Chapter

Nature-Inspired Processes and Structures: New Paradigms to Develop Highly Bioactive Devices for Hard Tissue Regeneration

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Abstract

Material scientists are increasingly looking to natural structures as inspiration for new-generation functional devices. Particularly in the medical field, the need to regenerate tissue defects claims, since decades, biomaterials with the ability to instruct cells toward formation and organization of new tissue. It is today increasingly accepted that biomimetics is a leading concept for biomaterials development. In fact, there is increasing evidence that the use of biomedical devices showing substantial mimicry of the composition and multi-scale structure of target native tissues have enhanced regenerative ability. As a relevant example, biomimetic materials have high potential to solve degenerative diseases affecting the musculoskeletal system, namely, bone, cartilage and articular tissues, which is of pivotal importance for most of human abilities, such as walking, running, manipulating, and chewing. In this respect, the adoption of nature-inspired processes and structures is an emerging fabrication concept, uniquely able to provide biomaterials with superior biological performance. The chapter will give an overview of the most recent results obtained in the field of hard tissue regeneration by using 3D biomaterials obtained by nature-inspired approaches. The main focus is given to porous hydroxyapatite-based ceramic or hybrid scaffolds for regeneration of bone and osteochondral tissues in neurosurgery and orthopedics.

Keywords: biomineralization, biomorphic transformation, biomimetic hydroxyapatite, bioactive porous scaffolds, bone regeneration, osteochondral regeneration

1. Introduction: The biomimetic concept in biomaterials science

Biomimicry in biomaterials science means examining nature, its models, systems, processes, and elements to emulate or take inspiration from in order to solve human problems. The scientific community has now realized that in spite of recent advances, many societal needs are still unmet. Biologically inspired approaches have been particularly attractive in several fields, in over 3.8 billion years of evolution.

Several solutions were introduced with increased functionality reducing energy and materials and with no impact on environment, exactly the targets faced by the actual technological challenges [1, 2]. Biomimicry has engaged several fields creating smart materials to solve those problems that nature has already solved. In that past 50 years, some examples of biologically inspired materials were developed. In particular, exploiting bioinspired technologies bone-like materials based on wood and tough ceramics based on mother-of-pearl were designed. Despite biomedical field, other kinds of materials were created such as self-cleaning structures based on flowers, underwater glues based on mussel adhesive, drag reduction based on dermal riblet on shark skin, flight mechanisms based on insect flight, etc. [3–6]. The most recent researches increasingly take inspiration from the nature trying to mimic complex behavior typical of natural structures; in particular, new synthesis methods enabling controlled crystal growth and organized structures at the multi-scale levels are paying close attention. In this way nature is studied not only to develop biomimetic material but also to mimic natural process to create new materials. A highly mimicked natural process is biomineralization useful to create biocompatible materials very close to natural tissue. Biomineralization is a natural process by which organisms form minerals and consists in a complex cascade of phenomena generating hybrid nanostructured materials hierarchically organized from the nanoscale to the macroscopic scale. This process is at the basis of load-bearing structures such as bones, shells, and exoskeletons and allows designing biocomposite with unique properties, not obtainable with any conventional approach, as the information's exchange with cells and the trigger of the bone regenerative cascade [7, 8].

2. Biomimetic nano-apatites

In biology, calcium phosphates are the major inorganic constituents of bones, teeth, fish enameloid, deer antlers, and some species of shells [9]. Human hard tissues are composed principally of calcium phosphates with the exception of small portions of the inner ear. They are poorly crystalline carbonate-substituted nanosized apatites, with the exception of enamel, which has a high degree of crystallinity. Nanocrystalline apatites are nonstoichiometric (Ca/P ratio less than 1.67) and calcium (and OH)-deficient and may incorporate substituted ions in the crystal lattice (Na^+, Mg^{2+}, K^+, Sr^{2+}, Zn^{2+}, etc.), in contrast to HA [$Ca_{10}(PO_4)_6(OH)_2$], which is the stoichiometric hydroxyapatite phase that is the most stable and least soluble calcium phosphate at physiological conditions. The nanocrystalline apatites exhibited higher solubility compared with HA; the responsible are calcium and hydroxide deficiencies. If they are submitted to humid environment, they are able to mature; as a result, "mature" bone crystals in vertebrates are less soluble and reactive than embryonic (young) bone mineral crystals [10].

The chemical composition of nanocrystalline apatites differs significantly from that of HA. The global chemical composition of biological apatites (or their synthetic analogues) can generally be described as

$$Ca_{10-x}(PO_4)_{6-x}(HPO_4 \text{ or } CO_3)_x(OH \text{ or } \tfrac{1}{2}CO_3)_{2-x} \text{ with } 0 \le x \le 2 \qquad (1)$$

Minor substitutions are also found in biological apatites that involve monovalent cations (especially Na^+ and K^+), for example. In this case, charge compensation mechanisms must be taken into account.

The nanocrystalline apatites (whether biological or their synthetic analogues prepared under close-to-physiological conditions) could be probably described as the composition of an apatitic core (often nonstoichiometric) and a hydrated

surface layer containing water molecules and relatively weakly bound ions (e.g., Ca^{2+}, HPO_4^{2-}, CO_3^{2-}, etc.) [11] occupying non-apatitic crystallographic sites.

The hydrated surface layer is responsible for most of the properties of biomimetic apatites. The role of bone mineral in homeostasis in vivo could be explained by the high surface reactivity of biomimetic apatites in relation to surrounding fluids (which is probably directly linked to a high mobility of ionic species contained within this layer). The ions inside the hydrated surface can potentially be exchanged by other ions from the surrounding solution or by small molecules, which may be exploited for couplings with proteins or drugs. It is interesting to remark that during the aging of the nanocrystals, the typical non-apatitic features mentioned above tend to be progressive. This process that has been related to the progressive growth of apatite domains at the expense of the surface hydrated layer is called "maturation" [12].

The metastability of such poorly crystallized nonstoichiometric apatites, which steadily evolve in solution toward stoichiometry and better crystallinity, is thought to be linked to the maturation process. This evolution can be, for example, witnessed by the decrease of the amount of non-apatitic HPO_4^{2-} ions upon aging or else by the decreased potentialities to undergo ion exchanges [12].

Synthetic HA exhibits excellent biological properties such as biocompatibility, bioactivity, lack of toxicity, absence of inflammatory and immune responses, and relatively high bioresorbability. Improving their biomimetism, that is, by preparing them with dimensions, morphology, and nanostructure, can significantly enhance these properties and chemical characteristics that are similar to those found in biological apatites [9]. In the recent years, many different strategies have been employed in the preparation of synthetic nanosized HA crystals, with the most common method being stoichiometric titration of calcium hydroxide slurry with phosphoric acid up to neutrality.

Several methods have been successfully employed in the synthesis of nanocrystalline apatites, including wet chemical precipitation, sol-gel synthesis, coprecipitation, electrodeposition, vapor diffusion, and a number of others [13]. The physicochemical characterizations carried out on several synthesized apatites at low temperatures have shown that they have the typical features of biological apatite, such as the size domain, the low degree of crystallinity, and the existence of surface compositions different from the bulk [14, 15].

The method of ionic substitution has been proposed for improving not only the biomimetic features of apatite but also the biological performance of apatite-based materials. Many attempts have been made to synthesize HA that contains carbonate as a raw material for the manufacture of biomaterials. Carbonate can substitute for OH (A-type substitution) or for PO_4^{3-} (B-type substitution). A and B carbonated apatites can be distinguished by the different positions of the carbonate infrared absorption bands and by their different lattice constants. In biological apatites, CO_3^{2-} substitutes mainly for PO_4^{3-} in B-type apatite. Charge compensation by a Ca^{2+} vacancy, together with an H atom that bonds to a neighboring PO_4^{3-}, has been established to be the most stable arrangement. The incorporation of carbonate usually results in poorly crystalline structures with increased solubility, because it inhibits apatite crystal growth [16].

Divalent ions, such as magnesium and strontium, that replace calcium are particularly active during the first stages of the remodeling and regenerative processes. In particular, magnesium enhances skeletal metabolism and bone growth, so is associated with the first stages of new bone formation. Like carbonate, magnesium decreases with the aging of the bone and with increasing calcification. In synthetic HA, the presence of magnesium increases the chemical-physical mimesis of the mineral bone. In fact, magnesium affects the kinetics of HA nucleation on collagen,

increasing it, and retards its crystallization, affecting the shape and size of mineral nuclei. The substitution of Ca^{2+} with Mg^{2+} into the HA structure leads to a continuous ion exchange from the outer hydrated layer to the well-crystallized apatite lattice, inducing a disordered state on the HA surface. Moreover, the incorporation of magnesium in surface crystal sites increases the number of molecular layers of coordinated water; all of these phenomena favor the adhesion of cells to the scaffold because the protein adsorption is increased. A greater osteoconductivity over time and higher material resorption, compared to stoichiometric HA, were detected in granulated Mg-HA powders that were implanted in a rabbit's femur, proving the increase of osteogenic activity in the presence of magnesium-substituted HA. A higher expression of specific markers of osteoblast differentiation and bone formation, which are associated with a lower osteoclastogenic potential, was revealed by studies of osteoblast gene expression profiles from Mg-HA grafts [17, 18].

The incorporation of strontium into the HA structure reduces bone resorption while enhancing osteogenesis; this effect improves physical stabilization of the new bone matrix, enhancing collagen synthesis, as shown in in vitro and in vivo studies. The incorporation of strontium ions into the HA lattice has been practiced in recent years, due to its potential as an anti-osteoporotic agent, and increasing effort is being dedicated to the development of strontium-containing bone cements [19].

Biomimetic HA powders can be synthesized and used as granules to fill bone defects of limited size, but if the regeneration of an extended bone part is necessary, the implantation of a 3D porous scaffold is required because the lack of mechanical stability and specific morphology of granulated bio-devices does not enable regeneration of extended bone segments; therefore, the porous scaffold must have, in addition to bioactivity and osteoconductivity characteristics, also biomechanical performance suitable for the specific implant site. The scaffolds must provide both the space for the new bone formation and the necessary support for the cells to proliferate and maintain their differential function. Furthermore, they should present suitable architectures for inducing the formation and maturation of well-organized tissue. The use of bioactive scaffolds aids the process of osteoconductivity that establish physical and mechanical integration with the surrounding bone, which in turn avoids micro-movements and the possibility of early mechanical loading in vivo [20].

3. Porous hydroxyapatite scaffolds for bone regeneration

Bone scaffolds are intended as 3D porous bodies that can allow efficient cell colonization and neovascularization of newly formed tissues throughout the whole implant [21], also giving tight mechanical attachment to the porous scaffold. This is a key achievement for the stabilization of the defect and the recovery of bone-like mechanical performance [22, 23].

Different technologies have been investigated for the development of bone scaffolds with bone-like porosity associated to adequate biomechanical strength [24]. All techniques are based on sintering processes for the consolidation of porous structures formed by processing of ceramic suspensions. Many of them make use of sacrificial phases that are later removed by controlled processes. Methods using sacrificial templates use porogenic agents, such as polymer components, mainly, but also natural sources and inorganic-soluble salts, dispersed into ceramic suspensions and then decomposed by thermal treatments or extracted by chemical processes. The replica method uses organic sacrificial templates but, in the form of 3D bodies, is also derived by natural sources such as cellulose sponges [25], which are eliminated by burning after being soaked into ceramic suspensions.

Other very efficient techniques use the formation of bubbles driven by chemical components dispersed in the suspensions or the direct introduction of gases in the ceramic slurries to obtain foamed powder suspensions, which are sintered after casting and drying [26]. The key aspect in such direct foaming methods is to accurately control the suspension rheology by the use of stabilizing agents (**Figure 1**).

Due to controlled macroporosity and pore interconnection obtained by this flexible method, scaffolds not only exhibit improved osteoconductive ability but also higher mechanical properties than those obtained using sacrificial templates [24]. A recent study reported a novel promising route based on a modified direct foaming method that gave HA bodies with 65% pore volume and a compressive strength $\sigma = 16.3 \pm 4.3$ MPa [27].

A relevant field of application for porous bone scaffolds is neurosurgery; here, cranial reconstruction often uses synthetic biomaterials implants (polymers, metals, and ceramics) instead of autologous bone [28], particularly for large bone defects. An important issue in this respect is the occurrence of bone resorption and infection, which can result in the removal of the implant and its replacement with other materials [29]. Nowadays, polymethyl methacrylate (PMMA) is the first option among synthetic materials for cranioplasty mainly because of its excellent tensile strength [30]; but its potential decomposition into the starting monomer may lead to fracture susceptibility, other than inflammation and infection [31]. To strengthen the prosthesis, titanium wire mesh is often used as a support for the acrylate thanks to its overall high strength and malleability [32]. Also, polyetheretherketone (PEEK), possessing mechanical strength and elasticity similar to natural bone, is involved as implant for cranial reconstruction [33].

However, all these synthetic materials have the limitation of being bioinert: they have poor osteogenic and osteoconductive ability, so their implants may not integrate tightly with the surrounding newly formed bone [28].

An interesting alternative is in the use of synthetic porous HA ceramic that, due to its good bioactivity deriving from biomimetic composition, can stimulate new bone formation and tight integration of bone to the prosthesis, with recovery of the original biomechanical performance [34, 35].

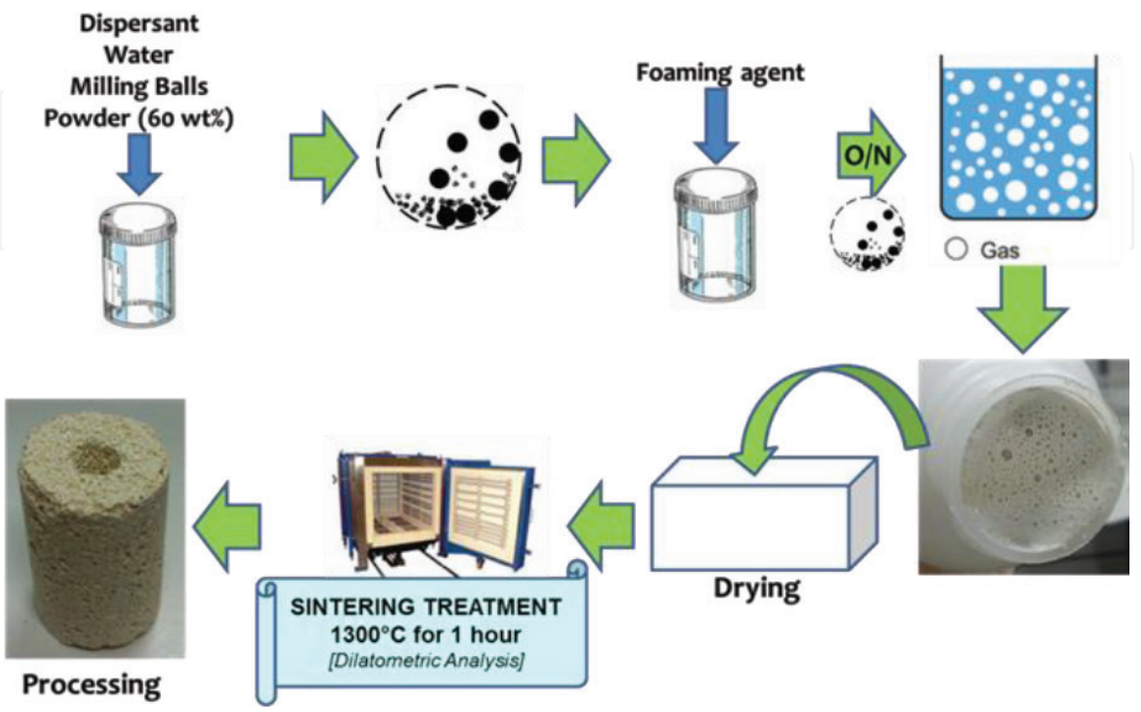

Figure 1.
Scheme of the direct foaming process to obtain 3D bioceramic porous scaffold.

Despite the advantages, HA is reported to have the tendency to fragmentation due to its brittle character, typical of ceramic materials [28], which do not allow its use for load-bearing bone (e.g., femur, tibia, and metatarsus) reconstruction. In this respect, the current research in scaffold materials is directed toward the design and development of bioactive ceramic composites, especially as biodegradable implants, with bone-like three-dimensional structure and improved mechanical performances. Several attempts were made to join a bioactive/bioresorbable component (particularly HA and other calcium phosphates, such as tricalcium phosphate (TCP)) and a bioinert/bioactive reinforcing phase (ZrO_2, calcium silicates, Al_2O_3, TiO_2, and others) [36–38]. Among them TCP/TiO_2 composites are considered very interesting for bone regeneration because β-TCP presents accelerated degradation and optimal reactivity with the bone tissue, thanks to its calcium to phosphorus ratio lower than that of HA [38], while TiO_2 can form a tightly bound superficial HA layer, thanks to its bioactivity, and presents high mechanical performances [39, 40].

It has been recently demonstrated that dense and porous TCP/TiO_2 bodies, obtained by optimized sintering process, display high values of flexural strength and fracture toughness, thanks to the presence of a reinforcing network made of TiO_2-coalesced nanoparticles [41]. Moreover, increased proliferation, colonization, and viability were found demonstrating good osteogenic properties, thus showing good potential as scaffolds for load-bearing bone reconstruction [42].

4. Injectable self-hardening bone cements with biomimetic composition and nanostructure

Bone diseases, such as hemangioma, multiple myeloma, osteolytic metastases, and osteoporosis, can yield bone weakening, thus commonly resulting in fractures in the vertebrae, femur, and radius, especially in the elderly [43]. Minimally invasive surgery procedures, such as vertebroplasty and kyphoplasty, are currently used to regenerate osteoporotic fractures with bone cements as bone defect fillers [44].

Ideally, bone cements should exhibit adequate mechanical support to withstand the early biomechanical loads and should establish effective integration with newly formed bone. The most common injectable cements are based on polymethyl methacrylate (PMMA), thanks to their favorable mechanical properties and robustness [45]. However, PMMA bone cements lack the necessary bioactivity and resorbability, for which it is a foreign body presenting excessive rigidity, in comparison with the bone, so to potentially provoke secondary fractures at adjacent vertebrae. Moreover, PMMA hardening occurs through an exothermic polymerization process, leading to the risk of thermal necrosis of the surrounding bone tissue [46]. In contrast with these drawbacks, calcium phosphate-based bone cements (CPCs) have attracted great attention due to their excellent bioactivity, deriving from the chemical similarity with the bone tissue, and bioresorbability, which lead to the formation of new bone that can replace the implant [47, 48].

Numerous CPC formulations with different initial reactants (which include α-tricalcium phosphate, β-tricalcium phosphate, anhydrous dicalcium phosphate, and monocalcium phosphate monohydrate), producing either an apatite-based or brushite-based cement [49], have been reported [50, 51]. In addition to their excellent biological behavior, CPCs are intrinsically microporous: pores in the size range of submicro-/micrometers are left by extra aqueous solution after hardening of CPCs [52]; such micropores are effective for the impregnation of biological fluids into the bone cements and help resorption and replacement of implants by bone.

One of the most critical issues of injectable CPCs is the control of the chemistry of setting reactions and rheological properties, to achieve adequate injectability, setting time, and mechanical properties [53]. A recent interesting approach involves the addition of natural polymers or their derivatives, such as sodium alginate [54], hydroxypropyl methylcellulose [55], hyaluronic acid [56], chitosan [57], and modified starch [58], into the starting powder or in solution into the cement paste [59]. Biopolymers can be developed as low-viscosity solutions for easy injection and have the ability to cross-link in situ after injection under physiological conditions (temperature or pH) or by the action of an initiator (light or cationic cross-linkers) [53]. In general, due to the higher viscosity of the CPC paste, the presence of polymers tends to increase setting time and to enhance CPC injectability and cohesion. Furthermore, the use of biopolymeric additives can be an effective method to improve the mechanical performance of CPCs [53]. Depending on the final CPC properties desired, different polymers may be incorporated into CPCs, and the polymeric solution may be altered by changing concentration, molecular weight, and polymer chain length [59].

Among the several approaches proposed for the synthesis of CPCs, the use of α-tricalcium phosphate (α-TCP) powder [60] as a metastable precursor is particularly of interest for introduction of foreign ions, such as Mg^{2+}, CO_3^{2-}, SiO_4^{4-}, and Sr^{2+}, enhancing bioactivity and providing efficient therapies against degenerative bone diseases [19, 20, 61].

In particular, CPC formulations based on Sr-doped apatitic cements are very interesting because of strontium ability to enhance cell proliferation and differentiation into bone-forming osteoblasts and decrease the resorbing activity of mature osteoclasts; this is a key achievement for the restoration of the bone turnover balance, especially when the cement is used to treat osteoporotic bone fractures [62]. Sr-substituted TCP was shown to slow down the cement setting as well as the transformation into Sr-doped HA. Moreover, due to apatite lattice expansion, the introduction of strontium in the apatite structure is associated with an increased solubility of the cements, leading to an increase of ions released, which in turn was found to have a positive effect on cell proliferation and osteogenic differentiation [62]. In particular, Sr-substituted CPCs previously tested in vivo exhibited increased new bone formation compared to Sr-free CPCs. Due to the different preparation routes and properties of the set samples, such as phase composition and porosity, contradicting results of Sr effect on the mechanical characteristics of substituted CPCs can be found. In most compositions setting into Sr-HA, strontium substitution either increased compressive strength or had no significant effect on the mechanical characteristics [63].

Recently, novel injectable, self-setting Sr-HA bone cements were prepared by mixing Sr-substituted α-TCP phases as unique inorganic precursors with disodium phosphate solutions enriched with alginate. In vitro tests showed that different concentrations of Sr^{2+} were able to promote an inductive effect on mesenchymal stem cell differentiation, especially at 2 mol% concentration, and on pre-osteoblast proliferation and an inhibitory effect on osteoclasts activity [64]. Moreover, the addition of alginate significantly improved both injectability and cohesion, leading also to significantly higher compression strength when compared with alginate-free cements, without affecting the hardening process and with the absence of cytotoxic effects. On the basis of these results, a selected Sr-HA cement formulation was further tested in vivo in a rabbit model by compositional, morphological, and histological/histomorphometric analysis. The cement exhibited complete transformation into HA, thus showing a biomimetic composition, and enhanced the ability to induce new bone formation and penetration, provided also by its porous microstructure [65].

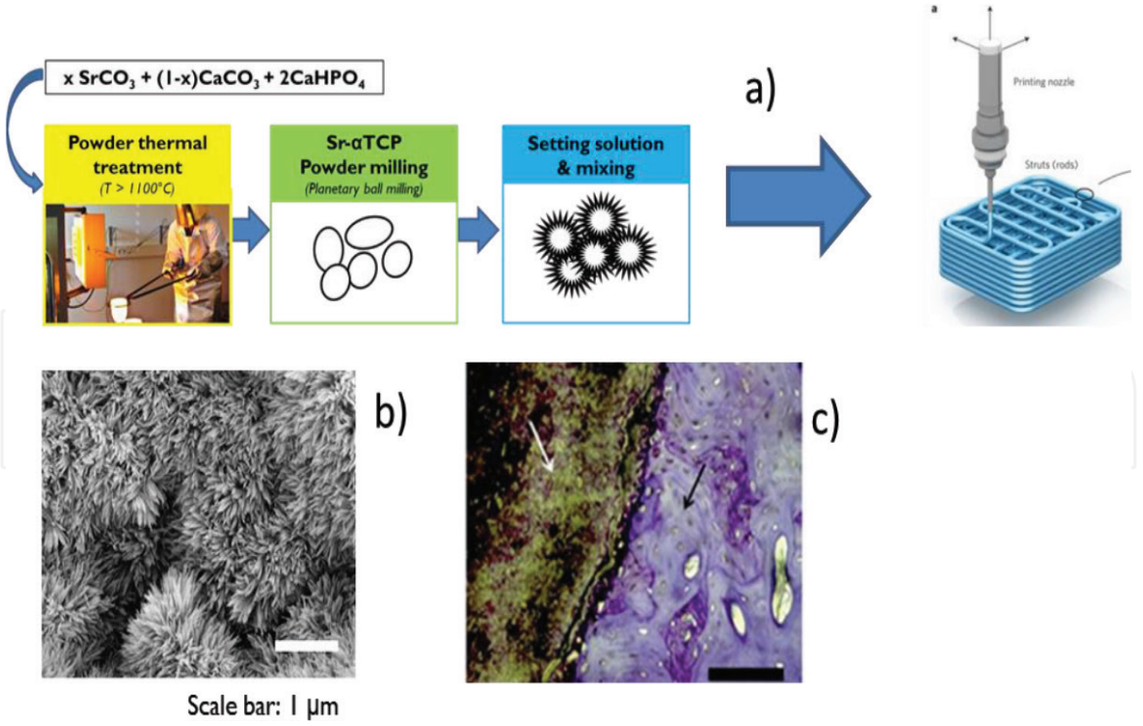

Figure 2.
(a) Scheme of the development of self-hardening formulations as printable pastes, (b) typical nanostructure of hardened apatite cements, and (c) very tight interface between Sr-substituted apatite cement and bone in rabbit test.

Ion-doped apatites obtained as bone cements offer interesting perspectives as a new class of injectable biomaterials that can found application as bioactive pastes for the regeneration of bone defects with complex geometry and not easily accessible by implantation of 3D solid scaffolds (e.g., femur head, tibial plateau, vertebral body, and maxilla). A very interesting perspective, further extending the possible application of bioactive pastes and cements, is the development of printable self-hardening biomaterials (**Figure 2**). Such pastes, to be prepared with rheologic properties enabling flowability, cohesion, and hardening in short times, to allow layer-by-layer deposition, can be processed by micro-extrusion to obtain solid scaffolds with enhanced bioactivity, thanks to the possibility to maintain biomimetic chemical composition, without the need of conclusive sintering process for consolidation.

5. Hybrid scaffolds obtained by bioinspired assembling/mineralization process for bone and osteochondral regeneration

Hard tissues are biological constructs incorporating minerals into soft matrix to create a protective shield or a structural support such as the bone, teeth, and cartilage [7]. The non-mineralized region, called also soft tissue, can be connective, muscular, nervous, or epithelial. Especially examining bone tissue, it is a highly dynamic and vascularized tissue which has an ability to self-heal and remodel through a well-orchestrated process; the bone remodeling is a constant process, targeting to replace odd bone through resorption by means of osteoclasts and to produce new bone by means of osteoblast which usually completes in 4–6 months. However, the high regenerative capacity is lost when there is a large segmental defect, severe non-unions, or bone tumor resection [66]. To overcome these issues, the concept of bone tissue engineering (BTE) has been developed producing

tailor-made scaffolds with the ability to fine-tune the tissue regeneration process. Four different biological prerequisites are necessary for BTE such as osteogenic cells, osteoinductive stimulus, osteoconductive matrix scaffolds, and mechanical environment which led to design scaffolds with appropriate macroporous structure, good degradability, and better osteoconductive properties [67]. A 3D structure is not enough to obtain a material with osteoinductive stimulus, but the chemical composition plays a decisive role. Both concepts (chemical composition and 3D architecture) are at the basis of biomimicry; hence, to obtain scaffolds with chemical composition very close to natural bone, a bioinspired synthesis method mimicking the natural biomineralization process was carried out [68].

In this respect, previous studies by Tampieri et al. exploiting the biomineralization process abovementioned developed biocomposites made of collagen and hydroxyapatite for bone and osteochondral regeneration [69–71].

Exactly as it happens in nature, collagen molecules promoted complex 3D arrangement and the heterogeneous nucleation of a low crystalline hydroxyapatite also due to the incorporation of foreign ions, usually present in human tissue, into the apatite phase. In details, biomineralization process was reproduced in the laboratory dropping an acid solution containing PO_4^{3-} ions mixed with collagen gel into an alkaline solution containing the Ca^{2+} ions exploiting a neutralization process. The pH of the suspension is increased up to neutral pH where two different mechanisms are simultaneously triggered; on the one hand, the collagen fibers reach the isoelectric point leading to their assembly into a 3D network; on the other hand, the mineral nucleation starts in correspondence to the carboxylic groups exposed by the collagen molecule that bind calcium ions [69–72]. One of the advantages of this material is the capability to entrap some foreign ions into HA lattice obtaining a hybrid material mimicking natural mineralized tissues. In particular, CO_3^{2-} ions can occupy two different sites of the apatite lattice. B-substitution occurs at the PO_4^{3-} site improving the osteoblasts adhesion and is typical of young and immature bones; conversely, carbonation in site A refers to partial substitution of OH^-, which increases the stability of mineral phase, and in fact it is more typical of mature bone tissue. Mg^{2+} promotes the HA nucleation and bioavailability decreasing the crystallinity. Sr^{2+} is able to restore the bone turnover balance; this is important for the treatment of osteoporotic bone fractures [73, 74].

The aptitude of the apatite lattice to host several isovalent and heterovalent ion substitutions permits to synthesize apatite nanocrystals with multiple substitutions that can be used in different applications in regenerative medicine and nanomedicine. Furthermore, besides the incorporation of foreign ions, also the control mechanisms exerted by the organic phase allow to produce a more biomimetic apatite thanks to nearly amorphous crystal state and crystal orientation; in this way, cells well recognize hybrid composite without any inflammatory reaction and start to interact with it promoting the adhesion and proliferation on its fibers [75, 76]. Therefore, the use of bioinspired mineralization process is a tool able to confer unique properties to hydroxyapatite otherwise impossible to find in stoichiometric hydroxyapatite as well as in composites where hydroxyapatite was simply mixed with collagen [70].

Among bone defects, large chondral articular defects represent a major problem in orthopedic practice [77], and tissue engineering is providing promising results [78]. However, the results for the treatment of cartilage lesions are still controversial, and osteochondral lesions are even more severe relating to two different tissues featuring different self-healing abilities and cell lineages involved. 3D scaffold, usually, is able to well regenerate a single tissue, as cartilage tissue, and in case of osteochondral damage, additional autologous bone grafting is often necessary [79]. To overcome these limitations and to increase the advantages for osteochondral

regeneration, biomaterials provide the template for tissue development that can be adjusted in shape, size, and orientation according to defect features [80].

For this reason, several authors have highlighted the need to modulate a multi-layered scaffold capable to reproduce different biological and functional environments of osteochondral region to promote regeneration [80, 81]. To create construct with more favorable integrative properties in the osteochondral site, bilayer or tri-layer composite is developed such as a polylactide-co-glycolide copolymer, the first scaffold reported for clinical use; however, it showed poor repair tissue quality at imaging, as well as unsatisfactory clinical outcomes [82, 83]. One of the difficult points in the osteochondral regeneration is the interface between material's layer and host tissue and between layers of host tissue; the cartilage repair should be followed by an adequate regeneration of the subchondral structure and by the effective union with surrounding host tissue [84]. Tampieri et al. designed a composite scaffold consisting of three different but integrated layers, corresponding to cartilage, calcified cartilage, and bone components [69]. It was developed to better mimic structure and composition of the whole osteochondral unit, showing promising clinical results even in challenging conditions, such as complex lesions or osteoarthritic knees [85, 86]. Exploiting biomineralization process, a different extent of mineralization was nucleated on collagen fibers developing a tri-layer with a gradient of hydroxyapatite ranging from a mineral content of 60–70% corresponding to subchondral bone and 30–40% corresponding to mineralized cartilage up to 0% corresponding to hyaline cartilage (**Figure 3**). Furthermore, in the top layer (hyaline cartilage), hyaluronic acid was added to create microstructural features improving the hydrophilic ability to reproduce columnar-like structure converging toward the external surface, where it formed horizontal flat ribbons, thus resembling the morphology of the *lamina splendens*.

Chemical-physical investigation highlighted that chemotactic information provided by collagen-induced unique features in the inorganic phase, promoting the nucleation of a biomimetic apatite very close to the biological one present in the bone [87]. In vivo evaluation demonstrates that it differentially supports cartilage and bone tissue formation in the different histological layers [88]. After 6 months from implantation of graded hybrid composites on femoral condyles of

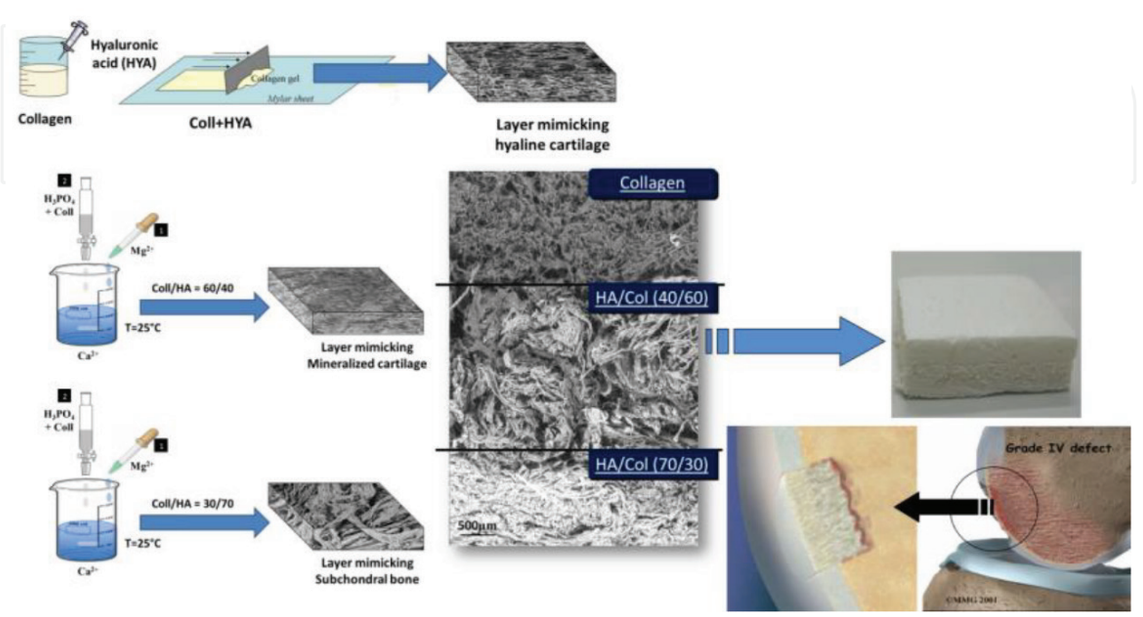

Figure 3.
Representation of multilayered hybrid scaffold obtained by in-lab biomineralization and its application in osteochondral defect.

sheep, a new hyaline-like tissue is formed, and a good integration of scaffolds with host cartilage is observed; furthermore, a strong proteoglycan staining, columnar rearrangement of chondrocytes, and an underlying well-structured subchondral trabecular bone are shown. Besides, hybrid scaffold was completely resorbed, and no remarkable difference was revealed with or without seeding of chondrocyte cells, highlighting as chemical-physical features of hybrid composite allow the recruitment of bone marrow stem cells directly from the underlying subchondral bone [88].

In conclusion, the ability of the scaffold to induce orderly osteochondral tissue repair without the introduction of cells makes it attractive for several reasons: (i) from a practical and commercial standpoint, because it could be used as an off-the-shelf graft in a one-step surgical procedure; (ii) from a surgical standpoint, it could be inserted under minimally invasive conditions due to its flexibility; and (iii) from a biological standpoint, because the problems related to the cell culture would be eliminated [89].

6. Biomorphic transformation of natural structures: a new way to obtain biomimetic scaffolds for regeneration of load-bearing segmental bones

Among the bone diseases, those affecting portions of long bone subjected to mechanical loads are the ones which most seriously impact on the quality of life of sufferers. The incidence of such pathologies is particularly relevant among the aged people (osteoporosis); anyway, more recently the number of relatively young patients affected by bone diseases has increased mainly owing to modern lifestyles (e.g., intense sport activity, obesity, etc.). In this case, pain and disability also impact on the psychological well-being, leading to anxiety, depression, fear for the future, and altered perception of the social role. Such feeling is nowadays shared by the aged people also, because of the increased expectation of an active life and well-being even among the elderly. For this reason, the abovementioned numbers in terms of socioeconomic costs and number of hospitalized people are likely to increase in the next future.

Due to the inability of the current manufacturing technologies to form mechanically strong porous inorganic structures with a hierarchic pore organization and complex morphological details in the submicron scale, the healing of load-bearing bone segments still relies on bioinert dense implants based on alumina, titanium, etc.

A significant change in engineering and ceramic processing is needed, thus greatly expanding the existing tools enabling the development of porous and massive ceramic bodies with designed smart functions. The current manufacturing approach in ceramic development is based on powder synthesis, forming, and thermal consolidation (sintering); the idea is to surpass the existing approach, by developing new "one-step synthesis/consolidation processes" to obtain new 3D ceramics with properties and functions not achievable with the current manufacturing approach. In particular, this is relevant when the ceramic phases with desired functional properties have low thermodynamic stability such as nanosized and atomic position, so that the existing ceramic process, particularly sintering, destroys labile phases increasing their stability but deleting its smart functional properties. Particularly, the sintering process, which is fundamental to consolidate ceramic bodies, impairs the maintenance of ceramic phases characterized by low crystallinity, nanosize, and nonstoichiometric composition. These features, relying on low thermodynamic stability, are very often the source of functions that cannot be expressed by a stable, sintered ceramic phase [20].

The main goal is the implantation of osteoinducting and osteoconducting scaffolds with spatially organized macroporosity and mechanical strength sufficient for early in vivo loading upon implantation and elastic properties close to those of the bone. This may enable scaffolds to respond to the biomechanical loads and activate mechano-transduction mechanisms, yielding remodeling and formation of new functional bone [90]. The complex structure of bones, hierarchically organized from the nano- to the macro-scale, and the interaction taking place across all levels of organization are the reasons of the outstanding mechanical performances of bones. For this reason, long-bone regeneration should be assisted by scaffolds endowed with bone-like composition and similar structural complexity; however, the common manufacturing methods do not produce mechanically resistant scaffolds with the required hierarchical pore organization and bioactivity. The chemical biomimesis in scaffolds for long-bone regeneration is influenced by the mechanical strength of HA-based materials. There are several studies about scaffolds based on composite materials, making use of strong bioactive or bioinert phases [36, 37] that were dispersed in a calcium-phosphate matrix. However, the limitation in the achievement of hierarchically organized structures still remains [8].

This problem can reside in nature, so the attention of scientists has been moved to find and observing complex morphologies that exist in nature, and then try to reproduce them. In particular, the ligneous structures strongly resemble bones in their structural organization and morphology which affect the mechanical performances [8].

Like bone, wood can be considered as a cellular material at the scale of hundred micrometers to centimeters (**Figure 4**). At the cell level, the mechanical properties are governed by the shape and diameter of the cell cross section, as well as by the thickness of the cell wall. In particular, the apparent density of wood, which in turn is a determining factor for the performance of lightweight structures, is directly related to the ratio of cell wall thickness to cell diameter. The particular hierarchical architecture of the cellular microstructure gives wood an exceptional combination of high stiffness, toughness, and strength at low density [91]. The alternation of channel-like porous and fiber bundle areas makes the wood an elective material to be used as a template in the preparation of a new bone substitute that is characterized by a biomimetic hierarchical structure [20].

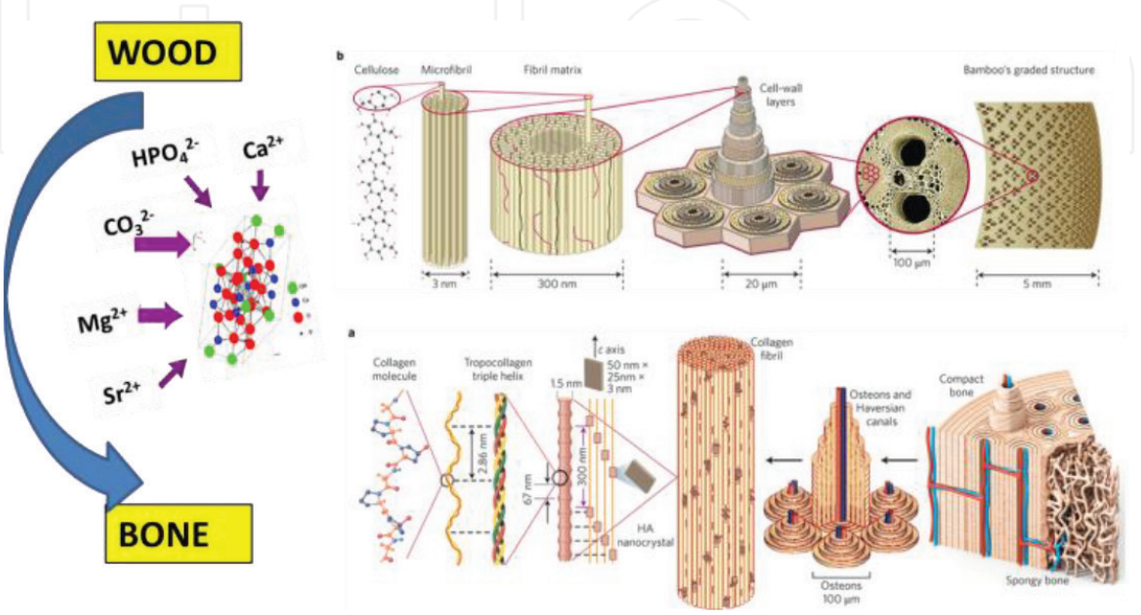

Figure 4.
Scheme of the multi-scale structure of wood and bone tissue.

A subject of investigation in the late 1990s was the transformation of wood into inorganic, hierarchically organized materials (e.g., oxidic ceramics such as Al_2O_3, ZrO_2, TiO_2, and MnO and nonoxidic ceramics such as SiC, TiC, and ZrC) [92–96]. The synthesis of hierarchically organized bone scaffolds made of SiC is a result of these studies [94], which have the advantage of offering bio-tolerated surfaces and very high fracture strength. Other kinds of biomorphic transformations, conceived recently, were used to manufacture hierarchically organized scaffolds made of HA [3]. The complexity of the apatite phase, in comparison with nitrides, carbides, and oxides, required the settling of a multistep process transformation, where the native wood was sequentially transformed into pure carbon, calcium carbide, calcium oxide, calcium carbonate, and finally HA. Due to their bone-mimicking hierarchical organization, microstructure and composition such a new generation of bioceramics scaffolds promise to offer enhanced integration, osteogenesis, and biomechanical behavior when implanted in vivo [8].

Woods such as rattan have strong similarities to 3D structure and morphology of cortical and spongy bone. Rattan is characterized by channel-like pores (simulating the Haversian system in bone), interconnected with a network of smaller channels (such as the Volkmann system) [3].

There is a precise control of the microstructure, crystallinity, and phase composition, during the multistep transformation process, in which different gas-solid reactions occur where the solid is the template. Calcium, oxygen, carbonate, and phosphate ions were progressively added in the different steps to finally get the HA molecules. The reaction kinetic is controlled throughout the different steps of the transformation process in order to have a precise control of the scaffold microstructure, composition, and bioactivity [95]. Importantly, even in the absence of thermal consolidation treatments, the scaffolds exhibit mechanical strengths comparable to those of spongy bone (~4 MPa) when measured along the channel direction, thanks to the maintenance of the original wood microstructure.

The establishment of biomorphic transformations that are able to transform woods into biomimetic bone scaffolds can provide solutions for long-bone regeneration and can be designed in a custom-made fashion. Selected wood structures could reproduce different bone portions that are characterized by different porosities and pore distributions, as occurring in cortical and spongy bones. Such devices may implement the formation of a biological chamber in vivo that contain a suitable environment that allows to promote and enhance bone formation and remodeling. The implant will thus function as an in vivo bioreactor, thus facing an unsolved clinical problem related to the disappearing of the regenerative process at distances far from the bone-implant interface [20].

7. Conclusions and future perspectives

The progressive population aging and the younger people modern behaviors, which expose to serious injuries and traumas, are concerns of large and continuously increasing socioeconomic impact. The continual advances in materials science and nanotechnology allowed great progress in biomedical device development for bone regeneration. Nevertheless, the development of bio-devices mimicking biological tissue structure and composition with high complexity and load-bearing properties, such as extended bone and osteochondral parts or segmental bones, still presents serious limitations. In fact, the related clinical needs remain unmet because of the absence of well-established regenerative devices for such applications. Many possibilities for solving these concerns are offered by the recent advances in materials science: the new-generation smart multifunctional device development could

be enabled by new fabrication approaches that get inspired from the multitude of outstanding biological structures and phenomena. The preliminary steps that have already been taken in this direction are very promising, and the development of bioinspired materials is rapidly becoming a priority to achieve nanotechnological solutions facing critical societal needs.

Conflict of interest

The authors declare no conflicts of interest.

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