Injectable macromolecule-based calcium phosphate bone substitutes

Hilel Moussi,†,‡ Pierre Weiss,§* Jean Le Bideau,‖ Hélène Gautier,¶ and Baptiste Charbonnier

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 indication24–29 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 procedures27,28 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 Schrötter 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...
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. 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 formulaion, 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 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.

#### 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. 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. More precisely, biphasic calcium phosphate macroporous granules (hydroxyapatite HAP/b-tricalcium phosphate β-TCP) were included in a hydroxypropyl methylcellulose (HPMC) matrix, thereby providing tailorable IBS (e.g., HAP/β-TCP ratio, % wet polymer). Once in vitro assays confirmed...

---

**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)

<table>
<thead>
<tr>
<th>Commercial name and manufacturer</th>
<th>Material class</th>
<th>Inorganic phase</th>
<th>Organic phase</th>
<th>Application</th>
<th>Characteristics</th>
<th>Clinical trial number and reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>MBCP-Gel/In’Oss®</td>
<td>I</td>
<td>CaP granules</td>
<td>HPMC polymer solution</td>
<td>Filling osseous defects of various origins</td>
<td>Ready to use, cohesively/putty</td>
<td>NCT00740311; (ref. 30)</td>
</tr>
<tr>
<td>ExcelOs-inject (CGBIO)</td>
<td>I</td>
<td>Beta TCP granules</td>
<td>Biodegradable hydrogel with or without rhBMP-2</td>
<td>Spinal fusion</td>
<td>Ready to use, injectable, cohesivity</td>
<td>NCT02714829; (ref. 31)</td>
</tr>
<tr>
<td>Mastergraft (Medtronic)</td>
<td>I</td>
<td>β-TCP + HA</td>
<td>Bovine collagen matrix</td>
<td>Spinal fusion</td>
<td>Putty</td>
<td>NCT01491542; (ref. 32)</td>
</tr>
<tr>
<td>Vitoss (Stryker)</td>
<td>I</td>
<td>β-TCP granules</td>
<td>Bone marrow aspirate</td>
<td>Spinal pathologies</td>
<td>Putty ultra-porous, flexible</td>
<td>NCT0309480; (ref. 33)</td>
</tr>
<tr>
<td>ChronOS (DePuy Synthes)</td>
<td>I</td>
<td>β-TCP granules</td>
<td>Autologous blood and/or bone marrow</td>
<td>Tibial plateau fractures</td>
<td>Injectable, degradable</td>
<td>NCT02056834; (ref. 34)</td>
</tr>
<tr>
<td>ChronOS Inject (DePuy Synthes)</td>
<td>I</td>
<td>Brushite matrix and (TCP) granules</td>
<td></td>
<td>Proximal tibial fractures</td>
<td>Injectable, degradable</td>
<td>NCT02056834; (ref. 35)</td>
</tr>
<tr>
<td>CERAMENT® (Bone Void Filler)</td>
<td>I</td>
<td>HA and calcium sulfate cement</td>
<td></td>
<td>Tibial plateau fractures</td>
<td>Injectable, degradable</td>
<td>NCT018288905; (ref. 36)</td>
</tr>
<tr>
<td>Norian® Drillable (DePuy Synthes)</td>
<td>II</td>
<td>Carbonated apatite cement</td>
<td>Bioresorbable polylactide/glycolide copolymer fibres</td>
<td>Tibial fractures</td>
<td>Mechanical resistance</td>
<td>NCT01132508; (ref. 37)</td>
</tr>
<tr>
<td>Graftys quickset/ HBS (Graftys)</td>
<td>II</td>
<td>CDHA cement</td>
<td>Powder of HPMC polymer</td>
<td>Bone disease, bone fractures</td>
<td>24 hours after injection</td>
<td>NCT02575352; (ref. 38)</td>
</tr>
</tbody>
</table>

---

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

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. 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.

For instance, a “self-hardening injectable calcium phosphate ceramics suspension” (SH-ICPCS) based on BCP-sintered granules associated with sialated HPMC (which reticulate at physiological pH) has been developed. Similarly, Hofmann et al., in 2007, 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. 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. 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.

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,
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 compounds76 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,77 Graftys® quickset/HBS™).

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](https://example.com/figure4.png) Modulation of IBS properties by a combination of polymers with calcium phosphate cement (Class II).
mechanical extrusion101 (a). Chitosan fibres or filtration in the needle with the formation of a "carpet" of solids102. 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 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

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

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

Young’s modulus

| Setting time
| Compression strength
| Young’s modulus
| Macroporosity
| Degradation rate
| Bone formation

Accelerate bone formation

Maintain mechanical integrity of the rate femoral defect
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, 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) has to apply while still maintaining sufficient control. Modulation of the CPC/polymer association can significantly reduce phase separation. Examples of such viscous binders include cellulosic derivatives (cellulose and hydroxypropyl methylcellulose), collagen/gelatin, hyaluronic acid, chitosan, and alginate, 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. More recently, Vojtova et al. 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., 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, 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.

**Fig. 5** Improvement of calcium phosphate cement physico-chemical properties by polymer addition.© 2022 The Author(s). Published by the Royal Society of Chemistry.

© 2022 The Author(s). Published by the Royal Society of Chemistry.
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$_2$PO$_4$) 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).  

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. demonstrated that the setting time of a brushitc 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). 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, 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. 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 by combining 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.

3.1.3 Mechanical properties. As shown in the clinical study performed by Blattert et al., CPCs still exhibit poor resistance to mechanical loading. Ideally, the CPC mechanical properties should be close to those of natural bone, 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). 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).

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). 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; 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. Of course, both strategies could be combined to optimize cement mechanical properties.

Gallinetti et al. and Gao et al. 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, 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. 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(g-glutamic acid) in the CPC, 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.

With the aim to increase young’s modulus, Aryaei et al. combined a cross-linked tripolypophosphate chitosan with z-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. 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.\textsuperscript{118,119} As an alternative to conventional drug therapies, researchers have tried loading drugs directly into the CPC.\textsuperscript{120–123} However, their release profile is often suboptimal and may not allow efficient treatment of the infection.\textsuperscript{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 \textit{et al.}\textsuperscript{125} associated penicillin-containing alginate microbeads with a chitosan/CPC composite (Fig. 6A) to increase the anti-bacterial properties of the chitosan by sustained drug release to inhibit the activity of bacteria (\textit{Staphylococcus aureus}). It was also shown that the addition of silver ions in the brushitite or apatitic cementitious phase allowed to present antibacterial properties with rapid release according to the nature and the solubility of the CPC.\textsuperscript{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 (\textit{i.e.}, quaternary nitrogen groups, halamines, and polyllysine).\textsuperscript{129} Fortunately, positively charged amphiphilic polymers do display antibacterial activity due to their ability to

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{fig6.png}
\caption{Improvement of calcium phosphate cement induction of osteogenesis and cellular response (CPC = calcium phosphate cement, CHI = chitosan, Si-HPMC = silanized hydroxypropylmethylcellulose, PLGA = poly(lactic-co-glycolic acid)).}
\end{figure}
penetrate the membrane and kill bacteria.\textsuperscript{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.\textsuperscript{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.\textsuperscript{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.\textsuperscript{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.\textsuperscript{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.\textsuperscript{137} As inherent features resulting from their formation process, CPCs display a very high contact surface with their \textit{in situ} environment due to the presence of a micro- and nano-porous network even for bulk samples (at the macro-scale).\textsuperscript{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\textsuperscript{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.\textsuperscript{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.\textsuperscript{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.\textsuperscript{141} Due to their chemical composition and micro-nano-structure, CPCs exhibited high osteoconductivity which allows for their perfect osteointegration within a bone defect.\textsuperscript{142} By modulating these features and also bringing their own biological benefits, macromolecules could be combined with CPCs to improve their osteoconductivity. Indeed, Kjalars-dottir \textit{et al.}\textsuperscript{99} 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 \textit{et al.}\textsuperscript{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.\textsuperscript{143} This could also be solved by the addition of macromolecules or polymers to CPCs, serving as a porogen\textsuperscript{84,86,103,133} or a foaming agent.\textsuperscript{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\textsuperscript{2+}) and phosphate (PO\textsubscript{4}\textsuperscript{3-}) which are essential to stimulate bone deposition.\textsuperscript{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.\textsuperscript{144,145} The acidic environment produced by these cells allows the local dissolution of the latter.\textsuperscript{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\textsuperscript{144} and bone formation. It has been widely demonstrated that macroporosity greater than 100 μm and interconnections\textsuperscript{20} are required to allow bone growth.\textsuperscript{23} Pore interconnection is essential to increase adhesion, cell colonization, and vascularization, as opposed to closed porosity, which only reduces the mechanical properties.\textsuperscript{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)\textsuperscript{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\textsuperscript{149} or microspheres\textsuperscript{86}). The porosity is classified by size with micropores (<1 μm), mesopores (1–100 μm) and macropores (>100 μm).\textsuperscript{146,150} Babo \textit{et al.}\textsuperscript{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 \textit{et al.}\textsuperscript{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). Kostun 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 osteoinduction 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 rhBMP-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 osteoinductive 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/collagen156 or hyaluronan95,157 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.
References


57 J. M. Kim, M. H. Kim, S. S. Kang, G. Kim and S. H. Choi, 
Comparative bone healing capacity of different bone graft 
matrices in a rabbit segmental defect model, J. Vet. Sci., 

58 H. Zhang, L. Yang, X. G. Yang, F. Wang, J. T. Feng and 
K. C. Hua, et al., Demineralized Bone Matrix Carriers and 
their Clinical Applications: An Overview, Orthop. Surg., 

59 P. Tourrier, J. Guicheux, A. Paré, A. Maltezeau, T. Blondy 
and J. Veziers, et al., A partially demineralized allogeneic 
bone graft: in vitro osteogenic potential and preclinical 
evaluation in two different intramembraneous bone healing 

60 M. Taz, P. Makkar, K. M. Imran, D. W. Jang, Y. S. Kim 
and B. T. Lee, Bone regeneration of multichannel biphasic 
calcium phosphate granules supplemented with hyaluronic 

61 O. Faruq, B. Kim, A. R. Padalhin, G. H. Lee and B. T. Lee, 
a hybrid composite system of biphasic calcium phosphate 
granules loaded with hyaluronic acid-gelatin hydrogel for 

62 J. L. Moreau, M. D. Weir and H. H. K. Xu, Self-setting 
collagen-calcium phosphate bone cement: Mechanical and 
91(2), 605–613.

63 R. O. Neill, H. O. Mccarthy, E. B. Montufar, M. Ginebra, 
Critical review: Injectablety of calcium phosphate pastes 

64 P. Weiss, L. Obadia, D. Magne, X. Bourges, C. Rau 
and T. Weitkamp, et al., Synchrotron X-ray microtomography 
(on a micron scale) provides three-dimensional imaging 
representation of bone ingrowth in calcium phosphate 

65 A. D. Speirs, T. R. Oxlund, B. A. Masri, A. Poursartip 
and C. P. Duncan, Calcium phosphate cement composites in 
revision hip arthroplasty, Biomaterials, 2005, 26(35), 
7310–7318.

66 V. Campana, G. Milano, E. Pagano, M. Barba, C. Cicione 
and G. Salonna, et al., Bone substitutes in orthopaedic surgery: 
from basic science to clinical practice, J. Mater. Sci.: Mater. 

67 K. Flégeau, R. Pace, H. Gautier, G. Rethore, J. Guicheux 
and C. Le Visage, et al., Toward the development of biomimetic 
injectable and macroporous biohydrogels for regenerative 

68 B. H. Fellah, P. Weiss, O. Gauthier, T. Rouillon, P. Pilet 
and G. Ducalusi, et al., Bone repair using a new injectable self-
628–635.

and M. Schieker, et al., A new biodegradable bone wax 
substitute with the potential to be used as a bone filling 

70 D. Apelt, F. Theiss, A. O. El-Warrak, K. Zlinszky, R. Betschart-
Wolfisberger and M. Bohner, et al., In vivo behavior of three 
different injectable hydraulic calcium phosphate cements, 

71 G. Daculsi, I. Khairoun, R. Z. LeGeros, F. Moreau, P. Pilet 
and X. Bourges, et al., Bone ingrowth at the Expense of a 
Novel Macroporous Calcium Phosphate Cement, Key Eng. 

72 J. Luo, H. Engqvist and C. Persson, A ready-to-use acidic, 
brushite-forming calcium phosphate cement, Acta Biomater., 
2018, 81, 304–314.

73 J. Zhang, F. Tancret and J. M. Bouler, Mechanical properties 
of Calcium Phosphate Cements (CPC) for bone substitution: Influence of fabrication and microstructure, Key Eng. 

74 I. Lodoso-Torrerella, N. A. P. van Gestel, L. Díaz-Gomez, 
E. C. Grosfeld, K. Laperre and J. G. C. Wolke, et al., Multi-
modal pore formation in calcium phosphate cements, 

75 A. Cheng, S. Takagi and L. C. Chow, Effects of hydroy-
propyl methylcellulose and other gelling agents on the 
handling properties of calcium phosphate cement, 

76 S. Takagi, L. C. Chow, S. Hirayama and A. Sugawara, 
Premixed Calcium-Phosphate Cement Pastes, J. Biomed. 

77 S. Takagi and L. C. Chow, Formation of macropores in 

78 W. J. E. M. Habraken, H. B. Liao, Z. Zhang, J. G. C. Wolke, 
D. W. Grijpma and A. G. Mikos, et al., In vivo degradation of calcium phosphate cement incorporated into biodegradable 
microspheres, Acta Biomater., 2010, 6(6), 2200–2211.

79 J. E. Barralet, L. Grover, T. Gaunt, A. J. Wright 
and I. R. Gibson, Preparation of macroporous calcium phos-
cphate cement tissue engineering scaffold, Biomaterials, 
2002, 23(15), 3063–3072.

80 M. Le Ferrer, C. Mellier, F. X. Lefèvre, F. Boukhechba, 
P. Janvier and G. Montavon, et al., In vivo resorption of 
injectable apatitic calcium phosphate cements: Critical 
role of the intergranular microstructure, J. Biomed. Mater. 

81 E. Cuzmar, R. A. Perez, M. C. Manzanares, M. P. Ginebra 
and J. Franch, In Vivo Osteogenic Potential of Biomimetic 
Hydroxyapatite/Collagen Microspheres: Comparison with 
Injectable Cement Pastes, PLoS One, 2015, 10(7), e0131188.

82 A. Bigi, P. Torricelli, M. Fini, B. Bracci, S. Panzavolta 
and L. Sturba, et al., A biomimetic gelatin-calcium phosphate 

83 A. Kvitun, M. J. Goeckelmann, A. A. Niclas, E. B. Montufar, 
M. P. Ginebra and J. A. Planell, et al., In vivo performance of 

84 M. Li, X. Liu and X. Liu, Calcium Phosphate Cement with 
BMP-2-loaded Gelatin Microspheres Enhances Bone Healing 
in Osteoporosis A Pilot Study.

85 X. Cui, C. Huang, Z. Chen, M. Zhang, C. Liu and K. Su, 
et al., Hyaluronic acid facilitates bone repair effects of


