A REVIEW OF BONE SUBSTITUTES IN BONE REMODELING: INFLUENCE OF MATERIALS CHEMISTRY AND POROSITY

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Abstract. Given the bone tissue's superb ability to adapt its mass and morphology to *in vivo* functional necessities, its aptitude to repair itself without leaving a scar, and its capacity to rapidly mobilize mineral supplies on metabolic demand, it is in fact the ultimate "smart" material in biological systems.

Scientific efforts which may eventually lead to the synthesis of materials that mimic the *natural bones* have started about four decades ago [1, 2], and it should be open-heartedly confessed now that the calcium phosphate-based synthetic bone substitute materials are still too far away from taking over the *golden standard* status of autologous bone chips/grafts which are harvested from the patient, in real time, during the surgery together with the bone marrow and living cells. The current paper tries to concisely bring together what perspectives are needed to develop new synthetic bone substitute materials exhibiting higher levels of participation in the bone remodeling process.

Requirements. Considering the ever-growing number of patients who suffer from devastating disorders of the skeleton, it becomes more critical for the material scientists to be able to design bone substitutes, which can:

1) readily take part in bone remodeling (i.e., *osteoconduction*: the direct anchorage of an implant by bony tissue surrounding it, without the onset and growth of fibrous tissue at the bone-implant interface),
2) itself cause the formation of bone tissues (i.e., *osteoinduction*), even if it is not in interfacial contact with natural bones,
3) maintain their mechanical strength even during the intermediate stages (3-4 months) of cellular (i.e., osteoclasts) or active resorption, and
4) be gradually but fully replaced, within the 48 to 52 weeks following the surgery, by new bone (i.e., *osseointegration* via osteoblastic activity) at the implantation site.

Unfortunately, until now, there are no synthetic biomaterials which simultaneously satisfy all of the above criteria required by the clinicians.

Bone Mineral. Bone mineral has commonly been referred to the perfectly stoichiometric compound *calcium hydroxyapatite* [$Ca_{10}(PO_4)_6(OH)_2$], but this can be a dangerously misleading oversimplification of this "smart material." Actually, it is a rather defective and complex substance (whose Ca sites were simultaneously doped (to a total percentage of about 1.5%) by several mono- or divalent cations (Na, K, Mg, Zn, Fe, Sr, Pb, Ba, Cu, etc.) and the hydroxyl and phosphate groups being doped with carbonate ions of around 5% by weight) with a generic formula of $Ca_{8.3}(PO_4)_{4.3}(HPO_4, CO_3)_{1.7}(OH, CO_3)_{0.3}$ [3-5]. The determination of the influence of collagen (and the organic molecules it supplies) on the formation (and dissolution and reprecipitation) of nanocrystals of bone mineral must be regarded as a task of colossal importance still needing the joint efforts of clinicians and the materials specialists. To summarize, bone mineral is not simply a hydroxyapatite ceramic, and it should be named as *biological, calcium-deficient carbonate apatite.*

Bone Remodeling. Bones contain three distinct types of cells: the matrix-forming *osteoblast*, the tissue-resorbing *osteoclast*, and the *osteocyte* [6]. Osteoblasts are the cells present in bones which actually build the extracellular matrix and regulate its mineralization. The lifespan of an osteoblast ranges up to 8 weeks in humans, during which time it lays down 0.5 to 1.5 μm osteoid per day [6, 7]. Cells named as osteoclasts, on the other hand, are able to resorb fully mineralized bone as they are equipped with a variety of enzymes which lower the local pH to values between 3 and 4 (i.e., cell-mediated acidification). Osteocytes are the principal (they account for about 90% of all cells in the adult skeleton) cells present in adult bones, and their special construction may actually orchestrate the spatial and temporal recruitment of the cells that form and resorb bone. Modeling is the processes whereby bone is laid down onto available surfaces, and in the case of *remodeling*, osteoclastic resorption of bone leaves pockets that are then filled by osteoblast activity [6]. When the bones no longer have any osteoblasts or osteoclasts, all the modeling/remodeling processes would cease.

Bone Substitutes. Calcium phosphate-based bone substitute materials should ideally be implanted with the design consideration that the osteoclastic resorption will be able to slowly and gradually degrade the implanted material, and in such pockets or crevices created by the osteclasts, new bone will simultaneously be deposited by the osteoblasts [6]. If a material is not resorbed by the osteoclasts (such as, crystalline alumina (mostly used as caps on metallic implants used for hip arthroplasty) or zirconia), then such bioinert materials may not be used as a successful bone substitute bioceramic, which can take part in bone turnover. On the other hand, if an implant material is rapidly eroded away in physiological fluids (such as, $CaSO_4 \cdot \frac{1}{2}H_2O$ or $Ca_4(PO_4)_2O$) by passive dissolution, then it also can not help much in the bone remodeling processes, due to the lack of that precise interaction and crosstalk which must be present between the *resorbing*

osteoclasts and *depositing* osteoblasts. Such a crosstalk is strictly essential for the successful replacement of the synthetic material by natural bone at the defect site.

Resorbability. It is known that perfectly stoichiometric (Ca/P=1.67), sintered synthetic hydroxyapatite ceramics do not actively participate in bone remodeling, but they can only display *osteoconductive* behavior [8]. In other words, bone can grow in apposition or in close contact with the hydroxyapatite implant interfaces, but hydroxyapatite ceramics can not be fully resorbed *in vivo* by the osteoclasts even after five years. On the other hand, in the course of natural biomineralization/calcification processes, the bone mineral forms (within a continuum of precipitation-dissolution sequences) petal- or needle-like calcium-deficient hydroxyapatite crystals 100-150 nm in length and 10-20 nm thick (Fig. 1), and since these are less perfect (as compared to stoichiometric apatite) in chemical and crystallographic structure, and since they are more reactive and soluble; these non-stoichiometric apatitic calcium phosphate nanocrystals facilitate chemical turnover or bone remodeling. Proteins and other organic macromolecules present in collagen of the bone tissues govern the dissolution/precipitation mechanisms for these nanocrystals.

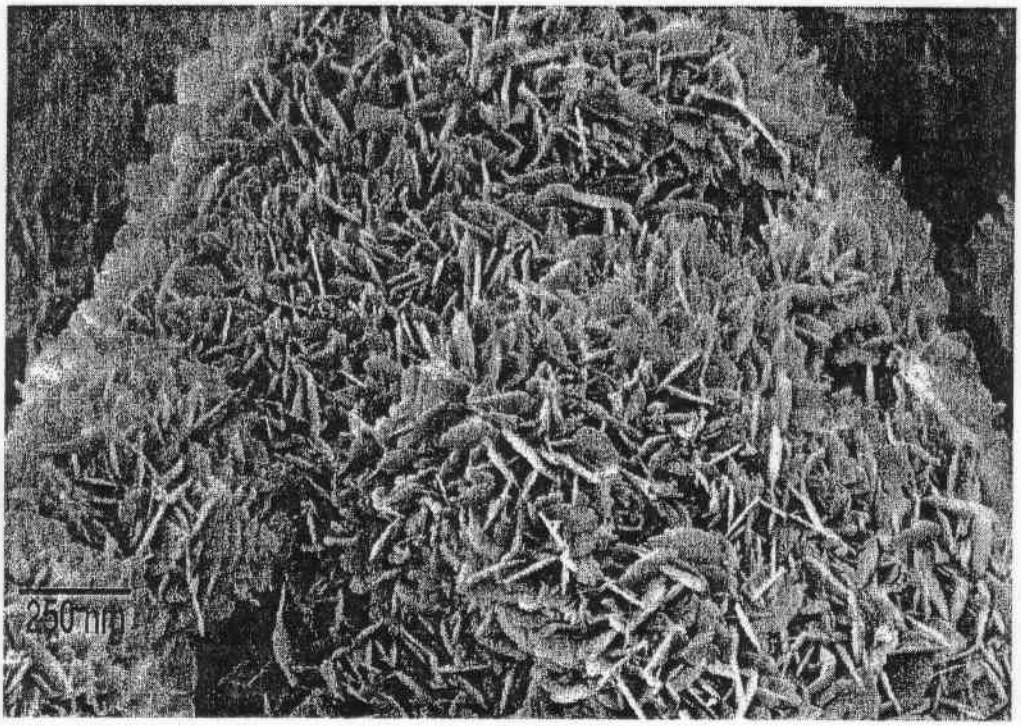

Fig.1 Calcium-deficient hydroxyapatite nanoplates grown on a calcium phosphate ceramic (with a Ca/P ratio of 1.30) immersed in synthetic body fluid

Active resorption of the bioceramic implant, by the osteoclastic action, is a crucial starting condition if the *in vivo* biological participation of the material in bone remodeling is desired. Therefore, any biomaterial left (without being resorbed) in the human body must be considered as a potential focus for infection or further clinical complications [9], especially if it is known to be a bioinert material (such as, bioinert ceramics or PMMA-polymers).

Material Form. Another important question which needs to be addressed in the design of calcium phosphate-based biomaterials is the form of the implant material. Nowadays, commercially available bioceramics (including the bioglasses) do typically come to the perusal of the clinician in the form of powders, granules, porous blocks (as either fully synthetic or of bovine-origin), pre-coated metallic implants or injectables. Powders may not find much of a widespread use in clinical practice, mainly because of the fact that defects which need to be filled are constantly wetted by blood, and the powders (especially if they are nanosize or submicron-particulated) to be applied to such a dynamic defect site would easily migrate and get washed away within a short time, and thus they would not function well. Injectable apatitic putties [10], which do not harden *in vivo*, do have the ability to readily penetrate the macropores of trabecular bones, and comprised of high surface area, nanosize apatite particles (in water) as a viscous paste, categorically fall in between the loose powders and hard calcium phosphates in terms of their applied forms. Such putties would, of course, have no applicability in load-bearing defect sites. Pre-shaped prismatic blocks, which are designed for use in load-bearing areas [11], do also have certain limitations, and they may require a certain extent of "machining/sizing-by-the-surgeon" on the operation table to fit those into the actual defects. However, granules within the size range of 1 to 5 mm, which can be *on site* impregnated with bone marrow cells harvested from the patient and packed into a gelated porous compact with the help of blood clotting, or granules which have previously been impregnated with certain antibiotics or growth factors (depending on the clinical application), can remedy some of the geometrical limitations associated with the use of dense or porous blocks. Injectable calcium phosphates (either in the form of a putty or self-setting cement), by quickly reaching quite high compressive strength values ($\geq$ 55 MPa) upon hardening *in vivo*, obviously present an attractive alternative [12-15] to the above-mentioned preshaped bone substitutes. On the other hand, the main concern for the injectable self-hardening cements still remains as their lack of macroporosity.

Porosity. For the osteoclasts to simultaneously attack even the bulk of the implanted ceramic, the material must have *interconnected* porosity (between 55 to 70%), and the pore sizes must be over the range of 150 to 700 μm [16], just like the natural bones. The presence of such a high porosity in the bone substitute materials facilitates the complete (i.e,, both bulk and the surface) invasion of the implant by the osteoclasts and osteoblasts from the very beginning, leading to

osseointegration and further *vascularization*. If the material does not have the stated porosity, osteoclasts can only degrade the external surface of the implant, and this initial surface attack lasts for a relatively short period of time and then it may totally stop, if the osteoblasts regard the material as a foreign body. By using the ceramic manufacturing technology it is not difficult at all to produce calcium phosphate-based bone subsitute materials as shown in Fig. 2 below. Ca/P molar ratio (over the range of 1.05 to 1.67), phase assemblage, as well as the percentages of the monovalent or divalent dopant elements (as mentioned previously), can again be easily adjusted or tailored in such porous bioceramics to control the *in vivo* response (cytotoxicity, resorption rate, the rate of bone ingrowth, mechanical strength following the first few weeks of implantation, etc.) to these implants.

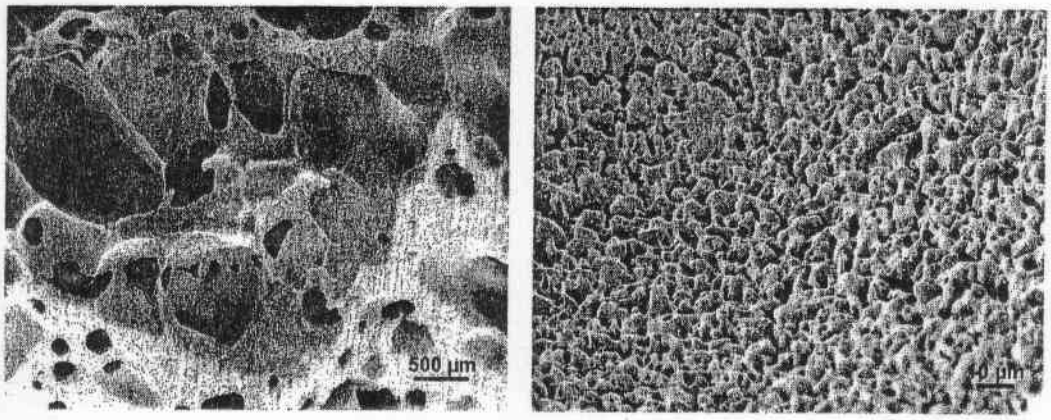

Fig. 2 Porous calcium phosphate scaffold material (Ca/P=1.45), after calcination at 1000°C; 35±2 MPa compressive strength: (*left*) interconnected macropores, (*right*) magnified view of the same sample, which thus reveals the microporous nature of those dense-looking struts/walls

Material Chemistry. However, physical factors like porosity and material form alone are not enough to allow a bone substitute implant to show the ability of full resorbability and participating in bone remodeling. A good example to this situation can be seen in the case of commercially available porous blocks or granules, which were manufactured from the trabecular bones of animal (bovine) origin [17, 18]. These materials are able to perfectly retain the magnificent porosity present in bovine bones, but since they are sintered at temperatures above 1200°C (to safely burn out the organic residues), they simply lose the material chemistry aspects of the original bones, and they convert into well-crystallized calcium hydroxyapatite, contaminated with only trace amounts of phases like CaO, $Ca_3(PO_4)_2$, and $Ca_4(PO_4)_2O$. Since the material after sintering is the *ceramic* and crystalline phase of hydroxyapatite with a Ca/P ratio slightly greater than 1.67, these samples were shown to be non-resorbable even after several years of

implantation. With these kinds of blocks used in defect-filling applications, it was seen that the bone ingrowth is perfect but the implant stayed as an almost inert material. Moreover, a ceramic bone substitute which sits in the bone for long years without being absorbed, could be a potential spot for the development of an inflammatory response. On the other hand, when the same porous bovine hydroxyapatite ceramic blocks were first blended with human bone marrow cells and then implanted *in vivo*, it was observed that the material showed positive and strong signs of participating in *osteogenesis* and remodeling processes [19].

As a different facet of the impact of material chemistry on the *in vivo* behavior of a calcium phosphate-based bone substitute, the surface chemistry of the implanted material becomes of extreme importance. In case of bone substitute materials with an acidic pH value at their interface they would form with the natural bone (for instance, when α–TCP powders are mixed with citric acid to form a cement for implantation [20]), the fibrous tissue formation and the onset of foreign body reactions are well-known. In contrast, in case of using implants with excessively alkaline surfaces, cell necrosis [21] may be the initial undesired *in vivo* response. Cytotoxicity of the bone substitute candidate materials must be carefully addressed and evaluated before their clinical use.

A delicate control to be achieved in the surface chemistry (for instance by the use of an acid phosphatase [22]) of the calcium phosphate-based bone substitute implants may in turn be used to adjust and monitor the extent of crosstalk between the osteoclasts and osteoblasts. As described by Lee, *et al.* [22], "the acid phosphatase coatings on porous hydroxyapatite bone substitutes serves to attract osteoclast progenitor cells from the bone marrow or bloodstream to the surface of the prosthetic device. The recruited osteoclast population then etches the bone mineral or hydroxyapatite surface of the implant and thereby provides the natural signals to recruit osteoblasts to lay down new bone that will abut and integrate with the graft or prosthetic surface mimicking the natural process of bone deposition on an osteoclast resorbed bone surface [22]. The acid phosphatase-induced stimulation of osteoclast recruitment results in osteointegration and enhanced bonding of the graft or prosthesis to the patient's bone. This, as claimed by Lee, *et al.*, reduces recovery time from the operation and lengthens the life of implants by reducing their tendency to loosen over several years [22]."

Challenges. The bone mineral which embrace about 70 wt% [23] of human bones

(1) is not a crystalline, simply stoichiometric ceramic,
(2) is a complex and rather defective material,
(3) is not soluble in physiological fluids, but can only be degraded, when necessary, by the osteoclastic environment,
(4) contains trace elements in differing but small percentages,
(5) resembles to *hydroxyapatite*, but both the A- and B-sites of the bone mineral are partially doped with carbonate ions,

(6) has its Ca-sites minimally doped with the above-mentioned cations,
(7) has a unique crystal structure [24] which places the hydroxyl and carbonate ions on its cell edges for easier chemical interaction with the surrounding cells and tissues, and
(8) the unit cell parameters of human bone mineral, as well as its overall Ca/P atomic ratio, display fluctuations as a function of bone maturation [25-27].

Predictions and Speculations. Hydroxyapatite-like bioceramics designed to mimic the bone mineral and intended for use in *in vivo* implantations should not possess steps of *heating/firing/calcination* at or above 650°C in any phase of their processing, manufacturing and shaping operations. The reason for this is clear that at or above 650°C carbonate ions which may be present in the apatitic structure are opt to readily leave the material [28]. The same also applies to the case of HPO_4^{2-} ions present in the bone mineral, and the materials chemist must face this challenge in preparing bioceramics which should resemble *the bone mineral* to the most possible extent. Sophisticated chemical techniques which involve the loading of several proteins, organic molecules, biopolymers or inorganic salts into the aqueous media of the synthesis reactors would become increasingly important in the manufacture of *next-generation macroporous* bioceramics. The total weight percentage of Na, K, and Mg, which altogether amount to a value greater than 1.25 wt% in the bone mineral, must be considered in preparing synthetic bioceramic bone substitute materials [4, 29]. Low-temperature (<100°C) chemical processing of Ca, Na, K, Mg and Sr phosphates and carbonates, to be selected from a tentative list of chemicals, such as α- or β-$Ca_3(PO_4)_2$, $Ca_4(PO_4)_2O$, $Ca_8(HPO_4)_4(HPO_4)_2 \cdot 5H_2O$, $Ca_2P_2O_7$, $CaHPO_4$, $CaHPO_4 \cdot 2H_2O$, $Ca(H_2PO_4)_2 \cdot H_2O$, $MgHPO_4 \cdot 3H_2O$, $Na_4P_2O_7$, Na_2HPO_4, NaH_2PO_4, KH_2PO_4, K_2HPO_4, $NaHCO_3$, Na_2CO_3, $CaCO_3$, $SrCO_3$, K_2CO_3, and to be later processed in synthetic body fluids [28], which contain trace amounts of Zn, Sr, Fe, and Cu ions [30], might yield bioceramics of higher resemblance to the bone mineral. Ca/P atomic ratio in the ideal synthetic bioceramic, as a tool of controlling resorbability, must be easily adjustable within the range of 1.05 to 1.67 by controlling the synthesis parameters. If the bioceramic undergoes *in vivo* osteoclastic resorption and osteoblastic deposition without a difficulty [31], then the Ca- and P-rich environment needed for *in situ* bone formation (i.e., *osteoinduction*) would have been provided even in cases of intramuscular implantation.

Summary. Stoichiometric calcium hydroxyapatite ceramic, especially if it is heated, prior to its clinical use, at elevated temperatures, does not take part in the bone remodeling process. Calcium-deficient hydroxyapatite (with a Ca/P ratio ranging from 1.3 to 1.6) doped with alkali and alkali-earth elements, such as Na, K, and Mg, resorb much faster and allow the natural bone to proceed with its remodeling process. Porosity (i.e., highly interconnected macropores) in bone substitute implant materials should be regarded as the bare necessity.

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