

We are IntechOpen, the world's leading publisher of Open Access books Built by scientists, for scientists

6,900

Open access books available

186,000

International authors and editors

200M

Downloads

Our authors are among the

154

Countries delivered to

TOP 1%

most cited scientists

12.2%

Contributors from top 500 universities



WEB OF SCIENCE™

Selection of our books indexed in the Book Citation Index
in Web of Science™ Core Collection (BKCI)

Interested in publishing with us?
Contact book.department@intechopen.com

Numbers displayed above are based on latest data collected.
For more information visit www.intechopen.com



Nature-Inspired Nanotechnology and Smart Magnetic Activation: Two Groundbreaking Approaches Toward a New Generation of Biomaterials for Hard Tissue Regeneration

Simone Sprio, Monica Sandri, Michele Iafisco,
Silvia Panseri, Monica Montesi, Andrea Ruffini,
Alessio Adamiano, Alberto Ballardini and
Anna Tampieri

Additional information is available at the end of the chapter

<http://dx.doi.org/10.5772/63229>

Abstract

Today, as the need of new regenerative solutions is steadily increasing, the demand for new bio-devices with smart functionality is pushing material scientists to develop new synthesis concepts. Indeed, the conventional approaches for biomaterials fail when it comes to generate nano-biocomposites with designed biomimetic composition and hierarchically organized architecture mimicking biologically relevant tissue features. In this respect, an emerging concept in material science is to draw inspiration from natural processes and products, which we may consider as the most advanced examples of smart nanotechnology. Natural processes of supramolecular assembly and mineralization of organic macromolecules, known as biomineralization, generate complex hybrid 3D constructs that are the basis of skeletons, exoskeletons, nacre and shells. On the other hand, natural structures such as woods and plants exhibit multi-scale hierarchic organization that is the source of smart and anisotropic mechanical properties associated with high porosity and lightness. The association of nature-inspired nano-technological products with smart functionalization can provide new advanced solutions to critical and still unmet clinical needs. In this respect, magnetic activation of biomaterials by the use of a recently developed biocompatible, resorbable magnetic apatite promises to represent a new safe and effective switching tool, enabling personalized applications in regenerative medicine and theranostics that so far were not feasible, due to the cytotoxicity of the currently used magnetic materials.

Keywords: bone regeneration, bioinspired materials, biomineralization, biomorphic transformation, magnetic activation

1. Introduction

Materials science today is experiencing a paradigmatic change in the development of new smart devices for biomedical applications. Particularly, the regeneration of hard tissues (i.e. bone, cartilage, tooth) is one of the most demanding issues in medicine and requires smart devices showing high mimicry of the host tissues and ability to instruct and drive progenitor cells to activate the regenerative cascade. Therefore, among the various approaches pursued so far for the synthesis of bone biomaterials, wide consensus is now consolidated around the concept of “*biomimetics*”. Such a definition indicates the ability of a synthetic material to closely reproduce the chemical composition, physical properties, and architecture of native tissues, with the purpose to create 3-D environments able to deliver signals stimulating cell chemotaxis and specific differentiation of autologous stem cells [1]. In this way, the main concept is that bone regeneration can be greatly aided by the fact that, by implantation of a biomimetic scaffold, the patient body acts as a natural bioreactor guiding proper tissue regeneration without the need of complicated tissue engineering procedures or of the use of biological factors, thus improving the safety of clinical approaches.

In this respect the chapter highlights some emerging concepts related to the development of bio-inspired materials addressed to hard tissue regeneration. In particular, the focus is on assembling/mineralization techniques that reproduce the cascade of phenomena acting in the formation of hybrid nanocomposites such as bone and shells, that can generate hybrid fibrous structures with excellent regenerative ability. This process, pinning on the exchange of information stored in the structure of natural polymers, is characterized by great versatility that enable the synthesis of smart multifunctional scaffolds for regeneration of tissue complexes such as joints and periodontium.

On the other side, the chapter is focused on the emerging concept of biomorphic transformations by which natural structures with hierarchic architecture are converted into apatitic biomaterials with unprecedented bioactivity and structure, by multi-step chemical processes. In fact, as the process bases on heterogeneous reactions at the interface between a solid template and a gaseous phase, the obtained scaffolds result well consolidated without the need of sintering treatments and exhibit enhanced mechanical properties, due to the hierarchical architecture, thus being very promising for regeneration of load-bearing bones such as those of the limbs. Finally, the chapter highlights the recent development of an iron-substituted hydroxyapatite (HA) nanophase that, thanks to its excellent biocompatibility and intrinsic magnetic properties, demonstrated ability to be activated by remote magnetic signalling, thus representing a new switching tool for the development of a multifunctional platform generating smart bio-devices for various applications in regenerative medicine and theranostics. This new material, overcoming the limitations of toxic iron oxide nanoparticles currently used

in nanomedicine, is very promising for the future establishment of new and more effective and personalized approaches for bone regeneration and cancer therapies. Moreover, the possibility of boosting bone regeneration by magnetic stimulation in patients with reduced endogenous potential is a key issue, in consideration of the progressive ageing of the population for which more effective and personalized regenerative therapies will be increasingly demanded in the incoming decades.

2. Bio-inspired synthesis processes: hybrid biomimetic scaffolds through biomineralization

In the last decade *bio-nanocomposites* emerged as a new class of materials including a natural polymer (biopolymer) in combination with an inorganic phase, rather than using synthetic polymers. Indeed the need of biomimetic materials and the limitations of the current fabrication methods are increasingly stimulating material scientists to explore this new class of compounds, thus benefitting of the presence of a polymer matrix that can be subjected to physiological, cell-mediated resorption *in vivo*, rather than to processes of chemical dissolution. In fact, the chemical leaching of polymeric scaffolds is one of the possible cause of failure *in vivo*, as the dissolution process can be too fast with respect to the new bone formation process, and also, the degradation products of many polymers can result in harmful effects, jeopardizing the regenerative cascade so that fibrous scars may form, rather than healthy, organized bone tissue. The inspiration for the design and development of bio-nanocomposites takes place from living organisms that are able to produce natural nanocomposites showing an amazing hierarchical arrangement of their organic and inorganic components from the nano to the macro scale (**Figure 1**).

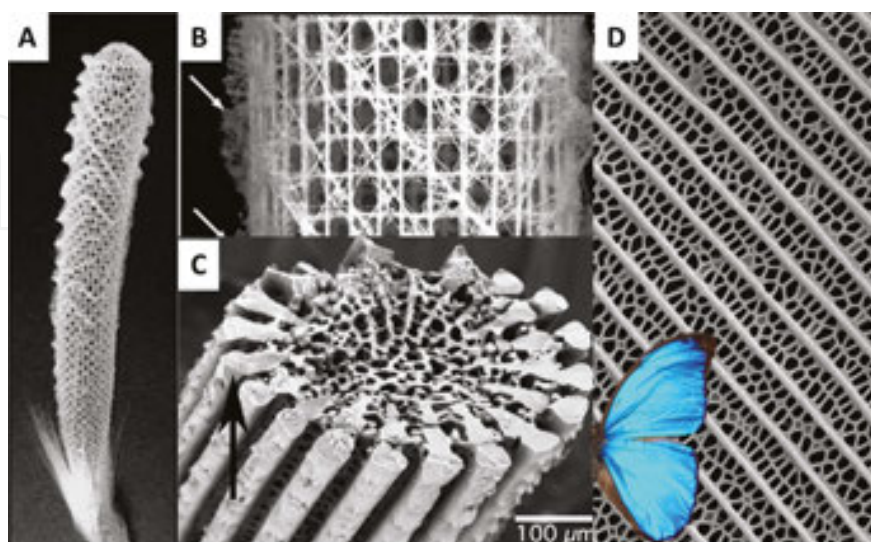


Figure 1. Natural bio-nanocomposites.

These outstanding architectures are the key of the insuperable performance of natural structures, particularly by a mechanic perspective: nacre, shells, bones, ligaments, tooth enamel, and dentine are just some examples of hierarchical, hybrid bio-nanocomposites found in nature. The mechanism at the basis of this outstanding structural arrangement is the establishment of hybrid building blocks formed upon heterogeneous nucleation of inorganic nanophases (such as carbonates and apatites) onto natural polymers, driven by several control mechanisms acting at the molecular scale [2]. In particular, during new bone formation, type I collagen, extruded by fibroblast cells, acts as a template for the nucleation of the mineral phase through a hierarchical assembly of collagen molecules into fibrils and ever thicker fibres, whereas HA nano-nuclei nucleate onto specific positively charged sites located in the collagen molecules. This process is governed by several control mechanisms inherent in the molecular structure of collagen that guide the formation of new bone at all scale sizes: a) the chemical interaction of HA with collagen prevents the crystallization and growth of the mineral phase, which results in a nearly amorphous material characterized by an apatite-like lattice; b) the growth of mineral nuclei is controlled by the organic matrix, so that the size of the nuclei are constrained up to few nanometers; c) the toptactic interaction induces specific crystal orientation of the mineral phase growing on the collagen fibers and evolving into lamellae; d) finally, lamellae are organized through different hierarchical levels to form the structure of the macroscopic bone [3–8]. The use of scaffolds able to guide cells to the re-growth of new bone tissue is an approach now considered as necessary for bone regeneration. Native extra-cellular matrix contains multiple signals whose presentation follows precise spatial and temporal patterns. In designing scaffolds for hard tissue regeneration, such signals must be reproduced so as to give chemical, physical, structural, and morphological information to cells and compel them to express specific phenotypes. Besides, ideal scaffolds guiding tissue regeneration should also have adequate properties with respect to degradation, cell binding, cellular uptake, non-immunogenicity, and mechanical performance. In particular, the essential characteristics of regenerative bone scaffolds are: surface activity enabling the establishment of a tight interface between the scaffold and the new tissue; osteoconductivity i.e. the ability to function as a template for 3D cell colonization; appropriate degradation profile without host tissue responses such as inflammation or fibrous encapsulation of the implant [9].

The reproduction of the bone biomineralization process in laboratory enabled the synthesis of hybrid HA/collagen composites reproducing most of the relevant features of newly formed bone and osteochondral tissues [10, 11]. Type I collagen extracted by equine tendon and dispersed into acetic acid in the form of nanofibrils can be subjected to controlled assembly in aqueous environment by pH variation, simultaneously to the mineralization with apatite nanophases where the content of foreign ions can be tailored to reach bio-competent compositions. In fact, the maintenance of a disordered crystal structure allows the entrapment of ions naturally present in the physiological environment (i.e. Mg^{2+} , CO_3^{2-} , Sr^{2+} , Na^+ , K^+ , SiO_4^{4-}) into the structure of the mineral phase. The molecular habitus of type I collagen acts as a 3D substrate for heterogeneous nucleation of the mineral phase but also as a constraint for the growth and long-range ordering of the mineral crystals (**Figure 2**).

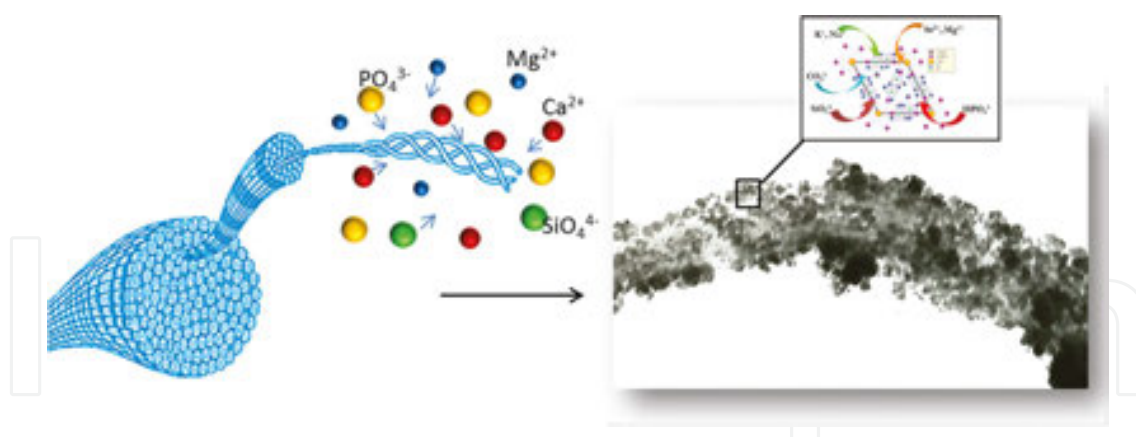


Figure 2. Scheme of collagen assembling and mineralization.

By this process CO_3^{2-} ions can be introduced to preferably occupy the phosphate site of the HA lattice (B type position) [4], thus providing the mineral phase with enhanced activity for cell adhesion and resorbability. Carbonate ions are abundant in young and newly formed bone tissue, and decrease in mature bone, thus evidencing their role in bone development. Among the foreign ions present in biologic apatite, Mg^{2+} have the marked property of increasing the nucleation kinetics of HA on collagen fibres but, in the meantime, hampering crystal growth, thus generating nano-size HA nuclei, strongly enhancing the bioavailability of the mineral phase. In fact, magnesium is found in much higher concentrations in young and newly formed mineralized tissues and is considered today as a fundamental element governing the first stages of bone formation [12]. Silicon is a minor element, essential for healthy skeletal development in higher biological organisms [6, 13], in particular for its role in the formation of crosslinks between collagen and proteoglycans [14], that provide stabilization of the new bone matrix and prevent enzymatic degradation.

The bone-like features of HA/Collagen hybrid composites reflect in bio-resorbability at physiological pH and high surface activity, particularly referred to the crystal size (i.e. ranging from 30–50 nm long, 15–30 nm wide, and 2–10 nm thick) [7, 8, 15–17] and to the specific orientation of the apatite nuclei, in respect to the long axis of collagen. The preferential growth of apatite nuclei along the *c* axis, as induced by the presence of particular functional chemical groups on the surface of the organic template, affects the surface polarity of the final hybrid composites, and consequently protein adhesion and cell attachment. The hierarchical assembly of these nano-size building blocks into macroscopic objects occurs upon supramolecular arrangement of collagen fibrils into thicker fibres, thus resulting into a final hybrid composite where, on a macroscopic scale, the mineral phase assumes a complex and hierarchical architecture, strictly dependent on the combination of the various above-described phenomena, which hierarchically occur on different dimensional scales in correspondence with the sites of heterogeneous nucleation.

The HA/Col composites assume a fibrous structure as well as high and interconnected porosity, the amount and morphology of which can be tailored by customized freeze drying processes (**Figure 3**).

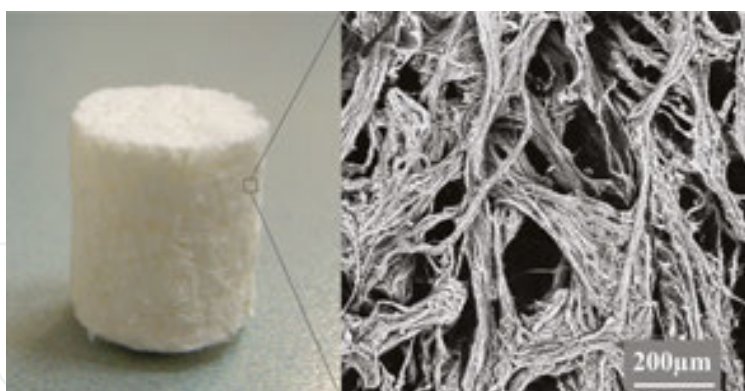


Figure 3. Bio-hybrid HA/Collagen composites.

The final dried scaffolds exhibit high activity towards cells; therefore they can be easily resorbed *in vivo* whereas new tissue forms. However, to limit the enzymatic degradation possibly preventing successful cell colonization and integration, cross-linking methods can be applied by using physical or chemical approaches addressing specific links among functional groups of collagen, thus enabling fibre bridging and tailored stability against resorption.

The in-lab reproduction of the phenomena occurring in biological processes can be considered as a conceptually new approach for nanotechnology and may pave the way to the development of new devices with outstanding properties. On the basis of the recognition of the different requirements to regenerate cartilaginous and bony part, such processes can be directed to graded scaffolds reproducing different histological areas in the osteochondral tissue by simply varying the degree of mineralization and the alignment of collagen fibres [11]. Therefore, hydrogels with designed features can be engineered into three-layered devices reproducing the sub-chondral bone (mineralization = 60-70 wt%), mineralized cartilage (mineralization = 30-40 wt%), and the hyaline cartilage (mineralization = 0 wt%) (**Figure 4**).

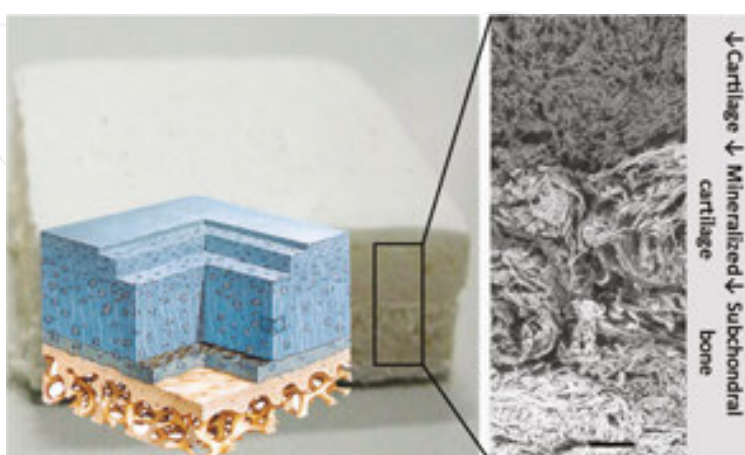


Figure 4. Scheme of osteochondral scaffolds.

In particular, the collagen-like layer, based on collagen and added with hyaluronic acid to create microstructural features improving the hydrophilic behaviour of the construct, reproduces some cartilaginous environmental cues such as the formation of a columnar-like structure converging towards the external surface where it forms horizontal flat ribbons, resembling the morphology of the *lamina splendens* [10, 11].

Such composites have demonstrated enhanced cell proliferation with very spread cell morphology, as well as high osteoinductivity and regenerative potential. The HA/collagen graded composites differentially support cartilage and bone tissue formation in the different histological layers, as demonstrated by comparative *in vivo* study carried out on adult sheep, where HA/collagen graded composites have been implanted on femoral condyles [18]. In particular, histological evaluation showed the formation of new hyaline-like tissue and good integration of scaffolds with host cartilage, with a strong proteoglycan staining and columnar rearrangement of chondrocytes, and an underlying well-ordered sub-chondral trabecular bone.

In this section it has been discussed that biologic processes pin on information exchanged at the molecular scale and on environmental boundary conditions that guide the process towards the establishment of 3-D hybrid composites with defined characteristics. This implies that bio-inspired syntheses are flexible processes that can be directed to fabricate specific devices *on demand*. In this respect, hybrid HA/Col composites can be developed to assume specific 3D morphologies, thus mimicking human multifunctional tissues such as periodontal regions. Indeed, human tooth is a tissue complex formed by the periodontium, in turn including alveolar bone and cementum, linked together by the periodontal ligament firmly bound to the root, and the dentin, a highly mineralized collagen matrix with tubular organization that is protected by the enamel (Figure 5).

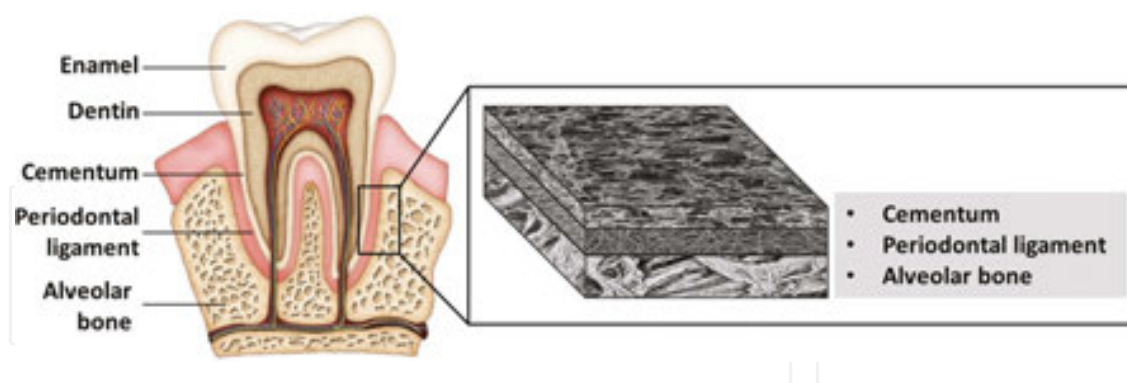


Figure 5. Scheme of dental tissues.

All the components of tooth form upon biologic phenomena close to those leading to formation of bone and cartilage [19]. The relevant differences are related to the mineralization extent of the different tissues (i.e. alveolar bone ~70 wt%, cementum ~50 wt%, dentine ~75 wt%, enamel ~98%), the degree of aggregation of collagen fibres, and the structural organization. Therefore, bio-inspired in-lab mineralization can be directed to develop new biomimetic scaffolds mimicking the different parts of the tooth by varying the concentration of calcium and phosphate ions with respect to collagen thus achieving the desired mineralization extent. Then,

oriented channel-like porosity mimicking the tubular organization of dentine can be obtained by ionotropic gelation techniques applied to the as-synthesized hydrogels (**Figure 6**).

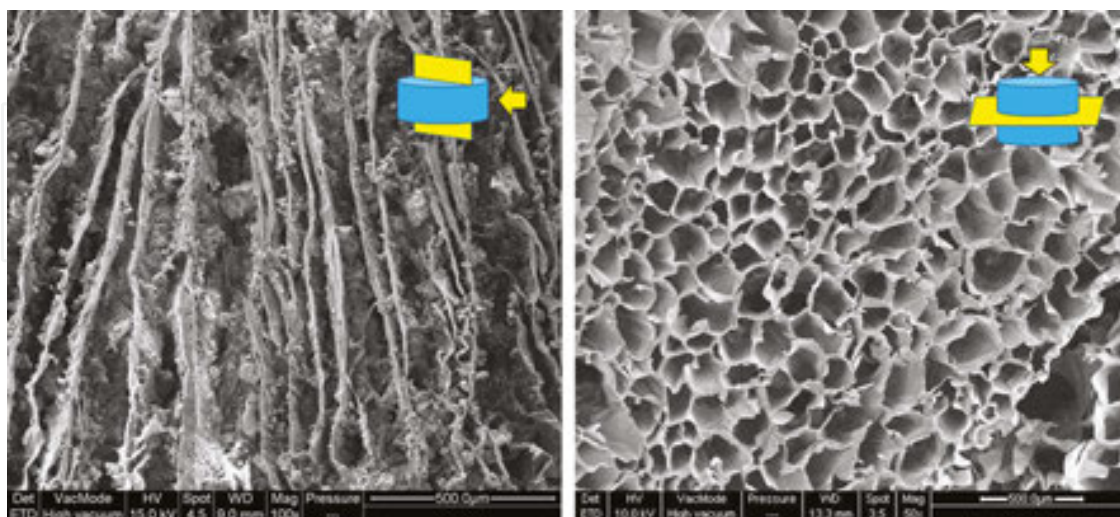


Figure 6. Dentin-like scaffolds.

Preliminary research shows that the application of bio-inspired synthesis techniques can enable the development of new implantable devices for the complete regeneration of dental tissues. This is a major and highly demanding clinical need and a target of high impact for materials science and medicine.

In perspective, the in-lab biomineralization process may be in principle translated to wider applications, possibly extending the range of natural polymers that can be combined to form composite matrices activating self-assembly and mineralization with specific inorganic phases. Non-mineralized constructs can be used as scaffolds for soft tissues and organs, where the biologic and mechanical performance can be tailored by combining various raw materials such as gelatin, nanocellulose, chitosan, alginate, and fibroin characterized by different hydrophilic behaviour and stiffness. On the other hand, the simultaneous mineralization of composite polymeric matrices with nano-apatites can generate scaffolds with improved mechanical performance, thus enabling wider applications in bone surgery, particularly referred to load-bearing applications where the soft nature of hybrid scaffolds does not allow to withstand strong biomechanical loads in the early stages of new bone formation.

3. Nature-derived biomaterials: biomorphic bone scaffolds with hierarchic architecture

The regeneration of load-bearing bone parts is still a high demanding challenge. Particularly, therapies to solve serious diseases involving the limbs, due to trauma or tissue degeneration, are today restricted to reconstructive approaches based on multiple surgery and the use of metallic parts that often give rise to secondary effects such as infections, pseudoarthrosis, and

non-unions which can also lead to the complete loss of the limb functionality and also to amputation [20–22]. The incidence of such events is great and steadily increasing globally due to the modern lifestyle and also to the progressive ageing of the population, thus leading to high disability with huge impact on the healthcare costs and the patient's life.

Today the use of grafts to assist the regeneration of long segmental bones is considered as a promising approach, with different alternatives including autologous vascularized bone grafts, homologous bone graft, heterologous bone graft (xenograft), or prostheses, each one of them dealing with both specific advantages and drawbacks, such as: donor site morbidity and limited available amount, possible immune response and viral transmission, possible animal-derived pathogen transmission and risk of immunogenic rejection, high invasiveness and surgery-related systemic risks, long recovery time and need of prostheses revision [23–27].

Due to these very serious drawbacks, the use of synthetic bone substitutes with osteogenic and osteoconductive ability may offer clear benefits compared to natural bone grafts. Adequate osteogenic ability is required to stimulate the formation of new bone by exhibiting highly exposed active surfaces, favouring cell adhesion and proliferation. Also, osteoconductivity enables the penetration of the scaffold by cells which is a key aspect to achieve early osseointegration in turn enabling adequate stability of the bone/implant construct and the possibility for the patient to stimulate bone regeneration by progressively increasing loading [28, 29]. Adequate osteoconductivity is provided by the presence of open and interconnected porosity in the bone scaffold, in association with high surface affinity with bone cells. However, most of the bio-devices today developed exhibits tortuous porosity that hampers the development of extensive angiogenesis and penetration of blood vessels in the inner parts of the scaffold; in consequence, even though a good surface integration occurred, bone penetration is limited thus penalizing the stability of the bone/implant construct and the mechanical performance. [30–32].

HA, and particularly ion-doped apatites are the golden materials for bone scaffolding. However, the feasibility of synthesizing large porous HA bodies with high bioactivity, osteoconductivity, and mechanical strength is hampered by the need of thermal consolidation that destroys the bioactivity features of HA, that means: segregation of the foreign ions outside the HA lattice, thermally-induced grain growth with strong reduction of the specific surface area and reduction of the hydrophilic character and surface reactivity. Moreover, the weak mechanical properties of HA make it difficult to develop large scaffolds with high porosity extent. However, it can be envisaged that early and extensive penetration of new bone into the scaffold pores may significantly enhance the strength of the bone/biomaterial construct and enable mechanical loading. This process may lead to the complete recovery of limb functionality by progressive and assisted stimulation of the implanted part [32].

Since the unique biomechanical properties of bone mainly depend on its hierarchically organized structure ranging from the molecular to the nano-, micro-, and macro-scales, only scaffolds endowed with a 3D structure capable of exhibiting complex biomechanical performances may activate mechano-transduction processes in a biological-like fashion and yield regeneration of well-organized bone [33–39].

In consideration to the limits imposed by the ceramic technology (particularly by means of the existing forming techniques and the sintering), new manufacturing approaches are required for synthesis of scaffolds with adequate requisites for regeneration of long segmental bones. In this respect, the complex structural organization exhibited by living beings such as woods and plants is an interesting source of inspiration for material scientists towards the generation of smart devices with strongly improved performances. Indeed, these structures possess a hierarchic organization on multiple size scales that provide high strength and lightness (**Figure 7**).

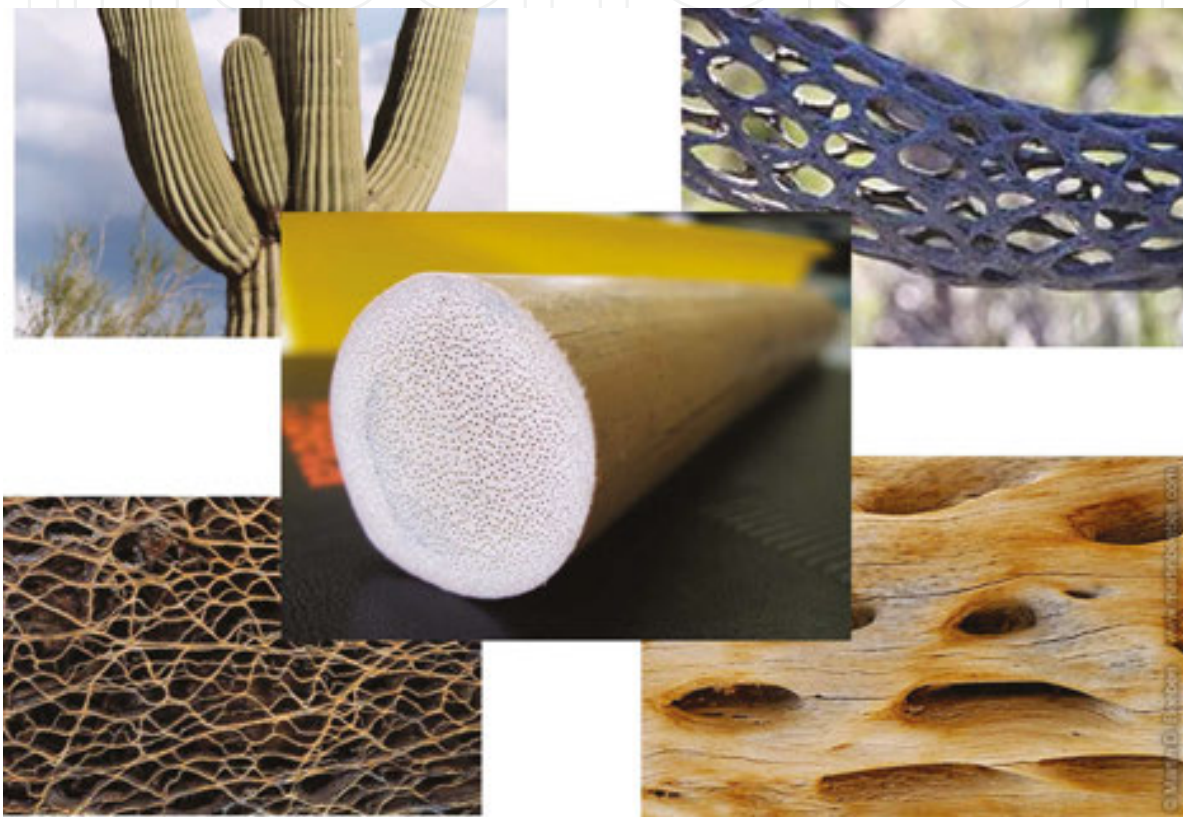


Figure 7. Structure of various woods and plants.

Among these, ligneous structures endowed with open porosity and suitable interconnection enabling extensive permeability to cells and fluids, as well as, at the same time, with adequate anisotropic mechanical behaviour, may be investigated as templates to develop new porous scaffolds with bone-like structural features [40–42].

Porous woods like pine and rattan were recently transformed into HA scaffolds with hierarchic organization, by a multi-step biomorphic transformation process (**Figure 8**) enabling precise control of phase composition and crystal ordering, as well as of the microstructure, since the different reactions occurred between a gas and the solid template at a molecular level, where calcium, oxygen, carbonate, and phosphate ions were progressively added, while building the HA molecules [43].

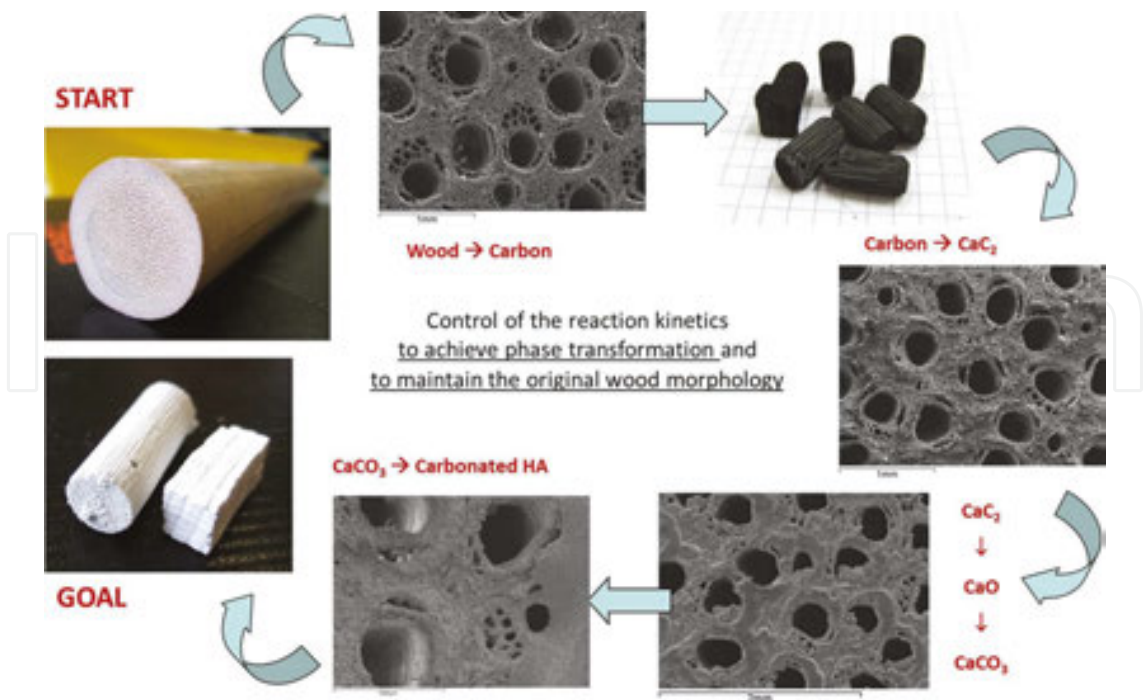


Figure 8. Biomorphic transformation process generating hydroxyapatite scaffolds.

By this process, it was possible to incorporate foreign ions, such as carbonate, in the final consolidated apatite scaffold [43] and to control the chemico-physical features related to the scaffold bioactivity and resorbability, such as the Ca/P ratio and the extent of crystal ordering of the HA phase. Among the existing ligneous sources rattan possesses a structure particularly suitable for bone scaffolding, i.e. a channel-like porosity very close to the Haversian structure with wide pores having diameter adequate for enhanced cell hosting and 3D colonization (**Figure 9**), thus being very promising for the activation of extensive vascularization throughout large volumes.

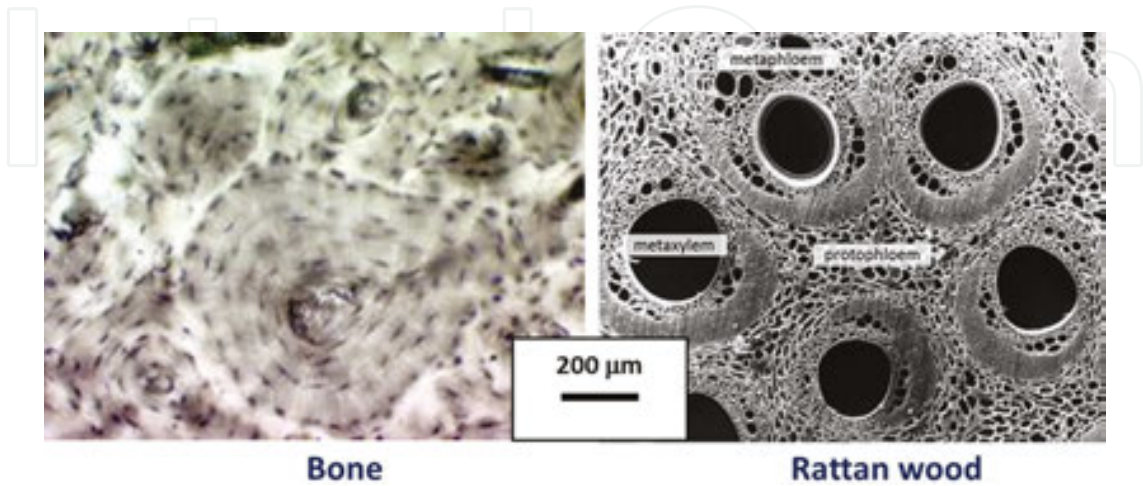


Figure 9. Bone mimicry of rattan wood.

Moreover, the endowment of the bone scaffolds with the channel-like structure of rattan resulted into anisotropic mechanical properties with values in the range of the trabecular bone, that reflect the complex bone response to directional loading. Preliminary biologic tests reported an outstanding affinity with cells, with complete coverage of the scaffolds by well spread cells (i.e. MG63 osteoblast-like cells) after 1 week and enhanced osteogenic ability compared to sintered HA scaffolds (**Figure 10**). Also, preliminary in vivo tests reported extensive bone formation and colonization in femoral bone defects, also showing good morphological organization after one month from implantation [20].

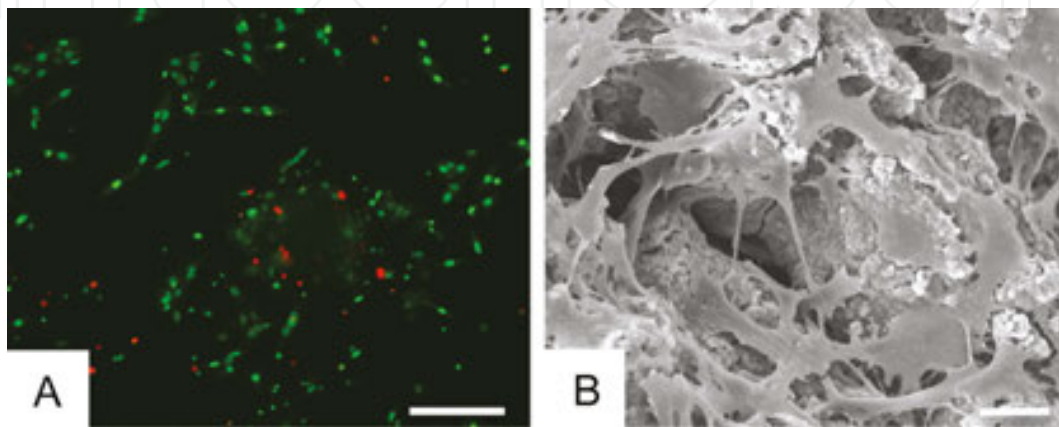


Figure 10. MG63 cells morphology in contact with wood-derived HA scaffold.

The first results obtained with this new type of bone scaffolds are very promising for further development and application into more clinically-relevant models, for assessing the feasibility of regenerating long segmental bone parts. In this respect the exploitation of natural sources as models for generation of new hierarchically organized scaffolds can be considered as a completely new synthesis approach that may open to still unexplored applications in the incoming years.

4. Biocompatible magnetic materials: a new smart, multifunctional tool in nanomedicine

The use of biomimetic scaffolds can be an effective approach for bone tissue regeneration, however the patients' metabolism plays an important role in the regulation of the kinetics and extent of new bone formation. Indeed, metabolic diseases, as well as degenerative conditions induced by aging, can seriously penalize new bone formation and fracture healing. In consideration of the ever increasing ageing of the world population, the occurrence of degenerative diseases is expected to steadily rise in the next decades, thus new therapeutic approaches are strongly required to boost and assist tissue regeneration in patients with reduced endogenous regenerative potential. Tissue engineering approaches and the use of drug delivery systems able to deliver growth factors are two main approaches for enhancing

tissue regeneration. Particularly, a great effort is being dedicated to the development of scaffolds with the ability of controlled biochemical stimulation that should be delivered in temporo-spatially defined fashion [44].

In this respect, recent advances in material science suggest that the use of weak magnetic fields is appealing as remote signalling for non-invasive controlling and *on demand* activation of biomedical devices in vivo [1]. The use of magnetic materials in nanomedicine is thus raising a steadily growing interest, as they can open to new personalized applications including cancer therapy by hyperthermia, magnetic resonance imaging, and other diagnostic approaches based on the guiding of such particles to specific targeted areas in vivo and their use as nano-probes [45–52]. A serious drawback in the use of magnetic materials in nanomedicine is their long term cytotoxicity [53, 54]. Intense effort is therefore dedicated to engineering SPIONs (i.e. superparamagnetic iron oxide nanoparticles) with surface treatments to achieve enhanced biocompatibility and affinity with cells [55–57]. A significant advance can be the development of magnetic materials with intrinsic biocompatibility and resorbability. In this respect, it has been shown that the doping of the apatite lattice with $\text{Fe}^{2+}/\text{Fe}^{3+}$ ions in specific calcium sites yields a new phase with intrinsic paramagnetic behaviour (FeHA) [58]. By virtue of its chemical composition very close to the one of mineral bone, FeHA is characterised by excellent biocompatibility, as also confirmed by in vitro studies revealing that FeHA nanoparticles do not reduce cell viability and at the same time enhance cell proliferation compared to undoped HA particles [59]. Moreover, a pilot animal study of bone repair (a rabbit critical bone defect model) demonstrated the in vivo biocompatibility and biodegradability of FeHA [59]. The achievement of biocompatible nano-biomaterials with magnetic properties opens new perspectives in regenerative medicine. Particularly, the development of bone scaffolds with the ability of remote magnetic activation is now an emerging concept in regenerative medicine [60], since it has been demonstrated that weak magnetic or pulsed electromagnetic fields are effective in promoting bone fracture healing, spinal fusion, and bone ingrowth in various animal models [61–65]. However, the incorporation of FeHA phase into ceramic bone scaffolds is made difficult by the need of consolidating green ceramic bodies by high temperature treatments provoking lattice destabilization and loss of magnetic properties [59]. In this respect FeHA can also be synthesized by suitable modification of the biomineralization process to induce heterogeneous nucleation of FeHA nanophase on Type I collagen [66]. This method yielded biomimetic hybrid scaffolds with paramagnetic ability and mineralization extent that could be tailored from cartilage to bone-like level. The presence of a mineral phase with bone-like features and ability to be activated by remote magnetic signal make this new biomaterial very promising to boost regeneration of extended bone and osteochondral regions, even in patients with reduced endogenous regenerative potential [67–70].

Besides, the use of biocompatible magnetic materials can open to further, different approaches for enhanced bone regeneration. It is accepted that a key limiting factor in the regeneration of extended bone defects is the inability of cells to self-propagate in the inner part of the scaffold and to establish new bone and vascular tissue [20]. Recent progresses show that it is possible to locally guide the migration of magnetic nanoparticles and nanoparticle-labelled cells through the use of an externally applied magnetic field gradient [71]. In this respect FeHA

nanoparticles can be easily incorporated into cells by endocytosis, thus obtaining “magnetic cells” without negatively affecting cell behavior (e.g. proliferation, morphology, differentiation). Through the application of an external magnetic field of low intensity, these cells can be guided within a scaffold, in order to have faster and more selective seeding for tissue engineering application (**Figure 11**).

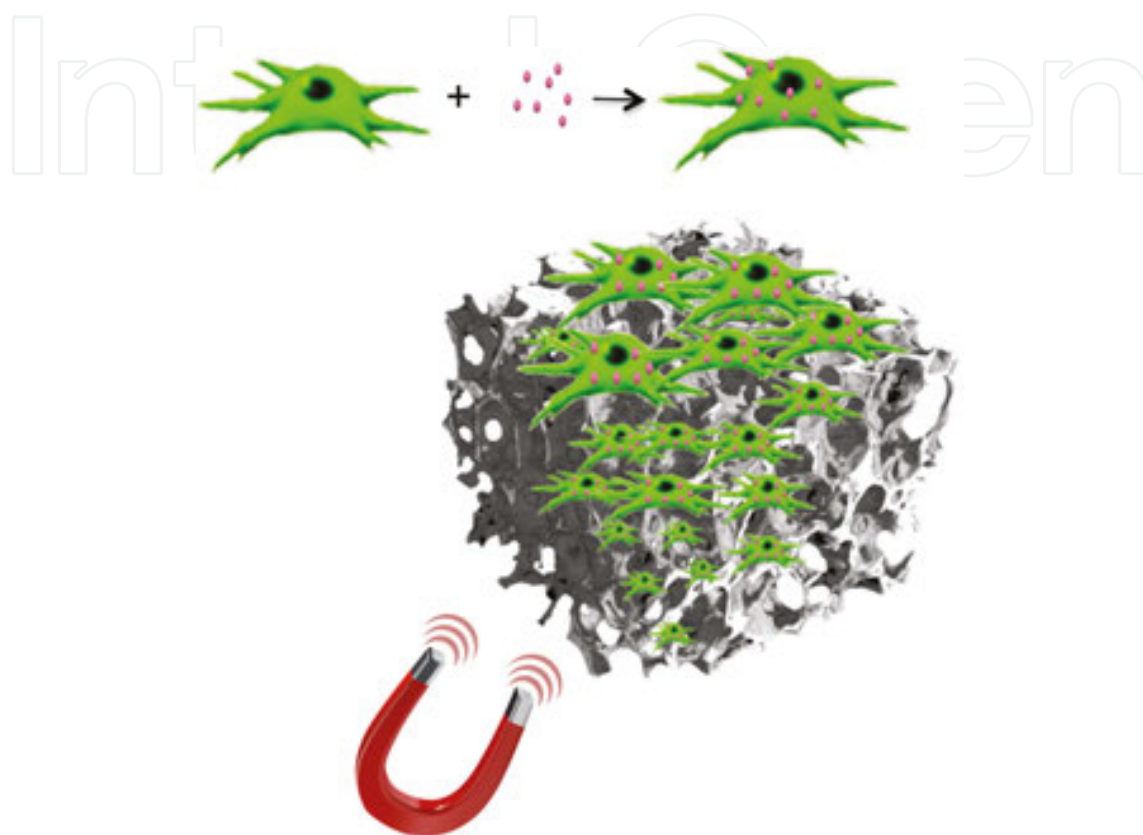


Figure 11. Scheme of magnetic guiding enabling enhanced scaffold colonization.

Biocompatible magnetic media can also be associated to polymeric or hybrid carriers to achieve new smart drug delivery systems with the ability of magnetic activation [72–77].

Hollow micro- and nano-spheres with controlled size and magnetization level, made of polycaprolacton coated with adequate amounts of FeHA displayed dose-dependent biocompatibility towards bone marrow mesenchymal stem cells, thus highlighting the positive effect of the mineralization extent on cell behaviour [78–80]. These carriers could be developed as magnetically-responsive drug delivery systems with activation and delivery kinetics modulated by phenomena of magnetoshaking or hyperthermia [81]. To explore these new approaches for controlled drug delivery, careful investigation is needed to investigate the most suitable conditions, by means of intensity and frequency of alternated magnetic fields that shall provide the energy needed for the release of the linked bioactive molecules. Therefore, in the incoming years further development of this approach may represent a new tool enabling the release of different chemical species under defined temporo-spatial patterns, thus opening to more advanced and personalized therapies.

5. Conclusions and future perspectives

The incoming decades will experience a growing role of smart biomaterials in therapies for bone regeneration. In this respect, a significant effort to develop nature-inspired synthesis approaches will generate new scaffolds endowed with high mimicry of host tissues and smart functions that will greatly improve the existing therapies and, might also generate new ones that were prevented so far by the inadequateness of the existing biomaterials. On the basis of some existing examples of nature-inspired biomaterials showing effective regenerative ability, and on the increasing effort of material scientists in the synthesis of biomimetic devices, it can be envisaged that significant advances will be reached in the next decade. In this respect, new emerging concepts of fabrication, such as biomineralization or biomorphic transformation, will overcome the limitations of current manufacturing techniques that, particularly in the case of ceramics, are not able to provide highly organized structures with details defined at the micron size. In this respect, due to the innumerable examples of natural structures exhibiting smart properties that are not achievable by conventional fabrication approaches, there are virtually no limits to the potential applications of biomorphic materials in various high-impact fields other than the biomedical one. Besides, the attainment of smart functionalization is another key topic that is engaging a significant part of the biomaterials researchers, particularly due to the increasing need to overcome the systemic drug administration and to provide more effectiveness and targeting to the existing therapies. In this respect, as safety, effectiveness, and targeting are key objectives for real applicability in nanomedicine, the use of magnetic stimulation can be considered as a promising concept that is raising ever increasing interest among scientists and will probably experience extended diffusion in the incoming years.

Acknowledgements

The research leading to these results has received funding from the European Union 7th Framework Programme under Grant agreement no. 310637 (SMILEY), the PNR-CNR Aging Program 2012–2014 and the Flagship project Nanomax (PNR-CNR 2011–2013).

Author details

Simone Sprio*, Monica Sandri, Michele Iafisco, Silvia Panseri, Monica Montesi, Andrea Ruffini, Alessio Adamiano, Alberto Ballardini and Anna Tampieri

*Address all correspondence to: simone.sprio@istec.cnr.it

Institute of Science and Technology for Ceramics, National Research Council, Faenza, Italy

References

- [1] Tampieri A, Sprio S, Sandri M, Valentini F. Mimicking natural bio-mineralization processes: A new tool for osteo-chondral scaffold development. *Trends in Biotech.* 2011; 29(10): 526–535. DOI: 10.1016/j.tibtech.2011.04.011.
- [2] Mann S. *Bio-Mineralization: Principles and Concepts in Bioinorganic Materials Chemistry*. Oxford University Press, Great Clarendon Street, Oxford, U.K. 2001. ISBN: 0198508824.
- [3] LeGeros RZ. *Calcium phosphate in oral biology and medicine*. Karger, Basel, Switzerland; 1991. DOI: 10.1159/000419232.
- [4] Boskey AL. Mineralization, structure and function of bone. In: Seibel MJ, Robins SP, Bilezikian JP, editors. *Dynamics of Bone and Cartilage Metabolism: Principles and Clinical Applications*. Academic Press, Theobald's road, London, U.K. 2006. pp. 201–212. ISBN: 9780120885626.
- [5] Bigi A, Foresti E, Gregorini R, Ripamonti A, Roveri N, Shah JS. The role of magnesium on the structure of biological apatite. *Calcif Tissue Int.* 1992; 50: 439–444. DOI: 10.1007/BF00296775.
- [6] Matsko NB, Žnidaršič N, Letofsky-Papst I, Dittrich M, Grogger W, Štrus J, Hofer F. Silicon: The key element in early stages of biocalcification. *J Struct Biol.* 2011; 174: 180–186. DOI: 10.1016/j.jsb.2010.09.025.
- [7] Lowenstam HA, Weiner S. *On Bio-Mineralization*. Oxford University Press, Great Clarendon Street, Oxford, U.K. 1989. ISBN: 9780195049770.
- [8] Jackson SA, Cartwright AG, Lewis D. The morphology of bone-mineral crystals. *Calcif Tissue Res* 1978; 25(1): 217–222. DOI: 10.1007/BF02010772.
- [9] Wagoner Johnson AJ, Herschler BA. A review of the mechanical behavior of CaP and CaP/polymer composites for applications in bone replacement and repair. *Acta Biomater.* 2011; 7(1): 16–30. DOI: 10.1016/j.actbio.2010.07.012.
- [10] Tampieri A, Celotti G, Landi E, Sandri M, Roveri N, Falini G. Biologically inspired synthesis of bone like composite: Self-assembled collagen fibers/hydroxyapatite nanocrystals. *J Biomed Mater Res.* 2003; 67A: 618–625.
- [11] Tampieri A, Sandri M, Landi E, Pressato D, Francioli S, Quarto R, Martin I. Design of graded biomimetic osteochondral composite scaffolds. *Biomaterials.* 2008; 29(26): 3539–3546. DOI: 10.1016/j.biomaterials.2008.05.008.
- [12] Bigi A, Foresti E, Gregorini R, Ripamonti A, Roveri N, Shah JS. The role of magnesium on the structure of biological apatite. *Calcif Tissue Int.* 1992; 50: 439–444. DOI: 10.1007/BF00296775.

- [13] Pietak AM, Reid JW, Stott MJ, Sayer M. Silicon substitution in the calcium phosphate bioceramics. *Biomaterials*. 2007; 28: 4023–4032. DOI: 10.1016/j.biomaterials.2007.05.003.
- [14] Schwarz K. A bound form of Si in glycosaminoglycans and polyuronides. *Proc Nat Acad Sci USA*. 1973; 70: 1608–1612. DOI: 10.1073/pnas.70.5.1608.
- [15] Eppell SJ, Tong WD, Katz JL, Kuhn L, Glimcher MJ. Shape and size of isolated bone mineralites measured using atomic force microscopy. *J Orthop Res*. 2001; 19: 1027–1034.
- [16] Weiner S, Price PA. Disaggregation of bone into crystals. *Calcif Tissue Int*. 1986; 39: 365–375.
- [17] Traub W, Arad T, Weiner S. Three-dimensional ordered distribution of crystals in turkey tendon collagen fibers. *Proc Natl Acad Sci* 1989; 86: 9822–9826.
- [18] Kon E, Delcogliano M, Filardo G, Fini M, Giavaresi G, Francioli S, Martin I, Pressato D, Arcangeli E, Quarto R, Sandri M, Marcacci M. Orderly osteochondral regeneration in a sheep model using a novel nano-composite multilayered biomaterial. *J Orthop Res*. 2010; 28: 116–124. DOI: 10.1002/jor.20958.
- [19] Linde A, Goldberg M. Dentinogenesis. *Crit Rev Oral Biol Med*. 1993; 4(5): 679–728. DOI: 10.1007/s00418-009-0556-6.
- [20] Sprio S, Sandri M, Iafisco M, Panseri S, Filardo G, Kon E, Marcacci M, Tampieri A. Composite biomedical foams for engineering bone tissue. In: Netti PA, editor. *Bio-medical Foams for Tissue Engineering Applications*. Cambridge (UK): Woodhead Publishing Limited; 2014. pp. 249–280. ISBN: 9780857096968.
- [21] Giannoudis PV, Atkins R. Management of long-bone non-unions. *Injury*. 2007; 38(Suppl. 2): S1–S2. DOI: 10.1016/j.injury.2011.03.036.
- [22] Kanakaris NK, Giannoudis PV. The health economics of the treatment of long-bone non-unions. *Injury*. 2007; 38(Suppl. 2): S77. DOI: 10.1016/S0020-1383(07)80012-X.
- [23] Stevenson S. The immune response to osteochondral allografts in dogs. *J Bone Joint Surg Am*. 1987; 69(4): 573–582. DOI: 10.1007/s11999-008-0444-8.
- [24] Lord CF, Gebhardt MC, Tomford WW, Mankin HJ. Infection in bone allografts. Incidence, nature, and treatment. *J Bone Joint Surg Am*. 1988; 70(3): 369–376.
- [25] Mankin HJ, Gebhardt MC, Tomford WW. The use of frozen cadaveric allografts in the management of patients with bone tumors of the extremities. *Orthop Clin North Am*. 1987; 18(2): 275–289.
- [26] Alman BA, De Bari A, Krajchich JI. Massive allografts in the treatment of osteosarcoma and Ewing sarcoma in children and adolescents. *J Bone Joint Surg Am* 1995; 77(1): 54–64. DOI: 10.1007/s11999-010-1362-0.
- [27] Berrey BH Jr, Lord CF, Gebhardt MC, Mankin HJ. Fractures of allografts. Frequency, treatment, and end-results. *J Bone Joint Surg Am*. 1990; 72(6): 825–833.

- [28] Daculsi G. Biphasic calcium phosphate concept applied to artificial bone, implant coating and injectable bone substitute. *Biomaterials*. 1998; 19: 1473–1478. DOI: 10.1016/S0142-9612(98)00061-1.
- [29] Kessler S, Mayr-Wohlfart U, Ignatius A, Puhl W, Claes L, Günther KP. The impact of bone morphogenetic protein-2 (BMP-2), vascular endothelial growth factor (VEGF) and basic fibroblast growth factor (b-FGF) on the osteointegration, the degradation and biomechanical properties of a synthetic bone substitute. *Z Orthop*. 2003; 141: 472–480. DOI: 10.1055/s-2003-41569.
- [30] Connor TB, O'Kelly KU. Fabrication and characterization of a porous multidomain hydroxyapatite scaffold for bone tissue engineering investigations. *J Biomed Mater Res Part B: Appl Biomater*. 2010; 93B: 459–467. DOI: 10.1002/jbm.b.31603.
- [31] Babis GC, Soucacos PN. Bone scaffolds: The role of mechanical stability and instrumentation. *Int J Care Injured*. 2005; 36S: S38–S44. DOI: 10.1016/j.injury.2005.10.009.
- [32] Sprio S, Ruffini A, Valentini F, D'Alessandro T, Sandri M, Panseri S, Tampieri A. Biomimesis and biomorphic transformations: New concepts applied to bone regeneration. *J Biotechnol*. 2011; 156(4): 347–355. DOI: 10.1016/j.jbiotec.2011.07.034.
- [33] Ingber DE. The architecture of life. *Sci Am*. 1998; 278(1): 48–57.
- [34] Li J, Han D, Zhao Y-P. Kinetic behaviour of the cells touching substrate: The interfacial stiffness guides cell spreading. *Sci Rep*. 2014; 4: 3910. DOI: 10.1038/srep03910.
- [35] Cameron AR, Frith JE, Cooper-White JJ. The influence of substrate creep on mesenchymal stem cell behaviour and phenotype. *Biomaterials*. 2011; 32(26): 5979–5993. DOI: 10.1016/j.biomaterials.2011.04.003.
- [36] Wells RG. The role of matrix stiffness in regulating cell behavior. *Hepatol*. 2008; 47: 1394–1400. DOI: 10.1002/hep.22193.
- [37] Pavalko FM, Norvell SM, Burr DB, Turner CH, Duncan RL, Bidwell JP. A model for mechanotransduction in bone cells: The load-bearing mechanosomes. *J Cell Biochem*. 2003; 88: 104–112. DOI: 10.1002/jcb.10284.
- [38] Sikavitsas VI. Biomaterials and bone mechanotransduction. *Biomaterials*. 2001; 22: 2581–2593. DOI: 10.1016/S0142-9612(01)00002-3.
- [39] Bilezikian JP, Raisz LG, Rodan GA. *Principles of Bone Biology*. 3rd ed., vol. 1. San Diego: Academic Press; 2002. ISBN: 0-12-098652-3.
- [40] Fratzl P, Weinkamer R. Nature's hierarchical materials. *Prog Mater Sci*. 2007; 52: 1263–1334. DOI: 10.1016/j.pmatsci.2007.06.001.
- [41] Wegst UGK, Ashby MF. The mechanical efficiency of natural materials. *Philos Mag*. 2004; 84: 2167–2181. DOI: 10.1080/14786430410001680935.
- [42] Gibson EJ. Wood: A natural fibre reinforced composite. *Met Mater*. 1992; 6: 333–336.

- [43] Tampieri A, Sprio S, Ruffini A, Celotti G, Lesci IG, Roveri N. From wood to bone: Multi-step process to convert wood hierarchical structures into biomimetic hydroxyapatite scaffolds for bone tissue engineering. *J Mater Chem.* 2009; 19(28): 4973–4980. DOI: 10.1039/b900333a.
- [44] Minardi S, Pandolfi L, Taraballi F, De Rosa E, Yazdi IK, Liu X, Ferrari M, Tasciotti E. PLGA-mesoporous silicon microspheres for the in vivo controlled temporospatial delivery of proteins. *ACS Appl Mater Interfaces.* 2015; 7(30): 16364–16373. DOI: 10.1021/acsami.5b03464.
- [45] Pankhurst QA, Connolly J, Jones SK, Dobson J. Applications of magnetic nanoparticles in biomedicine. *J Phys D Appl Phys.* 2003; 36: R167–81. DOI: 10.1088/0022-3727/36/13/201.
- [46] Ramchand CN, Priyadarshini P, Kopcansky P, Metha RV. Applications of magnetic fluids in medicine and biotechnology. *Indian J Pure Appl Phys.* 2001; 39: 683–689. DOI: 10.1016/j.jmmm.2006.10.1184.
- [47] Meng J, Xiao B, Zhang Y, Liu J, Xue H, Lei J, Kong H, Huang Y, Jin Z, Gu N, Xu H. Super-paramagnetic responsive nanofibrous scaffolds under static magnetic field enhance osteogenesis for bone repair in vivo. *Sci Rep.* 2013; 3: 2655–2662. DOI: 10.1038/srep02655.
- [48] Panseri S, Russo A, Sartori M, Giavaresi G, Sandri M, Fini M, Maltarello MC, Shelyakova T, Ortolani A, Visani A, Dediu V, Tampieri A, Marcacci M. Modifying bone scaffold architecture in vivo with permanent magnets to facilitate fixation of magnetic scaffolds. *Bone.* 2013; 56: 432–439. DOI: 10.1016/j.bone.2013.07.015.
- [49] Shan D, Shi Y, Duan S, Wei Y, Cai Q, Yang X. Electrospun magnetic poly(L-lactide) (PLLA) nanofibers by incorporating PLLA-stabilized Fe₃O₄ nanoparticles. *Mater Sci Eng C.* 2013; 33: 3498–3505. DOI: 10.1016/j.msec.2013.04.040.
- [50] Meng J, Zhang Y, Qi X, Kong H, Wang C, Xu Z, Xie S, Gu N, Xu H. Paramagnetic nanofibrous composite films enhance the osteogenic responses of pre-osteoblast cells. *Nanoscale.* 2010; 2: 2565–2569. DOI: 10.1039/c0nr00178c.
- [51] Hou R, Zhang G, Du G, Zhan D, Cong Y, Cheng Y, Fu J. Magnetic nanohydroxyapatite/PVA composite hydrogels for promoted osteoblast adhesion and proliferation. *Colloids Surf B.* 2013; 103: 318–325. DOI: 10.1016/j.colsurfb.2012.10.067.
- [52] Mertens ME, Hermann A, Bühren A, Olde-Damink L, Möckel D, Gremse F, Ehling J, Kiessling F, Lammers T. Iron oxide-labeled collagen scaffolds for non-invasive MR imaging in tissue engineering. *Adv Funct Mater.* 2014; 24: 754–762. DOI: 10.1002/adfm.201301275.
- [53] Lewinski N, Colvin V, Drezek R. Cytotoxicity of nanoparticles. *Small.* 2008; 4: 26–49. DOI: 10.1002/smll.200700595.

- [54] Singh N, Jenking GJS, Asadi R, Doak SH. Potential toxicity of superparamagnetic iron oxide nanoparticles (SPION). *Nano Reviews*. 2010; 1: 53–58. DOI: 10.3402/nano.v1i0.5358.
- [55] Fresnais J, Yan M, Courtois J, Bostelmann T, Bée A, Berret J-F. Poly(acrylic acid)-coated iron oxide nanoparticles: Quantitative evaluation of the coating properties and applications for the removal of a pollutant dye. *J Coll Interf Sci*. 2013; 395: 24–30. DOI: 10.1016/j.jcis.2012.12.011.
- [56] Castelló J, Gallardo M, Busquets MA, Estelrich J. Chitosan (or alginate)-coated iron oxide nanoparticles: A comparative study. *Coll Surf A: Physicochem Eng Asp*. 2015; 468: 151–158. DOI: 10.1016/j.colsurfa.2014.12.031.
- [57] Yue-Jian C, Juan T, Fei X, Jia-Bi Z, Ning G, Yi-Hua Z, Ye D, Liang G. Synthesis, self-assembly, and characterization of PEG-coated iron oxide nanoparticles as potential MRI contrast agent. *Drug Dev Ind Pharm*. 2010;36(10): 1235–1244. DOI: 10.3109/03639041003710151.
- [58] Tampieri A, D'Alessandro T, Sandri M, Sprio S, Landi E, Bertinetti L, Panseri S, Pepponi G, Goettlicher J, Bañobre-López M, Rivas J. Intrinsic magnetism and hyperthermia in bioactive Fe-doped hydroxyapatite. *Acta Biomater*. 2012; 8(2): 843–851. DOI: 10.1016/j.actbio.2011.09.032.
- [59] Panseri S, Cunha C, D'Alessandro T, Sandri M, Giavaresi G, Marcacci M, Hung CT, Tampieri A. Intrinsically superparamagnetic Fe-hydroxyapatite nanoparticles positively influence osteoblast-like cell behavior. *J Nanobiotechnol*. 2012; 10: 32–42. DOI: 10.1186/1477-3155-10-32.
- [60] Clavijo-Jordan V, Kodibagkar VD, Beeman SC, Hann BD, Bennett KM. Principles and emerging applications of nanomagnetic materials in medicine. *WIREs Nanomed Nanobiotechnol*. 2012; 4: 345–365. DOI: 10.1002/wnan.1169.
- [61] Grace KLR, Revell WJ, Brookes M. The effects of pulsed electromagnetism on fresh fracture healing: Osteochondral repair in the rat femoral groove. *Orthopedics*. 1998; 21: 297–302.
- [62] Glazer PA, Heilmann MR, Lotz JC, Bradford DS. Use of electromagnetic fields in a spinal fusion: A rabbit model. *Spine*. 1997; 22(20): 2351–2356.
- [63] Yan QC, Tomita N, Ikada Y. Effects of static magnetic field on bone formation of rat femurs. *Med Eng Phys*. 1998; 20: 397–402. DOI: 10.1016/S1350-4533(98)00051-4.
- [64] Assiotis A, Sachinis NP, Chalidis BE. Pulsed electromagnetic fields for the treatment of tibial delayed unions and nonunions. A prospective clinical study and review of the literature. *J Orthop Sur Res*. 2012; 7: 24. DOI: 10.1186/1749-799X-7-24.
- [65] Chalidis B, Sachinis N, Assiotis A, Maccauro G. Stimulation of bone formation and fracture healing with pulsed electromagnetic fields: Biologic responses and clinical implications. *Int J Immunopathol Pharmacol*. 2011; 24: 17–20.

- [66] Tampieri A, Iafisco M, Sandri M, Panseri S, Cunha C, Sprio S, Savini E, Uhlarz M, Herrmannsdörfer T. Magnetic bio-inspired hybrid nanostructured collagen-hydroxyapatite scaffolds supporting cell proliferation and tuning regenerative process. *ACS Appl Mater Interf.* 2014; 6(18): 15697–15707. DOI: 10.1021/am5050967.
- [67] Torbet J, Ronziere MC. Magnetic alignment of collagen during self-assembly. *Biochem J.* 1984; 219: 1057–1059.
- [68] Higashi T, Yamagishi A, Takeuchi T, Kawaguchi N, Sagawa S, Onishi S, Date M. Orientation of erythrocytes in a strong static magnetic field. *Blood.* 1993; 82: 1328–1334. DOI: 10.1007/s11626-0030005-0.
- [69] Kotani H, Kawaguchi H, Shimoaka T, Iwasaka M, Ueno S, Ozawa H, Nakamura K, Hoshi K. Strong static magnetic field stimulates bone formation to a definite orientation in vitro and in Vivo. *J Bone Miner Res.* 2002; 17: 1814–1821. DOI: 10.1359/jbmr.2002.17.10.1814.
- [70] Xu H-Y, Gu N. Magnetic responsive scaffolds and magnetic fields in bone repair and regeneration. *Front Mater Sci.* 2014; 8: 20–31. DOI: 10.1007/s11706-014-0232-1.
- [71] Ganguly R, Puri IK. Microfluidic transport in magnetic MEMS and bioMEMS. *WIREs Nanomed Nanobiotechnol.* 2010; 2: 382–399. DOI: 10.1002/wnan.92.
- [72] Veisheh O, Gunn JW, Zhang MQ. Design and fabrication of magnetic nanoparticles for targeted drug delivery and imaging. *Adv Drug Deliv Rev.* 2010; 62: 284–304. DOI: 10.1016/j.addr.2009.11.002.
- [73] Sperling RA, Parak WJ. Surface modification, functionalization and bioconjugation of colloidal inorganic nanoparticles. *Philos Transact A Math Phys Eng Sci.* 2010; 368: 1333–1383. DOI: 10.1098/rsta.2009.0273.
- [74] Yiu HH, McBain SC, Lethbridge ZA, Lees MR, Dobson J. Preparation and characterization of polyethylenimine-coated Fe₃O₄-MCM-48 nanocomposite particles as a novel agent for magnet assisted transfection. *J Biomed Mater Res A.* 2010; 92: 386–392.
- [75] Schellenberger E, Schnorr J, Reutelingsperger C, Ungethum L, Meyer W, Taupitz M, Hamm B. Linking proteins with anionic nanoparticles via protamine: Ultrasmall protein-coupled probes for magnetic resonance imaging of apoptosis. *Small.* 2008; 4: 225–230. DOI: 10.1002/smll.200700847.
- [76] Yu MK, Jeong YY, Park J, Park S, Kim JW, Min JJ, Kim K, Jon S. Drug-loaded superparamagnetic iron oxide nanoparticles for combined cancer imaging and therapy in vivo. *Angew Chem Int Ed Engl.* 2008; 47: 5362–5365. DOI: 10.1002/anie.200800857.
- [77] Dilnawaz F, Singh A, Mohanty C, Sahoo SK. Dual drug loaded superparamagnetic iron oxide nanoparticles for targeted cancer therapy. *Biomaterials.* 2010; 31: 3694–3706. DOI: 10.1016/j.biomaterials.2010.01.057.

- [78] Bañobre-Lopez M, Piñeiro-Redondo Y, De Santis R, Gloria A, Ambrosio L, Tampieri A, Dediu V, Rivas J. Poly(caprolactone) based magnetic scaffolds for bone tissue engineering. *J Appl Phys.* 2011; 109: 07B313. DOI: 10.1063/1.3561149.
- [79] Gloria A, Russo T, D'Amora U, Zeppetelli S, D'Alessandro T, Sandri M, Bañobre-Lopez M, Piñeiro-Redondo Y, Uhlarz M, Tampieri A, Rivas J, Herrmannsdörfer T, Dediu VA, Ambrosio L, De Santis R. Magnetic poly(ϵ -caprolactone)/iron-doped hydroxyapatite nanocomposite substrates for advanced bone tissue engineering. *J R Soc Interf.* 2013; 10: 20120833. DOI: 10.1098/rsif.2012.0833.
- [80] Iafisco M, Sandri M, Panseri S, Delgado-Lopez JM, Gomez-Morales J, Tampieri A. Magnetic bioactive and biodegradable hollow Fe-doped hydroxyapatite coated poly(L-lactic) acid micro-nanospheres. *Chem Mater.* 2013; 25: 2610–2617. DOI: 10.1021/cm4007298.
- [81] Nappini S, Bonini M, Bombelli FB, Pineider F, Sangregorio CL, Baglioi P, Norden B. Controlled drug release under a low frequency magnetic field: Effect of the citrate coating on magnetoliposomes stability. *Soft Matter.* 2011; 7: 1025–1037. DOI: 10.1039/C0SM00789G.