Materials Research. 2020; 23(1): e20190487
DOI: https://doi.org/10.1590/1980-5373-MR-2019-0487

Additive Manufactured Nanocomposites for Bone Tissue Engineering Applications: an Overview

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Received: August 28, 2019; Revised: January 20, 2020; Accepted: January 29, 2020

Tissue Engineering aims to repair, regenerate or restore damaged tissues. Structures known as scaffolds can be manufactured in different ways and with various materials such as synthetic and natural polymers as well as inorganic materials. Additive Manufacturing (AM) has been used to produce porous scaffolds. Particularly, Selective Laser Sintering (SLS) produces materials and composites through a selective solidification of a variety of fine powders. AM-made biomaterials may be suitable for different affected or injured parts by stimulating the biological system around the implant. The present work aims to review relevant concepts concerning the nanostructure for regeneration of the bone tissue when in contact with the native tissue, as well as the suitable techniques and materials to fabricate it.

Keywords: *Tissue Engineering; Scaffolds; Selective Laser Sintering.*

1. Introduction

Tissue engineering is based on the development of scaffolds that permit regeneration of the tissue with defect. The development of scaffolds allows the regeneration of damaged bone tissue, which usually can be derived from a natural bone defect or removed from the tumors and/or fractures[1]. Therefore, there is a great call for materials and procedures for bone regeneration, such as spontaneous regeneration and/or autologous/allogeneic transplantation. A variety of materials and techniques have been investigated over the last two decades to produce these scaffolds[2-5].

Scaffolds must have a structure that try to mimic the extracellular matrix (ECM) found in native tissue[3]. These structures should facilitate the migration, proliferation and adhesion of cells, thus playing the role of a "real" native tissue. Scaffolds must also be biocompatible, have mechanical strength, controlled porosity, and interconnected pore network, among other characteristics that differ from tissue to tissue[6].

In search of the improvement of these characteristics of the scaffolds in recent years, alternatives are been studied with greater intensity. In this context arise the techniques of Additive Manufacturing (AM), which produce scaffolds with controlled geometry and 100% interconnectivity through the progressive application of layers of material by combination of CAD software with the use of inorganic and polymeric materials[7].

Still in the perspective of improved scaffolds, some substances can be incorporated in their structure, or deposited as a coating in the production process, also in order to increase mechanical strength, flexibility, porosity and cell culture[8,9].

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In this overview, a search procedure was performed using Scopus, NCBI PubMed and Web of Science platforms, with the following combination of keywords: "bone tissue" and nanomaterials; "features of a scaffolds" and "biomaterials"; "additive manufacturing techniques" and "scaffolds"; "tissue engineering" and "nanomaterials"; "materials for production of scaffolds"; "biocomposites" and "bone regeneration"; "bioceramics" and "scaffolds"; "polymers" and "ceramic scaffolds"; "bone morphogenetic". These keywords were searched amongst article title, abstract and keywords. Since the objective of this research was to identify recent nanocomposites for bone tissue, articles published 2005 until 2019 were searched. Open access and CAPES-consortium available publications were downloaded and analyzed. By selecting only research articles and excluding duplicates, we found a total of 200 articles. The main concepts and findings are summarized in the following sections.

2. Bone Tissue Engineering

Bone tissue is chemically composed of two parts: an organic part and an inorganic part. The organic part consists basically of cells (osteoclasts and osteoblasts) and collagen fibers; the inorganic part is formed by calcium phosphates such as hydroxyapatite, equivalent to 70% of the total bone mass[10].

Structurally, the bone tissue is composed of two parts: one part corresponding to the cortical bone and the other part to the trabecular bone (Figure 1). The most compact bone that is involving bone marrow and attaches to mechanical strength to bone is known as the cortical bone, which can reach 30% porosity volume and with pore sizes of 10-20 μm. The more spongy bone, due to its higher porosity, which

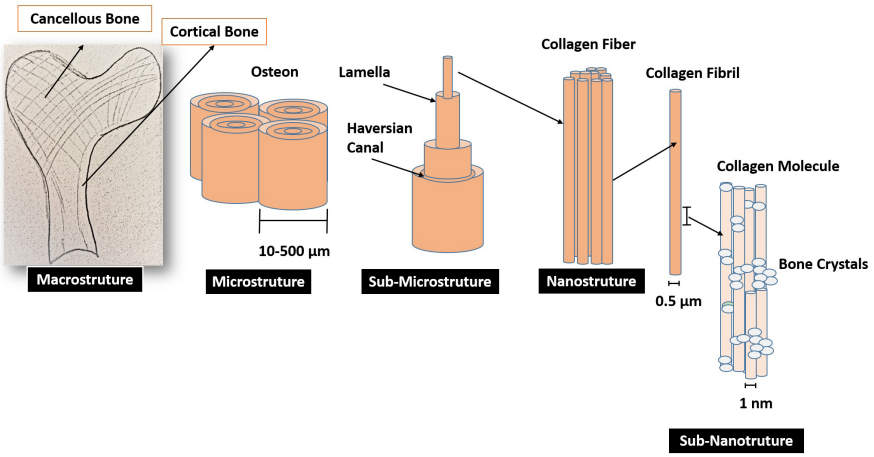

Figure 1. Anatomy of bone. (Adapted of Bao C, Teo E, Chong M., 2013; Clarke B, 2008).

Table 1. Properties of cortical and trabecular bones.

Type of bone	Porosity (%)	Apparent density (g·cm^{-3})	Modulus of elasticity (GPa)	Mechanical Strength (MPa)		
				Compression	Traction	Flexion
Cortical bone	5-30	1.8-2.0	7-30	100-230	78-150	50-150
Trabecular bone	30-90	0.1-0.9	0.05-0.5	2-12	-	-

Font: Adapted of Bao; Teo; Chong, 2013; Kokubo; Kim; Kawashita, 2003.

can vary from 30-90% with pore sizes of 100 µm, is known as the trabecular bone[10-12]. Some common properties of the cortical and trabecular bones are presented in Table 1.

2.1. Regeneration of bone tissue

The most common bone fracture healing is indirect or secondary consolidation. This bone healing consists of endochondral and intramembranous, which is characterized by the formation of intermediate callus before the formation of the bone callus[13].

Bone regeneration is composed of four steps. The first phase is the immediate inflammatory response, leading to signaling to stem cells, which will differentiate into chondrocytes that will produce cartilage and osteoblasts, forming a new bone. After the already formed cartilaginous matrix, it will be mineralized, and then it will be resorbed forming the bone. This formation generates the primary bone, which undergoes a remodeling of the preformed bone callus, occurring a second resorption, which restores the anatomical structure[13].

From the beginning of the process of regeneration of injured bone tissue, tumor necrosis factors (TNF-α), interleukins, as well as factors that recruit inflammatory cells[13], as well as stimulating the synthesis of Extracellular Matrix (ECM) and angiogenesis at the lesion site[14]. During injury repair, morphological bone proteins (BMPs) are produced, which function independently or in collaboration with other cells. The BMPs stimulate a cascade of events that will promote the formation of cartilage and bone[14].

Direct consolidation is not a natural process of regeneration that commonly occurs. Direct regeneration of the injured site will not result in periosteal or endosteal callus formation. This consolidation takes place with the reduction of the interfragmentary tension, by the action of an internal fixation surgery. Later it initiates the process of regeneration, and it may take years for the formation of the new bone to occur completely[14].

In the perspective of efficient bone regeneration, in the last years biomaterials are being investigated. Their composition normally comprises an osteocondutor ceramic material, conjugated with a biodegradable, bioactive polymer. Current research is focused in adding proteins to those materials as well as activating the surface thorough a surface treatment, with the intention of developing a material capable of being used as a permanent replacement[2,3,15-18].

BMPs are pleiotropic molecules that are involved in chemotaxis, mitosis and differentiation of mesenchymal cells in bone tissue, but these proteins need a carrier for effective osteoconduction. BMPs belong to the superfamily of the Tumor Growth Factor β (TGF-β), and were discovered by Urist (1965), who verified that bone arrays implanted at ectopic sites promoted bone formation[19,20].

The activity of the BMPs involves a complex system of signals to the coupling of enzymes and of factors in the repair ways[21]. Since the formation of bone tissue cannot occur simply by injecting an aqueous solution of recombinant human BMP-2 (rhBMP-2) into the target site, as this would be rapidly eliminated from the site of application[22]. In this way, a biomaterial with osteoconductive and biodegradable characteristics can serve as a carrier for the BMPs in the host tissue.

BMP-2 can promote significant bone cell differentiation, mesenchymal cells into chondrocytes and osteoblasts, and induces precursor cells of osteoblast to differentiate into osteocytes, as well the formation of the blood vessels[23]. During the fracture healing, bone formation and cartilage formation also can be promoted by BMP-2 to induce bone formation by stimulating osteoblast differentiation and chondrogenic differentiation separately[24,25].

2.2. Features of a scaffold

Scaffolds must modulate the healing process by providing a mechanical support and stimulate the onset of cell colonization, by action of the physiological and biological process forming a new tissue[3], promoting regeneration without training necrosis or scarring at the site of implantation[3].

Scaffolds depend on some important requirements when it comes to materials for their development. Firstly, biocompatibility of the substrate materials is indispensable. The material must not educe an unresolved inflammatory response nor demonstrate immunogenicity or cytotoxicity[26-28]. In addition, the mechanical properties of the scaffold must be sufficient to prevent structural failure during handling and during the patient's normal activities. An important requirement in scaffolds for bones is porosity, which directs the cells in their physical structure and serves as support for vascularization. A typical porosity of 90% as well as a pore diameter of at least 100 µm is known to be required for cell penetration and a proper vascularization of the ingrown tissue[18,28] (Figure 2).

Several materials in the development of scaffolds have been explored for the regeneration of tissues, based on physical, chemical and biological properties that are most suitable to stimulate the production of new tissues. Natural and synthetic polymers, as well as inorganic materials are major sources of raw materials for scaffolds in the regeneration of tissues, such as bone, skin, ligaments, etc[3,29].

Natural polymers such as chitosan, chitin, collagen, glycosaminoglycans, gelatin, elastin, and bacterial cellulose have been used in various applications in tissue engineering[30-34]. Some of these polymers are present in the ECM of many native tissues and enhances the adhesion and the functionality of the cells[3].

Bioactive inorganic materials, such as hydroxyapatite (HAp), and tricalcium phosphate (TCP), are biocompatible and osteoconductive. These properties are due to the chemical composition of these materials that are close to the inorganic mineral phase found in the bone tissue[15,16].

In recent years, methodologies for producing three-dimensional (3D) porous structures are being investigated. In this case, it is possible to use natural sources (plants and bacteria) and synthetic polymers combined. These 3D structures intended to simulate the environment the of ECM of the native tissue, facilitating the regeneration of injured tissues[35]. Thus, scaffolds need to be produced with the aim to remedy the biological requirements seeking to mimic the cellular microenvironment of the ECM and technical requirements of implementation and low cost[36].

Scaffolds must have basic features to play its role literally. The main characteristics of a scaffold include: biocompatibility, bioactivity, biodegradability, biorreabsorption, mechanical compatibility, porosity, and non-toxic nature[6,37].

These characteristics need to be connected with the aim desired of this scaffold, because scaffolds have specific properties depending on the application. For example, if the goal is bone tissue regeneration, a scaffold must be biocompatible and have similar mechanical properties to those of a natural bone[37]. For comparison, cortical and trabecular bone compressive strength are 100-230 and 2-12 MPa and for bending strength are 50-150 and 10-20 MPa, respectively[38]. Furthermore, the scaffold must have an environment that promotes the growth, proliferation and differentiation of cells. This environment must have a 3D structure with porosity >40-60% and an interconnected network for cellular growth, which favors the rapid diffusion of nutrients and metabolic waste as well as cell migration[6,39]. The implants of this material take between 6 and 15 weeks to be partially bioreabsorbed, time that depends mainly on the porosity of the implant[40].

Many scaffolds are being developed for different purposes, which have different chemical compositions and different physical and chemical properties, being produced by distinct methods. In the search of controlled structure and porosity, the method of 3D printing (3DP) stands out, allowing to produce scaffolds with different components and controlled geometry, interconnectivity , and disposition of layers with different components[37].

3. Additive Manufacturing Techniques for Scaffolds

Additive Manufacturing (AM) is a technique that produces uniform, complex shaped 3D scaffolds, promoting an improvement in the structural characteristics, as controlled connectivity and porosity. Furthermore, AM can produce scaffolds from customized images, obtained by computer tomography or magnetic resonance, together with CAD techniques[41,42].

Many processes of AM can be used for production of scaffolds aimed to prostheses. Typically, five processes are

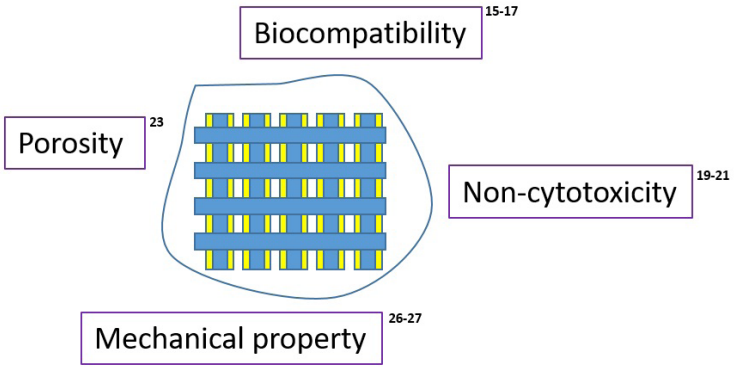

Figure 2. Main features of scaffolds.

used to build 3D scaffolds: 3D Printing (3DP), selective laser sintering (SLS), Stereolithography (SLA), Robocasting (RC) and Fused Modeling Deposition (FDM).

Selective Laser Sintering (SLS) is a solid freeform fabrication technique, developed by Carl Deckard for his master's thesis[43] at the University of Texas, patented in 1989. SLS is a technique that produces physical models through a selective solidification of a variety of fine powders. The physical aim is manufactured layer-by-layer, transforming the three-dimensional problem in a bi-dimensional one. Scaffolds are build layer-by-layer from CAD data files exported in the industry-standard exchange file format standard triangulation language (STL)[44]. (Figure 3).

The morphology and the particle size of the powder are well known as crucial parameters in SLS[45,46]. These properties have an impact on the powder bed density and on the powder flowability. The flowability of the powder is considered a critical point, because the powder must be uniformly spread at an elevated temperature and need to form layers having a thickness of about 100 μm. The powders used in SLS have specific granulometry and good sphericity. Commercially available SLS powders have grain size with a size distribution of 60 μm and a low percentage of fine particles below 10 μm[47].

The advantages of SLS are related to fast and economical process; durable, functional, large and complex parts; small series produced in one manufacturing process; as well as sterilizable parts, high part accuracy, and material versatility[48]. Disadvantages may be found in parts that have rough, grainy and porous surface finish, which is not as smooth as SLA but acceptable for most of applications[44].

High-quality lasers were introduced so that a partial melting of SLS has been taken over by complete melting giving rise a new development of metal laser sintering (MLS) or Selective Laser Melting (SLM). SLM is SLS done at high laser powers with an aim to achieve complete melting of metallic powders[49,50]. The working principle is based on fusing metal powder into a solid and melting it locally using a focused laser beam.

Polymer-based scaffolds containing bioactive bioceramics can be manufactured in which the bioceramics can serve two purposes: (a) making the scaffolds osteoconductive and (b) reinforcing the scaffolds. With this composite strategy, there are two approaches for making bioceramic–polymer composite scaffolds: (1) incorporating bioceramic particles in the scaffold through a variety of techniques and (2) coating a polymer scaffold with a thin layer of apatite through biomimetic processes[44,51].

Such polymers are saturated poly(alpha-hydroxy ester), including poly(lactic acid) (PLA) and poly(glycolic acid) (PGA), as well as poly(lactic-co-glycolide) (PLGA) copolymers. PLA exists in three forms: L-PLA (PLLA), D-PLA (PDLA), and racemic mixture of D, L-PLA (PDLLA). The chemical properties of these polymers allow hydrolytic degradation through de-esterification. Once degraded, the monomeric components of each polymer are removed by natural pathways.

Poly(alpha-hydroxy ester) have been extensively studied for the fabrication of scaffolds via SLS for applications in tissue engineering (TE). Tan and collaborators[52] proposed the use of a biocomposite blend comprising of PLLA and HA in SLS. Results observed from the scanning electron SEM micrographs indicate the viability of the blend used for building TE scaffolds and ascertain the capabilities of the SLS process for creating highly porous scaffolds for TE applications. Simpson and collaborators[53] investigated 95/5 PLLGA for the role of a porous scaffold, using the SLS fabrication process, with powder sizes of 50–125 and 125–250 μm.

For the production of objects, an algorithm is applyied in the printing system[54]. 3DP was considered a highly flexible process when considering the geometry, materials and desired properties. In further studies, Sachs et al. began to use metallic materials for 3DP. In addition, automation

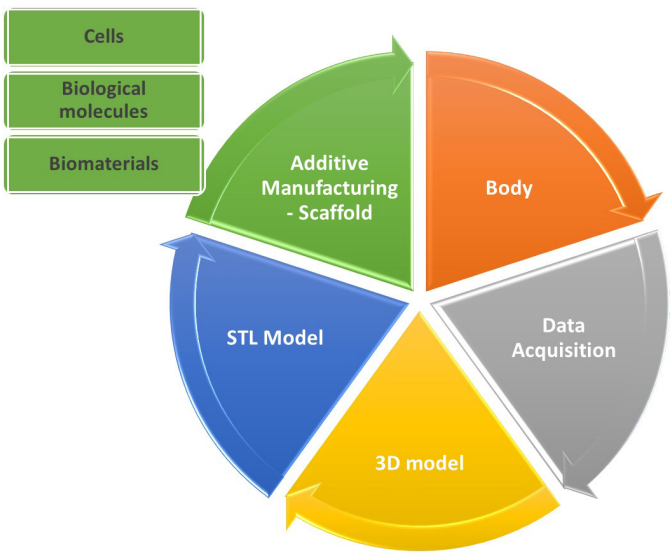

Figure 3. Schematic representation of the main steps required to produce TE scaffolds using AM technique.

was incorporated in the production flow from design to finished product[55].

In recent years, studies of 3DP were expanded, and different inorganic materials were employed as well as polymers and/or even combination thereof[37,56,57]. Moreover, proteins have been incorporated in their structures, where the response of the interaction of cells is enhanced with the environment formed of in these 3D structures[58-60].

4. Supports and Coatings for Scaffolds

4.1. Biocomposites

The bone tissue by nature presents biological systems, which have the capacity to stimulate the regeneration of fracture. However, major defects and complex fractures have some limitations regenerating properly. In pursuit of to minimize these regenerative processes, biomaterials are being developed in different compositions, using materials of various natures seeking resemblance to native tissue which will regenerate, and even be replaced[61].

Metallic materials such as titanium and stainless steel alloys are widely used in cases where there is an immediate need for stability and structural support of bone. These materials have excellent mechanical properties and corrosion resistance due to the presence of chromium, molybdenum and low carbon[62-64].

To improve the bioactivity the surface of these metal materials, some surface treatments can be applied, such as coating, and cold plasma. Research using groups of chemicals added to the surface can increase the inducement and the growth of bone cells, beyond accelerating the regenerative process and also reducing the rejection of these materials in native tissue[61].

Although metals have important properties, they have drawbacks, such as non-degradability and high stiffness, being not suitable for bone tissue regeneration. Promising results have been demonstrated with osteoconductive biomaterials, which remodel the native tissue and are capable of degrading at the same time that a new bone is obtained[65].

Various types of polymers are used in bone tissue regeneration. Polymers can be divided into natural and synthetic and natural derived from a natural source such as collagen, chitosan, hyaluronic acid. Natural polymers are biocompatible and bioactive, since synthetics derived from chemical reactions, such as polifumarates, polycarbonates, can cause toxicity when in contact with the native tissue[5].

4.2. Bioceramics

Ceramics comprises inorganic materials usually obtained after of a heat treatment at elevated temperatures[66]. In the filling and/or bone, replacement bioceramics are being used, while the process naturally renews functions of the native tissue. They can also be used for covering other structures in implantation as well as combined with other materials to enhance the biochemical and mechanical properties thereof[67].

For about 40 years, there is a constant increase in the use of bioceramics, which actively stimulate osseointegration between the implant and native tissue[68]. Alumina (α-Al_2O_3) was the first bioceramic to be used due to the bioinert property[69]. It presents biocompatibility and high mechanical strength, and has been used in orthopedic prostheses, replacing bones or parts of them[70-72].

Beyond alumina, other ceramics have been used such as zirconia (ZrO_2)[73], titanium dioxide (TiO_2)[74], calcium phosphates[75] and silica glass[76]/calcium phosphate[77], for instance. The use of bioceramics has been enlarged from the employment of the material to other uses, such as in the coating of metal prosthesis or in combination with polymeric materials such as collagen[75], policapralactone (PCL)[78], among other materials.

Bioceramics induce a specific biological activity, such as hydroxyapatite [$Ca_{10}(PO_4)_6(OH)_2$][79], tricalcium phosphate [$Ca_3(PO_4)_2$][77], bioglasses[80] and gypsum[81].

Different methods are used for production of bioceramics, which involve different stages of synthesis[82-86]. A point to emphasize is the sintering at temperatures than can reach 1000 °C thus leads melting of the particles in the surface, causing them to agglomerate and form a solid dense block. This procedure results in ceramics with pores size in the micrometer (μm) range including some interesting materials used in bone implants[87,88].

Hulbert in 1970[89] showed that pores greater than 100 μm promote bone growth through the material. The pore size for cell colonization in bioceramics is around 100 μm. This pore size enables flow of growth factors as well as cell adhesion and proliferation, thus allowing new bone formation and developing a capillary system connected to the ceramic implant[90].

To obtain ceramics with pores of this size 100 μm, the techniques commonly used consist in mixing a polymer or organic substance with the ceramic powder, which is subsequently eliminated during thermal processing, or wetting the powder with a volatile material that later decomposes with the release of gas forming the pores[91].

β-TCP has been widely used in bone tissue engineering due to its superior oseteoconductivity, cellular adhesion, and mediation in accelerated differentiation[92]. Furthermore, β-TCP is more quickly degraded than crystalline hydroxyapatite[93]. However, β-TCP has low mechanical strength. On the other hand, biocompatible synthetic or natural polymers such as poly (L-lactide acid) (PLLA), poly (lactic-co-glycolic acid) (PLGA) and collagen have been also used as biomaterials for scaffold construction due to their biodegradability, non-biotoxic characteristics, and moderate mechanical and physical properties[94,95].

4.3. Polymers

Ceramic scaffolds have low mechanical strength and weakness in its handling[96]. Polymers might be added to the ceramic to improve these characteristics. Polymers can be incorporated into these 3D structures, or even by coating the scaffolds in the production process, Collagen (COL), hyaluronic acid (HA) and poly(lactic acid) (PLA) are common polymers used for this purpose.

COL is the most abundant protein in the human body, corresponding to about 30% of the body's proteins. Its function is to maintain the integrity of tissue structures and give strength to various tissues and organs. Collagens differ in amino acid composition in each molecule and structural

arrangements[97]. Type I collagen is the most found in blood vessels, skin, tendons and bones. Collagen on tissue is organized into fibers that are linked by hydrogen bonds, electrostatic interactions and is hydrophobic[97].

HA is composed of two sugar molecules (D-glucuronic acid (known as uric acid) or DN-acetyl glucosamine), thus being considered a glycosaminoglycan[98,99]and is a basic component of the ECM, which can be found in most tissues and body fluids, such as synovial fluid[100]. HA is a biodegradable, hydrophilic, and high molecular weight (105-107 Da) polymer, which acts as a scaffold. It also has important biological functions, such as adhesion control, mobility, differentiation and cell proliferation[100].

PLA is produced from lactic acid polycondensation or by ring-opening polymerization of the lactide cyclic dimer. It was first synthesized by Carothers in 1932[101,102]. PLA can be degraded by non-enzymatic hydrolysis and its by-products are eliminated through normal cell metabolism[103]. PLA is an ideal candidate for implantable devices because it presents important characteristics such as cytocompatibility and biodegradability[103,104]. The regulation of PLA-based devices by the Food and Drug Administration (FDA) has raised further interest in the use of PLA in the field of tissue engineering. Tissue engineering aims to restore, sustain, or improve tissue function through the combination of three components: scaffolds, bioactive molecules, and/or cells[1]. PLA has a chirality of lactic acid (L- and D-lactic acid) that can be leveraged to synthesize PLA with different stereoregularities. Stereoregularity influences the physicochemical properties of the material, such as mechanical and thermal properties and degradation characteristics. Consequently, PLA has been widely used in tissue engineering applications, both as scaffolds and as drug delivery systems[105-108].

The development of biomaterials that mimic the environment in which it will be implanted is in continuous research over the years. Different materials and several characteristics must be attributed in this development and the combination of materials is always a possibility, which tends to approach the native environment.

4.4. Bone morphogenetic proteins

Bone morphogenetic protein 2 (BMP-2) can promote healing in bone defects. This molecule stimulates the activity of osteoblasts and other cells to promote bone formation. The release of recombinant human bone morphogenetic protein 2 (rhBMP-2) to the local tissue should be controlled by delivery from osteoconductive biomaterials[109].

Short BMP-2 treatment stimulated the expression of a panel of factors in hASCs that may play a role in the conditioning of the environment to facilitate bone repair *in vivo*. Short incubation with BMP-2 may thus be a promising treatment to enhance both osteogenic differentiation of stem cells as well as modulation of the wound environment[110].

BMP-2 incorporated in gelatin sponge with calcium beta-tri-phosphate accelerated a bone rule in *in vivo* tests. The *in vivo* test was monitored for 16 weeks by radiography and historical analysis, where the results are demonstrated in a regeneration of the host tissue[111].

The effect of nano-scaled surface on the adsorption and bioactivity of BMP-2 were investigated with a series of hydroxyapatite surfaces (HAp). This study indicated that the nano-scaled HAPs had obvious influences on the conformation and availability to BMPs of the adsorbed rhBMP-2, which in turn governed the bioactivity of rhBMP-2[112].

A hybrid of chitosan-xerogel membrane was combined with bone morphogenetic protein-2 (BMP-2) for enhancing bone regeneration. The ability of the hybrid membrane as a BMP-2 delivery carrier and its biological properties were examined by *in vitro* and *in vivo* tests, in comparison with the pure chitosan membrane. According to the results, the hybrid membrane exhibited higher affinity for BMP-2 than the pure chitosan membrane, thereby greatly improving its cellular responses. Moreover, the *in vivo* test demonstrated that the healing process was accelerated by the hybrid membrane with BMP-2[113].

Scaffold porous TCP for bone tissue engineering used a chitosan/BMP-2 coating on the surface of the scaffold, which has a good biocompatibility and osteoconductive activity. There was appreciable bone tissue formation and in growth for rhBMP-2-loaded ceramics 3 weeks after implantation. Thus, this combination could be considered an interesting approach for bone tissue engineering application[114].

In a pilot study using rabbit calvarial defect model, more new bone formation was detected in rhBMP-2 impregnated groups. In another pilot study, new bone formation was increased in time-dependent manner after the graft of BCP (calcium biophosphate) and BCP-collagen composite impregnated with rhBMP-2. BCP with 30% hydroxyapatite (HAp) showed a faster resorption rate than BCP with 20% HA. At 8 weeks, BCP-collagen composite with 30% HAp formed more new bones than BCP-collagen with 20% HAp and BCP-collagen composite showed more new bone formation than BCP without collagen. From the results of these two pilot studies, it was concluded that rhBMP-2 played positive roles in new bone formation. Moreover, BCP-collagen composite block bone showed a superior bone forming capacity on early stage and BCP-collagen with 30% HAp could be more appropriate for rhBMP-2 carrier than the others[115] (Figure 4).

Dadsetan et al. evaluated the role of calcium phosphate coating and simultaneous delivery of recombinant human bone morphogenetic protein-2 (rhBMP-2) on the *in vivo* bone regeneration capacity of biodegradable, porous poly(propylene fumarate) (PPF) scaffolds. *In vivo* bone regeneration was analysed by implantation of scaffolds in a critical-sized rabbit cal- varial defect loaded with different doses of rhBMP-2. The data demonstrated that scaffolds with each of the calcium phosphate coatings were capable of sustaining rhBMP-2 release and retained an open porous structure. After 6 weeks of implantation, micro-computed tomography revealed that the rhBMP-2 dose had a significant effect on bone formation within the scaffolds and that the SBM-coated scaffolds regenerated significantly greater bone than BCP-coated scaffolds. Mechanical testing of the defects also indicated restoration of strength in the SBM and b-TCMP with rhBMP-2 delivery. Histology results demonstrated bone growth immediately adjacent to the scaffold surface, indicating good osteointegration and osteoconductivity for coated scaffolds. The results obtained in this study suggest that the coated scaffold platform demonstrated a synergistic effect between calcium phosphate coatings and rhBMP-2 delivery and may provide a promising platform for the functional restoration of large bone defects[116].

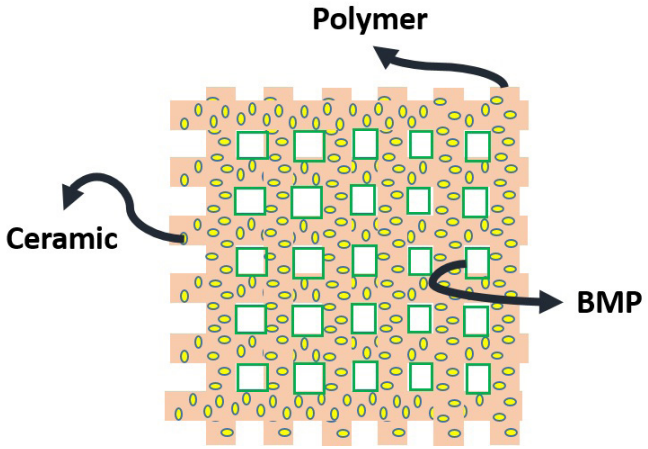

Figure 4. Scaffolds for rhBMP-2 carrier.

5. Conclusions

The human bone features are specific and peculiar to each bone. The bone composition consists of an inorganic part, apatite, and an organic substances, collagen fibers and cells, enabling specific production of structures. Bone regeneration occurs along with different biological pathways involved. If these processes are not in tune, they will regenerate the tissue inappropriately. Tissue engineering then arises to aggregate structures, which enable the regeneration of injured bone tissue effectively. Different techniques and materials, are in use to produce scaffolds in the regeneration of injured bone tissue. Using additive manufacturing, it is possible to customize these structures for use at the injured site. Natural and synthetic polymers are commonly employed, as well as materials that stimulate osteointegration, such as hydroxyapatite and β-TCP, eventually including growth factors. The prospect of application of this technique is very promising, as 3D-printing equipment can be easily installed in existing structures to permit customized solutions for tissue engineering.

6. Acknowledgments

The authors want to acknowledge the financial support from Brazilian agencies CAPES and CNPq.

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