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Journal of the European Ceramic Society 32 (2012) 2663–2671

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# Calcium phosphate bone graft substitutes: Failures and hopes

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Available online 12 March 2012

**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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doi:10.1016/j.jeurceramsoc.2012.02.028

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

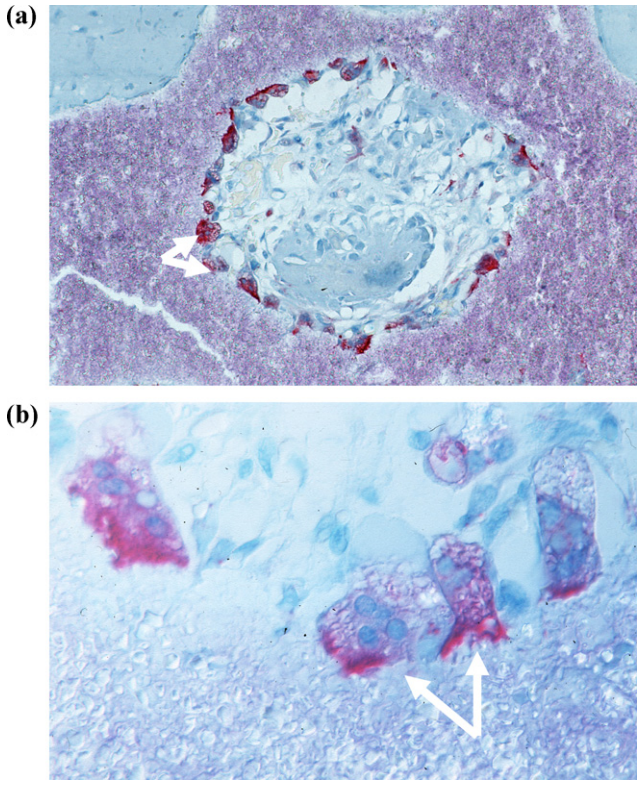


Fig. 1. Histological section of a β-TCP porous scaffold after 6-month implantation in a baboon (reproduced from [6]; courtesy of Prof Olah, University of Berne). Two enlargements are presented. On top (a), the purple zone corresponds to the ceramic. The two white arrows show some osteoclasts ("bone resorbing 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

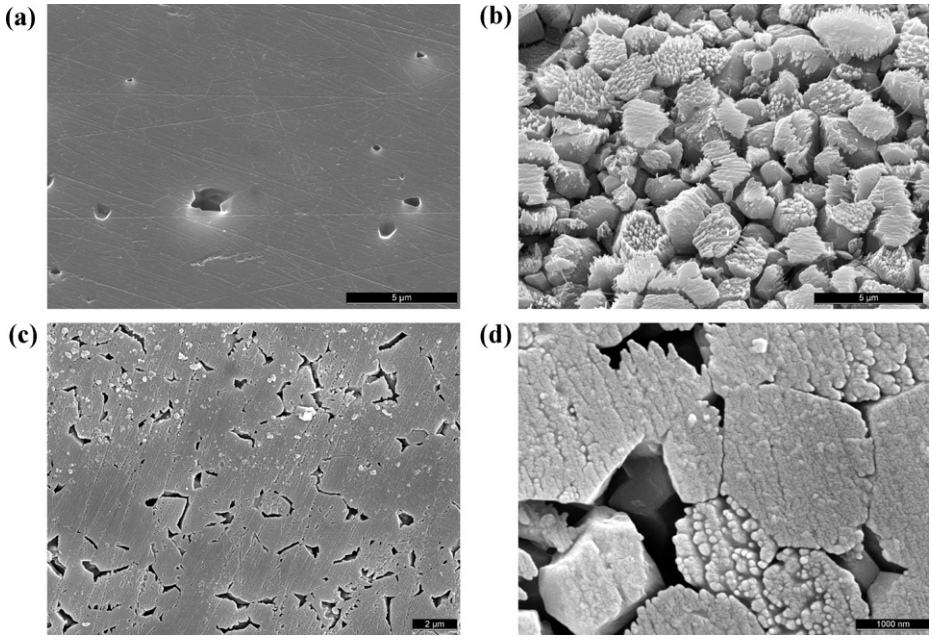


Fig. 2. surface of a dense β-TCP sample (a) before, and (b) after osteoclast culture (more details about the experimental procedure can be found elsewhere[37]). The grain boundaries of dense β-TCP samples are attacked by 10-day incubation in the cell culture medium despite the fact that the solution 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

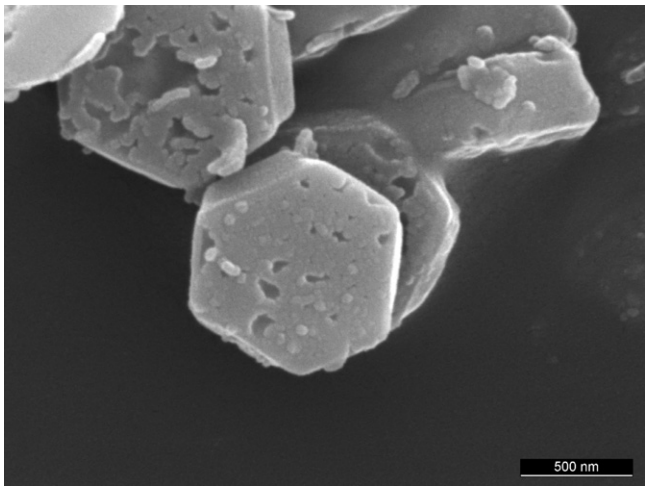


Fig. 3. β-Tricalcium phosphate plates obtained according to the method of Tao et al.[100]

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.

## Acknowledgements

The author would like to thank Mathys Ltd for their financial support, as well as Rainer Egli and Michel Descamps for Fig. 2.

## References

1. Sullivan F. European Markets for bone grafts and bone. 2008.
2. Banwart JC, Asher MA, Hassanein RS. Iliac crest bone graft harvest donor site morbidity. A statistical evaluation. *Spine* 1995;**20**(May (9)): 1055–60.
3. Younger EM, Chapman MW. Morbidity at bone graft donor sites. *J Orthop Trauma* 1989;**3**(3):192–5.
4. Arrington ED, Smith WJ, Chambers HG, Bucknell AL, Davino NA. Complications of iliac crest bone graft harvesting. *Clin Orthop* 1996;(August (329)):300–9.
5. Hofmann GO, Kirschner MH, Wangemann T, Falk C, Mempel W, Hammer C. Infections and immunological hazards of allogeneic bone transplantation. *Arch Orthop Trauma Surg* 1995;**114**(3):159–66.
6. Bohner M. Resorbable biomaterials as bone graft substitutes. *Materials Today* 2010;**13**(1–2):24–30.
7. Teitelbaum SL. Bone resorption by osteoclasts. *Science* 2000;**289**(September (5484)):1504–8.
8. Koerten HK, van der Meulen J. Degradation of calcium phosphate ceramics. *J Biomed Mater Res* 1999;**44**(January (1)):78–86.
9. Zaidi M, Datta HK, Patchell A, Moonga B, MacIntyre I. 'Calcium-activated' intracellular calcium elevation: a novel mechanism of osteoclast regulation. *Biochem Biophys Res Commun* 1989;**163**(September (3)):1461–5.
10. Kanatani M, Sugimoto T, Fukase M, Fujita T. Effect of elevated extracellular calcium on the proliferation of osteoblastic MC3T3-E1 cells: its direct and indirect effects via monocytes. *Biochem Biophys Res Commun* 1991;**181**(December (3)):1425–30.
11. Metsger DS, DePhilip RM, Hayes TG. An autoradiographic study of calcium phosphate ceramic bone implants in turkeys. *Clin Orthop* 1993;(June (291)):283–94.
12. Schilling AF, Linhart W, Filke S, Gebauer M, Schinke T, Rueger JM, et al. Resorbability of bone substitute biomaterials by human osteoclasts. *Biomaterials* 2004;**25**(August (18)):3963–72.
13. Bohner M. Bioresorbable ceramics. In: Buchanan F, editor. *Degradation rate of bioresorbable materials*. Cambridge: Woodhead Publishing Limited; 2008. p. 95–114.
14. Driessens FCM. Physiology of hard tissues in comparison with the solubility of synthetic calcium phosphates. *Ann N Y Acad Sci* 1988;**523**: 131–6.
15. Driessens FCM, Verbeeck RM, editors. Relation between physicochemical solubility and biodegradability of calcium phosphates. Amsterdam: Elsevier Science Publishers; 1988.
16. Ducheyne P, de Groot K. In vivo surface activity of a hydroxyapatite alveolar bone substitute. *J Biomed Mater Res* 1981;**15**(May (3)): 441–5.
17. Rabalais Jr ML, Yukna RA, Mayer ET. Evaluation of durapatite ceramic as an alloplastic implant in periodontal osseous defects. I. Initial six-month results. *J Periodontol* 1981;**52**(November (11)):680–9.
18. Linhart W, Briem D, Amling M, Rueger JM, Windolf J. Mechanical failure of a porous hydroxyapatite ceramic 7.5 years after treatment of a fracture of the proximal tibia. *Unfallchirurg* 2004;**107**(February (2)):154–7.
19. Passuti N, Daculsi G, Rogez JM, Martin S, Bainvel JV. Macroporous calcium phosphate ceramic performance in human spine fusion. *Clin Orthop Relat Res* 1989;(248):169–76.
20. Eggli PS, Muller W, Schenk RK. Porous hydroxyapatite and tricalcium phosphate cylinders with two different pore size ranges implanted in the cancellous bone of rabbits. A comparative histomorphometric and histologic study of bony ingrowth and implant substitution. *Clin Orthop* 1988;(July (232)):127–38.
21. Merten HA, Wiltfang J, Grohmann U, Hoenig JF. Intraindividual comparative animal study of alpha- and beta-tricalcium phosphate degradation in conjunction with simultaneous insertion of dental implants. *J Craniofacial Surg* 2001;**12**(1):59–68.
22. Sasano Y, Kamakura S, Nakamura M, Suzuki O, Mizoguchi I, Akita H, et al. Subperiosteal implantation of octacalcium phosphate (OCP) stimulates both chondrogenesis and osteogenesis in the tibia, but only osteogenesis in the parietal bone of a rat. *Anat Rec* 1995;**242**(May (1)):40–6.

23. Habibovic P, Gbureck U, Doillon CJ, Bassett DC, van Blitterswijk CA, Barralet JE. Osteoconduction and osteoinduction of low-temperature 3D printed bioceramic implants. *Biomaterials* 2008;**29**(March (7)):944–53.
24. Munting E, Mirtchi A, Lemaître J. Bone repair of defects filled with a phosphocalcic hydraulic cement: and in vivo study. *J Mater Sci Mater Med* 1993;**4**:337–44.
25. Theiss F, Apelt D, Brand B, Kutter A, Zlinszky K, Bohner M, et al. Biocompatibility and resorption of a brushite calcium phosphate cement. *Biomaterials* 2005;**26**(July (21)):4383–94.
26. Constantz BR, Barr BM, Ison IC, Fulmer MT, Baker J, McKinney L, et al. Histological, chemical, and crystallographic analysis of four calcium phosphate cements in different rabbit osseous sites. *J Biomed Mater Res* 1998;**43**(Winter (4)):451–61.
27. Boanini E, Gazzano M, Bigi A. Ionic substitutions in calcium phosphates synthesized at low temperature. *Acta Biomater* 2010;**6**(6):1882–94.
28. Yoshida K, Hyuga H, Kondo N, Kita H, Sasaki M, Mitamura M, et al. Substitution model of monovalent (Li, Na, and K), divalent (Mg), and trivalent (Al) metal ions for β-tricalcium phosphate. *J Am Ceram Soc* 2006;**89**(2):688–90.
29. Habibovic P, Barralet JE. Bioinorganics and biomaterials: bone repair. *Acta Biomater* 2011;**7**(August (8)):3013–26.
30. Bohner M. Silicon-substituted calcium phosphates—a critical view. *Biomaterials* 2009;**30**(November (32)):6403–6.
31. Bohner M, Loosli Y, Baroud G, Lacroix D. Deciphering the link between architecture and biological response of a bone graft substitute. *Acta Biomater* 2011;**7**(2):478–84.
32. Bohner M, Baumgart F. Theoretical model to determine the effects of geometrical factors on the resorption of calcium phosphate bone substitutes. *Biomaterials* 2004;**25**(August (17)):3569–82.
33. Karageorgiou V, Kaplan D. Porosity of 3D biomaterial scaffolds and osteogenesis. *Biomaterials* 2005;**26**(September (27)):5474–91.
34. Klawitter JJ, Hulbert SF. Application of porous ceramics for the attachment of load bearing internal orthopedic applications. *J Biomed Mater Res Symp* 1971;**2**(1):161–229.
35. Klein CP, de Groot K, Driessen AA, van der Lubbe HB. Interaction of biodegradable beta-whitlockite ceramics with bone tissue: an in vivo study. *Biomaterials* 1985;**6**(May (3)):189–92.
36. Ito A, Senda K, Sogo Y, Oyane A, Yamazaki A, LeGeros RZ. Dissolution rate of zinc-containing b-tricalcium phosphate ceramics. *Biomed Mater* 2006;**1**:134–9.
37. Egli RJ, Gruenenfelder S, Doebelin N, Hofstetter W, Luginbuehl R, Bohner M. Thermal treatments of calcium phosphate biomaterials to tune the physico-chemical properties and modify the in vitro osteoclast response. *Adv Eng Mater* 2011;**13**(3):B102–7.
38. Bohner M, Luginbuhl R, Reber C, Doebelin N, Baroud G, Conforto E. A physical approach to modify the hydraulic reactivity of alpha-tricalcium phosphate powder. *Acta Biomater* 2009;**5**(September (9)):3524–35.
39. Bohner M. Design of ceramic-based cements and putties for bone graft substitution. *Eur Cells Mater* 2010;**20**:1–12.
40. Miyamoto Y, Ishikawa K, Takechi M, Toh T, Yuasa T, Nagayama M, et al. Histological and compositional evaluations of three types of calcium phosphate cements when implanted in subcutaneous tissue immediately after mixing. *J Biomed Mater Res* 1999;**48**(Spring (1)):36–42.
41. Pioletti DP, Takei H, Lin T, Van Landuyt P, Ma QJ, Kwon SY, et al. The effects of calcium phosphate cement particles on osteoblast functions. *Biomaterials* 2000;**21**(June (11)):1103–14.
42. Bashoor-Zadeh M, Baroud G, Bohner M. Simulation of the in vivo resorption rate of beta-tricalcium phosphate bone graft substitutes implanted in a sheep model. *Biomaterials* 2011;**32**(September (27)):6362–73.
43. Ohura K, Bohner M, Hardouin P, Lemaitre J, Pasquier G, Flautre B. Resorption of, and bone formation from, new beta-tricalcium phosphate-monocalcium phosphate cements: an in vivo study. *J Biomed Mater Res* 1996;**30**(February (2)):193–200.
44. Vereecke G, Lemaitre J. Calculation of the solubility diagrams in the system $Ca(OH)_2$-$H_3PO_4$-KOH-$HNO_3$-$CO_2$-$H_2O$. *J Crystal Growth* 1990;**104**:820–32.
45. Hollister SJ. Porous scaffold design for tissue engineering. *Nat Mater* 2005;**4**(July (7)):518–24.
46. Komlev VS, Mastrogiacomo M, Pereira RC, Peyrin F, Rustichelli F, Cancedda R. Biodegradation of porous calcium phosphate scaffolds in an ectopic bone formation model studied by X-ray computed microtomography. *Eur Cells Mater* 2010;**19**:136–46.
47. Komlev VS, Mastrogiacomo M, Peyrin F, Cancedda R, Rustichelli F. X-ray synchrotron radiation pseudo-holotomography as a new imaging technique to investigate angio- and microvasculogenesis with no usage of contrast agents. *Tissue Eng Part C: Methods* 2009;**15**(3):425–30.
48. Kokubo T, Ito S, Shigematsu M, Sakka S, Yamamuro T. Fatigue and lifetime of bioactive glass-ceramic A-W containing apatite and wollastonite. *J Mater Sci* 1987;**22**:4067–70.
49. Nakamura T, Yamamuro T, Higashi S, Kokubo T, Itoo S. A new glass-ceramic for bone replacement: evaluation of its bonding to bone tissue. *J Biomed Mater Res* 1985;**19**(July–August (6)):685–98.
50. Zberg B, Uggowitzer PJ, Loffler JF. MgZnCa glasses without clinically observable hydrogen evolution for biodegradable implants. *Nat Mater* 2009;**8**(November (11)):887–91.
51. Suwanprateeb J, Tanner KE, Turner S, Bonfield W. Influence of sterilization by gamma irradiation and of thermal annealing on creep of hydroxyapatite-reinforced polyethylene composites. *J Biomed Mater Res* 1997;**39**(1):16–22.
52. Martinez-Vazquez FJ, Perera FH, Miranda P, Pajares A, Guiberteau F. Improving the compressive strength of bioceramic robocast scaffolds by polymer infiltration. *Acta Biomater* 2011;**6**(11):4361–8.
53. Wagoner Johnson AJ, Herschler BA. A review of the mechanical behavior of CaP and CaP/polymer composites for applications in bone replacement and repair. *Acta Biomater* 2011;**7**(1):16–30.
54. Munch E, Launey ME, Alsem DH, Saiz E, Tomsia AP, Ritchie RO. Tough, bio-inspired hybrid materials. *Science* 2008;**322**(5907):1516–20.
55. Gao H, Ji B, Jäger IL, Arzt E, Fratzl P. Materials become insensitive to flaws at nanoscale: lessons from nature. *Proc Natl Acad Sci U S A* 2003;**100**(10):5597–600.
56. Hartgerink JD, Beniash E, Stupp SI. Self-assembly and mineralization of peptide-amphiphile nanofibers. *Science* 2001;**294**(November (5547)):1684–8.
57. Deville S, Saiz E, Nalla RK, Tomsia AP. Freezing as a path to build complex composites. *Science* 2006;**311**(January (5760)):515–8.
58. Koester KJ, Ager III JW, Ritchie RO. The true toughness of human cortical bone measured with realistically short cracks. *Nat Mater* 2008;**7**(8):672–7.
59. Currey JD. The mechanical properties of bone. *Clin Orthop Relat Res* 1970;**73**:209–31.
60. Lefort M, Popa G, Seyrek E, Szamocki R, Felix O, Hemmerlé J, et al. Spray-on organic/inorganic films: a general method for the formation of functional nano- to microscale coatings. *Angew Chem Int Ed* 2010;**49**(52):10110–3.
61. Podsiadlo P, Kaushik AK, Arruda EM, Waas AM, Shim BS, Xu J, et al. Ultrastrong and stiff layered polymer nanocomposites. *Science* 2007;**318**(5847):80–3.
62. Bonderer LJ, Studart AR, Gauckler LJ. Bioinspired design and assembly of platelet reinforced polymer films. *Science* 2008;**319**(February (5866)):1069–73.
63. Seeherman H, Li R, Bouxsein M, Kim H, Li XJ, Smith-Adaline EA, et al. rhBMP-2/calcium phosphate matrix accelerates osteotomy-site healing in a nonhuman primate model at multiple treatment times and concentrations. *J Bone Joint Surg Am* 2006;**88**(January (1)):144–60.
64. Seeherman HJ, Azari K, Bidic S, Rogers L, Li XJ, Hollinger JO, et al. rhBMP-2 delivered in a calcium phosphate cement accelerates bridging of critical-sized defects in rabbit radii. *J Bone Joint Surg Am* 2006;**88**(July (7)):1553–65.
65. Axelrad TW, Einhorn TA. Bone morphogenetic proteins in orthopaedic surgery. *Cytokine Growth Factor Rev* 2009;**20**(5–6):481–8.
66. Mroz TE, Wang JC, Hashimoto R, Norvell DC. Complications related to osteobiologics use in spine surgery: a systematic review. *Spine* 2010;**35**(SUPPL. 9S):S86–104.
67. Williams BJ, Smith JS, Fu KMG, Hamilton DK, Polly Jr DW, Ames CP, et al. Does bone morphogenetic protein increase the incidence of perioperative complications in spinal fusion? A comparison of 55,862 cases of spinal fusion with and without bone morphogenetic protein. *Spine* 2011;**36**(20):1685–9.

68. Wong DA, Kumar A, Jatana S, Ghiselli G, Wong K. Neurologic impairment from ectopic bone in the lumbar canal: a potential complication of off-label PLIF/TLIF use of bone morphogenetic protein-2 (BMP-2). *Spine J* 2008;**8**(6):1011–8.
69. Yaremchuk KL, Toma MS, Somers ML, Peterson E. Acute airway obstruction in cervical spinal procedures with bone morphogenetic proteins. *Laryngoscope* 2010;**120**(10):1954–7.
70. Yoshinari M, Oda Y, Inoue T, Matsuzaka K, Shimono M. Bone response to calcium phosphate-coated and bisphosphonate-immobilized titanium implants. *Biomaterials* 2002;**23**(July (14)):2879–85.
71. Lindley EM, Guerra FA, Krauser JT, Matos SM, Burger EL, Patel VV. Small peptide (P-15) bone substitute efficacy in a rabbit cancellous bone model. *J Biomed Mater Res - Part B Appl Biomater* 2010;**94**(2):463–8.
72. Tian X, Jee WSS, Li X, Paszty C, Ke HZ. Sclerostin antibody increases bone mass by stimulating bone formation and inhibiting bone resorption in a hindlimb-immobilization rat model. *Bone* 2011;**48**(2):197–201.
73. Marx RE, Carlson ER, Eichstaedt RM, Schimmele SR, Strauss JE, Georgeff KR. Platelet-rich plasma: growth factor enhancement for bone grafts. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod* 1998;**85**(June (6)):638–46.
74. Hernigou P, Poignard A, Manicom O, Mathieu G, Rouard H. The use of percutaneous autologous bone marrow transplantation in nonunion and avascular necrosis of bone. *J Bone Joint Surg Br* 2005;**87**(July (7)):896–902.
75. de Bruijn JD, van den Brink I, Mendes S, Dekker R, Bovell YP, van Blitterswijk CA. Bone induction by implants coated with cultured osteogenic bone marrow cells. *Adv Dent Res* 1999;**June (13)**:74–81.
76. Intini G. The use of platelet-rich plasma in bone reconstruction therapy. *Biomaterials* 2009;**30**(28):4956–66.
77. Kruyt MC, Dhert WJ, Yuan H, Wilson CE, van Blitterswijk CA, Verbout AJ, et al. Bone tissue engineering in a critical size defect compared to ectopic implantations in the goat. *J Orthop Res* 2004;**22**(May (3)):544–51.
78. Stoll T, Maissen O, Meury T, Becker S. New aspects in osteoinduction. *Materialwiss Werkst* 2004;**35**(April (4)):198–202.
79. Ripamonti U. The morphogenesis of bone in replicas of porous hydroxyapatite obtained from conversion of calcium carbonate exoskeletons of coral. *J Bone Joint Surg Am* 1991;**73**(June (5)):692–703.
80. Winter GD, Simpson BJ. Heterotopic bone formed in a synthetic sponge in the skin of young pigs. *Nature* 1969;**223**(July (5201)):88–90.
81. Yamasaki H. Heterotopic bone formation around porous hydroxyapatite ceramics in the subcutis of dogs. *Jpn J Oral Biol* 1990;**32**:190–2.
82. Habibovic P, de Groot K. Osteoinductive biomaterials—properties and relevance in bone repair. *J Tissue Eng Regen Med* 2007;**1**(January–February (1)):25–32.
83. LeGeros RZ. Calcium phosphate-based osteoinductive materials. *Chem Rev* 2008;**108**(11):4742–53.
84. Ripamonti U, Crooks J, Kirkbride AN. Sintered porous hydroxyapatites with intrinsic osteoinductive activity: Geometric induction of bone formation. *S Afr J Sci* 1999;**95**(8):335–43.
85. Habibovic P, Yuan H, van der Valk CM, Meijer G, van Blitterswijk CA, de Groot K. 3D microenvironment as essential element for osteoinduction by biomaterials. *Biomaterials* 2005;**26**(June (17)):3565–75.
86. Yuan H, Fernandes H, Habibovic P, de Boer J, Barradas AM, de Ruiter A, et al. Osteoinductive ceramics as a synthetic alternative to autologous bone grafting. *Proc Natl Acad Sci U S A* 2010;**107**(August (31)):13614–9.
87. Takemoto M, Fujibayashi S, Neo M, Suzuki J, Matsushita T, Kokubo T, et al. Osteoinductive porous titanium implants: effect of sodium removal by dilute HCl treatment. *Biomaterials* 2006;**27**(May (13)):2682–91.
88. Meleti Z, Shapiro IM, Adams CS. Inorganic phosphate induces apoptosis of osteoblast-like cells in culture. *Bone* 2000;**27**(September (3)):359–66.
89. Wu X, Itoh N, Taniguchi T, Nakanishi T, Tanaka K. Requirement of calcium and phosphate ions in expression of sodium-dependent vitamin C transporter 2 and osteopontin in MC3T3-E1 osteoblastic cells. *Biochim Biophys Acta - Mol Cell Res* 2003;**1641**(1):65–70.
90. Wang D, Christensen K, Chawla K, Xiao G, Krebsbach PH, Franceschi RT. Isolation and characterization of MC3T3-E1 preosteoblast subclones with distinct in vitro and in vivo differentiation/mineralization potential. *J Bone Miner Res* 1999;**14**(June (6)):893–903.
91. Habibovic P, Bassett DC, Doillon CJ, Gerard C, McKee MD, Barralet JE. Collagen biomineralization in vivo by sustained release of inorganic phosphate ions. *Adv Mater* 2010;**22**(16):1858–62.
92. Yang L, Perez-Amodio S, Barrère-de Groot FYF, Everts V, van Blitterswijk CA, Habibovic P. The effects of inorganic additives to calcium phosphate on in vitro behavior of osteoblasts and osteoclasts. *Biomaterials* 2010;**31**(11):2976–89.
93. Patntirapong S, Habibovic P, Hauschka PV. Effects of soluble cobalt and cobalt incorporated into calcium phosphate layers on osteoclast differentiation and activation. *Biomaterials* 2009;**30**(February (4)):548–55.
94. Barralet J, Gbureck U, Habibovic P, Vorndran E, Gerard C, Doillon CJ. Angiogenesis in calcium phosphate scaffolds by inorganic copper ion release. *Tissue Eng Part A* 2009;**15**(July (7)):1601–9.
95. Roshan-Ghias A, Terrier A, Bourban PE, Pioletti DP. In vivo cyclic loading as a potent stimulatory signal for bone formation inside tissue engineering scaffolds. *Eur Cells Mater* 2010;**19**:41–9.
96. Frankenburg EP, Goldstein SA, Bauer TW, Harris SA, Poser RD. Biomechanical and histological evaluation of a calcium phosphate cement. *J Bone Joint Surg Am* 1998;**80**(August (8)):1112–24.
97. Vatsa A, Mizuno D, Smit TH, Schmidt CF, MacKintosh FC, Klein-Nulend J. Bio imaging of intracellular NO production in single bone cells after mechanical stimulation. *J Bone Miner Res* 2006;**21**(11):1722–8.
98. Nakade O, Takahashi K, Takuma T, Aoki T, Kaku T. Effect of extracellular calcium on the gene expression of bone morphogenetic protein-2 and -4 of normal human bone cells. *J Bone Miner Metab* 2001;**19**(1):13–9.
99. Ignatius AA, Betz O, Augat P, Claes LE. In vivo investigations on composites made of resorbable ceramics and poly(lactide) used as bone graft substitutes. *J Biomed Mater Res* 2001;**58**(6):701–9.
100. Tao J, Jiang W, Zhai H, Pan H, Xu R, Tang R. Structural components and anisotropic dissolution behaviors in one hexagonal single crystal of β-tricalcium phosphate. *Cryst Growth Design* 2008;**8**(7):2227–34.
101. Pan HB, Darvell BW. Calcium phosphate solubility: the need for re-evaluation. *Cryst Growth Design* 2009;**9**(2):639–45.
102. Dorozhkin SV. A review on the dissolution models of calcium apatites. *Prog Cryst Growth Ch* 2002;**44**(1):45–61.
103. Hench LL, Hench JW, Greenspan DC. Bioglass: a short history and bibliography. *J Australasian Ceram Soc* 2004;**40**(1):1–42.
104. Peltier LF. The use of plaster of paris to fill defects in bone. *Clin Orthop* 1961;**21**:1–31.
105. Ikenaga M, Hardouin P, Lemaitre J, Andrianjatovo H, Flautre B. Biomechanical characterization of a biodegradable calcium phosphate hydraulic cement: a comparison with porous biphasic calcium phosphate ceramics. *J Biomed Mater Res* 1998;**40**(April (1)):139–44.
106. Tamimi F, Torres J, Kathan C, Baca R, Clemente C, Blanco L, et al. Bone regeneration in rabbit calvaria with novel monetite granules. *J Biomed Mater Res* 2008;**87**(4):980–5.
107. Suzuki O, Imaizumi H, Kamakura S, Katagiri T. Bone regeneration by synthetic octacalcium phosphate and its role in biological mineralization. *Curr Med Chem* 2008;**15**(3):305–13.
108. Driskell T. Calcium phosphate resorbable ceramics: a potential alternative to bone grafting. *J Dental Res* 1973;**52**:123.
109. Yamada S, Heymann D, Bouler JM, Daculsi G. Osteoclastic resorption of calcium phosphate ceramics with different hydroxyapatite/β-tricalcium phosphate ratios. *Biomaterials* 1997;**18**(15):1037–41.
110. Witte F, Fischer J, Nellesen J, Crostack HA, Kaese V, Pisch A, et al. In vitro and in vivo corrosion measurements of magnesium alloys. *Biomaterials* 2006;**27**(March (7)):1013–8.
111. Peuster M, Hesse C, Schloo T, Fink C, Beerbaum P, von Schnakenburg C. Long-term biocompatibility of a corrodible peripheral iron stent in the porcine descending aorta. *Biomaterials* 2006;**27**(October (28)):4955–62.
112. Gogolewski S, Jovanovic M, Perren SM, Dillon JG, Hughes MK. Tissue response and in vivo degradation of selected polyhydroxyacids: polylactides (PLA), poly(3-hydroxybutyrate) (PHB), and poly(3-hydroxybutyrate-co-3-hydroxyvalerate) (PHB/VA). *J Biomed Mater Res* 1993;**27**(September (9)):1135–48.
113. Vert M. Polymeric biomaterials: strategies of the past vs. strategies of the future. *Progr Polym Sci (Oxford)* 2007;**32**(8–9):755–61.

114. Fraser JR, Laurent TC, Engstrom-Laurent A, Laurent UG. Elimination of hyaluronic acid from the blood stream in the human. *Clin Exp Pharmacol Physiol* 1984;**11**(January–February (1)):17–25.
115. West JL, Hubbell JA. Polymeric biomaterials with degradation sites for proteases involved in cell migration. *Macromolecules* 1999;**32**(January (1)):241–4.
116. Suh JK, Matthew HW. Application of chitosan-based polysaccharide biomaterials in cartilage tissue engineering: a review. *Biomaterials* 2000;**21**(December (24)):2589–98.
117. Hirano S, Tsuchida H, Nagao N. N-acetylation in chitosan and the rate of its enzymic hydrolysis. *Biomaterials* 1989;**10**(October (8)):574–6.