Advancements in Brushite cement formulations for bone repair

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Chapter 2: Innovations for Brushite Cements toward applications in Bone Regeneration and Drug Delivery

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Introduction

Due to the increase in human life expectancy, a growing number of people are facing various health challenges, particularly those associated with aging [52-54]. In the Netherlands alone, >13,000 people were diagnosed with a bone disease in 2021 [55], while larger populations, such as that of the United States, have reported 124.1 million adults to be affected by a musculoskeletal medical condition in 2015 [56]. These numbers of patients are projected to reach 15-24% of the population over 65 years old by 2060 [55, 56]. Among all diseases and disorders patients face, bone-related problems have become one of the most severe conditions: traumatic fractures, deteriorating joints, age-related osteoporosis, and bone defects resulting from different pathologies have significantly and increasingly impacted individuals' daily lives. The treatment of large bone defects, which cannot heal spontaneously and hence require the implantation of suitable bone grafts at the defect site, has become a challenge [52, 53]. Aside from obvious orthopaedic examples comprising spinal and extremity bone defects, also oral surgeons are confronted with multiple pathologies causing bone defects in the oral and maxillofacial region that need grafting for treatment: alveolar bone reconstruction [57], continuity defects [58], midface/orbital reconstruction [59], and congenital craniofacial deformities like Treacher-Collins syndrome. In addition to only causing bone defects, these pathologies also affect aesthetics and functionality [60].

In surgical procedures, a range of bone substitutes are available for use, including autografts, allografts, and synthetic materials. Over the last decades, there has been a substantial increase in the number of new bone substitutes available as alternatives for autografts in bone regenerative treatment. Despite this expanded range of bone substitutes, autologous bone grafting is still considered the gold standard, as it provides the essential elements for bone growth to achieve the biological performance reflected by osteoconduction, osteoinduction, and bioactivity (Table 1).

Table 2. Biological performance parameters for bone grafting materials.

Parameter	Definition	Reference
Bioactivity	Ability of a material to achieve direct bone apposition without an intervening layer of soft tissue	[61, 62]
Osteoconduction	Capacity of a material to allow bone growth along its surface, which in the biomaterial field involves providing a biocompatible surface structure that promotes the migration of cells on it (for example: mesenchymal cells)	[63] [64]
Osteoinduction	Capacity of a material to initiate *de novo* bone formation	[64]
Osteogenesis	Synthesis of new bone by cells obtained from the graft or the host	[65]

In more detail, bone tissue is the unique material to use for bone regenerative treatment, as the tissue contains the 'house' (extracellular matrix (ECM) with the proper composition), 'guests' (all relevant cell types normally present in bone tissue), and 'guidance' (growth factors and instructive elements in ECM components) [1, 66]. A major disadvantage of autografts is donor site morbidity [67]. While the harvest of donor bone, commonly at the iliac crest, is considered a safe procedure, it can lead to chronic pain, superficial infection, bruising, and lateral femoral cutaneous nerve injury, resulting in paraesthesia and gait disturbances [67].

The associated morbidity and the limited supply of autografts has led to the search for synthetic materials as an alternative [30]. Among these alternatives, calcium phosphates (CaPs) have gained prominence due to their biocompatibility and bioactive properties, making CaPs a viable option in the field of bone regeneration [68, 69]. CaPs can be used in various applications of orthopaedic and maxillofacial surgery [70, 71], whether for bone substitution in bone defects, augmentation of the alveolar ridge, middle ear implants, or fusion of spinal vertebrae [72]. CaPs can be applied in different forms, such as granules, variously shaped blocks, or as cements that set during their application [73, 74]. Notably, calcium phosphate cements (CPCs) offer a distinct advantage, as these can be prepared as a paste that can be injected and sets in a few minutes. This means that such injectable pastes can be applied via minimally invasive surgical techniques and moulded to easily adapt to the shape of the bone defect [75]. On top of that, CPCs have shown their potential as drug delivery systems for a variety of active compounds, including antibiotics that are critical after any surgical intervention. Such local application avoids the challenges of systemic antibiotic treatments, which are less effective at specifically targeting the infected surgical site. [76]. CPCs with brushite as the main component (bCPCs) are even more advantageous than apatitic CPCs, as brushite is faster resorbed, which is interesting from a regenerative medicine perspective to achieve complete replacement of the material by newly formed bone tissue [77].

Bone

Bone is composed of a unique extracellular matrix and several types of bone cells therein. Hierarchically, bone is present either as dense, cortical bone, or as spongious, cancellous bone. The characteristics of the bone are given by the composition of the bone matrix [78]. The bone matrix consists mainly of two components: organic material (approximately 35 wt% of the matrix) and inorganic material (constituting about 65 wt% of the matrix). The organic material includes predominantly collagen and proteoglycans, providing flexibility and strength to the bone matrix. On the other hand, the inorganic component comprises predominantly a non-stoichiometric

hydroxyapatite [79] (stoichiometric formula: $Ca_{10}(PO_4)_6(OH)_2$) which is calcium-deficient and contains carbonate. This combination of organic and inorganic constituents is responsible for the main characteristics of bone. [78, 80]. Additionally, bone marrow occupies the intramedullary space and the intertrabecular space in cancellous bone and regulates the origin and activity of the bone cells [81]. The bone marrow contains mesenchymal stem cells that differentiate into bone-regenerative osteoblastic cells under the influence of specific bone growth factors, cytokines, and hormones [81]. There are three main types of specialized cells in bone tissue: osteoblasts, osteocytes, and osteoclasts, each with distinct functions and origins.

Osteoblasts are of mesenchymal origin and characterized by the presence of an extensive endoplasmic reticulum and numerous ribosomes. Their primary role is producing collagen and proteoglycans, essential components of the bone ECM. Additionally, osteoblasts form vesicles that accumulate calcium ions (Ca^{2+}), phosphate ions (PO_4^{3-}), and various enzymes, which are used to start and propagate the formation of hydroxyapatite crystals. These processes result in the formation of a mineralized bone matrix, in which the mineral aligns with collagen fibrils [82]. Osteoblasts actively participate in bone formation, known as ossification or osteogenesis.
Osteocytes, derived from osteoblasts, represent the fully matured form of osteoblasts, but with the function of orchestrating bone homeostasis: osteocytes are mechanosensitive and capable of transducing mechanical signals into chemical signals through their lacuna-canalicular system within the bone extracellular matrix [83] [84]. This interconnected system of osteocytes regulates both bone formation and resorption during bone remodelling, meaning that osteocytes control the activity of both osteoblasts and osteoclasts. The osteocytes form a large network of cells that are connected through canaliculi [85]. They respond to mechanical stimulation to balance bone remodelling and formation in such a way that they make the bone stronger where necessary.
Osteoclasts are large, multi-nucleated cells responsible for bone resorption or bone breakdown. Originating from hematopoietic stem cells, osteoclasts form by the fusion of 2 or multiple macrophages. In the areas where the plasma membrane of osteoclasts comes into contact with the bone matrix, they form ruffled borders. These borders facilitate the pumping of hydrogen ions through this edge and cause a local decrease in pH of a secluded area that triggers the decalcification of the bone matrix. Osteoclasts are also involved in the resorption of bone by releasing enzymes that normally break down the thin layer of organic matrix covering the bone. Once this layer is removed, the osteoclasts come into contact with the mineralized bone, accelerating resorption [78]. Recently, osteoclasts have been implicated with a role in stimulating bone formation via secretion of extracellular vesicles that signal to bone forming osteoblasts via receptors on the surface [86] or their cargo [87].

Bone repair process

Following tissue damage resulting in a bone defect, a repair process is initiated. This process consists of four basic stages, in which multiple cell types are involved (see Figure 1 for a schematic representation of the bone repair process).

1. Following a bone defect, the damage of the blood vessels provokes the formation of a localized mass of blood confined within an organ or space, known as a hematoma. Normally this blood forms a clot, which consists of fibrous proteins that stop bleeding, however, the tissue adjacent to the defect site is further affected due to inadequate blood circulation.
2. At the bone defect site, a callus is formed, surrounding the defect. The formation of this callus is established via blood vessels growing into the blood clot, disintegration of the blood clot, and fibroblasts producing collagen and other extracellular molecules to form granulation tissue. The chondroblasts begin to produce cartilage in the fibrous network, while the osteoblasts produce new bone that contributes to the internal callus.
3. Callus ossification occurs when the cartilage in the external callus is replaced by cancellous bone tissue. Finally, this process occurs in the internal callus, which is replaced by cancellous bone tissue that further stabilizing the bone defect.
4. Bone remodelling is a crucial step in the bone repair process. Even after the internal callus or cancellous tissue is formed, the bone remains structurally unstable, so the final process of bone repair is only completed when the cancellous tissue is replaced by cortical bone [53, 78, 88]. This remodelling process takes time, and even after a year, it may not be complete.

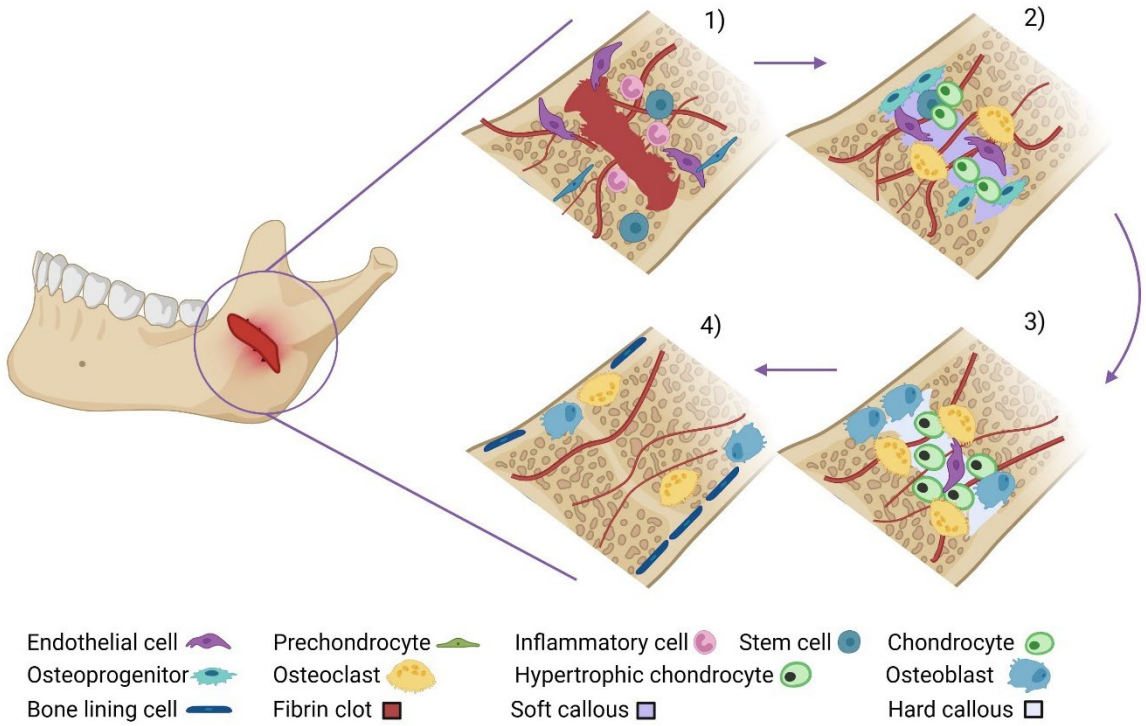


Figure 1. Schematic representation of the fundamental steps of bone healing and cellular activity therein: 1) Hematoma formation; 2) Soft callus formation; 3) Callus ossification; 4) Bone remodelling. (Created with BioRender.com)

Materials for bone regenerative applications

Regenerative materials for bone substitution are categorized as materials of natural origin and synthetic materials [89]. The natural materials, especially autografts, provide a conducive matrix for bone regeneration. One of the main natural origin materials used is demineralized bone matrix (DBM), which is an allograft [90] that consists of acid-treated bone that still contains the organic matrix and embedded growth factors. Due to the presence of the growth factors, DBM has shown the capacity to retain osteoinductive capacity [91]. However, the application of natural origin materials is limited by the relative scarcity of bone tissue, particularly for autografts [92]. On the other hand, in the case of allogeneic or xenogeneic bone tissue (i.e. human or animal, respectively), it also implies the possibility of immunological rejection, and can serve as a transmission route for diseases such as AIDS and hepatitis [93].

Significant research efforts have been dedicated to the development of synthetic materials that can effectively replace or repair bone defects by eliciting a favourable biological response and maintaining functionality. Among the multiple types of synthetic materials used in bone regeneration are calcium phosphate ceramics (CaPs) (Table 2), polymers, and CPCs. Synthetic materials used to regenerate bone defects must meet some essential minimal requirements to

obtain a favourable response from the body: biocompatibility and bioactivity. In addition, it can be required that the material possesses mechanical properties appropriate for the site where it will be implanted while maintaining the functionality of the skeletal system [94]. The material must be able to undergo osseointegration and complete resorption to facilitate full regeneration of the affected area in the most efficient way possible [95].

Table 3. Calcium phosphate compounds and their major properties (modified from [53])

Name and Abbreviation	Chemical Formula	Ca/P Ratio	Solubility at 25^{O}C (mg/L)
Amorphous calcium phosphate (ACP)	-	1.5	25.6-32.8
Hydroxyapatite (HA)	$Ca_{10}(PO_4)_6(OH)_2$	1.67	~0.3
Calcium-deficient hydroxyapatite (CDHA)	$Ca_{10-x}(HPO_4)_x(PO4)_{6-x}(OH)_{2-x}$	1.5-1.67	~9.4
Dicalcium phosphate anhydrous (DCPA)	$CaHPO_4$	1	~48
Dicalcium phosphate dihydrate (DCPD)	$CaHPO_4{\cdot}2H_2O$	1	~88
Monocalcium phosphate monohydrate (MCPM)	$Ca(H_2PO_4)_2{\cdot}H_2O$	0.5	~18,000
α-Tricalcium phosphate (α -TCP)	α -$Ca_3(PO_4)_2$	1.5	~2.5
β-Tricalcium phosphate (β -TCP)	β -$Ca_3(PO_4)_2$	1.5	~0.5
Fluorapatite (FAp)	$Ca_{10}(PO_4)_6F_2$.	5.5-6.0 [96]	~0.092 [96]

Calcium phosphate cements (CPCs)

CPCs have garnered significant interest for biomedical applications, particularly as bone regenerative materials. Unlike other biomaterials, CPCs can trigger the repair of bone defects in a more permanent way [21, 22] by promoting the formation of new bone tissue in tight balance with the CPC being degraded [23]. CPCs also present biocompatibility and osteoconductivity [24-26]. The most common CPCs on the market include HA-based (Norian SRS® (Synthes, USA) [27], Kyphos® (Kyphon, USA) [28], or Biopex-R® (Hoya Corp., Japan) [29]) [30, 31] and brushite-based CPCs [18] (ChronOs® Inject (Synthes, USA) [32], Eurobone (Group FH ORTHO, France) [33]).

Compared to other CaP ceramics, the use of CPCs offers several advantages. CPCs adapt perfectly to the bone defect dimensions regardless of their shape and can be injected into the body using minimally invasive techniques [16, 18, 19, 51]. However, the CPC formulation must be carefully chosen to ensure appropriate paste cohesion and proper injectability, which guarantees that the CPC moulds itself to the site where it is applied [20].

The final product of apatitic CPCs is hydroxyapatite (HA) or calcium-deficient hydroxyapatite (CDHA). This transformation occurs in an aqueous medium with a pH above 4.2. The resulting apatite, characterised by low crystallinity, closely mimics the mineral phase of bone [12, 35, 36]. Alpha-tricalcium phosphate (α-TCP) typically acts as the main constituent in these apatitic CPCs, where the particle size and crystallinity determine the degree of reactivity. Notably, lower crystallinity results in higher reactivity [13]. Reducing the particle size increases the contact surface, thereby enhancing the material's reactivity [12, 14, 15]. The solubility of the apatitic CPCs is relatively similar to the mineral component of bone. However, although they degrade faster than stoichiometric HA, the rate of degradation is slower than that exhibited by brushite CPCs [97, 98].

Another widely explored material in the field of dental treatment is fluorapatite (FAp) [99-101]. Fluoride ions are commonly included in toothpaste to enhance enamel strength and provide protection against cavities [99-101]. Several studies have shown evidence that the release of fluoride ions directly influences the differentiation, morphology and attachment of the osteoblast cells [102]. Furthermore, fluoride ions in the FAp crystal lattice structure have shown to prevent bacterial growth [102, 103].

Brushite CPCs

Contrary to apatite, brushite is characterised by being metastable in a physiological environment, which results in a much faster resorption [35, 41, 104]. CPCs are usually resorbed through two mechanisms: active or passive resorption. In the former, cells such as osteoclasts or macrophages intervene, while the latter is based on dissolution in body fluids [35].

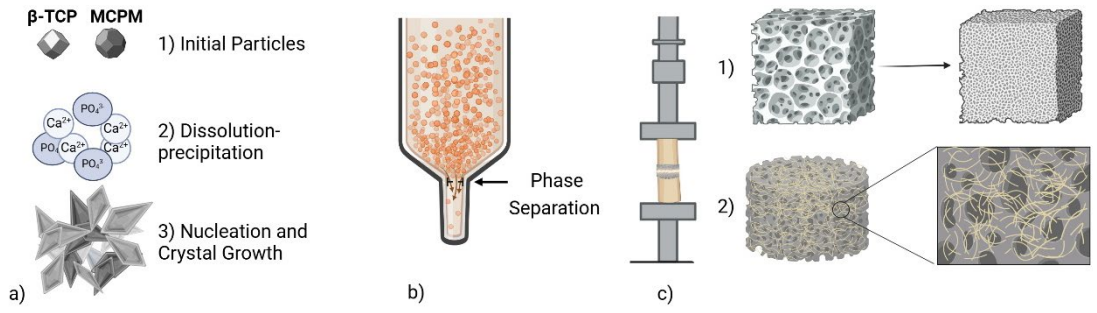


Figure 2. Handling properties of the bCPCs: a) Setting process from initial particles until the growth of the new phase crystals; b) Injectability (liquid separation during the extrusion process); c) Mechanical properties, methods to improve it, 1) Decreasing the CPC pores size; 2) Adding polymeric materials for reinforcement (Created with BioRender.com).

Setting reaction of bCPCs

In 1989, Mirtchi *et al*. [21] mixed monocalcium phosphate monohydrate (MCPM) and β-tricalcium phosphate (β-TCP) with water, obtaining brushite, chemically known as dicalcium phosphate dihydrate (DCPD) (Equation 1) [16, 21, 48], with a tensile strength between 0.1 and 1.1 MPa.

$$\beta\text{-}Ca_3(PO_4)_{2\,(s)} + Ca(H_2PO_4)_2{\cdot}H_2O_{\,(s)} + 7H_2O = 4CaHPO_4{\cdot}2H_2O_{\,(s)} \quad (1)$$

The setting reaction that gives rise to the solid consistency of bCPC occurs in three stages: dissolution of the reagents, nucleation of a new phase (brushite) and the growth of crystals (Figure 2a). Consequently, setting of bCPC is a dissolution-precipitation reaction. During dissolution, calcium and phosphate ions are released which generates a supersaturation of the solution and when the critical concentration value is reached, nucleation occurs in a new phase, forming crystals, generally around the precursors particles [35, 36].

Setting time

In practical terms, the setting time is crucial as it reflects the amount of time a surgeon has to apply and manipulate a CPC prior to its setting. Therefore, for effective use of CPCs, the initial setting time (which indicates when the CPCs paste starts to lose its plasticity) should be between 3-8 minutes, while the final setting time (the complete hardening of the CPCs) should not exceed 15 to 18 minutes for proper clinical implementation [37-40].

In this regard, one of the main limitations of bCPCs is their short initial setting time, generally between 30 and 60 seconds. In order to increase this time for suitability in surgical procedures, several strategies have been adopted, usually in combination. The first approach involves increasing the liquid-to-powder ratio (L/P). This, however, can affect the mechanical properties of the material, as it is related to the porosity that the set bCPC will have (Figure 3). The second strategy engages the incorporation of a CPC setting retarder in the liquid phase, such as sodium pyrophosphate, sodium citrate, pyrophosphoric acid, tartaric acid, or citric acid [16, 105]. These additives modify the setting time by delaying the formation of brushite crystals, affecting the dissolution of the reagents, or both. Organic acids such as citric and tartaric acid, have the ability to bind to calcium ions and inhibit the formation of crystals, slowing down the setting speed. On the other hand, citrate ions can interact with the β-TCP particles, limiting their dissolution, and hence delaying CPC setting [17]. The optimal balance between the L/P ratio, the concentration of the retarder, and the mechanical properties of the obtained bCPC has been extensively investigated. The results of studies on this topic have led to the commercialization of bCPCs, such as ChronOS™ Inject and Eurobone [33].

Injectability

Injectability is a significant property of bCPCs, as it enables a surgical application via minimally invasive procedures and ensures full conformity to the bone defect dimensions [36, 106]. The standard for measuring the injectability of a CPC relies on testing a paste's ability to be extruded through a syringe in a testing machine with a force of 100 N. Injectability is quantified by calculating the percentage by weight of the extruded paste [107-110].

The injectability of CPCs is limited by a phenomenon identified as liquid phase separation (Figure 2b), which results in the separation of the liquid phase from the solid phase during extrusion. Consequently, only the liquid passes through, leaving the majority of the solid material behind in the syringe [50, 75]. For bCPCs to be optimally injectable, it is important that the L/P ratio stays consistent during injection. It has been reported that several bCPC formulations exhibit phase separation upon injection, resulting in limited injectability [54, 107, 109]. This phase separation alters the final composition of the CPCs by changing the L/P ratio, which in turn impacts other properties [50, 111].

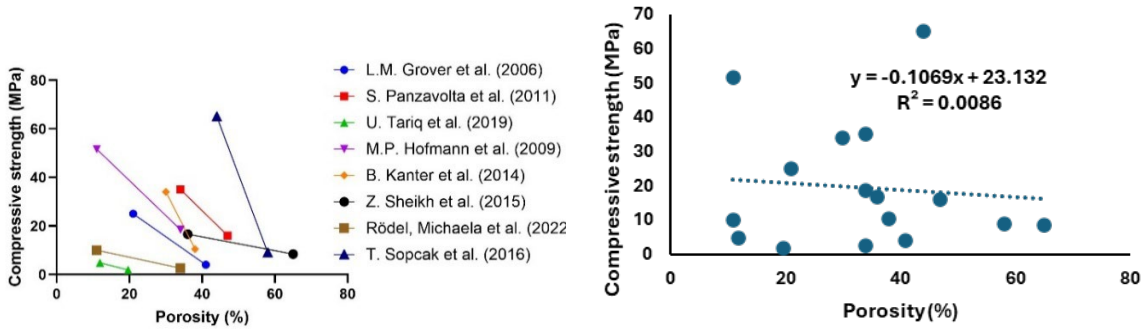
To enhance injectability, adjustments can be made both in the injection process and the bCPC composition. These include using different needle diameters or gauges [109], depending on the application. The factors associated with the composition of the bCPC encompass increasing the L/P ratio and the viscosity of the mixture, reducing particle-particle interactions or the particle size of the powder, and integrating sodium citrate or citric acid into the liquid phase [106]. Achieving a smaller particle size and a more spherical shape of the powder phase has proven to be effective [54]. However, these modifications may impact other important bCPCs properties. For example, an increased L/P ratio can significantly reduce the mechanical properties of the resulting bCPC after complete setting [112].

Mechanical properties and their optimization

Mechanical properties of bCPCs are known to be limited compared to cancellous bone [49]. Extensive research efforts have focused on improving mechanical properties. The use of several crystal growth retardants simultaneously causes higher compaction of the crystal, and hence an improvement in mechanical properties (Figure 2c), 1)) [113]. This densification comes with a reduction of porosity and several studies have probed an inverse relationship between porosity and mechanical properties (Figure 3). A search in PubMed[1] resulted in data used for the analyses on the relation between porosity and compressive strength are presented in Figure 3 and Figure

[1] PubMed search key words “brushite cements porosity” revealed a total of 49 results from which 40 articles were useful.

4 based on the criteria of showing numeric results of the porosity studies performed in each article.



A) B)

Figure 3. Correlation between porosity and mechanical properties of bCPCs within studies. A) Intra-study relation between porosity and compressive strength for bCPCs.)

Figure 3 shows the inverse correlation between porosity and mechanical properties for bCPCs across different studies. Each individual study shows that higher porosity leads to lower mechanical strength, which is a crucial consideration in the design and application of bCPCs [114-121]. In all retrieved data from the literature here, the main factor affecting the porosity and, ultimately the mechanical properties of the bCPCs, was the L/P ratio. However, this is not the only factor affecting porosity. After a wider search we found more elements to consider.

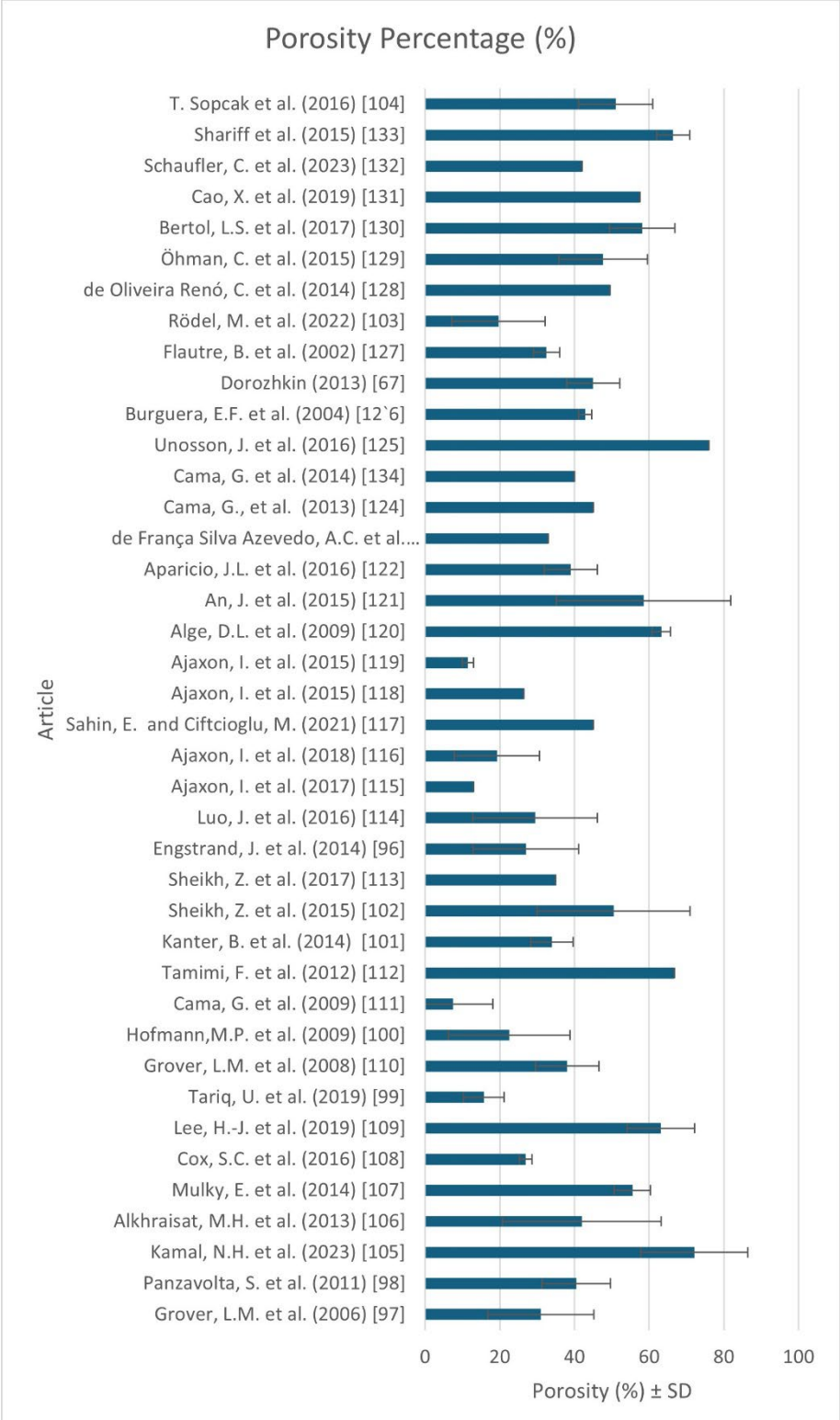


Figure 4. Porosity percentage in bCPCs analysed through a total of 40 articles. (Created consulting the references listed in the graph: [36, 47, 113-150], the percentages presented are the average of all porosity data for each publication, including standard deviation as error bar)

The average porosity across numerous studies ranges widely from 5% to 76% (Figure 4). This indicates significant variability in the porosity of bCPCs used in different contexts and

formulations. Many studies (e.g., Grover et al. [114], Panzavolta, et al. [115] , Tariq et al. [116]) consistently report that high porosity is related with low values of mechanical properties. This suggests a trade-off between porosity and mechanical strength that must be balanced depending on the application requirements. Additives such as chitosan-alginate, gentamicin, and adjustments in particle size significantly affect porosity. Lee et al. [126] found that addition of chitosan-alginate decreased porosity. Treatments like fiber reinforcement also impact porosity. For instance, Mulky et al. [124] observed a reduction in porosity after fiber reinforcement. Specific conditions like different degradation performance due to environmental exposure (e.g., medium been unstirred, stirred, or perfused) also influence porosity, as noted by An et al. [137].

Certain studies highlight ideal porosity levels for specific applications. For instance, de França Silva Azevedo et al. [139] found 33% porosity to be ideal for load-bearing applications in bone tissue injuries.

In general, we can infer that reducing the porosity of a bCPC improves the mechanical properties by increasing bCPC density. However, from a biological perspective, the reduction on the porosity could lead to a detrimental effect on cell migration into the inner parts of the bCPC.

Polymeric materials have been added to CPCs and specifically to bCPCs to improve some of their properties: injectability (as lubricants), cohesion, and mechanical properties (as reinforcement; Figure 2, c),2)) [18] [151]. Polymers, encompassing both natural and synthetic materials, are known for their versatile properties. Despite their structural complexity, polymers offer the advantage of being highly similar, and often nearly identical to the macromolecular substances found in the biological environment of the (bone) ECM [152]. They are or can be made degradable by the natural enzymes of living organisms, ensuring that permanent implants made from materials like cellulose, collagen, hyaluronic acid, and sodium alginate are metabolized through the body's physiological mechanisms [153]. Polymers can be integrated in bCPC in different states, as part of the liquid phase, or in the solid phase as powder, microparticles or fibers [154]. Several studies collectively demonstrate that the addition of specific polymers or polymeric fibers to CPCs/bCPCs significantly enhances their mechanical properties and suitability for medical applications such as vertebroplasty and kyphoplasty. Hyaluronic acid, especially of high molecular weight, improves the cohesion of bCPCs without negatively impacting their mechanical strength, particularly during the cement paste phase [155]. Other polymers, like xanthan and alginate, also contribute to better CPC cohesion by acting as flocculant agents, which influences properties like setting time and injectability, but increases the mechanical properties of CPCs [155, 156]. Composite bCPCs, particularly those reinforced with partially

acrylic matrices, provide a balance of mechanical strength and osteoconductivity, comparable to commercial bCPCs [157]. Additionally, the integration of poly(lactic-co-glycolic acid) (PLGA) fibers into bCPCs not only avoids adverse effects but also significantly boosts bone formation and mechanical properties, suggesting their potential for use in load-bearing bone defect treatments [158]. Poly(vinyl alcohol) (PVA) fiber reinforcement, particularly with decoated hydrophilic fibers, markedly improves the toughness and flexural strength of CPCs, introducing crack-arresting mechanisms that enhance their durability. However, the mechanical strength of these fiber-reinforced CPCs decreases over time due to biodegradation, although they continue to exhibit extensive plastic deformation [159, 160]. Finally, fiber-reinforced CPCs combined with carboxymethyl cellulose (CMC) demonstrate compressive strengths closer to natural vertebral bone, significantly outperforming traditional materials like PMMA, thereby making them more suitable for vertebral augmentation procedures [161]. Overall, these findings underscore the importance of selecting appropriate polymer or polymeric fibre additives to optimize the mechanical performance and clinical efficacy of CPCs in various orthopedic applications.

Collagen has been combined with bioceramics, hyaluronic acid and synthetic polymers on several occasions to improve mechanical properties and reduce susceptibility to degradation [162]. Collagen is the most abundant protein in mammalian tissues, making up a third of the entire protein mass in mammals. Its fibre forms the basis of the ECM in human bone, where minerals are incorporated in its structure. Additionally, collagen is a significant component of tendon and skin tissue, primarily providing mechanical strength to the tissue [162, 163]. This type of collagen is an organic component that makes up the ECM of bone and is widely accepted as a biocompatible substance that does not show any immunological effect [164-166]. For this reason, collagen has been widely used in support (biological scaffold) formulations including sponges, fibres, microspheres, and hydrogels. Its main applications are aimed at the regeneration of bone tissue, tendons, cartilage, and skin.

Sodium alginate has been utilized in CPCs due to its biocompatibility, biodegradability, and its ability to function as a reinforcement. The addition of sodium alginate improves both the cohesion and the injectability of a CPC paste, as well as its mechanical properties, raising the compressive strength to values of 20 MPa, compared to 14 MPa for the CPC without alginate [167]. Alginates are constituents of the cell wall of brown algae and are composed of linear block copolymers of β-D-mannuronic acid linked by 1-4 (M) and α-L-guluronic acid (G) [168]. Divalent ions form cross-links in the alginate joining the guluronic residues, which induces a sol-gel transition in the material [169]. Due to their abundance and affordability, alginates have been widely used in the food and pharmaceutical industries as thickeners, emulsifying agents,

binders, and disintegrating agents for tablet and capsule formulations. Due to their biocompatibility, alginates have been used in medical applications such as wound dressings, scaffolds for tissue engineering and hepatocyte cultures and surgical or dental impression materials, drug delivery, and cell transplantation, taking into account their ability to form a gel in the presence of divalent cations, for example, Ca^{2+} or Zn^{2+} [163, 169], forming what is called the egg-box model [170]. Alginate is commonly used as an encapsulation vehicle for cell xenotransplantation, particularly in therapies for conditions like diabetes, and as such serves as a drug delivery system. This acts as a semipermeable barrier that allows the diffusion of growth factors and other cellular signals to the host, although it protects the transplanted cells from immunological processes [171].

Degradation of bCPCs *in vivo*

Beside biocompatibility and adequate mechanical strength, CPCs should also possess the appropriate degradation rates for effective bone repair and regeneration. Numerous studies have shown that bCPCs can either remain stable or dissolve, releasing calcium and phosphate ions, depending on various conditions [172]. Table 3 provides characteristics of in vivo degradation studies of bCPCs resulting from a PubMed search[2].

Some bCPCs show rapid initial degradation within days to weeks, especially those modified with additives like PEG [173]. For instance, bCPCs implanted in vivo typically show substantial resorption within 8 weeks [105] (Table 3). However, complete resorption and replacement by newly-formed bone requires several months. Experimental work has shown nearly half resorption of the bCPC within 6 months [174].

bCPC degradation rates can vary depending on the implantation site and the specific conditions within the body, affecting the interaction with surrounding tissues and the rate of resorption [175]. bCPCs undergo bioresorption primarily through cell-mediated processes involving macrophages and osteoclasts [176]. This mechanism plays a crucial role in the gradual replacement of the bCPC with newly formed bone tissue. Understanding the degradation kinetics of bCPCs is essential for optimizing their clinical use. The ability to control degradation rates through formulation adjustments (e.g., additives) can impact the suitability of these bCPCs for specific applications, such as bone regeneration or drug delivery**.**

[2] A PubMed search with the key words “brushite cements degradation rate in vivo” retrieved a total of 10 results, from which 6 articles were selected. The selection process was based on presentation of quantitative results.

Table 4. In vivo degradation rate over time of bCPCs in different studies (for the calculations of the degradation rates per day, a constant degradation rate over time was assumed).

Study	Degradation rate (% per day)	Remaining amount of cement (% of total)	Time	Notes
Alkhraisat et al. (2010) [105]	1.5%	17% (bCPC with glycolic acid silica gel)	8 weeks	Slower resorption compared to bCPC with glycolic acid
	1.7%	3% (bCPC with glycolic acid)	8 weeks	Faster resorption
Rentsch et al. (2018) [174]	0.3%	53% (Cr 50)	6 months	Highest resorption among modified cements
	0.2%	72% (Unmodified cement)	6 months	Slower resorption
Kowalewicz et al. (2022) [177]	0.6%	4.63% (bCPC modified with Mg)	24 weeks	Significant material loss observed
	0.4%	35.14% (TCP)	24 weeks	Slower degradation
Gao, et al. (2024) [173]	7.4%	63% (bCPC@0.0% PEG)	5 days	Fastest degradation
	6.2%	69% (bCPC with 1.5% PEG)	5 days	Moderate degradation
	4.4%	78% (bCPC with PEG modified by carboxy acid)	5 days	Slowest degradation
Thao Le et al. (2022) [178]	0.5%	78% (CPC)	4 weeks	Lower degradation compared to CPC/CDHA20
	0.8%	85% (CPC/CDHA20)	4 weeks	Higher degradation
Jayasree et al. (2019) [176]	0.5%	86% (PB - normal bCPC)	28 days	Lower degradation compared to EB
	0.57%	84% (EB - egg shell bCPC)	28 days	Higher degradation

Drug delivery via bCPC

For several decades, the main forms of drug administration to the human body have included pills, capsules, potions, and injections [179]. Nonetheless, most of the treatments that aim to administer pharmaceutical compounds are based on the administration of these preparations systemically, resulting in abrupt fluctuations in drug levels in the bloodstream [180].

In recent years, considerable progress has been made in the development of new techniques for drug administration. These advancements not only control dosage and prolong therapeutic effects but also allow for precise targeting or "microlocation" of drug delivery to specific sites within the body [180]. This represents unquestionable advantages by drastically reducing the amount of drug delivered to the body and the repetition of unnecessary repetitive doses that could lead to an increase in adverse side effects in the patient as well as trigger the resistance of bacteria to antibiotics [76, 181].

CPCs are appealing as local delivery systems for drugs partially due to the fact that CPCs harden without requiring high temperatures, thereby preventing heat-induced drug denaturation [35, 38] [182]. Additionally, the porosity of CPCs helps to contain substances, including drugs, biologically active molecules and cells, and release them in the biological environment [20, 106] [49, 183]. For instance, CPCs can be used to locally deliver drugs that stimulate bone regeneration or to direct the action of the material to specific disorders or pathologies [35, 38]. To use CPCs as a drug delivery system, a wide range of factors must be considered, including the solubility of the drug and interactions with the CPC matrix, possible deterioration of this matrix, and the microstructure of the material, i.e. permeability, porosity and specific surface area [35].

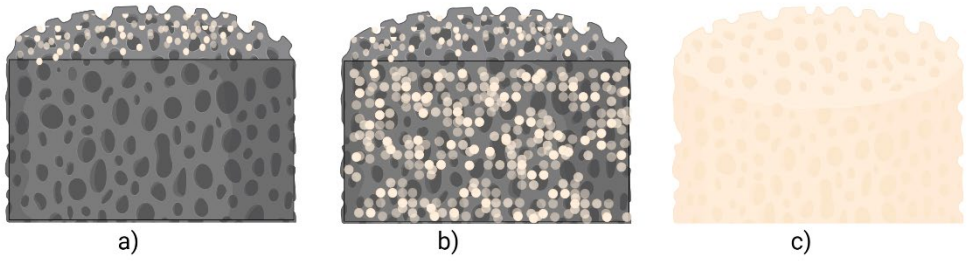


Figure 5. Distribution of drug within a CPC material. a) Only on the surface of the CPC, b) Distributed only in the inside of the CPC, generally while added in the solid phase, and c) Complete homogeneous distribution in all the CPC, generally while added in the liquid phase. (Created with BioRender.com)

The method of drug incorporation into the CPCs presents a challenge as it determines both the drug's distribution and its interaction with the matrix [184]. Typically, drugs are incorporated into CPCs either by mixing the drug powder with the solid phase or by dissolving it in the liquid phase. In both methods, the drug is distributed throughout the entire volume of the material, though incorporating it in the liquid phase tends to result in a more uniform distribution. [19, 35, 185] (Figure 5).

On the other hand, the addition of a drug can influence the setting reaction of the CPCs, whether in the liquid or solid phase, which affects the setting time and the development of the CPCs microstructure [35]. Obviously, the microstructure of the CPCs can also affect how the drug will be released after implantation [184].

Moreover, the mechanical properties of CPCs can be significantly affected by the introduction of a drug, potentially restricting their applications. However, this impact varies depending on the chemical nature of the drug molecule, making it difficult to predict the final effect on the material's mechanical properties [35]. Other properties can also be affected, such as release kinetics, as changes can occur in crystallization, crystal size and porosity [35].

Finally, the stability of the drug must be evaluated since the precipitation dissolution processes that occur during the CPCs setting process can affect the local pH and ionic concentrations of the solution. These changes may affect the drug's functionality and, consequently, influence its release process [35].

Another crucial aspect to consider is the drug release kinetics in CPCs, particularly because the release rate is often significantly lower than the rate of drug release in other systems. It is generally assumed that the release process occurs primarily through diffusion within the CPC matrix [35, 184]. A determining issue in the release kinetics is the distribution of the drug in the CPCs. It is not expected that the drug will be incorporated into the interior of the crystal lattice of the precipitated crystals. Only a minimal amount of drug would be incorporated in this manner, ensuring that the majority of the drug becomes entrapped within the entangled crystals [35, 184].

As any surgical intervention imposes the risk of bacterial infection, every intervention requires the use of antibiotics, generally in high doses and for prolonged times. Antibiotics are used prophylactically in the post-surgical period to combat potential infectious conditions, such as osteomyelitis, periodontal diseases or dental caries [186, 187]. Conventional approaches for antibiotic administration cause a range of side effects and an increase in bacterial resistance. These aspects have led to explorations on the use of CPCs as delivery systems of antibiotics in surgery for bone replacement and the repair of small bone defects in the field of traumatology or orthodontics [186]. Among the antibiotics most studied for these applications are the families of aminoglycosides, cephalosporins, glycopeptides, quinolones, and tetracyclines. For prophylactic treatment, it is important that the release of the antibiotic is fast enough so that it remains above the minimum inhibitory concentration (MIC), although the MIC is generally determined using *in vitro* experimental conditions that do not resemble clinical antibiotic treatment of infections. Concentrations below the MIC should be avoided for extended periods of time, as this can cause bacterial resistance. In contrast to acute release for prophylactic purposes, the treatment of infections such as osteomyelitis and periodontitis requires a long-lasting and sustained release period [35].

The incorporation of antibiotics into the CPC affects the physical, chemical, and mechanical properties. This causes prolonged setting times, and a decrease in mechanical properties in some cases. More specifically, tetracyclines can cause a decrease in the size of the crystals, affecting the microstructure of the CPCs [35]. Consequently, it is crucial to achieve a balance between the drug load and release rate to ensure effective bacterial eradication without compromising the overall performance of the CPCs.

For the use of bCPCs as local antibiotic drug delivery systems, Table 4 shows the results of a PubMed search[3]

Table 5. Antibiotics loaded in bCPCs and their release profiles.

Study	Antibiotic	Method of incorporation of the antibiotic in the bCPCs	Release Profile Summary
Taha et al. (2017) **[188]**	Gentamicin sulphate, Amoxicillin, Ampicillin trihydrate	Powder phase	Initial release: 47-65%. Strontium doping increased release to 73-96% within 72 hours. Bimodal release: burst followed by sustained release.
Cabrejos-Azama et al. (2016) **[189]**	Vancomycin	Method 1: Adsorption	Fast initial burst, then levels off over 6+ days. Cements with magnesium-modified TCP showed slightly faster release, with up to 81% release after 140 hours.
		Method 2: Powder phase	Two-stage release: high initial, then sustained. Magnesium modification resulted in a faster release, up to 98% within 72 hours.
Cox et al. (2016) **[125]**	Gentamicin sulphate	Liquid phase	~37% release from cement without implant. Lower burst release with antibiotic-loaded cement inserted in the implant.
Hofmann et al. (2009) **[117]**	Vancomycin, Ciprofloxacin	Powder phase	High porosity: 60-80% release within 24 hours. Lower porosity: slower, more sustained release.
Ren et al. (2021) **[190]**	Vancomycin, Tobramycin	Polymeric gel	Vancomycin: sustained release up to 28 days (~76%). Tobramycin: much lower release.
Jiang et al. (2010) **[191]**	Vancomycin Hydrochloride	Powder phase	~60% release in 120 hours.
Tamimi et al. (2008) **[192]**	Doxycycline	Liquid phase	80% release within 4 days, after an initial burst of 50% in 5 hours.
Dabiri et al. (2019) **[48]**	Gentamicin sulphate	Liquid and Powder phase	Three-phase release: initial burst (38-59%), secondary burst, plateau. ~91.5% release after 2 weeks.
Guardia et al. (2021) **[193]**	Erythromycin	Polymeric gel	Reduced burst release (32%), within the first 72 h. Sustained release up to 28 days (~90%).
Morilla et al. (2021) **[156]**	Tetracycline	Powder phase	The drug shows a burst release in the first 8 hours with the fastest release from the samples without alginate.

As shown in Table 4, different antibiotics have been incorporated into bCPCs, showing how different methods of incorporation and composition adjustments affect the drug release. For instance, Taha et al. [188] found that incorporating gentamicin sulphate, amoxicillin, and ampicillin trihydrate into the powder phase with strontium doping significantly increased the

[3] PubMed was searched with the key words “brushite cements antibiotic release;” this retrieved a total of 16 results from which 10 articles were included. The rest of the articles were discarded due to the use of other antibacterial agents, not traditional antibiotics.

release rates compare to the non-doped formulation, with gentamicin sulphate reaching 96% release within the first 72 hours. Cabrejos-Azama et al. [189] explored vancomycin release, noting that incorporation into the powder phase led to a two-stage release pattern with a rapid burst release followed by a sustained release, which varied depending on magnesium content. Similarly, Cox et al. [125] observed that gentamicin sulphate had a reduced initial burst release when incorporated via the liquid phase. Studies like those by Hofmann et al. [117] and Ren et al. [190] highlighted how porosity and the use of polymeric gels, respectively, influence release kinetics, with higher porosity leading to faster release and polymeric gels providing a more sustained release.

Adding tetracycline to bCPCs has several notable effects. Gbureck et al. (2007) [194] investigated the adsorption and desorption behaviour of various antibiotics, including tetracycline hydrochloride, with different CaP-based materials. They found that bCPC exhibited higher tetracycline loading (13 mg/sample), compared to monetite and HA. bCPC released about 25% of the drug over 5 days, following an initial burst release. The tetracycline forms stable chelates complexes with calcium ions, leading to a prolonged release compared to other antibiotics [156]. This release behaviour is influenced by physical properties like porosity and surface area, with brushite showing the highest release rate among the materials studied due to the fast degradation. Additionally, Tamimi et al. (2008) [192] reported that the addition of doxycycline to bCPC affects the final setting time by inhibiting crystallization, which can be beneficial for applications requiring extended working times. Doxycycline release patterns showed a substantial burst release (~50%), followed by a slower during 4 days. Still, 30% of the doxycycline demonstrated retention in the bCPC matrix due to chelate formation.

Overall, these studies demonstrate that antibiotics incorporated into bCPCs typically exhibit an initial burst release followed by a slower, sustained release, which can be tailored by altering the CPC's composition, porosity, or the incorporation method. This customization potential allows for bCPCs to be optimized for specific clinical needs, whether for rapid infection control or sustained antibiotic delivery.

Key Findings

Bone regeneration materials often encounter significant challenges in achieving optimal healing and complete defect regeneration. Among these materials, bCPCs stand out due to their notable biocompatibility and bioactivity. There has been growing interest in bCPCs, particularly because of their rapid resorption rates, which are considered to potentially enhance long-term clinical outcomes.

However, they also face significant challenges that have persisted over time, including rapid setting times that can compromise injectability, and suboptimal mechanical properties that may not meet the demands of bone regeneration. A range of strategies has been employed to improve these properties. These include the use of setting retarders to modulate the hardening process and the integration of polymers to enhance both mechanical integrity and handling characteristics. Moreover, the potential of bCPCs as drug delivery systems has been increasingly recognized. This feature is particularly advantageous for addressing complications related to surgical interventions, such as the risk of bacterial infections.

The ability of bCPCs to serve as localized drug delivery systems offers a strategic advantage, especially in the context of rising global concerns over antibiotic resistance. By enabling targeted antibiotic delivery locally at the surgical site, bCPCs reduce the need for systemic antibiotic therapy, thereby decreasing the potential for adverse side effects and mitigating the risk of developing antibiotic resistance.

In summary, the rapid resorption rates, and drug delivery capabilities of bCPCs significantly widen their importance in the field of bone regeneration. These properties not only support immediate post-surgical needs but also promise enhanced long-term outcomes through improved integration with host tissue and targeted therapeutic actions. As research progresses, optimizing the formulation and application of bCPCs remains pivotal to harnessing their full potential in clinical settings.

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