Bone regeneration: molecular and cellular interactions with calcium phosphate ceramics

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Abstract: Calcium phosphate bioceramics are widely used in orthopedic and dental applications and porous scaffolds made of them are serious candidates in the field of bone tissue engineering. They have superior properties for the stimulation of bone formation and bone bonding, both related to the specific interactions of their surface with the extracellular fluids and cells, ie, ionic exchanges, superficial molecular rearrangement and cellular activity.

Keywords: calcium phosphate, biomaterials, tissue engineering, osteoprogenitor cells, osteoconduction, osteoinduction

Introduction

Calcium phosphate bioceramics have a unique characteristic for bone substitution compared with other biomaterials. They have such compositional resemblance to bone mineral that they induce a biological response similar to the one generated during bone remodeling. Bone remodeling or renewal consists of the resorption of old bone mineral coupled with the formation of new bone. During resorption, the degradation products of calcium phosphate bioceramics (calcium and phosphate ions) are naturally metabolized and they do not induce abnormal calcium or phosphate levels in urine, serum, or organs (liver, skin, brain, heart, kidney, lung, and intestine) (den Hollander et al 1991). Calcium phosphate biomaterials are successfully used in cranio-maxillofacial, dental, and orthopedic surgery. They are of synthetic origin (obtained after aqueous precipitation or after sintering) or natural origin (freeze-dried or banked bone and derived coral hydroxyapatite), and they are used as bone fillers in the form of cement or granules. As they cannot replace as such the load-bearing functions of bone because of their lower mechanical properties, they are also successfully used as coatings on metallic hip and dental implants in clinics (Epinette and Manley 2004). Naturally porous calcium phosphates biomaterials have been selected as relevant scaffold candidates in bone tissue engineering (Kruyt et al 2004; Arinzeh et al 2005). Technically, several procedures have been developed to tailor the scaffolds, such as rapid prototyping (Wilson et al 2004), phase mixing (Li et al 2002), use of porogenic agents (Barralet et al 2002), or shape replication (Tancred et al 1998). In addition, by selecting and/or combining calcium phosphate phases, one can tailor the resorption kinetics, and also their stimulating effect on bone formation (Dhert et al 1991; Dhert et al 1993; Barrere et al 2003a; Rahbek et al 2004; Habibovic et al 2005b; Schopper et al 2005).

In this review, we address the molecular and cellular, interactions that take place at the calcium phosphate surfaces and we correlate them with their relevance in bone regeneration.

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Bone

Bone is the component of the skeletal system that is involved in body protection, support, and motion. Bone is a protection and production site for specialized tissues such as the blood-forming system, ie, bone marrow. Heart, lungs, and other organs and structures in the chest are protected by the rib cage. The function of these organs involving motion, expansion, and contraction requires flexibility and elasticity of the protective rib cage. Bone supports structurally the mechanical action of soft tissues, like the contraction of muscles or expansion of lungs. Finally, it is a mineral reservoir, whereby endocrine systems regulate the level of calcium and phosphate ions in the circulating body fluids.

Bone structure

Bone macro- and microscopic structures are affected by genetic, metabolic, and mechanical factors. These intrinsic factors are the main cause of bone macrostructural diversity. For example, broad, flat plates, such as the scapula, anchor large muscle masses, whereas hollow and thick-walled tubes, such as the femur or radius, support weight. All bone consists of a basic double structure, the importance of which varies with the function. An external layer, or cortex, covers the bone; it is smooth, dense, and continuous. In the interior, cancellous bone is arranged in a network of intersecting plates and spicules varying in amount and enclosing spaces. These cavities are filled with blood vessels and marrow, either red, hematopoietic or yellow, adipose, its character varying with age and site.

Microscopically, bone is a highly complex and specialized form of connective tissue. It is a mineralized tissue, which is composed of an organic matrix strengthened by deposits of calcium phosphate crystals; in other words bone is a natural composite material. The organic matrix is composed of collagen type I fibers (approximately 95%) and of proteoglycans and numerous non-collagenous proteins (5%). This organic matrix, calcified by calcium phosphate minerals, embeds bone cells, which participate in the maintenance and organization of bone, namely osteoprogenitor cells, osteoblasts, osteocytes, and osteoclasts.

Mechanical properties of bone

The intimate blend of hard inorganic and resilient organic components results in excellent mechanical properties. For example, compact bone specimens have been found to have tensile strength in the range of 700 to 1400 kg/cm^2, and compressive strength in the range of 1400 to 2100 kg/cm^2. These values are within the same magnitude as for aluminum or mild steel, but bone is much lighter. The great strength of bone exists principally along its axis and hence roughly parallel both to the collagen fiber axis and to the long axis of mineral crystals. Although apparently stiff, bones exhibit a considerable degree of elasticity, which is important in the skeleton's ability to withstand impact. Estimates of modulus of elasticity of bone samples are of the order of 420 to 700 kg/cm^2, values very much less than steel, for example, indicating much greater elasticity of bone.

Bone renewal

Bone is a dynamic tissue. In the first year of life, the rate of turnover of the skeleton approaches 100% per year. This rate declines to about 10% per year in late childhood, and then usually continues at approximately this rate or more slowly throughout life, up to hundred years. After the completion of skeletal growth, bone turnover results primarily from remodeling: a coordinated cycle of tissue resorption and formation over extensive regions of bone and prolonged periods. Throughout life, physiological remodeling, removal, and replacement of bone, at roughly the same location, occur without affecting the shape or density of the bone, through a sequence of events that include (i) osteoclasts activation, (ii) resorption of bone, (iii) osteoblasts activation, and (iv) formation of new bone at the site of resorption (Buckwalter et al 1996). Owing to these remodeling properties, defects and fractures are easily repaired up to sizes called critical defects, defined as defects of a size that will not heal during the lifetime of the animal (Schmitz and Hollinger 1986). For larger defects, human interventions are necessary in order to help or stimulate the healing.

Bone mineral

Bone mineral, or enamel (biological apatite) have structure close to a type AB carbonated calcium phosphate apatitic structure more or less deficient, which can be tentatively represented by the formula (Rey 1990)

$$Ca_{8.3}\square_{1.7}(PO_4)_{4.3}(CO_3)_1(HPO_4)_{0.7}(OH,CO_3)_{0.3}\square_{1.7}$$

$\square$ = vacancy

However, bone mineral apatite contains non-apatitic carbonate and phosphate groups, which are, structurally and physically, unstable and very reactive. This high reactivity provides certain physicochemical, biological, functional, and chemical features important in the formation and dissolution of the crystals in biological tissues. Furthermore, bone contains minor or trace elements, which are not indicated

in the above formula, and which are difficult to attribute to either the mineral phase or the organic matrix (Rey 1990). In bone and other mineralized tissues, the mineral crystals are formed of thin plates of irregular shapes. Their sizes range in length from 20 Å for the smallest particles, to 1100 Å for the largest particles (Moradianoldak et al 1991; Kim et al 1995). These bone crystals expose a very large surface area to the extracellular fluids, which is critically important for the rapid exchange of ions with these fluids.

Bone mineral starts to nucleate into the holes and pores present in the collagen fibrils (Glimcher 1987). This heterogeneous nucleation is catalyzed by the presence of phosphated esters groups (Glimcher et al 1984) and carboxylate groups (Rhee et al 2000) present in the collagen fibrils. Thereafter, the growth, or mineralization, takes place along the collagen fibrils, eventually interconnecting all of the collagen fibrils.

The nature of the primary mineral phase formed prior to mature bone mineral apatite remains controversial. According to Posner, bone mineral apatite derives from calcium phosphate clusters ($Ca_9(PO_4)_6$) packing randomly with interfacial water to form amorphous calcium phosphate precursor (Posner 1985). This theory is supported by the presence of several calcium phosphate growth inhibitors such as magnesium that stabilize the amorphous state. However, dicalcium phosphate (DCPD) (Grynpas et al 1984; Roberts et al 1992) and octacalcium phosphate (OCP) phases have been also proposed as bone mineral precursors because of the partial similarities, especially between apatite and OCP (Brown et al 1987). DCDP and OCP are kinetically favored compared with apatitic phases, supporting their role as precursors in bone apatite formation (Nancollas and Wu 2000). However, Kuhn et al (2000) have shown that the apatitic phase seems to be the largely dominant one compared with other possible transient phases even at the earliest stages of mineralization.

Biologically relevant calcium phosphates

Synthetic calcium phosphates

Calcium phosphates, or more accurately calcium orthophosphates, are salts of orthophosphoric acid (H_3PO_4), and thus can form compounds that contain $H_2PO_4^-$, HPO_3^{2-} or PO_4^{3-}. The calcium phosphate salts constitute a wide group of compounds (Elliot 1994). Table 1 summarizes the chemical name, the formula, the abbreviation, and the calcium to phosphorus ratio (Ca/P) of some calcium phosphate compounds. Calcium phosphate salts vary by their composition and their crystal structures, leading to specific physicochemical properties.

Table 1 Main biologically relevant calcium phosphate salts

Name	Formula	Abbreviation	Ca/P
Dicalcium phosphate anhydrate or monetite	$CaHPO_4$	DCPA	1.00
Dicalcium phosphate dihydrate or brushite	$CaHPO_4.2H_2O$	DCPD	1.00
Octacalcium phosphate	$Ca_8(PO_4)_4(HPO_4)_2.5H_2O$	OCP	1.33
Tricalcium phosphate	$Ca_3(PO_4)_2$	TCP	1.50
Hydroxyapatite	$Ca_{10}(PO_4)_6(OH)_2$	HA	1.67

Apatites

Stoichiometric hydroxyapatite (HA) belongs to the general and wide apatitic group, represented by the formula:

$$Me_{10}(XO_4)_6Y_2$$

Where Me is a divalent metal (Ca^{2+}, Sr^{2+}, Ba^{2+}, Pb^{2+} …), XO_4 is a trivalent anion (PO_4^{3-}, AsO_4^{3-}, VO_4^{3-} …), and Y is a monovalent anion (F^- Cl^-, Br^-, I^-, OH^-…). Apatitic crystal structure has usually a hexagonal lattice, having a strong ability to form solid solutions, and to accept numerous substitutions.

The structure of biological apatites, namely bone mineral, dentine, or tooth enamel, is far different from stoichiometric HA because of numerous substitutions (i) by hydrogenophosphate (HPO_4^{2-}) of XO_4 groups and (ii) by carbonate (CO_3^{2-}) of Y_2 and XO_4 groups. In addition, Rey (1990) has shown that bone mineral apatites contains non-apatitic carbonate and phosphate groups, which are, structurally and physically, unstable and very reactive (Rey 1990). This high reactivity provides certain physicochemical, biological, functional, and chemical features important in the formation and dissolution of the crystals in biological tissues. The substitutions affect the apatitic lattice parameters: the crystal size is decreased, and thereby the surface area is increased compared with stoichiometric HA (Legeros 1991). Biological apatites contain various trace elements from intrinsic origins, for example, fluoride is present in dental apatite and confers to enamel its low dissolution properties to resist acidic attacks; or from extrinsic origins, for example, water pollution is a straightforward intake of trace elements for bone due to its high hosting capacity (Cazalbou et al 2004). In addition these trace elements present in extracellular fluids and in bone apatite may have a specific role on bone quality and health. Table 2 presents the trace elements known to have an effect on bone.

Table 2 Selection of some trace elements having an effect on bone as such or in calcium phosphate biomaterials

Trace element	Mechanism of action	Evidences in combination with calcium phosphates bioceramics
Copper	Cross-linking of collagen and elastin; Increasing bone strength (Hunt 1998; Lowe et al 2002)	No
Zinc	Stimulating osteoblastic activity in vitro; Inhibiting bone resorption in vivo (Lowe et al 2002)	Reducing bone loss at the zinc-containing biomaterial (Kawamura et al 2003)
Manganese	Stimulating alkaline phosphatase activity in vitro (Leone et al 1995), and in vivo (Pabbruwe et al 2004)	No
Fluoride	Stimulating alkaline phosphatase activity Increasing of bone mass (Modrowski et al 1992)	Stabilizing bone bonding Reducing biomaterial's resorption (Dhert et al 1991, 1993)
Strontium	Increasing bone mass: stimulating bone formation and reducing bone resorption (Marie 2005)	No
Lithium	Stimulating human mesenchymal stem cells proliferation (de Boer et al 2004)	No
Borate	Possibly interacting with mineral and vitamin D metabolism (Hunt 1998)	No
Silicate	Stimulating extracellular matrix formation and mineralization (Carlisle 1988)	Stimulating bone remodeling (Porter et al 2004b)

HA-based biomaterials have been until now the most abundant materials used in modern bone substitution (Epinette and Manley 2004). Clinically, apatitic biomaterials are widely used in the form sintered macroporous granules, cement in non-load-bearing applications, and in the form or coatings on metallic prostheses. More recently, carbonated apatite biomaterials have been developed as their composition and structure characterics are closer to those of bone mineral. But their superiority compared with pure apatite is not yet proven.

Tricalcium phosphate

Two major distinct phases of anhydrous tricalcium phosphate (TCP) crystals exist: α-TCP and β-TCP phases. The α-TCP crystallizes in the monoclinic space group, and β-TCP crystallizes in the rhombohedral space group. Despite their similar chemical composition, their different crystallographic features confer different resorption features: α-TCP is more soluble than β-TCP, and it is obtained after heating the β-TCP to more than 1170ºC. Clinically, α-TCP is a major reagent in the composition of cements as they hydrolyze into apatitic structures, but it is also sold under the form of powder, blocks, or granules, like β-TCP. In pre-clinical studies, TCP coatings on hip prostheses have been compared with HA coatings for bone formation and bone fixation (Dhert et al 1991, 1993).

Dicalcium phosphate dihydrate

DCPD crystals ($CaHPO_4.2H_2O$) are monoclinic. There are four formulas per unit cell with an asymmetric unit $CaHPO_4.2H_2O$. DCPD crystals are one of the most soluble of the calcium phosphate salts, and are the most stable at pH=5.0. They can be the end product of brushite calcium phosphate cement. However, in clinical applications, DCPD crystals are used as an initial components for bone cements.

Octacalcium phosphate

Octacalcium phosphate (OCP, $Ca_8H_2(PO_4)_6.5H_2O$) crystals are triclinic and they consist of alternating "apatite layers" (arrangement of calcium and phosphate groups similar to that of apatite) and "hydrated layers". These two layers are linked to each other by Van der Walls and hydrogen bonds. OCP often occurs as a transient intermediate in the precipitation of the thermodynamically more stable HA and biological apatites. The close relationship between OCP and HA has been used to explain the incorporation (via hydrolysis) of impurities, particularly carbonate, magnesium, and sodium ions, and hence the non-stoichiometry of precipitated apatites (Legeros 1991). OCP is biocompatible, resorbable, and osteoconductive in the form of compacted powder or in the form of biomimetic coating (Barrere et al 1999, 2003a; Sasano et al 1999). In goat muscles, biomimetic OCP-coated

porous materials were found to be osteoinductive (Barrere et al 2003a). However these materials have not been used clinically.

Amorphous calcium phosphates

Amorphous calcium phosphates vary widely in composition because of the possible insertion of several secondary ions. They are characterized by the broad X-ray diffraction bumps, and by infrared mono-component PO_4 bands. The basic structure unit of amorphous calcium phosphates is a cluster of ions comprising $Ca_9(PO_4)_6$ packed with interfacial water to form bigger entities. Amorphous calcium phosphates are the most soluble calcium phosphate salts; they are used as initial components of some cement.

Biphasic calcium phosphate

Biphasic calcium phosphate (BCP) is a mixture of two different calcium phosphate phases: the sparingly soluble HA and highly soluble tricalcium phosphate at different ratios. BCPs are obtained by sintering of deficient apatites ($Ca_{10-x}M_x(PO_4)_{6-y}(HPO_4)_y(OH)_2$, Ca/P<1.67) at or above 700°C according to the following reaction (Legeros et al 2003):

$$Ca_{10-x}M_x(PO_4)_{6-y}(HPO_4)_y(OH)_2 \rightarrow Ca_{10}(PO_4)_6(OH)_2 + Ca_3(PO_4)_2$$

The combination these two different calcium phosphate phases with different solubility in one biomaterial allows its degradation kinetics to be tuned in vitro and in vivo. Clinically, BCPs are used as bone fillers.

Stability of calcium phosphates

Thermal stability

We present here a summary on the thermal transformations behavior, extensively developed by Elliot (Elliot 1994).

TCP and **HA** are the only calcium phosphate salts fully stable up to 1000°C. For TCP, up to 1125ºC, β-TCP is the most stable phase, whereas above this and up to 1430ºC α-TCP phase is the most stable. For stoichiometric HA, above 1000ºC, some hydroxyl groups may condense to produce water following the reaction:

$$Ca_{10}(PO_4)_6(OH)_2 \leftrightarrow Ca_{10}(PO_4)_6O\square + H_2O$$

$\square$ = vacancy

DCPD, **OCP**, **carbonates,** and/or **deficient apatite** are subjected to thermal transformations:

The water-containing phases such as DCPD or OCP dehydrate at approximately 180°C. The HPO_4 containing calcium phosphate phases such as deficient apatite, OCP or DCPD sustain a condensation resulting in the formation of pyrophosphates ions ($P_2O_7^{4-}$) at approximately 300°C according to the reaction:

$$2HPO_4^{2-} \rightarrow P_2O_7^{4-} + H_2O$$

Depending on the substitution mode, the thermal treatment of carbonated apatite may lead to the release of CO_2 gas with subsequent rearrangements.

As a result of a thermal treatment, carbonated and/or deficient apatite, OCP, and DCPD will transform in a mixture of sub-products: pyrophosphate, HA, and TCP.

Stability in physiological systems

The formation, dissolution, and transformation of calcium phosphates depend on the nature of the calcium phosphate body (particle size, crystallographic features, density) and the nature of the solution (composition, pH, temperature).

Most of calcium phosphates are sparingly soluble in water, and some are very insoluble, but all dissolve in acids. Their solubility, defined as the amount of dissolved solute contained in a saturated solution when particles of solute are continually passing into solution (dissolving) while other particles are returning to the solid solute phase (growth) at exactly the same rate (Wu and Nancollas 1998), decreases with the increase in temperature and in pH (de Groot 1983).

Each calcium phosphate phase possesses its own thermodynamical solubility. For example, at pH=7 and 37ºC, HA is the most stable phase, followed by TCP, OCP, and finally DCPD. However, these thermodynamical considerations are under equilibrium conditions, and therefore they do not take into account kinetics that dictates the formation of one or the other phase under dynamic conditions.

The second important factor in the stability of the calcium phosphates is the characteristics of the solution in which these salts are formed or placed, namely the solution supersaturation in free calcium and phosphates ions (Tang et al 2001). At a given pH and temperature, a free calcium and phosphate ion containing solution can be categorized in three different states: (i) the stable (undersaturated) zone, where crystallization is impossible, (ii) the metastable zone (supersaturated), where spontaneous crystallization of calcium phosphate salt is improbable, although the concentrations are higher than the ones corresponding to the salt solubility. If a crystal seed were placed in such a metastable solution, growth would occur on the seed; (iii) the unstable or labile (supersaturated) zone, where spontaneous crystallization of calcium phosphate is probable, but not inevitable (Mullin 1993).

Extracellular fluids that are supersaturated for calcium and phosphate may induce the nucleation and growth of new calcium phosphate crystals.

Biological milieus

In vivo, the interactions between the implant and its "biological surrounding" are highly complex due to the non-equilibrium conditions and due to the undefined amount of compounds playing a role in these interactions. Dhert et al (1998) addressed the kinetic of the early events taking place within the first month at the interface between calcium phosphate-coated implants in a press fit model, ie, implant and defect dimensions are equal, implicating a maximum contact between the hosting bone tissue and the biomaterial. Regardless of the nature of the biomaterial, its biological surrounding evolves with time. In the first 3 days, blood will have invaded all of the empty spaces between the original bone and the implant. Thereafter, at the end of the first week of implantation, callus and mesenchymal tissues will have entirely replaced blood while host bone resorption has started. Finally, between the second and fourth week of implantation, callus, mesenchymal tissues, and host bone will have gradually disappeared in favor of newly formed bone while bone remodeling takes place (Dhert et al 1998). In a nutshell, components of biological fluids and cells will interact with the biomaterial. The biomaterials features can affect the molecular and cellular interactions at their surface and consequently can affect the process of bone formation.

The molecular components

The body can be divided into two major aqueous compartments: 2/3 of intracellular fluid within cells, and 1/3 of extracellular fluid. The extracellular fluid is divided into smaller compartments that are distinguished by different locations and different kinetic characteristics: (i) the interstitial fluids lie in the interstices of all body tissues that bathes all the cells in the body and is the link between the intracellular fluid and the intravascular compartment. Oxygen, nutrients, wastes, and chemical messengers all pass through the interstitial fluid; (ii) the plasma is the only major fluid compartment that exists as a real fluid collection all in one location. It differs from interstitial fluid in its much higher protein content and its high bulk flow (transport function). Plasma is the liquid in which red blood cells and white blood cells are suspended. The water of the plasma is freely exchangeable with that of body cells and other extracellular fluids and is available to maintain the normal state of hydration of all tissues; (iii) the transcellular fluid is a small compartment that represents all those body fluids which are formed from the transport activities of cells; (iv) bone and dense connective tissues represent 15% of body water (intracellular:extracellular, 55:45). This water is mobilized much more slowly than the other components of the extracellular fluids. Extracellular fluids are composed of water-soluble molecules: organic (amino acids, sugars, fatty acids, coenzymes, vitamins, hormones, neurotransmitters) and inorganic (calcium, phosphates, potassium, carbonate, sulfate, magnesium, chloride, sodium, copper, zinc) compounds as well as the waste products from the cellular metabolism.

The cellular components

As bone formation takes place in an organism during embryonic development, growth remodeling, and fracture healing, and after ectopic implantation of osteoinductive matrixes, a large reservoir of cells in the body is capable of osteogenesis throughout life (Aubin 2001). Although the biomaterials are subjected to numerous cellular interactions of diverse origin in vivo, we will consider only the cells that are related most directly to bone formation and regeneration.

The osteoblasts

Osteoblasts are derived from pluripotential mesenchymal stem cells that have the capacity to differentiate into various lineages. Mature osteoblasts are non-migratory, highly differentiated cells that can differ substantially in their properties depending on their stage of development. Their function and phenotype vary and four categories of cells can be described. First, the active osteoblasts are cuboidal in shape, mononuclear and they are rich in alkaline phosphatase activity. They synthesize and secrete collagen type I and glycoproteins (osteopontin, osteocalcin), cytokines, and growth factors into a region of unmineralized matrix (osteoid) between the cell body and the mineralized matrix (Kartsogiannis and Ng 2004). Osteoblasts produce also calcium phosphate minerals extra- and intracellularly within vesicles (Annaz et al 2004b). Second, osteocytes are mature osteoblasts which have become trapped within the bone matrix and are responsible for its maintenance. Third, bone-lining cells are found along the bone surfaces that are undergoing neither bone formation nor resorption. Finally, inactive osteoblasts are elongated cells, undistinguishable morphologically from the bone-lining cells.

The osteoclasts

Osteoclasts are derived from hematopoietic stem cells that differentiate along the monocyte–macrophage lineage. They are responsible for bone resorption by acidification of bone mineral leading to its dissolution and by enzymatic degradation of demineralized extracellular bone matrix. The mature osteoclast is a functionally polarized cell that attaches via its apical pole to the mineralized bone matrix by forming a tight ring-like zone of adhesion, the sealing zone. This attachment involves the specific interaction between the cell membrane and some bone matrix proteins via integrins (transmembrane adhesion proteins mediating cell–substratum and cell–cell interactions). In the resorbing compartment, situated under the cell and delimited by the sealing zone, osteoclasts generate a milieu acidification resulting in the dissolution of bone mineral. This osteoclastic acidification is mediated by the action of carbonate anhydrase that produces carbon dioxide, water, and protons that are extruded across the cell membrane into the resorbing compartment (Kartsogiannis and Ng 2004).

The mesenchymal stem cells and osteoprogenitor cells

Adult mesenchymal stem cells can be isolated from bone marrow, adipose tissues, or amniotic membrane. Mesenchymal stem cells are, by definition, of self-renewal capacity and able to repopulate all the appropriate differentiation lineages. They are multipotent cells that can differentiate into osteoblastic, myoblastic, adipocytic, chondrocytic, endothelial, and neurogenic lineage through a multi-step differentiation sequence as follows: proliferation, commitment, lineage progression, maturation, and differentiation. For the osteogenic lineage, mesenchymal stem cells sustain a cascade of differentiation steps as described by the following sequence: Mesenchymal stem cell → immature osteoprogenitor → mature osteoprogenitor → preosteoblast → mature osteoblast → osteocyte or lining cells or apoptosis. The later the differentiation stage, the lower the cell self-renewal and proliferation capacity (Aubin 2001). In bone marrow, osteoprogenitor cells represent a very small percentage (eg <0.005%) of nucleated cell types in healthy adult bone (Block 2005). Embryonic stem cells are also a potential source due to their pluripotentiallity and therefore their ability to differentiate into osteogenic lineage.

Osteoprogenitor–stem cell differentiation is controlled by the "osteogenic mastergene" Cbfa1/Osf2 that intervenes in skeleton and tooth mineralization (Sodek and Cheifetz 2001). The differentiating osteoprogenitor cells express several bone matrix macromolecules, namely alkaline phosphatase, collagene type I, Cbfa1, bone sialoprotein, osteocalcin, and osteopontin (Sodek and Cheifetz 2001).

Biological events at the calcium phosphate substrates

Ionic exchanges at calcium phosphate surfaces

Physico-chemically, calcium phosphate surfaces sustain dissolution–reprecipitation cascades as the result of exchanges at a solid–liquid interface in supersaturated conditions. In biological systems, this physico-chemical phenomenon is the result of a multi-component dynamic process involving ions and proteins.

In terms of surface reactivity, ionic transfers occur from the solid phase to the aqueous liquid via surface hydration of calcium, inorganic phosphate species, and possible impurities like carbonate, fluoride, or chloride present in the biomaterial. Under physiological conditions, this dissolution process is highly dependent on the nature of the calcium phosphate substrate (Okazaki et al 1982; Dhert et al 1993; Ducheyne et al 1993; Radin and Ducheyne 1993; Christoffersen et al 1997; Doi et al 1999; Barrere et al 2003b), and on the composition and supersaturation of the environment in vitro (Hyakuna et al 1990; Raynaud et al 1998; Tang et al 2001), or the implantation site in vivo (Daculsi et al 1990; Barralet et al 2000). Ionic transfers occur also from the surrounding fluids to the calcium phosphate substrate in vitro and in vivo, as illustrated by the formation of carbonated apatite nanocrystals as a result of surface transformation (Heughebaert et al 1988; Daculsi et al 1989, 1990; Johnsson et al 1991; de Bruijn et al 1992a; Radin and Ducheyne 1993; Barrere et al 2003b). The presence of magnesium and carbonate contributes to the formation of a poorly crystallized carbonated apatite that has similar features to the bone mineral phase (Furedi-Milhofer et al 1976; Legeros 1991; Elliot 1994). In the presence of proteins, this newly formed mineral phase is also associated with organic compounds (Heughebaert et al 1988; de Bruijn et al 1995; Barrere et al 2003b). This phase transformation occurs for all of the calcium phosphate bioceramics, even the stable apatitic structures, since they have a strong ability to adapt to their environment by hosting foreign ions and subsequently to sustain atomic rearrangements (Cazalbou et al 2004). However, crystalline hydroxyapatitic substrates are often too stable to transform.

The result of these ionic exchanges favoring either phase transformation or dissolution follows the thermodynamical

stability order, ie HA>TCP>OCP>DCPD from the least soluble to the most soluble. This surface reactivity has pivotal implications in the biological relevance of calcium phosphate bioceramics and will be discussed further later in this review. However, for a given calcium phosphate phase, the crystalline feature, eg, the presence of defaults in the crystal lattice or the decrease of crystal size, accelerates the dissolution. Eventually amorphous biomaterials dissolve faster than crystalline ones (Legeros 1991; de Bruijn et al 1992b; Barrere et al 2000). In addition, the presence of some additives of mineral origins within the calcium phosphate structure can affect the crystal lattice, and therefore can accelerate the dissolution, eg carbonate, silicate, or strontium in HA. On the other hand, some additives reduce in vitro and in vivo the dissolution process, eg fluoride in HA, magnesium or zinc in beta-TCP (Okazaki et al 1982; Legeros 1991; Dhert et al 1993; Elliot 1994; Christoffersen et al 1997; Ito et al 2002; Porter et al 2004a). The incorporation of proteins into calcium phosphate can also affect the dissolution kinetics (Liu et al 2003). Last but not least, micro- and macroporosity play an important role in the physicochemical dissolution process of calcium phosphates. The larger the exposed surface to the environment, the faster the biomaterial dissolves, simply because larger quantities of exchanges can take place (Radin and Ducheyne 1994).

The proteins from the surrounding fluids are also involved in the ionic exchange mechanisms as observed in vitro. Their interaction with calcium phosphate substrates depend on the bioceramics characteristics (such as phase, crystallinity, composition, texture) (Sharpe et al 1997; El-Ghannam et al 1999; Rouahi et al 2006) and on the protein's features (such as conformation, isoelectric point, composition), their concentration, and whether they act in solution or on substrates (Johnsson et al 1991; Hunter et al 1996; Koutsopoulos and Dalas 2000; Combes and Rey 2002; Ofir et al 2004). Firstly, in suspension, proteins can inhibit or support calcium phosphate nucleation and growth (Johnsson et al 1991; Hunter et al 1996; Boskey and Paschalis 2001). For phosphorylated proteins, such as collagen, ostepontin, osteonectin, bone sialoprotein, or ostecalcin, phosphorylated entities have demonstrated their ability to nucleate and grow calcium phosphate crystals (Boskey and Paschalis 2001). However, not all of the phosphorylated proteins induce calcium phosphate formation; osteopontin especially is a strong crystallization inhibitor (Hunter et al 1996) . Secondly, when proteins adsorb onto calcium phosphate substrates, their charge, their concentration, and the presence of calcium in the surrounding fluids influence the surface-coverage kinetics and pattern that can evolve with time (Kawasaki et al 2003). These adsorbed proteins can thereafter influence the new formation of calcium phosphate crystals by blocking or not blocking the substrate's nucleation sites (Koutsopoulos and Dalas 2000; Combes and Rey 2002; Ofir et al 2004), irrespective of the protein's isoelectric point (Ofir et al 2004). These results obtained from in vitro experiments differ strongly from in vivo experiments, as hundreds of proteins are present in biological fluids, and their global effect on calcium phosphate reactivity is insufficiently understood. It is, however, clear that they play a significant role in the ionic exchanges and their subsequent effect on their biological activity, since proteins are detected in close association with the nanocrystalline carbonated apatite formed on the surface of calcium phosphate bioceramics in vitro and in vivo (Heughebaert et al 1988; Johnsson et al 1991; de Bruijn et al 1992b; de Bruijn et al 1994b; Radin et al 1998; Barrere et al 2003b). Consequently, the nature, quantity, and conformation of these proteins at the biomaterial surface will determine the cellular activity (El-Ghannam et al 1999; Rouahi et al 2006).

Cellular interactions with calcium phosphate surfaces

In general, cell–biomaterial interactions depend on surface characteristics such as topography, chemistry, and surface physics. As mentioned above, surface characteristics determine the ionic exchange dynamics and the protein adsorption. They also affect the cellular activity, namely their attachment, proliferation, and differentiation.

Calcium phosphates – osteoblasts and osteoprogenitor cells

In vitro, cell–biomaterial interactions are assayed by primary osteoblasts, osteosarcoma cell lines, and mesenchymal–osteoprogenitor cells issued from bone marrow. More and more research currently addresses the interaction between mesenchymal stem–progenitor cells and scaffolds, as these cells participate at the early stage of new bone tissue formation in vivo (Dhert et al 1998; Davies and Hosseini 2001; Devlin and Sloan 2002). In contrast with differentiated osteoblasts, osteoprogenitor cells are migratory, highly proliferative cells and they have a greater differentiation potential. They can migrate on a substrate by generating cycles of weak adhesion, traction, movement, and detachment. At the end of the migration phase, like osteoblasts, mesenchymal–osteoprogenitor cells adhere to the substrate by developing strong focal adhesion with substrates in order to start

their differentiation phase. The migration and adhesion of bone cells in general are mediated via integrins which are transmembrane proteins (Anselme 2000). Among the integrin superfamily, fibronectin and vitronectin are proteins involved in osteoblasts adhesion on biomaterials in vitro (El-Ghannam et al 1999; Anselme 2000). Regarding their interaction with calcium phosphates, osteoblasts are intimately in contact with calcium phosphate surfaces thanks to the production of extracellular collagen firmly attached perpendicular or parallel to the substrate (Annaz et al 2004b).

The osteogenic differentiation phase comprises three principal biological periods: cellular proliferation, cellular maturation, and matrix mineralization. Through these periods, the osteoblasts are known to synthesize and secrete subsequently type I collagen, alkaline phosphatase, and other non-collagenous extracellular bone matrix proteins such as osteocalcin, osteopontin, osteonectin, and bone sialoprotein. Type I collagen is expressed during the initial period of proliferation and extracellular matrix biosynthesis, whereas alkaline phosphatase is expressed during the post-proliferative period of extracellular matrix maturation, and the expression of osteopontin, osteocalcin, and bone sialoprotein occurs later during the third period of extracellular-matrix mineralization (Sodek and Cheifetz 2001). Consequently, because there is no specific single marker for osteoblasts, the cellular expression of a range of non-collagenous and collagenous proteins as well as alkaline phosphatase and Cbfa1 has to be investigated, when examining osteoblastic differentiation. Finally, osteoblasts participate actively to the biomaterial mineralization (Radin et al 1998) by producing calcium phosphate containing vesicles (Annaz et al 2004b). In practice, the mineralization of osteoblasts cannot be assayed on calcium phosphates biomaterials because of the similarities in composition between the substrate and the extracellular mineral.

Several calcium phosphate parameters can affect the cellular activity:

(i) Dissolution. Depending on the calcium phosphate reactivity (ie, dissolution–precipitation behavior) osteoblast function can be affected, in their proliferation, their differentiation, and their maturation phenotypes (de Bruijn et al 1992a, 1994b; Midy et al 2001; Knabe et al 2004; Siebers et al 2004; Wang et al 2004; Arinzeh et al 2005; Berube et al 2005). De Bruijn et al demonstrated a clear link between dissolution rate and early bone formation in vivo and the osteogenic differentiation in vitro of osteoprogenitor cells, suggesting therefore the influence of free calcium and inorganic phosphates on bone formation (de Bruijn et al 1992a, 1994b). As explained earlier the calcium and phosphate concentrations of the environment may increase or decrease when calcium phosphate ceramics are immersed. This level of modification induced by immersing calcium phosphate ceramics can even induce cell death (Hyakuna et al 1989). Changes of calcium or phosphate contents in culture medium also affect directly the osteoblastic activities (Bellows et al 1992; Meleti et al 2000; Adams et al 2001; Dvorak et al 2004). As a consequence of surface transformation the cellular activity might be further affected as the substrate has significantly changed (Anselme et al 1997);

(ii) Composition. A change in calcium to phosphorus ratio in the bioceramics means a change in phase composition and consequently a direct effect on the ionic exchanges mechanisms, as discussed earlier. The incorporation of mineral ions such as zinc (to a certain extent) or silicate in calcium phosphate bioceramics showed an increase of osteoblasts attachment and proliferation (Ishikaw et al 2002; Thian et al 2005). While the presence of carbonate in the apatitic network of the biomaterial had contradictory effects on osteoblastic proliferation and alkaline phosphatase production compared with the HA substrate (Anselme et al 1997; Redey et al 2000; Midy et al 2001);

(iii) Topography. On one hand, grooved calcium phosphate surfaces influence the osteoblastic guidance, and the groove profile independently of nature of the substrate (Lu and Leng 2003). On the other hand, in contact with micro- and macroporous calcium phosphate ceramic osteoblasts sense the surface microporosity and they can bridge even large pores many times larger than fully flattened osteoblasts (Annaz et al 2004a). For osteoblasts differentiation, Chou et al have demonstrated in vitro that MC3T3 cells were sensitive to the crystal shape: large apatite crystals induced more bone sialoprotein and osteocalcin expression after 3 weeks of culture than small apatite crystals (Chou et al 2005b).

(iv) Surface energy. The study by Redey et al (2000) showed that the surface energy strongly affected the initial osteoblastic activity for proliferation and function, while at a later time the less favorable surface exhibited a comparable osteoblastic activity to the most favorable surface.

Calcium-phosphates – osteoclasts

Heymann et al (1999) have extensively addressed the cellular degradation of calcium phosphate ceramics in vitro and in vivo. After colonization of the substrate by monocytes–macrophages that are recruited during the inflammatory reaction following surgery (Basle et al 1993;

Heymann et al 1999), osteoclasts are responsible for bone mineral degradation, ie, bone resorption. It has been observed that osteoclasts degrade calcium phosphate ceramics in a similar way to bone mineral: osteoclasts attach firmly to the substrate sealing zone. In the center of this sealing zone, they secrete H^+ leading to a local pH=4–5. In vivo, osteoclasts participate partially in the degradation of calcium phosphate ceramics (Gauthier et al 1999; Lu et al 2002; Ooms et al 2002; Wenisch et al 2003; Zerbo et al 2005). In contact with osteoclasts, implanted bioceramics exhibited resorption pits associated with newly formed bone (Gauthier et al 1999; Ooms et al 2002; Wenisch et al 2003). However, the in vivo degradation of calcium phosphate materials is also associated with a dissolution phenomenon (Gauthier et al 1999; Lu et al 2002). The osteoclastic activity versus dissolution process of a calcium phosphate ceramic depends on the nature of the calcium phosphate (such as, cement, bulk ceramic, particles, highly soluble TCP, sparingly soluble HA) (Gauthier et al 1999; Lu et al 2002). In the the degradation of highly soluble TCP ceramics in vivo, Zerbo et al (2005) have shown that physicochemical dissolution took place to a larger extent than osteoclastic resorption. Osteoclasts can also degrade calcium phosphates by phagocytic processes, ie, incorporation in the cytoplasm of the cell and thereafter dissolved by acid attack or enzymatic process when biomaterials particles are generated as the result of either local dissolution at grain boundaries or by mechanical stress generating debris (Heymann et al 1999). The osteoclastic activity is usually determined by a specific osteoclastic enzyme (tartrate-resistant acid phosphatase) depends on intrinsic properties of the calcium phosphate ceramics that can vary from study to stud. However, some general trends can be outlined:

(i) Physicochemical dissolution kinetics of the biomaterial: calcium phosphate ceramics do not all interact in the same way with osteoclasts. The release of calcium ions from the biomaterial seems to play a critical role in the osteoclastic activity; above a critical range of calcium ions levels, osteoclastic resorption is inhibited (de Bruijn et al 1994a; Yamada et al 1997; Doi et al 1999). Together with the dissolution behavior, the structure of the calcium phosphate ceramic and the crystallinity influence the osteoclastic activity (de Bruijn et al 1994a; Yamada et al 1997; Leeuwenburgh et al 2001).

(ii) Carbonates and other mineral ions: carbonated apatitic salts, eg dentin, bone mineral, synthetic carbonated apatite, and calcium carbonate structures (ie aragonite, calcite), are resorbed by osteoclasts. It has been proposed that the carbonate content may stimulate the carbonic anhydrase activity known to promote the osteoclastic acidic secretion in vitro (Doi et al 1999; Leeuwenburgh et al 2001). On the other hand, zinc and fluoride included in calcium phosphate biomaterials have shown also an inhibiting effect on osteoclastic resorption in vitro (Sakae et al 2000; Ito et al 2002) and in vivo (Kawamura et al 2003; Sakae et al 2003) for specific concentration in zinc-containing calcium phosphates;

(iii) Surface energy of the calcium phosphate biomaterial: the polar component of the surface energy was found to modulate the osteoclastic adhesion in vitro. However, the further spreading and resorption was not influenced by surface energy considerations (Redey et al 1999);

(iv) Surface roughness is known in general to influence the cell attachment in vitro and it was shown also in vitro for osteoclast attachment. Rough apatitic surfaces appear to enhance osteoclastic attachment compared with smooth ones (Gomi et al 1993).

Future directions in cell–calcium phosphate interactions

Cell sources At present, the reactivity of calcium phosphate substrates is evaluated from primary osteoblasts, osteosarcoma cell lines, pre-osteoblasts, and osteoprogenitor cells. Although human osteoprogenitor cells seem to be the most adapted for bone tissue engineering, very few reports deal with the culture of human osteoprogenitor cells. These cells are donor- and culture-dependent (Mendes et al 2002) and their pluripotency decreases with passage numbers. Unless many donors' cells are used to screen calcium phosphate biomaterials, human osteoprogenitor cells are not a handy tool for understanding their interactions with calcium phosphate, as another donor's cell may act very differently. To our knowledge, no extensive studies on different human osteoprogenitor cells have been performed in general on biomaterials. On the other hand, cell lines giving more reproducible results are "manipulated" and may not represent the real situation, as different cell lines have demonstrated a variation in responsiveness when cultured on a similar calcium phosphate materials (Rochet et al 2003). Embryonic stem cells are also a potential source in tissue engineering and they are investigated on calcium phosphate biomaterials (Both et al 2005; Melville et al 2006).

The question remains open of which cell type to focus on to understand the cell–calcium phosphate interactions for a tissue engineering approach.

Co-culture systems, ie, culturing two types of cells, are the new center of interest in the domain of bone tissue engineering. For example, bone tissue-engineered constructs

lack viability due to insufficient vascularization. As angiogenesis in the bone marrow is closely associated with osteogenesis in developing and mature bone, co-cultures of osteoprogenitor cells with endothelial cells are initiated in order to create simultaneously vascularization and bone formation. However, to our knowledge there are no reports including the influence of a calcium phosphate biomaterial (or any other biomaterial) with osteoprogenitor–endothelial cell co-cultures. This should be taken into account in future, as it was shown that a calcium phosphate substrate, in contact with a hematopoietic–osteoblasts co-culture system, induced a entirely specific differentiation pathway than tissue culture-treated plastic (Rochet et al 2002). Could the prior osteoclastic resorption of the calcium phosphate biomaterial influence the osteoblastic activity as observed in natural bone remodeling?

Finally, live observation of transgenic luminescent cell cultures offers great opportunities to evaluate long-term activities on the same sample in vivo and in vitro (Cao et al 2005; Kotobuki et al 2005; de Boer et al 2006). More specifically for bone, de Boer et al has recently addressed the application of transgenic luminescent cell cultures coupled with osteogenic reporters (de Boer et al 2006).

Limitations of in vitro tests Calcium phosphates are reactive and their reactivity depend on their characteristics (such as composition, dissolution, sintering temperature, microstructure). As a consequence, the calcium and phosphate levels in the cell culture medium can vary substantially without being regulated and this can affect cellular function (Bellows et al 1992; Meleti et al 2000; Adams et al 2001; Midy et al 2001; Dvorak et al 2004; Wang et al 2004; Arinzeh et al 2005; Berube et al 2005); while in vivo, these variations are automatically and rapidly regulated. Therefore the in vitro predictability of the biomaterial as such is often inconclusive (Habibovic et al 2005a). In the context of bone tissue engineering, scaffolds and cells are in contact in vitro for several days before being implanted, hence testing the in vitro cellular activity remains pertinent. However, Kruyt et al (2004) did not find any differences in vivo between the bone tissue engineering constructs cultured in stimulating or non-stimulating osteogenic differentiation medium for 6 days, while they measured a significant difference in alkaline phosphatase activity in vitro between these two conditions. On the other hand, the number of biological assays increases with time, but they are still in the developmental stage. It is sometimes difficult to understand the regulations of some proteins as reported by Chou et al (Chou et al 2005a, 2005b). The quantification of alkaline phosphatase activity is the most common and straightforward biological assay performed on osteogenic differentiation, but the presence of inorganic phosphate may inhibit the production by the cells of alkaline phosphatase activity without inhibiting the further mineralization process by osteoblasts (Bellows et al 1992). So measuring alkaline phosphatase activity as a unique differentiation assay on reactive calcium phosphate bioceramics may not represent a valid quantification of osteogenic differentiation.

Relevance of calcium phosphates for bone tissue regeneration

Bone-bonding ability

Ionic exchange phenomena occurring with calcium phosphate bioceramics are associated with reactivity towards bone bonding, ie, the formation of an interfacial mineralized layer between bioceramics and bone tissue that insures their cohesion. Structurally, this layer is comparable to the films grown in vitro by dissolution–precipitation mechanisms, ie, nanocrystals of carbonated apatite in simulated body fluids that mimic the mineral composition of blood plasma. When formed in the presence of osteogenic cells experiments, this mineralized layer is comparable with the cement lines synthesized in vivo (de Bruijn et al 1995; Davies 1996). In vivo (osseous and non-osseous environment), physico-chemical, and crystallographic continuity are observed between the calcium phosphate implant and the newly formed mineralized layer (Daculsi et al 1989; de Bruijn et al 1992b; Neo et al 1993). Its occurrence and thickness are related to the reactivity (dissolution–precipitation) of the calcium phosphate substrate (Neo et al 1993), the so-called bioactivity (Hench and Wilson 1984). This mineralized interface insures a physicochemical and mechanical cohesion between the implant and the host bone. It is particularly relevant for load-bearing applications, ie, hip metallic prostheses coated with calcium phosphate which undoubtedly improves the mechanical stability of the implant by augmenting and accelerating the bone apposition (Geesink et al 1987; Dhert et al 1993; Rahbek et al 2004).

Osteoinduction by calcium phosphates

Osteoinductive materials are biomaterials that have intrinsic properties to induce bone formation in a non-osseous environment. Recently, it has been demonstrated in goats, that osteoinductive macroporous calcium phosphates stimulate more bone formation as tissue-engineered constructs

ectopically (Kruyt et al 2004) and as such in critical-sized orthopedic defect models (Habibovic et al 2005b). Although the mechanism of osteoinduction remains unclear, the ionic exchanges properties of the calcium phosphate scaffolds with the surrounding milieu have been pointed out as a relevant parameter, among others (Yuan et al 2001). By implanting intramuscularly in goats two macroporous calcium phosphate scaffolds identical in composition, crystallinity, and porosity but with different microporosities, Habibovic et al (2005a) have demonstrated that an elevated microporosity was responsible for ectopic bone formation. This high microporosity is directly correlated with the exposed surface, and therefore an elevated dissolution in the pores where the level of stable critical level of free calcium ions and possibly free orthophosphate ions might trigger cell differentiation into osteogenic lineage. In addition, through a dissolution–precipitation process, the development of a bone-like mineral layer might initiate bone formation either by mimicry with the bone mineral structure or by the presence of osteogenic compounds (for example bone morphogenetic proteins, BMPs) contained naturally in body fluids that might have concentrated at the newly formed mineral layer (Ripamonti 1996).

Tailoring the resorption kinetics of calcium phosphates

In large defects that cannot be healed naturally by bone, adjusting the degradation kinetics of the calcium phosphate bone filler with the kinetics of bone formation rate remains a great challenge in bone tissue regeneration. While contributing to bone formation, the scaffold should degrade in a controlled fashion to leave gradually more space for newly formed bone until full tissue regeneration. Mixing at various ratios a low soluble phase (HA) with a highly soluble phase (amorphous TCP) resulting in biphasic calcium phosphates ceramics (BCP), including additives (magnesium, carbonate, fluoride) in a given crystalline phase, or selecting different calcium phosphate phases (amorphous, DCPD, OCP, HA, TCP), are the options to tailor the degradation kinetics of calcium ceramics from a few weeks to a few years. In theory and in practice, one can change the resorption kinetics of calcium phosphate ceramics; however, no universal degradable scaffolds have been developed yet, and their degradation properties should be designed depending on their application. Below, two types of degradation patterns are described, one for load-bearing and non-load bearing applications.

Load-bearing applications

Calcium phosphate applied as coatings on metallic prostheses have a highly successful clinical record for hip arthroplasty (Epinette and Manley 2004). These coatings significantly accelerate bone growth onto the metallic implant, improve fixation of the implants, and extend the prostheses' longevity. Extensive animal studies have been conducted taking into account bone formation rate versus resorption rate and mechanical stability. By changing the coating's parameters (technique, temperature, composition) one can change the coatings' degradation characteristics. For hip prostheses, the calcium phosphate coatings' resorption kinetic has a certain paradigm. On the one hand, soluble coatings enhance bone formation at the early implantation stage, inducing a fast early fixation, but soluble coatings may lead to a second stage mechanical instability between the metallic implant and the surrounding bone. On the other hand, insoluble coatings delay bone fixation, ie, the first stage mechanical stability, but insoluble coatings stabilize the long-term fixation of the implant with the surrounding tissues (Dhert et al 1993, 1998; de Bruijn et al 1994b; Rahbek et al 2004).

Non-load bearing applications

In non-loading situations, bare calcium phosphates are used as granules or cement. Their composition can have virtually limitless variations with regard to their structure, the incorporation of additives, and the mixture of phases. The current scaffolds either degrade too fast (and therefore do not allow sufficient stability for new bone formation), or scaffolds do not resorb fully (and remain encapsulated by the newly formed bone, avoiding the entire restoration of natural bone mechanical strength). A common way to tune the degradation properties of calcium phosphate scaffolds is to combine a highly soluble phase (TCP) with a non-soluble phase (HA), to create so called BCP ceramics (Legeros et al 2003). Depending on the HA/TCP ratio, bone formation versus biomaterial resorption can be significantly improved or decreased (Schopper et al 2005).

Functionalization of calcium phosphates for triggering bone regeneration

In view of the dissolution properties of calcium phosphate, several groups have used calcium phosphate ceramics as delivery systems by (i) adsorption on powder followed by compaction, (ii) co-precipitation, or (iii) adding the cement paste applied in bone regeneration and related fields of gene therapy (Shen et al 2004), or local drug delivery (Urist et al 1987; Lebugle et al 2002; Stigter et al 2004; Kroese-

Deutman et al 2005; Liu et al 2005). For stimulation of bone regeneration, specific proteins have been administrated via calcium phosphate carriers. Bone morphogenic proteins (BMP, especially BMP-2) adsorbed onto ceramics (Urist et al 1987; Kroese-Deutman et al 2005) or co-precipitated into biomimetic carbonated apatite coatings (Liu et al 2005) induce more bone formation than ceramics alone in vivo. Recently, incorporation of silicate and zinc ions, in TCP and HA ceramics respectively, were reported to have a significant influence on osteogenesis in vitro and in vivo (Kawamura et al 2000; Ikeuchi et al 2003; Porter et al 2004b).

Conclusion and future perspectives

Calcium phosphates have intrinsic properties that stimulate bone regeneration. However, at present, their stimulating mechanisms remain insufficiently understood. The potential of the nanotechnologies applied in tissue regeneration has been demonstrated in different ways, eg, (i) the incorporation of luminescent genes in cells and animals (Cao et al 2005; de Boer et al 2006), allowing live molecular imaging and therefore the follow up on several time points on one animal and one type of biomaterials; (ii) the micro-arraying of biomaterials at the nano-liter scale (Anderson et al 2004) rendering possible the screening of thousands of relevant biomaterials in a significantly reduced amount of time, consumables and facilities; (iii) the surface nanotopography-regulating cell differentiation (McBeath et al 2004). Further developments of the nanotechnologies applied in the fields of biomaterials, and cellular and molecular biology will facilitate closer insights and more profound understandings at the cell–biomaterial interaction levels. Thanks to the contributions from nanotechnology, we can therefore expect breakthroughs in the development of novel biomaterials and scaffolds for treating bone defects more efficiently.

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