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Injectable macromolecule-based calcium phosphate bone substitutes

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Injectable bone substitutes (IBS) represent compelling options for bone regenerative medicine as they can be used to optimally fill a complex bone defect through minimally invasive intervention. Since their discovery, calcium phosphate (CaP) based IBS have never stopped evolving to match the diverse clinical needs. The main challenge is to combine the desired physico-chemical and handling properties of the IBS to an optimal induced biological response. This cannot unfortunately be achieved with CaP biomaterials alone, hence a growing use of polymers and organic macromolecules as additives. To properly understand the ins and outs, a didactic classification of IBS is proposed in this review, which compiles the past, present and future developments of IBS. Class I IBS, taking advantage of ceramic particles or granules as the support for bone formation, are already commercialized and widely employed in clinics. In contrast, Class II IBS, where cements serve as a stiff matrix for the development of mineralized tissues, associated with polymers, are still in their early stages but have shown significant improvements *versus* Class I products. These innovative Class II IBS will be the second focal point of this review.

1. Introduction

Despite its inert appearance, bone is a highly dynamic organ that exerts important mechanical (*e.g.*, a supportive frame, locomotion, and protection), metabolic (*e.g.*, homeostasis), and synthetic (*e.g.*, haematopoiesis) functions.[1] Unlike skin and other soft organs, bone has the innate ability to regenerate without scarring by means of complex biological cascades.[2,3] However, bone maintenance and/or healing can be impaired as a result of age,[4,5] lifestyle,[6–8] pathological conditions,[9–11] medical treatment,[12–14] and injury.[15–17] To help bone regeneration, strengthen skeletal integrity, stabilize an implanted bone prosthesis, or relieve joint pain, surgical strategies that are often coupled to bone grafting have been developed. As such, bone represents the second most frequently transplanted tissue after blood,[18,19] with musculoskeletal pathologic conditions affecting more than 100 and 120 million adults in the U.S.[20] and Europe,[21] respectively, (*i.e.* ≈50% and 25%, respectively, of the adult populations). To date, the vast majority of bone grafting procedures have been performed with autologous bone (*i.e.*, harvesting of the patient's own bone from a healthy site), with well-known associated limitations: additional burden for patients whose health is already compromised, infection risk, morbidity, graft resorption, weakness of the harvesting site, variable quality, limited availability, *etc.*[22,23] To improve the overall care of patients, most research has been geared toward the development of alternative treatments. Therefore, in the past 20 years, there has been increasing interest in synthetic bone substitutes designed for a given medical indication[24–29] or even a specific patient(s). Among these substitutes, injectable bone substitutes (IBS) have garnered considerable attention due to their tailorability and consequently a wide range of potential clinical applications.[24] Unlike the most common synthetic substitutes (*i.e.*, calcium phosphate porous blocks and granules), IBS have significant advantages of being fully adapted for minimally invasive procedures,[27,28] to perfectly fill complex defects and to self-set *in situ*, to eventually display "biomimetic" features, and to eventually include active molecules of cells in their formulations.

Most of the commercial IBS currently in clinical use are based on calcium phosphate (CaP) ceramic particles or sintered microporous granules often blended with the viscous solution of macromolecules (Table 1). Unfortunately, these formulations (referred hereafter as Class I) suffer from poor mechanical properties and can leak into the trabecular bone or outside the defect, as documented in the informative recent reviews of Lodoso-Torrecilla *et al.*[20] and Schröter *et al.*[24] To tackle these issues, extensive research has been carried out on composite self-setting, coupling inorganic cementitious phases (*e.g.*, calcium phosphates, CaP) to innovative biopolymers, which we

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Table 1 Class I and Class II injectable and extrudable commercialized materials currently on the market (TCP = tricalcium phosphate, HA = hydroxyapatite, CaP = calcium phosphate, CDHA = calcium deficient hydroxyapatite, HPMC = hydropropylmethylcellulose, rhBMP = recombinant human bone morphogenetic protein)

Commercial name and manufacturer	Material class	Inorganic phase	Organic phase	Application	Characteristics	Clinical trial number and reference
MBCP-Gel/In'Oss™ (Biomatlante)	I	CaP granules	HPMC polymer solution	Filling osseous defects of various origins	Ready to use, cohesivity/ putty	NCT00740311; (ref. 30)
ExcelOs-inject (CGBIO)	I	Beta TCP granules	Biodegradable hydrogel with or without rhBMP-2	Spinal fusion	Ready to use, injectable, cohesivity	NCT02714829; (ref. 31)
Mastergraft (Medtronic)	I	β-TCP + HA	Bovine collagen matrix	Spinal fusion	Putty	NCT01491542; (ref. 32)
Vitoss (Stryker)	I	β-TCP granules	Bone marrow aspirate	Spinal pathologies	Putty ultra-porous, flexible	NCT03509480; (ref. 33)
ChronOS (DePuy Synthes)	I	β-TCP granules	Autologous blood and/or bone marrow	Tibial plateau fractures	Putty	NCT02056834; (ref. 34)
ChronOS Inject (DePuy Synthes)	I	Brushite matrix and (TCP) granules		Proximal tibial fractures	Injectable, degradable	NCT02056834; (ref. 35)
CERAMENT® (Bone Void Filler)	I	HA and calcium sulfate cement		Tibial plateau fractures	Injectable, degradable	NCT01828905; (ref. 36)
Norian® Drillable (DePuy Synthes)	II	Carbonated apatite cement	Bioresorbable polylactide/ glycolide copolymer fibres	Tibial fractures	Mechanical resistance 24 hours after injection	NCT01132508; (ref. 37)
Graftys quickset/ HBS (Graftys)	II	CDHA cement	Powder of HPMC polymer	Bone disease, bone fractures	Porosity	NCT02575352; (ref. 38)

refer to as Class II. Very few Class II materials are currently in clinical use (Table 1), although the number of preclinical and research studies is increasing as a result of the use of "biomimetic" cement phases.

In this review, we discriminated Class I and Class II IBS and detailed their specifications and features in the first section. Besides, a particular focus is on Class II IBS and their future developments. IBS improvements achieved through the addition of polymers and macromolecules will specifically be highlighted.

2. Injectable bone substitutes (IBS): classifications

There is currently no classification to rank the existing and developing CaP IBS composites for bone regeneration. There are several classification options:

– The first way to sort them may be by their final formulation, *i.e.*, the composition of the inorganic phases (after setting for hydraulic cements) and the composition of a possible organic phase.

– The second way to classify them relies on their ability to remain in a cohesive viscous state (non-hardening IBS) or to set (hardening IBS); whether or not the organic (reticulation, physicochemical interactions) or/and inorganic phase (acid–base reaction) is responsible for the hardening mechanism.

– The third way may be their interface with the host environment, *i.e.*, the presence or absence of interconnected macropores (*i.e.*, pores >100 μm) in addition to micro- and nano-porosity.

– Finally, the fourth and more clinically oriented way could be based on their practicality in the operating room: 'ready-to-use' *versus* 'preparation required before use' composite IBS.

In an effort to harmonize the current nomenclature, we humbly propose the following classification based on the notion of Class I and Class II materials. Class I IBS, which are already in widespread clinical use, include CaP particles and sintered granules (ceramic) embedded in a non-hardening (polymeric liquid viscous solution) or hardening (polymeric viscous solution that becomes a hydrogel or mineral fast resorbable cement) matrix. Often, the matrix tends to be rapidly washed off or biodegraded, leaving only the particles as a scaffold for the development of new bone tissue. Still in preclinical development, Class II IBS associate the calcium phosphate cement (CPC) with organic phases; the cementitious inorganic phase setting *in situ* provides a mechanically stable and porous environment that becomes replaced by new bone over time. Detailed information about Class I and Class II composite IBS is provided in the sections below, as illustrated in Fig. 1, and their main characteristics are summed up in Table 2.

2.1 Class I IBS: ceramic particles in a matrix

2.1.1 Ceramic particles with a non-hardening organic matrix. This Class I subclass comprises ceramic granules of calcium phosphate (thermally sintered), which support osteoconduction, associated with a non-hardening organic matrix (polymer solution). The design of these injectable bone substitute (IBS) composites was first reported in early 1995 by Weiss *et al.*,[39] who aimed to combine the relevant biological properties of CaP-sintered granules with the viscous features of a hydrophilic polymer in solution, resulting in an injectable formulation.[40] More precisely, biphasic calcium phosphate macroporous granules (hydroxyapatite HAP/β-tricalcium phosphate β-TCP) were included in a hydroxypropyl methylcellulose (HPMC) matrix, thereby providing tailorable IBS (*e.g.*, HAP/β-TCP ratio, $\%_{wt}$ polymer).[41–43] Once *in vitro* assays confirmed

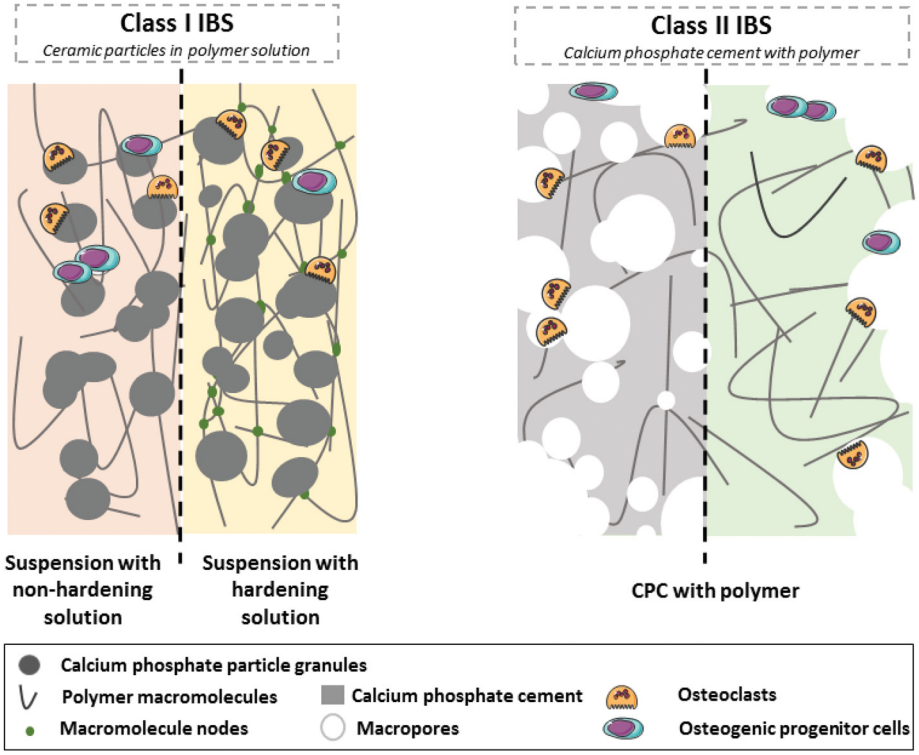

Fig. 1 The two classes of IBS present different osteoconduction mechanisms: for Class I, calcium phosphate supports are made of divided ceramics (thermally-sintered particles, resulting in high density materials); for Class II, calcium phosphate supports are made of bulk cements (soft chemistry route to interlinked particles, resulting in low-density materials showing macropores).

Table 2 Advantages and disadvantages of Class I and II injectable bone substitutes

Class	Class I			Class II
	Ceramic particles in the polymer solution			Calcium phosphate cement with polymer
		Hardening matrix		
Associated with...	Non-hardening matrix	Organic matrix	Resorbable mineral matrix	Polymer (hydrogel or solution)
Advantages[a]	• ↑ Injectability • ↑ Cell-material interactions • ↑ Cohesivity	• ↑↑ Injectability • ↑ Cell-material interactions • ↑↑ Cohesivity • No material leakage • Controlled release of substances	• ↑ Injectability • No material leakage • Stiff bioactive formulations • Biodegradation of the matrix • ↑↑ Mechanical properties	• ↑↑ Injectability • No material leakage • Stiff bioactive formulations • ↑↑ Mechanical properties • ↑↑ Osteoconductivity (*e.g.*, due to macroporosity) • ↑ Potential to stimulate bone growth • Controlled release of substances
Disadvantages[a]	• Material leakage • Low potential to simulate bone formation • Limited particle (bio)degradation • Poor mechanical properties	• Limited particle (bio)degradation • Low potential to simulate bone formation • Poor mechanical properties	• Low potential to simulate bone • Limited particle (bio)degradation	• Inadequate mechanical properties due to macroporous network

[a] Comparison *versus* pure inorganic cement.

the biocompatibility of this formulation,[44] the biological response induced by this ready-to-use injectable calcium phosphate ceramic suspension (ICPCS) was evaluated in various animal models.[26,45–55] Bone developed rapidly within the interconnected macroporous network of granules (as illustrated in Fig. 2), rapidly providing sufficient mechanical properties to

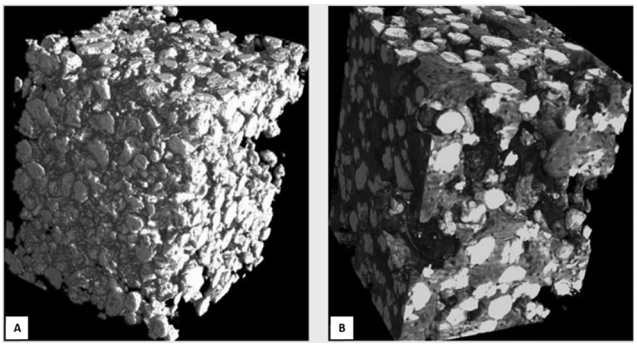

Fig. 2 (A) Reconstructed microtomographic images of IBS Class I BCP (size 40–80 μm) in the hydroxypropyl methylcellulose suspension before implantation and (B) after 8 weeks of implantation in a rabbit femoral defect. Newly formed bone (dark grey) developed extensively in the intergranular macropores between the BCP particles (white).[64]

this initially weak construct.[56] Alternative formulations have been developed, such as by either switching the inorganic filler with another natural version of synthetic calcium phosphate (*e.g.*), demineralized bone matrix[57–59] or by changing the nature of the viscous carrier (*e.g.*, hyaluronic acid,[60] gelatin,[61] or collagen[62]). Comparable overall bone regeneration was observed. Despite the interesting osteogenic properties, the main issues with suspension-based CaP particles are the problems related to cohesiveness (with potential material leakage), low mechanical properties, sterilization, injectability, and sedimentation.[63]

2.1.2 Ceramic particles with a hardening matrix. The Class I subclass comprises ceramic granules of calcium phosphate (thermally sintered), which support osteoconduction, associated with the hardening of an organic or mineral matrix. To overcome the limited initial cohesivity (leakage of the solution) and mechanical properties of non-hardening injectable bone substitute (IBS) composites, CaP sintered particles can be embedded in an organic material that hardens after injection (hydrogels). This hardening matrix can also be a mineral with quick resorption properties (*e.g.*, calcium phosphate[65] or calcium sulphate[66]).

2.1.2.1 Ceramic particles in an organic hardening matrix. The concept of using self-hardening hydrogels for the formulation of hardening IBS arose in the late 2000s, more particularly with the addition of pH- or temperature-sensitive reactive groups to polymeric macromolecules or modification of their chemistry through physicochemical processes.[67] Highly pure preparations of these organic macromolecules can be obtained by synthetic means (*e.g.*, polyethylene glycol) or biosourced (*e.g.*, chitosan, HPMC), with the chain length and modification (*e.g.*, nature, substitution rate) allowing tailored-self-setting hydrogels to be designed according to the intended applications.[68] For instance, a ''self-hardening injectable calcium phosphate ceramics suspension'' (SH-ICPCS) based on BCP-sintered granules associated with silated HPMC (which reticulate at physiological pH) has been developed.[39] Similarly, Hofmann *et al.*, in 2007,[69] formulated an SH-ICPCS with hydroxyapatite powders mixed with deacetylated chitosan and oxidized starch that forms a putty upon the addition of water. This has a significant advantage that the paste viscosity can be adjusted on the fly by the surgeon as required by the surgical procedure.

2.1.2.2 Ceramic particles in a resorbable mineral matrix. Calcium phosphate cements (CPCs) are well-known and widely used setting IBS, as described by the informative reviews of Schröter *et al.*[24] The hardening process often occurs within 20 min after mixing the reagents, leading either to a calcium-deficient apatitic phase or to a brushitic phase.[70] As such, CPCs can be used as a carrier for the injection of sintered CaP particles and serve as a hardening matrix after implantation (Fig. 3B). In addition to providing a more mechanically stable environment and preventing IBS washout from the filled bone defect, the high bioreactivity of CPCs allows them to serve as a primary anchor for bone formation. Indeed, the CPC matrix is intended to be replaced by bone after a few weeks, leaving sintered CaP granules as scaffolds for the long-term regeneration of the defect. Although minor differences have been observed, this strategy has proven to be clinically relevant with brushite-forming CPCs but failed with apatite-forming CPCs, mainly due to a much lower capacity to be biodegraded.[24]

2.2 Class II IBS: calcium phosphate cement associated with polymers

As outlined previously, one of the main issues with Class I IBS relates to the use of calcium phosphate particles, which tend to remain even after several months or even years of implantation,

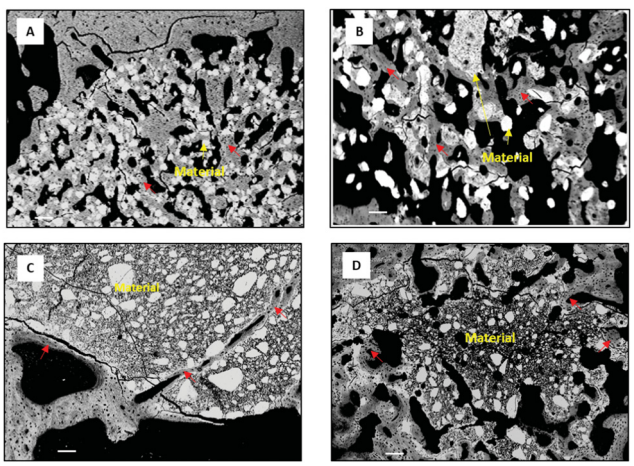

Fig. 3 SEM representative images of IBS implanted in a critical model of rabbit femur after (A) 3 weeks and (B–D) 6 weeks of implantation. Scale bars represent 100 μm (red arrows: newly formed bone). Class I IBS: (A) non-hardening suspension of BCP ceramics in the hydroxypropyl methylcellulose (HPMC) solution (bone formation in contact with BCP particles); (B) hardening suspension of BCP ceramics in a mineral resorbable matrix (MPCP) (bone formation in contact with BCP and the remaining cement).[71] Class II IBS: (C) calcium-deficient hydroxyapatite (CDHA) cement (new bone formation occurred in close contact with the surface of the CDHA cement) and (D) macroporous foam of the CDHA cement containing hyaluronic acid hydrogel (the macroporosity and biocompatibility of the material allow its biodegradation and new bone formation).

thereby hindering the proper repair of the defect. On the other hand, calcium phosphate cements (CPCs) and especially brushitic cements have shown interesting biological responses, as they appear to biodegrade at a sufficient rate but suffer among other things from a thick consistency before injection (causing injectability and filling issues but also limiting the addition of porogens),[72] poor mechanical properties once set (high risk of implant failure),[73] and the absence of a macroporous interconnected network (limiting the biological response by acting as a barrier).[74]

To address this, Class II IBS have been developed, coupling the advantage of CPCs with viscous polymer solutions or self-setting hydrogels (Fig. 4). Viscous-polymer-solution-based Class II IBS appeared in the mid-1990s, such as in the work of Cherng *et al.*,[75] who investigated the injectability and handling of the hydroxyapatite-forming calcium phosphate cement mixed with various "gelling agents" (HPMC, carboxymethyl cellulose CMCs, chitosan acetate, and chitosan lactate). Since then, a large number of substances such as glycerin, silicone gel, polyethylene glycol, liquid paraffin, glycerol, and cellulosic compounds[76] have been used to improve the rheological properties and handling of composite CPCs. Concurrently, as the viscosity of such formulations decreases, macroporous injectable CPCs have been developed by the incorporation of porogens in their formulations.[72,76–79] However, the generation of a macroporous network in already mechanically weak cements remains a major issue as the possible mechanical benefits induced by the presence of an organic compound are not able to counterbalance the decrease in mechanical properties due to the presence of macropores. To overcome the brittleness of these macroporous Class II IBS, new formulations based on self-setting hydrogels to replace the viscous polymeric solution have recently been developed (for example, with silanized hyaluronic acid, see Fig. 3D). To the author's knowledge, no Class II IBS using reticulating hydrogels are on the market to date, and only a few using a viscous macromolecule solution are commercially available (*e.g.*, Norian® Drillable,[37] Graftys® quickset/HBS[80]).

3. Optimization of calcium phosphate cement Class II by polymer addition

In the following sections, the main research trends are presented, with a specific focus on the benefits resulting from the combination of calcium phosphate cement (CPC) and polymer macromolecules (Fig. 4 and Table 3). Although improvement of IBS properties (*e.g.*, cohesivity and handling) and induction of an *in vivo* response are essential, it should be noted that sterilization, stability, and storage concerns for these new hybrid formulations are key to their commercialization.

3.1 Improvement of calcium phosphate cement physico-chemical properties by polymer addition

3.1.1 Injectability. The capacity of calcium phosphate cement (CPC) systems to remain homogeneous during

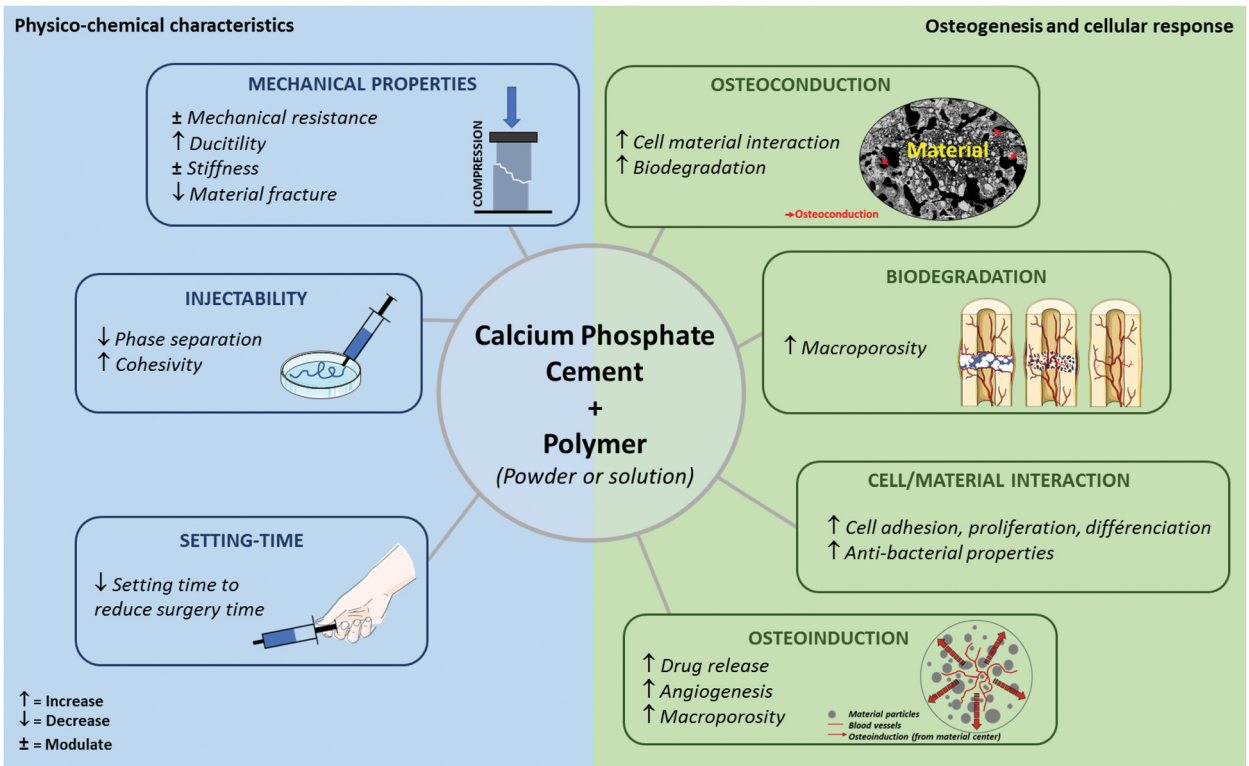

Fig. 4 Modulation of IBS properties by a combination of polymers with calcium phosphate cement (Class II).

Table 3 Impact of macromolecules or polymeric loading on calcium phosphate bone cement: a few examples

Polymeric phase	Calcium phosphate cement phase	Physico-chemical improvement	Biological improvement	Ref.
Collagen microsphere	Calcium deficient hydroxyapatite; α-tricalcium phosphate-based cement		↑ Macroporosity ↑ Cell accessibility ↑ Surface area available for osteoconduction *in vivo*	81
Gelatin	α-Tricalcium phosphate-based cement	↓ Setting time ↑ Compressive strength		82
Gelatin foam	α-Tricalcium phosphate-based cement		↑Macroporosity ↑ Material degradation *in vivo*	83
Gelatin microsphere	Rebone biomaterials, Shanghai, China	↑ Drug release with microsphere charged with rhBMP2	↑ Bone mineralization rate	84
Hyaluronic acid	Tetra-calcium phosphate (TTCP) and dicalcium phosphate anhydrous (DCPA) in an equimolar ratio		↑ Bone formation after implantation in a rat tibial defect	85
Hyaluronic acid microsphere	α-Tricalcium phosphate-based cement	↑ Drug release with microsphere charged with platelet lysate	↓ Epithelial growth ↑ Osteoconduction	86
Hydroxypropyl methylcellulose foam	α-Tricalcium phosphate-based cement	↑ Injectability ↑ Cohesion ↓ Brittleness	↑ Macroporosity	87
Alginate–chitosan complex (microencapsulated MC3T3-E1 cells)	Brushitic-based cement (β-tricalcium phosphate and monocalcium phosphate monohydrate)	↓ Compressive strength	↑ Scaffold remaining ↓ Lamellar-bone-like mineral structure ↑ Newly formed collagen ↑ Mineralisation rate	88
Alginate microbeads	Chitosan–Calcium phosphate composite	↑ Drug release with penicillin	↑ Increase the antibacterial properties ↓ Bacteria activity	89
Alginate	Brushitic cement	↓ Setting time		90
Alginate	Tetra-calcium phosphate (TTCP) and dicalcium phosphate anhydrous (DCPA) in an equimolar ratio	↑ Injectability ↑ Cohesion ↑ Compressive strength ↑ Young's modulus		91
Chitosan fibres	α-Tricalcium phosphate-based cement	↑ Mechanical reinforcement		92
Chitosan	α-Tricalcium phosphate-based cement	↑Young's modulus		93
Chitosan	Tetracalcium phosphate/a-tri-calcium phosphate		↑ Osteoconduction of the material in mandibular rat model	94
Chitosan with arginine–glycine–aspartate motif	Tetra-calcium phosphate (TTCP) and dicalcium phosphate anhydrous (DCPA) in a equimolar ratio	↑ Flexural strength	↑ New bone volume in Bone defect at femoral condyles of New Zealand white rabbits	95
Strontium-poly(γ-glutamic acid)	α-Tricalcium phosphate-based cement	↑ Mechanical resistance		96
Poly(lactic-*co*-glycolic acid)	α-Tricalcium phosphate-based cement		↑ Macroporosity ↑ Degradation rate ↑ Bone formation	74
Lactide modified poly(ethylene glycol) dimethacrylate (PEG-PLLA-DMA)	Brushitic cement	↓ Compressive strength ↑ Mechanical deformation		97
Poly(lactic-*co*-glycolic acid)-*b*-poly(ethylene glycol)-*b*-poly(lactic-*co*-glycolic acid) (PELGA)	Hydroxyapatite	↑ Drug release with rhBMP	↑ Accelerate bone formation Maintain mechanical integrity of the rate femoral defect	98

injection[99] may be limited by their injectability. Phase separation during injection can be caused by the size, shape, and distribution of powder grains,[100] paste homogeneity, and mechanical extrusion[101] (*i.e.*, pressure exerted by the syringe plunger, which causes the liquid phase to drain and the solids to consolidate; suction of the powder network during its flow; or filtration in the needle with the formation of a ''carpet'' of solids[102]). These limitations restrict their potential application and especially their use in minimally invasive surgery. These disadvantages that influence phase segregation can be explained by the low viscosity of the liquid phase (generally with a viscosity close to that of water), which flows more readily than the solid phase. Different methods to reduce the phase separation of CPC materials during extrusion have been

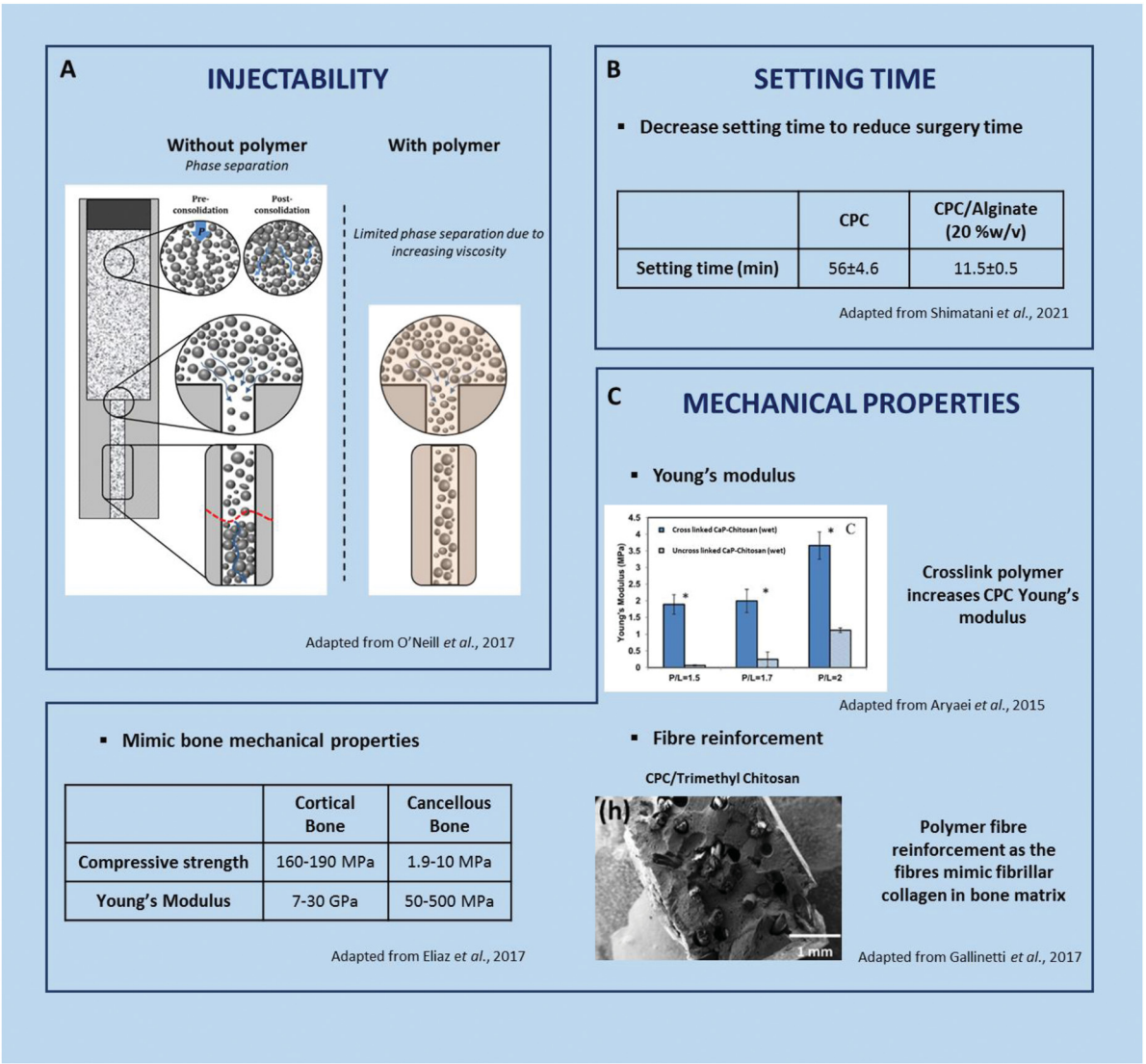

Fig. 5 Improvement of calcium phosphate cement physico-chemical properties by polymer addition.[90,92,93,102,111]

devised, including increasing the powder/liquid ratio and injection rate, reducing the plastic limit, increasing the viscosity of the liquid component, and optimising the cylinder geometry. Adding a viscous liquid has been shown to reduce phase separation during the injection/extrusion of CaP pastes and cements,[99] but an excessive increase in liquid viscosity increases the extrusion force, which may exceed the force that the surgeon (limit of manual injection: 100–300 N[78,103]) has to apply while still maintaining sufficient control. Modulation of the CPC/polymer association (Fig. 5A) can significantly reduce phase separation.[104] Examples of such viscous binders include cellulosic derivatives (cellulose and hydroxypropyl methylcellulose),[105] collagen/gelatin, hyaluronic acid,[106] chitosan,[107,108] and alginate,[109] which create strong attractive forces between the polymer and CaP particles. Similarly, the addition of beads (*e.g.* poly(lactic-*co*-glycolic) acid (PLGA)) may help to modulate the paste injectability depending on their size and concentration.[99,100] More recently, Vojtova *et al.*[110] associated a hydrogel forming copolymer composed of poly(lactic acid)–poly(glycolic acid)–poly(ethylene glycol) with α-TCP-based cement, thereby increasing the viscosity of the composite due to the reticulation of the macromolecule chains. They demonstrated that the pastes that retained a high level of shear stress did not exhibit phase separation during either the rheological or the injection tests.

Caballero *et al.*[9], associated poly(L-lysine) dendrigrafts polyethylene glycol homobifunctionalized with *N*-hydroxysuccinimide hydrogel with α-TCP-based cement, which had an excellent injectability, with total paste extruded at low force (7 ± 1 N), correlated with the cohesivity improvement.

In short, the addition of macromolecules to hydraulic cements represents a fantastic tool to tailor their injectability. They however affect the physico-chemical features of the IBS such as the setting time. On a purely technical note, this is why (i) injectability studies should not be done independently of setting time studies and (ii) the injectability of a cement should be measured after a time interval related to the setting time.[99]

3.1.2 Setting time. In brief, the setting of a hydraulic cement paste results in the dissolution of a reactive inorganic powder and the re-precipitation of a new inorganic phase, providing the final structure and features of the cement. Today, it is well-known that the setting time of such cements, including CPC, can be modulated by (i) the particle size and the crystallinity of the reactive powder, (ii) retarders (*e.g.*, citric acid) or accelerators (*e.g.*, NaH_2PO_4) as admixtures in the liquid or solid phase, (iii) the setting temperature and surrounding humidity and (iv) the reactive powder to liquid ratio (*P*/*L* ratio).[100,112]

For a given *P*/*L* ratio and defined reactive species (both powder and liquid phases), the addition of macromolecules (or polymers) may significantly affect in various ways the cement-forming reaction, from steric hindrance, physico-chemical adsorption onto the reactive powder to bonding with the released ions. Although much remains to be investigated and understood about the effect of macromolecules on CPC setting time, it is evident that their nature, features, and functionalization are key parameters.

For instance, Shimatani *et al.*[90] demonstrated that the setting time of a brushitic cement decreased with the increasing addition of low viscosity sodium alginate (from 56.0 to 11.5 min with 0 and 20% w/v, respectively, Fig. 5B).[90] Similarly, the addition of silanized HPMC seemed to reduce the setting time of an apatitic cement (*e.g.*, *P*/*L* =1.25), from 26 to 18 min with 0 and 4% w/v Si-HPMC,[105] whereas the addition of gelatin tends to increase it (*e.g.*), *P*/*L* = 2.5, from 10 to 25 min with 0 and 10% w/v gelatin.[113] It should be noted that it is of prime importance not to modify the reactive powder to liquid ratio to conclude on the influence of a given additive, which may not be that obvious.

Optimizing both setting time and injectability of the formulation can be achieved to match the intended clinical application. In the end, it is essential to prepare an easily injectable IBS with an appropriate setting time so that it sets slowly enough to give the surgeon time to inject it but quickly enough to prevent any material leakage out of the defect and to limit operating time.[112]

3.1.3 Mechanical properties. As shown in the clinical study performed by Blattert *et al.*,[114] CPCs still exhibit poor resistance to mechanical loading. Ideally, the CPC mechanical properties should be close to those of natural bone,[24] cortical bone (*E* = 7–30 GPa per compressive strength = 160–190 MPa) or the cancellous bone (*E* = 50–500 MPa per compressive strength = 1.9–10 MPa).[111,115] However, CPCs' specifications should be adapted to their intended clinical applications, and their final mechanical properties should be modulated as the function of the nature of the bone to treat and its localization (loading *versus* non-loading site).[111,112,114,116]

Current research revolves around 3 main focal points, which are (i) the improvement of their resistance to compression, traction and shear stress, (ii) the development of stiff but non-brittle formulations after setting, and (iii) the enhancement of their fracture toughness through the use of macromolecules or polymers as additives.

The addition of water soluble macromolecules is known to affect the setting reaction through a wide range of interactions as explained in Section 3.1.2. As a direct consequence, the cement's nano- to micro-structure, which greatly affects the properties of a set cement, is modulated, as illustrated later on. In addition to the nano- to micro-structural changes of the inorganic phase, the mechanical enhancement could also be achieved through the binding of macromolecular chains to the CaP crystal surface (calcium ions providing a preferential target of many anionic chains).[20] However, it is important to note that an excessive concentration of macromolecules could strongly decrease the setting time or even prevent it; hence a suitable balance is required to be found. On the other hand, reinforcing cement with polymeric fibers is a common strategy in other fields;[21] the nature of the polymer fiber along with other factors such as the length of the fiber, the volume fraction, the orientation and the fiber/matrix adhesion have a relevant effect.[22] Of course, both strategies could be combined to optimize cement mechanical properties.

Gallinetti *et al.*[92] and Gao *et al.*[96] have shown the benefits of adding macromolecules in their CPC formulation to reinforce the material, indeed, with the aim to reinforce the material mechanically. For example, with the addition of trimethyl chitosan fibre reinforced in the CPC,[92] when the matrix starts to fissure, fibres bridge the crack to prevent it from opening and propagating any further. Moreover, crack deflection by the fibres extends the distance over which the crack propagates, consuming more energy in newly formed surfaces.[117] This can be compared to the cortical bone, where the fibrillar collagen architecture allows the bone to be reinforced. Or, with the addition of strontium-poly(γ-glutamic acid) in the CPC,[96] during the setting time, a compact microstructure was created by the surrounding calcium particles and γ-PGA that enhanced the mechanical resistance of the material. Another way to mimic the bone mechanical properties with their fracture resistance is to associate anionic and cationic polymers with a cement to obtain heterogeneous agglomeration in the CPC.[88]

With the aim to increase young's modulus, Aryaei *et al.*[93] combined a cross-linked tripolyphosphate chitosan with α-TCP-based cement. In the wet solution, Young's modulus increased 2 to 4 times according to the powder/liquid mass ratio. The material reinforcement was greater as the polymeric chain was lengthened. The authors hence suggested that increasing the concentration and the cross-linking time of the polymer increases the modulus value (Fig. 5C).

Finally, another important aspect for the biomaterial implantation in a bone defect is the consideration of the effect of the mechanical stress exerted by the host tissue on the ability of the material to be deformed under the stress. The ductility of the cement is characterized by a higher deformation before rupture. Here, the polymer will allow the cement to support a load better than the CPC alone. With the addition of polymers, the curves of compression are diminished, and the deformation is greater than that for the cement alone, at 30% with PEG-PLLA-DMA/brushite compared to 5% with brushite alone.[97] It has been suggested that the crystals can grow more readily and

become entangled in the hydrogel network and hence provide high mechanical performance.

3.2 Improvement of induced osteogenesis and cellular response

3.2.1 Anti-bacterial properties. Bone infection after implantation of biomaterials in an injured site is among the greatest challenges faced in the field.[118,119] As an alternative to conventional drug therapies, researchers have tried loading drugs directly into the CPC.[120–123] However, their release profile is often suboptimal and may not allow efficient treatment of the infection.[124] This is because (i) it may be difficult to reach a sufficient drug load over a relevant time period and (ii) the CPC/drug interactions may inactivate the drugs due to pH, ion binding, or other factors. Therefore, polymers, used as vectors for protection and controlled delivery of active substances, are clinically relevant to topically fight infection. For instance, Wu *et al.*[125] associated penicillin-containing alginate microbeads with a chitosan/CPC composite (Fig. 6A) to increase the antibacterial properties of the chitosan by sustained drug release to inhibit the activity of bacteria (*Staphylococcus aureus*). It was also shown that the addition of silver ions in the brushitic or apatitic cementitious phase allowed to present antibacterial properties with rapid release according to the nature and the solubility of the CPC.[126]

Another attractive alternative to standard drugs may rely on the inherent antibacterial properties of certain polymers. Antimicrobial polymers have been described in terms of their capacity to inhibit or kill bacteria due to their chemical structure (*i.e.*, quaternary nitrogen groups, halamines, and polylysine).[129] Fortunately, positively charged amphiphilic polymers do display antibacterial activity due to their ability to

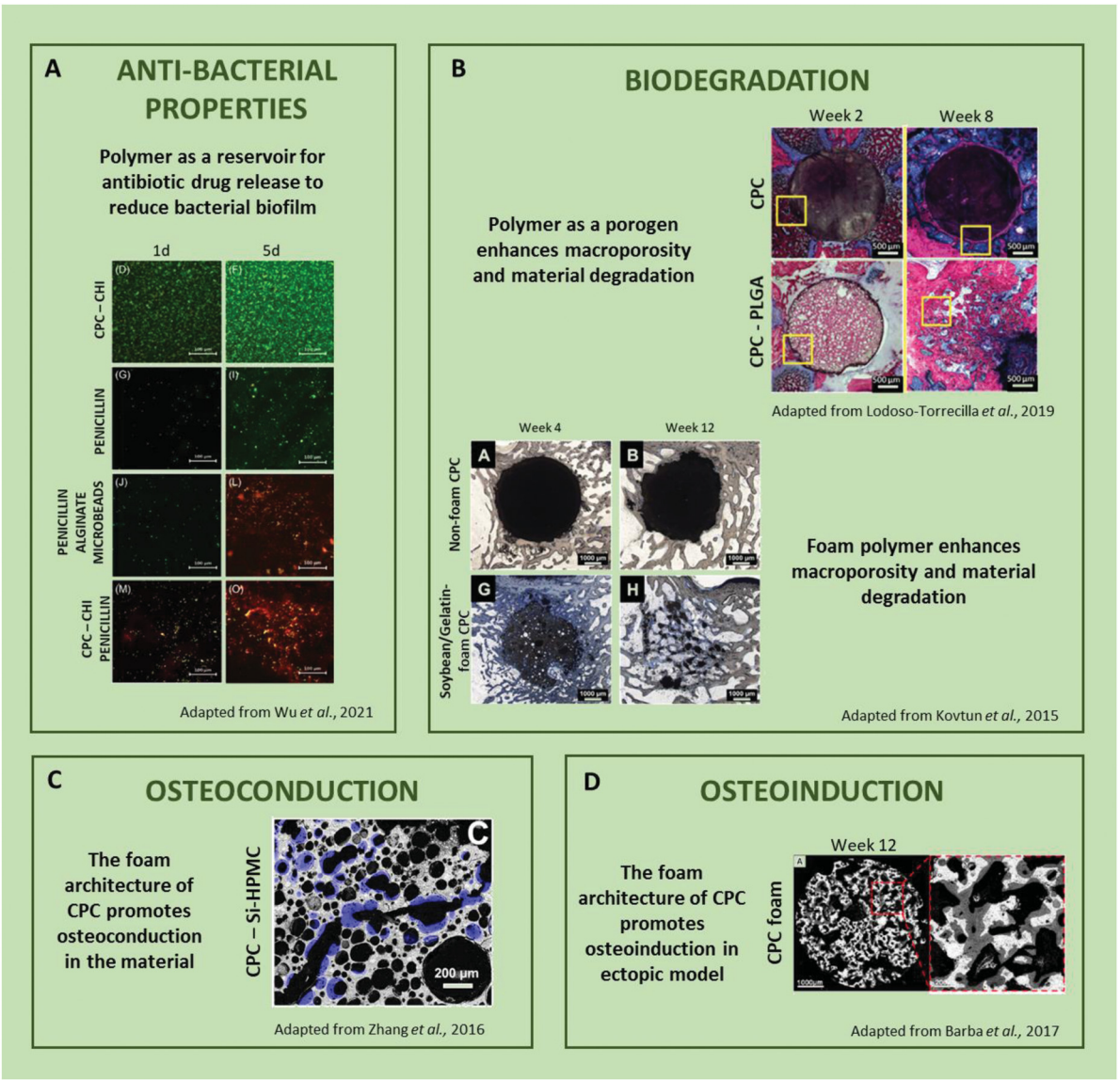

Fig. 6 Improvement of calcium phosphate cement induction of osteogenesis and cellular response (CPC = calcium phosphate cement, CHI = chitosan, Si-HPMC = silanized hydroxypropylméthylcellulose, PLGA = poly(lactic-*co*-glycolic acid),[83,103,127,128]).

penetrate the membrane and kill bacteria.[130] For example, *N*-(2-hydroxypropyl)-3-trimethyl chitosan chloride (HTCC) and the bioadhesive poly(dextran-aldehyde) hydrogels exhibited activity against Gram-positive and Gram-negative bacteria and also promoted the wound healing in a rat model.[131] The formulation of IBS coupling CPCs and these hydrogels would be of clinical interest to greatly limit the potential infection after implantation. For instance, several studies have already reported the association of CPC with chitosan, hence little effort may be required to formulate an innovative drug-free antibacterial solution.[127,129–134]

3.2.2 Host-material interactions and osteoconductivity. The implantation of a foreign body automatically triggers a reaction of the host biological system.[135] Biomaterials, even those deemed 100% biocompatible such as CPCs, are no exception; hence controlling host-material interactions to positively stimulate the formation of a new tissue is the key.[136] The biomaterial surface, serving as an interface, presents a wide range of physico-chemical cues (*e.g.*, chemistry, topography, wettability, porosity, electrical charges) which will impact cell recruitment, adhesion, proliferation, and differentiation.[137] As inherent features resulting from their formation process, CPCs display a very high contact surface with their *in situ* environment due to the presence of a micro- and nano-porous network even for bulk samples (at the macro-scale).[20] Consequently, they are considered as more ''bioactive'' than their ceramic counterparts with the same composition by maximizing the exchanges (*e.g.*, the release of ions[138,139]) with their biological surroundings. This enhanced bioactivity (*versus* ceramics of comparable composition) could also be attributed to CPC surface topology, often composed of petal or needle-like microstructures which is known to enhance cell adhesion and fate.[140] Taking advantage of physico-chemical and biological features of macromolecules could be a relevant strategy to further the biomaterial-induced biological response of CPCs, modulating their biodegradation, osteoconduction and osteoinduction.[85,94,133] These specific points will be detailed in the following sections.

Osteoconductivity was determined by the physicochemical characteristics of the material to support tissue ingrowth, osteoprogenitor cell growth, and development for bone formation to occur.[141] Due to their chemical composition and micro-nano-structure, CPCs exhibited high osteoconduction which allows for their perfect osteointegration within a bone defect.[142] By modulating these features and also bringing their own biological benefits, macromolecules could be combined with CPCs to improve their osteoconductivity. Indeed, Kjalarsdóttir *et al.*[94] injected a chitosan/CPC in a mandibular rat model. After 14 days of implantation, new bone growth formed as an outgrowth from the periosteum, covering the surface of the bone, located along specific areas of the mandible. The authors suggested that the biomechanical weakening of the polymer/cement implant structure stimulates the osteoconduction of the material. Similarly, Cui *et al.*[85] showed that an increase in the hyaluronic acid concentration from 0% to 4% in calcium phosphate cement resulted in more bone formation after 12 weeks of implantation in a rat tibial defect. In this case, they suggested that the incorporation of hyaluronic acid may promote higher osteogenicity by the secretion of stimulatory factors and osteogenic gene expression.

However, as most CPCs are often lacking an interconnected macroporous network, osteoconduction is often still limited to the bone defect/material interface.[143] This could also be solved by the addition of macromolecules or polymers to CPCs, serving as a porogen[84,86,103,133] or a foaming agent.[83,87] This directly affects their ability to be invaded by a newly formed bone tissue up to their core, as well as their ability to be biodegraded.

3.2.3 Biodegradation. A relevant biodegradation rate of the CPC is clearly an important feature as it both provides free space for the formation of new bone and allows for the release of bioactive ions such calcium (Ca^{2+}) and phosphate (PO_4^{3-}) which are essential to stimulate bone deposition.[138,139] This degradation is not only produced by (i) a passive dissolution of the material, especially in the case of apatite, but it is mainly resorbed by (ii) the biological activity of immune cells and osteoclasts.[144,145] The acidic environment produced by these cells allows the local dissolution of the latter.[144] However, without a proper macro-architecture, biodegradation of the CPC remains peripheral. That is why, the presence of an interconnected macroporous network had been deemed essential for both material biodegradation[146] and bone formation. It has been widely demonstrated that macroporosity greater than 100 μm and interconnections[20] are required to allow bone growth.[23] Pore interconnection is essential to increase adhesion, cell colonization, and vascularization, as opposed to closed porosity, which only reduces the mechanical properties.[147] Furthermore, it has been assumed that the accumulation of phosphate and calcium ions mostly occurs in the core of the materials, in materials with high surface areas, and in concave pores (as opposed to convex pores)[148] as this makes these pores a very favourable environment for the cells.

One of the possible ways to create macroporosity in CPCs from polymers is to incorporate sacrificial porogens (particles[149] or microspheres[86]). The porosity is classified by size with micropores (<1 μm), mesopores (1–100 μm) and macropores (>100 μm).[146,150] Babo *et al.*[86] associated hyaluronic acid microspheres loaded with platelet lysate with a calcium phosphate cement as an osteointegrative biodegradable system. In this study, after 6 weeks of implantation in bilateral intrabony defects in maxillary first molars of rats, the material resulted in a beneficial decrease in the epithelial growth and overall periodontal healing. Despite these beneficial properties, the mineral phase of the material was degraded too fast compared to the hyaluronic particles and a lack of mechanical properties was observed. This is an interesting example that highlights the importance of controlled and simultaneous biodegradation and osteoconduction to allow consolidated bone regrowth. Lodoso-Torrecilla *et al.*[74] developed CPC/PLGA combined with sucrose particles. The 60 μm microspheres of PLGA and 400 μm microspheres of sucrose improved the

macroporosity, increasing the degradation rate and bone formation (20% newly formed bone with the composite compared to 0.1% with the CPC at 8 weeks). PLGA hydrolysis that was supposed to represent the late stage (6–8 weeks) induced as early as 2 weeks after implantation of the second intended degradation of the CPC matrix *via* local acidification, which may have been beneficial for bone formation.

Another way to generate macroporosity in CPCs relies on the surfactant properties of certain macromolecules. For instance, Zhang *et al.*[87] developed an injectable cement foam, based on a mixture of Si-HMPC hydrogel and α-TCP. After setting, a hydroxyapatite-deficient calcium (CDHA) structure displaying interconnected macropores from 10 to 200 μm in size was observed. As simple ways to introduce macroporosity in injectable systems, forming stable foam is increasingly being studied.[87,151,152] The heterogeneity of the porosity and the interconnection created using the foam techniques provide an interesting environment for the cells to adhere, proliferate, differentiate, and migrate by nesting in the material interconnected with macropores (Fig. 3D). Kovtun *et al.*[83] associated gelatin-soybean with a CPC foam. The foams were formed with a manual system and the results revealed a high degree of degradation of the material, with 60% more degraded material than the non-polymer cement. Therefore, the microporosity of materials that allows invasiveness of cells in the material remains one of the most important properties in the development of such injectable materials.

3.2.4 Osteoinduction. Osteoinduction is the ability of a material to form new bone in an ectopic site.[153] Bohner *et al.*[154] described the material prerequisites for intrinsic osteoinduction as (i) material mineralization with a bioactive apatite layer consuming released ions; (ii) a porous material; (iii) pores large enough to allow vessel growth and cell transport (minimum interconnection 50 μm); (iv) a blood supply to maintain physiological concentrations of phosphate and calcium. The architecture of materials could be responsible for the osteoinductive properties. In order to compare and study the osteoinduction of the materials in ectopic sites with different architectures, Barba *et al.*[128] performed intramuscular-implantation in a beagle dog model (Fig. 6D). The calcium phosphate cement (calcium phosphate-deficient hydroxyapatite) foam porosity characteristic compared to a 3D porosity architecture showed a clear difference in terms of an increase in bone growth in the ectopic zone. The high reactivity of the biomimetic CDHA, which is due to its poor crystallinity, nanostructured nature, combined with the concave macroporosity produced by the foaming process, resulted in accelerated osteoinduction compared with conventional sintered BCP ceramics with the same macroporous architecture. This strategy is very promising for the development of an efficient injectable cement. The association of the polymer with a CPC as a foam would allow the creation of an injectable material with the right mechanical strength and macroporosity to promote osteoconduction and osteoinduction of the material.[86,87,128,155]

Some authors augmented this phenomenon by incorporating growth factors such as bone morphogenic protein (BPM) in the biomaterial. For example, Li *et al.*[84] added rhBPM-2 (recombinant human bone morphogenetic protein) encapsulated in gelatin microspheres associated with calcium phosphate cement. The role of the polymer was to control the release of the factor to repair bone defects. Growth factors should be included in bioactive apatite layers on the surface, and their release is caused by inflammatory cell action on the material surface. Similarly, Zhang *et al.*[98] highlighted that the addition of 400 ng of rhBMP (recombinant human bone morphogenetic protein-2/7 heterodimer) in macroporous cement composed of the degradable amphiphilic polymer PELGA (poly(lactic-*co*-glycolic acid)-*b*-poly(ethylene glycol)-*b*-poly(lactic-*co*-glycolic acid)) and osteoconductive HA (hydroxyapatite) resulted in significantly higher osteoinduction compared to the material without rhBMP. The addition of rhBMP-2/7 to this material accelerated robust bone formation and achieved the full functional restoration of the mechanical integrity of the rat femoral defect.

In another way, without using osteoinductive molecules, polymers such as chitosan/collagen[156] or hyaluronan[95,157] have shown osteoinductive properties in ectopic sites and *in vitro* (*e.g.* osteogenic differentiation).

The nature, dose and molecular weight of the macromolecule incorporated in the CPC appear to be relevant parameters for osteoinduction. Although this point remains largely unexplored, the hypothetic osteogenic potential of macromolecules represents an additional reason for incorporating them in CPC in addition to all the above benefits.

4. Conclusion

The association of polymers with calcium phosphate cement or granules is increasingly being used in the development of injectable bone substitutes for bone tissue engineering. It is important to establish a classification for the nomenclature of biomaterials that are being used and that are currently under development. The Class I IBS ceramics currently on the market, which tend to remain in place even after several months or even years of implantation, have poor initial mechanical properties and tend to leak out of the defect during injection. On the other hand, Class II calcium phosphate cements have been shown to lead to interesting biological responses, and they appear to biodegrade at a suitable rate, while nevertheless suffering from limitations. In this review, several means are proposed to overcome these limitations to make these calcium phosphate cements better materials for achieving better bone growth. Improved parameters are presented here individually, but the real challenge is being able to combine all the required properties for an injectable material. Presently, it should be pointed out that foaming techniques appear to be very promising, with the potential to create injectable, ductile, macroporous, biodegradable materials that result in the pronounced biological responses required for bone repair.

Conflicts of interest

There are no conflicts to declare.

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