Composites of Calcium Phosphate and Polymers as Bone Substitution Materials

Markus Neumann, Matthias Epple[1]

Abstract
The state of the art of calcium phosphate/polymer composites for bone substitution is reviewed. Many combinations are possible which were proposed to improve the mechanical properties and the biocompatibility. However, the way from the laboratory to the clinical application is long, and potential candidates for new bone substitution materials have to meet many different requirements.

Key Words
Bone substitution materials · Calcium phosphates · Hydroxyapatite · Tricalcium phosphate · Polymers

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Introduction

There is a high clinical demand for synthetic bone substitution materials, due to the drawbacks associated with biological bone grafts. Xenografts are generally associated with potential infections. The same is true for allografts where concerns about infections are also strong and lead to high demands to ensure a safe implantation. These demands make such allografts, e.g. from bone banks, increasingly expensive. Autografts are still the "gold standard" of all bone substitution materials, but the available amount is generally limited, and the explantation requires a second, sometimes painful, operation. Fully synthetic bone substitution materials can – in principle – be prepared in unlimited amounts and there are no concerns about a potential infectiveness.

According to Rueger [1], there are different classes of bone substitution materials. Very prominent are ceramics, usually on the basis of calcium phosphate, due to their good biocompatibility. This is directly related to the fact that calcium phosphate forms the inorganic material of hard tissue, i.e. of bone and teeth [2–4]. Several calcium phosphates with different chemical composition are on the market (see [5] for a review), the most prominent ones being hydroxyapatite, $Ca_5(PO_4)_3OH$, and tricalcium phosphate, $Ca_3(PO_4)_2$. As with any ceramic material, these compounds suffer from a low elasticity and a high brittleness which leads to concerns about their mechanical performance after implantation. A possible solution is the combination of calcium phosphates with polymers to suitable composites which have both a high degree of elasticity and a good biocompatibility. As the number of available polymers is almost unlimited, there is a wide potential for such combinations. Another point is the acidity of polyesters like polylactide during degradation which may be compensated by basic calcium phosphates. The different reasons why such composites are prepared are summarized in Figure 1. Here, we review the state of the art of such calcium phosphate/polymer composites and draw some conclusions for potential clinical application.

Composites of Calcium Phosphate and Polymers

In principle, many combinations are possible (Figure 2). There is a range of available calcium phosphates, and an even greater choice of biocompatible polymers. However, two main groups of polymers can be distinguished: synthetic polymers like polyesters (polyglycolide, polylactide) and polymers of biological origin

[1] Department of Inorganic Chemistry, University of Duisburg-Essen, Essen, Germany.

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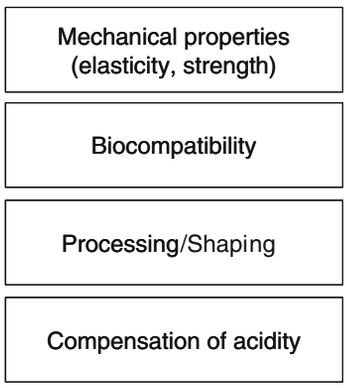

Figure 1. Common reasons to prepare calcium phosphate/polymer composites as bone substitution materials.

like collagen, gelatin, or alginate. Different ways were realized to bring these two components together into a potential implant, like simple mechanical mixing or co-precipitation. By special techniques, it is also possible to introduce porosity into the implant which is advantageous for most applications as bone substitution material. In the following, we review the different combinations under the headline of the corresponding calcium phosphate.

Hydroxyapatite-based Composites

Hydroxyapatite (HAP) is known as the biomineral in bone and teeth. However, it must be taken into account that all biological apatites contain substitutions of other ions, with carbonate instead of phosphate being the most prominent substitution [4], leading to the so-called carbonated apatite, $Ca_{5-x}(PO_4)_{3-x}(CO_3)_xOH$ (approximated formula), or bio-apatite. In bone, dentin, and tendon the crystals of carbonated apatite have a size of a few nanometers ("nano-apatite") [3]. Consequently, hydroxyapatite has been widely used to prepare composite materials, usually with the aim to improve the bioactivity.

Lickorish et al. [6] prepared collagen sponges with an open porosity (30–100 µm) by freeze-drying and coated the surface with a 10-µm layer of calcium phosphate from simulated body fluid. They found a good performance in in vitro fibroblast cell culture. Kikuchi et al. [7] prepared collagen/HAP composites whose mechanical performance was increased by cross-linking the collagen fibers with glutaraldehyde. The materials were tested in vivo (rabbits) and showed a good biological performance, including osteoconductivity and biodegradation. A similar approach was selected by Wu et al. [8] who prepared hydroxyapatite/collagen microspheres (diameter: 5 µm) by a water–oil emulsion technique in which the surface was cross-linked by glutaraldehyde. The material showed a good performance in in vitro osteoblast cell culture. Gelatin foams were successfully mechanically reinforced by hydroxyapatite and then crosslinked by a carbodiimide derivative by Kim et al. [9]. It was also shown to be a good carrier for the antibiotic tetracycline [10].

A further increase in biocompatibility is predicted by the addition of silicon, and Lynn et al. [11] proposed Si-substituted hydroxyapatite-collagen composites with silicon being preferentially in the collagen phase for enhanced bone substitution.

Porous alginate/hydroxyapatite composites on the basis of hydrogels were prepared by Lin et al. [12] by freeze-drying. The biocompatibility in cell culture studies and the in vitro biodegradability were high; how-ever, the mechanical strength was limited. A composite of hydroxyapatite with chitosan and poly (methylmethacrylate), PMMA, was developed by Kim et al. [13]. Thereby, a non-biodegradable polymer (PM-

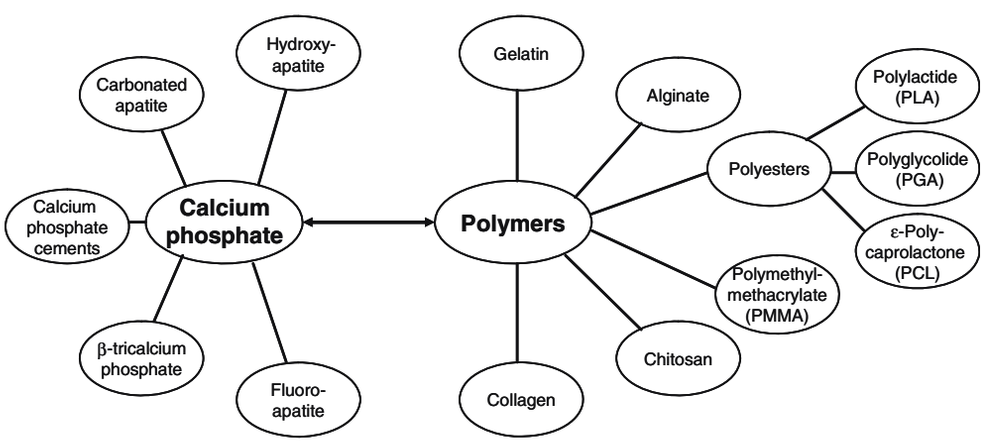

Figure 2. Some possible combinations of calcium phosphates and polymers.

MA) was made more bioactive and osteoconductive, yielding a well-processable cement. The biocompatibility was tested in vivo in rabbits, and a higher degree of new bone formation compared to pure PMMA was observed.

Biodegradable polyesters on the basis of polyglycolic acid (PGA) and polylactic acid (PLA) are well established in clinical medicine. Therefore, they make a good choice when a suitable polymeric filler material is sought. However, on their own they degrade to acidic products (glycolic acid and lactic acid) and can lead to acidosis and osteolysis in bone contact. Linhart et al. [14] reported extensive cell culture experiments on pH-stabilized composites of PGA and carbonated calcium phosphate which were later supported by extensive in vitro pH-studies. These experiments showed that calcium phosphate alone is not able to buffer this acidic degradation, but that the carbonate content is responsible for the pH compensation [15]. A consequent application of these principles led to the construction of functionally graded skull implants consisting of polylactides, carbonated apatites, and calcium carbonate [16]. The mechanical properties of hydroxyapatite can be improved by the addition of polylactide, as shown by Ignjatovic et al. [17] who prepared hot-pressed composites which consisted of 80% HAP and 20% PLLA. Hasegawa et al. [18] prepared porous scaffolds of poly-DL-lactide (PDLLA) and hydroxyapatite. Upon implantation into rabbit femora, newly formed bone was observed and the biodegradation was significantly enhanced compared to pure hydroxyapatite. This may be due to the local release of lactic acid which in turn dissolves hydroxyapatite. The higher homologues poly(3-hydroxybutyrate), 3-PHB, and poly(3-hydroxyvalerate), 3-PHV, show almost no biodegradation. Nevertheless, composites of these polymers with calcium phosphate showed a good biocompatibility in vitro and in vitro [19–21].

Along this line, poly(ε-caprolactone) (PCL) is also used as slowly biodegradable, but well biocompatible polymer. Causa et al. showed that its mechanical performance can be strongly increased if hydroxyapatite is added and that the biocompatibility in osteoblast cell culture is also significantly increased by the presence of hydroxyapatite [54]. Drug release studies were reported by Kim et al. who coated porous hydroxyapatite blocks with PCL and hydroxyapatite from dichloromethane solution. The antibiotic tetracycline hydrochloride was added into this layer, yielding a bioactive implant with drug release for longer than a week [22].

Tricalcium Phosphate-based Composites

β-Tricalcium phosphate (β-TCP) is another synthetically prepared calcium phosphate. It has a higher solubility than hydroxyapatite [4]. Therefore a number of authors used this compound instead of hydroxyapatite to prepare biodegradable composites. However, there were also reports about an absent biodegradation after implantation in calvarial defects [23]. Kikuchi et al. [24] prepared a composite of β-TCP and a co-polyester (lactide-co-glycolide-co-ε-caprolactone, PLGC). The added β-TCP was able to counter the acidic degradation of the polyester to some extent, but did not prevent a pH drop down to about 6. An implantation study in beagles' mandibular bones was successful (12 weeks). Yao et al. [25] prepared composites of crosslinked gelative with β-TCP and found a good biocompatibility and bone formation upon subcutaneous implantation in rats. Zou et al. [26] prepared porous composites of collagen (crosslinked with glutaraldehyde) and β-TCP by freeze-drying and sublimation of the solvent (porosity about 95% with interconnected pores of 50–100 μm) which showed a good biocompatibility upon implantation in the rabbit jaw. This was extended by Yang et al. [27] to porous β-TCP/gelatin composites (porosity about 75%) which also contained BMP-4. An in vitro study with primary rat calvarial osteoblasts showed an increased cellular activity in the BMP-loaded samples, confirmed by Takahashi et al. [28] with BMP-2-loaded porous composites (porosity 95%) of gelatin/β-TCP. The so-called biphasic calcium phosphates (BCP) are a mixture of HAP and β-TCP. The biodegradability of these lies between the pure compounds, i.e. it can be fine tuned according to the requirements at the implantation site [29]. Collagen-coated BCP ceramics were studied by Brodie et al. [30], and it was found that the biocompatibility towards osteoblasts increased upon coating with collagen.

Another polymorphic phase of tricalcium phosphate is α-TCP. This is metastable compared to β-TCP, i.e. it is a solid phase with the same composition but with a different crystal structure and a higher specific energy. Consequently, its solubility is higher [4] and it is faster resorbed in vivo. A long-term implantation study of PDLLA/α-TCP composites in a loaded sheep implant model by Ignatius et al. [31] showed good results after 12 months but a strong osteolytic reaction after 24 months. This was ascribed to the almost complete dissolution of α-TCP at this time and an adverse reaction (acidosis?) of the remaining polylactide.

Calcium Phosphate Cement-based Composites

Inorganic cements on the basis of different calcium phosphates which harden in the body were introduced by Constantz et al. [32] in 1995. Chemically, it is a precipitation reaction which leads to bone mineral-like nanocrystals of calcium phosphate if the compounds are mixed in a suitable way [33–36]. However, these cements are brittle after hardening and the setting time is sometimes unsuitable for clinical procedures. Therefore, it was attempted to modify these materials by adding other substances, e.g. hydroxyl carboxylic acids, to control the setting time [37], gelatin to improve the mechanical parameters and the setting time [38], or osteocalcin/collagen to increase the bioactivity [39]. A porosity of 42–80% was introduced into calcium phosphate/chitosan cements by addition of the water-soluble sugar mannitol by Xu et al. [40]. Chitosan significantly improved the mechanical strength of the cement. A similar approach was followed by Ruhe et al. [41] who studied the effect of the addition of PLGA microparticles (which can also be loaded with drugs) before setting to a calcium phosphate cement. This composite was implanted into cranial defects in rats, and it was found that a content of about 30 wt% of polylactide led to the most favorable results.

Fluoroapatite-based Composites

Chemically, fluoroapatite (FAP) is hydroxyapatite in which all hydroxyl groups were substituted by fluoride: $Ca_5(PO_4)_3F$ instead of $Ca_5(PO_4)_3OH$. This compound has a lower solubility and a higher hardness than hydroxyapatite and occurs in nature, e.g. in shark teeth [4] and in some mollusk shells [42]. It also occurs in the outer layer of teeth [43] after brushing with fluoride-containing toothpaste. The higher mechanical and chemical stability of fluoroapatite as compared to hydroxyapatite was investigated by Yoon et al. [44] who prepared FAP/collagen composites and studied their effect in osteoblast-like cell culture. They found an increased cellular activity in FAP composites compared to HAP composites. This was confirmed by Kim et al. [45] who made FAP-HAP/polycaprolactone composites with variable fluoride content. Fluoride seemed to have a stimulating effect on osteoblasts in vitro.

Nanoapatite-based Composites

Natural bone mineral consists of nanocrystals of carbonated hydroxyapatite ("bioapatite"; see above). Given the fact that the organic phase of bone is collagen, i.e. a natural polymer, it is obvious that a composite of such a bone mineral-like phase with a biodegradable polymer should be advantageous as bone substitution material. The mineral phase would be responsible for the mechanical strength (hardness) and the polymer phase for the elasticity. In addition, the solubility of calcium phosphates depends on their crystallite size (smaller crystals have a higher solubility) and on their carbonate content (a higher carbonate content increases the solubility) [46].

Liao et al. [47] prepared porous composites of nano-HAP with collagen and PLA (of unspecified tacticity) by precipitation and freeze-drying (porosity 85%) which did not show a drop in pH upon in vitro degradation. They were implanted in the radius of rabbits and showed a high biocompatibility and partial resorption after 12 weeks. Chitosan/HAP composites with improved mechanical stability were prepared on the basis of chitosan/HAP nanorods by Hu et al. [48]. Composites

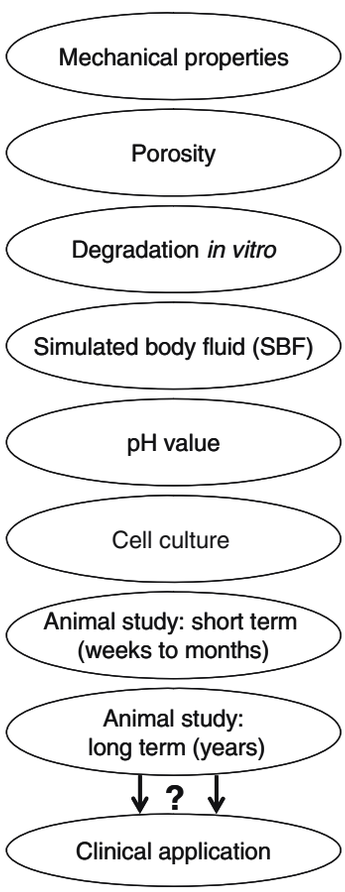

Figure 3. Parameters to be checked and steps to be taken before a new composite can be clinically applied.

Table 1. Mechanical properties of some composites, compared to natural bone, are highly variable, depending on the composition and the processing.

Material	Compressive strength (MPa)	Elasticity modulus (GPa)
HAP/PLLA (hotpressed) [53]	90–123	4–9.7
HAP/PCL coating on polyurethane template [22]	0.24–0.45	1–1.43
HAP/Alginate [12]	100–160	
HAP/Gelatin [9,10]		0.0023–0.004
HAP/PCL [54]		0.0214–0.0279
CaP/gelatin cement [38]	10.7–14	
HAP/collagen [39]	25	
Nano-HAP/collagen/PLA [47]	1.3–1.8	0.020–0.0473
Compact human bone [55]	133–193	11.5–27
Diaphyseal cortical bone [56]		20.1 ± 5.4
Trabecular bone [56]		11.4 ± 5.6
Morsellized particles of bone [57]		0.085–0.135

of nano-HAP and PLLA with high porosity (90%) were prepared using thermally-induced phase separation (TIPS) by Wei and Ma [49]. Liao et al. [50] succeeded in mimicking the bone structure by blending carbonated apatite with collagen. A similar material (mineralized collagen) was implanted by Yokoyama et al. [51] into the femur of rats. Excellent clinical results were observed after 12 weeks. The biocompatibility of chitosan in osteoblast cell culture was also significantly improved by the addition of nano-HAP [52].

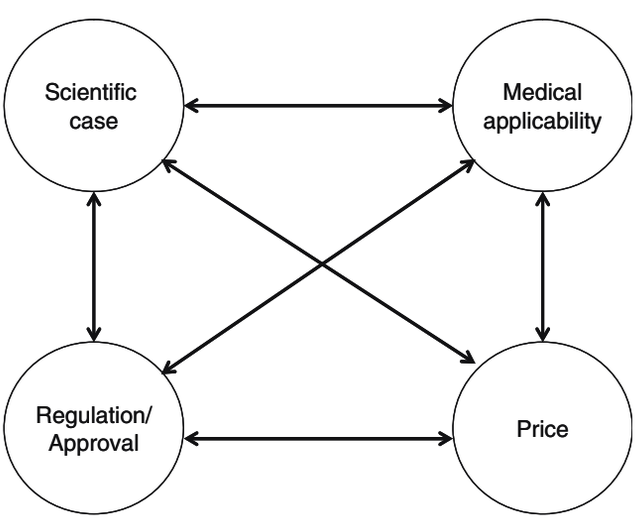

Figure 4. Apart from the scientific case, other factors are equally important for a new biomaterial. The scientific case is only one important property.

Conclusions

Many combinations of calcium phosphate and polymers were proposed, and their biocompatibility was tested up to different stages. In Figure 3, we summarize the different steps which such a new biomaterial has to undergo before it can be clinically applied. The properties of such composites are highly variable, depending on the combination, the shape, and the microstructure (e.g., porous or compact) of the implant. In Table 1, we list some of the mechanical properties. It is clear that the range is wide, from very elastic to rather strong and inelastic.

The properties of such new biomaterials have to be adapted to the clinical and biomechanical requirements. Clinically, an excellent biocompatibility, a biodegradability which are adjusted to the growth of new bone, a sufficient mechanical stability, and a good applicability during operation are necessary. Of course, any material must also undergo sterilization without significant change of its properties. All these requirements can be tested in vitro and also in vivo. The final outcome can only be predicted from long-term animal studies (as the work of Ignatius et al. showed impressively [31]), because the growth of bone is slow, and the degradation of an artificial bone substitution material will have to be observed for the same time. The steps before certainly have to be undertaken, but they may not be sufficient for a full assessment of a biomaterial's clinical properties (Figure 4).

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Address for Correspondence
Prof. Matthias Epple, MD
Institute of Inorganic Chemistry
University of Duisburg-Gießen
Universitätsstraße 5–7
45117 Gießen
Germany
Phone (+49/201) 183-2413, Fax -2621
e-mail: matthias.epple@uni-due.de