



Review article

Calcium phosphate cements: Optimization toward biodegradability

I. Lodoso-Torrecilla^{a,b}, J.J.P. van den Beucken^a, J.A. Jansen^{a,*}^a Dept. of Dentistry - Biomaterials, Radboudumc, Nijmegen, The Netherlands^b Biomaterials, Biomechanics and Tissue Engineering Group, Department Materials Science and Metallurgy, Technical University of Catalonia (UPC), Escola d'Enginyeria Barcelona Est (EEBE), Barcelona, Spain

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ABSTRACT

Synthetic calcium phosphate (CaP) ceramics represent the most widely used biomaterials for bone regenerative treatments due to their biological performance that is characterized by bioactivity and osteoconductive properties. From a clinical perspective, injectable CaP cements (CPCs) are highly appealing, as CPCs can be applied using minimally invasive surgery and can be molded to optimally fill irregular bone defects. Such CPCs are prepared from a powder and a liquid component, which upon mixing form a paste that can be injected into a bone defect and hardens in situ within an appropriate clinical time window. However, a major drawback of CPCs is their poor degradability. Ideally, CPCs should degrade at a suitable pace to allow for concomitant new bone to form. To overcome this shortcoming, control over CPC degradation has been explored using multiple approaches that introduce macroporosity within CPCs. This strategy enables faster degradation of CPC by increasing the surface area available to interact with the biological surroundings, leading to accelerated new bone formation. For a comprehensive overview of the path to degradable CPCs, this review presents the experimental procedures followed for their development with specific emphasis on (bio)material properties and biological performance in pre-clinical bone defect models.

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Statement of significance

Calcium phosphate cements (CPCs) represent one of the most widely studied materials for bone regeneration due to their numerous benefits such as injectability or moldability. However, their slow degradation rate has put the focus of many researches on improving their degradation mainly by enhancing their macroporosity. This article reviews the main techniques to enhance macroporosity of CPCs, the benefits and drawbacks of each technique as well as the recent advances in enhancing this macroporosity.

1. Introduction

1.1. Bone regeneration

Bone regeneration is a complex physiological process, which requires surgical intervention for patients with bone defects and/or bone fractures that do not spontaneously heal. Often, these surgi-

cal interventions include grafting procedures to fill bone voids. After the surgical intervention, the physiological process starts with an inflammatory stage to remove debris, like blood clot, dead cells and damaged bone matrix, then proceeds with a proliferation stage in which granulation tissue is formed with revascularization and formation of primary, plexiform bone (i.e. callus). If the blood supply is sufficient, trabeculae will be formed in the maturation stage, in which the conversion of plexiform bone into lamellar bone takes place. Bone possesses the intrinsic capacity for regeneration as part of the repair process in response to injury [1,2], but only if a defect is below the so-called critical size [3] and if bone healing is not impaired by local or systemic comorbidities (e.g. osteoporosis, diabetes, and cancers) [4–6]. Unlike other tissues, bone heals without the formation of scar tissue, and bone is regenerated with its pre-existing properties largely restored [7]. At the end of the healing process, the newly formed bone is even indistinguishable from the adjacent uninjured bone [2].

1.2. Bone grafting

The annual average proportion of the U.S. adult population suffering from a musculoskeletal pathologic condition in 2012 was 50% (absolute number of patients: 126.6 million), being double the

* Corresponding author.

E-mail address: john.jansen@radboudumc.nl (J.A. Jansen).

proportion of patients with chronic heart and lung conditions [8]. Impaired bone regeneration is one of these musculoskeletal conditions. The healing of fractures is a physiological process that results in bone union [9]. When in fracture healing bone regeneration is impaired, this results in a delayed union or even non-union. Studies have estimated that 5–10% of all fractures are associated with impaired healing, resulting in delayed union or non-union [10–12]. Audigé et al. [13] performed a prospective observational study in 41 trauma centers, where 416 patients with tibial shaft fractures were treated operatively and followed up for at least 6 months. They reported a delayed union or non-union in 13% of the cases. Beside impaired bone regeneration, there are conditions in which bone regeneration is required in large quantity, beyond the normal potential for self-healing, such as for skeletal reconstruction of large bone defects caused by trauma, infection, tumor resection or skeletal abnormalities. Additionally, there are cases in which bone regeneration is not sufficient because of a compromised regenerative process due to, for example, avascular necrosis or due to systemic diseases such as osteoporosis and diabetes mellitus [7].

In the abovementioned cases, bone defects have to be reconstructed surgically using bone grafting materials in order to fill the defect and aid in bone regeneration or augmentation. Bone grafting is a common surgical procedure, with bone being the second most common transplanted tissue after blood [14,15]. The need for bone grafting procedures is increasing worldwide. The number of bone grafting procedures ranges from 0.5 million in the US to 2.2 million worldwide annually [16]. Amongst other causes, this increase is the result of an increased population suffering from systemic diseases that negatively affect the bone regenerative capacity of the body, predominantly being osteoporosis and diabetes mellitus [17,18]. Trauma, plastic/reconstructive, orthopedic and maxillofacial surgeons face the complexity of reconstructing bone defects every day. Different bone regenerative treatments are currently clinically applied. Autologous bone grafting, in which the patient's own bone is used to regenerate the bone defect, is mostly used as standard-of-care procedure for bone regenerative treatment worldwide because of the osteoinductive, osteoconductive, osteogenic and non-immunogenic properties of autologous bone. Osteoinductivity is the ability to actively induce (de novo) bone formation [19]. Osteoconductivity is a property that allows the colonization and ingrowth of new bone cells over a surface. Osteoconduction is mainly determined by the chemical and physical properties that promote adhesion and cell growth [20,21]. Osteogenicity is related to the presence of bone-forming cells within the bone graft [22]. On the other side, autologous bone grafting has a major disadvantage; being the need for an additional surgical procedure to harvest donor bone. This autologous donor bone is commonly harvested from the iliac crest, which is easily accessible and contains a relatively large amount of corticocancellous bone [22]. The harvest of autologous donor bone from the iliac crest is associated with minor complications in 10% of the cases and with major complications in 5.8% of the cases [23]. Minor complications include superficial infections, superficial seromas, and minor hematomas. Major complications include vascular injuries, deep hematomas that require a surgical intervention, deep infections at the donor site, iliac wing fractures, neurological injuries [23] (causing gait disturbances, deviations in form, meralgia paresthetica (neuropathy of the *N. cutaneus femoralis lateralis*)), and herniation of intestines through a defect in the abdominal wall.

An alternative treatment modality is the use of allogeneous bone, in which processed human cadaver bone is transplanted into the patient. However, allogeneous bone is not always accepted as a bone substitute for several reasons, including (i) undesired graft-versus-host reactions, (ii) graft necrosis, (iii) delayed incorporation, and (iv) relatively high costs [22]. Finally, similar to autologous bone grafts, allogeneous bone grafts suffer from limited availability [24].

In view of the drawbacks of autologous and allogeneous bone grafts, synthetic bone graft materials have become heavily explored as alternatives. As synthetic graft materials are off-the-shelf available, no second surgical procedure for tissue harvest is necessary and no donor site complications can occur. Most importantly, synthetic bone grafts do not suffer from limited availability. However, synthetic bone grafts do not possess all the characteristics of natural, autologous bone and hence are subject to continuous explorations by researchers for improvement to generate the ideal bone substitute material. The diamond concept [25,26] suggests that in order to achieve uneventful fracture healing, four parameters are mandatory: osteogenic cells, an osteoconductive scaffold, growth factors, and a stable mechanical environment. Later, also vascularity at the defect site was added as an important factor in the fracture healing process [16]. An ideal bone graft material should support that these criteria can be met. In view of this, a variety of synthetic graft materials have already been evaluated as scaffolds in bone repair, of which bioceramics are highly appealing. These bioceramics can be categorized into bioinert (i.e. alumina or zirconia) and bioactive/bioresorbable. Calcium phosphate (CaP)-based bioceramics, calcium sulphate-based bioceramics, or silica-based bioactive glasses are amongst the most studied bioactive/bioresorbable bioceramics. Habraken et al. [27] described the characteristics of an ideal bioceramic material for bone tissue engineering as follows:

- 1 biodegradable to enable bone remodeling;
- 2 macroporous to enable tissue ingrowth;
- 3 mechanically stable and easy to handle;
- 4 osteoconductive to guide bone growth around and inside the material;
- 5 suitable to function as a carrier for growth factors or cells.

CaP-based bone graft materials represent the most widely used synthetic biomaterials for bone regenerative treatments due to their biological performance that is characterized by biocompatibility, bioactivity and osteoconductive properties [28]. CaP-based bone substitutes allow attachment, proliferation, migration, and phenotypic expression of bone cells leading to formation of new bone in direct apposition to the biomaterial [19]. CaP-based bone graft materials are commonly available as granules, blocks and, more recently, as cements. CaP cements (CPCs) are the most appealing CaPs for clinical application, due to their ability to be injected and molded as a paste, enabling minimally invasive application and optimal filling of irregular bone defects [29,30]. In contrast, when granules, commonly applied mixed with a liquid (i.e. blood), or blocks are implanted, the bone-to-implant contact is not optimal (Fig. 1). Further, a block cannot be applied by minimally invasive surgery and the size of the block has to be matched to the defect size by carving, cutting or drilling.

2. Calcium phosphate cements

Calcium phosphate cements (CPCs) are synthetic, self-setting bone substitute materials. As mentioned before, the advantage of CPCs is that they are injectable, moldable and harden *in situ*, so the contact between the tissue and the implant will be optimal, even when the defect dimensions are irregular. Moldability and *in situ* hardening of CPCs along with their biocompatibility make CPCs a promising alternative for current synthetic bone substitute materials. Moreover, CPCs have shown to be osteoconductive and their bioactive behavior allows integration within the tissue by the same processes involved in remodeling healthy bone [31,32].

CPCs are systems that consist of a CaP-based powder and a liquid phase, which upon mixing undergo a chemical reaction resulting in setting of the formed material in a crystalline solid at body temperature. Upon mixing the powder with the liquid phase, the CPC particles partly dissolve superficially and a setting reaction is

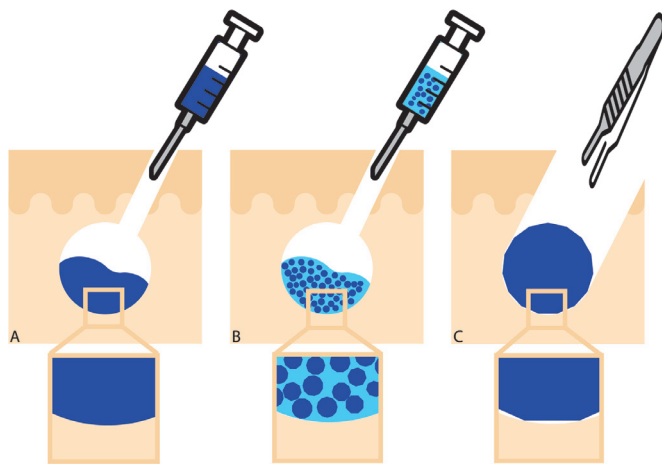


Fig. 1. Schematic representation of modes of application of the different CaP-based bone graft materials. A) CaP cements (CPCs) can be applied by minimally invasive surgery and bone-to-implant contact is optimal (magnification). B) CaP granules can be applied by minimally invasive surgery (pre-mixed with a liquid) but bone-to-implant contact is suboptimal (magnification). C) CaP blocks cannot be applied by minimally invasive surgery and bone-to-implant contact is suboptimal (magnification).

initiated based on the entanglement of CaP crystals, which leads to a hardened micro- or nanoporous structure [33]. This setting reaction is neither toxic nor exothermal [34], which represents a benefit over poly(methyl methacrylate) (PMMA) cements, highly used in orthopedic surgeries. The chemical reaction that occurs during setting of CPCs can be of two types, namely an hydrolysis reaction or an acid-base interaction, and will be determined by the CaP compounds present in the powder phase. The hydrolysis reaction involves only one CaP precursor compound that, when mixed with the liquid phase, becomes hydrated. For example, in case of α -tricalcium phosphate (α -TCP) being the CaP precursor, the reaction is controlled through the dissolution of PO_4^{3-} and Ca^{2+} ions at the surface of the particles and the simultaneous formation of an entangled network of precipitated calcium-deficient hydroxyapatite (CDHA) crystals [35,36]. On the other hand, the acid-base interaction involves multiple CaP precursor compounds, some acidic and other basic, that interact and react into a neutral end-product [37]. The most common basic components are either tetracalcium phosphate (TTCP) or β -TCP and the most widely studied acidic reactants are dicalcium phosphate anhydrous (DCPA) or monocalcium phosphate monohydrate (MCPM) [38–40].

CPCs can be divided in two categories: apatitic and brushite CPCs. The final end-product of the reaction between the powder and liquid phase defines whether a CPC is apatitic or brushite. This end-product of the reaction is, in turn, determined from the solubility of CaP precursor compounds used as powder phase of the CPC and the pH of the setting reaction. Apatitic CPCs have as end-product precipitated hydroxyapatite (HA) or CDHA [41], which is highly similar to the mineral phase of bone and teeth. Likewise, they also show similar solubility behavior; apatitic CPCs are relatively insoluble at neutral pH, but their solubility increases with at more acidic pH, so they can be resorbed by osteoclasts during bone remodeling [42]. Apatitic CPCs can be formed by a hydrolysis reaction (when only one CaP component is used) or by an acid-base interaction (when multiple CaP components are used). Brushite CPCs, on the other hand, precipitate into dicalcium phosphate dehydrate (DCPD). DCPD can only appear as end-product when the pH of the solution is below 6, so brushite CPCs are relatively acidic and reactions may occur at pH as low as 2.5 [43,44]. Further, by dehydration of brushite, these CPCs can also precipitate into anhydrous DCP (i.e. monetite) [45]. This can occur in water-deficient condi-

tions and at low pH [46]. In contrast to apatitic CPCs, brushite CPCs can only be formed by and acid-base interaction. The main difference between the end products of apatitic and brushite CPCs is their solubility and resorption rate. Brushite CPCs are more soluble than apatitic CPCs, and hence more quickly resorbed *in vivo* [33,47,48]. However, brushite CPCs have very short setting times and in order to have a workable CPC, high liquid-to-powder (L/P) ratios have to be used. This increase in liquid phase results in a highly porous and relatively weak CPC, which limits its clinical application [49,50]. Therefore, most research efforts have focused on apatitic CPCs [51]. In general, by changing different parameters such as the L/P ratio, porosity or phase composition, the strength and degradation rate can be adjusted for different clinical applications. These parameters should be modified in such a way that the kinetics of biodegradation are consistent with the rate of bone formation. An overview of the various commercially available CPCs is given in Table 1. It can be observed that the majority are apatitic CPCs due to their superior performance over brushite CPCs. However, the fact that apatitic CPCs are relatively non degradable has hindered their more widespread use in clinical applications. Therefore, the enhancement of apatitic CPC degradation is of utmost importance to increase their clinical use. Details regarding the enhancement of degradation of apatitic CPCs are discussed below and are the main focus of this review.

In a clinical situation, the ability of a surgeon to properly mix the CPC and place it in the defect before it hardens is a crucial factor in achieving optimal results. Therefore, the **setting time** of CPCs is one of their most important properties, as it determines the hardening time as well as the nature of the end-products, which will influence the physical and biological properties of the CPC. Initial and final setting times refer to the time periods between addition of the liquid phase and reaching a certain mechanical property (i.e. the indentation of a Gillmore needle with tip diameter 2.12 mm and mass of 113.4 g for initial setting time and 1.06 mm and 453.6 g for final setting time; ASTM C266-18)¹ [54]. The clinical meaning of this material property is that a CPC has a clinical window (i.e. working time) during which it is possible to inject the CPC and mold it to desire prior to initial setting as well as a certain stability prior to wound closure [55]. The hardening of the CPC can be related to one of three different reaction processes. It can be the result of reactions among the different CaP compounds in an aqueous environment, producing a dissolution/re-precipitation reaction. Because HA is the most stable CaP phase above pH 4.4 (and hence also in the body), most CPC formulations form HA or a closely related apatitic phase as the end-product [56,57]. Another option for CPC hardening is the mixture of the powder phase with a liquid solution that contains a carboxylic acid, which reacts with the calcium compounds and produces fast hardening cements [29,58,59]. Finally, CaP compounds can also produce a hardened cement when mixed with aqueous solutions of a number of different polymers, such as poly(methyl vinyl ether-maleic acid) [60], chitosan [61,62] or gelatin [63]. The three different setting reactions are not mutually exclusive, and can be combined to obtain a cement with the optimal setting properties for the desired application.

In order to be injectable for *in vivo* applications, CPCs must have two features: injectability and cohesion. **Injectability** is the ability of a CPC to pass through a syringe of a certain diameter and length at a given load (e.g. 100N) maintaining the homogeneity of the paste [51,64]. The quantitative measurement can be related to the relative amount of cement extruded from the syringe. The injectability of CPCs depends on a large number of parameters of the CPC composition; e.g. particle size and shape, particle size distri-

¹ <https://www.astm.org/Standards/C266.htm>.

Table 1
List of commercially available CPCs. Partially reproduced from Bohner (2010) [52].

Producer	Product name	Powder phase	Liquid phase	Product
Berkeley Advanced Biomaterials (US)	Cem-Ostetic™	Calcium phosphate powder (details unknown)	Sterile water	Apatite
Biomatlante (FR)	MBCP® putty, In'Oss™	HA, β -TCP (BCP ¹)	Hydrogel	Apatite
Biomet (US)	Calcibon®	α -TCP (61%), DCPA (26%), CaCO ₃ (10%), pHA (3%)	H ₂ O, Na ₂ HPO ₄	Apatite
ETEX ² (US)	α -BSM (marketed as N-Force Blue®); β -BSM®; γ -BSM® ³	ACP (50%), DCPD (50%)	Unbuffered aqueous saline solution	Apatite
Exactech Biologics(US)	Ossilix® MP	Calcium phosphate powder (details unknown) and CaSO ₄ granules	Unknown	Apatite
Graftys (FR)	Graftys® HBS Graftys® Quickset	TCP, ACP, BCP Calcium phosphate powder, hydroxypropylmethylcellulose (HPMC)	Phosphate buffered solution Na ₂ HPO ₄ -based aqueous solution	Apatite Apatite
Kasios ⁴ (FR)	JectOS®	TCP (45%), DCPD (55 %)	Unknown	Brushite
Kyeron (NL)	Axoz QS®	Calcium phosphate powder (details unknown)	Unknown	Apatite
Kyphon ⁵ (US)	KyphOs™ FS	TCP (77%), Mg ₃ (PO ₄) ₂ (14%), MgHPO ₄ (5%), SrCO ₃ (4%)	H ₂ O, (NH ₄) ₂ HPO ₄ (3.5M, 46 w/v%)	Apatite
Medacta International SA (CH)	Medacta biologics	Calcium phosphate powder (details unknown)	Unknown	Apatite
Mitsubishi Materials ⁶ (JP)	Biopex®-R	α -TCP, TTCP, DCPD, HA, Mg ₃ (PO ₄) ₂ , NaHSO	H ₂ O, Sodium succinate, sodium chondroitin sulfate	Apatite
NGK Spark Plug Co (JP)	Cerapaste	TTCP, DCPA	Sodium dextran sulfate sulfur 5	Apatite
Shanghai Rebone Biomaterials Co (CN)	Rebone Gutai	TTCP, DCPA	H ₂ O	Apatite
Skeletal Kinetics (US)	SKaffold™ SKaffold™ ReNu	α -TCP and unknown compounds α -TCP, CaSO ₄ granules and unknown compounds	Unknown Unknown	Apatite Apatite
Stryker (US)	DirectInject	TTCP (73%), DCPA (27%)	H ₂ O, mixture of Na ₂ HPO ₄ and NaH ₂ PO ₄	Apatite
DePuy Synthes (US)	HydroSet™ ⁷ Cranios reinforced® Fast Set Putty and Rotary Mix Norian Drillable	TTCP, DCPD, trisodium citrate Calcium phosphate powder and bioresorbable PLGA ⁹ fibers α -TCP, CaCO ₃ , MCPM and bioresorbable PLGA ⁹ fibers	H ₂ O, PVP ⁸ , sodium phosphate Sodium hyaluronate solution Sodium hyaluronate solution	Apatite Apatite Apatite
Teknimed (FR)	Nanogel®	HA nanoparticles	H ₂ O	Apatite
Walter Lorenz Surgical ² (DE)	Mimix™ Quick Set Mimix™	TTCP, α -TCP, trisodium citrate (C ₆ H ₅ O ₇ Na ₃ ·2H ₂ O) Calcium phosphate powders, Na ₃ C ₆ H ₅ O ₇ ·2H ₂ O	H ₂ O, citric acid (C ₆ H ₈ O ₇) Citric acid aqueous solution	Apatite Apatite

¹ BCP = biphasic calcium phosphate (composite between HA and β -TCP);

² Nowadays part of Zimmer Biomet (US);

³ β -BSM (injectable) and γ -BSM (putty) are formulated based on the α -BSM chemistry but differ in powder processing[53];

⁴ Nowadays part of Ipmagna (AR);

⁵ Nowadays Medtronic (US);

⁶ Nowadays HOYA Technosurgical Corporation (JP);

⁷ Previously commercialized by Leibinger (DE);

⁸ PVP= poly (N-vinylpyrrolidone);

⁹ PLGA=poly(lactic-co-glycolic acid).

bution, L/P ratio, and liquid viscosity [51,64–66]. High L/P ratios lead to increased injectability, but decreased mechanical properties. Therefore, minimizing L/P ratios while keeping high injectability levels is the key point to develop high strength CPCs. **Cohesion**, on the other hand, is the property of the paste that determines whether the cement is able to set in a fluid without disintegration. It has been described that CPCs have poor cohesion properties, which can lead to leakage of CPC particles into bloodstream or blood clotting. CPC cohesion is related to the interaction between the CaP particles within the CPC. Different approaches to improve CPC cohesion include reduction of the CaP particle size [67] or using a CPC paste with high viscosity [33,68]. The latter can be achieved by incorporating water-soluble polymers into the CPC paste, such as carboxymethyl cellulose (CMC) [69] or poly (N-vinylpyrrolidone) (PVP) [70].

One of the main drawbacks of CPCs is the poor **mechanical properties**. A comparison between the mechanical properties of bone and CPC is shown in Table 2. The strength values given there show that the mechanical properties of CPCs are consider-

ably lower than those of bone, especially when comparing to cortical bone. Furthermore, CPCs have relatively low bending/flexural strengths. Due to their brittleness, CPCs undergo a sudden and total failure at a relatively small strain. These problems have led to the use of CPCs mainly for non-load bearing applications. In order to be possible to use CPCs in load bearing applications, research efforts are put into the improvement of their mechanical properties. Since they can harden at room temperature, reinforcement of the cements by incorporation of different biocompatible fibers and meshes into the CPC formulation is possible. One of the most successful approaches to toughen CPCs is the reinforcement with fibers. These fibers are usually made of resorbable materials such as polylactic acid (PLA) [71], poly(lactic-co-glycolic acid) (PLGA) [72], poly(vinyl alcohol)(PVA) [73], gelatin [74] or chitosan [74], but non-resorbable materials such as glass [75] or carbon [76] are also occasionally used.

Further, a general disadvantage of CPCs is their lack of macroporosity. Consequently, their biodegradation is very slow and takes place gradually from the outer surface to the central part. In view

Table 2
Overview of mechanical properties of bone and CPCs [77–81].

	Cortical bone	Trabecular bone	Apatitic CPC	Brushite CPC
Compressive strength	90–230 MPa	2–45 MPa	20–83 MPa	1–24 MPa
Tensile strength	90–190 MPa	~ 50 MPa	<15 MPa	0.7–4.5 MPa

of this, the incorporation of macropores and improvement of their biodegradability is a key factor for the clinical application of these CPCs.

3. Biodegradability of CPCs

3.1. Degradation and porosity of CPCs

When CPC is applied in bone regenerative procedures, its complete degradation and replacement by new living bone is preferred. However, as mentioned earlier, the biodegradability of CPCs is quite poor. Ideally, the rate of biodegradation of CPCs is almost similar to the rate of new bone formation to allow for gradual restoration of the mechanical properties by the new bone tissue.

In vivo degradation can be achieved via two different routes; (i) passive degradation by dissolution of the ceramic matrix into the extracellular liquid, and (ii) active degradation due to cellular activity (i.e. osteoclasts, giant cells, macrophages). The rate of dissolution of the matrix (i.e. passive degradation) by the extracellular liquid depends on the properties of the CPC, such as the surface area, Ca/P ratio, the crystallinity or the solubility [82], or on local properties like pH or perfusion with bodily liquids [83]. Previous work [84,85] showed that the physical breakdown of CPCs can be related to ion dissolution and to particulate fragmentation due to loss in mechanical integrity. On the other hand, active degradation of CPCs is mainly mediated by giant cells and osteoclasts [86,87], but also macrophages are involved in the phagocytosis of the fragmented particles of the CPC [88]. Rae [89] reported that macrophages are among the first cells colonizing the surface of the CPC after implantation and he hypothesized that macrophages play a crucial role in biodegradation. Additionally, it is known that the biomaterial particles that are liberated from the CPC interact with immune cells leading to the release of inflammatory mediators [90] and when the macrophages encounter CaP particles, they attach and get activated to phagocytose these particles [82]. While macrophages play an important role in phagocytosis of small fragments and particles, osteoclasts are the cells responsible for active biodegradation of the CPC. These cells reduce the pH locally in the vicinity of the biomaterial, which results in degradation of CPCs *in vivo* [31].

Presence of pores in the CPC is beneficial for its degradation. Pores will allow fluid flow (perfusion in case of interconnected porosity), migration and proliferation of osteoblasts into the CPC, as well as vascularization. Furthermore, the presence of pores will improve the stability of the tissue-implant interface, as cells will have more surface area to proliferate and regenerate new tissue. Pores in CPCs can be classified on basis of their pore size, i.e. micropores (internal pore width <1 μm), mesopores (internal pore width 1–100 μm), and macropores (internal pore width >100 μm) [91,92]. CPCs present intrinsic porosity, due to their hardening mechanism, where a crystal growth occurs into needle-like or plate-like structures that create a microporous structure [93]. Microporosity of CPCs can be up to 60%, and it enlarges the surface area of the CPC, enables fluid flow into the CPC, and contributes to protein adsorption [94]. It has been shown that the microporosity can be tuned by adjusting specific processing parameters, such as particle size of the powder phase and L/P ratio. Espanol et al.

(2009) prepared cements with different percentages of open porosity (35–55%) by modifying the liquid-to-powder ratio and by using α -TCP powder of two different sizes (fine and coarse; 2.8 and 5.2 μm , respectively). They found that the reactivity of the powder increased with decreasing particle size, leading to a smaller pore size than when using bigger particle size. Additionally, maintaining the particle size, it was observed that at low L/P ratios the pore size diminishes due to the decrease of the space between particles in the blend [95]. Microporosity also depends on sintering temperature of the powder phase. It has been shown that CaP materials sintered at 1200°C show considerably less microporosity than those sintered at 1000°C and a significant change in crystal size [96,97].

Meso- and macroporosity, on the other hand, are considered when the pore size is greater than 1 or 100 μm , respectively. However, CPC does not intrinsically possess meso- or macroporosity, for which different techniques should be used to introduce porosity at these scales. These types of porosity are required, because they allow cell migration and proliferation of osteoblasts and mesenchymal cells as well as vascularization [98]. High porosity and large pore sizes are known to enhance bone ingrowth within CPC. For instance, Kuboki et al. demonstrated the need of porosity in synthetic materials for bone regeneration using porous and non-porous hydroxyapatite; while in porous CPCs osteogenesis occurred, no bone formation was observed when using solid particles [98]. The pore size generally considered adequate for bone regeneration is 100 μm , as smaller pores may result in ingrowth of unmineralized bone tissue or fibrous tissue and do not allow the ingrowth of blood vessels [99]. Nevertheless, it has been demonstrated that pores greater than 300 μm show enhanced osteogenesis in some cases [100,101]. On the other hand, others have confirmed that pores smaller than 100 μm also allow bone formation or ingrowth into a synthetic material [102–104].

Other important parameter regarding porosity of the CPC is the so-called “interconnectivity” of the pores. This parameter indicates to what extent the pores introduced into the CPC connect to each other. The pores may be interconnected or contain a “dead-end” [99]. Generally, CPCs with high interconnectivity are advantageous over those containing “dead-end” pores. The reason behind this is that interconnectivity provides an efficient way of fluid flow, and as a result migration and distribution of cells into the CPC, as well as facilitates blood vessel formation necessary for new bone formation and remodeling [105–107].

Porosity and interconnectivity calculations have been performed by different approaches that can be categorized in image-based and physical methods. Among the image-based methods, analysis by Scanning Electron Microscopy (SEM) or microcomputed tomography (micro-CT) images have been most commonly used. SEM images are analyzed with different software applications in order to measure porosity and pore size [108,109]. Micro-CT images have been used to transform 2D X-ray images of the CPC into 3D models, from which quantitative morphological data can be extracted [110,111]. Physical methods include e.g. gravimetry or mercury intrusion porosimetry. Total porosity can be calculated by gravimetry by relating the density of the material of which the CPC is fabricated and the apparent density of the CPC [112,113]. Mercury intrusion porosimetry involves infusing the CPC constructs with mercury under increasing pressure. This method can be used to measure open and closed porosity (by means of the volume of

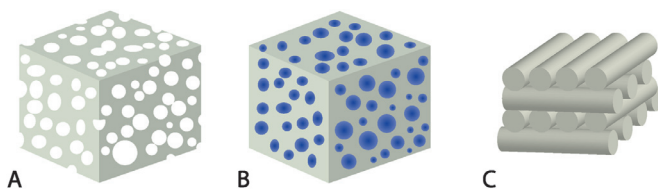


Fig. 2. Schematic representation of the different macroporosity creating techniques. A) Foaming agents; B) Incorporation of water-soluble or polymeric porogens and C) rapid prototyping.

intrusion of the mercury into the CPC) and pore size (using the rationale that as the pressure applied to the mercury increases, the radius of the pores that can be filled decreases) [113–115].

3.2. Enhancement of macroporosity of CPCs

As previously stated, CPC degradation is limited and this restrains the clinical use of CPCs. It is known that the presence of macroporosity enhances bone regeneration as well as favors CPC degradation. Therefore, there is a need for introducing macropores into CPCs. This can be achieved by foaming agents, rapid prototyping techniques or introduction of porogens (Fig. 2). However, it is important to mention that while an increase of macroporosity is crucial for bone regeneration, macroporosity simultaneously leads to a decrease in mechanical properties and, usually, to a modification of its handling properties. Therefore, this should be considered when designing a macroporous CPC in order to obtain a trade-off between macroporosity (and hence, bone regeneration) and other important material properties.

• Foaming agents

One of the possible methods to introduce macroporosity in CPC is through foaming (Fig. 2A), which is usually achieved by gas generation via a chemical reaction. Almirall et al. developed a technique to introduce oxygen macropores in an α -TCP cement paste by applying low temperature to hydrogen peroxide in order to decompose it into water and oxygen. They obtained high porosity (up to 66%) by controlling different processing parameters, such as L/P ratio and the concentration of the hydrogen peroxide solution [116]. Real et al. used an acid-based reaction between NaH_2PO_4 and NaHCO_3 to form CO_2 bubbles into CPCs. With this method, a porosity of up to a 50% was achieved [115]. A study in tibial metaphyses in goats showed significantly more bone formation for macroporous CPCs compared to controls (CPCs with no macroporosity). At 10 weeks, while the control CPCs still maintained their integrity, macroporous CPCs were almost completely degraded and new bone was formed [117]. Additional studies have demonstrated the bone regeneration capabilities of foamed CPCs in animal models, such as rats [118] or rabbits [119,120]. CO_2 bubbles can also be introduced into CPCs by producing an acid-base reaction between NaHCO_3 and citric acid monohydrate ($\text{C}_6\text{H}_8\text{O}_7 \cdot \text{H}_2\text{O}$). This method produced macropores of 100 μm in size and a macroporosity up to 21% [121]. Additionally, several studies have successfully used this technique and have created a prevascularized CPC via coculture of endothelial cells and osteoblasts that can be further used for bone regenerative applications [122,123]. Another possibility for obtaining foamed CPCs is by incorporation of surface active agents, which can be either natural (i.e. albumen [124] or gelatin [125]) or synthetic (i.e. polysorbates [126]). The latter have been thoroughly investigated and have shown to produce CPCs with high porosities (~70% [127]) that are osteoconductive [128] and osteoinductive [129].

The main benefit of introducing macropores by foaming agents is the easiness of the method. However, the final CPC constructs have in general low initial strength and it is difficult to produce

scaffolds with uniform characteristics. In most cases, because of the lower density of the gas in respect to the CPC, there is a risk of obtaining higher porosities at the top part of the CPC than at the bottom. Furthermore, direct *in vivo* injection is not recommended as the gasses can diffuse to the surrounding tissue and initiate adverse reactions.

• Rapid prototyping techniques

While most of the methods have issues regarding interconnectivity of the pores and reproducibility of the scaffolds, these limitations are overcome with rapid prototyping (RP) (Fig. 2C). RP includes different advanced manufacturing techniques that are based on an additive process in which complex structures are constructed layer-by-layer according to a computer model. Using this method, CPC scaffolds with highly controlled internal pore architectures have been obtained. One of the techniques that has attracted a lot of interest is 3D printing. While most 3D printed CaPs need a hardening treatment after printing [130], the self-setting capabilities of CPCs have been exploited to generate macroporous, 3D printed CPC scaffolds. Maazouz et al. (2014) developed 3D printed CPCs with porosities of 60–65 % and macroporosities of ~20 % by incorporating gelatin to improve printability [131]. Most recently, Barba et al. (2020) created 3D printed CPCs with similar porosity values and much higher macroporosities (~50 %) and implanted these into femoral bone defects. They observed that after 12 weeks, new bone formation was above 50%. However, material degradation was limited (i.e. ~10 %) [128].

The main benefit of rapid prototyping techniques, and most specifically, of 3D printing, is the high accuracy of the 3D model and the possibility of controlling the macroporosity to high extent. However, 3D printed CPC scaffolds are not injectable and hence cannot be implanted by minimally invasive surgery. Further, the initial bone-to-implant contact depends on the accuracy by which the bone defect is transformed into a 3D model, and the subsequent resolution of the 3D printing equipment; this likely leads to inferiority compared to injectable CPCs.

• Leaching of water-soluble porogens

A widely used method for enhancement of macroporosity in CPCs is the introduction of water-soluble particles in the CPC paste as porogens (Fig. 2B). These water-soluble porogens can be either salts, sugars or water-soluble polymers. The rationale of this technique is that after CPC injection into the defect, the porogen dissolves and generates porosity in the ceramic matrix. Depending on the solubility, interconnectivity and size of the particles, the porogen will be dissolved just after implantation or will gradually dissolve from the periphery to the center. The solubility of the porogen will also affect the final porosity and pore size. This was clearly observed when PVP, a water-soluble polymer, was incorporated as a porogen in different sizes and molecular weights. Smaller particle size and lower molecular weight led to an increased porogen dissolution and an earlier macropore formation [70].

One of the most common water-soluble porogens used is mannitol. Xu et al. introduced mannitol crystals into CPC to produce highly porous CPC constructs with a porosity up to 70%. However, as a consequence of obtaining such a porous matrix, the mechanical properties were 7 times lower than in the non-porous controls [132]. Therefore, a trade-off between porosity and mechanical properties should be achieved. Further studies demonstrated the biocompatibility of these CPC/mannitol systems *in vitro* [133] as well as *in vivo* [134]. Calcium sulfate (CS) has also been incorporated as a water-soluble porogen in CPCs. CS can be applied as bone substitute material in its own, but its high resorption rate has hindered its clinical use. This high resorption rate is the rationale to use CS as a water-soluble porogen that can rapidly dissolve and

create a macroporous CPC [135]. Interestingly, of all the commercialized CPCs (Table 1) only two of them were macroporous (i.e. Ossilix® MP, from Exactech® Biologics [136] and SKaffold™ ReNu from Skeletal Kinetics [137] and both used CS granules as porogens. Apart from these, other water-soluble porogens used are sucrose [138,139], glucose [140,141], NaCl [142], or Na₂HPO₄ [143].

The advantages of using water-soluble porogens is the ease as well as the possibility of directly injecting the CPC with the porogens. One important limitation is the need of incorporating high amounts of porogen in order to assure interconnectivity of the pores. This might compromise the biocompatibility of the CPC as well as worsen its mechanical and handling properties.

- Degradation of degradable polymeric porogens

Probably one of the mostly used methods for creating macroporosity is the addition of polymeric porogens to the CPC paste as a second solid phase (Fig. 2B). These polymeric porogens will start degrading after the CPC is set and will produce a macroporous CPC composite. The degradation of polymers will occur gradually throughout the matrix until the polymer is fully broken down into individual monomers. This degradation will depend on different parameters, including the rate of degradation or the molecular weight (Mw) of the polymer used. Polymers can be added as powder, microspheres or fibers and can be either of natural (i.e. gelatin, collagen, chitin or alginate) or synthetic origin (i.e. polylactic acid (PLA), polyglycolic acid (PGA), poly(lactic-co-glycolic acid) (PLGA) or poly-ε-caprolactone (PCL)).

Because collagen is the main organic component of the extracellular matrix of bone, the use of collagen as a porogen is of great interest. However, the degradation of collagen may take longer than the degradation of other polymers, so it is usually preferred to use alternatives. For example, gelatin is a polymer produced by partial hydrolysis of collagen extracted from skin, bones, and connective tissue. Among the natural polymers available, gelatin is one of the mostly used. CPC-gelatin composites have been produced in order to improve macroporosity and CPC degradation, and hence promote bone regeneration. Different amounts and sizes of gelatin microspheres have been studied. Gelatin microspheres were found to be able to degrade and provided in this way space for cells to penetrate and for new bone ingrowth [144–148]. In *in vivo* studies, gelatin microspheres incorporated into CPC have shown a gradual degradation, where microparticles located at the outside of the

composite were degraded before the inner microparticles. Such an erosive degradation mechanism fits the natural process of bone regeneration, while keeping the mechanical stability of the composite [146].

Synthetic polymers have also been explored as porogens. The polylactic/glycolic acid groups that appear in some of these polymers are interesting due to their biocompatible nature and degradation properties. In general, CPCs with different degradation rates can be obtained due to the possibility of tailoring the degradation rate of the polymer and the CPC/polymer ratio. The main drawbacks of these CPC/polymer composites are the low interconnectivity of the porosity as well as the poor mechanical strength. An approach that aims to overcome these limitations is the incorporation of resorbable fibers. Zuo et al. [71] introduced PCL and PLLA fibers into CPC, which produced macroporosity up to 30% once degraded. Other studies proved that the length of the fibers have an effect on degradation, with longer fibers providing a higher interconnectivity once resorbed [149,150].

Currently, the introduction of PLGA porogens within the CPC paste is one of the most promising approaches to enhance CPC degradation. The rationale for the incorporation of PLGA porogens in CPC is the generation of porosity upon their hydrolytic degradation into lactic and glycolic acid. These acidic monomers decrease the pH locally, which accelerates CPC degradation [110,151]. Depending on the type of PLGA, degradation starts at around 10 days after implantation and from then its molecular weight (Mw) decreases continuously until it is fully degraded 12 weeks after implantation [146]. In Fig. 3, a comparison on tissue regeneration capacity between plain CPC and a CPC/PLGA composites is presented. In Fig. 4, CPC/PLGA is compared to CPC and CPCs with different pore forming agents regarding their CPC degradation and bone regeneration ability. These data show a clear difference regarding bone regeneration and, specifically CPC degradation, depending on the implantation site, with trabecular implantation sites being more favorable for CPC degradation than cortical sites.

Multiple studies have already been done focusing on CPC/PLGA degradation both *in vitro* and *in vivo*. Several parameters of the PLGA porogens have been investigated in their role of CPC degradation. Link et al. and Lopez-Heredia et al. compared different porogen sizes in *in vivo* [153] and *in vitro* [154] models. The effect of PLGA Mw has also been investigated, showing PLGA with lower

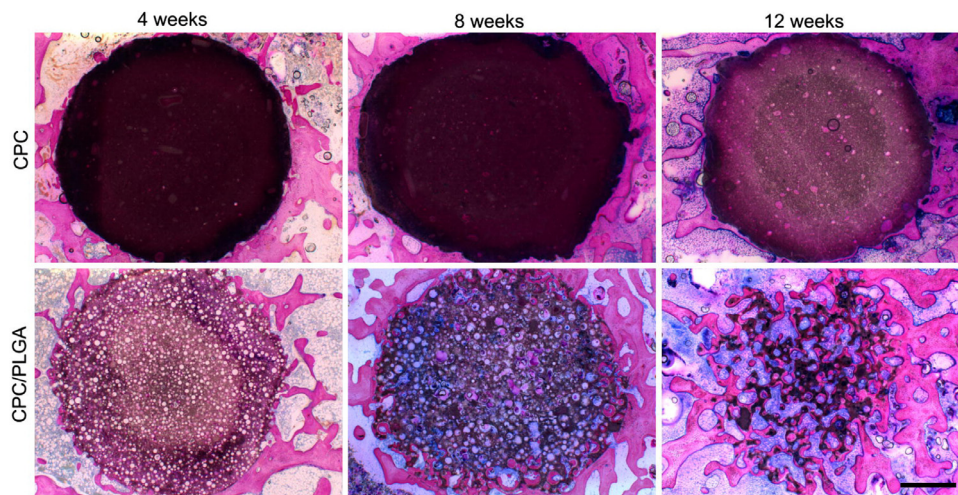


Fig. 3. Histological sections of a femoral condyle of a rabbit injected with plain CPC or CPC/PLGA composites, after 4, 8 and 12 weeks of implantation time. In the plain CPC implant, degradation and bone ingrowth is limited. In contrast, CPC/PLGA construct shows an increase in implant degradation and bone ingrowth from 4 weeks implantation onwards. At 12 weeks, all the PLGA microspheres have disappeared and most of the pores have been replaced by newly formed bone tissue. Scale bar represents 1 mm. Modified from Liao et al. (2011) [152].

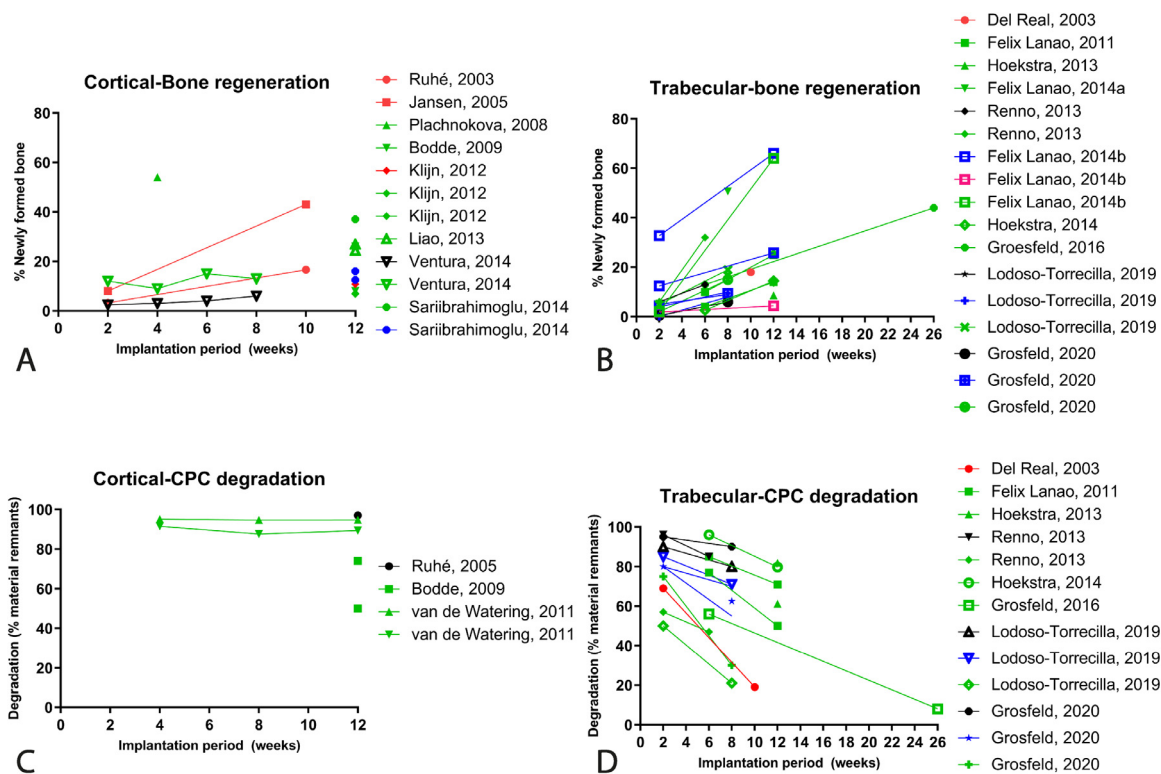


Fig. 4. Bone regeneration (%) (A and B) and CPC degradation (%) (C and B) compiled from different studies as a function of implantation period in weeks. Studies were differentiated depending on the implantation site of the CPC, cortical (A and C) or trabecular (B and D) bone. Different colors state for different CPC construct types; dense CPC (no porogens), CPC with porosity formed by CO₂, PLGA, water-soluble porogens and other degradable polymeric porogens (apart from PLGA). Data extracted from [102–104,111,117,119,138,140,151,155,158–167].

Mw more favorable for bone regeneration [155]. Félix-Lanao et al. investigated the effect of PLGA termination (i.e. acid-terminated vs. end-capped), PLGA morphology (i.e. hollow vs. dense porogens) and PLGA Mw on CPC degradation *in vitro* [110] and *in vivo* [151]. Furthermore, several studies have been dedicated to determine the optimal CPC/PLGA ratio to obtain a highly degradable and porous CPC while maintaining the biocompatibility. While in initial studies relatively low PLGA contents were used (e.g. 10–20 wt.%) [155,156], later studies incorporated up to 40 wt.% of PLGA, being still able to promote CPC degradation and enhance bone tissue formation [69,157,158].

Since the combination of PLGA porogens and CPC has already been established as a highly promising approach to enhance CPC degradation and bone regeneration, recent studies have focused on improving other aspects of CPCs, such as injectability [69], cohesion [168], mechanical properties [73] or short-term CPC degradation [162]. This short-term CPC degradation has been mainly investigated by incorporation of water-soluble porogens to these CPC/PLGA systems. It has been observed that this multimodal platforms achieve early-stage macroporosity thanks to water-soluble porogen dissolution and later stage macroporosity and CPC degradation due to PLGA degradation [70,139,169].

Finally, an additional advantage of PLGA porogens is that bone growth stimulating drugs can be incorporated into the microparticles, which will be released during the microparticle degradation and pore formation. Ruhé et al. were one of the first who introduced PLGA into CPC as both a growth factor delivery vehicle [159,165] and a porogen [170].

• Other methods

While enhancement of macroporosity is most commonly achieved by foaming, incorporation of porogens or, more recently, by rapid prototyping techniques, other methods have been ex-

plored. For example, macroporosity can also be introduced by creating an emulsion of the cement paste (hydrophilic) and an oil (hydrophobic) [171]. Depending on the volume fraction of each phase a water-in-oil (w/o) or an oil-in-water (o/w) emulsion can be formed, being the latter option the desired to generate a porous scaffold. Many studies show the feasibility of this process using different types of oil, such as olive oil [172], sunflower oil [172] or a paraffin-containing oil [173], among others. However, interconnectivity of the pores is not possible. Another possibility is the incorporation of cell-laden hydrogel porogens in the CPC matrix. These cell-laden hydrogels act in a dual manner; on the one hand they create a macroporous structure once the porogen has degraded, and on the other hand release cells that further enhance CPC degradation [174].

4. Concluding remarks

CPCs are highly appealing bone substitute materials, as they can be applied using minimally invasive surgery and molded to fill irregular bone defects. As such, there are some commercially available CPCs. However, the lack of macroporosity is an important drawback of CPCs and limits their clinical use, especially regarding apatitic CPCs. Therefore, extensive work has been performed in the last years in order to enhance this macroporosity. Efforts have been focused on the following approaches; foaming agents, rapid prototyping or incorporation of water-soluble or polymeric porogens. While all techniques can create macroporosity, incorporation of PLGA seems the most appealing approach. However, PLGA hydrolysis requires days-weeks to initiate, for which water-soluble porogens could be combined with PLGA to obtain a 2-type porogens CPC composite with a 2-stage degradation profile. Nevertheless, it is important to emphasize that while enhancement of CPC macroporosity is of utmost importance for bone regeneration, it

may lead to a decrease in handling and/or mechanical properties. Therefore, a correct fine-tuning between macroporosity and other important material properties must be performed.

Declaration of Competing Interest

The authors declare no conflict of interest and no benefit of any kind was received directly or indirectly.

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