Chapter 3

Bioceramic Scaffolds

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Additional information is available at the end of the chapter

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Abstract

Millions of peoples in the world suffer from their bone damage tissues by disease or trauma. Every day, thousands of surgical procedures are performed to replace or repair these tissues. The availability of these tissues is a big problem, and their costs are expensive. The repair of these defects has become a major clinical and socioeconomic need with the increase of aging population and social development. The emerge of tissue engineering (TE) is considered as a glimmer of hope to contribute in solving this problem. It aims at the regeneration of damaged tissues with restoring and maintaining the function of human bone tissues using the combination of cell biology, materials science, and engineering principles. In this chapter, the current state of the tissue engineering in particular bioceramic scaffolds was discussed. Concept of tissue engineering was explored. Bioceramic scaffold materials, their processing techniques, challenges taken into consideration the design of the scaffolds, and their in-vitro and in-vivo studies were highlighted. The scaffolds with extra-functionalities such as drug release ability and clinical applications were mentioned.

Keywords: bioceramics, scaffolds, classifications, processing, in-vivo, in-vitro, applications, challenges

1. Introduction

Bone defects and its functional disturbance have become a huge health care problem in the worldwide [1, 2]. The repair of these defects has become a major clinical and socioeconomic need with the increase of aging population and social development [3, 4]. Every day, thousands of surgical procedures are performed to replace or repair tissue that has been damaged through disease or trauma. The availability of these tissues is a big problem and their cost is expensive. The emerge of tissue engineering (TE) is considered as a glimmer of hope to contribute in solving this problem. It aims to regenerate damaged tissues. Through this approach, the regeneration of damaged tissue is achieved by combining cells from the human body with highly porous

biomaterial scaffolds. These biomaterials scaffolds are metal, polymer, or ceramics which act as templates for growing the new viable tissues [5]. The suggested materials for tissue engineering (scaffolds) must interact with cells and culture media from the in-vitro stage to their implantation. They have to (i) host a sufficient amount of cells and (ii) support their viability for several weeks. Like any implanted biomaterials, there are some characteristics that must be found in the ideal scaffolds such as: (1) they are porous structure to allow cell penetration, tissue in-growth, (2) biocompatible, i.e., compatible without causing any toxic reactions or an inflammatory response, (3) bioactive, i.e., form strong bonding with the host bone, (4) has sufficient mechanical properties. A major difficulty in the design of scaffolds is to simultaneously tailor these requirements due to their competing nature in fulfilling host tissue demands. Namely, if a specific requisite is achieved, another one is negatively affected. It can be said, although some scaffolds have a highly porous structure with interconnected pores and have features like degradability, biocompatibility, and bioactivity, they cannot be used under a heavy load because of their poor mechanical properties. Thus, the biomaterials scaffolds with suitable mechanical properties, bioactivity, biocompatibility, and biodegradability in order to become the bottleneck. Therefore, recently many researchers have tried to find new solutions that tackle this bottleneck for achieving abovementioned requirements. They have tried to achieve that through many ways for example, (i) proposing new biomaterials like bioceramics, or composite based bioceramics (bioceramic-polymers composites, or bioceramic metal composites), (ii) using new processing techniques or developing the current processing techniques [6–9].

To verify the bone job of TE tissue engineering in restoring and maintaining the function of human bone tissues using the combination of cell biology, materials science, and engineering principles, the scaffolds must have the following criteria:

Biocompatibility: The biocompatibility of the scaffolds means that their ability to perform as the 3D substrates that will have surface chemistry (with the facilitation of molecular and mechanical signaling system) to promote cell adhesion, proliferation, and migration in vitro [10]. And after implantation, the scaffold must not induce any undesirable immune reaction that may reduce healing or cause rejection by the body [11].

Biodegradability: The gradual degradation of scaffolds helps to make space for new growing tissues to deposit their own matrix and hence avoids the necessity of second surgery to remove the implant [12]. Thus, it is one of the crucial factors for scaffolds. The degradation of an ideal scaffold must occur with time in-vivo, and its rate must proportional to the rate of the tissue formation. The biodegradation products should be nontoxic to other tissues in-vivo.

Bioactivity: Stimulation of rapid tissue attachment to the implant surface (without formation of fibrous tissue) and creation of a stable long-term bonding that prevents micromotion at the interface and the onset of an inflammatory response [13].

Structural requirements: An ideal scaffold should have void volume for vascularization, neo tissue formation and remodeling, necessary to facilitate host tissue integration on implantation [14]. Biomaterials should be processed to provide a highly porous structure with interconnected porosity for transporting oxygen, nutrients, and waste metabolites in and out of the scaffold without significantly compromising the mechanical stability of the scaffold [14]. If a scaffold has too small pore size, it may enact the cells to penetrate the scaffold initially and

subsequently to migrate through these pores to the other regions of the scaffolds. But, if it has too large pore size, it may inhibit the effective neo-tissue regeneration by disabling the cells to bridge pores during cell proliferation [13].

Manufacturing technology/commercialization potential: The fabrication technique of the scaffolds is a crucial factor in the production of TE scaffolds. It is a challenge to produce a large quantity of scaffolds at a relatively low or reasonable cost, i.e., to be easily offered to the market [13, 14].

This chapter defines the current state of tissue engineering regarding bioceramic scaffolds. In addition, the complexity of this field. In other words, the following items will be highlighted in details:

It discusses

- Concept of tissue engineering
- Bioceramic scaffold materials
- Processing techniques
- Challenges
- In vitro and in vivo studies of bioceramic scaffold materials
- Scaffolds with extra-functionalities such as drug release ability
- Clinical applications

2. Concept of tissue engineering

Over 8 million surgical operations for treating the organ failure or tissue loss are performed annually in the US [15]. Despite of the success made by the organ transplantation and reconstruction surgery in the life quality, and in some cases the life save, there are still problems associated with the patients. These operations, in most cases, need either organ donation from donor individual or tissue transplantation from a second surgical site in the individual being treated. The generated problems from the use of organ transplantation are the drastic shortage of donor organs. For example, in 1996, 20,000 donor organs were only available, and the number of patients waiting the organs were 50,000. This means that patients are more likely to die while they are waiting for a human donor than in the first 2 years after transplantation [15]. The problems associated with the second surgical sites are pain and morbidity. Accordingly, organs development, tissues, and synthetic materials outside of the body ready for future transplant use have emerged [5, 15–21]. The estimated market of these products is approximately $5 billion worldwide [16].

Tissue engineering may be defined as the application of biological, chemical, and engineering principles toward the repair, restoration, or regeneration of living tissue by using biomaterials, cells, and growth factors alone or in combination. In the early 1990s, the emerged tissue engineering started to address the limitations of tissue grafting and alloplastic tissue repair. The concept was to transplant a biofactor like cells, genes and/or proteins within a porous degradable material known as a scaffold. The biofactors are used to stimulate tissue repair. They include

stem cells and gene therapy approaches. The tissue/organ repair has been considered the ultimate goal of surgery from ancient times until now. Generally, repair is achieved through two approaches: (i) tissue grafting and organ transplantation and (ii) alloplastic or synthetic material replacement. Since 2000 BC gold has been used in cranial defects as repair; however, the grafting of the tissue has been used at the earlier of 1660s. Both approaches mentioned above have limitations. The grafting needs second surgical sites with associated morbidity and is confined by finite amounts of material, especially for organ replacement. One of the efforts made to solve the problems associated with the use of the autologous allograft, and bone cements is the finding appropriate materials to replace lost or missing tissues from the human body [22, 23].

The definition of 'Tissue Engineering' term according to the NSF workshop held in 1988 is "the application of principles and methods of engineering and life sciences toward the fundamental understanding of structure-function relationships in normal and pathological mammalian tissues and the development of biological substitutes to restore, maintain, or improve tissue function" [24]. This definition hold some promises such as (1) driving out the re-operations by using biodegradable biological substitutes, (2) encourage the use of biological substitutes as a natural regeneration process to repair or replace lost or damaged tissues i.e. to provide long term solutions, (3) solving the generated problems from the immune rejection of implants, infections or diseases transmission pertinent allografts and xenografts, and organ donation shortage, (4) offering practical solutions for currently untreatable cases [25, 26].

Since emergence of tissue engineering in the mid-1980s, it has continued to evolve as an exciting and multidisciplinary field that aiming to develop biological substitutes to restore, replace, or regenerate defective tissues. Key component of tissue engineering are cells, scaffolds, and growth-stimulating signals which are generally referred to as the tissue engineering triad (**Figure 1**) [27]. Porous 3D scaffolds are generally seeded with cells and occasionally with signaling molecules or subjected to biophysical stimuli in the form of a bioreactor [28]. These cell-seeded scaffolds are either subjected to a pre-implantation differentiation culture in-vitro to synthesize tissues and then transplanted or are directly implanted into the injured site, using the body's own systems, where tissue regeneration is induced in-vivo [11]. These approaches with porous scaffolds are shown in below by **Figure 2** [29].

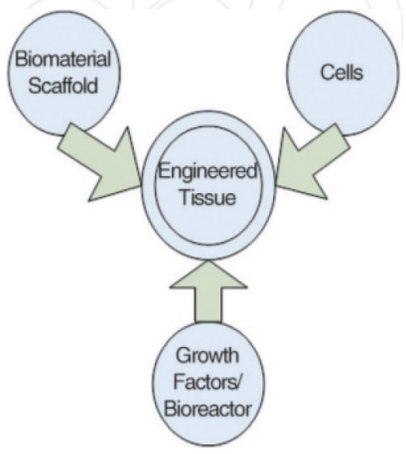

Figure 1. Tissue engineering triad [11].

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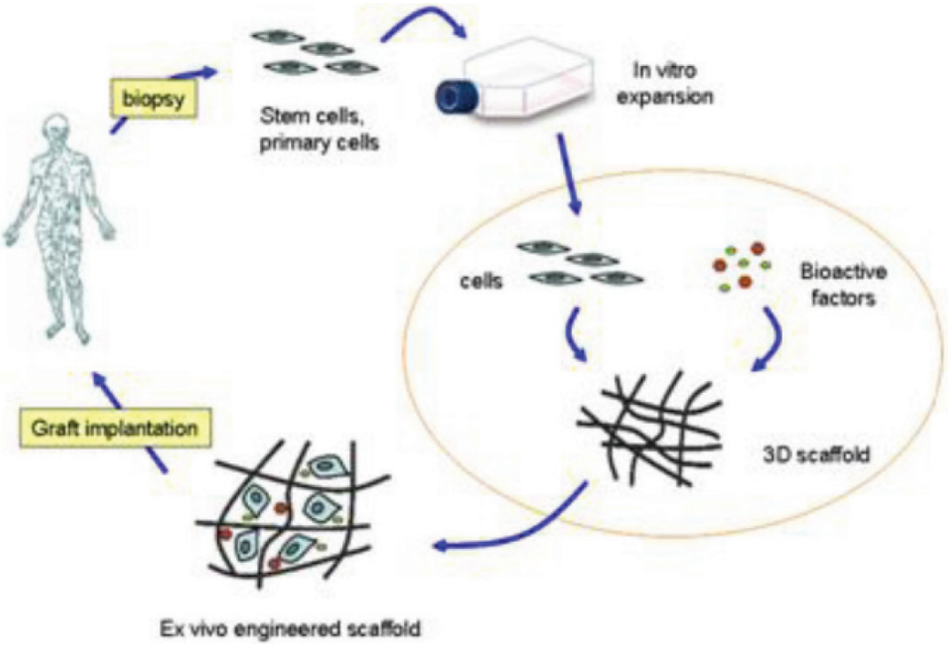

Figure 2. Tissue engineering approaches with porous scaffolds [30].

3. Bioceramic scaffold materials

Today, tissue engineering has emerged as a rapidly expanding approach to overcome the drawbacks of the classical treatments by regenerating damaged tissues, instead of replacing them [11, 30]. This approach leads to the development of biomaterials to prepare porous 3D scaffolds as biological substitutes to restore, maintain, or improve defective tissues [14]. Various materials have been proposed for tissue engineering including different types of biomaterials (metal, polymers, and ceramics) to overcome the problems associated with natural bone grafts in reconstructive surgery. Although porous metallic scaffolds are considered as the most suitable implants for hard tissue engineering in load bearing areas, they have some limitations such as (1) lacking of biological recognition or bioactivity [31, 32], (2) lacking of the integration of biomolecules [33], (3) nonbiodegradable [33], (4) releasing of toxic ions [33], (5) corrosion or wear, and (6) The architecture control [34]. One of the essential requirements for using a biocompatible metal in tissue engineering scaffold is the surface modification by coating with bioactive ceramic materials, where it reduces some of the limitations of metallic scaffolds.

Another primary materials studied mostly to fabricate scaffolds are polymers such as polylactic acid [35, 36], polyglycolic acid [37], polyurethane [38], and a number of copolymers [39–41]. Natural polymer-based scaffolds have excellent bioactivity, biodegradability but poor mechanical properties. These characteristics reveal their successful use in soft tissue engineering and limit their use in the load bearing applications. Moreover, there is still immunological concern associated with naturally derived polymers. An additional problem limits the application of the natural polymeric materials scaffolds, there are still a question mark on their structure homogeneity and reproducibility [11, 35–37, 42–48]. Metal and polymer drawbacks

mentioned above lead to emerging of a new type of materials prevent the production of the wear debris and can be designed to more closely match the material properties of natural bone. Such materials must be mechanically stronger than polymers and play a critical role in providing mechanical stability to construct prior to synthesis of new bone matrix by cells. The materials nominated to fit this purpose are called bioceramics.

Ceramic scaffold possesses many aspects like being bioactive, biocompatible, biodegradable, mechanically stiff (Young's modulus) [49], less elastic and brittle. They also exhibit shaping difficulties. Bioceramics can be classified into three groups as given in the following **Table 1** [50]:

Groups	Phases
1. Bioinert	e.g., Aluminum oxide (Al_2O_3) and zirconium oxide (ZrO_2).
2. Surface bioactive	e.g., Sintered hydroxyapatite(s-HA) at high temperature, bioglass
3. Bioresorbable	e.g., Sintered hydroxyapatite(u-HA) at low temperature, tricalcium phosphate (α-TCP and β-TCP), tetracalcium phosphate (TTCP), octacalcium phosphate (OCP).

Table 1. Bioceramics classification.

Later group (3) is used in bone tissue engineering, various calcium phosphates (CaPs) specially HA, β-TCP, and biphasic calcium phosphate, BCP (mixture of HA and β-TCP) has long been studied as porous scaffold materials. As natural bone composed of large amounts of HA ($Ca_{10}(PO_4)_6(OH)_2$), so it might be useful to use HA, β-TCP as they closely simulate the chemical and crystalline nature of the mineral phase of the native bone [51, 52], and hence, they will be biocompatible. Hydroxyapatite (HA) is known by its bioactivity, biocompatibility, nontoxicity, noninflammatory, osteoconductivity, and biodegradability. By its comparison with β-TCP, it degrades slowly in following order: OCP > α-TCP > β-TCP > u-HA> > s-HA [53] and after implantation, it undergoes little conversion to a bone like material [54]. For the same porosity, β-TCP scaffolds often exhibit lower mechanical strength than HA scaffolds, limiting their use in the load bearing applications [55]. The degradation rate and other properties can be influenced by varying HA to β-TCP ratios in BCP. Interestingly, researchers have shown that dopant addition in the scaffolds of CaPs can control the biocompatibility, densification behavior, dissolution rates, and mechanical strength [33, 56].

Recently, calcium phosphates containing materials used for tissue engineering are nominated as bioactive glasses, silicate bioactive glasses, borate bioactive glasses, phosphate bioactive glasses, and akermanite. Each one will be explained as the following:

Bioactive glasses have already shown their excellence as promising biomaterials for tissue engineering due to their ability to enhance bone cell growth, bonding to both hard and soft tissues [27], ability to restore defect sites and controllable degradation rate in vivo. Glass compositions and of the scaffolds and their microstructure play an important role in the determination of the degradation rate and conversion to an HA-like material, mechanical properties, and response to cells.

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Recently, doped bioglasses with various elements, such as Cu, Zn, and Sr, promote the healthy bone growth that have been developed [58]. These types of the bioglass showed an enhancement of angiogenesis (formation of blood vessels) [55, 59] and soft tissue wound healing [55]. And this capacity of bioglasses has provided an alternative approach to the use of expensive growth factors for stimulating neovascularization of engineered tissues [55].

45S5 glass has long been established as highly bioactive, biocompatible [60], and biodegradable. The composition of 45S5 glass is 45% SiO_2, 6% P_2O_5, 24.5% CaO, 24.5% Na_2O and the low SiO_2 content (<55% SiO_2), high content of network modifiers like Na_2O and CaO, high CaO/P_2O_5 ratio contributes to the bioactivity of 45S5 glass. The immersion of this form of glass is body fluid, and it forms HCA layer (carbonate substituted HA, typical bone composition) on its surface that significantly promotes osteoblast activity. However, there is a difficulty in the processing of this glass into a porous 3D scaffold due to its low mechanical strength, slow degradation rate, and conversion to an HA mineral [52]. Recently, it was found that by heating this type of glass to high temperatures (>950°C), its phases crystallize with strong mechanical strength and become bioactive glasses. In addition, it converts to a biodegradable, amorphous calcium phosphate at the body temperature, and in a biological environment [61]. This process enables the mechanical competence and biodegradability to be incorporated in a single scaffold, making it promising as tissue engineering scaffold [51].

Borate bioactive glass: Researchers have indicated that borate or borosilicate bioactive glasses promote cell proliferation and differentiation in vitro, as well as tissue infiltration in vivo [55]. Borate bioactive glasses degrade as faster as than 45S5 glasses. They completely convert to an HA-like material because of their lower chemical stability [62]. The degradation rate can be controlled by manipulating the glass composition [62, 63]. Besides, there is a concern about the toxicity of boron released into the solution as borate ions $(BO_3)_3$ [55].

Phosphate Bioactive glass: [55] It forms networks, where CaO and Na_2O act as network modifiers. It shows a chemical affinity toward bone due to the existed ions in the organic mineral phase of the bone. The degradability of this glass can be controlled by modifying their composition. Its flexibility displayed has made it potential resorbable biomaterials for tissue engineering.

Akermanite ($Ca_2MgSi_2O_7$): Recently, it has received more attention due to its controllable mechanical properties and degradation rate [64, 65]. In previous studies, marrow-derived or adipose-derived stem cells and osteoblasts have displayed good activities of proliferation and osteogenesis on akermanite compared by β-TCP [66–70]. The recent studies suggest that this Mg-containing silicate ceramic as a bone graft material may meet the requirement of bone regeneration than b-TCP. However, the mechanism of akermanite's bioactivity is still unknown. The materials chemistry of biomaterials is one of the main factors in the proliferation and differentiation of various cells.

In tissue engineering, biomaterials play a critical role. They act as a 3D template, supply mechanical support and allow artificial extracellular matrix environment (ECM) for neotissue formation. This means that one type of biomaterials is not sufficient to compromise hard and soft tissue engineering. Therefore, each type from the biomaterial types such as metals, ceramics, and polymers has its own importance in making tissue engineering scaffolds. Therefore, the composite materials have been emerged. Sometimes biocompatibility

and biodegradability of ceramics are not sufficient. Moreover, ceramics are very brittle, and too stiff, while the polymers are found to be biocompatible and biodegradable with low mechanical strength. So, this biological and mechanical mismatch can be overcome by blending the ceramics with natural or synthetic polymers or metal. Recently, various composites were explored such as synthetic polymers/natural polymers, synthetic polymers/bioceramics, polymers/metals, metals/ceramics, …etc. However, novel metal/polymer/ceramic composites have also been suggested for load bearing applications [34]. Composite materials are necessary approach to obtain optimal biological, structural, mechanical, and chemical properties of scaffolds. Thus, bioceramics/polymers are commonly used composites.

Finally, needless to say, yet, there exists no polymers or metals that can effectively bond to bone. On the other hand, it does not exist ceramic materials that can sufficiently in mechanical properties. Therefore, composites of biodegradable polymers and hard metals with bioactive ceramic composites are still a promising approach.

4. Processing techniques

It is a key point to obtain porous structure with proper mechanical properties to create a microenvironment for cell adhesion and proliferation. Nature bone has multi-level 3D pore structure size ranging from several nano to hundreds of micrometers [71]. This level of pore sizes meets the requirements of a tissue growth. Pore sizes in the range of 150–800 μm prevent the growth of the bone tissue and the vessels of the blood. However, pore sizes in the range of 10–100 μm are useful for the growth of the blood capillaries, nutrients exchange, and waste products excretion. Nano pores are larger specific surface area and more active targets. They are good for the formation of apatite and the attachment of protein or osteoblast [72]. Meanwhile, they are also important for the adjustment of cell adhesion and proliferates.

Many fabrication techniques are available to produce ceramic scaffolds with varying architectural features. There are two main types of fabrication techniques: conventional techniques and advanced techniques. Conventional techniques for the fabrication of porous structure mainly include replica; sacrificial template; and direct foaming as seen in **Figure 3** [3].

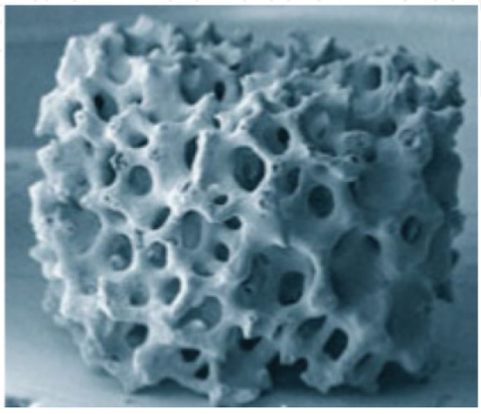

Figure 3. Calcium phosphate–based scaffold (Willis [57]).

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The replica technique employs a synthetic or natural template that is impregnated with a ceramic suspension. After drying, the template is removed thus creating a replica of the original template structure [73]. Many synthetic and natural cellular structures can be used as templates to fabricate macroporous ceramics through the replica technique [74].

The sacrificial template method incorporates some sort of pore former or sacrificial material to act as a place holder within the ceramic powder or slurry. Once the green body is formed, the pore former is removed to leave behind pores which are empty (**Figure 1(b)**) [73]. This method leads to porous materials displaying a negative replica of the original sacrificial template, as opposed to the positive morphology obtained from the replica technique described above [74].

Direct foaming is a process where gas bubbles are incorporated into a ceramic suspension, and once the slurry is set and dried, the ceramic retains the resulting spherical pores (**Figure 4(c)**) [73]. To obtain high-strength ceramic foams, the dried objects are then sintered at high temperatures. The total porosity of the obtained foam is proportional to the amount of gas incorporated into the suspension or liquid medium during the foaming process. The sizes of the pores depend on the stability of the wet foam before setting [74].

Freeze casting is considered as one of the promising techniques for manufacturing of porous structure. It utilizes growing ice crystals in a ceramic slurry to form the pores in a ceramic

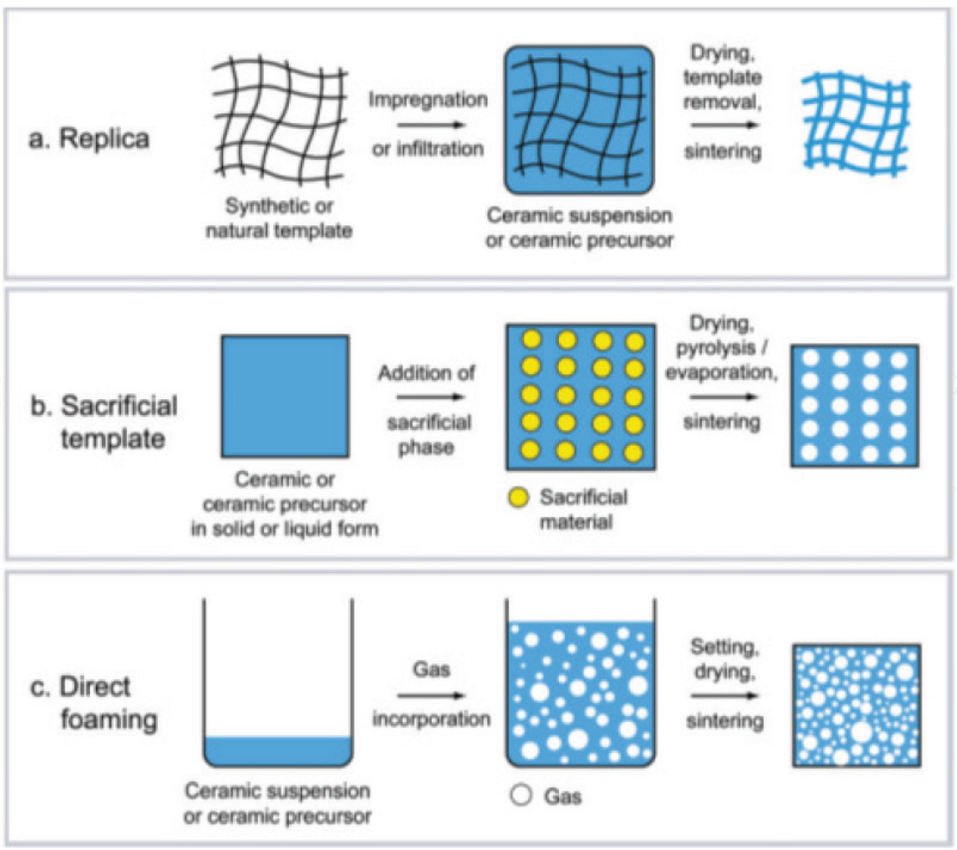

Figure 4. Scheme of possible processing routes used for the production of macroporous ceramics [77].

body. It is a simple technique to produce porous complex-shaped ceramic or polymeric parts. It has first been developed as a near net shape forming technique, yielding dense ceramics parts with fine replicate of the mold details. In this technique, a ceramic suspension is poured into a mold and then frozen. The frozen solvent acts as a temporary binder to hold the part together, see **Figure 5**. The de-molded part is subjected to freeze drying to sublimate the frozen solvent under vacuum, avoiding the stresses and shrinkage that might lead to cracks and warping during normal drying. After drying, the parts are sintered to obtain a scaffold with (1) a complex and often anisotropic porous microstructure and (2) proper strength and stiffness. By controlling the direction of ice crystals growth direction, it is possible to tailor a preferential orientation for the porosity in the ultimate products [75].

Human cortical bone has a compressive strength of 100–150 MPa and toughness of 2–12 MPa $m^{1/2}$, while human trabecular bone has a compressive strength of 2–12 MPa and toughness of 0.1–0.8 MPa $m^{1/2}$. The question is: How can design ceramic scaffolds and mimic the structure and properties of natural bone as closely as possible? This means the scaffold fabrication must be carried out with high accuracy taking into consideration the effective and functional properties such as the microstructure, mechanical properties as well as the biocompatibility [73].

In some cases, it is difficult to achieve the targeted pore for tissue growth such as interconnectivity, pore size, and pore geometry when traditional processing techniques are used. The limited control over the pore characteristics generates closed pores and leads to lack of interconnecting pores. In addition, it gives low strength and variable properties. Recently, additive

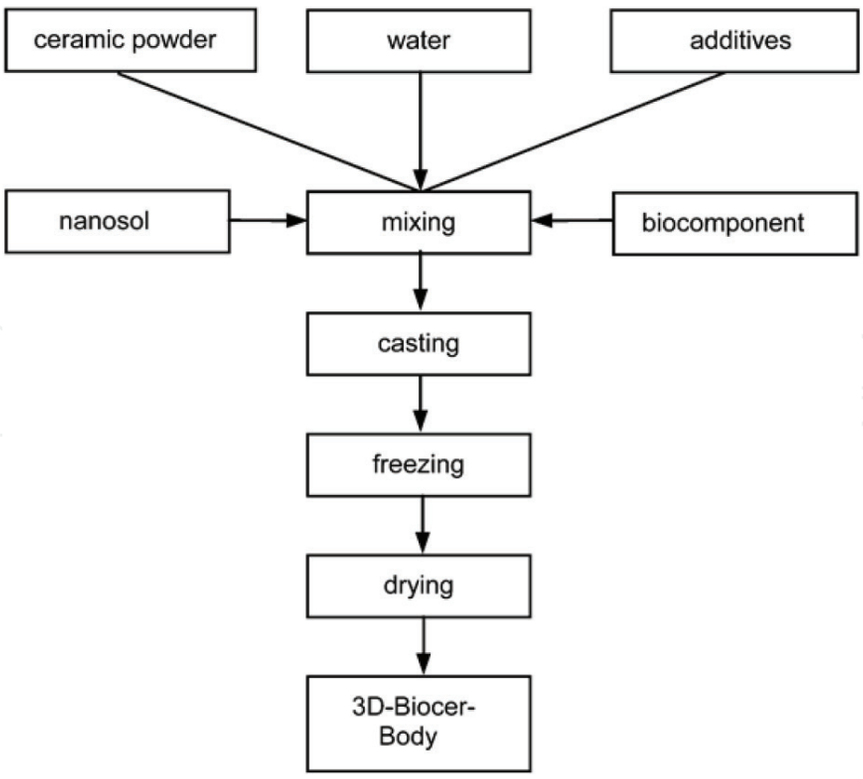

Figure 5. Freeze cast process for bioceramic preparation [78].

manufacturing (AM) techniques, the rapid prototyping (RP) and electrospinning, have been proposed for the fabrication of porous ceramic scaffolds. They have several potential benefits over traditional techniques [73].

The rapid prototyping (RP) techniques: RP techniques are referred to as a solid free-form (SFF) manufacturing. They are precise and reproducible for controlling the internal pore size, porosity, pore interconnectivity, mechanical performance, and overall dimensions of tissue engineering scaffolds [76, 77]. Based on programmed 3D images, they are defined as automated deposition of each tomographic layer sequence into the desired architecture through an additive layer-by-layer method [78]. One of the main requirements for translational applications is a high productivity using automated method and possibility to produce patient-specific constructs; therefore, an RP-based method can potentially be used to fabricate such customized tissues [79]. The benefits of RP technology are numerous, e.g. the versatility of modeling software allows for the fabrication of the desired parts without the need for expensive molds, and the process is usually achieved in only a few steps. It could easily be used in the manufacturing bio-scaffolds with fit needs of a specific individual and match the surrounding bone which may vary from person to another depending on the age, health, condition of the surrounding bone, or location within the intended recipient, see **Figure 6** [73].

The most relevant RP techniques in the design of 3D scaffolds for tissue engineering are 3D printing (3DP), selective laser sintering (SLS), stereolithography (SLA), robocasting (RC), and

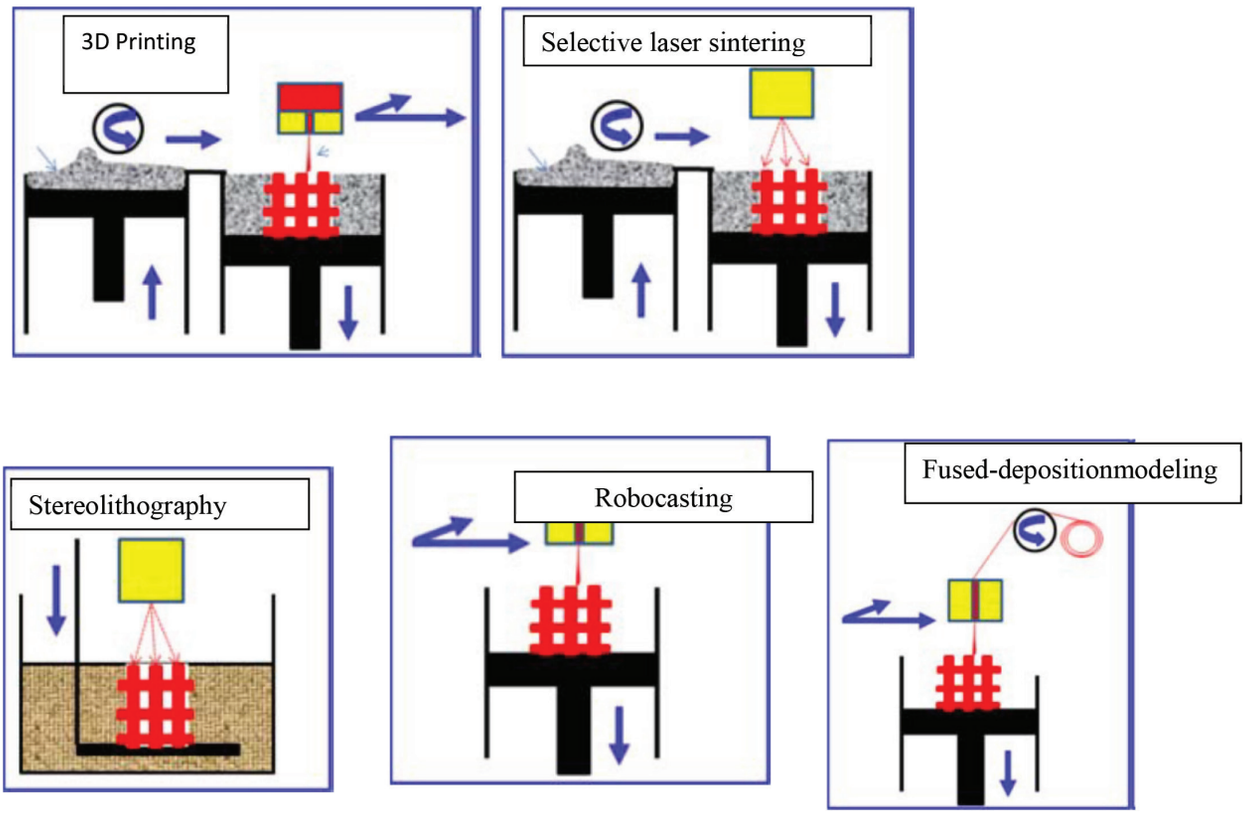

Figure 6. Main RP-based techniques relevant for tissue engineering applications [93].

fused deposition modeling (FDM). **Figure 6** shows a schematic diagram of the processing of each RP based-technique [80].

3D printing (3DP): The inkjet head of this device prints droplets of a binder fluid onto a powder bed. This process is repeated for every layer until the 3D scaffold structure is printed, and the remaining powder is removed. It has been used to create scaffolds for use in bone tissue engineering. One of the important benefits of this method is the powder bed support by itself for each successive layer. The fragility of the obtained parts is considered a drawback [81–88].

Selective laser sintering (SLS): It is a heat dependent. This method uses a CO_2 laser beam to selectively sinter polymer or composite powders to form material layers. The laser beam is directed onto the powder bed by a high precision laser scanning system [89].

Stereolithography (SLA): This technique is light dependent. The laser beam selectively initiates solidification in a thin layer of liquid photopolymer [90].

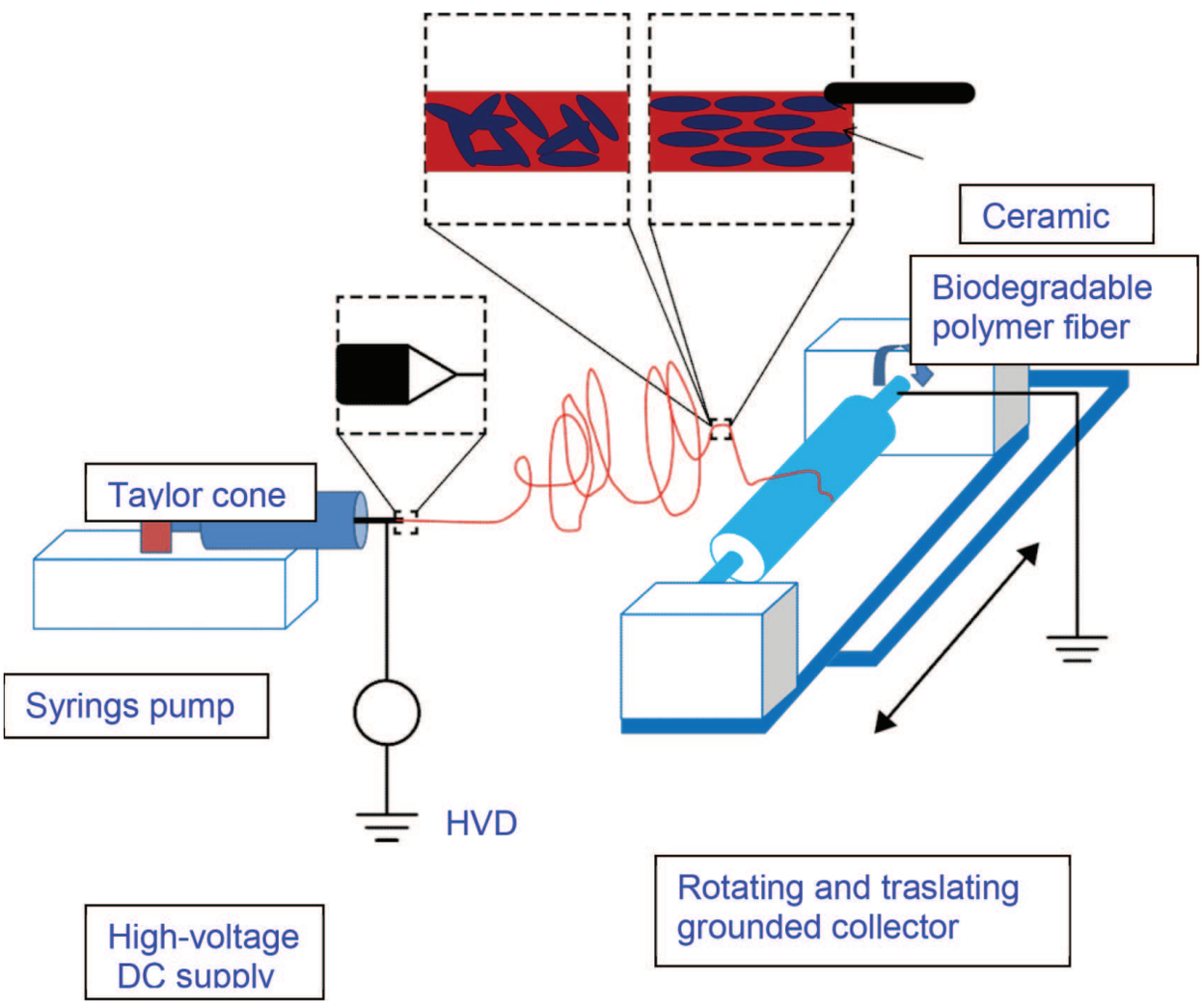

Figure 7. Schematic of a typical electrospinning system.

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Robocasting (RC): This technique is slurry dependent. It consists of the robotic deposition of a highly concentrated colloidal suspension (inks) [91, 92].

Electrospinning: This technique is easy to use. It is used to produce nano to microfibers by subjection of a solution of polymeric materials or ceramic/polymeric composites to an electric field. Solid fibers are produced from electrified jets using high voltage. These fibers are continuously elongated because of the electrostatic repulsion between the surface charges and the solvent evaporation, see **Figure 7** [94].

This technique allows obtaining high surface area scaffolds, which simulate the size scale of fibrous proteins found in the natural ECM [95–97]. One of the great interests in this methodology is the capacity to easily produce materials at the biological length scale for tissue engineering and drug delivery applications [98]. Also, this technique is able to form nonwoven fibrous mats, which ensure fiber production from a broad range of precursor materials including synthetic polymers, natural polymers, semiconductors, ceramics, or their combinations [99, 100]. As mentioned above, the strength and the great merit of electrospinning technology are the ability to conduct fiber size, porosity, and shape using processing variables, such as applied voltage, polymer melt flow rate, capillary/collector distance, polymer/ceramic concentration, and solvent conductivity and volatility [94]. **Figure 7** displays schematic of a typical electrospinning system.

Recently, inorganic nanoparticles, such as HA, bioactive glass, and carbon nanotubes, have been widely co-electrospun into polymer nano fibers to enhance their mechanical properties and their biocompatibility response [101–106]. Although the bioceramic needle oriented in polymer fiber is challenging, but it is very important for enhancing the mechanical properties of the scaffolds.

5. Challenges

The design and fabrication of the synthetic tissue scaffold and the engineering of tissue constructs in vitro and in vivo are big challenges. Various materials like metals, ceramics, natural and synthetic polymers, and even their composites have been explored as TE scaffolds. Bioceramics and polymers are suitable for bone TE, where the native bone composed mainly of a naturally occurring polymer and biological apatite. Since ceramics are brittle and the mechanical properties of the polymers are not sufficient, the applications of these materials are limited, in particular, in load-bearing areas. Although metals have high mechanical strength are suitable for load-bearing applications, but they are bio-inert and their biodegradability are none. Therefore, they are not suitable for soft tissue engineering. Generally, the most important challenge of tissue engineering is to mimic what happens in nature. Attempts are being made to engineer in vitro practically every tissue and organ in the body [11]. In addition, the following items are playing an important role in the properties of the bioceramic scaffolds:

(1) **Macrostructure:** It is important to determine the geometry of the regenerating tissue and in turns to be capable to re-shape it.

(2) **Mechanical properties:** the scaffolds should have sufficient mechanical strength to provide temporary function in a defect until the tissue regenerates. If the mechanical properties

of the native bone are used as a guideline in the scaffolds designing, they must exhibit linear elastic properties with a moduli of hundreds of megapascals and microstructures of preferred orientations due to bone anisotropy. In addition, it is important to know that the scaffold mechanical properties will decrease with the scaffold degradation. Thus, if the scaffolds have sufficient mechanical properties at the time of implantation, the change of their mechanical properties during the degradation could be expected and affected on the function within the tissue defect [107, 108].

(3) **Pore size, porosity and interconnectivity:** The pore size is an important variable to stimulate cell ingrowth and new bone formation [109, 110], while the interconnected porous network and porosity are critical in maintaining spatially uniform cell distribution, cell survival, proliferation, and migration in vitro. Moreover, the scaffold's porosity (exceeding 60%) and degree of pore interconnectivity directly affects the diffusion of physiological nutrients and gases [111, 112]. Interestingly, the pores with smaller sizes than 1 μm are appropriate to interact with proteins and are mainly responsible for inducing the formation of an apatite-like layer in contact with simulated blood fluids. Pores of sizes from 1 to 20 μm are important in cellular development, where the cells are attached and the orientation and directionality of cellular in-growth. Pore of sizes between 100 and 1000 μm are essential to assure nutrient supply, waste removal of cells and promoting the in-growth of bone cells. Finally, the presence of pores of sizes >1000 μm will play an important role in the implant functionality [113, 114].

6. In-vitro and in-vivo studies of bioceramic scaffolds

The in-vitro and in-vivo responses of bioceramic scaffolds are dependent on their composition and their pore architecture (microstructure). Various studies were handled biological response of the bioceramic scaffolds, some of them discussed below.

6.1. In-vitro and in-vivo studies of bioactive glass scaffolds

The ability of bioactive glass scaffolds to support cell proliferation and function in-vitro and tissue ingrowth in-vivo has been shown in numerous studies [115–122]. Fu et al. showed that 13–93 bioactive glass scaffolds prepared using a polymer foam by replica method supported the attachment and proliferation of MC3T3-E1 preosteoblastic cells both on the surface and within the interior pores of the scaffold [115].

Poh et al. prepared and estimated the in-vitro response of two different types of bioactive glass composite scaffolds. They are polycaprolactone with 45S5 glass (PCL/45S5) and strontium-substituted glass with polycaprolactone (PCL/SrBG). These two types of bioactive glasses were incorporated into polycaprolactone (PCL) and fabricated by additive manufacturing technology. The in vitro results showed that the rates of degradation of these scaffolds were PCL/SrBG > PCL/45S5 > PCL scaffolds. It was found that the degradation rate of PCL/SrBG scaffolds was faster than PCL/45S5 scaffolds. This is due to the substitution of Sr^{2+} of larger ionic radius (1.12A°) by Ca^{2+} of lower ionic radius (0.99 A°) leading to the expansion of the silicate glass network [7, 35, 36]. Such expansion weakens the glass network and increases the

dissolution rates of the SrBG. The cytotoxicity test indicated that all scaffolds (PCL, PCL/45S5, and PCL/SrBG) were noncytotoxic and are able to support cell attachment, growth, and proliferation; at day 7 and 14, PCL/SrBG (control and osteo group) show a significantly higher degree of mineralization compared to all other groups (PCL/45S5 and PCL); indicating that PCL/SrBG can stimulate earlier matrix mineralization [123]. Melchers et al. investigated the effect of alumina from 0.5 to 15 mol% in mesoporous bioactive glasses based on composition 80% SiO_2–15%CaO -5% P_2O_5. Sol–gel method in combination with a structure directing agent for the formation of mesopores was used. It was found that the incorporation of Al_2O_3 in a range of 1 to 10 mol% reduces the order of the mesostructure, while the further increase of doped amount of Al_2O_3 to 15 mol% creates well-ordered mesopores again. In addition, pore diameter, pore volume, and specific surface area decrease only slightly on the incorporation of Al_2O_3. In-vitro bioactivity tests of these glasses, a decrease in their bioactivities upon the incorporation of small amounts of alumina was observed, while a sudden drop was noticed beyond the addition of 3 mol% of Al_2O_3. These results back to the strong interaction of Al^{3+} and PO_4^{3-}, which could be proven by multinuclear single and double resonance solid state nuclear magnetic resonance (NMR) spectroscopy [124].

6.2. In-vitro and in-vivo studies of hydroxyapatite scaffolds

Generally, hydroxyapatite (HA) is a material which most often induces osteogenesis both in-vivo and in-vitro. Adding HA to other materials (either natural or synthetic) could, therefore, modulate the osteogenic potential and mechanical properties of the subsequent mixture. Therefore, many papers have been devoted for a combinatorial approach of HA with another supporting material [45]. The choice of supporting material is often paramount. The main reasons of combination of HA with another material are improved strength, increased porosity, altered cell binding abilities, and so forth. These materials are divided to natural materials and synthetic materials. Natural materials (collagen, gelatin, fibrinogen) tend to have good cellular adhesion remodeling properties but can also carry a high risk of immune response. On the other hand, synthetic materials, however, are less immunogenic and more customizable but carry higher risks of toxicity. Furthermore, MSCs can be included in such scaffolds for differentiation to osteogenic lineages and/or implantation for bone defects purposes, although differentiation media are often required. Therefore, HA scaffolds containing MSCs can be used as a combinatorial modality for treating bone disease and degeneration. The combining of stem cells, in particular, MSCs, into the various HA-based scaffolds increases the scaffolds potential use for bone regeneration. Adding the benefits of MSCs immunomodulatory, immune inert, and immune-privileged state to a synthetically or naturally enhanced HA scaffold has demonstrated superior results than the scaffolds alone [125].

In another study, porous chitosan/hydroxyapatite (C/HA) scaffolds were fabricated via freeze-drying with desired pore size. The in-vitro proliferation of Human osteoblasts (hOBs) on the scaffolds were evaluated. Then, these scaffolds were combined with the adenoviral vector encoding vascular endothelial growth factor and green fluorescence protein (Ad-VEGF). In-vivo studies were conducted by subcutaneously implanting inactivated and gene-activated C/HA sponges containing hOBs into the epigastric fasciovascular flaps of Wistar rats. The results show that, in the in-vitro investigation, the adenovirus encoding VEGF gene-activated macroporous C/HA composite scaffold supports transfection of human primary osteoblasts

and bone-like tissue formation. In-vivo findings demonstrate that C/HA + AdVEGF + hOBs promote abundant neovascularization during ectopic bone formation, while viral gene therapy has some drawbacks. Generally, all findings support the notion that gene-activated C/HA scaffold could have potential in vascularized bone tissue engineering [126].

Campos et al. studied the synthesis of three-dimensional (3D) scaffolds composed of 50 wt.%HA and 50 wt.% collagen for bone tissue engineering. Self-assembly method with a 0.125% glutaraldehyde solution as cross-linked was used a synthetic route. The in-vitro evaluations are cytotoxicity using MC3T3 cells, proliferation and differentiation. Proliferation and differentiation were tested using STRO-1A human stromal cells for time up to 21 days. The results show that no cytotoxicity was observed in the scaffold by MC3T3 cells. STRO-1A cells were found to adhere, proliferate, and differentiate on the 3-D scaffold, but limited cell penetration was observed [127].

Porous composite bioceramics of hydroxyapatite and dicalcium phosphate dehydrate (HAp/DCPD) were prepared using polyurethane foam. They were designed for application in osteoconductive and osteoinductive scaffolds. In-vitro and in-vivo examinations were performed to evaluate the biological responses of the prepared porous composites. In-vitro studies were performed by immersion of the samples in SBF. The in-vivo test was conducted by inserted porous composite samples into defects in the medial femoral condyle of rabbits. From in vitro and in-vivo studies, it can be concluded that the scaffolds are biocompatible without inflammation. After implementation, necroses or rejection of the tissue was noticed. The application success of the combined HAp and DCPD scaffolds for generating a new bone tissue is attributed to the merge of the biocompatibility property and the formation ability of a favorable 3D matrix for human osteoblast cells to adhere and spread, taking into consideration the advantage of TCP osteoinduction to the superior bioactivity and osteoconduction of HAp. Finally, the prepared scaffolds seem to be a promising biomaterial for low-weight-bearing orthopedic applications [128, 129].

The obtained data from literature indicate that biphasic calcium phosphate is the optimal cell-supporting material. Biphasic calcium phosphate should be recommended as the most suitable matrix for osteogenic cells expansion and differentiation in tissue engineered systems [130]. I.E. biphasic calcium phosphate (BCP) ceramics composed of HA and ß-TCP with varying HA/ß-TCP ratios have been extensively studied in the past two decades, because they combine the excellent biocompatibility and bioactivity of HA and a degradation rate of ß-TCP that matches the growth rate of newly formed bon.

6.3. In-vitro and in-vivo studies of calcium phosphate cement (CPC) scaffolds

It has been reported by Kent et al. in vitro and in vivo measurements of commercial calcium phosphate cement is considerably stronger in vivo compared to in vitro, the cause of this attributed to bone formation within the cement pores and a high degree of osseointegration [131].

Chen et al., 2013 proved that when hUCMSCs and hBMSCs were seeded onto a CPC scaffold. Then, they implanted into the defects, new bone formation increased with time. Compared with CPC control without cells, 88% and 57% increases in new bone were achieved when hBMSCs and hUCMSCs were seeded with CPC, respectively (see **Figure 8**) [132]. These results confirmed by Zeng et al., 2012, whereas he found when h-BMSCs seeding with CPC, the greater new bone was generated greater than in a model without cells in a rabbit maxillary sinus floor elevation model [133].

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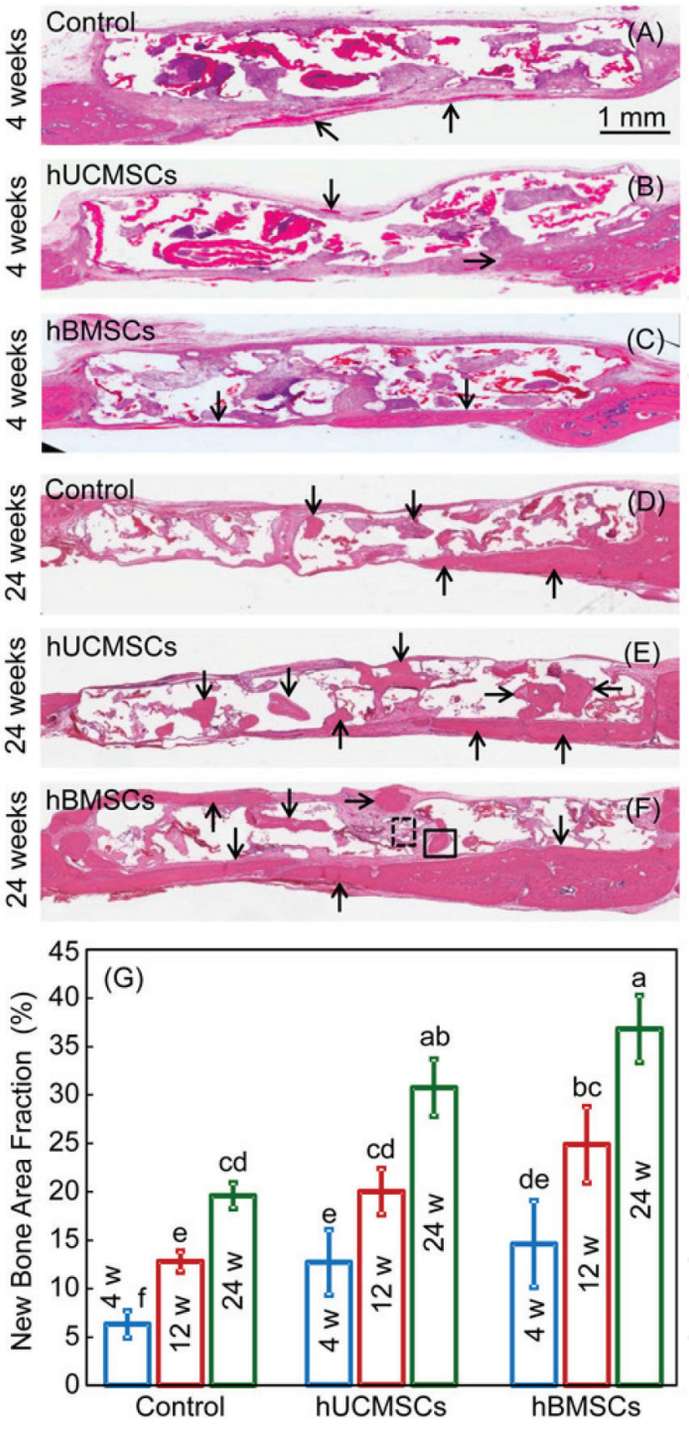

Figure 8. Bone regeneration in critical-sized cranial defects in nude rats with 3 groups (CPC control without cells; CPC with hUCMSCs; CPC with hBMSCs).

7. Scaffolds with extra-functionalities such as drug release ability

Scaffolds are implants or injects, which are used to deliver cells, drugs, and genes into the body. Scaffold matrices can be used to achieve drug delivery with high loading and efficiency to specific sites. This means that the scaffolds must be designed to provide not only the structure integrity required for bone regeneration but also for controlling dose of drug release.

Zhao et al. designed and prepared porous scaffold, which composed of a newly designed polylactone, poly(e-caprolactone)-block-poly(lactic-co-glycolic acid) (b-PLGC) copolymer, and β-tricalcium phosphate (β-TCP). Then, this scaffold was loaded with an antituberculous drug (rifampicin, RFP) to cure serious bone tuberculosis from two points of views (bone regeneration and antituberculous drug therapy). The in-vitro drug release experiment showed that hydrophobic RFP could be released from the b-PLGC/TCP scaffold in a sustained manner for 84 days. Accordingly, RFP concentrations obtained in blood and tissues surrounding the implant could reach a high value in 12 weeks, which was above the effective level needed for the treatment of tuberculosis. The cytological assay proved that the RFP-loaded scaffold has a good cell cytocompatibility. In another trial, it was found that the composite system gave a good regeneration ability for bone. Therefore, the b-PLGC/TCP scaffold can perform a local long-term drug release as well as osteogenesis capability. These achievements are suitable for clinical applications [134].

In another study, Zhu et al. proved that a mesoporous silica nanoparticulate/β-TCP/bioactive glass (BG) composite drug delivery system for osteoarticular tuberculosis therapy much higher antituberculous drugs (rifampicin (INH) and isoniazid (RFP)) loading capacities than pure β-TCP scaffold. The best concentrations of drugs (INH and RFP) for treating tuberculosis (TB) in-vivo can be maintained for an extra-long duration over 42 days without significant long-term lesions to liver and kidney [135].

It has been reported by Kundu et al. that HAp exhibited better drug release than β-TCP when CFS (ceftriaxone sodium and sulbactum sodium in 2:1 w/w ratio) drug was used. HAp and pure β-TCP based porous scaffolds were prepared by applying together starch consolidation and foaming techniques. A bilayered coating was also applied to the pore surfaces of some samples using chitosan and b-lactamase–cephalosporin derivative to assess their effect on sustained drug releasing. The result of bilayered coating of chitosan with CFS provided prolonged release pattern for more than 5 weeks irrespective of the scaffold material, a period that is considered to be sufficient for local drug delivery to combat osteomyelitis [136].

8. Clinical applications

Ceramics include a broad range of inorganic and non-metallic compounds. Although their applications in tissue engineering is recent, they demonstrate good results, whatever they are a single phase or composite. Herein, we will explored the clinical application of the most common examples of bioceramic (HA, TCP, BCP, and bioactive glasses) that are used as in tissue engineering applications.

Hydroxyapatite: By insertion porous HA wedges into the tibias of ten knees in seven patients having high tibial osteotomies, it was found that pores located at the interface, at the time of hardware removal, were completely filled with bone, and the bone depth formed increased consistently with time [137]. In another study, the transverse sections of porous HA implants placed in rabbit tibias demonstrated a new bone growth through the pores. After 8 weeks, a formed concentric lamellae by osteon structure was observed around a single vessel in the

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pores of sizes 50 and 100 μm of the cylindrical HA implants. Also, similar structures were displayed around the multiple vessels in the pores of sizes 300 and 500 μm of the implants [114]. The examination of 103 patients suffering from cranial defects in which Bone SourceTM was used, a success rate of 97% was recorded [138]. Such high record was achieved by the implant maintenance for 24 months.

Tricalcium phosphate (TCP): TCP has become one of the first calcium phosphates to be used in bioceramics for bone substitution and repair. Thanks to its stability at high temperature and ease of processing as tricalcium phosphate–based ceramics 339 ceramics. β-TCP containing ceramics are one of the major bioresorbable synthetic bone that used daily by orthopedic surgeons and dentists. They are used in the form of porous ceramic pieces and granules to reconstruct all kinds of bone defects, from augmentation of alveolar ridge defects after a tooth extraction and before implant positioning to sinus reconstruction correction of various deformities and bone reconstruction following injury or disease. Recently, α-TCP has also been proposed as ceramic materials for similar applications [139].

Tricalcium phosphate/hydroxyapatite biphasic ceramics (BCP): The BCP concept is based on an optimum balance between the more stable phase (HA) and the more soluble phase (β-TCP). BCP bioceramics of various sizes and shapes are used in maxillofacial surgery, dentistry, ear, nose and throat (ENT) surgeries, and orthopedics. For example, BCP granules with HA/TCP of 60/40 were placed in the alveolar cavity immediately after tooth extraction and followed up radiographically from 0 to 5 years [140].

Bioactive glasses: Bioactive glasses have a wide range of clinical applications in both medicine and dentistry. It is used as bone graft material, a coating materials, and disinfectants.

As bone graft material: Bioglass has been used clinically as a synthetic bone graft material for over 10 years under two different product names: Novabone_ for orthopedics and Perioglass for maxillofacial surgery. The first reported clinical application of bioactive glass was the treatment of conductive hearing loss for the reconstruction of the bony ossicular chain of the middle ear.

As a coating materials: More researchers use bioactive glasses as a coating materials for dental implants. Bioactive silicate glass has also been used for implant coatings, as a bone graft, in dentifrices, and as air-abrasive particles to remove carious enamel and dentin. Goudouri et al. indicated that bioactive glass could be used as a dental material to improve the bonding of the restorative material to dentin [139].

As disinfectants: Bioactive glasses can serve as topical endodontic disinfectants with no effects on dentin stability. Bioactive glass can raise the pH of an aqueous environment to produce its antimicrobial effects. For example, when implanted in areas of periodontal defects, Bioglass can inhibit bacterial colonization at the surgical site by increasing the pH and calcium levels.

As a bone regeneration: Bioactive glass can promote bone regeneration, with osteostimulatory effects in vitro. Similarly, in primate models, bioactive glass filled bony defects by stimulating osteoproduction. Felipe et al. reported that bioactive glass particles were able to treat periodontal defects and triggered the development of mineralized bone in dogs [141, 142].

9. Conclusion

This chapter discussed the bioceramic scaffolds which considered as one-third of the tissue engineering triad. It dealt with the most effective materials that were used in the bioceramic scaffolds. All traditional and advanced techniques for scaffolds manufacturing, their requirements, and challenges taken into consideration the design of the scaffolds were explored. In additions, several examples of the most common bioceramic scaffolds were highlighted from different corners, e.g., their testing in in-vitro and in-vivo, their extra functionalities such as drug release ability and their clinical applications.

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