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Invited Review

Cellular mechanisms of calcium phosphate ceramic degradation

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Summary. Calcium phosphate (CaP) ceramics are widely used for bone substitution in orthopedic, maxillo-facial and dental surgery. Many environmental factors are involved in the gradual degradation of calcium phosphate ceramic after implantation, including physicochemical processes (dissolution-precipitation) and the effects of various cell types. Several of these cell types degrade ceramics by phagocytotic mechanisms (fibroblasts, osteoblasts, monocytes/macrophages) or by an acidic mechanism with a proton pump to reduce the pH of the microenvironment and resorb these synthetic substrates (osteoclasts). Various mesenchymal cells located at the implantation sites can induce the solubilization of CaP ceramics. Crystal-cell contacts were required to induce such crystal dissolution. Mesenchymal cells such as fibroblastic cells are also actively involved in the ceramic degradation process. In this context, CaP crystals underwent dissolution into the phagosome. If osteoclasts resorb CaP ceramics similarly to the natural bone, they possess a phagocytic capability. This phagocytosis mechanism consisted of three steps: crystal phagocytosis, disappearance of the endo-phagosome envelope membrane and fragmentation of phagocytosed crystals within the cytoplasm. Similar phenomenons have been observed during the phagocytic mechanism induced by monocytes/macrophages. The cellular mechanisms of CaP ceramic degradation are modulated by various parameters, such as the properties of the ceramic itself, the implantation sites and the presence of various proteins (cytokines, hormones, vitamins, ions, etc.). The cells involved in these mechanisms could intervene directly or indirectly through their cytokine/growth factor secretions and their sensitivity to the same molecules. This article reviews recent knowledge on the cellular mechanisms of calcium phosphate ceramic degradation.

Key words: Calcium phosphate ceramic, Resorption, Phagocytosis, Osteoclast, Monocyte/Macrophage

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Biological properties of calcium phosphate ceramics

Bone grafts are frequently used in orthopedic and maxillo-facial surgery. Although autogenous bone grafts are well-adapted bone substitutes in terms of tolerance, mechanical properties and the quality of new bone-forming, difficulties in bone harvesting, the potential risk of associated morbidity, the small volume available and risks of contamination (bacteria, virus, prions) limit their clinical use (Berrey et al., 1990; Friedlaender, 1987; Lord et al., 1988). These difficulties have led to the development of new synthetic bone substitution materials (Jarcho, 1981). Thus, bone substitutes such as porous calcium phosphate (CaP) and their composites (synthetic polymer, collagen, fibrin glue) appear to be suitable alternatives to autogenous and allogenous bone grafts (Passuti et al., 1997).

Among synthetic bone substitutes, CaP ceramics are bioactive in contrast to bioinert ceramics such as alumina (bioactivity being defined as the property inducing specific biological reactions). The physico-chemical structure of CaP ceramics, which is close to that of the mineral phase of bone, provides bioactivity (Klein et al., 1983, 1989). CaP ceramics can induce the formation of apatite similar to the biological apatite observed during physiological mineralization processes. The two main components of CaP ceramics are hydroxy-apatite (HA) and ß-tricalcium phosphate (ß-TCP), which can be used separately or together. The biocompatibility of such materials has been described *in vitro* and *in vivo* (Winter et al., 1981). After implantation, CaP ceramics undergo numerous dissolution/precipitation processes induced by biological fluids. This first mechanism begins to degrade the ceramic and influence later cellular degradation (i.e. by adsorption of numerous extracellular matrix proteins). Shortly after cellular degradation,

osteoconduction begins inside the structure of the ceramic which is then progressively replaced by true bone characterized by a mineralized extracellular matrix, osteoblasts, osteocytes, osteoclastic cells and neovascularization. Haversian remodeling subsequently takes place, and physiological turnover with resorption-apposition steps can begin (Passuti et al., 1989; Daculsi et al., 1990). However, though CaP ceramics are conductive to new bone formation (osteoconduction), they cannot induce this formation themselves (osteoinduction). In this context, close contact with bone tissue for conduction of new bone formation is essential to osteointegration of the implants.

Numerous parameters influence the kinetics of substitution-colonization of CaP ceramics: physicochemical parameters (composition, density, porosity, granulometry, specific surface, etc.), implantation sites, biological fluids and their chemical changes in the implant environment (pH), and cellular mechanisms (fibroblasts, macrophages, osteoclasts) (Legeros, 1983, 1991). The cellular mechanisms of calcium phosphate ceramic degradation are considered in the following paragraphs.

Mesenchymal cells participate in the degradation of calcium phosphate ceramics

Various mesenchymal cells are present at the implantation of ceramics (e.g. fibroblasts, endothelial cells, osteoblasts, bone-marrow stromal cells). These mesenchymal cells participate actively in the fibrous encapsulation of implanted ceramics not adequately immobilized to prevent micromovements. This fibrous encapsulation limits the formation of true bone ingrowth and the ceramic degradation processes (Daculsi et al., 1990).

Mesenchymal cells can induce the solubilization of CaP ceramics (Evans et al., 1984a,b; Owens et al., 1986; Kwong et al., 1989). Kwong et al. (1989) demonstrated that crystal-cell contact was required to induce ceramic dissolution and that crystal dissolution was inhibited by lysosomotropic agents. The dissolution process did not appear to be modulated by bone-resorption agents such as parathyroid hormone, prostaglandin E2, and 1,25-dihydroxyvitamin D3. However, this dissolution process needs to be elucidated.

Mesenchymal cells are also actively involved in the ceramic degradation process. Thus, many studies have shown the capability of osteoblastic cells to phagocytose CaP crystals. Grégoire et al. (1990) described the presence of phagosomes containing CaP particles ingested by human bone cells and the murine MC3T3-E1 osteogenic cell line. This phagocytic activity was associated with cellular activation marked by protein synthesis and stimulation of RNA transcription. CaP crystals underwent dissolution into the phagosome. Similar results had been noted with fetal mouse calvaria (Takahashi et al., 1986) and rat ROS 17/2.8 osteosarcoma (Alliot-Licht et al., 1991). If osteoblastic cells can internalize CaP crystals, then fibroblasts possess the same ability (Kallenberger, 1978; Cheung et al., 1984, 1986; Evans et al., 1984a,b; Grégoire et al., 1987; Orly et al., 1989; Alliot-Licht et al., 1994). In all cases, the phagocytosis of CaP ceramics induced extensive alterations of cellular metabolism (proliferation, alkaline phosphatase production).

Mesenchymal cells are not the only ones to degrade CaP ceramics. The main cells involved in calcified tissue degradation, such as monocytes/macrophages and osteoclasts, are also implicated the degradation of bone substitutes.

Calcium phosphate ceramic degradation by monocytes/macrophages

Calcium phosphate ceramics, like all implanted biomaterials, induce an inflammatory reaction caused by the wound inflicted during the surgical act. The first factors of biomaterial-associated inflammation concern the physicochemical characteristics of the materials. Though some authors have shown that biomaterials can alter the number and ratio of macrophages and foreign-body giant cells at the implantation site, it is not known whether these differences originate from structural or chemical parameters (Behling and Spector, 1986; van Blitterswijk and Grote, 1989). The implantation site is also known to influence the inflammation process relative to a biomaterial (Kaminski et al., 1968; van Blitterswijk et al., 1985; Bakker et al., 1988).

Monocytes/macrophages, which are among the first cells to colonize the biomaterial surface after *in vivo* implantation, could play a crucial role during biomaterial degradation (Rae, 1986). The most important role of monocytes/macrophages is their phagocytic capability. Numerous authors have described the presence of monocytes/macrophages at the implantation site of calcium phosphate ceramics in *in vivo* experiments (Harms and Mausle, 1979; Howie et al., 1990; Ikami et al., 1990; Hashimoto-Uoshima et al., 1995; Lin et al., 1997; Overgaard et al., 1998). The many particles observed in the cytoplasm by electron microscopy are indicative of the phagocytosis of ceramics by monocytes/macrophages. Recently, the phagocytic activity of monocytes/macrophages has been demonstrated in *in vitro* models (Ushida et al., 1990; Benahmed et al., 1994, 1996a,b; Blottière et al., 1995; Catelas et al., 1997). Thus, Blottière et al. (1995) showed that a human U937 monocytic leukemia cell line adhered to the ceramics (hydroxyapatite or ß-tricalcium phosphate), remaining active and viable. The U937 macrophage lineage activated by vitamin D3 or phorbol ester degraded the ceramic surface. Benahmed et al. (1996b), who conducted an in vitro study on an ultrasructural scale to determine the behavior of human monocytes/macrophages with regard to CaP ceramic, noted the existence of two types of phagocytosis when cells came into contact with biomaterials. The first mechanism concerned the internalization of CaP crystals

together with a small amount of culture medium by monocytes/macrophages closely attached to the ceramic surface. In this condition, the envelope membrane of the phagosomes disappears, releasing CaP crystals into the cytoplasm and thereby facilitating interaction with the organelles. The phagocytosed particles then undergo dissolution. The second mechanism concerned CaP crystals detached from the ceramic surface and internalized together with a large amount of culture medium. Endophagosomes then form heterophagosomes after fusion with primary lysosomes. The dissolution of the crystals occurs in the phagosome. During these two mechanisms, the monocytes/macrophages undergo spontaneous differentiation (accumulation of residual bodies, large fat droplets) accelerated by intense phagocytosis. Subsequent to these mechanisms, differentiated macrophages appear incapable of evacuating the debris formed and finally die in culture.

Catelas et al. (1997) used an *in vitro* model with the murine J774 macrophage cell line to analyze the parameters controlling phagocytosis. Their results indicate that phagocytosis increased with the size and concentration of particles up to 2 μm. With a larger particle size (up to 4.5 μm), phagocytotic activity reached a plateau, suggesting a saturation dependent on the overall particle volume ingested. In these conditions; the mortality of macrophages was similarly increased with size (up to 2 μm) and concentration. Smaller particles (0.6 μm) induced cell mortality only at high concentrations. These results show that macrophage response is dependent on size and concentration but independent of ceramic composition, as previously reported by Shanbhag et al. (1994). Catelas et al. (1997), like Benahmed et al. (1996b), observed that the phagocytosis of ceramic particles begins very early after cell exposure.

Monocytes/macrophages, like fibroblasts, cause dissolution of calcium phosphate ceramics, a process which can be associated with phagocytosis activity (Evans et al., 1984a,b; Owens et al., 1986; Kwong et al., 1989).

Multinucleated giant cells known as macrophage-polykaryons, Langhans' cells or foreign body giant cells (Mariano and Spectror, 1974), observed in chronic inflammatory tissue reaction (granuloma), have shown a limited capacity to resorb calcified matrix (Heymann et al., 1998). The size of giant cells depends on the intensity of the inflammatory reaction (Chambers and Spectror, 1982; Damien and Parsons, 1991). Macrophage-polykaryons do not develop a ruffled border or TRAP activity (*in vivo*) and are formed by fusion of mature monocytes/macrophages. Macrophage-polykaryons containing a large accumulation of mineral crystals in vacuoles have been described in close association with the implanted ceramic (van Blitterswijk et al., 1985; Ikami et al., 1990; Baslé et al., 1993; Dersot et al., 1995). Baslé et al. (1993) showed that CaP ceramics implanted in bone induce the recruitment of two multinucleated populations able to degrade the ceramic. The first, associated with the inflammatory reaction (macrophage-polykaryons), intervenes early at the implantation site and then disappears. The second, corresponding to physiological polykaryons called osteoclasts that are involved in calcified matrix resorption, is recruited progressively after implantation.

Osteoclastic resorption of calcium phosphate ceramics

The nature and origin of multinucleated cell populations is still uncertain and controversial. It has been reported that synthetic hydroxyapatite or ceramic implanted into bone induces the recruitment of multinucleated cells with certain morphological and functional features of osteoclasts (tartrate-resistant acid phosphatase activity, ruffled border-like with clear zones) (Weber et al., 1990; Takeshita et al., 1992; Dersot et al., 1995). Conversely, Ogilvie et al. (1987), Kamakura et al. (1997) and Wada et al. (1989) have shown that implants of hydroxyapatite into human periodontium, octacalcium phosphate into rat bone marrow or beta-tricalcium phosphate into dog periodontium induce the recruitment of multinucleated giant cells lacking TRAP activity and the morphological features of osteoclasts. Similar observations have been done by Bauer et al. (1991), Eggli et al. (1988) and Holtrop et al. (1982). Today, there is consensus that both macrophage-polykaryons and osteoclasts are involved in the degradation of CaP ceramic degradation. *In vitro* studies have confirmed that osteoclasts are capable of resorbing ceramic (Jones et al., 1984; Kawaguchi et al., 1992; Davies et al., 1993; Gomi et al., 1993; de Bruijn et al., 1994; Yamada et al., 1994, 1997a,b).

Yamada et al. (1997a,b), using an unfractioned rabbit bone cell model, showed that osteoclasts are capable of forming resorption lacunae on the CaP ceramic surface. The morphology of ceramic crystals inside lacunae was intensively modified (series of spikes aligned in a single direction) compared to that of crystals outside, showing similarities with ceramic crystals treated by an acidic solution. They hypothesized that crystal degradation within the lacunae was induced by dissolution in a highly acidic microenvironment located under the ruffled border of the osteoclastic cell type. This mechanism appears to be similar to that used by osteoclasts to resorb natural calcified tissues (Suda et al., 1992; Kukita and Kukita, 1996). Yamada et al. (1997b) developed four types of ceramic (HA 100%, HA 75%/ß-TCP 25%, HA 25%/ß-TCP 75%, ß-TCP 100%) to study the influence of CaP ceramic solubility on osteoclastic resorption. Solubility was regulated by varying the ratio of less-soluble HA and more soluble ß-TCP. After two days of culture, the two ceramics composed of HA 100% and HA 75%/ß-TCP 25% were not resorbed by osteoclasts, whereas osteoclasts resorbed the other two ceramics. Moreover, when the resorption area was measured, it was found that osteoclats resorbed the HA 25%/ß-TCP 75% mixture more extensively than did pure

ß-TCP. This suggests that the resorption capability of osteoclasts depends on ceramic composition and is thus directly related to ceramic solubility, whereas the monocyte/macrophage lineage is responsible for degradation of the ceramic.

When osteoclasts resorb calcified tissues by extracellular acidification, they phagocytose various biomaterial particles (latex, titanium, polymethylmethacrylate) (Chambers, 1978; Wang et al., 1997a,b). Osteoclastic phagocytosis of porous HA has also been reported after implantation in sheep mandible (Ylinen et al., 1991). More recently, we demonstrated *in vitro* that osteoclasts cultured on CaP ceramic develop typical ultrastructural features of bone osteoclasts and are able to degrade ceramic by simultaneous resorption and phagocytosis. The phagocytotic mechanism was similar to that observed in the presence of monocytes/macrophages (Heymann et al., submitted paper).

These results show that all CaP ceramics are degradable by osteoclasts, although the kinetics depends on the physicochemical characteristics of the ceramics and the implantation site.

Proteins involved in the degradation of calcium phosphate ceramic

Many proteins (growth factors, extracellular matrix proteins) are involved in the differentiation of monocytes/macrophages and osteoclasts and the activation of their cells (Roodman, 1993; Rowe et al., 1996; Heymann et al., 1998). Several lines of investigation have shown potentially stimulatory effects of ceramics on cells. Thus, HA particles induced the production of superoxide by human polymorphonuclear cells (Nagase et al., 1993) and the production of cytokines and prostaglandin E2 by human bone-marrow mononuclear cells (Kim et al., 1993). Moreover, the physicochemical properties of ceramics can influence response. Thus, Harada et al. (1996) suggested that the biochemical and crystalline structural properties of particles affect the capacity of human monocytes/macrophages to produce interleukin-1ß, interleukin-6, tumor necrosis factor-α and prostaglandin E2, which are highly involved in inflammatory reaction and osteoclast and monocyte activation (Heymann et al., 1998). An increase in CaP ceramic degradation induced by lipopolysaccharides intensified inflammatory reaction during the early implantation stage (Benahmed et al., 1997; Kimakhe et al., 1998). Other molecules inhibit the degradation of CaP ceramic by human monocytes/macrophages. For instance, leukemia inhibitory factor, which is involved in bone remodeling and inflammatory reaction (Benahmed et al., 1996b), induced a powerful inhibition of the differentiation of monocytes into macrophages and

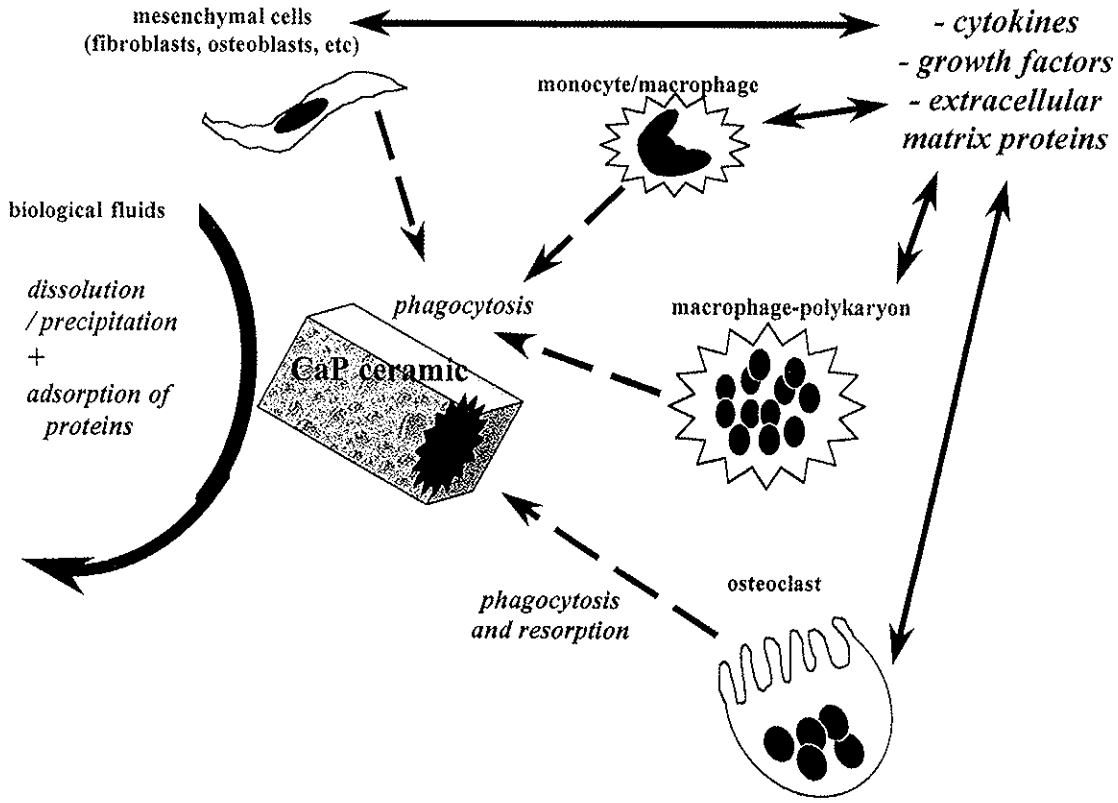

Fig. 1. Summary diagram of ceramic mechanisms involved in CaP ceramic degradation. Various cell types (mesenchymal cells, macrophages, osteoclasts) involved in the degradation of CaP ceramic phagocytose and/or resorb (acidic mechanism) the ceramic. Various molecules (extracellular matrix proteins, growth factors) produced by degradation cells or carried by biological fluids influence ceramic degradation, cellular differentiation and cellular activities.

therefore of endocytic activity, phagocytosis and autophagy. Conversely, growth hormone, a key substance affecting bone metabolism, increased the degradation of CaP ceramic by human monocytes/macrophages and increased the resorption of ceramic implanted into a rabbit bone site (Guicheux et al., 1998a,b).

CaP ceramics can also adsorb various proteins (extracellular matrix proteins, serum proteins, soluble growth factors) which are key protagonists in bone remodeling (mineralization/resorption). McCarthy et al. (1992) have shown that CaP ceramics (HA and TCP) induce collagenase and metalloprotease release from cells which are then activated.

Conclusion

CaP ceramics are bioactive products with physicochemical characteristics closely related to the mineral phases of calcified tissues. After implantation, CaP ceramics undergo physicochemical and cellular degradation and are progressively replaced by lamelar true bone characterized by physiological bone remodeling. Cells involved in degradation/resorption of CaP ceramics intervene via two main mechanisms: phagocytosis and extracellular acidification (resorption). These two processess are modulated by various parameters, such as the properties of the ceramic itself, the implantation sites and the presence of various proteins (cytokines, extracellular matrix proteins). The cells implicated in this degradation process (mesenchymal cells, monocytes/macrophages, osteoclasts) could intervene directly or indirectly through their cytokine/growth factor secretions and their sensitivity to the same substances which modulate cellular activities (Fig. 1). The particles of biomaterials ingested by degradation cells may also influence the differentiation program of these cells (Sabokbar et al., 1996).

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