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Calcium phosphate bone graft substitutes: Failures and hopes

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Abstract

Despite 40 years of efforts, researchers have failed to provide calcium phosphate bone graft substitutes performing well enough to replace bone grafting procedures: their osteogenesis potential is limited, and calcium phosphates are too brittle. However, there is hope to solve the two aforementioned problems. First, it is now clear why nacre and bone are very tough despite a high ceramic load. Also, recent studies suggest that calcium and phosphate ions can trigger osteoinduction. The present article aims: (i) to review our current knowledge in the field of synthetic bone graft substitutes, (ii) to explain why ceramics and in particular calcium phosphates are still the most promising materials for bone graft substitution, and (iii) finally to describe the strategy to obtain osteoinductive calcium phosphate bone graft substitutes as strong as cortical bone.

Keywords: Biomaterial; Bone; Physico-chemical; Biological; Calcium phosphate

1. Introduction

Yearly, a few million patients need a bone graft or bone graft substitute. The most frequent causes are bone cysts and tumours, revisions of orthopaedic implants, spine fusion, and traumatic fractures.[1] Even though bone grafts provide the best biological results, there is an increasing fraction of these defects that are filled with bone graft substitutes.[1] This is partly related to the drawbacks of bone grafting, such as donor site morbidity or additional surgical time,[2–4] and partly related to the advantages of bone graft substitutes, such as availability.

Bone graft substitutes may be human-derived (allogenic), animal-derived (xenogenic) or synthetic. Since the use of human-derived and animal-derived products is ethically questionable and may lead to complications such as disease transmission,[5] it is of great interest to study and improve synthetic materials. In fact, it is to be expected that sooner or later, synthetic materials will perform better and be cheaper than allogenic and xenogenic materials.

Synthetic bone graft substitutes may consist of metals, polymers, and ceramics (Table 1).[6] The broad range of materials used for this purpose can be explained by the fact that none of these materials unite three essential criteria: (i) they should have mechanical properties as high or better than those of cortical bone ("load-bearing" property), (ii) they should be resorbable (or degradable) to prevent fatigue fractures at long implantation times, and (iii) they should promote bone formation ("osteoinductivity"). In other words, despite 40 years of research, the scientific community is still looking for more adequate bone graft substitutes. The aim of this manuscript is to recapitulate our knowledge in this field and highlight current research directions. A special emphasis is set on ceramics, in particular calcium phosphates, because it is likely that the first resorbable, strong and osteoinductive bone graft substitute will consist of a polymer-calcium phosphate composite.

2. Resorbable bone graft substitutes

Many resorption mechanisms have been reported in the past, including dissolution, cell-mediated dissolution, hydrolysis, enzymatic decomposition, or corrosion (Table 1).[6] However, the cell-mediated dissolution is probably the most interesting resorption mechanism for bone graft substitutes because it is controlled by the host cells. In other words, it is not a random process as for polylactide hydrolysis, magnesium corrosion, or calcium sulphate dissolution. During cell-mediated resorption, osteoclasts dock themselves to the material surface, open their membrane and secrete hydrochloric acid in a pouch defined between the cell and the material[7] (Fig. 1). The pH value in this zone drops down to roughly 4.5,[7] which is in the case of calcium

Abbreviations: HA, hydroxyapatite; β-TCP, β-tricalcium phosphate; α-TCP, α-tricalcium phosphate; LBL, layer-by-layer; PMMA, polymethyl methacrylate; PVA, poly(vinyl alcohol); Na-MTM, sodium montmorillonite.

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Table 1
Resorption mechanism of selected bone graft substitutes (this table is a modified version of Table 1 in Reference6).

Material type	Material	Resorption mechanism
Ceramic	Ceramic glasses (e.g. bioglass)	Very limited to complete resorption through partial dissolution[103]
	Plaster of paris[104] (=calcium sulphate hemihydrate, CSH)	Dissolution
	Gypsum[104]	
	Dicalcium phosphate dihydrate[13,26,105] (=calcium sulphate dihydrate, CSD)	Dissolution and/or conversion into an apatite
	Calcium carbonate[13]	Dissolution
	Dicalcium phosphate (DCP)[23,106]	Cell-mediated dissolution
	Octacalcium phosphate (OCP)[107]	
	β-Tricalcium phosphate (β-TCP)[20,108]	
	Biphasic calcium phosphate (BCP)[109]	
	Precipitated hydroxyapatite crystals[13,105]	
	β-Calcium pyrophosphate (β-CPP; β-$Ca_2P_2O_7$)[13]	
	Sintered hydroxyapatite	Practically no resorption
Metal	Magnesium (alloy)	Corrosion[110]
	Iron (alloy)	Corrosion[111]
	Tantalum, titanium	Practically no resorption
Polymer	Polylactides, polyglycolides[112]	Hydrolysis
	Polycaprolactone[113]	
	Cellulose	Transport to lymph nodes
	Hyaluronan	Enzymatic decomposition with hyaluronidase[114]
	Fibrin	Enzymatic decomposition with plasmin[115]
	Collagen	Enzymatic decomposition with collagenase[115]
	Chitosan	Enzymatic decomposition with lysozyme[116,117]

phosphates a value low enough to provoke calcium phosphate dissolution. The ions released during this dissolution are generally evacuated at the back of the osteoclasts and may either precipitate there[8] or mediate the activity of bone cells.[9,10] If the bone graft substitute consists of calcium phosphates, the ions may also be used as raw materials for new bone formation.[11]

Considering the importance of a cell-mediated resorption, it is important to find methods to determine by which mechanism a bone graft substitute is resorbed. In general, the cell-mediated resorption only occurs with inorganic materials. Identifying which ones are resorbed by cells can be done using in vitro tests.[12] For that purpose, osteoclasts or osteoclast-like cells are cultivated on the material and the presence of "dissolution pits" or etched crystals are indicative of a cell-mediated resorption (Fig. 2). Another potential approach is to look at the material solubility in physiological fluid[13]: the material should not be soluble in physiological fluids at pH 7.4, because spontaneous rather than osteoclastic dissolution would occur, but should be soluble at a slightly lower pH value, typically between the pH value present at the osteoclast interface (pH 4–5) and pH 7.4. However, even though solubility data is a good predictor of the in vivo behaviour,[14,15] the only way to really assess the in vivo behaviour is to implant the material.

Since the solubility of an inorganic material is so important to determine its in vivo fate, many studies have been devoted to the synthesis of materials with new compositions. In the 1970s and 1980s, a strong focus was set on hydroxyapatite (HA; $Ca_5(PO_4)_3OH$), due to its chemical similarity to bone mineral.[16,17] However, it soon appeared that the resorption rate of sintered HA was far too low, and might in some cases provoke complications due to the mechanical mismatch between HA and bone.[18] As a result, there has been a continuous evolution towards the use of more soluble calcium phosphates, from HA to the so-called "biphasic calcium phosphates" (HA–β-tricalcium phosphate mixtures (β-TCP; β-$Ca_3(PO_4)_2$)),[19] β-TCP,[20] α-tricalcium phosphate (α-TCP; α-$Ca_3(PO_4)_2$),[21] octacalcium phosphate (OCP; $Ca_8H_2(PO_4)_6{\cdot}5H_2O$),[22] anhydrous dicalcium phosphate (DCP; $CaHPO_4$)[23] and dicalcium phosphate dihydrate (DCPD; $CaHPO_4{\cdot}2H_2O$).[24] Whereas HA, β-TCP, α-TCP and OCP are mostly resorbed by osteoclasts, at least DCPD[25,26] but perhaps also DCP might be resorbed by simple dissolution.

Another approach in controlling calcium phosphate resorption consists in creating ion-substituted calcium phosphates,[27,28] which do not only have a different solubility than the undoped material, but may provide a therapeutic effect due to the release of the doping agent (e.g. Sr, Si, Mg, K, CO_3^{2-}) during resorption. Many ions are indeed known to be potent drugs.[29] However, there is presently no scientific evidence demonstrating that a doping agent has a direct (positive) effect on the in vivo performance of a doped calcium phosphate material, as explained in a recent article on Si-substituted HA.[30]

Apart from chemical approaches, physical approaches can also be used to control the resorption rate of inorganic materials used as bone graft substitutes.[31] The most important and common one is to modify the material architecture.[32,33] It is indeed known that the presence of "macropores" (in the biomaterials field, "macropores" are pores that are large enough to allow cell and blood vessel invasion; typically larger than 50 μm) can accelerate resorption and bone ingrowth.[34] Also, Klein et al.[35] underlined in the 1980s the importance of "micropores" (in

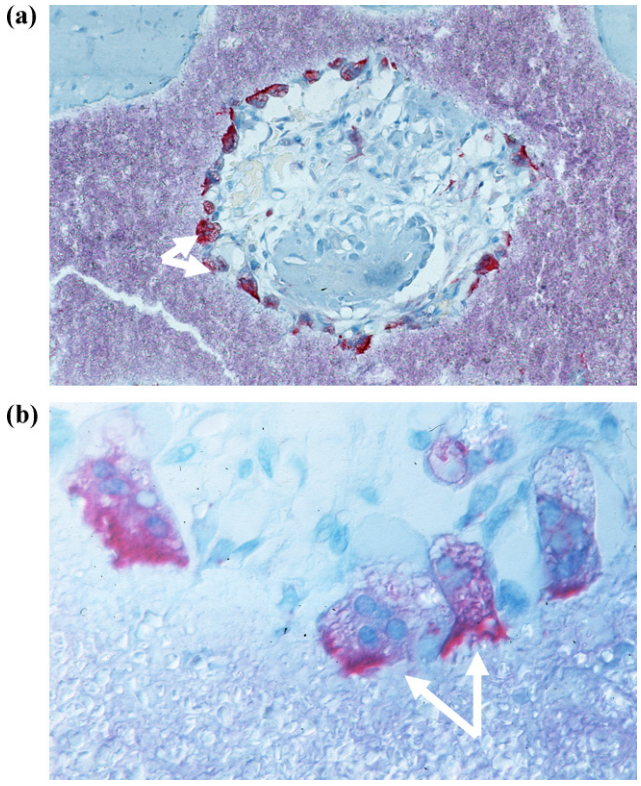

Fig. 1. Histological section of a β-TCP porous scaffold after 6-month implantation in a baboon (reproduced from [6]; courtesy of Prof Olah, University of Berne). Two enlargements are presented. On top (a), the purple zone corresponds to the ceramic. The two white arrows show some osteoclasts ("bone resorbing cells") present at the ceramic surface. The central "hole" is a ceramic pore (≈0.3 mm in diameter). An enlargement of the interface ceramic–pore is presented in (b). The two arrows show again two osteoclasts.

the biomaterials field, "micropores" are pores that are present between sintered particles; typically in the range of 0.1–10 μm). However, it is still unclear what an ideal architecture should look like and whether there is an ideal architecture.[31]

Beside a change of bone graft substitute architecture, other physical aspects are expected to modify their in vivo behaviour. For example, when a ceramic is dissolved in vitro (Fig. 2) or in vivo, resorption takes place preferentially at the grain boundaries.[8,36] Thus, a change in grain size should affect its resorption rate. However, to the best of our knowledge there is no study yet demonstrating a change of resorption rate with a change of grain size (all other factors being the same). Interestingly, a recent study of Egli et al.[37] suggests that the way a grain–air interface is formed may affect its reactivity. Indeed, these authors crushed large α-TCP blocks to produce 0.125–0.180 mm particles and tested their hydraulic reactivity before and after applying at thermal treatment at 500 °C: while the crystalline composition remained the same, the time to reach 10% of the reaction increased from a few minutes to a few hours.[38] Also, the activity of osteoclast cells was up-regulated.[37] In other words, a grain–air interface created by mechanical forces appears to be much more reactive than a grain–air interface obtained by sintering.

Since synthetic bone graft substitutes have poor mechanical properties, it is recommended to use them in mechanically stable locations (e.g. fixed with an osteosynthesis plate and screws). However, local deformations may still occur and provoke surface wear or comminution of the calcium phosphate bone graft substitute. Beside the fact that local deformations can lead to pseudo-arthrosis, the presence of loose particles should be addressed carefully. Some authors have reported

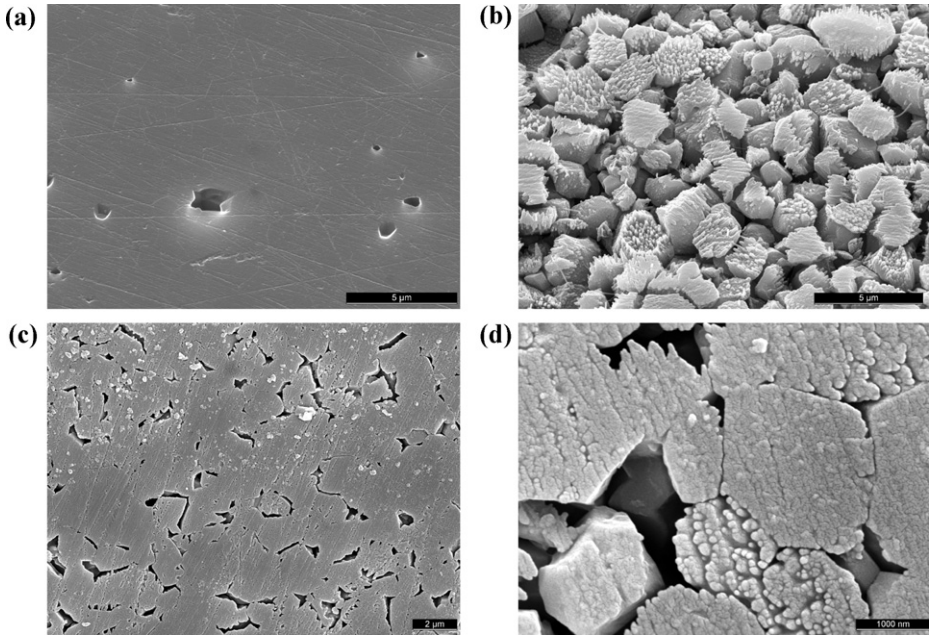

Fig. 2. surface of a dense β-TCP sample (a) before, and (b) after osteoclast culture (more details about the experimental procedure can be found elsewhere[37]). The grain boundaries of dense β-TCP samples are attacked by 10-day incubation in the cell culture medium despite the fact that the solution is supersaturated towards β-TCP (c). Simultaneously, since the medium is also supersaturated towards all calcium phosphate phases, a thin precipitated layer is observed on the sample surface, as evidence by the presence of a ruffled surface (d). The scale bar has a length of 5 μm in (a) and (b), 2 μm in (c) (same enlargement as in a and b), and 1 μm in (d).

biocompatibility problems due to the release of calcium phosphate particles,[40] whereas cytocompatibility tests suggest that size of these particles might be critical for cytotoxicity.[41] However, there is currently no indication that such loose particles may migrate and no further indication that they may be harmful. Also, calcium phosphate particles from nanometer to millimeter scale have been used successfully as bone graft substitutes.[39] Moreover, it is known that calcium phosphate resorption leads to the formation and accumulation of amorphous calcium phosphate extra-cellular deposits between macrophages or multinucleated giant cells and the ceramic.[8]

To design the most adequate bone graft substitute, it would be essential to have an idea about the rate of bone graft resorption, its mechanism, and the link between graft resorption and bone formation. Unfortunately, very little is known in this field because most in vivo studies are only descriptive. Nevertheless, a model was proposed a few years ago to determine the effect of geometry on the cell-mediated resorption rate of a bone graft substitute.[32] This model was applied to convex and concave calcium phosphate bone graft substitutes and proved to provide good to excellent fits.[32,42] However, the results also indicated that the resorption rate in sheep may vary fourfold (from 2.5 to 10 μm/week) for the same material just due to a change of macropore size.[42] Interestingly, this value is only slightly lower than the value of 25 μm/week reported for brushite (=DCPD) cements in rabbits,[43] despite the fact that sheep metabolism is slower than that of rabbits and DCPD is much more soluble than β-TCP.[44]

In summary, there are many studies devoted to the resorption of inorganic bone graft substitutes, but it is still unclear how fast bone graft substitute resorption should be. Whereas HA resorption appears too slow (in the order of years to dozen of years), possibly leading to mechanical instabilities and bone fractures,[18] DCP resorption might be too fast. Moreover, there is currently no good understanding of the interplay between material composition, architecture, resorption and bone formation.[31] So, it is not possible to design the most adequate material for a given application. However, since it is getting easier to design materials with well-controlled compositions and architectures using rapid-prototyping methods,[45] as well as to monitor their in vivo fate using advanced scanning approaches,[46,47] there is hope that enough understanding will be gained in the next few years to design much more efficient inorganic bone graft substitutes.

3. Strong bone graft substitutes

As mentioned in Section 1, several materials possessing very high mechanical properties have been proposed as bone graft substitutes.[48–50] For example, a wollastonite–hydroxyapatite composite was reported to have a bending strength of 157 MPa[49] and a life-time of 10 years in vivo at a bending strength of 65 MPa.[48] Zberg et al.[50] prepared MgZnCa glasses with a tensile strength close to 800 MPa. Unfortunately, the wollastonite–hydroxyapatite composites are not resorbable and there are biocompatibility issues related to the resorption of magnesium alloys.[50] In the field of calcium phosphates, attempts have been made over the last 40 years to improve calcium phosphate mechanical properties with polymers. The most famous case is represented by blends of hydroxyapatite and polyethylene ("HAPEX^{TM}").[51] More recently, there has been a trend towards the development of structured composites. For example, Martinez-Vazquez et al.[52] infiltrated a robocast β-TCP scaffold with polycaprolacone or polylactic acid to obtain a compressive strength close to 100 MPa. However, none of the proposed material has tensile, shear and fatigue properties similar to those of cortical bone.[53] In other words, all proposed materials eventually fail and/or do not repair the bone defect adequately. So other alternatives have to be searched for.

Interestingly, Mother Nature has developed approaches to produce strong and tough composite materials using mechanically weak materials such as collagen, chitosan, calcium carbonate, or calcium phosphates. The solution to this problem lies in a perfect structural and morphological design of the composite material.[54,55] For example, nacre consists of ceramic single crystals which have a perfectly controlled size and shape and which are "glued" together by thin organic layers.[54] Indeed, it is known that below a critical size, ceramics become tolerant to flaws and hence reach the theoretical strength of a perfect crystal.[55] So, to obtain bone graft substitutes with outstanding mechanical properties, the key is to produce ceramic–polymer composites with ceramic platelets or fibres thin enough to reach the theoretical strength of a perfect crystal and long enough (aspect ratio ≈ 20–30) to reinforce efficiently a polymer matrix. Unfortunately, mimicking natural structures remains a challenging problem due to their complexity, high level of perfection, and small scale.

Two main strategies have been used so far: (i) produce a scaffold with the first phase and reinforce it with the second phase, or (ii) produce the composite using a layer-by-layer (LBL) strategy. Applying the first strategy, Hartgerink et al.[56] reported that specific polymers could self-assemble to form long fibres and that oriented hydroxyapatite crystals could precipitate within and around these fibres. However, it is unclear how these fibres would self-assemble to form large and spatially well-organized solids and how the ceramic content could be increased to suitable levels—a too high polymer content is likely to cause biocompatibility problems. More recently, Deville et al. proposed a way to produce oriented pores in aluminium oxide solids via the so-called freeze-casting approach.[57] By impregnating such porous structures with polymethyl methacrylate (PMMA), samples with a toughness (in energy terms) 300 times larger than that of their constituents were obtained.[54] More importantly, these materials presented markedly better mechanical properties than nacre. Numerically, the final product had a yield strength and fracture toughness of 200 MPa and 30 MPa $m^{1/2}$ (human bone: yield strength 100–150 MPa, toughness > 20 MPa $m^{1/2}$[58,59]). Promising results have also been obtained with the second strategy (LBL).[60,61] For example, Podsiadlo et al.[61] dipped a substrate alternately into a poly(vinyl alcohol) (PVA) solution and into a sodium montmorillonite (Na-MTM) solution and obtained PVA–Na-MTM composites with a tensile strength of 150 MPa. Similarly, Bonderer et al. combined dip-coating and spin-coating to obtain alumina–chitosan composites with a tensile strength close to 300 MPa at an elongation of 25%.[62] Despite these very

promising results, none of the proposed materials unite adequate resorbability and strength. For example, PMMA, alumina and Na-MTM are not resorbable, and PVA readily dissolves in water. Nevertheless, it seems that only a small incremental improvement is needed to obtain the first load-bearing resorbable bone graft substitute.

4. Osteoinductive bone graft substitutes

The third important property of a bone graft substitute is its osteoinduction, i.e. its ability to stimulate bone formation. The standard approach to obtain an osteoinductive bone graft substitute is to combine it with a drug, typically a growth factor such as BMP-2[63,64] or BMP-7[65] but there are concerns related to their price and safety.[66–69] Therefore, the scientific community is actively looking for alternatives, such as bisphosphonates,[70] peptides,[71] or antibodies.[72] Another approach consists in combining bone graft substitutes with patient extracts such as platelet-rich plasma,[73] bone marrow,[74] or expanded cell extracts,[75] but the efficacy of these approaches is disputed.[76–78] In this context, the observation that materials implanted subcutaneously or in muscles[79–81] trigger bone formation has received much attention.[82,83]

Presently, the mechanism(s) involved in this process is/are unknown[82] but general rules can be drawn: more and faster ectopic bone formation is obtained in samples presenting concavities[84] and micropores.[85,86] Also, "osteoinduction" is not limited to calcium phosphates, but has also been reported for polymers,[80] and metals.[87] Even though neither polymers nor metals contain calcium phosphates, it is speculated that calcium phosphates are responsible for polymer and metal osteoinduction.[82] Indeed, since the human body is saturated towards apatite crystals, spontaneous calcium phosphate precipitation might occur at polymer and metal surfaces, hence binding growth factors and/or modifying the local concentrations of calcium and phosphate ions.

The importance of calcium and phosphate ions for osteoinduction is suggested in many studies. Phosphate ions are known to regulate osteoblast apoptosis,[88] osteopontin production,[89] and mineralization rate,[90] and calcium ions have been reported to have a profound effect on osteoblast proliferation[10] and regulation.[9] The most striking study underlining the importance of phosphate ions was presented recently by Habibovic et al.[91] These authors demonstrated that phosphate ions could have an osteogenic effect when slowly released into the thoracic muscles (erector spinae muscle) of CD1 (20–25 g) mice. Contrary to studies devoted to ion-substituted calcium phosphates, these authors did not simply implant a poorly-soluble calcium phosphate material hoping that during resorption, ions would be released, but used a drug delivery approach: fairly-soluble sodium phosphate salt crystals were squeezed between two polycaprolactone layers. Upon implantation, the salt crystals dissolved and phosphate release proceeded by diffusion. Recently, the same group of authors demonstrated that the release of other ions, such as cobalt, copper, zinc, strontium, fluoride and carbonates, could provoke interesting biological reactions.[92–94] Considering the potency of many ions, in particular calcium and phosphate ions, and the possibility to apply various strategies to control their release rate, there is great hope that the use of an "ionic therapeutic" approach might lead to an osteoinductive bone graft substitute.[29]

5. Discussion

In Section 1, it was mentioned that the scientific community is still looking for bone graft substitutes uniting the following three properties: (i) resorbable, (ii) strong, and (iii) osteoinductive. From the present review of the literature, it appears that the goal is much closer than in the past: (i) resorbable materials exist, (ii) strong materials can be produced, and (iii) calcium phosphate ceramics and phosphate-loaded polycaprolactones are osteoinductive. Unfortunately, uniting these three properties is much more complex than producing materials with one of the three properties because material resorption, bone formation and mechanical properties are self-dependent. Indeed, it is known that mechanical loading affect bone graft resorption and bone formation,[95,96] probably in the same way as bone metabolism is affected by loading.[97] Also, considering the potent activity of calcium and phosphate ions[9,10,88–90,98] or the necrotic effects provoked by certain degradation by-products,[99] it is likely that the resorption of a bone graft substitute affects its ability to be replaced by new bone. Finally, these reactions depend on a range of additional parameters such as patient age, gender, or social habits (sport, addictions), as well as implant location.[26] To explain the latter observation, Constantz et al.[26] mentioned factors such as changes of metabolic state, blood flow rate and implant–bone interface at the implantation site.

Considering the excellent biological properties of ceramics, such as calcium phosphates, it is most likely that the first resorbable, osteoinductive and strong synthetic bone graft substitute will contain a large ceramic fraction. As a result, it is extremely important to control ceramic synthesis and understand what factors may affect its physical, chemical and biological properties. Surprisingly, there are still many ceramic-related aspects that remain fragmental or unknown as demonstrated by three examples. First, despite thousands of studies on calcium phosphate and calcium carbonate synthesis, there is to our knowledge not a single study describing how the raw materials of nacre or nacre-like structure can be produced. For that purpose, non-agglomerated platelets with a controlled shape (e.g. hexagon), size (typically $\pm$ 20%) and a large aspect ratio (>10) should be produced. A step in this direction was taken by Tao et al. who produced hexagonal β-TCP platelets with a fairly homogeneous size and an aspect ratio close to 3 (Fig. 3).[100] The second example is related to ceramic grain size. In more details, it is known that the solubility of a ceramic bone graft substitute defines its resorption rate, and are playing a role during ceramic resorption.[8] It is also known that grain boundaries are generally more soluble than grains. For example, there is evidence that grain boundaries of β-TCP are dissolved in cell culture media even though β-TCP is insoluble in such a media (Fig. 2). However, there is no study relating composition, grain size, grain solubility, and in vivo response. The third

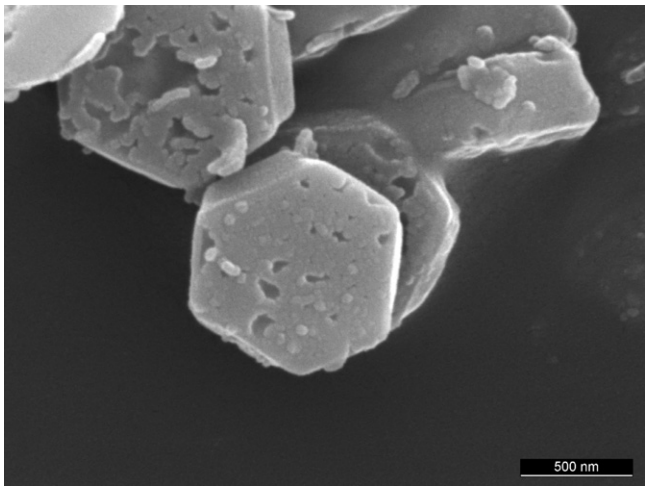

Fig. 3. β-Tricalcium phosphate plates obtained according to the method of Tao et al.[100]

and last example given here is related to the observation made in Section 4 that a controlled delivery rate of ions such as phosphates might lead to osteogenesis. This observation implies a good knowledge of the solubility and dissolution of calcium phosphates. In fact, the solubility values of calcium phosphates have been questioned.[101] Moreover, dissolution mechanisms of calcium phosphates are still poorly understood.[102]

6. Conclusion

Bone graft substitute research started four decades ago. Thousands of studies have been devoted to the search of THE ideal bone graft substitute, but efforts have failed so far: there is no material that is mechanically and biologically as good as human bone. As a result, the clinical indications of bone graft substitutes are still limited. The ideal bone graft substitute should be (i) resorbable, (ii) osteoinductive, and (iii) mechanically as strong as cortical bone. The present review of the literature in these three research fields reveals that there is a good understanding of the needs and routes to reach these goals. In other words, despite the complexity of the problem, one may hope to see the first resorbable, osteoinductive, and strong bone graft substitute soon. Looking at the present state of knowledge, it is highly likely that such a material will contain a large ceramic fraction, most likely a calcium phosphate. But since there are many aspects related to calcium phosphate synthesis and properties that are unknown, there is a great need for further research. This may include the synthesis of calcium phosphate single crystals with controlled shape, size and aspect ratio, or an improvement of our understanding of the link between calcium phosphate synthesis, properties and in vivo behaviour.

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