Technical notes

Potential applications of synthetic bioceramic bone graft substitute in spinal surgery

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SUMMARY: *The number of spine operations is increasing sharply both in the United States, which is at the forefront of the field, and in Europe. Reconstruction of bone loss has been an important clinical challenge for many years, and the various surgical approaches involve, among other things, the use of bone substitutes. To this end a large variety of techniques and materials are available to the surgeon, each with its own advantages and disadvantages. Over the last 30 years, more and more synthetic bone graft substitutes have been used to minimise complications associated with the use of autologous and homologous bone, namely morbidity, limited availability, significant incidence of postoperative infections, and potential risk of disease transmission. Synthetic bone grafts have the decided advantages of ready availability, sterility and reduced morbidity. However, at the same time their cost is relatively high, and not all are fully biomimetic. The aim of the present work was to verify the possible applications of bone substitutes in spinal surgery, and to assess their characteristics from the surgeons' point of view. This article examines the pathologies that require the use of bone substitutes in spinal surgery, in which the focus is more and more on quality and adequate quantity. The main synthetic bioceramic bone graft substitutes, which, owing to their chemical, physical and mechanical properties, can be used in spinal surgery are then described, citing some example applications.*

KEY WORDS: *Bioceramics, Bone graft substitute, Innovation, Spine.*

INTRODUCTION

Bone is the second most transplanted tissue after blood, and it is required in case of large trauma, not necessarily fractures, that cause bone lacunae. In the specific case of spinal surgery, we can cite osteoporotic vertebral fractures, cases of osteomyelitis (severe bone infections), and lacunae in bones resulting from the removal of tumours and cysts. In these cases the bone can be replaced with an autograft (autologous bone), allograft (homologous bone), or xenograft (heterologous bone), respectively harvested from the patient - generally from the ribs or iliac crest - or cadavers or animals. The outcome of a bone graft depends on many factors linked to both the nature of the graft itself and the host. Its osteointegration is a continuous dynamic process involving the extracellular microenvironment, the mechanisms of inflammation, neoangiogenesis, the resorption of the bone graft and its "substitution" through remodelling. Foremost amongst the local factors is the blood supply, which requires limited detachment of the soft tissues, an absolute mechanical stability to promote integrity of the bone callus[2,17], and correct asepsis. Tobacco ad-

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LIST OF ACRONYMS AND ABBREVIATIONS: **HA** = HydroxyApatite; **Mg** = Magnesium; **Na** = Sodium.

diction, diabetes and chronic inflammatory diseases stand out among the systemic factors that can jeopardise neovascularisation and thus adequate tissue oxygenation.

Autologous bone remains the gold standard in terms of regenerative capabilities, but has certain disadvantages in terms of availability. Indeed, if more bone than can be harvested is required, or if the bone itself is not of excellent quality (for example, it might be osteoporotic), another solution needs to be sought. However, the use of bone from cadavers or animals involves a series of risks, including rejection or inadequate performance, caused by the very different remodelling mechanisms from those in humans (when animal bone is used) or a reduction in quality due to the aggressive treatments they are subjected to minimise the risk of disease transmission. For these reasons surgeons are resorting ever more often to the use of synthetic (or alloplastic) bone graft substitutes for bone regeneration[18].

□ DESIRABLE PROPERTIES IN BONE SUBSTITUTES

The market offers many types of bone substitutes. Among these, the biomaterials used most often are the bioceramics, like hydroxyapatite, and the calcium phosphates (which have the same structure as the mineral component of bone). The new generation of bone substitutes, besides biochemical biomimetism, reproduce the porosity of spongy bone with a view to improving the structural biomimetism of the graft. Spongy bone porosity ranges from 50% to around 80%, based on several factors. In bone substitutes, like in bone, these pores need to be interconnected and of sufficient dimensions to allow both cell migration and the passage of blood vessels.

Obviously, bone substitutes must also offer mechanical properties similar to those of autologous bone and meet a series of biological requirements, namely osteogenesis, osteoconductivity and osteoinductivity[1,25]. Osteogenesis is the capacity to produce bone in situ, and is determined by the presence of specific cellular elements, among which the osteoprogenitor cells stand out. Following the activation of these cells, mediated by specific biohumoral signals (cytokines, growth factors, extracellular matrix), they start a migration, proliferation and differentiation process whose objective is osteogenesis and subsequent remodelling. Osteogenesis is normally obtained by fresh, richly vascularised bone grafts, at times loaded with autologous bone marrow cells. Osteoconductivity is the ability to promote osteogenesis by providing a structural support. Indeed, the presence of a three-dimensional porous scaffold fosters adequate capillary invasion (neoangiogenesis) and promotes the activity of the various cell lines involved. Osteoinductivity is the capacity of a graft to stimulate the activation of osteogenesis. Osteoinductive capability is enhanced by certain molecules within the superfamily of the cytokines, and growth factors, which are capable of transmitting a clear signal to the target cells. Structural organic molecules of the extracellular macroenvironment, such as collagen, also appear capable of promoting cellular activity, and thereby enhancing osteoinduction[20]. The main target cells are the pluripotent mesenchymal cells, found in large concentration in the bone marrow and in smaller amounts in the circulating blood[6]. The complex and not yet entirely understood molecular biology of osteogenesis seems to indicate that these three properties are not independent but that they are instead closely interconnected.

□ SPINE PATHOLOGIES AND SURGICAL PROCEDURES THAT COULD BENEFIT FROM BONE SUBSTITUTES

Modern spinal surgery has benefited from the integration of classical biomechanical and pathophysiological concepts with effective and readily available prosthetic systems. The use of materials like titanium and tantalum has significantly improved the biomechanical performance of the different constructs and expanded the population of treatable patients, extending it to elderly subjects with intrinsic poor bone quality.

Although prosthetic substitutes and synthetic systems based on screws and rods make it possible to carry out internal fixations with extreme ease - also serving as support for decompressive procedures - the need to integrate or replace bone components is still extremely important today. Screw-based stabilisation systems (transpedicular/lateral masses) can be integrated with

the apposition of bone material along the rods and at the articular processes. This technique, called posterolateral arthrodesis, is applicable both in the case of degenerative disease (instability) and, naturally, traumatic pathology.

As previously mentioned, autologous bone material, harvested through stripping of the laminae to obtain a certain number of "lamellae", is preferable, sometimes supplemented with grafts from the iliac crest or tibia. However, this is a costly procedure in terms of time, and has certain limitations connected with morbidity and the relatively limited availability of autologous bone. Hence, pre-shaped bone substitutes may be useful in the course of these procedures, making it possible to guarantee more effective arthrodesis and to shorten the length of the surgical procedure.

With regard to trauma, in the case of cervical instabilities caused by simple disc lesion, the intersomatic fusion achieved using autologous bone has guaranteed satisfactory results in a relatively short space of time for years. However, today's intersomatic arthrodesis devices (cages, mesh, somatic substitutes) require integration with bone substitutes capable of guaranteeing rapid osteointegration or even osteoinduction.

Circumferential arthrodesis procedures are a special case. In complex cases, when there is a considerable degree of deformity (e.g., in spondylodiscitis) the structural rigidity of somatic prostheses can be a significant limitation. However, this can be overcome by intervening directly through the preparation and modelling of bone components like ribs, or harvesting from the iliac crest. Thus it appears clear that the use of bone today, even more than in the past, is decisive in modern spinal surgery techniques.

It follows that quality bone substitutes to make up for any lack of usable autologous bone are necessary, not least because they can decrease morbidity and increase the effectiveness of the different surgical procedures. Today, for example, vertebral trauma is still a significant cause of morbidity. The need to guarantee immediate recovery of the structural stability of the spinal column is associated, in some cases, with the urgent need to guarantee suitable decompression of the neural structures. In many cases, especially in the presence of osteoporotic or abnormal bone (ankylosing spondylitis), the possibility of having synthetic "grafts" available may be an important factor in relation to medium and long-term prognosis.

With regard to degenerative spine disease, the modern concepts of macro- and, above all, micro-instability have led to an increase in arthrodesis procedures. The prevalence of this type of disease within the general population and the expected increase in surgical procedures in this sector will create a growing demand for quality bone substitutes in the future. Infections and metabolic disorders, which are on the rise, will also require complex reconstruction procedures. Furthermore, oncology patients are being treated with ever greater aggressiveness, and there will therefore be an increasing need for available and effective bone substitutes that can integrate safely with other therapeutic procedures (chemo/radiotherapy).

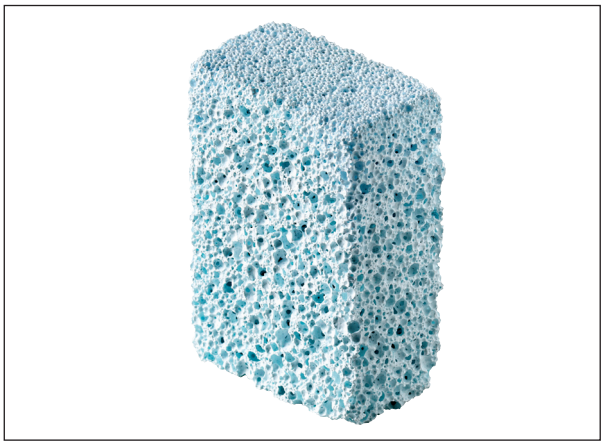

Figure 1. Porous HA bone substitute in block form: ENGIpore®.

☐ THE TYPES OF BONE GRAFT SUBSTITUTES AVAILABLE

Among the synthetic bioceramic bone graft substitutes currently in use, at least three can be used successfully in spinal surgery. The first, porous HA bone substitute in block form - ENGIpore® - is characterised by high porosity, between 80 and 90%, and a trabecular structure entirely similar to that of natural bone, thereby maximising the chances of osteointegration and neo-ossification. Indeed, porosity controlled at the macro-, micro- and pore interconnection level is known to foster rapid absorption of biological fluids and of signal molecules, thereby promoting the rapid cell colonization essential for good bone regeneration[4,5,10,11]. In spite of its high degree of porosity, this biomaterial is capable of withstanding compression loads to a similar extent as spongy bone (Figure 1).

Figure 2. Bone substitute composed of nanocrystals of Mg-substituted HA in putty, paste and granular form: SINTlife®.

Figure 3. Composite bone substitute made of Mg-substituted HA nucleated on equine collagen fibres (type I): RegenOss®.

The second available bone substitute, SINTlife®, promotes cell-mediated resorption and is composed of nanocrystals of Mg-substituted HA in putty, paste or granule form. The putty is composed of saline solution (around 44%), Mg-substituted HA powder and 600-900 µm and 900-1200 µm granules of the same; the paste is made up of physiological solution (46%) and Mg-substituted HA powder; and the granules are essentially 600-900 µm Mg-substituted HA microgranules. In these formulations, the Mg ions are placed in the crystalline HA cell in the same position and percentage found in the mineral phase of human bone. Indeed, it has been demonstrated that the presence of Mg^{2+} deforms the crystalline structure of the HA cell, making it unstable and biologically active and thereby aiding bone formation, remodelling, and rapid resorption of the cell-mediated material (Figure 2). In addition, MgHA changes the chemico-physical surface properties of the biomaterial, which actively interacts with the water molecules to quickly capture the key proteins involved in osteogenesis[21].
This latter type of bone substitute is therefore able to interact with the cells that form bone and fosters the deposition of new bone tissue. Owing to its specific chemical and biomimetic composition, its nano-structure and surface properties, it is remodelled and resorbed by the cellular action in a physiologically adequate amount of time (6-18 months), remaining at the application site during growth and maturation of the new bone. During the remodelling phase we can observe osteoclast resorption activity around the particles of material, until complete bone regeneration. This type of bioceramic bone substitute promotes physiological, rapid and effective bone reconstruction.
The third type of bone substitute, the composite RegenOss®, also exploits the useful properties of Mg-substituted HA, this time nucleated onto equine collagen fibres (type I). This type of material is engineered at the macro-, micro- and nanostructural level in order to foster bone regeneration. In the intra-surgical phase, this biomimetic and biodegradable scaffold can be easily adapted to the size of the defect, saving time and facilitating positioning. It is a completely biomimetic scaffold - its distinctive structure and chemical composition give the product characteristics entirely similar to human bone. Using nature as a model, the patented process of nucleation of the Mg-HA nanocrystals in the type I collagen fibres mimics the process that occurs in nature during neo-ossification[7,8,9,16]. Thanks to its high hydrophilicity, this type of bioceramic bone substitute is also capable of rapidly absorbing the biological fluids, molecules and cells that foster bone regeneration. Its architecture therefore promotes attachment and cell proliferation (Figure 3).
Due to their favourable properties, these bone substitutes are already beginning to find favour with surgeons, whether alone or as an adjunct to autologous bone, as the following example applications will testify:

- *Example application 1*: use of a bone substitute composed of nanocrystals of Mg-substituted HA in paste form plus autologous bone as posterolateral arthrodesis in a posterolateral stabilization (Figure 4 and 5);
- *Example application 2*: use of porous HA bone substitute in block form as filler for an intersomatic cervical cage (Figure 6 and 7).

□ DISCUSSION

In recent years we have arrived at a better understanding of bone repair. This has led to significant steps forward for the future generations of bone substitutes. The identification of osteoinductive components and other factors involved in the promotion

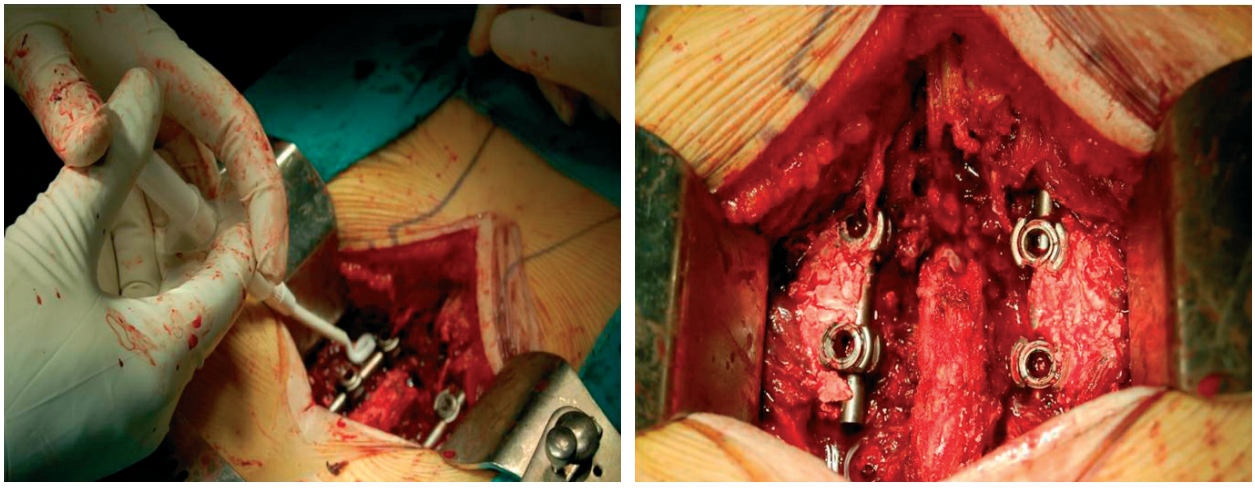

Figure 4. Examples of intraoperative view of bone substitute composed of nanocrystals of Mg-substituted HA in paste form plus autologous bone as postero-lateral arthrodesis in a postero-lateral stabilisation.

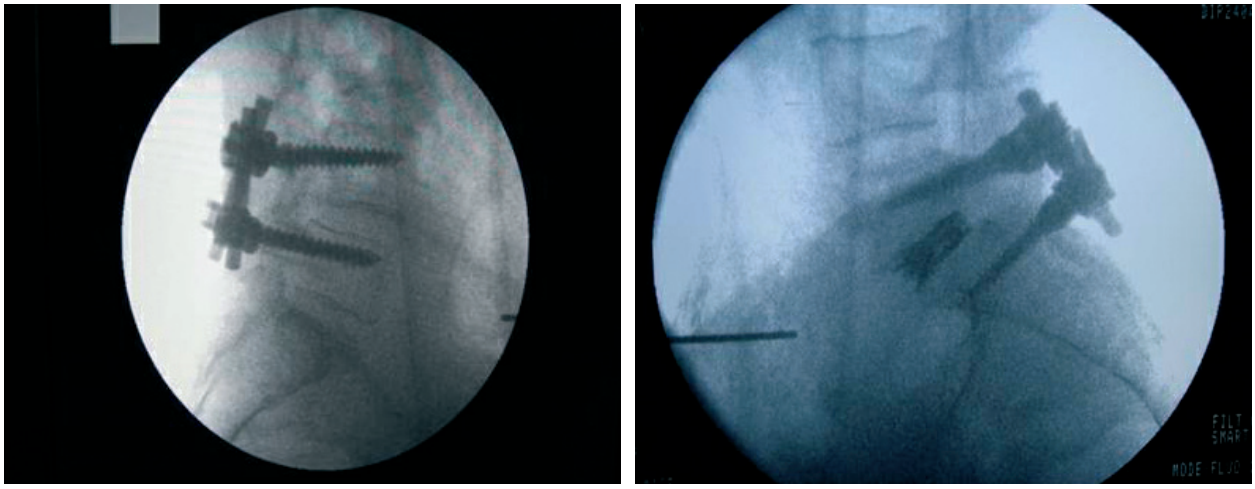

Figure 5. Examples of postoperative X-ray of bone substitute composed of nanocrystals of Mg-substituted HA in paste form plus autologous bone as postero-lateral arthrodesis in a postero-lateral stabilisation.

of osteoblast proliferation has brought about marked improvements in the capabilities of bone substitutes[23,24].

The cascade of biological events leading to bone regeneration is a complex process. It involves stimulation of an osteoblast response and development of a biological environment capable of supporting this response. In addition, adequate vascularisation and the ability to keep the system stable and controlled during the repair process are decisive for attaining a satisfactory outcome. In order to obtain excellent results, a combination of strategies is therefore required to manage this process, depending on the clinical situation. As we have seen, traumas, tumours and osteoporotic deficits are pathologies that differ quite a lot from each other, and therefore require different solutions. Nevertheless, it is predicted that bone substitutes will have a very important role to play in each. Currently the majority of bone substitutes have little biological activity. They act as fillers and have limited osteointegrative and conductive properties. Ideally the bone substitutes of the future will be entirely biomimetic structures acting as intelligent scaffolds capable of transmitting and differentiating the different bone responses. These processes will inevitably be accompanied by controlled resorption,

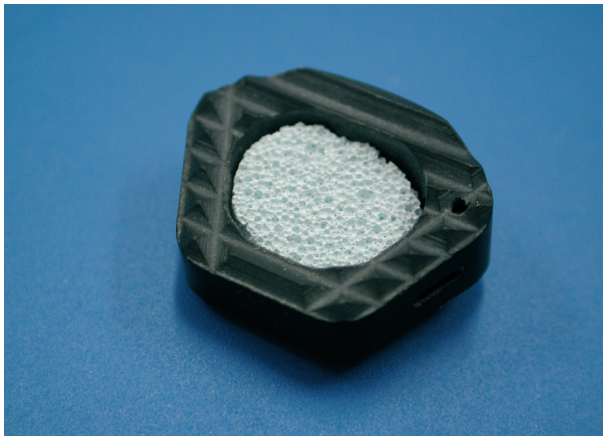

Figure 6. Porous HA bone substitute in block form as filler for an intersomatic cervical cage - material in the cage.

which is necessary to ensure a timely and predictable release of factors intrinsic to the material but decisive for the regeneration process. Furthermore, recent studies on regeneration have made it possible to foresee a combined approach between bone substitutes and the patient's blood[13,15], which contains precursors that are particularly suitable for reconstructive stimulation. This combination of an appropriate bioactive bone substitute and the autologous component will help optimize and accelerate repair and bone regeneration to the utmost.

❒ Design considerations for future generations of bone substitutes

Recently the real composition of natural bone tissue has been clarified and described as a dynamic system made of many interacting agents in a complex environment and the functionality of the active groups contained therein[14,19]. Bone mineral has the structure of a calcium-deficient apatite, with a Ca:P ratio less than 1.67, which is the theoretical value for pure HA, $Ca_5(PO_4)_3(OH)$ (HA). As bone is a living tissue that is continuously undergoing remodelling and repair, the small size and/or non-stoichiometry of the crystals presumably endows the mineral phase with the solubility needed for resorption of the bone by osteoclasts and allows bone mineral to act as an ion "reservoir" capable of either capturing or releasing ions (or small molecules), a two-way process regulated by homeostasis[12,22].

In developing a bone scaffold made of biomimetic HA, it should be considered that bioreabsorbability is strictly related to solubility in biological fluids. The solubility of HA under physiological conditions can be increased by reducing the extent of HA crystallinity up to nano-structured nuclei, and introducing doping ions, substituting calcium and phosphate groups that twist the crystalline structure up to an amorphous state, thereby mimicking natural apatite.

Careful examination of the stoichiometry of the apa-

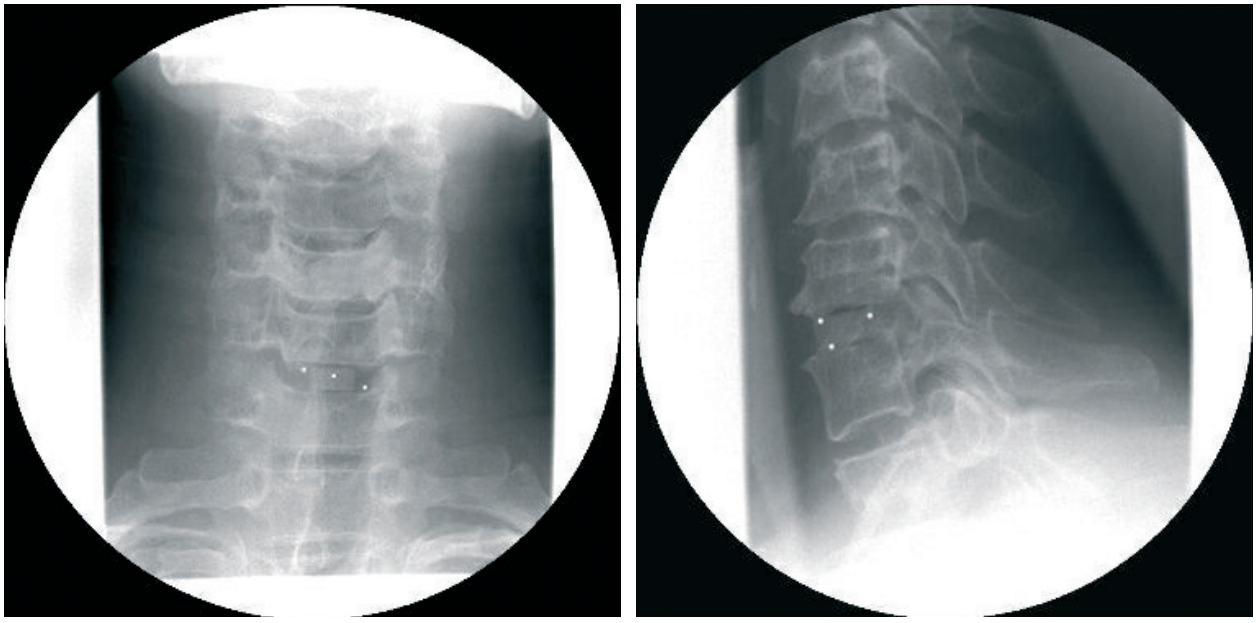

Figure 7. Examples of postoperative x-ray of porous HA bone substitute in block form as filler for an intersomatic cervical cage.

tite present in human bone tissue brought to light the presence of anionic substitutions (doping groups) such as HPO_4^{3-} and CO_3^{2-}, and cationic substituents such as Mg^{2+} and Na^+, which are able to alter drastically the solubility and therefore the bioreabsorbability of the material. The substitution of carbonate ions for phosphate ions is the major source of structural disorder in bone mineral, and consequently increases its chemical reactivity (dissolution and thermal decomposition). In particular, B-type carbonation enhances the solubility of apatite without altering the surface polarity or the affinity of the osteoblast cells.

CO_3^{2-} ions also exist in non-apatitic domains, mainly located in a hydrated layer surrounding apatite crystals. They are more abundant in young bone, and therefore appear to represent a reservoir of ions that promote remodelling processes.

In addition to anionic substitution, foreign cations are also present in natural apatite. The presence of Mg-substituting Calcium (5-6 mol%) increases the nucleation kinetic of HA on collagen fibres but retards its crystallization, giving rise to small mineral nuclei (< 20 nm), thereby increasing the bioavailability of the mineral phase. Moreover, the replacement of calcium by Mg in surface crystal sites increases the number of molecular layers of coordinated water, generating the so called " *maturation layer*" - a layer a few nanometers thick at the solid-liquid interface where the ions dissolve and crystallize. All these phenomena favour protein adsorption and cell adhesion to the scaffold[3].

Silicon, even if present in traces, is an essential element for healthy skeletal and cartilage growth and development in higher biological organisms. It is especially high in the metabolically active state of the cell, and is known to bind to glycosaminoglycan macromolecules. It has been shown to play a role in the formation of cross-links between collagen and proteoglycans, thereby resulting in the stabilization of bone matrix molecules and preventing their enzymatic degradation.

Biomimetic apatite can be prepared in the lab with the same composition as that in natural bone and used to develop 3D scaffolds with physico-chemical-microstructural features in compliance with human mineralized tissues. This will enable it to recruit cells from the bloodstream and to direct their differentiation to the desired phenotype (e.g., osteoblasts, odontoblasts, ameloblasts).

□ CONCLUSIONS

At present, autologous bone and allogenic bone are the main sources for bone graft procedures, where autologous bone is by far the one with the greatest osteogenic potential. Nevertheless, it is available in finite quantities, which has led to the development of synthetic alternatives. Current synthetic bone substitutes possess good biomechanical characteristics and osteointegrative/osteoconductive properties. In future, however, an ideal bone substitute would imitate native bone in all aspects with regard to both biomechanical characteristics and osteogenic properties. The continual evolution of synthetic bioceramic composites like the ones described above is bringing us closer and closer to this goal.

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