Instructional Lecture: General Orthopaedics

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Bioceramics and bone healing

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- Calcium phosphates have long been used as synthetic bone grafts. Recent studies have shown that the modulation of composition and textural properties, such as nano-, micro- and macro-porosity, is a powerful strategy to control and synchronize material resorption and bone formation.
- Biomimetic calcium phosphates, which closely mimic the composition and structure of bone mineral, can be produced using low-temperature processing routes, and offer the possibility to modulate the material properties to a larger extent than conventional high temperature sintering processes.
- Advanced technologies open up new possibilities in the design of bioceramics for bone regeneration; 3D-printing technologies, in combination with the development of hybrid materials with enhanced mechanical properties, supported by finite element modelling tools, are expected to enable the design and fabrication of mechanically competent patient-specific bone grafts.
- The association of ions, drugs and cells allows leveraging of the osteogenic potential of bioceramic scaffolds in compromised clinical situations, where the intrinsic bone regeneration potential is impaired.

Keywords: Bioceramics; bone healing; bone graft; calcium phosphate

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Bone as a living tissue: the bone healing process

Bone composition: the role of bone mineral

Bone is a metabolically active tissue, containing several types of cells in a unique extracellular matrix. A distinctive feature of the extracellular matrix of bone is its composite nature, as it is made of a network of collagen fibres reinforced with a mineral phase, i.e. calcium phosphate (CaP) crystals. The mineral phase, hydroxyapatite (HA) represents approximately 65% of the weight of the bone tissue. However, the properties of bone are not explained simply by its composition, but also by its complex structure. In fact, from a mechanical point of view, the properties of collagen and HA are poor compared with some engineering materials. Bone tissue mechanical properties are remarkable owing to the way both components are arranged and structured together. Bone formation follows a bottom-up approach, making it a hierarchically organized nanocomposite.[1] This provides bone with a unique combination of properties such as high strength and low Young's modulus, resulting in high toughness.

However, the mineral phase of bone cannot be regarded only as the reinforcing phase of a composite material. In addition to its mechanical function bone fulfils a series of metabolic and physiological functions. Here also the mineral phase plays a very relevant role. The apatite nanocrystals function as a chemical reservoir of both calcium (Ca) and phosphorus (P) in the body. In the case that levels of calcium or phosphorus are too low in body fluids, osteoclasts can resorb bone to resolve ion deficiency quickly.[2] Biological apatite is significantly different in composition from stoichiometric HA ($Ca_{10}(PO_4)_6(OH)_2$). In addition to being calcium-deficient, it contains large amounts of carbonate (up to 8% of its weight) and acidic phosphate groups, and it accommodates many other ions in its structure like sodium (Na), magnesium (Mg), potassium (K), strontium (Sr), fluor (F) and chloride (Cl), in addition to structural water. The presence of these ionic substitutions and lattice vacancies, together with the nanometric size of the biological apatite, results in a high reactivity, enabling its capacity to maintain the ionic balance in the body fluids.[3,4]

Bone formation and remodelling

When addressing the challenge of designing bioceramics for bone regeneration it is important to keep in mind the process of bone formation, healing and remodelling.

Bone is a dynamic tissue that is constantly being remodelled.[4] Our entire skeleton is renewed every five to ten years. Old bone is resorbed by the action of osteoclasts and new bone is laid down by osteoblasts. This is a responsive process: bone is able to shift the balance between osteoblastic and osteoclastic activity according to external stimuli.[5] Indeed, biomechanical stimuli can foster the activity of osteoblasts, thus increasing bone mass under increased loads (mechanotransduction). Alternatively, when the load-bearing requirements are reduced, osteoclastic activity is promoted, leading to bone resorption. In other circumstances, the triggering signal in this equilibrium is chemical rather than mechanical, and osteoclasts are deployed to resorb bone in order to release calcium or phosphate to the body fluid that is necessary for specific metabolic reasons.

As previously mentioned, osteoblasts are responsible for the synthesis, deposition and mineralization of bone extracellular matrix. They not only produce the organic molecules, i.e. collagen type I, glycosaminoglycans, transforming growth factors or bone morphogenetic proteins (BMPs), but also control the mineralization of collagen. After fulfilling their secretory activity, osteoblasts undergo either apoptosis (about 80%) or terminal differentiation to osteocytes (about 20%).

Osteocytes are quiescent osteoblasts embedded in the mineralized matrix. They communicate with each other by cytoplasmic processes within interconnecting channels (canaliculi) in the matrix. They are crucial for maintaining the osseous matrix, participate in extracellular exchanges and are involved in the mechanotransduction process.[6]

Osteoclasts are derived from the monocyte-macrophage lineage and are responsible for bone resorption.[7,8] They are able to degrade both the inorganic phase of bone by releasing acidic species, i.e. protons, and the organic phase through specific enzymes that digest the organic components. Monocyte-macrophage lineage cells play an important role in the triggering of bone repair processes; it is believed that the fusion-differentiation of monocytes in osteoclasts and subsequent osteoclastic activity is induced through chemokines – chemotaxis events on osteoprogenitors cells.[9] Understanding and controlling the response of osteoclasts to bioceramics by tuning chemical and structural features is of paramount importance for bone tissue regeneration.[10,11]

Bone grafting

As described in the previous section, bone has the capacity to regenerate in specific circumstances. However, this capacity is not unlimited; it is restricted to small bone defects. There is no biological mechanism for large-scale repair of bone. This is the case, for example, in large bone defects caused by open trauma or by resection of tumours. In other cases, the bone regeneration fails due to other factors such as in the case of fracture nonunions; or it is necessary to increase the amount of bone prior or posterior to the placement of implants. In these clinical situations, the use of materials acting as a bridge to support and, if possible, stimulate bone growth is needed. According to Giannoudis et al,[12] more than 2.2 million bone grafting procedures are performed annually in the world. Clinically, the most common strategy is the use of autografts. While biologically ideal, they present important drawbacks such as the need for a second surgical intervention, the limited amount that is harvestable and sometimes residual pain over time at the harvesting site. Grafts from bone banks or other animal species are still subject to risks, such as immunological reactions or disease transmission.[13]

The development of synthetic materials emerges as a distinct strategy, allowing the aforementioned limitations to be overcome. Although they currently only account for around 20% of the market for bone regeneration materials, their use is increasing. Synthetic biomaterials are obtained from chemical reagents by controlled synthetic processes, allowing the tuning of their properties in order to fit the specific requirements of different clinical situations,[14] even in compromised scenarios as, for instance, in osteoporotic patients.[15] In this latter situation, where the bone remodelling mechanisms are impaired, the osteogenic potency of CaP bone grafts can be fostered by combining them with drugs, growth factors or gene delivery strategies, as well as with cell therapies.[15] There is a large variety of biomaterials for bone regeneration on the market (e.g. granules, blocks, putties, cements, etc.), which will be discussed in the following sections.

Synthetic bone grafts: state of the art

Main compositions and historical evolution

A biomaterial is a material designed to interact with biological systems for either a therapeutic or diagnostic medical purpose. Different biomaterials have been proposed as synthetic bone graft substitutes, ranging from metals, like titanium or tantalum, to polymers like polylactides or hydrogel-based materials.[16] However, the most extensively used are bioceramics, and among them, calcium orthophosphates due to their similarity to the mineral phase of bone.[17-19] There are several calcium orthophosphate compounds, as displayed in Table 1, which can be obtained either by precipitation at room or body temperature in aqueous solutions (the first six compounds), or by solid state reactions at high temperatures (the last four compounds). Interestingly, HA is the only CaP that can be obtained both by precipitation in aqueous systems at low temperature (precipitated HA) and by solid state reaction (sintered HA).

Table 1. Main calcium phosphates used as biomaterials[13,14,17]

Calcium/phosphorus ratio	Name	Symbol/mineral name	Chemical formula	Solubility*
0.5	Monocalcium phosphate monohydrate	MCPM	$Ca(H_2PO_4)_2{\cdot}H_2O$	~18
1.0	Dicalcium phosphate dihydrate	DCPD/brushite	$CaHPO_4{\cdot}2H_2O$	~0.088
1.0	Dicalcium phosphate anhydrous	DCPA/monetite	$CaHPO_4$	~0.048
1.3-2.5	Amorphous calcium phosphate	ACP	$(Ca,X)_x(PO_4,Y)_y{\cdot}nH_2O$ $X = Mg^{2+}, Zn^{2+}, Sn^{2+}, Al^{3+}$; $Y = (CO_3)^{2-}, (P_2O_7)^{4-}$	N.A.
1.33	Octacalcium phosphate	OCP	$Ca_8H_2(PO_4)_6{\cdot}5H_2O$	~0.0081
1.5-1.67	Precipitated hydroxyapatite†	PHA, CDHA	$Ca_{10-X}(HPO_4)_X(PO_4)_{6-X}(OH)_{2-X}$ $0 \leqslant x < 1$	~0.0094
1.5	α-Tricalcium phosphate	α-TCP	$\alpha\text{-}Ca_3(PO4)_2$	~0.0025
1.5	β-Tricalcium phosphate	β-TCP	$\beta\text{-}Ca_3(PO4)_2$	~0.0005
1.67	Sintered hydroxyapatite	SHA/hydroxyapatite	$Ca_{10}(PO_4)_6(OH)_2$	~0.0003
2.0	Tetracalcium phosphate	TTCP/hilgenstockite	$Ca_4(PO_4)_2O$	~0.0007

*solubility in g/L in water at 25 °C
†when $x > 0$ one talks about calcium deficient hydroxyapatite, CDHA. It is common to have $x = 1$, which leads to the composition $Ca_9(HPO_4)(PO_4)_5(OH)$

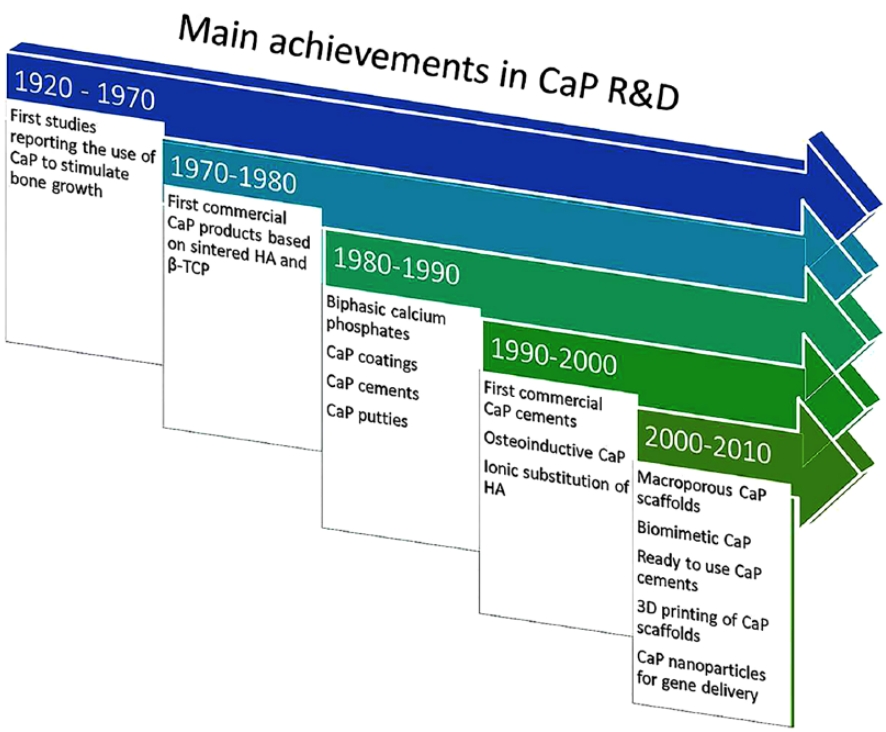

Fig. 1 Historical overview of relevant milestones in the research and development (R&D) of calcium phosphate (CaP) biomaterials (HA, hydroxyapatite; β-TCP, beta tricalcium phosphate).

In general, the lower the calcium/phosphorus ratio, the more acidic and soluble in water. The exception is tetracalcium phosphate (TTCP), which in spite of having the highest calcium/phosphorus ratio is more soluble than HA and beta tricalcium phosphate (β-TCP).

The first documented application of a CaP to stimulate bone regeneration dates from 1920. Albee[20] reported faster healing in surgically-induced gaps in dog's bones when aliquots of 'triple calcium phosphate' were injected into the defect.[20] However, it was not until the 1970s that the first CaP synthetic bone grafts were introduced to the market. Their use spread in the 1990s due to increasing concerns about disease transmission by xeno- and allografts, associated with the social awareness caused by some diseases like bovine encephalitis and acquired immune deficiency syndrome. Since then, important improvements have been achieved, both on the technical side and in the biological performance, as summarized in Figure 1.

Sintered bioceramics

Historically, the first bioceramics used as synthetic bone grafts were HA and β-TCP, both obtained at high temperature by a sintering process. Special attention was paid to HA, due to its similarity with the mineral phase of bone. The

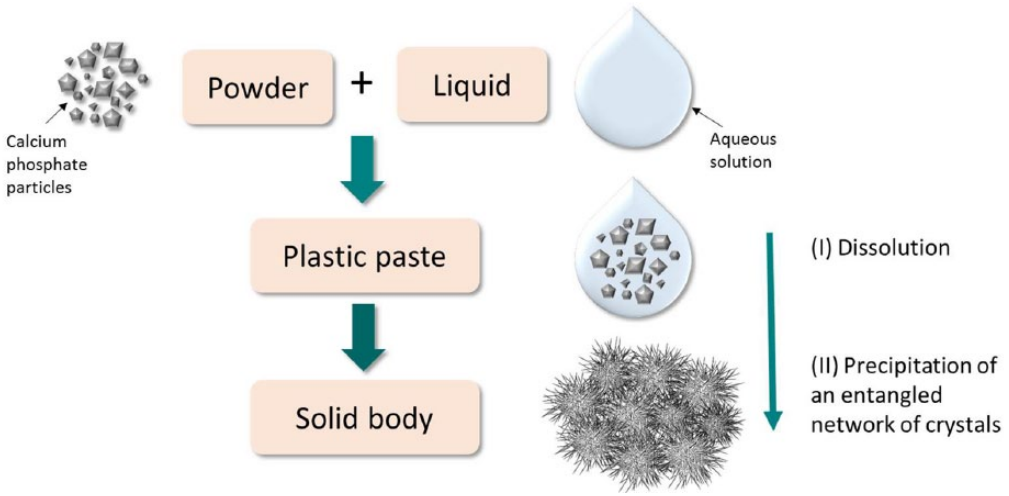

Fig. 2 Calcium phosphate cements: processing and microstructure.

initial idea of developing dental implants or osteosynthesis devices made of HA required the optimization of the mechanical properties to ensure stability. Therefore, much effort was devoted to producing high temperature sintered HA with low porosity and high crystallinity to minimize excessive reactivity. However, it was soon evident that sintered HA, in addition to being too brittle to be reliable for load-bearing applications, was non-biodegradable and hardly bioactive. Osteoclasts are unable to rapidly dissolve the much more stable, stoichiometric and highly crystalline sintered HA. In an attempt to increase the degradation rate, in the late 1980s Daculsi et al[21] introduced an approach based on the combination of HA with the more soluble ceramic phase β-TCP: the biphasic CaPs (BCP). Since then, BCPs with different relative phase proportions and thus different resorbabilities have been extensively used in the form of blocks and granules.[22]

CaP cements (CPCs)

The introduction of CPCs in the early 1980s by LeGeros[23] and Brown and Chow[24] represented a breakthrough in CaP research. It provided clinicians with mouldable and even injectable pastes that were able to harden within the body. The initial formulation was based on TTCP and dicalcium phosphate (DCP) (Table 1), which reacted to set into HA. Later on, other formulations were developed and apatitic cements were proposed based on other compounds like alpha TCP (α-TCP), and also cements that produce other final phases like brushite (DCP dihydrate, DCPD) or monetite (DCP anhydrous, DCPA).[25,26] The wide range of formulations allows users to adapt its properties to specific clinical needs and requirements for different degrees of resorbability. The hardening of the cement is based on a dissolution and precipitation reaction and the formation of a porous network of micrometric/nanometric CaP crystals (Fig. 2).[27] Unlike in acrylic bone cements, widely used in orthopaedic surgery for arthroplasty fixation and vertebroplasty, the setting reaction in CPC is not exothermic,[28] and therefore allows the incorporation of drugs and biologically active molecules, like antibiotics or growth factors, which can provide additional functionalities or increase the osteogenic capacity.[29]

Different issues remain to be improved in CPC, such as mechanical properties – to more closely mimic those of natural bone. Modification with soluble polymers[30] and reinforcement with polymer and ceramic fibres[31,32] represent a step forward in this direction. Another aspect that has been the subject of extensive research is the adjustment of porosity as a strategy to enhance biological performance.[33] Although self-setting CPCs are intrinsically porous, with porosities in the nano/micron range, they still require interconnected macropores $>$ 100 μm to allow tissue ingrowth and neovascularization and to ensure a fast healing response. This has been accomplished using different strategies, such as the introduction of biocompatible surfactants to the formulation, which allows the production of self-setting apatitic foams[34,35] and the development of self-setting inks that can be used in additive manufacturing techniques, such as 3D microextrusion.[36,37]

Synthesis of ion-substituted apatites

One of the specific features of biological apatites is the presence of a number of foreign ions which, in addition to modifying the structure and reactivity of the mineral, play an important metabolic role.[38] On this basis, HA substituted with biologically relevant ions has been synthesized and proposed as an attractive biomaterial for bone grafting, prompting a large number of studies.[39] Atomic substitutions in synthetic apatites can be produced by small changes in the composition of the reactants in the

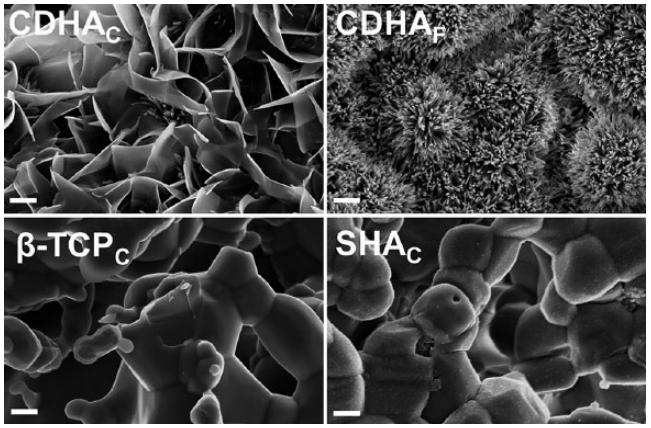

Fig. 3 Scanning electron micrographs of different microstructures of calcium phosphates. Top: Biomimetic calcium-deficient hydroxyapatite (CDHA) obtained by a self-setting reaction of alpha TCP, using a coarse powder (CDHA$_C$) or a fine powder (CDHA$_F$). Bottom: Sintered calcium phosphates, beta tricalcium phosphate (β-TCP) and sintered hydroxyapatite (SHA). Scale bar: 500 nm. Adapted from Diez Escudero et al,[55] with permission.

synthesis. Traditionally the most studied ions were carbonates, due to their abundant presence in bone mineral, and fluoride in particular for dental applications as it was associated with the stabilization of the apatite phase in enamel (which proved to be an effective prevention strategy for tooth decay). More recently, other ions have attracted a great deal of attention due to their specific biological effects. This has fostered not only the use of ion substituted HA, but also that of silicate and phosphate glasses, as matrices for the controlled delivery of ions.[40,41] For example, strontium has been used for the treatment of osteoporosis, based on its stimulation of osteoblastic differentiation and inhibition of osteoclastogenesis;[42,43] silicon has been incorporated into CaPs taking advantage of their osteogenic properties due to its role in bone development;[44] and silver has been used due to its antimicrobial activity.[45]

Osteoinductive ceramics

The development of bioceramics with intrinsic osteoinductive properties is one of the most important achievements in the field of synthetic CaPs in past decades. A range of CaPs have revealed osteoinductive properties, in the sense that they have the ability to trigger the differentiation of non-differentiated cells towards the osteogenic lineage. This mechanism leads to bone formation, even in the absence of exogenous BMPs, and is also associated with a higher bone healing capacity when implanted orthotopically, compared with non-osteoinductive ceramics.[46] It has also been reported that osteoinductive ceramics perform similarly to autologous bone graft and rBMP2 (recombinant bone morphogenetic protein 2) in repairing critical-size bone defects.[47] Although the mechanism underlying bone induction is still not fully understood, it seems clear that it is the result of the combination of several parameters rather than of a single one. Chemical composition, macropore size and geometry, microporosity, surface microstructure and specific surface area have been shown to play key roles.[19,46] One of the most plausible hypotheses behind osteoinduction combines the natural ability of CaP to bind BMPs with the presence of concavities within the scaffold that helps the retaining and concentrating of BMPs and ions in the vicinity of the scaffold, creating a favourable niche for the differentiation of mesenchymal stem cells (MSCs).[48-51] The identification of these properties and the mechanisms involved in osteoinduction will definitely lead to the design of synthetic bone grafts with higher osteogenic potential, initiating solutions to compromised clinical situations. Recently, it has been shown that the nanostructured nature of biomimetic HA leads to an accelerated osteoinduction when compared with microstructured sintered ceramics with analogous composition.[52]

Sintered versus biomimetic CaPs

In recent years attention has been focused on the enhancement of the biological properties of synthetic bone grafts. In order to design synthetic bone grafts able to perform as well as or even outperform autografts, it is necessary to establish the appropriate interactions between the graft, the osseous cells and the extracellular matrix. The final goal is to obtain materials that can be recognized and processed by osteoclasts in a similar way to the natural bone extracellular matrix. In other words, biomaterials are sought that can enter the physiological bone remodelling cycle. In this sense, it seems counterintuitive to continue relying on the traditional high temperature processing strategies that are so far from the mild processes involved in bone formation.[18] It is important to highlight the fact that the synthetic process determines not only the composition of a material, but also the final properties that this material will have, such as solubility, morphology, porosity, crystallite size and specific surface area. In the case of ceramics, the high-temperature treatment (sintering process) generates a final structure consisting of large crystals with low specific surface area and a low nano-/microporosity and, therefore, low reactivity.

Considerable research efforts have been devoted to biomimetic processing methods of CaP as they result in materials with composition, morphology, crystallinity and solubility much closer to the biological apatite.[53,54] The processing techniques associated with CPCs allow fulfilment of this objective. They result in fabricated scaffolds, pre-set granules or macroporous blocks using mild consolidation methods through low-temperature dissolution–precipitation reactions that mimic the biomineralization phenomena (Fig. 2).[53] The differences between the

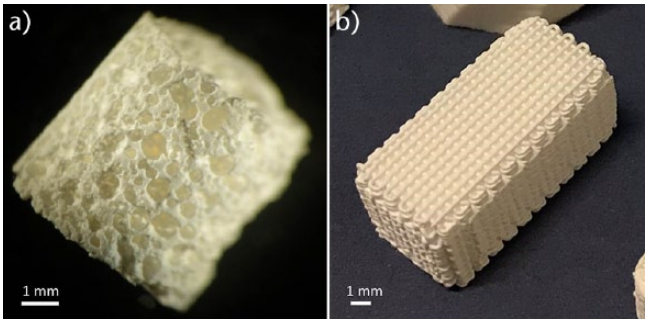

Fig. 4 Images of macroporous scaffolds obtained with biomimetic hydroxyapatite: a) injectable self-setting hydroxyapatite foam; b) structure obtained by 3D micro-extrusion of a self-setting hydroxyapatite ink.

microstructures of sintered and biomimetic CaPs can be appreciated in the scanning electron microscope images displayed in Figure 3.[55]

Architecture and porosity

Following the principles of tissue engineering, porosity has become a key feature in the design of biomaterials for bone regeneration. There is increasing evidence that some crucial aspects regarding the clinical success of bioceramics, such as the rate of resorption and the extent of angiogenesis and tissue colonization, depend not only on the intrinsic properties of the material but also on the amount, size and shape of the pores it contains.[56,57] Thus, while porosity can be a limitation for the use of these materials in high-load bearing applications, it is vital for other applications. Porosity is sought to enhance a material's resorbability and the extent of bioactivity by increasing the surface area available for reaction.[58]

Three pore size regions are often distinguished when dealing with biomaterials or scaffolds for tissue engineering: macropores (pores $>$ 100 μm), micropores (in the range of 0.1 μm to 10 μm) and nanopores ($<$ 0.1 μm). The role of macroporosity in an ideal bone graft is to guide and support tissue ingrowth within the material so that colonization and angiogenesis can take place along with the progressive bioresorption of the scaffold. When using granulated materials, the space in between individual granules defines a macroporous network even if there is no mechanical continuity in the material. Alternatively, the use of macroporous blocks or foams is proposed, as a means to promote tissue ingrowth.

But it is not just large pores that are important; the control of the micro- and nanostructure of a ceramic, and therefore the micro- and nanoporosity, has been shown to play a very relevant a role in material resorption and bone formation. Small-size pores, of micrometric or nanometric size, have a critical effect on the biological response by influencing protein adsorption, cell adhesion and the permeability of the biomaterial to the physiological fluids. It is well known, for example, that CaPs with a microporous structure have a higher osteogenic capacity and even greater osteoinduction capacity than their non-microporous analogues.[46] This trend is even clearer in nanostructured ceramics, both *in vitro* and *in vivo*.[52,59]

In contrast to high-temperature ceramics, presenting pores in the micrometric range, the low-temperature routes allow fabrication of CaP nanostructured materials (Fig. 3), extending the pore size range to much smaller sizes and thus increasing substantially the specific surface area and reactivity of the materials. The modulation of the specific surface area and the porosity at a multiscale level (nano-, micro- and macro-porosity) has become a powerful tool that allows fine-tuning of the degradation of CaPs.[55]

Open challenges in the design of high performance synthetic bone grafts

In the previous sections, an overview of the important developments in CaP research over 40 years was provided. Significant advances made in the last few decades coupled with recent technological developments ensure a bright future for these materials. In this last section we have spotted some promising topics, which may be of interest in the coming years.

Enhancement of mechanical properties

CaP ceramics have poor mechanical properties and are brittle in nature. Therefore, they are not suitable for load-bearing applications. Although there have been several attempts to improve the mechanical properties of CaP ceramics by combining them with polymers,[60] as of today no biodegradable composites have been obtained with a combination of strength, ductility and toughness close to that of cortical bone. In this sense, one of the most promising lines of research is the one based on a biomimetic approach, applying bottom-up strategies and trying to reproduce hierarchical structures at multiple length scales (from the molecular to the macroscopic scale), to have hybrid materials displaying the desired combinations of properties (strength, toughness, ductility, density, etc). This approach, based on the combination of architectural gradients, is constantly used in nature to build natural structural materials with exceptional properties.[1]

Bioceramics as drug delivery systems: bio-inorganics and biological molecules

The design of synthetic bone grafts combining both osteogenic and antimicrobial properties would represent a major breakthrough. Recent studies have shown that low temperature biomimetic CaPs offer an excellent platform to incorporate antibiotics, as they offer the possibility to

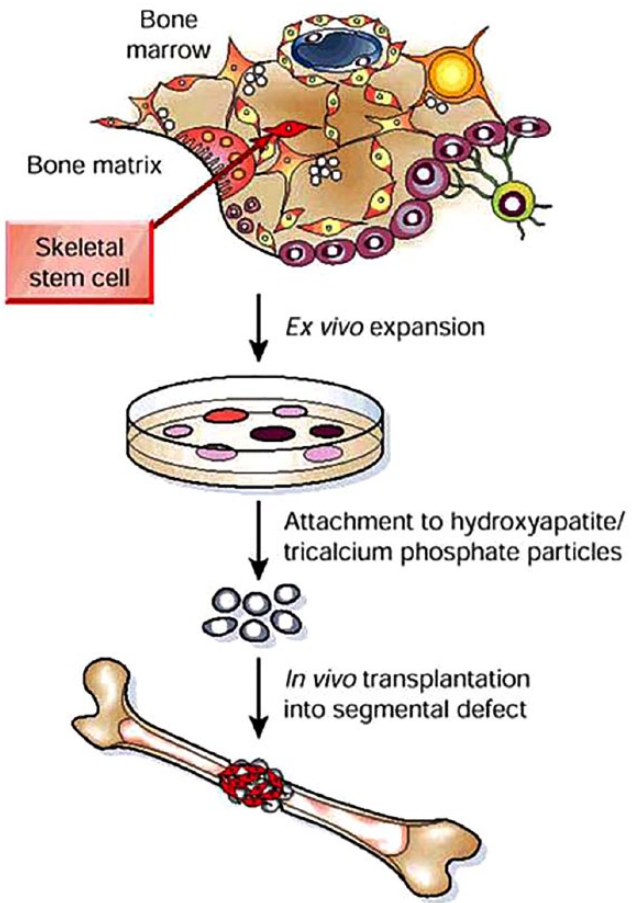

Fig. 5 Bone tissue engineering requires *ex vivo* expansion of marrow-derived skeletal stem cells and their attachment to 3D scaffolds, such as calcium phosphate ceramic particles. This hybrid construct can be transplanted into segmental defects and will subsequently regenerate an appropriate 3D structure *in vivo*. Adapted from Bianco et al,[70] with permission.

control the release kinetics by adapting the textural properties of the biomaterial, preserving intact the activity of the drug.[61,62] Similarly, other active principles can be incorporated, like anti-inflammatories or anti-cancer drugs, or even growth factors, like BMPs, which can enhance the bone graft's osteogenic potential.[63] However, some challenges remain, such as the control of the release kinetics over an appropriate time period, monitoring the release *in vivo* or ensuring a reproducible performance in different locations, clinical situations and patient specificities.

In addition to the delivery of antibiotics or other biomolecules, bioceramics can be used as vehicles for the local delivery of active ions, able to trigger specific biological responses.[41,64,65] Thus, ions such as copper, strontium, zinc, cobalt, silicon and boron have the potential to stimulate osteogenesis and angiogenesis while copper, zinc and silver have also demonstrated anti-inflammatory and antibiotic capabilities. Important advantages of this approach are those associated with lower cost and better stability.[66]

Injectable porous biomaterials

Injectable materials have many advantages in surgical practice.[67] The possibility of having an injectable biomaterial combining both multiscale porosity and the capacity to harden *in situ* would bring significant benefits to clinical practices, such as the compatibility with minimally-invasive surgical techniques, the geometrical fit to the defect thanks to the moldability of the material, superior reactivity and drug delivery capability through the presence of nano-/micropores and the possibility to be colonized by tissues due to the presence of a macropore network. Recent studies show the feasibility of designing such materials[68,69] (Fig. 4). Moreover, the possibility of using these materials as drug delivery matrices is very attractive, as recent studies have shown that, in addition to enhancing osteoconduction and material resorption, the multiscale porosity presented by these materials enables tuning the local delivery of drugs.[62]

Bioceramics and cell-based therapies

Bioceramics can also play a significant role in bone tissue engineering. Since bioceramics were introduced in the 1990s, they are being increasingly used as a bone grafting strategy,[70] using hybrid constructs that combine bone marrow cells or MSCs with synthetic scaffolds, typically made from porous ceramics and molecular signals[71] (Fig. 5). This strategy is particularly suited to complex skeletal injuries lacking sufficient osteogenic potential. Although significant progress has been made in this field in recent years and several clinical studies are under way both in Europe and the United States,[76] a better understanding of the role of each of the components and the interactions amongst them remains to be reached. In particular, the development of scaffolds that are able not only to support cell attachment and growth, but to also provide the adequate temporary niche to the cells and instruct them in the right direction, may represent a significant step forward for the efficacy of this therapeutic approach.[77] One aspect to be aware of is the myriad of possibilities that can be considered in the cell therapy field and the lack of sufficient reported data allowing for the correlation of results. Thus, the creation of international databases on clinical trials to help spread information becomes an urgent need for both researchers and surgeons.[78]

Still another challenging aspect associated with tissue engineering strategies is obtaining regulatory approval of products to conduct clinical studies. Indeed, tissue engineering approaches combining materials with cells are

considered biological devices and the regulatory path imposed by agencies both in Europe and United States is far more strict than for non-biological products.[79] This also applies to scaffolds combined with biological entities if their proposed mechanism of action is considered to be biological. Thus, any translation of a discovery in the laboratory into a tissue engineering product not only faces technical challenges but also needs to guarantee regulatory approval in order to safely and effectively bring the product to the market.

Bioceramics for personalized implants

The latest 3D-printing technologies are particularly suited for the design of personalized bone grafts[36,80,81] as they provide an accurate control of the geometry. The design of the implant shape, based on x-ray computed tomography (CT) data, ensures a perfect fit between the graft and the anatomical defect. Significant advances have been reached in the printing of CaP structures using technologies such as powder bed fusion (a laser beam melts and fuses material powder together),[82] binder jetting (a liquid binder is dispensed to join powder particles),[83] fused deposition modelling (a temperature-controlled head extrudes a thermoplastic polymeric-ceramic composite followed by a quick solidification),[84] solvent evaporation-assisted printing (a concentrated polymer-ceramic solution is extruded following rapid solvent evaporation)[85] and micro-extrusion (extrusion of a thixotropic ink to form self-supportive structures), the latter being the most widely employed (Fig. 4).[86] Typical ink formulations, which consist of a suspension of ceramic powders, like HA or β-TCP, using polymeric binders, require a sintering step to consolidate the printed green body.[86] More recently self-hardening CaP inks have been developed based on reactive CaPs like α-TCP, which allow skipping of the sintering step and, since they harden at a low temperature, this making them extremely versatile for the incorporation of biological molecules.[87,88] The possibility of working at low temperatures broadens the applicability of this technique to potentially have in-hospital patient specific bone graft manufacturing facilities, allowing surgeons to obtain ready to use bone grafts at low temperature in a few hours, revolutionizing the treatment of emergency cases and changing the way surgical planning is done for non-urgent cases.[89]

One important aspect associated with 3D printing is the possibility of adjusting the geometry and microstructure of the scaffold to tune its final mechanical properties.[90] To this end, finite element analysis is an extremely powerful tool to better understand and predict the mechanical properties of the scaffold. Furthermore, the use of *in silico* models that combine biomechanical requirements with cell fate predictions can further aid in the design of functional patient-specific 3D scaffolds at reduced cost.[91]

Conclusions

Bone is a self-repairing material. However, in some compromised clinical situations the self-regeneration mechanism fails, and it is necessary to induce bone formation beyond the capacity of the host tissue. The development of biomimetic synthetic materials with composition and structural features closer to natural bone stands as a promising strategy to improve the synchronization between bone formation and material resorption. Ideal bone grafts should tightly balance resorption and new bone deposition across different conditions imposed by the patient age, gender and social habits (sport, addictions), as well as the specific loads at the implantation site. New technologies allow great versatility and a more precise control in the design of scaffolds. However, the issue is now to decide which architecture and features the graft should have to spur regeneration in each particular situation. Understanding the biological mechanism of interaction between graft at the bony site is crucial to progress in the development of high performance, patient-specific bioceramics.

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