin are less ordered than those of more mature dentin.^[136] Therefore, maturation of dentin is a slow transformation of a poorly crystallized CDHA into a better crystallized HA.

The development of individual enamel and dentin crystals was studied by high-resolution transmission electron microscopy.^[165–167] 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 CDHA. They are followed by formation of ribbonlike crystals, which until recently was considered to be the first step of biological crystal formation in the tooth.^[165–167] These complicated processes, starting with the heterogeneous nucleation of inorganic calcium phosphate on an organic extracellular matrix, are controlled in both tissues by the organic matrix and are under cellular control (odontoblasts and ameloblasts).^[168] 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.^[169] Organic molecules (e.g. amelogenin)^[169] at physiological solution pH values appear to bind on the charged surface domains of CDHA.

On the other hand, dentin and enamel share a common starting location: the dentin–enamel junction.^[170–172] The steps of enamel crystal growth at the junction are a matter of current debate. 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.^[173–175] Others have shown that enamel crystals are formed at a given distance from the dentin surface^[155–157, 176] and could either

reach dentin crystals by a subsequent growth^[177] or remain distant.^[176, 178] Thus, both structure and formation of the teeth appear to be more complicated than those of the bone.

A physicochemical mineralization occurs every day on our teeth. Enamel is only formed during dentinogenesis in the jaw, that is, it will never be repaired by cellular action. If it is etched, for example, by acidic food or beverages, CDHA is dissolved. Fortunately, the saliva in the mouth is supersaturated with respect to CDHA deposition (as is the blood serum), and after a while, the surface layer is restored again. This process does not involve any biological action and therefore can be classified as “passive mineralization” (see also the discussion above on bone formation). Replacement of some hydroxide ions with fluoride ions (which leads to fluorohydroxyapatite) lowers the solubility and therefore improves the acid resistance.^[154]

3.3. Cartilage

Cartilage is usually (but not exclusively) part of the endoskeleton of animals^[1, 179] and exists both in mineralized and unmineralized forms. Only vertebrates develop mineralized cartilage, in some cases in the central portions of the vertebra and close to the surface of jaws. Except for pathological cases, the mineralization of cartilage occurs in two situations in the body: First, during bone formation in the endochondral plate (in almost all vertebrates) and second, as final mineralized product (only in sharks and certain other fishes^[180]).^[1]

Mineralized cartilage consists of the unmineralized cartilage plus crystals of CDHA, as well as considerable amounts of amino acids, phosphoserine, and other biological compounds. The molecular organization of macromolecules of cartilage and CDHA crystals is still not fully understood. Mineralized cartilage and bone coexist in close proximity in the endochondral plate during bone formation. They have similar macromolecular constituents, and both contain CDHA.^[1] However, the shape of the CDHA crystals in mineralized cartilage, in general, resembles that in enamel: the crystals were found to be needlelike (CDHA crystals of bone are platelike),^[1, 105, 108–112] but much shorter (25–75 nm^[181] or 50–160 nm^[182]) than those of enamel (up to 100 μm ^[146–150]). The average thickness of the CDHA crystals in mineralized cartilage was reported as 5–7.5 nm^[181] and 1.8 nm.^[182]

The process of cartilage mineralization has been well-described elsewhere.^[183–185] Before the crystal formation, the organic matrix (consisting of proteoglycans, type II collagen and water)^[1] first takes up calcium and then phosphate.^[185] The first crystals of CDHA, those formed in cartilage, were needlelike and located inside cellular matrix vesicles.^[183, 184] After growth within the vesicles, the crystals extend out of these containers into the surrounding organic matrix. They aggregate into clusters of randomly oriented crystals. In a second step, these clusters further aggregate to form the mature mineralized-cartilage structure with a random arrangement of crystals.^[181] Physicochemical investigations of the crystals revealed their very poor crystallinity and the

presence of significant amounts of nonapatitic calcium phosphates. The concentration of such nonapatitic phosphates was found to increase during the early stages of cartilage mineralization but then decreased as the mineral content steadily rose, until full mineralization was achieved.^[186] Therefore, the CDHA crystals in the vesicles act as centers of cartilage mineralization. However, a detailed understanding of the mechanisms of crystal nucleation and growth in these vesicles is not yet available.^[1]

3.4. Shells

Rarely, calcium phosphates are encountered in mollusk shells (that in most cases consist of calcium carbonate).^[187, 188] When biomineralization was “invented” by nature about 570 million years ago, there were both mollusks with calcium carbonate and calcium phosphate shells. Over time, the ones with calcium phosphate shells mostly disappeared (so-called “problematica”), and today the overwhelming majority of mollusk has shells of calcium carbonate.^[1] Figure 7 shows fossilized shells of the species *Lingula* that consist of calcium phosphate (apatite).^[116]



Figure 7. Fossilized shells of the brachiopod *Lingula* from the Lower Triassic, consisting of calcium phosphate (taken from <http://inyo.topcities.com/ef/lingula.html> with permission).

4. Pathological Crystallization of Calcium Phosphates

Unwanted deposition of calcium phosphates in the body can lead to severe diseases. Calcium phosphate depositions are responsible, among other things, for urinary stones,^[15, 189, 190] atherosclerosis,^[141, 191–193] dental calculus,^[45, 46] calcification of artificial heart valves,^[194–198] and calcified menisci (“chondrocalcinosis”).^[199, 200] Figure 8 shows an example of atherosclerotic depositions of calcium phosphate (together with cholesterol) that was isolated from arteries.^[193, 201] Blockage of arteries by such deposits is the major cause of death in developed countries.

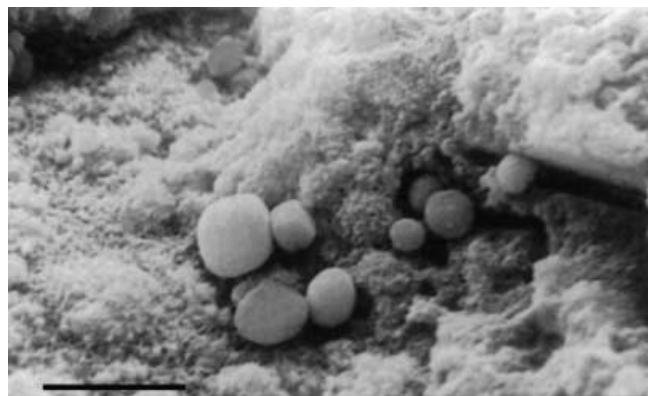


Figure 8. Spherical calcium phosphate particles isolated from an atherosclerotic lesion. Scale bar: 4 μm (taken from ref. [193] with permission).

As many body fluids (blood, saliva) are supersaturated with respect to HA precipitation,^[141] we may conclude that calcification is thermodynamically feasible but kinetically hindered in most parts of the body. Therefore, suitable inhibitory mechanisms must be at work to prevent an unwanted mineralization in the body. The mechanisms of this inhibition are a topic of current research in molecular medicine, as it can be concluded that disruptions of this inhibition are probably the cause of pathological calcifications. In addition, the fine-tuned equilibrium of bone resorption and formation may be based on such processes. For instance, in mice in which the genes that are responsible for the production of the specific blood proteins (fetuin, ^[142, 144] matrix Gla protein^[143, 202]) were knocked out, uncontrolled calcification in the arteries occurs. Obviously, these proteins serve as inhibitors of calcium phosphate precipitation by suitable complexation of the dissolved ions or by effectively preventing formed nuclei from further growth by preferential adsorption.^[203–205]

On the other hand, some mechanisms have been identified that enhance crystallization.^[141] Currently discussed, especially for the case of atherosclerosis, are:

- the heterogeneous nucleation of calcium phosphates on the membranes of dead cells that contain phospholipids (phosphate groups act as nucleators),^[192, 195]
- nucleation by antibodies that are specific for cholesterol,^[205, 206] and
- cellular action of osteoblast-like cells (so-called pericytes) within arteries that form bonelike tissue.^[207]

For the case of atherosclerosis, obviously a number of effects are responsible for the pathological calcification; these range from purely physicochemical effects (supersaturation)^[141] over biologically induced nucleation to the biologically controlled deposition of calcium phosphates by specialized cells.^[141]

Similar effects exist during the calcification of artificial heart valves. The replacement of heart valves by implants of either biological (porcine heart valves) or synthetic origin is now a common procedure in cardiosurgery (about 150 000 are implanted every year worldwide).^[196] However, the implanted devices tend to calcify after implantation (in some cases even after a few months), that is, they become stiff because of

deposition of calcium phosphate. The origin of this behavior is not yet clear but, at least with heart valves of biological origin, a nucleation by membranes of dead cells (phospholipids) appears likely.^[194–198]

5. Calcium Phosphates as Biomaterials

The treatment of injuries or diseases often requires surgical action. For the past 50 years, biomaterials have increasingly been applied to improve surgical procedures or to restore lost body functions. Bone fractures are usually treated with metallic wires, nails, screws, and plates, joints are replaced by artificial endoprostheses (hip or knee), and lost teeth are replaced by metallic implants in the jaw, to name a few examples. As soon as foreign materials come into internal contact with the body, the question of biocompatibility becomes paramount, as any adverse effect (namely toxicity, allergy, inflammation, corrosion, and mechanical failure) must be strictly avoided. The search for optimally designed biomaterials is still ongoing as a joint effort of physicians, engineers, chemists, and physicists.^[15, 194, 208–213]

Calcium phosphates generally have an excellent biocompatibility, that is, they are well-accepted by the body and integrate well, for example, into bone upon implantation. This is because of their almost ubiquitous presence in the body in either the dissolved or solid form. Consequently, they have found important applications as biomaterials, particularly for hard-tissue regeneration.^[21, 47, 66, 214–221]

In the bulk form, calcium phosphates are used as artificial bone-substitution material for surgical treatment of bone defects by orthopedic surgeons and maxillofacial surgeons.^[15, 16, 219, 222] A bone defect that is caused, for example, by tumor extraction, complicated fracture, or inflammation must be filled with a suitable material to permit growth of new bone into this defect. Otherwise, ingrowth of fibrous tissue would prevent bone formation within the defect. Because the ideal substitute (the “golden standard”), a patient’s own spongy bone from the *Iliac crest* (hip) is usually not available in sufficient quantities, and as materials of biological origin are critically discussed because of possible infections or immune reactions, the need for a fully synthetic material is evident. Today, many different calcium phosphate ceramics are on the market for the treatment of bone defects (see, for example, refs. [15, 16, 219, 222] for overviews).

Chemically, synthetic bone-substitution materials are usually based on HA, β -TCP, or BCP (i.e. a composite of HA and β -TCP).^[15, 16, 219, 222, 223] The requirements for an ideal substitute are usually:

- a porosity with a pore diameter of some 100 μm size (to permit ingrowth of bone cells; see Figures 2 and 3),
- a biodegradation rate comparable to the formation of bone tissue (i.e. between a few months and about two years), and
- a sufficient mechanical stability.^[15, 16, 219, 222]

HA is more stable than α - and β -TCP under physiological conditions, as it has a lower solubility and slower resorption kinetics.^[15, 16, 219, 222] Implants of calcined HA of high crystallinity are present in a defect even years after implantation in a virtually unchanged form, therefore β -

TCP^[218] or BCP^[21, 61, 63, 64] ceramics are favored today. An ideal material should be degraded inside the defect simultaneously with the formation of a new bone, that is, the full restoration of the defect with biological material is desired. Figure 9 shows three examples of calcium phosphate-based bone-substitution materials of different origins. Implant porosity is a very important property to allow cell invasion and bone ingrowth.

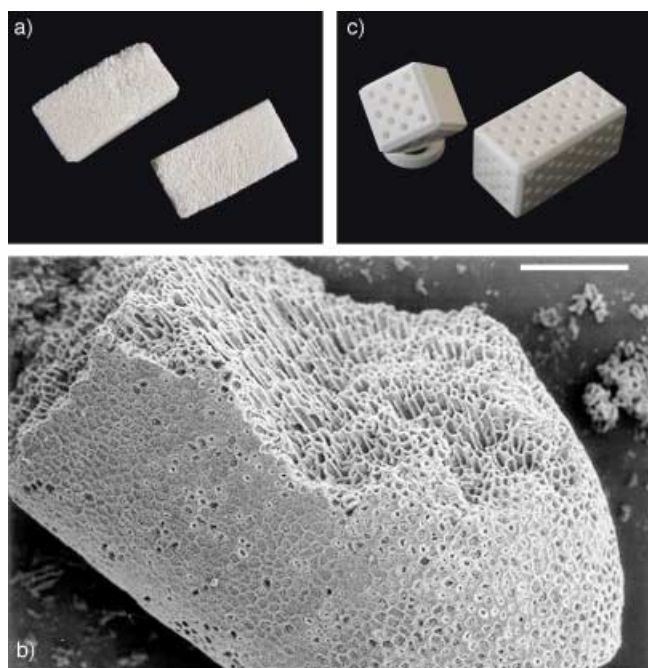
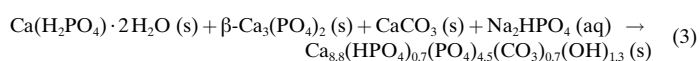


Figure 9. Examples of porous calcium phosphate-based bone-substitution materials: a) Cerabone (hydroxyapatite) from spongy calcined bovine bone (about $3 \times 1 \times 1 \text{ cm}^3$); b) Aligpore (hydroxyapatite) from hydrothermal processing of calcium carbonate-containing algae with ammonium phosphate. Scale bar: 100 μm ; c) Cerasorb (synthetic phase-pure β -TCP) with CNC (computer numerical control)-drilled holes (about $1 \times 1 \times 2 \text{ cm}^3$).

A new concept in the treatment of bone defects was introduced with bone cements based on calcium phosphates, which harden inside the defect. Although different formulations are on the market (see the discussion of the different calcium phosphates above), they usually consist of solid calcium phosphates that are mixed with a solution to induce the precipitation of a CDHA-like phase [Eq. (3), not stoichiometrically balanced]:^[66, 102–104, 211, 212]



The advantage of this procedure is that the cement adapts better to the defect geometry than ceramic materials that are implanted as solids. The structure and composition of the hardened calcium phosphate is close to that of bone mineral; therefore, a facilitated resorption is observed.^[66]

Calcium phosphate coatings on metals are often applied in medicine. Metallic implants are encountered in endoprostheses (total hip-joint replacements) and artificial tooth sockets. The requirement for mechanical stability necessitates the use

of a metallic body for such devices. As metals usually do not undergo bone bonding, that is, they do not form a mechanically stable bond between implant and bone tissue, ways have been sought to improve the mechanical contact at the interface.^[194, 208, 224] One possibility is to coat the metal with calcium phosphate ceramics; these increase the roughness of the bone surface and thereby facilitate bone bonding, and may therefore serve as a “glue” between the metal and bone (Figure 10).



Figure 10. Calcium phosphates in hip endoprostheses: a ceramic ball joint (Al_2O_3), a calcium-phosphate coated endoprosthesis (“cementless endoprosthesis”) and an uncoated endoprosthesis that must be fixed in place with PMMA bone cement.

Two methods of bone coating are currently applied: Application of molten calcium phosphate by high-temperature plasma spraying and precipitation from a supersaturated calcium phosphate solution. The first approach^[94, 95, 216, 224] is very rough from a chemical point of view. Solid calcium phosphate is injected into a plasma flame and directed towards an implant that is appropriately rotated to achieve a uniform coating. This extremely fast quenching leads to the formation of a mixture of calcium phosphates on the implant surface. Metal and calcium phosphate are strongly joined after this procedure.^[94, 95, 216, 224]

The second approach involves dipping metallic implants into supersaturated calcium phosphate solutions. This method was strongly promoted by the work of Kokubo and co-workers and van Blitterswijk and co-workers, who showed that after appropriate surface etching, a stable interface evolves between metal and ceramic.^[225–230] The method also permits coating of internal surfaces (difficult with plasma spraying) and the incorporation of biologically active substances, for example, proteins or antibiotics into the coating.^[231] A special case is surface coating with a biomimetic defect apatite by dipping into simulated body fluid (SBF), a solution that contains the inorganic ions of human blood plasma in almost natural concentrations.^[225–228, 232–235] Figure 10 shows both a calcium phosphate-coated and an uncoated hip endoprosthesis. The latter has to be fixed in the femur bone by a suitable bone cement based on poly(me-

thylmethacrylate) (PMMA). Note that this polymer is not biodegradable and remains in the operation site.^[224, 236]

The same principles are valid for tooth implant systems that are fixed into the jawbone, onto which artificial teeth are attached. In general, the mechanical contact between implant and bone is crucial, as considerable forces have to be withstood. Coating of such dental implants with calcium phosphates (usually by plasma spraying) leads to better and faster bone attachment. Figure 11 shows such a plasma-spray-coated tooth implant in low and high magnification. Finally, Figure 12 shows the surface of a nickel-titanium shape-memory alloy (NiTi, “Nitinol”) that was coated with calcium phosphate from solution to improve its biocompatibility.^[237]

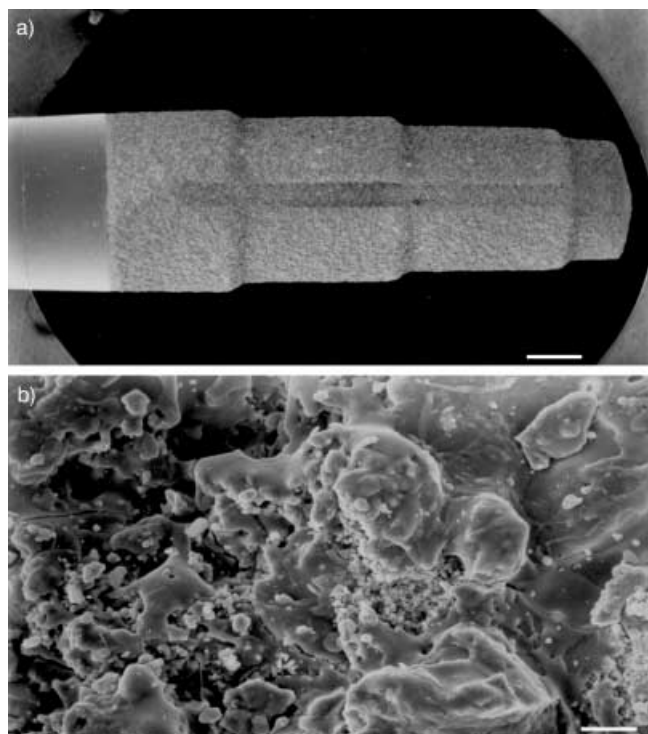


Figure 11. Dental implants (by Friadent) coated with calcium phosphate by a plasma-spray process. a: $\times 10$, b: $\times 1000$. Note the irregular, rough structure of the deposited calcium phosphate at the higher magnification. Scale bars 1 mm and 10 μm , respectively.



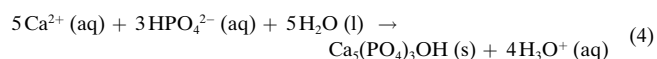
Figure 12. The surface of a nickel–titanium shape-memory alloy (“Nitinol”) that was coated with a calcium phosphate layer by dipping into an supersaturated calcium phosphate solution. The front part shows the etched metal surface from which the calcium phosphate layer has been mechanically removed. Scale bar: 2 μm .

6. Biomimetic Crystallization of Calcium Phosphates

Nature’s ability to assemble inorganic compounds into the biological structures (shells, spicules, teeth, bone, skeletons) is still not reproducible by synthetic procedures. Because of its potential benefits for materials science, research groups around the world are increasingly addressing the question of biomineralization. When considering calcium phosphates, the demand of clinical medicine to design biocompatible implants and to treat diseases related to crystallization phenomena adds a strong practical impetus to understanding these processes. The fundamentals of biomineralization have been reviewed extensively.^[1, 3–7, 238–241] We will limit ourselves to considerations of biologically inspired crystallization of calcium phosphates and present a few examples that demonstrate the current possibilities.

An approach to the preparation of biomimetic bone-substitution materials was made by Pompe et al., who crystallized HA on collagen to obtain a bonelike composite.^[242] Although the ultrastructure of bone could not be realized, such collagen–HA tapes are currently under investigation for clinical use. Note that the final step to make bone out of artificial implants is up to the body’s own remodeling function. Ozin et al. precipitated HA in the presence of surfactants, to obtain a biomimetic lamellar product.^[243] Stupp et al. have prepared so-called “organoapatites” with a bone-like crystallinity by precipitation of calcium phosphate in the presence of organic polyelectrolytes.^[214, 217, 244, 245] Kokubo and co-workers and van Blitterswijk and co-workers were successful in coating different substrates with a bonelike apatite layer (see refs. [229, 234] and those given above on coated metal prostheses). We have recently prepared bulk samples of bonelike apatite and composites of it with biodegradable polymers.^[84–86, 246]

Nancollas and co-workers invented the “Constant-Composition Technique” to monitor and control the external conditions (mainly solution pH value and concentrations of participating ions) during a crystallization experiment.^[22, 247] Generally, during precipitation of calcium phosphates from a neutral solution, the pH value decreases because of the release of protons that were formerly bound to hydrogen phosphate or dihydrogen phosphate [Eq. (4)].



One of the main differences between chemical and biological crystallization is the rate of precipitation. Usually in chemistry, precipitation occurs fast whereas in biology the crystals need days, weeks, or months to grow. A suitable simulation of this process, especially in the presence of (bio)organic additives, must therefore slow down the crystallization. This can be achieved by separating the two components with a suitable membrane or medium that acts as a diffusion barrier (a double-diffusion technique). If this medium itself contains some biomimetic functional groups, it can have a templating influence on the growing crystals. Work along this line has been carried out by Iijima et al.

(collagen matrix from bovine achilles tendon,^[248] and membranes in the presence of bovine^[249] and murine^[250] amelogenins), Kniep and co-workers (matrix of denaturated collagen),^[154, 251, 252] Epple and co-workers (matrix of microporous polyglycolide),^[253–256] Falini et al. (matrix of collagen),^[257] and Stupp and co-workers (carbon-coated TEM grid).^[113] Work on the crystallization from SBF under static and dynamic conditions to yield bonelike apatite was also reported recently by Vallet-Regi and co-workers^[258, 259] and by Epple and co-workers.^[256, 260]

Interactions between collagen and growing fluoroapatite crystals are responsible for a fractal growth of fluoroapatite into dumbbell shapes that finally close to give spheres.^[154, 251, 252] Figure 13 shows this special morphology.



Figure 13. A biomimetically grown aggregate of fluoroapatite that was crystallized in a gelatin matrix. The crystal shape can be explained and simulated by a fractal growth mechanism. Scale bar: 10 μm (taken from ref. [252] with permission).

By combining the constant-composition technique with the double-diffusion setup, we were able to identify different crystal morphologies of fluoroapatite as functions of overall concentration (i.e. supersaturation), pH value, and fluoride ion concentration.^[254, 255] Figure 14 shows a uniform crystal population that was prepared by this method.

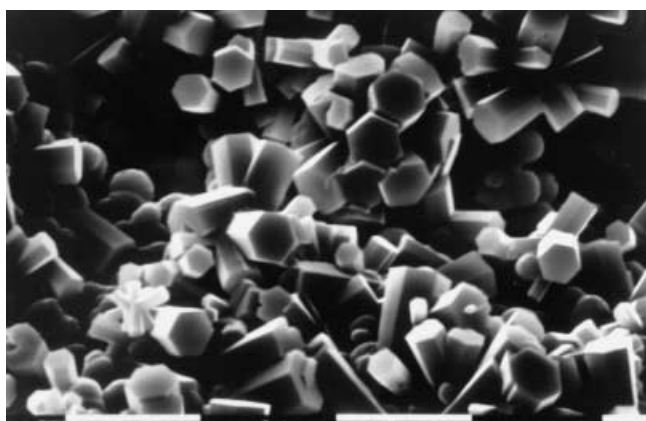


Figure 14. Hexagonal fluoroapatite crystals that were grown by a double-diffusion technique under controlled conditions (pH 7.4, 37 °C, constant ion concentrations, 7 days). Note the well-shaped crystals and their uniform size and morphology. Scale bar: 10 μm .

7. Summary and Outlook

Although it may appear surprising to the nonspecialist, there are still many open questions within the area of calcium phosphate chemistry. The basic questions concerning crystallography, thermodynamics, and phase relationships have been answered. Nevertheless, when it comes to the biological formation of calcium phosphates, issues including rate of crystallization, control of morphology, incorporation of foreign ions, and interaction with biomolecules remain hot topics that are not well understood even today. A better understanding of structure, formation, and dissolution of such biominerals will lead to improved biomaterials that can substitute bone and teeth. This knowledge will also help to counter widespread pathological calcifications such as atherosclerosis, stone formation, or dental calculus. Further progress of unforeseeable impact will come from modern genetics, where gene structures are currently related to hard-tissue formation.

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