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Calcium phosphate biomaterials as bone drug delivery systems: a review

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A short review is proposed on the existing literature for the research performed in calcium phosphate (CaP) biomaterials used as drug delivery systems. In the first part, a brief update is given on the performance of both CaP ceramics and CaP cements. Second, a review of the research and clinical situation is developed for CaP materials already used as drug delivery systems. Experimental works performed for local delivery are reported. In particular, a description is given of the *in vitro* and *in vivo* studies in which these materials are loaded with various proteins and drugs.

Introduction

Local drug delivery devices could reduce side-effects, improve the efficacy of existing drugs and open the door to entire classes of new treatments. Such combined systems are able to precisely control the timing of a drug release by adjusting the properties of the carriers. Synthetic polymers are widely used as carriers [1] because they do not cause any considerable inflammation to tissues at the implantation site. In that case, the rate of drug release can be controlled by various mechanisms: diffusion out of the matrix that remains intact, simultaneous drug release and degradation of the matrix, or drug expulsion by osmotic pressure. The incorporation of therapeutic agents into inorganic materials, such as silica gel mainly obtained by sol–gel methods, has also been extensively studied [2]. In this context, calcium phosphates (CaPs), commonly used as implants for bone reconstruction [3–6], seem to be good candidates as bioactive carriers available in various forms including ceramics, cements, and composite and thin coatings. Considered presently as the more reliable alternative to bone grafting, CaPs can be resorbed by cells, present evidenced osteoconductive properties and are efficient in most non-load bearing clinical situations in orthopedics, dental, and ear, nose and throat surgeries.

Any pathological situation (such as an infection, irradiation or a disease such as osteoporosis), however, unfavorably affects the performance of the implant in terms of the substitution and/or resorption process. Nowadays, efforts are therefore focused on developing mixed systems that combine CaP bone substitutes with active molecules. Current research on these bone drug delivery systems aims to improve the osteogenic potential of bone substitutes in healthy bone sites and to provide a bone response in pathological ones. The local administration of active agents has numerous advantages compared with systemic treatments in terms of therapeutic efficiency and tolerance.

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Calcium phosphates

Bone substitutes are largely inorganic compounds and in some cases are inorganic–polymer composites. These inorganic materials are mainly divided into three chemical families: calcium phosphates, calcium sulfates and calcium carbonates. These represent the most current alternatives to biological bone grafts and exist in different forms such as powders, granules, ceramic, cement and coatings. The mineral phase of bone tissues in vertebrates is composed mainly of CaPs, which explains why these CaP materials have chemical properties suitable for bone-remodeling kinetics. Because the other two families are considered too soluble to enable quality *de novo* bone formation, this article focuses on the CaP biomaterials.

Ceramics and unsintered apatites

On the basis of composition, synthetic CaPs presently used as biomaterials are classified as calcium hydroxyapatite (HA), $Ca_{10}(PO_4)_6(OH)_2$; alpha- or beta-tricalcium phosphate (α- or β-TCP), $Ca_3(PO_4)_2$; biphasic calcium phosphates (BCPs) for mixtures

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of HA and β-TCP; and unsintered apatites or calcium-deficient apatites (CDAs). These mixtures have to be subsequently sintered at more than 1100 °C. CaP biomaterials differ in their solubility [5]; the comparative extent of dissolution is: α-TCP ≫ CDAs > β-TCP ≫ HA. For BCPs, extent of dissolution depends on the β-TCP/HA ratio: the higher the ratio, the higher the extent of dissolution [5,6].

Calcium phosphate ceramics (CaPCs) are available in the form of granules or blocks, depending on the bone defect to be filled. Macroporosity (pore size >80–100 μm) is defined by its capacity to be colonized by cells. It can be induced in the material by the addition of organic substances (e.g. naphthalene or sucrose particles) that are sublimated or calcinated before sintering at higher temperatures [7]. Microporosity (pore size <10 μm) is defined by its capacity to be impregnated by biological fluids. It results from the sintering process, and the size of microporosity depends mainly on the material composition and the thermal cycle used. Solubility and biological properties of these CaP materials depend strongly on crystal size, ionic impurities, specific surface area, and both macroporosity and microporosity [6,7]. All these parameters also have a specific influence on the bioceramics' final mechanical properties [8].

Biological performances of porous CaPCs can be evaluated through three fundamental properties that will govern the potential bone substitution at the expense of the implanted bioceramic: biocompatibility [9,10], bioactivity (for example, resorption and/or substitution of CaPC process resulting in interaction between biological fluids) [11,12], and biofunctionality (mechanical properties) [13,14].

Cements

The concept of apatitic calcium phosphate cements (CPCs) was first introduced by LeGeros in 1982, and the first patent on self-setting CPC was obtained by Brown and Chow in 1986 [15]. Currently, several CPCs – with varying compositions of the powder and liquid components – are commercially available, and many more are in experimental stages [16,17]. Unlike the CaP bioceramics in granules or pre-shaped form, CPC has the major advantage of being able to readily adapt to the shape of the bone defect, rapidly integrate into the bone structure and be transformed into new bone by the cellular action of bone cells responsible for the local bone remodeling [18,19]. In spite of these good properties, however, CPCs have limitations owing to their poor mechanical properties and slow *in vivo* biodegradation. The current commercial CPCs remain dense after implantation, lacking the macroporosity that will enable 3D cell colonization and tissue ingrowth. In this attempt, new CPCs have been designed incorporating polysaccharides [20,21] or resorbable fibers (Vicryl®) [22]. The dissolution of these particles or fibers is also supposed to develop channels suitable for bone ingrowth [22]; however, both mechanical and osteoconductive properties remain non-optimal at present.

Because of their excellent biocompatibility and their non-exothermic behavior, it is possible to incorporate organic molecules in these cements, making them potential vector materials for the therapeutic agent delivery [23].

Advantages of combined systems in bone reconstruction surgery

Improving bioactivity of bone substitutes in healthy bone sites

Bone defects can occur after trauma, prosthetic revisions, and tumor-induced osteolysis of a tumor source. Because of the increase in the number of accidents, multiple trauma victims, and ageing of the population, the demand for bone reconstruction is constantly growing.

Under normal conditions, bone tissue has the capacity to regenerate itself. The repair of bone tissue is a complex phenomenon requiring the involvement of cytokines, hormones, and growth factors [24]. This biological cascade recruits and activates the inflammatory cell progenitors necessary for efficient repair of the damaged tissue. This spontaneous repair is valid only for small-sized, 'non-critical' defects. For larger defects, an osteogenesis ability is required, notably when using the previously mentioned bone substitutes. These bone substitutes, however, have a low osteogenic potential. Improving the osteogenic potential of bone substitutes is the objective of using combined growth factors–CaP matrices systems. The factors involved in bone regeneration should be considered as candidates for the development of combined systems (Table 1).

Specific interest of local release

All these growth factors are characterized by their short half-life (60–240 min), their instability, and generally their pleiotropism of action [25]. These characteristics explain the low efficiency of a systemic administration of these factors. Their administration *in situ* enables the obtention of a specific tissue response and an optimal bioavailability [25]. The parameters to be controlled,

TABLE 1

The growth factors involved in bone regeneration

Growth factors	Functions	Refs
Growth hormone	Bone remodeling Proliferation and differentiation of osteoblasts Stimulation of osteoclastic resorption activity	[67]
Bone morphogenetic protein	Proliferation and differentiation of mesenchymal stem cells (MSCs) and osteoprogenitor cells Ectopic bone formation	[68]
Transforming growth factor beta	Recruitment, proliferation, and differentiation of MSCs and osteoprogenitor cells Extracellular matrix production Angiogenic and inflammation properties	[69]
Insulin growth factor	Proliferation and migration of MSC and osteoprogenitor cells New bone formation and mineralization	[70]

within a suitable clinical use, are the conservation of their bioactivity, their release kinetics, their distribution zone, and their migration out of the region of interest that conditioned the tolerance of these growth factors. Undesirable effects can appear in the event of distribution to the surrounding tissues; for example, angioma, fibrosis, ectopic mineralization, and so on [26].

In vivo applications

Numerous studies have shown the benefit of associating growth factors with CaP during the filling of bone defects [25,27–30].

Growth hormone

BCP cylinders (HA 60/β-TCP 40) loaded with growth hormone (GH) were implanted in a femoral site in rabbits. After three weeks of implantation, GH, loaded at 1 μg/implant, increased bone growth by 65% and ceramic resorption [31] by 140% compared to cylinders not loaded with GH.

Bone morphogenetic protein

Haddad *et al.* [30] evaluated the bone repair of a critical-sized calvarial vault defect' in rabbits, after implantation of apatitic cement loaded with bone morphogenetic protein-2 (BMP-2; 25 mg/mL). After 12 weeks of implantation, they observed a bone formation of 45.8% more than control.

In another study, Namikawa reported the efficiency of a composite material (β-TCP + copolymers PLA-DX-PEG) associated to rh-BMP-2 on vertebral fusion (L4–L5) in rabbits [32]. After six weeks of implantation, there was a full vertebral fusion. The doses of rh-BMP-2 (15–30 μg) used in this study were largely inferior to the doses generally used in systemic (>100 μg).

Studies led by Seeherman *et al.* [27,28] confirm the efficiency of these combined systems. For example, the injection of a combined apatitic cement with rh-BMP-2 in primates, in an osteotomy model of the fibula, accelerated the filling of the bone defect by 40% after 14 weeks of implantation compared to an unloaded cement [27]. This combined rh-BMP-2/apatitic cement (0.166 mg/mL) was also implanted in a critical bone defect in rabbits [28]. After four weeks of implantation, an acceleration of combined cement resorption, as well as the filling of the defect, was observed compared with the unloaded control cement. Due to this acceleration of bone remodeling, the defect was fully filled with *de novo* bone eight weeks after implantation.

A clinical study has also shown encouraging results. This study compared the efficiency on a vertebral fusion model of an iliac crest autograft versus BCP combined with BMP-2 [33]. The implantation of combined BCP gave a clear improvement compared to the iliac crest bone graft in terms of vertebral fusion rate (88% vs 73%), operatory conditions (duration of the intervention 2.4 h vs 2.9 h and hemorrhages 273 cm^3 vs 465 cm^3) and morbidity (leg and back pain, standardized scores for pain such as SF36, OLBPD index, and so on).

Transforming growth factor-beta

Sumner *et al.* [29] have shown that the release *in situ* of transforming growth factor-beta (TGF-β)2 from a BCP, loaded with 120 μg of TGF-β2, stimulates bone growth by increasing its amount twofold after a four week implantation period on the proximal humerus in dogs.

Calvaria critical-sized defects on adult rats were filled with 53 mg of cement loaded with 0, 10 and 20 ng of TGF-β1 [34]. Eight weeks after implantation, the combined cement stimulated bone formation by 50% and improved the bone–cement contact by 65% compared with the control cement. The presence of 10 and 20 ng of TGF-β1 accelerated resorption of the cement by 10% and 20%, respectively.

Insulin-like growth factor

A preliminary study evaluated the *in vitro* effect of a composite substance: alginate, TCP, poly(lactide-co-glycolide) microspheres loaded with insulin-like growth factor (IGF)-1 [35]. The presence of IGF increased the proliferation of MG-63 cells (X7) and the phosphatase alkaline activity of SaOS-2. These beneficial *in vitro* effects must be confirmed by *in vivo* studies.

Extending the biofunctionality of bone CaP substitutes in pathological bone sites

Osteoporotic situation

The clinical context

Post-menopausal osteoporosis manifests itself clinically through the development of bone fractures. These fractures mainly affect the proximal femur, the vertebral spine and the wrist. This specificity of osteoporotic sites justifies the use of a local approach for the prevention of osteoporotic fractures. One of the strategies [36–38] has been to locally reinforce these bone sites, by releasing BPs *in situ* from CaP biomaterials. This local approach is doubly interesting because the CaP matrix implanted in the osteoporotic site will mechanically reinforce the weakened bone and will act as a bone substitute, which will serve as a support for new bone formation, and because the BP released *in situ* will locally regulate osteoclastic hyperactivity, a characteristic of osteoporosis.

In vitro and in vivo studies

The affinity of BPs for HA has been used to develop new CaP systems for the controlled release of BP [39–42]. Seshima *et al.* [40] envisaged HA as a potential vector for alendronate and have studied the influence of crystallinity, the specific surface, and the solubility on the release profile of BPs. Similarly, Boanini *et al.* [41] synthesized HA nanocrystals loaded with alendronate at 7 wt%. The *in vitro* evaluation of their materials showed a reduction in the number of osteoclasts of approximately 30% and an increase in osteoblastic activity, characterized by doubling in synthesis of alkalin phosphatase (ALP), osteocalcin, and type I collagen [41]. This team has developed an apatitic cement loaded with BP [42]. In late 2002, a patent was filed by Yayon [43] relating to a bone-enhancing composite, comprising synthetic apatite and at least one bioactive compound (including an anti-resorptive agent) and suitable for use as bone graft implants. In a typical example, a CDA was precipitated in the presence of alendronate; however, neither the final BP content nor the association mode between the BP and the CaP were described.

Surprisingly, very few studies related to the interaction of BPs with CaP materials, other than HA, are present in the literature, whereas a variety of synthetic CaPs are currently developed for their use as bone substitutes (i.e. BCPs, α- or β-TCP, CDAs, and dicalcium phosphate dehydrated) because in contrast to the case of HA, which is highly stable under physiological conditions, they

can be degraded in bone defects simultaneously with the formation of new bone [44]. Because of notable differences in the solubility and chemical composition of these CaPs, possible variation in their reactivity towards bisphosphonates could be anticipated.

In a first study, CDAs were suspended in aqueous solution of bisphosphonates. Under these conditions, a surface adsorption of the drug took place, driven by PO_3 for PO_4 exchange [36,37]. A simple mathematical model was designed [45] that correctly described the bisphosphonate–CDA interaction at equilibrium, in simplified media such as ultrapure water or phosphate buffers. The chemical binding of the bisphosphonate onto the CDA can be depicted as in the following equation [45]:

$$BP + P - X \equiv \underset{k-}{\overset{k+}{\rightleftarrows}} BP - X \equiv + P$$

where X≡ corresponds to the surface binding sites of the CDA that are in interaction with either a bisphosphonate (BP) or a phosphate (P) moiety. The maximum bisphosphonate uptake, which corresponds to the saturation of the exchangeable sites on the CDA surface, was found to be similar for various bisphosphonates, including last-generation clinically used molecules such as alendronate (monosodium form ~0.26 mmol g^{-1}) and zoledronate (disodium form ~0.23 mmol g^{-1}). On the other side, the reaction of aqueous zoledronate solutions with β-TCP resulted in the precipitation of a crystalline zoledronate complex on the surface of the CaP. This complex ($CaNa[(HO)(C_4H_5N_2)C(PO_3)(PO_3H)]\cdot xH_2O$) was found to be metastable, leading to a pure calcium complex ($[Ca_3[(HO)(C_4H_5N_2)C(PO_3)(PO_3H)]_2\cdot xH_2O]$ upon washing with water [46]. *In vitro* evaluations highlighted that the two combined systems offer differing BP release at biologically active doses [38,46]. For example, after four days of incubation, the BP-loaded CDA releases 10^{-6} M of zoledronate, leading *in vitro* to a statistically significant inhibition of osteoclastic resorption activity without consequences on osteoblast viability and activity [46]. Osteoclastic inhibition was indirectly quantified by measuring pits of resorbed dentin using image analysis system – see Figure 1 and Ref. [47].

In another study, zoledronate was grafted onto HA coatings of titanium implants. The implants were then inserted into either healthy or osteoporotic female rat condyles with various zoledronate concentrations (0, 0.2, 2.1, 8.5 and 16 μg/implant) [48,49]. In both cases, the effectiveness of the concept of using a local bisphosphonate delivery from a CaP coating was demonstrated because a statistically significant increase in the peri-implant bone volume fraction was observed.

BP-loaded HA/PLGA microsphere composites were developed and tested *in vitro* [50]. During the first four days, 20–40% of BPs were released from the matrix. This release continued progressively and after 30 days of incubation, 70–90% of BPs had been released. *In vitro* tests carried out from human fetus osteoblast cultures showed that released alendronate stimulated osteoblast proliferation and activity and reduced the viability and the proliferation of macrophages.

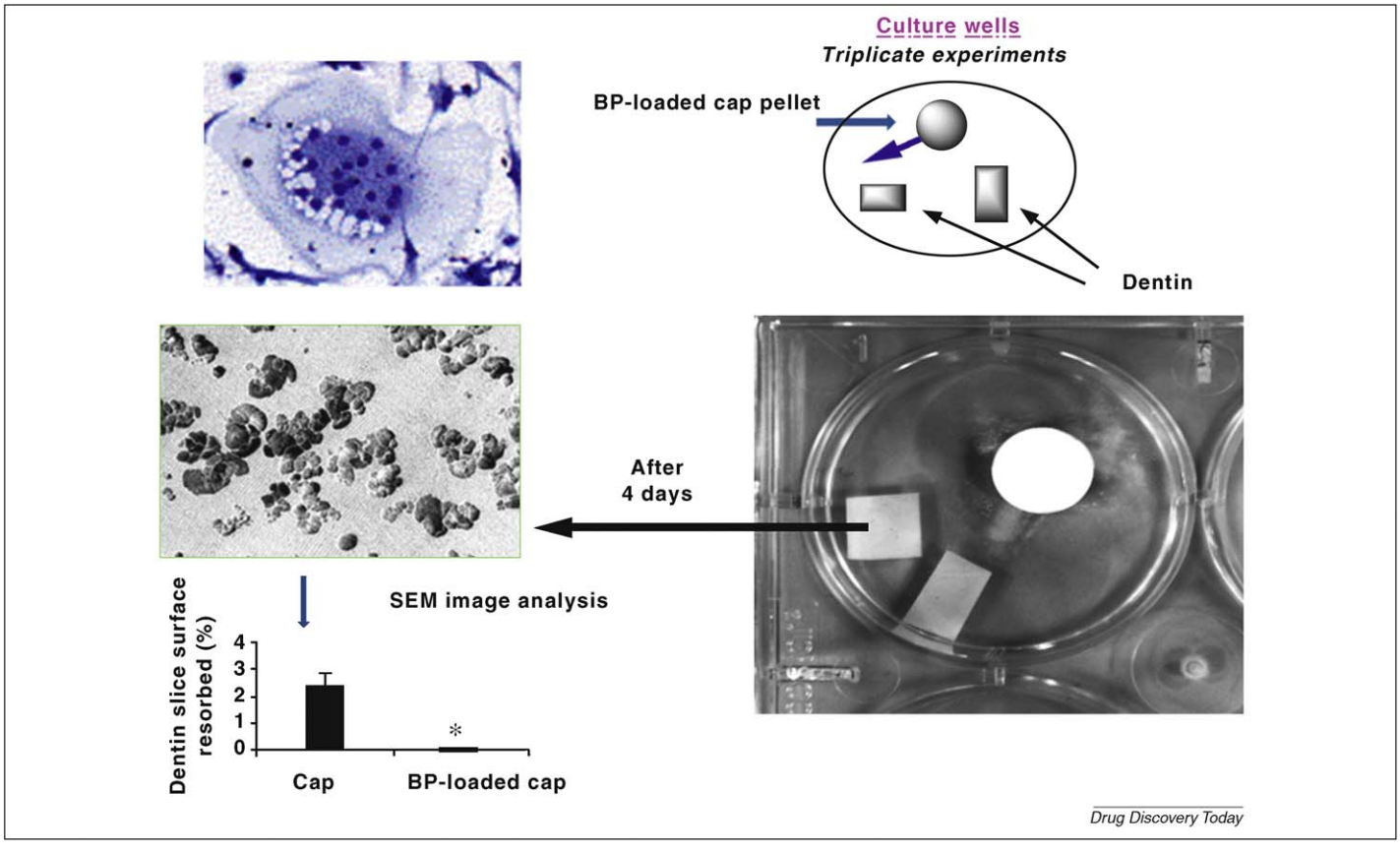

FIGURE 1

Quantitative and reliable *in vitro* method combining scanning electron microscopy and image analysis for the screening of osteoclastic inhibitors.

Bone infections

Although rare (1–2%), the risk of infection associated with the implantation or the revision of a medical device is undesirable because of the morbidity that it can generate [51,52]. In its most extreme occurrence, osteomyelitis, whether acute or chronic, can compromise the vital prognosis of the patient [51].

After implantation of HA blocks saturated with antibiotics, six of the seven patients who had contracted an infection after hip arthroplasty did not contract an infection during the five years of monitoring [53]. CPC, used as an antibiotic vector, gives good clinical results [54–56]. A meta-analysis of 19 studies, covering 35 659 patients, confirmed the usefulness of loaded cements in treating bone infections efficiently [57]. They reduce the bone infection rate by half in primary prevention and by approximately 40% in secondary prevention [57].

Resistance of some strains has appeared, however [58]. Because of this, certain clinicians recommend avoiding the routine use of these loaded cements and restricting their use only to multi-resistant strains [59,60].

Cancerology

Although it represents only 0.2% of all malignant tumors, osteosarcoma is the main primitive malignant tumor of the skeleton. In 80% of cases, osteosarcoma found at the time of diagnosis is generally treated with pre- and post-operative chemotherapy and surgery; the healing rate varies between 60% and 70% [61,62]. Surgery is a conservative procedure (preservation of the member) for more than 90% of patients [62]. Giant cell tumors, which represent 5–10% of primitive bone tumors, are reputed to be the most recurring tumors. These recurrences mainly occur during the first two or three years of the first appearance of the tumor [63].

Wide excision of the bone-localized tumor and the curing of surrounding tissues must be systematically associated with chemotherapy. This association reduces the risk of developing, for example, pulmonary metastases by half in patients with a bone-localized tumor [62]. Before the introduction of chemotherapy, survival at five years did not exceed 20% [62]. Faced with this finding, several teams looked into developing combined systems with the goal of finding anticancer drug releases in bone sites [64–66]. The objectives of these combined CaP systems are to fill the bone defect, created by excision of the tumor, with a bone substitute (reconstructive surgery) and to release, on the bone site, a local, high, and sustained concentration of chemotherapeutic agents to prevent the risk of recurrence. Furthermore, thanks to local release, the high doses administered are better tolerated by the patient. This is important given that these treatments have considerable side-effects (such as digestive complications, hematotoxicity, nephrotoxicity, hepatotoxicity, and so on) that are a cause of interruption of the treatment.

Itokazu *et al.* [65] characterized the release of methotrexate from two CaPs, HA (0.625 mg/block) and β-TCP (2.25 mg/block). These combined systems released, in the first days, approximately 1 mg/mL of methotrexate. This release reduced progressively to reach, on the 12th day, a local concentration of methotrexate that remained efficient, of approximately 0.1–1 μg/mL. Abe *et al.* [64] evaluated *in vivo* the release efficiency on bone sites of paclitaxel from a composite material (HA/alginate beads with 2.4 wt% of paclitaxel). They used a rat model of bone metastases on the vertebral column. Compared to the untreated control group, the local approach using their combined biomaterial slowed the appearance of paralysis linked to bone metastases by 140%. Furthermore, their combined system increased the survival rate by 150%. The release of paclitaxel by this combined system gave better results compared to systemic administration, even with doses 30 times stronger.

Concluding remarks

The integration of drugs and devices is a growing force in the medical industry. The incorporation of pharmaceutical products promises not only to expand the therapeutic scope of device technology but also to access combination products whose therapeutic value stems equally from the structural attributes of the device and the intrinsic therapy of the drug. Although the use of implants as a drug delivery system is well developed for cardiovascular applications (drug-eluting stents) and entering the market for diabetic management (insulin pump), this approach is still in infancy for bone applications. This is mainly because of regulatory aspects that have to be enfaced by orthopedic industries. Indeed, such bone drug delivery systems are considered as combination devices by the FDA and, correspondingly, the registration of these new systems follows a longer process than that of traditional orthopedic implants.

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