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Review

Current Options and Future Perspectives on Bone Graft and Biomaterials Substitutes for Bone Repair, from Clinical Needs to Advanced Biomaterials Research

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Abstract: The ideal biomaterials substitute for bone repair should possess the following characteristics: provide osteogenic, osteoinductive and osteoconductive properties; stimulate the neoangiogenesis process; absence of antigenic, teratogenic or carcinogenic reactions; avoid the systemic toxicity complications; assure satisfactory support and stability from mechanical properties point of view; hydrophilic nature of the surface properties and good interface with human bone; good handling in clinical condition and ability to be easy sterilized; and able to be supplied in sufficient quantities with reduced costs. Despite years of effort, the perfect bone reconstruction material has not yet been developed; further effort is required to make this objective feasible. The aim of this article is to provide a contemporary and comprehensive overview of the grafting materials that can be applied for the treatment of bone defects by the clinicians from orthopedics surgery, neurosurgery and dentistry, discussing their properties, advantages and disadvantages, and illuminating present and future perspectives in the field of bone graft and biomaterials substitutes for bone repair, from clinical needs to advanced biomaterials research.

Keywords: bone; grafting; advanced biomaterials; biocomposites; nanostructures; in-vivo; in-vitro; laser cutting

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1. Introduction

In recent decades, orthopedics has become one of the most dynamic medical specialties, a fact confirmed by the huge increase in the number of surgical interventions, as well as their complexity. Changes in lifestyle, which has become much more active and exposed to different types of accidents, the increase in life expectancy and, last but not least, significant technological advances are responsible for this advance. This fact leads to special situations, more and more frequent, characterized by different amounts of bone loss, i.e., situations that require the use of bone grafts or substitutes, depending on different circumstances. The used bone grafts can be natural or synthetic, each one with their advantages and disadvantages and special indications as well. The present work tries to review the most frequently used substitutes in order to obtain an overview that will assist choice making for the situations encountered in everyday practice.

Bone healing is a complex process, conditioned by the interaction between physical factors and the biological response. A defining element of the unique bone healing process is that it is not achieved by creating a fibrous scar but by a process of tissue regeneration. Bone healing is of two kinds: direct and indirect.

Direct bone healing can occur only when anatomical reduction of the bone fragments and their rigid fixation is achieved, which means a minimal interfragmentary movement. Bone healing in this case is conducted in a special way, i.e., by contact or gap healing.

Indirect healing is most common in the natural evolution of fractures; it does not require anatomical reduction of the fracture or special stability of the fixation; moreover, the healing speed through this mechanism is improved by micromovements and especially by direct axial loading.

If this process is disturbed by different mechanical or biological issues, the evolution is towards a delay in consolidation or pseudoarthrosis, situations with a profound negative effect on the functionality of the affected bone segment, as well as on the limb of which it is a part [1]. Understanding this mechanism is crucial in taking the best therapeutic decisions in aid of healing as quickly as possible and without sequelae.

Pseudarthrosis is defined as failure to achieve bone healing 9 months after trauma, or lack of clinical and radiological signs of healing progression for 3 months [2]. Other authors consider 6 months after trauma the time of the onset of pseudarthrosis. For this condition, bone grafts, bone substitutes and bioactive factors can enhance the healing process [3].

They are many other pathologic conditions that requires the use of bone grafts or substitutes with or without bioactive factors: open fractures with loss of bone substance; revision arthroplasties with important reduction of local bone capital after prosthetic component loosening; surgical treatment of infections and tumors by resections [4]; and arthrodesis (joint fusion). Filling the spaces created for the purpose of bone axis restoration (osteotomies) is another potential indication for bone grafts.

Bone defects can be segmental or cavitary. For segmental defects, the bone graft must be structured in order to ensure the primary mechanical strength of the replaced segment. In cavitary defects, the graft needs only to fill the cavity, without offering mechanical strength. The morselized, cancellous graft, without any mechanical strength, is much faster integrated than the structured one due to its large trabecular surface area which promotes revascularization and incorporation at the recipient site.

2. Bone Grafting Physiology

The integration of a bone graft is conditioned by three distinct processes: osteoinduction, osteoconduction and osteogenic capacities. Depending on the type of graft, these processes may interfere or may not be present one or another.

Osteoconduction is the process of the ingrowth of the host capillaries, connective tissue and mesenchymal stem cells (MSCs) in an implanted scaffold with a structure similar to that of host bone. Consequently to this ingrowth process, the graft is incorporated into the host's bone [4]. There are different models of this process according to the type of implanted scaffold. For fresh bone autograft, osteoconduction is facilitated by associated osteoinduction. Allograft is treated mechanically (removal of soft tissues), chemically (ethanol) and physically (gamma radiation) in order to sterilize it, so any osteogenic and osteoinductive properties are compromised. For this kind of graft, osteoconduction is the main process of integration, similar to synthetic bone substitutes which lack osteoinductive factors as well [5]. The difference between nonviable biologic scaffolds and synthetic structures is about the speed of integration, which is higher for the first ones.

Osteoinduction represents the recruiting process of the MSCs from the host in order to differentiate into chondroblasts and osteoblasts, which produce new bone by endosteal ossification. Osteoinduction is initiated and controlled by exogenous growth-factors, natural proteins or hormones that stimulate cellular differentiation, proliferation and growth [6]. The most important are -2, -4 and -7 bone morphogenetic proteins (BMP); platelet derived growth factor (PDGF); interleukins; fibroblast growth factors (FGF); granulocyte-macrophage colony-stimulating factors; and vascular endothelial growth factor (VEGF) [7].

Osteogenic capabilities of bone grafts are their ability to synthesize new bone by donor cells from either the host or graft donor; the cells involved are MSCs, osteoblasts and osteocytes. Only fresh autologous grafts or allografts and bone marrow have this

property [6]. Cancellous bone has more osteogenic elements compared with cortical bone, so the osteogenic capabilities are higher.

Bone graft incorporation has two phases: the first phase is represented by the formation of hematoma at the level of the graft–host tissue interface, and the release of inflammatory factors (cytokines and growth factors) at this level, responsible for recruitments of MSCs and macrophages; the second stage is represented by inflammatory processes with the development of fibrovascular tissue. Due to their structural difference and the vascularization of cancellous and cortical bone, the speed and the amount of integration is can differ.

For cancellous autograft, the necrotic graft tissue is slowly removed by the macrophages, a neovascularization is developed, and the osteoblasts derived from MSCs are aligned at the host graft interface, producing osteoid. After a complex process that takes 6 to 12 months, this osteoid is mineralized, which generates new bone [6]. For cancellous allograft, the inflammatory response of the host produces a fibrous layer around the graft, making it more challenging for the surrounding bone host to deposit new osteoid and bone at this level [7].

The mechanism of cortical bone incorporation, mediated predominantly by osteoclasts as opposed to osteoblasts, is defined as creeping substitution. This process of almost complete resorption of the graft, with simultaneous deposition of new, viable bone, begins at the graft–host tissue interface, progressing along the long axis of the cortical graft. It is defined by a quick loss of mechanical strength of the graft in the initial period of resorption and a long period (years) before completion [8].

3. Characteristics of an Ideal Bone Grafting Material

An ideal bone graft has all three of the aforementioned properties (osteoinduction, osteoconduction and osteogenic capabilities) as well as being easy to harvest and available in the desired quantity, with minimal risks for infectious disease transmission and low cost.

The autograft has all bioactive properties, being the easiest to incorporate and infectious risk free, but is associated with significative donor site morbidity and is available in a limited quantity.

Vascularized bone graft is a form of bone transplant, in which both the arteries and veins of the transplanted bone segment are anastomosed to a nearby host. In this kind of graft, all osteocytes and osteoprogenitor cells are preserved and graft incorporation is realized by primary or secondary bone healing, not by creeping substitution like for a fracture [9]. The main advantage of this graft type is the maintaining of bone strength during the entire integration process [10].

Allografts are harvested from living donors or (most of the time) from cadavers. The advantages of this kind of graft are related to the easiness of harvesting and to the disposable quantity and forms (structural or morselized). Graft processing decreases the immune response and removes substances that may transmit different diseases. On the other hand, this process, especially gamma radiation, affects the mechanical properties of the graft by polypeptide chain splitting and water molecule radiolysis. At the same time, its ability to stimulate bone healing by osteogenic and osteoinductive properties are compromised due to the destruction of all cellular elements [11]. Demineralized bone matrix (DBM) is a form of processed allograft consisting of collagens, non-collagenous proteins, BMPs and other growth factors which are responsible for osteoinduction and osteoconduction. The osteoinductive properties of DBM are greater than those of allografts. Improved osteoconductive properties can be obtained by mixing DBM with bone chips [8].

Synthetic calcium salt-based bone substitutes need to be similar in structure and strength to human bone. They are strictly osteoconductive, so the role in bone healing is limited. Adding collagen, growth factors and even MSCs to these synthetic substitutes may add osteoinductive or even osteogenic properties to these materials. The advantages are related to the quantity, different forms (powders, pellets, blocks or coatings on implants), lack of risk of infection and availability. Bone tissue engineering and three-dimensional

printing are generating promising products which could provide new perspectives in the future.

Trabecular metal is a 3D-processed tantalum with excellent biocompatibility and resistance to corrosion. It is structurally similar to cancellous bone with very high porosity [12]. Due to the high friction coefficient, the primary stability of this kind of metal is very high, which makes it widely used in revision arthroplasties with large structural bone defects.

4. Bone Grafts and Substitutes for Bone Repair

There is a wide spectrum of natural and synthetic materials used for bone repair (Table 1) whose chemical composition is based on calcium phosphate. Depending on the provider, the delivery form and corresponding properties allow medical doctors to use them in different applications.

Table 1. Current major commercial products for bone repair.

Product (Commercial)	Company	Composition	Delivery Form	Properties
Xenograft	Bio-oss	Chemically and thermally treated cancellous bovine bone	small or large granules	Good mechanical support; osteoconduction facilitates the development of new blood vessels
Accufill	Zimmer	Amorphous calcium phosphate (ACP) and dicalcium phosphate dihydrate (DCPD)	Syringe filled with flowable material	Undergoes cell-mediated remodeling into natural bone; physical properties comparable to cancellous bone
HydroSet	Stryker	Tetra-calcium phosphate (TeCP)	Syringe filled with flowable material	Osteoconductive and osteointegrative properties
Vitoss	Stryker	Highly porous (up to 90%) beta tricalcium –phosphate (β-TCP)	Foam strips Foam pack Granules	Regeneration of bone by providing a scaffold for bone remodeling that allows cell penetration and attachment, as opposed to creeping substitution
Actifuse	ApaTech Ltd.	Silicate-substituted calcium phosphate (Si-CaP)	Granules Syringe filled with flowable material	Osteoconductive/osteostimulative silicate
Ceraform	Teknimed	Hydroxyapatite HA and beta tricalcium phosphate (β-TCP)	Granules Preformed shapes	Osteoconductive Resorbable after 2 years
Surgibon	Surgival	Hydroxyapatite HA and beta tricalcium phosphate (β-TCP)	Granules Preformed shapes	Osteoconductive; resorbable
ChronOs	DePuy Synthes	Porous beta tricalcium –phosphate (β-TCP) calcium-phosphorus	Granules Preformed shapes	Osteoconductive; resorbable
NovaBone	Novabone	Sodium–silicate (Bioglass) particles mixed with a synthetic binder	Putty Granules	Osteoconductive; osteoinductive
Hydroxyapatite	Fluidinova	mineral with formula $Ca_5(PO_4)_3OH$ containing calcium and phosphoric acid	Powders Clocks Beads	Osteointegrative; osteoconductive; its porous structure resembles native bone

4.1. Natural Bone Grafts and Substitute Materials

Materials of natural origin are defined as those materials that have been derived from a living source without modification. These materials can be divided into four categories: autologous materials, from the same individual—autografts; homologous materials, from another individual of the same species—allografts; heterologous materials from another species—xerographs and phytogenic materials [13].

4.1.1. Autografts

Autografts are considered the "gold standard" among the different materials for bone augmentation [14,15]. This status is due to their osteogenic character, which keeps bone cell structures from the harvesting site alive [16], but also to their osteoinductive properties which favor the differentiation of mesenchymal stem cells in osteoblasts due to their growth factors content [17,18]. Having an identical origin with the affected tissue means that the

possibility of an unwanted immune rejection reaction is eliminated, achieving a success rate of over 95% [19,20].

The most frequent harvesting site for autografts is the iliac crest; from this region, both cortical and spongious graft can be harvested, depending on the necessities. On the other hand, the obtained bone can be arranged to adapt as best as possible to the receiving site. The other sites are distal femur (especially for a spongious graft), the peroneal shaft for structural graft, ribs and distal radius. In dentistry, autologous augmentation materials are usually obtained from the same individual, such as the mandibular symphysis, mandibular branch and external oblique ridge, but also from other donor places such as the iliac crest or distal area of the ulna due to good cortical and spongious bone resources [21]. Autograft bone harvested from the mandibular branch presents low risks compared to other areas of the oral cavity. It must be taken into account that this harvest may endanger the inferior alveolar nerve. Tissue harvesting from the mandibular branch is suitable to use when the area of the receptor is less than 4 mm thick and extends to at most four teeth [22].

Even though there are also other augmentation materials commonly used to manage bone defects, block autologous grafts are still commonly used in more complex oral augmentation procedures, as very few other augmentation materials can produce a volume of new bone tissue similar to those obtained after the use of autografts. This is due to the fact that these autogenous bone blocks improve the repaired bone structure both in terms of volume and quality, favoring the use of implants with a larger diameter that facilitate the proper distribution of forces [23–25].

Moreover, in orthopedic treatments, autografts are considered the "gold standard". Cancellous autografts are the most frequently used as they bring to the receiving site mesenchymal stem cells which provide the capacity to stimulate the formation of new tissue in the affected area [26]. Cortical autografts offer an integral and rigid structure with special mechanical properties With a reamer–irrigator–aspirator system, the autologous material can be obtained directly from an intramedullary canal of long bones [27,28].

A series of disadvantages, such as the morbidity of the harvesting place and the reduced volume of tissue that can be obtained, limit the autografts used both in orthopedics and dentistry [29]. The second surgical trauma frequently has an increased morbidity, sometimes even affecting the patient's general condition, when it involves large tissue structures to be collected. Reduced bone supply is mentioned when harvesting areas are selected from the oral cavity, which also frequently correlates with local problems related to healing after the surgical act [30,31].

4.1.2. Allografts

Allografts are natural materials that come from an individual of the same species and can be obtained from a compatible living donor or from cadaveric bone sources. Allograft materials can be prepared in three main forms—fresh, frozen or freeze-dried [32]. Fresh and frozen homologous materials have superior osteoinductive properties but are rarely used today due to the increased risk of a host immune response, limited vitality and increased risk of disease transmission [33].

Allografts are commonly used in the United States, being preferred by orthopedic surgeons, and there are currently four bone tissue banks that deal with the procurement and processing of osteochondral tissue [34]. The disadvantages of using allografts are generally reduced by lyophilization but also by other methods of tissue processing, such as mechanical debridement, ultrasonic washing and especially sterilization using gamma radiation [35,36].

In Europe, increased regulatory restrictions on the use of materials from other people have led to a shift in the frequency of use from these materials to synthetic augmentation materials [37].

Allografts have good histocompatibility and are available in various forms, from whole bone segments, cortical–spongious segments and cortical pieces, to small pieces in

the form of bone chips, powder and demineralized bone matrices. They can be produced in custom forms to meet the requirements of the receiving sites [38].

Homologous bone blocks from bone banks have been commonly used to rehabilitate bone supply in cases of severe atrophy of the alveolar process; they allow a sufficient volume of bone to be obtained for implant placement. Although their use compared to autologous materials avoids the existence of a second operating field, the integration time to be used in implant surgery exceeds 12 months [39].

The use of fragmented bone in the form of small pieces of spongy or cortical bone is indicated with encouraging results due to the increased osteoconductive potential, especially in the case of larger defects in the posterior maxillary area that require performing operations to lift the sinus membrane. These small fragments of spongy and cortical bone are usually used in a mixture with each other or with other categories of augmentation materials due to the increased risk of resorption of the spongy bone [40]. Demineralized bone matrix (DBM) is a decalcified product for which obtaining an acidic solution is used to remove mineral components, leaving behind collagen, other proteins, bone morphogenic proteins (BMP), variable percentages of calcium phosphate and a small percentage of cell debris.

After decalcification, BMPs are released from the surrounding mineral structure and can fully exercise their osteoinductive potential, while the remaining collagen proteins in the matrix can provide a 3D configuration for the growth of host tissue capillaries, perivascular tissue and osteoprogenitor cells. In the meantime, the original cells and any bacteria in the allogeneic bone are removed, which could reduce the risk of immune rejection and infection [41,42].

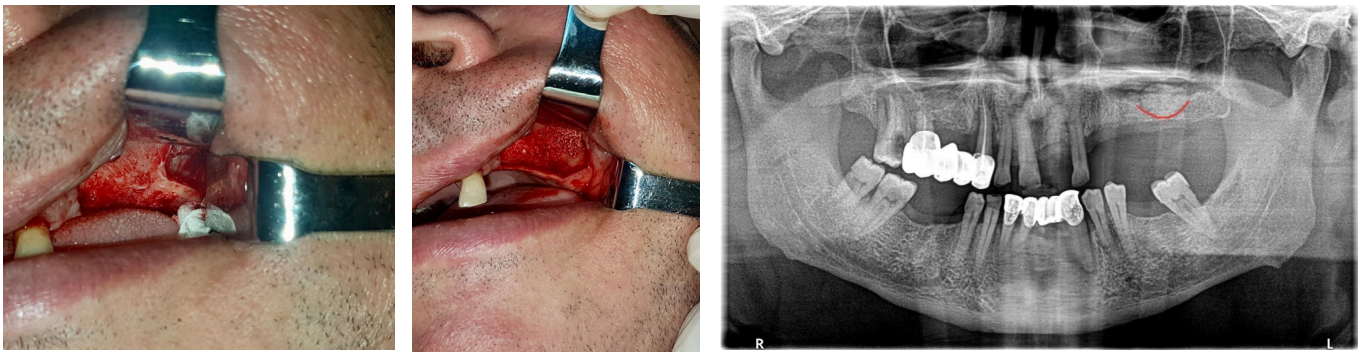
The release of BMP-type osteoinductive growth factors have cause demineralized bone tissue allografts to be considered a gold standard in periodontal regeneration surgical techniques [43,44].

DBM-based augmentation materials have been used successfully both to improve the bone substrate prior to dental implant insertion [45] and for peri-implant repair surgery [46]; however, they are especially used as a supportive material for a number of biologically active substances [47]. Moreover, the increasing use of DBM in dental applications is related to its use as a transport vehicle for a number of added excipients, such as glycerol, starch, hyaluronic acid or saline, which allows for good maneuverability and improved adaptability [48]. Moreover, the use of DBM in the form of putties to preserve the height and thickness of the alveolar processes immediately after tooth extraction provided good clinical results both in terms of accessibility of application and obtaining a bone substrate favorable to implantation only 6 months after extraction [49].

4.1.3. Xenografts

Xenografts are materials that are derived from a genetically different species from the host species.

In dentistry, the most common source of xenograft material is deproteinized bovine bone tissue that is commercially available as Bio-Oss. Bovine bone is treated to produce a hydroxyapatite-based porous material that contains only the inorganic component of bovine bone tissue. The resulting porous structure closely resembles that of human bone and can provide good mechanical support and stimulate the healing process by osteoconduction. This porous structure facilitates the development of new blood vessels through angiogenesis, which underlies the formation of new bone tissue [50]. Bone xenografts of bovine origin have been used extensively in procedures for lifting the floor of the maxillary sinus and obtaining implant support due to their superior stability and low immunogenicity (Figure 1) [51,52].



(**a**) (**b**) (**c**)

Figure 1. Clinical aspects of the lateral bone window created in a direct sinus lift surgical intervention before (**a**) and after augmentation (**b**) with particles from a xenograft material, followed by the post-op radiological examination (**c**) (Courtesy of Dr. Salan Alex, University of Medicine and Pharmacy of Craiova, Romania).

Statistical studies have suggested that the effectiveness of Bio-Oss in stimulating the formation of new bone tissue is similar if not superior to autografts [53,54]. It was also found that the volume and quality of the newly obtained bone tissue allow for the foreseeable simultaneous placement of the implants, thus performing procedures for augmentation of the maxillary sinus in one stage [55]. Clinically, Bio-Oss has been shown to be a valuable bone replacement material, providing good-quality newly formed bone structures and promising rates of long-term survival of inserted dental implants [56]. Of course, there are other commercial products, such as OsteoGraf, Cerabone [57] or Lumina-Porus [58], which have very similar structures and biochemical properties favorable to human bone and which can act as an effective osteoconductive graft [59].

Another xenograft material with promising results in the recent studies is chitosan, which is a polymer derived from crustacean exoskeletons capable of stimulating bone regeneration by providing a structural skeleton that supports osteoblastic activity, mineralization of bone matrix and induction of mesenchymal cell differentiation into osteoblasts [60]. Due to its poor mechanical properties, chitosan is usually combined with other materials in order to obtain the desired properties. However, its structural versatility and hydrophilic surface make this material a viable alternative to autografts and allografts [61].

In the following, some phytogenic materials acting as bone tissue substitute biomaterials that are preferred in the field of dentistry will be presented, given the smaller volume of the bone defect compared to orthopedic applications.

4.1.4. Phytogenic Material (Plant, Coral, Marine Algae)

Phytogenic materials are bone augmentation materials from plant sources. A number of in vitro studies have suggested that plant-derived substances may induce osteogenic differentiation of stem cells and also have angiogenic potential. Moreover, in the field of tissue engineering, plant-derived compounds or plant extracts can be easily incorporated as biomaterials. However, the lack of predictive use, clinical efficacy and quality control are currently the major impediments to their widespread use [62].

Corals, due to their chemical and structural characteristics similar to those of human spongious bone, have a high potential for use as a bone augmentation material, but the clinical data presented so far are ambiguous, with both positive and negative results reported. They have porous structures of different sizes, good compressive strength, low immunogenicity and good bonding with bone tissue, but have relatively low tensile strength, brittleness and a pattern of resorption that does not seem appropriate [63].

The product of Frios AlgiPore is a seaweed hydroxyapatite that has been used clinically as a bone augmentation material since 1988, considered a favorable bone substitute material due to its excellent biocompatibility, low immunogenicity, biodegradability and bone

binding capacity, but which was used more like a space maintainer after tooth extraction to maintain bone volume and avoid deformation of the edentulous ridge [64].

4.1.5. Bone Graft Material Derived from Extracted Tooth Used in Dentistry

Bones, dentin and enamel have a similar composition to hydroxyapatite in the inorganic component as well as type 1 collagen and other proteins in the organic component but with different percentages [65,66]. The potential osteoinduction characteristics of the demineralized dentin matrix have been demonstrated in several studies, as well as the presence of bone-morphogenetic proteins in the human dentin matrix after a demineralization process [67,68].

In 2017, Rijal theorized how the process of dentin demineralization of autologous extracted teeth allows better bone augmentation through the increased availability of bone morphogenetic proteins [69]. Other studies have confirmed the efficacy of a partially demineralized autologous dentin matrix prepared in real time for human bone regeneration clinical procedures [70,71].

Since bone and dentin are mineralized tissues with an almost similar chemical composition, and the morphogenetic proteins in dentin and bone have a major stimulating effect with osteoinductive properties, the regenerative properties of autogenous demineralized dentin matrix (DDM) have been highlighted in several studies. It was found that the dentinal collagen matrix, similar to the bone matrix, can also induce bone formation. They are currently in development, and there have already been a few clinical uses of such demineralized dentin (DDM) matrices, produced from the patient's extracted teeth, to repair alveolar bone defects. The materials obtained are processed and then applied in the form of powders, bone blocks or moldable pastes, and in the future, they could be an option for use as a vehicle for growth factors and stem cells [72,73].

4.2. Synthetic Bone Substitute Materials

4.2.1. Calcium Phosphate Ceramics (CaP Ceramics)

Hydroxyapatite (HA) is the most widely used ceramic material for human bone augmentation because it has a chemical composition and a crystalline structure similar to that of bone. Its bioactivity is related to osteoconductive properties, which allow the apposition and migration of osteoblasts to the surface of the material [74–76]. HA, alone or in combination with an auto-/allo-/xenograft, has been used with adequate clinical success rates in dentistry and orthopedics to support bone regeneration [77]. However, the quality and quantity of newly formed bone following augmentation with synthetic HA only was often considered insufficient. This is why recent research has focused on the production of HA particles with nanometric dimensions, which improves the biomechanical properties and better mimics the composition of natural bone [78,79]. The nanostructure allows a higher surface-to-volume ratio, favoring the adhesion, proliferation and differentiation of osteogenic progenitor cells [80–82].

Tricalcium phosphate (TCP) has two crystallographic forms: α-TCP and β -TCP. β-TCP is a material that has been widely used as a bone replacement material for many years. It has a faster biodegradation and absorption compared to HA due to its low level of Ca/P ratio, but it also has many desirable properties, such as ease of handling, radiopacity that allows monitoring of healing, good osteoconductivity due to macroporosity that promotes fibrovascular growth and osteogenic cell adhesion, good resorbability compared to bovine bone grafts, low immunogenicity and no risk of disease transmission [83]. While the interconnected porous structure of β-TCP allows for improved vascularity, it also causes poor mechanical strength which makes it suitable as a bone substitute only with other materials, especially hydroxyapatite [84].

That is why β-TCP and HA are frequently used in combination today, developing biphasic commercial products (Figure 2).

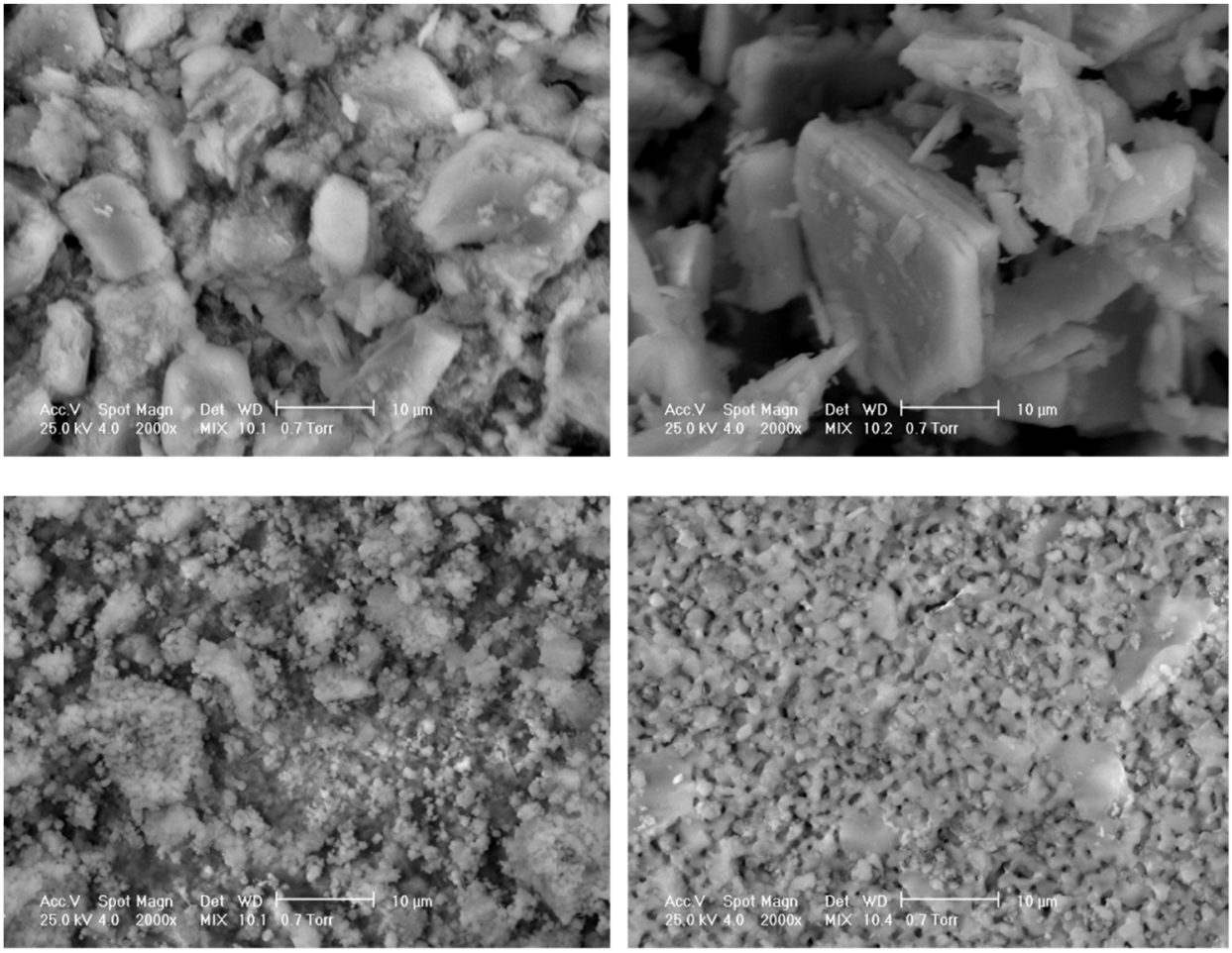


Figure 2. Scanning electron microscopy images of different commercial bone substitutes based on calcium phosphate ceramics (Courtesy of Prof. I.V. Antoniac, University Politehnica of Bucharest, Faculty of Materials Science and Engineering).

Therefore, faster and higher bone regeneration rates were obtained compared to using only HA, but also better mechanical properties than β-TCP used alone. In addition, the resorption and osteoconductivity of these biphasic calcium phosphate ceramics can be controlled by changing the HA/β-TCP ratio (Figures 3 and 4) [85].

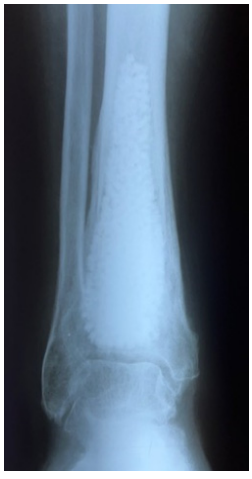


Figure 3. Filling the remaining cavity after intralesional curettage of a tumor with granules of synthetic biphasic ceramic (hydroxyapatite HA and beta tricalcium phosphate β-TCP).

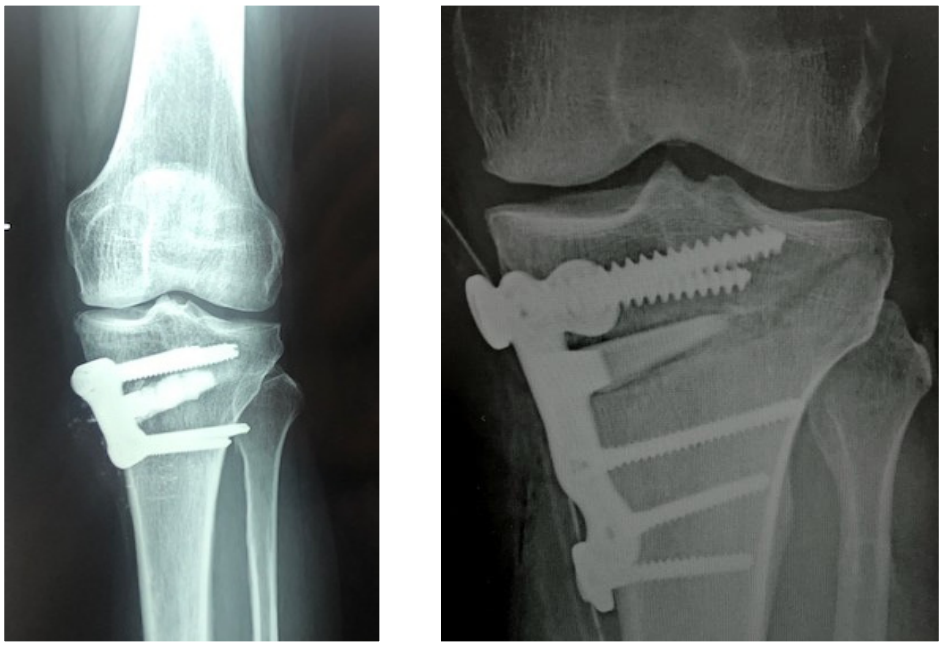


Figure 4. Synthetic biphasic ceramic (hydroxyapatite HA and beta tricalcium phosphate β-TCP) granules (**a**) vs. wedge (**b**) inserted into the high tibial osteotomy gap. The structural graft provides greater stability and strength to the construct.

4.2.2. Calcium Phosphate Cements (CPC)

Calcium phosphate cements are two- or three-component systems that typically contain materials such as TCP and HA. The mixing of the components results in a paste that hardens in situ to form HA nanocrystals at room temperature. The main advantages of these cements include their ability to form a pasty consistency instead of the defect, their ability to replicate the structure and composition of the bone in a repeatable manner, their high biocompatibility, their availability in different forms, for different types of bone defects and properties, and their osteoconductivity. However, they lack a macroporous structure which limits the rate of cell adhesion, fluid exchange and restoration capacity. In addition, the risk of incomplete setting may lead to a local inflammatory reaction. Recent research proposes the development of 3D-printed structures prefabricated from these cements and their improvement through various mechanisms including the addition of viscous binders such as chitosan, gelatin and hyaluronic acid, optimizing the size, distribution and particle shape or optimizing the setting reaction [86,87].

4.2.3. Composite Bone Substitute Materials

Composites are one of the advanced materials used in various kinds of bioapplications. By definition, they represent a mixture between one continuous component, named "matrix", and one or more components (continuous or discontinuous), named "reinforcement"(s). The symbol of a composite is generally represented by the formula: matrix material/reinforcement material/reinforcing content (wt.%). For instance, HA/HDPE/27 means a composite material made of hydroxyapatite as a matrix, which is reinforced by high-density polyethylene of 27% wt.

The choice to select a biocomposite or a biomaterial for a specific application, i.e., bone substitute materials, is made according to functional, technological and (not the least) economic reasons. The properties provided by the conventional biomaterials (metallic or ceramic or polymers) are generated during their processing (by physical–chemical reactions between the chemical elements) but especially by post-processing operations (such as

mechanical, thermal, biofunctional, etc.) involving extra-time and energy consumption costs, respectively.

Graphically, the recommendations to use the composites for BS may be expressed as functions of the bone density and the driven mechanical properties governing the alloplastic composite selection, respectively (Figure 5).

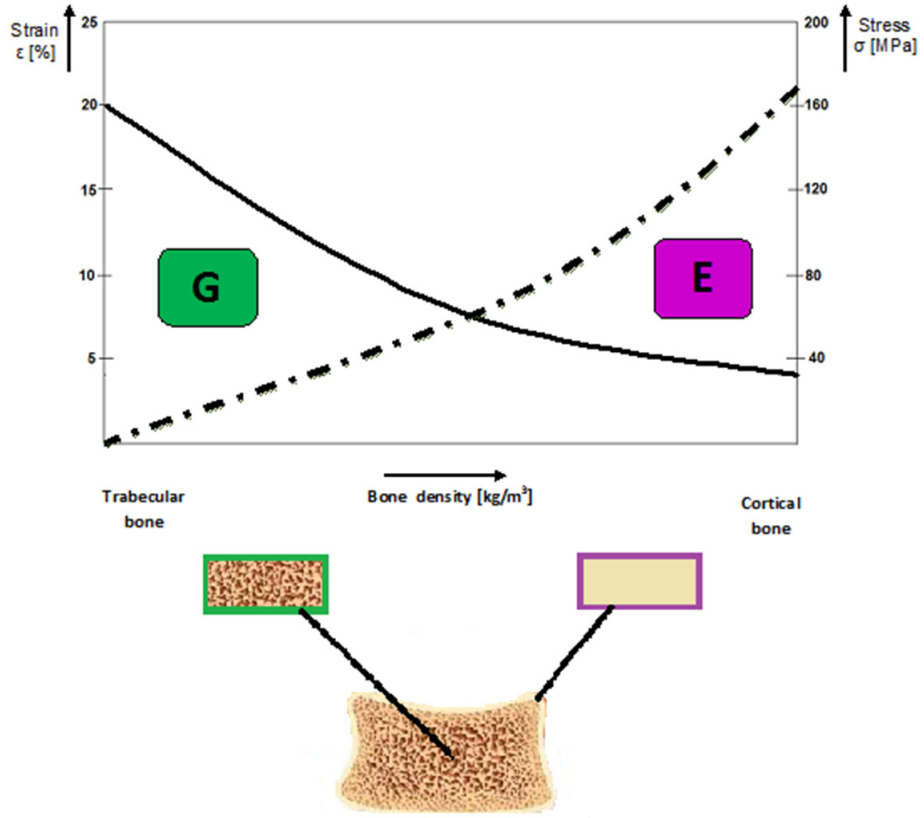


Figure 5. Graphical representation on the general prediction concerning the relationship between the structural features of the bone tissues and the governing mechanical properties for a specific grafting application (Courtesy of Prof. Oana Gingu, University of Craiova, Faculty of Mechanics).

On the other hand, in order to obtain a particular property for a bone substitute material, the matrix and reinforcements are optimally selected from different points of view (structural, functional, market availability, biocompatible and environmentally friendly) to fulfil the most efficient ratio between the expected performance and necessary processing costs. The most important feature in designing a biocomposite is to create a working interface between the matrix and reinforcements, i.e., one able to efficiently transfer the loads (mechanical, thermal, etc.) during its functioning without any components' physical and chemical degradation.

As far as the bone substitutes are concerned, biocomposites provide a wide range of possibilities to significantly improve the properties of the above-mentioned conventional solutions (autologous grafts, allografts and xenografts). Different types of biocomposites are further presented—But also, their selection criteria for a specific bone substitute.

The mechanical properties of HA may be significantly improved by its reinforcing with carbon nanotubes (CNTs, 1–3 wt.%). Most of such applications concern dentistry applications [88]. The obtained properties were superior vs. the pure HA, such as the flexural strength, 83 MPa (1.6 times higher); and the fracture toughness, 2.4 MPa $m^{1/2}$ (2 times higher), because of the special interface geometry between the matrix and the reinforcing element, schematically represented in Figure 6. The higher is the CNT roughness, the more efficient is the interlocking effect towards the HA particles whose properties are provided by the processing technology.

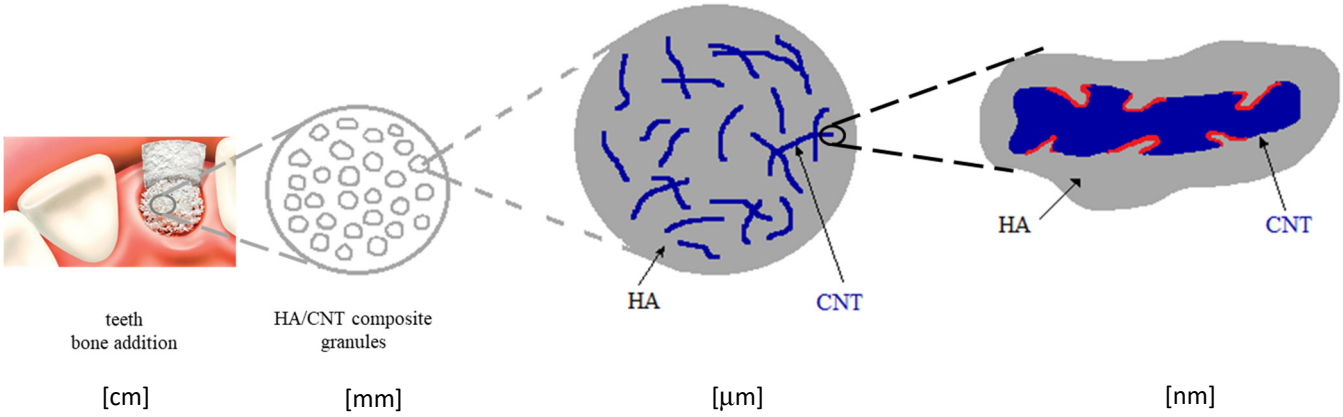


Figure 6. Schematic representation of the micromechanical interlock model of the interface between the HA and CNTs, providing a physical bonding at the interface level (red curves).

The processing of such interface morphology was released by means of the double in situ CNTs synthesis within the HA matrix by chemical vapor deposition (CVD) using Fe catalysts [89].

An alternative approach to reinforce the matrix of a biocomposite is to use precursors, leading to in situ synthesis of the reinforcements. Among the processing technologies for bone substitutes, powder metallurgy (PM) is one of the most versatile. The reasons lie in its flexibility to select the adequate materials for the matrix and the reinforcing precursor, the reinforcing content and the particle size of both components. Another important aspect is the selection of the sintering processes and parameters, providing the physical chemical conditions for the precursor's reactions and leading to the optimal reinforcing effect of the matrix.

Recent research regarding advanced materials for alloplastic bone substitutes highlighted the PM biocomposites based on submicronic HA particles reinforced by titanium hydride micrometric powders (100–150 µm; 25% wt.) as the precursor. The TiH_2 dehydrogenation reaction [89] occurring during the two-step sintering (TSS) treatment leads to the biocompatible TiO_2-rutile allotropic phase synthesis [90,91], which acts as a reinforcement for the HA/TiO_2 sintered biocomposite (Figure 7).

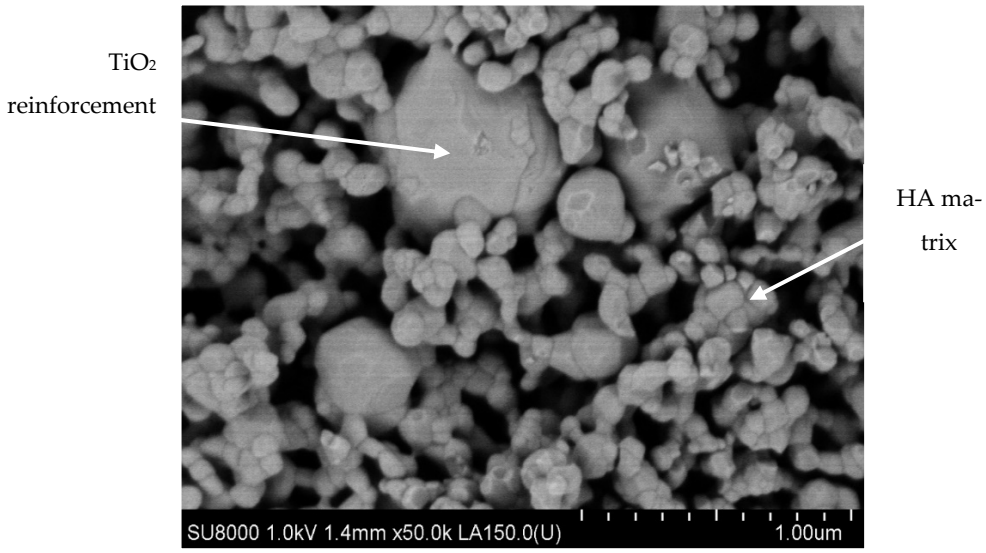


Figure 7. SEM micrograph of HA/TiO_2 biocomposite processed by TSS technology (Courtesy of Prof. Oana Gingu, University of Craiova, Faculty of Mechanics).

On the other hand, the hydrogen releasing during dehydrogenation reaction determines a specific porosity; thus, alloplastic bone substitutes or trabecular bone tissues may be designed using this technological approaching corroborated with the reinforcing content. Moreover, the PM biocomposites' porosity could be increased (30–60%) using different foaming agents such as calcium carbonate ($CaCO_3$) and ammonium hydrogen carbonate (NH_4HCO_3) in the initial powder mixture. The TSS technology allows for the monitoring of the foaming reactions; thus, each foaming agent contributes to creating the specific morphology of the pores [92].

For the cortical bone tissue, BS applications could be manufactured by PM biocomposites obtained through spark plasma sintering (SPS) technology. The same dehydrogenation reaction mentioned above for HA/TiO_2 composite is restricted by the compaction developing simultaneously with the sintering treatment, so the porosity is much lower (5–12%) than in the case of the same biocomposite made via TSS (Figure 8) [93].

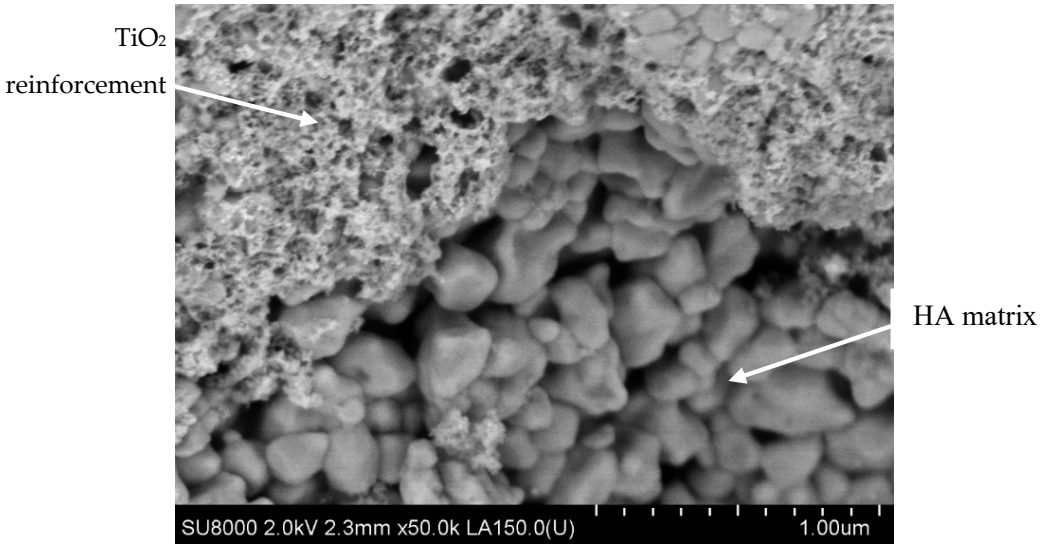


Figure 8. SEM micrograph of HA/TiO_2 biocomposite processed by SPS technology (Courtesy of Prof. Oana Gingu, University of Craiova, Faculty of Mechanics).

For some clinical cases, the necessity for geometrical matching between the alloplastic bone substitutes product and the bone defect arises. Recent research proved that the biocomposite type HA/TiO_2 manufactured via TSS technology presents high potential to be processed by laser micromachining in order to fit the bone defect (Figure 9). Using a pulsed Nd:YAG solid state laser with 1064 nm wave length, 4 mm laser beam diameter, pulse energy of 15 J max., average power of 1000 W and specific cutting regimes (pulse length, 0.02–20 ms; pulse frequency, 0.1–1000 Hz; and voltage, 240–320 V), the outer surface of the graft presents a variable roughness (Ra = 4.6–18.2 μm), assuring good physical interface with the adjacent natural bone without cracks/fractures in the bulk composite bone substitutes [94].

The bone substitutes' biocompatibility is significantly increased using submicronic HA powder particles as ceramic matrix. According to Varut et al., different antibiotics (such as ciprofloxacin, gentamicin) may be adsorbed on the nanostructured HA/Ti biocomposites, and their releasing in a few days provides a significant osteointegration support under antibacterial protection.

Furthermore, these effects are more intense in the case of calcium fructoborate (CaFB) adsorption on the HA/TiO_2 PM biocomposites [95,96].

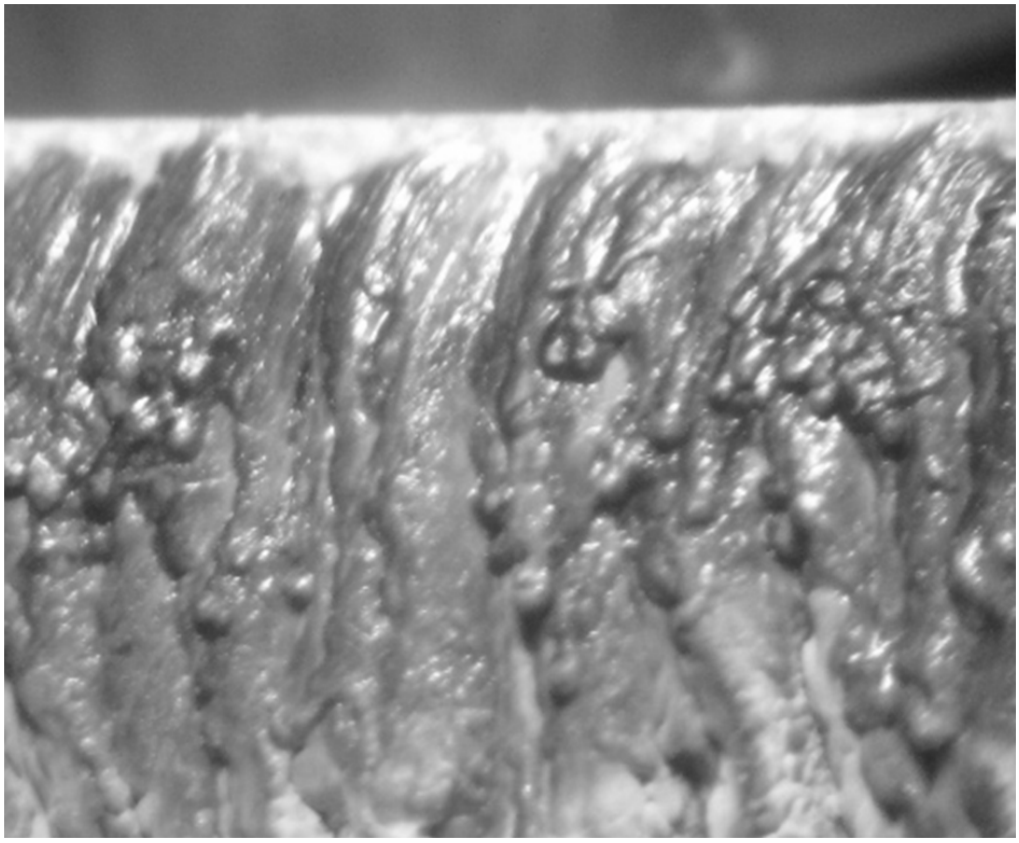


Figure 9. Macroscopic aspect (×8 magnification) of HAP/TiO_2 biocomposites after laser micromachining at 280 V, 60 Hz and 0.35 ms as pulse duration. The average obtained roughness Ra = 15.4 μm [94].

The nanostructured HA matrix, which is TiO_2-rutile reinforced by the TSS treatment, is responsible for other improved biocompatible performances. The cell viability of L929 fibroblast cells, assessed via quantitative (MTT assay) and qualitative (Giemsa staining) tests in connection with morphological analysis, demonstrated increased biocompatibility in comparison with HA-based ceramics. Moreover, the nanostructured HA proved to be stable, i.e., its decomposition to phosphates is avoided during the TSS treatment [90]. Other in vitro tests using mesenchymal stem cell culture confirms an improved biocompatibility of HA/TiO_2 PM biocomposites [97], and CaFB functionalization increases this property, i.e., the osteointegration process [98] (Figure 10).

Another category of composite materials with interesting properties in this field starts from the natural mammalian bone matrix formed from hydroxyapatite and collagen. This structure represents a matrix on which various other components, such as Ag, Sr and Zn, can be added. The addition of magnesium can also reduce the rate of bone substitutes resorption, an important aspect in a number of clinical situations [99] (Figure 11).

Synthetic materials with a polymer matrix to which hydroxyapatite powder can be added also demonstrate an interesting potential [100].

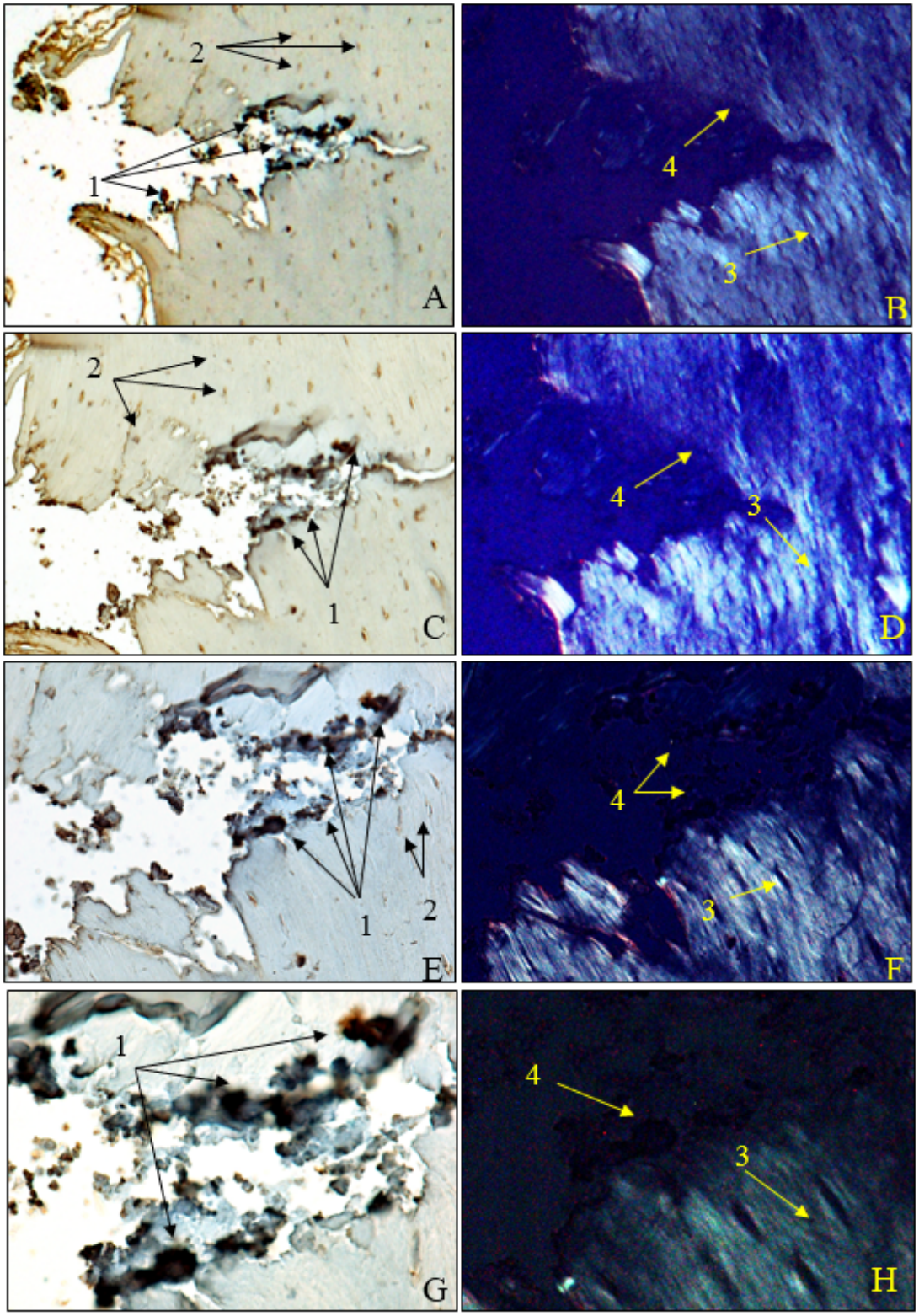


Figure 10. Morphogenesis of implant integration into adjacent bone tissue; (**A**–**H**) tissue surrounding the HApTiCaFb implant in the right femur was immunostained for OPN. 1: adherent fragments of adjacent bone-bearing tissue; 2: osteocytes; 3: birefringent collagen fibers under polarized light examination; 4: implant incorporation zone without the birefringence phenomenon. Anti-OPN antibody immunostaining: (**A**,**B**) ×28; (**C**,**D**) ×140; (**E**,**F**) ×210; (**G**,**H**) ×280. HApTi: Hydroxyapatite-coated titanium; HApTiCaFb: Calcium fructoborate coating on a HApTi; OPN: Osteopontin (Courtesy of [98]).

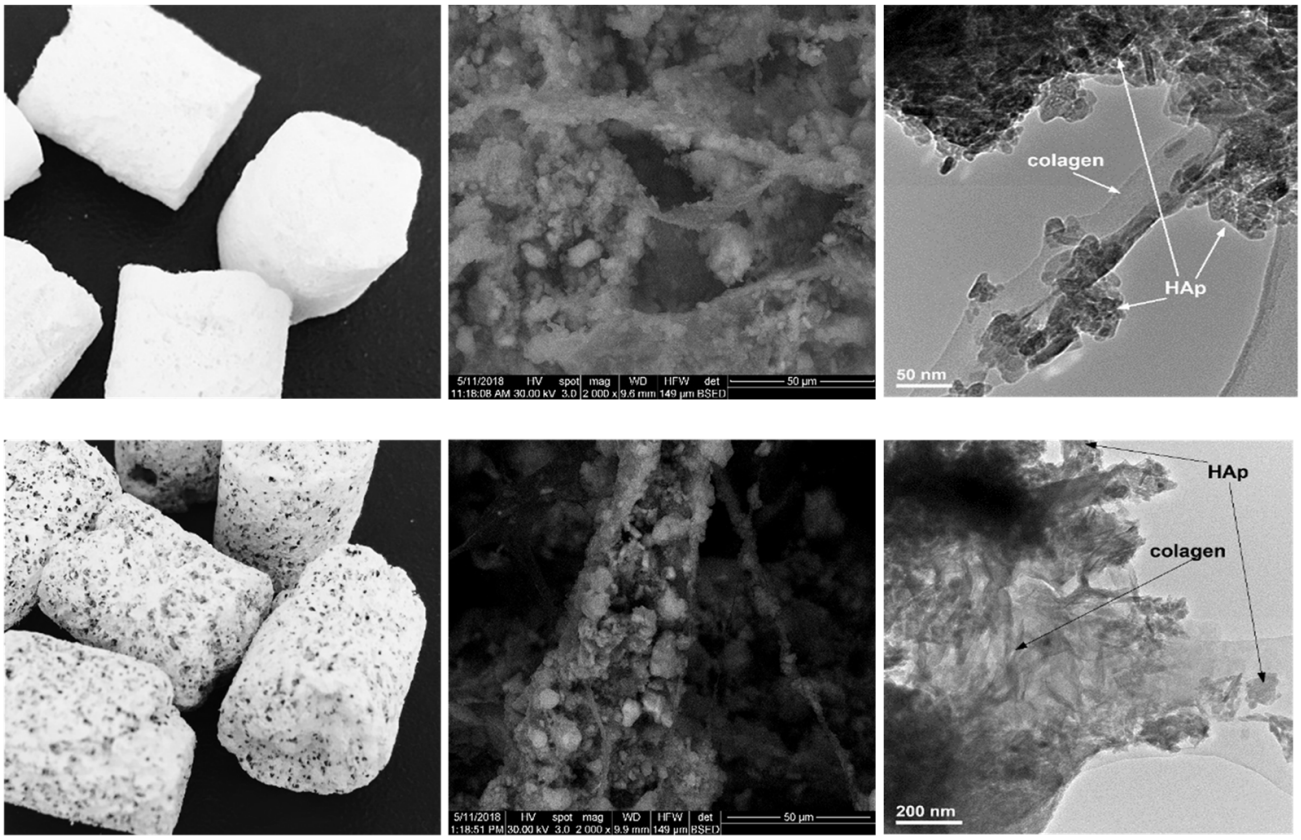


Figure 11. Experimental composite materials for bone substitutes type collagen–hydroxyapatite (first line) and collagen–hydroxyapatite–magnesium (second line): left row (up and bottom) macroscopic view, middle row (up and bottom) scanning electron microscopy image, right row (up and bottom) transmission electron microscopy image [99].

5. Adjunctive Materials to Synthetic Bone Graft Substitutes (Growth Factors)

Normal bone healing is conditioned by the equilibrium between biomechanical and biological factors. Orthopedic implants are used to ensure mechanical support. Sometimes, the biological factors that are involved in fracture healing are inadequate; hence, additional biological enhancement is sometimes needed.

Bone substitutes are used many times to facilitate bone healing through osteoconductive capacities; however, only relatively recently have bioactive molecules been utilized to stimulate fracture repair by their osteoinductive capacities. The most used products are bone morphogenetic proteins (BMPs), transforming growth factors (TGF), platelet derived growth factor (PDGF), vascular endothelial growth factors (VEGF) and parathyroide hormone (PTH).

5.1. Bone Morphogenetic Proteins (BMPs)

In 1965, Urist described bone formation after the implantation of demineralized bone matrix in soft tissues of rabbits [101] and discovered bone morphogenetic proteins to be important elements in this complex process. Since then, BMPs have become a major subject of orthopedic research, and at the same time, have been intensively used clinically in different forms of human recombinant BMPs [102].

BMPs are members of the TGF-beta superfamily, a class of molecules deeply associated with the complex signaling pathways of osteoblastic differentiation and osteogenesis. Today, more than twenty BMPs have been identified. They have osteoinductive activity and promote cartilage formation and angiogenesis [103]. BMP-2, -4, -6, -7, -9 and -14 have significant osteogenic properties [104]. BMP-2 is especially associated with osteoblastic differentiation from mesenchymal stem cells and has the capacity to promote local neo-

vascularization. BMP-7 has potent osteoinductive properties and the potential to promote angiogenesis, similar to BMP-2 [8]. Recent studies show that BMP-6 may be superior to BMP-2 and BMP-7 in promoting osteoblast differentiation in vitro and inducing bone formation in vivo, and, secondarily, it is involved in iron metabolism regulation [105].

One of the main characteristics of this class of substances is their solubility. This makes both transport and fixation to the desired bone level very difficult. The concentration of the BMPs decreases due to dilution, which leads to a significant decrease in their local healing effects. On the other hand, heterotopic ossifications may occur after diffusion in the surrounding soft tissues, with significant associated morbidity. One of the major research directions is the development of transporting and fixing vehicles of BMPs capable of ensuring a maximum concentration at the desired bone site and a prolonged time of releasing [106]. The main categories of carriers are as follows: natural polymers (collagen, hyaluronic acid, gelatin, fibrin), synthetic polymers (polylactic acid, polyglycolic acid, polyethylene glycol, poly-E-caprolactone), inorganic materials (calcium phosphate or calcium sulphate ceramics, bioglass) and combinations between these groups (composites containing either natural or synthetic polymers with ceramics) [107].

The main advantage of natural polymers is their biocompatibility; on the other hand, their animal origin brings an important immunogenicity and disease transmission potential, which are clear disadvantages for clinical use.

Synthetic polymers are moldable into porous three-dimensional scaffolds, such as blocks or chips, without any immune potential [108]. These structured carriers have the ability to replace certain bone defects while the attached BMPs facilitate osteoconduction at this level.

Non-structured carriers are used especially on the surface of various implants, favoring their fixation to the surrounding host bone.

5.2. Fibroblast Growth Factors (FGFs)

Fibroblast growth factors (FGFs) are important molecules that regulate many stages of endochondral ossification. FGF-9 and FGF-18 signal chondrocyte differentiation, skeletal vascularization and osteoblast/osteoclast recruitment to the growth plate. FGFRs also function in osteogenic differentiation and osteoblast maturation [109].

5.3. Vascular Endothelial Growth Factor (VEGF)

Vascular endothelial growth factor (VEGF) is a growth factor that stimulates vasculogenesis (embryonic development of the circulatory system) and angiogenesis (development of new blood vessels from existing ones). VEGF plays an essential role in fracture healing by promoting the development of a vascular network in the cartilaginous callus, acting on the endothelial cells. This process is followed by the transformation of the fibrous callus into the primitive bone callus. VEGF is released from platelets, inflammatory cells and hypertrophic chondrocytes. Due to their origins in platelets, high concentrations of VEGF have been found in PRPs, theoretically explaining the repairing effect of these preparations. Recent studies show that VEGF may enhances bone formation by other synergistic mechanisms as well; VEGF enhances the activity of cultured osteoblasts (osteoblastogenesis) [110], modulates osteoclastogenesis by upregulating RANK expression in osteoclast precursors and increases the activity of osteoclasts [111,112].

Despite these theoretical considerations and in vitro studies, the effectiveness of these growth factors in accelerating healing in humans is not yet fully certified.

5.4. Parathyroid Hormone (PTH)

Parathyroid hormone (PTH) is an endocrine mediator of calcium and phosphate human metabolism with an important anabolic effect achieved by favoring osteoblast-mediated bone deposition compared to osteoclast-mediated bone resorption, being an important regulator in the process of bone remodeling.

At the same time, PTH stimulates vitamin D synthesis in the kidneys and increases absorption of calcium from the intestines and mineralization of the bone matrix.

Due to these mechanisms of action, PTH is used in the treatment of severe forms of osteoporosis. Proving to be effective in treating osteoporosis, the question has been asked as to whether it could be useful in stimulating bone healing too.

Animal studies demonstrate that PTH increases both the quantity and strength of calluses. The main effect is osteoinductivity, which stimulates the differentiation of osteoblasts from mesenchymal stem cells (MSCs), as well as the acceleration of their maturation by stimulating the expression of BMPs [113].

5.5. Platelet-Rich Plasma (PRP)

Platelet-rich plasma (PRP) is an autologous suspension of high concentration platelet-rich plasma (PRP) is a high concentration autologous suspension of platelets resulting from centrifugation of blood. The fold concentration of thrombocytes after preparation by a single- or double-centrifugation process ranged from 2- to 33-fold. Platelets are rich in many growth factors involved in different healing processes: platelet-derived growth factors (PDGF-aa, PDGF-ab and PDFG-bb isomers), transforming growth factor-beta (TGF-β), VEGF, interleukin-1, platelet-derived angiogenesis factor, platelet-derived endothelial growth factor, insulin-like growth factor (IGF-1), osteocalcin and osteopontin [8,114]. Platelets are the first elements that release these growth factors at the fracture site, followed by macrophages, whose chemotaxis is stimulated by the factors released from the platelets. The above-mentioned growth factors are responsible for the osteoinductive property of PRP, promoting the angiogenesis, proliferation and differentiation of pluripotent mesenchymal cells on the lines of osteoblasts and chondrocytes [115]. There are several controversies regarding the clinical use of PRP: whether or not the platelets should be activated before application, the method of application (injection or direct application) and the possible combination with other elements that may stimulate bone healing (demineralized bone matrix—DBM; concentrated bone marrow aspirate—cBMA; bone allograft).

Despite extensive research and widespread clinical use in various types of conditions, there are currently no data to certify the effectiveness of these preparations on accelerating bone healing; thus, the routine use of PRP for improving fracture healing rates and speed is not recommended.

6. Future of Bone Substitute Materials from Advanced Biomaterials Research Perspective

New trends in the advanced development of biocomposite materials with a bone substitution/reconstructive role can be found in the current EU strategies, i.e, the objectives of the Cluster 1—HEALTH of the Horizon Europe program. One of the priorities in the scientific research of biocomposites is to support the development of new technologies and methodologies to reduce, as much as possible, in-vivo tests, the use of animals for scientific purposes. There are mentioned omics-type approaches and other high-throughput procedures based on human-derived cells, organoids, micro-physiological systems and in silico models [116].

The multidisciplinarity of research teams in the field of biocomposites is all the more complex as the replicated biological models are more faithful to natural biological tissues. Regarding bone tissue and one specific goal, i.e., the rare and refractory bone cancer especially in children and adolescents (<24 years), he specialized literature offers a wide spectrum of information about alloplastic grafts made of advanced biocomposites which were recently developed for this purpose [117]. These twin models serve for the in-depth and multidisciplinary research of the reactions, less known until now, that take place at the bone tumor–natural bone tissue interface. Starting from this objective, researchers from the fields of general and biomolecular medicine, biology, biochemistry, hard tissue engineering, food science, social sciences and psychology are concentrating their efforts in order to innovate minimally invasive care and treatment approaches for the targeted patients.

A key element of this assembly is represented by artificial intelligence (A.I., golden highlighted box in Figure 12), which, with the help of digital tools (golden highlighted box in Figure 12), optimally combines the input data in order to create specific algorithms to predict the evolution of bone tumors as accurately as possible in a personalized way (Figure 12). The expected impact of the research–development–innovation policy of the European Union, through the development of ex vivo (twin-type) and in silico models is concatenated from the following components: (i) to improve the understanding of the rare and refractory bone cancers in the broad framework of the working and living conditions of patients in the widest possible sense; (ii) the improvement of cancer prevention strategies will be followed with the help of policy makers; (iii) it will be possible to reach the level of optimization of the diagnosis and treatment of the rare and refractory bone cancers based on the principle of fair access; (iv) the quality of life of cancer patients, survivors and their families will be improved in conjunction with the large-scale analysis of all key factors and needs that are related to quality of life; and (v) advanced levels of the digital transformation of research in the field of rare and refractory bone cancers can be reached by innovating specific tools of the health systems [118].

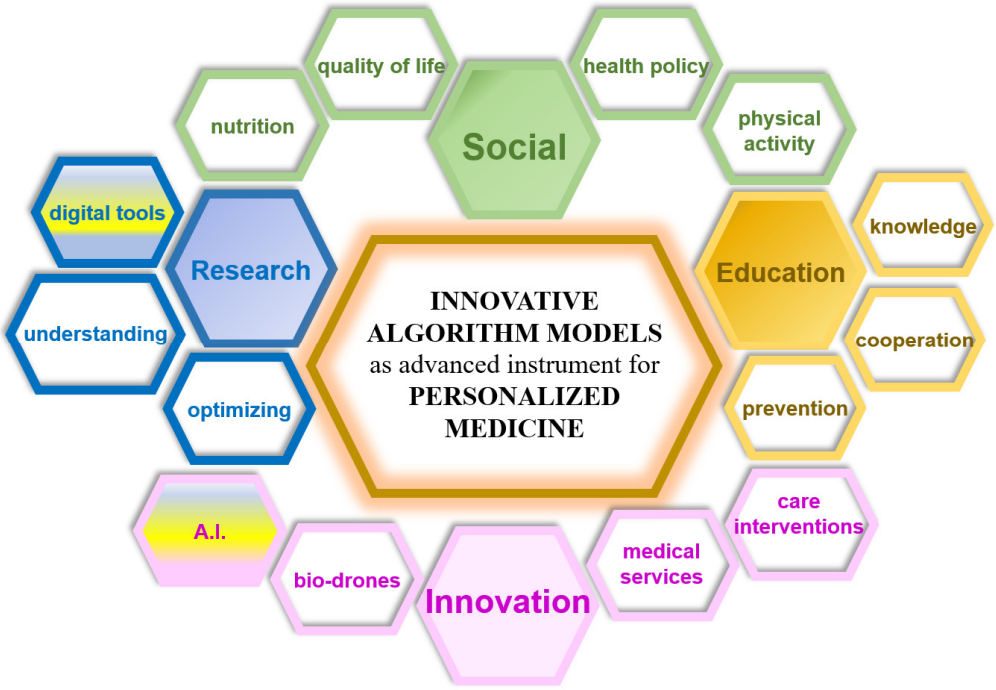


Figure 12. The multidisciplinary approach to the research of advanced biocomposites for bone replacement/reconstruction from the perspective of personalized medicine.

A first stage in the entire course of development of such an algorithm is represented by finite element modeling (FEM) and simulation of the behavior of biocomposites under certain demands of the grafted/implanted bone tissue. Although classical, the FEM, applied in the context mentioned above, contributes essentially to the selection of the chemical composition of the studied biocomposites as well as the technological parameters of their manufacture so that the new bone substitutes optimally correspond to the simulated demand conditions. The results offered by FEM contribute significantly to the increase in the technology readiness level (TRL), i.e., from the scientific research level (TRL1-3) to the development level (TRL4-6), with an impact on the deployment stage (TRL7-9).

In this respect, there are published research data regarding the mechanical behavior of biocomposites bone substitutes, tested by modeling and simulation using FEM (Figure 13).

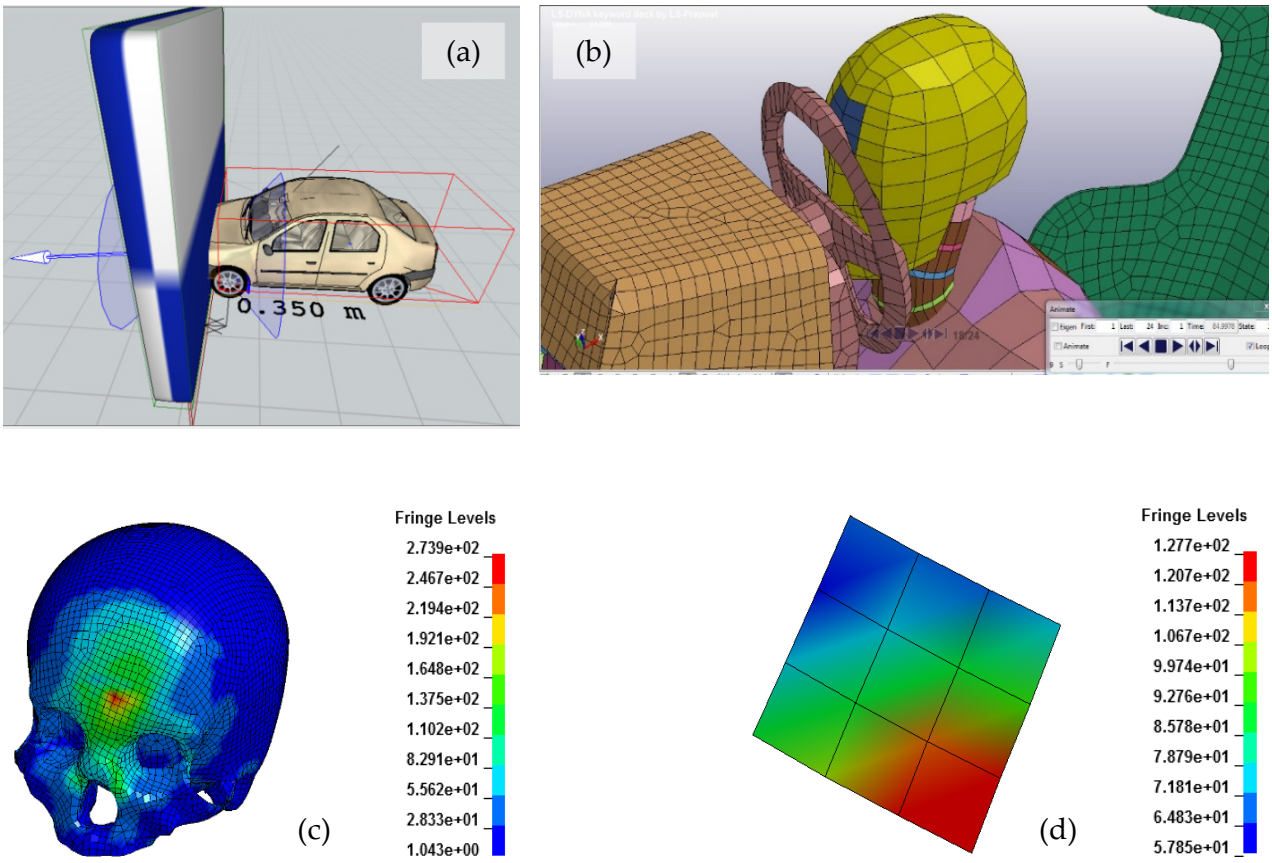


Figure 13. FEM simulation of (**a**) one sedan frontal impact with (**b**) the "pilot" having an alloplastic bone graft in frontal-cranial position; (**c**) von-Mises stress map for the skull and (**d**) von-Mises stress map for the graft (Courtesy of Prof. Oana Gingu, University of Craiova, Faculty of Mechanics).

One of the most attractive application fields envisages the prevention of traumatic brain injuries resulting from a car crash [119]. Finite element analysis of the skull implant is used to predict mechanical behavior of the skull BS made of HA/TiO_2 under various static and dynamic loading conditions. Using ANSYS software (2015-2016) and LS-DYNA software, promising results were obtained after FEM simulation of a frontal car crash considering a medium class sedan driving at 50 km/h speed (EURO NCAP standard condition) in the worse conditions: the "pilot" (dummy virtual model Hybrid III) does not wear the safety belt and the air-bag system is OFF. The biocomposite BS presented good mechanical results after this frontal impact (Figure 13). The FEM analysis was applied in order to identify the optimal chemical composition of the biocomposites that meet the minimum impact resistance conditions according to the conditions described above. Thus, biocomposites with a ceramic matrix made of submicronic particles of hydroxyapatite were identified as appropriate compared to biocomposites with a matrix of HA, but with particles of micrometric size [89]. At the same time, FEM contributed to the identification of the optimal manufacturing parameters of nanostructured biocomposites through powder metallurgy technology [120].

Composite grafts combine scaffolding properties with biological elements to stimulate cell proliferation and differentiation and eventually osteogenesis. Autograft is considered ideal for grafting procedures, providing osteoinductive growth factors, osteogenic cells and an osteoconductive scaffold. Synthetic graft substitutes offer structural support but lack osteoinductive or osteogenic properties. Calcium sulfate has an osteoconductive crystalline structure which resorbs rapidly (1–3 months), creating porosity in capillaries, and perivascular mesenchymal tissue can grow [121].

β-tri-calcium phosphate (β-TCP) is considered as the "gold standard" for synthetic bone grafts, with properties similar to the inorganic phase of bone. Like other bone substitutes, β-TCP's main property is osteoconduction. Its resorption is slower than the resorption of calcium sulfate, being made in 13–20 weeks after implantation and then completely replaced by remodeled bone. Due to this property, most of the time, it is used to fill large, contained defects in association with bone marrow aspirate [122]. Using this method, the healing rates vary from 90% to 100% [123].

Biphasic calcium phosphates (HA and β-TCP ceramics) can be used in association with autologous, expanded, bone marrow-derived mesenchymal stromal cells at a dose up to 200 million cells. The efficiency and safety of their use has been set by ORTHO—1, a European, multicentric, human clinical trial. The results were promising, with HA and β-TCP ceramics, in a ratio of 20/80 in weight, being the most efficient support for autologous cells, compared to the equivalent macro and microstructure of different calcium phosphate bioceramics. The radiological success rate (in at least three views) was 74%, and the combined clinical criteria success rate was 85% [124].

Another direction is bioactive factors releasing. Bone scaffolds may deliver bioactive molecules or cells to accelerate healing and tissue regeneration. Administration of growth factors and other bioactive molecules to promote bone formation and repair has achieved promising results. There are different administration methods, such as surface adsorbed protein release, osmotic pumps and controlled release from biodegradable scaffolds.

Unlike the metallic biomaterials [125], the ceramic materials have the ability to biodegrade and release bioactive molecules at a controlled rate [113,126]. Natural polymers such as collagen, fibrin and gelatin have been used as drug delivery vehicles in bone tissue engineering. The most commonly utilized copolymer is PLGA (poly lactic acid-*co*-glycolic acid). Its clinical utility is limited due to its poor mechanical properties compared with cancellous bone; to improve its strength, it is combined with other materials [127]. The bioactive molecules include TGF-β, BMPs, IGFs, VEGF, NGF and DNA [128,129].

7. Conclusions

The last years have represented a period of uninterrupted progress in terms of solving different types of pathology of the musculoskeletal system due to improvements in surgical techniques improvements and technological advancements. Dentistry, furthermore, introduced various innovative techniques. Due to various aspects, the restoration of bone stock is a continuous challenge. For a long time, bone autograft and allograft were the most widely used methods in the context of various clinical situations that involved a bone defect that had to be grafted. Autografts are still considered the "gold standard" due to their osteogenic properties, their maintaining and transferring viable cells from the donor site to the recipient site, as well as their osteoinductive characteristics. Allograft is harvested from cadavers and requires the sterilization and deactivation of proteins and other substances normally found in bone. The mineral content of the bone is degraded by using a demineralizing agent such as hydrochloric acid. The final result of this complex process is a demineralized bone matrix which contains osteoinductive agents.

The various limitations of this types of bone grafts, as well as recent technological advances, have led to the presence on the market of various types of bone substitutes, with different chemical structures, mechanical and biological properties adapted to different types of bone defects.

Most of the currently available bone substitutes (calcium phosphate ceramics, polymers) display only osteointegrative and osteoconductive properties.

Composite bone substitute materials combine two or more materials, improving the mechanical properties of each component and their osteoconductive properties as well. In order to add osteoinductive properties, some synthetic bone substitutes can be combined with bone marrow or act as carriers for BMPs, growth factors or modified living osteogenic progenitor cells. These hybrid grafts, which utilize growth factors and living osteogenic cells capable of inducing bone regeneration, present the future of bone generation technologies.

On the other hand, the cost of these new bone substitutes is another important aspect, with a strong impact on their use in current practice; therefore, future research directions must also take this aspect into account.

The European strategy in the field of advanced biomaterials for bone grafting/reconstruction envisages the approach of new technologies and design, manufacturing and testing methodologies involving digital tools specific to artificial intelligence. The main goal is the continuous improvement of the patients' quality of life through the innovative development of minimally invasive treatment technologies. The trend of personalized medicine is supported by the creation of "twin"-type bone grafts that will be set up in personalized mini-laboratories in order to identify, with minimal medical risk, non-invasive treatments and care interventions.

In order to achieve this objective, natural and/or synthetic biomaterials will contribute together with alloplastic biocomposites, which, in conjunction with the materials specific to bone growth factors, will constitute the most faithful scaffold to human bone tissue. Together, they form a mini-laboratory dedicated to the innovation of treatments for specific diseases adapted to patients' particularities.

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