CHAPTER

12 Production of polymer–bioactive glass nanocomposites for bone repair and substitution

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12.1 INTRODUCTION

An ideal synthetic bone graft is a porous material that can act as a temporary 3D-scaffold to support and promote bone growth. Its functions and performance should mimic that of porous cancellous bone (autograft) by stimulating the adhesion, proliferation, and differentiation mechanisms that are inherent to the structural complexity of natural tissues (Fillingham and Jacobs, 2016; Boyan et al., 1996). In face of the complexity that characterizes the human body, the requirements for scaffold materials for tissue engineering applications are many and very challenging. They should (1) be biocompatible and bioactive to promote osteogenesis and osteointegration; (2) bond to the host bone without inducing an inflammatory response, immunogenicity, or cytotoxicity; (3) possess interconnected porous structure that allows vascularization, bone ingrowth, and cell and fluids migration; (4) be flexible in terms of shape and size (fit the defect); (5) degrade at a specific rate and eventually be remodeled by osteoclast action; (6) display mechanical performance similar to the local host tissue; (7) be cost-effective for mass production purposes; and, finally, (8) be able to be sterilized and meet the requirements for clinical use (Jones, 2013; Hench and Kokubo, 2016).

Since their discovery in the 1970s by Hench, bioactive glasses (BGs) have been the subject of intense investigation as biomaterials for bone tissue repair and replacement (Hench, 2006; Hench et al., 1971). Their atomic structure allows BG to bind with the host tissues at a chemical level, and the gradual release of their ions into the surrounding media promotes the formation of a carbonate hydroxyapatite-like (HAC) layer on its surface, which increases their biocompatibility and osteointegration abilities (Hench, 1991; Jones et al., 2006; Hench and Paschall, 1973). Despite the impressive bioactivity revealed by the many BGs, these glasses cannot fulfill all the criteria for bone regeneration. Their low

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fracture toughness and mechanical strength, especially in a porous form, limit their clinical application. As a result, BG cannot be used alone in applications where significant stress or cyclic load-bearing demands are applied (Roohani-Esfahani et al., 2011; Jones, 2013). To overcome these challenges, composites that incorporate BG in biopolymer matrices have been designed and investigated. The goal is not only to improve the scaffold mechanical properties, while maintaining the polymer flexibility and capacity to deform under load, but also to create a better environment for cell attachment and growth (Ding et al., 2016; Rezwan et al., 2006).

In this chapter, we introduced and categorized the different types of BG, highlighted some of the most common polymers used as matrices for composite production applied in bone tissue regeneration, and depicted the techniques used in the production of BG composites.

12.2 BIOACTIVE GLASS

Discovered in 1969 by Hench et al., BGs were first characterized as "materials that elicit specific biological responses that result in the bond between the tissues and the material" (Hench, 2006; Hench et al., 1971). Since then, they have been extensively studied for medical applications, namely as bone graft substitutes. BGs are amorphous and biologically active glasses with osteostimulative properties. They are composed of calcium and phosphate in a proportion that is similar to the bone hydroxyapatite, which accounts for its biocompatibility (Kaur et al., 2014). BG can react with physiological fluids to form tenacious bonds to bone through the formation of bone-like hydroxyapatite layers (Kaur et al., 2014). When implanted in living tissue, effective biological interactions and fixation of bone tissue with the material surface occurs, inducing specific intracellular and extracellular responses that ultimately stimulate rapid bone formation (Hench, 1991, 2006; Hench et al., 1971).

Many functional BGs have been developed over the years with proven capacity to maintain growth of osteoblasts (Hattar et al., 2002), fibroblasts (Alcaide et al., 2010), and chondroblasts (Bal et al., 2010) and to increase bone formation. The osteoactivity of each glass correlates with the solubility and composition of the glass (Penttinen, 2011). In Table 12.1 a list of the most common BG and respective composition is provided. Depending on chemical elements present, BG can be classified in silicate-based (SiO_2), phosphate-based (P_2O_5), and borate-based (B_2O_3, less common).

12.2.1 SILICATE-BASED BIOACTIVE GLASS

Since its discovery nearly 40 years ago, the now designated 45S5 or Bioglass (commercial name), which possesses unique bone-bonding properties, has been

extensively researched for biomedical applications (Hench et al., 1971; Hench, 1991). 45S5 is capable of forming a HAC layer on its surface when in contact with bodily fluids. Hench et al. has described a sequence of five reactions that precede the formation of a HAC layer (Table 12.2) (Hench, 1991). This layer resembles in composition the mineral portion of bone and is responsible for the BG bone-bonding abilities (Hoppe et al., 2011). Indeed, it attracts and promotes the adsorption of growth factors, which is quickly followed by attachment, proliferation, and differentiation of osteoprogenitor cells. Osteoblasts are then

Table 12.1 Composition of the Most Common BG (Rahaman et al., 2011; Hoppe et al., 2011)

Bioactive Glass	Na_2O	K_2O	MgO	CaO	SiO_2	P_2O_5	B_2O_3
Bioglass or 45S5	24.5	0	0	24.5	45.0	6.0	0
S520	21.0	7.0	0	18.0	52.0	2.0	0
6P53B	10.3	2.8	10.2	18.0	52.7	6.0	0
13–93	6.0	12.0	5.0	20.0	53	4.0	0
13–93B1	5.8	11.7	4.9	19.5	34.4	3.8	19.9
13–93B3	5.5	11.1	4.6	18.5	0	3.7	56.6
58S	0	0	0	32.6	58.2	9.1	0
60S	0	0	0	38.4	59.9	1.7	0
70S30C	0	0	0	28.6	71.4	0	0
77S	0	0	0	16.0	80.0	4.0	0
$P_{50}C_{35}N_{15}$	9.3	0	0	19.7	0	71.0	0

Table 12.2 Sequence of Events Preceding the Formation of a HAC Layer on the BG's Surface (Hench, 1991)

Stage 1	Rapid exchange of cations such as Na^+ or Ca^{2+} with H^+ or H_3O^+ from solution, leading to the hydrolysis of the silica groups and the creation of silanol (Si–OH) groups on the glass surface. pH increases due to consumption of H^+.
Stage 2	Loss of soluble silica in the form of $Si(OH)_4$ to the solution and continued formation of Si–OH groups on the glass surface. Concentration of Si increases in solution.
Stage 3	Condensation and polymerization of an amorphous SiO_2-rich layer on the surface of the glass depleted of Na^+ and Ca_2^+ ions.
Stage 4	Migration of Ca_2^+ and PO_4^{3-} ions from the glass through the SiO_2^- layer and from the solution to the surface, forming CaO-PO_4^{3-} clusters on the top of the SiO_2^--rich layer. Growth of an amorphous calcium phosphate (CaP) layer.
Stage 5	Crystallization of the amorphous CaP by incorporation of OH^- and CO_3^{2-} anions from the solution, resulting in the formation of a HAC layer.

responsible for the formation of the extracellular matrix, which mineralizes to form a nanocrystalline mineral and collagen on the surface of the BG (Hench and Polak, 2002; Ducheyne and Qiu, 1999). While these changes are taking place, the BG undergoes degradation and conversion.

The ionic dissolution products of silicon based BG have been shown to change the intracellular ionic concentrations and as a result to mediate cell metabolism (Fig. 12.1) (Ducheyne and Qiu, 1999; Jell and Stevens, 2006). By adding 45S5 to the culture medium the concentration of Ca in osteoblasts increases and so does the amount of ATP generated (Silver et al., 2001). Dissolution products of 45S5 are capable of upregulating (up to fivefold) gene expression in osteoblast-like cells, including cell metabolism, proliferation and cell–cell or matrix–cell adhesion (Xynos et al., 2001). They have also been described as upregulators of osteogenic markers such as bone sialoprotein or alkaline phosphatase, and as instigators of collagen type I formation and cell differentiation (Jell et al., 2008; Effah Kaufmann et al., 2000). Similar results have been acquired with other BGs (i.e., 13–93, 58S, 77S, etc.).

It has become clear that successful clinical application of scaffolds in bone tissue engineering highly depends on a functional vascularized network that is in

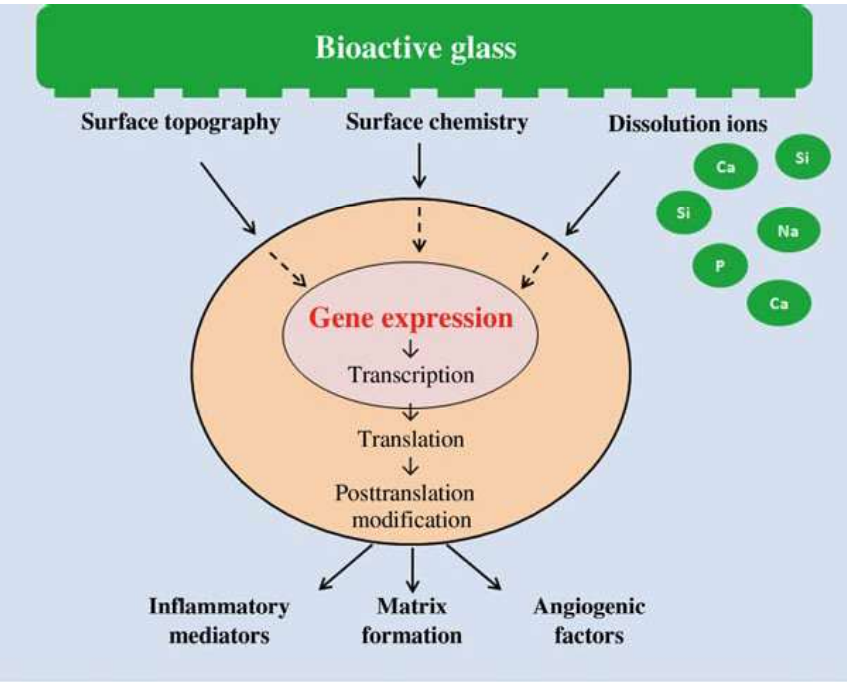

FIGURE 12.1

Gene expression mechanisms activated by BG.

Adapted from Jell, G., Stevens, M.M., 2006. Gene activation by bioactive glasses. J. Mater. Sci. Mater. Med. 17, 997–1002.

perfect harmony with the tissue regeneration rate. A strong porous structure that degrades at the same rate as the tissue is formed is desirable (Kaur et al., 2014). 45S5 is considered the BG gold standard; yet, there are restrictions to its processing in the form of porous 3D scaffolds. 45S5 has limited ability to sinter by viscous flow above its glass transition temperature (T_g), and the narrow window between T_g and the onset of crystallization, raises severe difficulties in sintering the particles into a dense network, thus resulting in weak structures (Chen et al., 2006). In addition, 45S5 degrades very slowly, which makes it difficult to match the degradation rate of the scaffold with the rate of tissue formation (Huang et al., 2006). To overcome these limitations, BGs have been designed based on the 45S5 composition. The 13–93 is the most common example. Aside from the elements composing 45S5, it possesses traces of K_2O and MgO that provide the required stability to undergo viscous flow sintering and form 3D scaffolds without crystallization. Analyses of cell response did not detect differences in behavior between the 45S5 and the 13–93 BG (Brown et al., 2008).

12.2.2 PHOSPHATE-BASED BIOACTIVE GLASS

Phosphate-based BG is mostly used as bone filling materials and in the fabrication of scaffolds for bone tissue engineering. Its chemical similarities with the inorganic phase of bone, which increases its affinity and compatibility, make the BG from this class very desirable in bone substitution (Lakhkar et al., 2013). Yet, it is the BG's high solubility and variety of compositions that confers additional potential as resorbable materials, and that most affects cell behavior. Cell response varies according to the glasses' ionic dissolution rate; however, there is no universal predictable behavioral rule (Ahmed et al., 2004b,c; Khan et al., 2014). It has been shown that BGs with high dissolution rates inhibit osteoblastic cell growth and bone antigen expression, while BGs with low dissolution rates upregulate the osteoblasts proliferation and expression of bone sialoprotein, osteonectin, and fibronectin genes (Salih et al., 2000). The opposite has been reported as well (Skelton et al., 2007). To better control/predict the cell response and thus stabilize the glass network and degradation rate of the phosphate-based glasses, different oxides have been used as additives, for example, TiO_2, B_2O_3, ZnO, MgO, CuO, etc. (Navarro et al., 2003; Saranti et al., 2006; Ahmed et al., 2004a; Neel et al., 2005; Shu et al., 2010).

12.2.3 BORATE-BASED BIOACTIVE GLASS

Borate-based BGs are a more recent class of BGs. These have gained much interest due to their fast degradation rate that allows the formation of a more complete HAC layer than, for instance, 45S5 or 13–93 (Fu et al., 2010). Borate BG can be used as substrate for drug release in the treatment of bone infections and has been shown to support cell proliferation and differentiation in vitro

(Marion et al., 2005; Fu et al., 2009; Liu et al., 2010). It should be pointed out, however, that the release of borate ions represents a great limitation to its use in in vitro cultures, due to the ions' toxicity (Brown et al., 2009). Unexpectedly, in vivo reports have been more favorable with no cell toxicity being detected while using these BGs to support new tissue infiltration (Zhang et al., 2010). The greatest advantage of using borate-based BG is the ease of manufacture and the ability to control the composition and, thus, the degradation rate to match bone regeneration.

12.2.4 FABRICATION OF BIOACTIVE GLASS SCAFFOLDS

The greatest advantage of preparing scaffolds using BG is the possibility to control the chemical composition and, consequently, the degradation rate. The scaffold's structure and chemistry can be tailored by changing the composition or processing conditions (Jones, 2013). Thus, scaffolds can be prepared with variable degradation rates that can both match the bone ingrowth and the remodeling. By optimizing the composition, processing, and sintering conditions, BG scaffolds can be produced with characteristics comparable to human trabecular and cortical bones (Gorustovich et al., 2009; Hoppe et al., 2011).

BGs were initially obtained via melting at high temperatures. Later, the sol–gel technique was defined as most suitable since it required lower processing temperatures and the resulting BG possessed increased bioactivity. Sol–gel process is a chemistry-based synthesis technique in which a solution containing the elemental precursors undergoes gelation reactions at room temperature to form a gel. The gel, formed of a wet inorganic network, is then subjected to aging processes to increase its strength, drying to remove the liquid byproduct, and sintering to form a porous 3D architecture (Brinker and Scherer, 2013; Valliant and Jones, 2011). Aside from requiring lower temperatures than the melting process, the sol–gel-derived BGs also possess an inherent nanoporosity and higher specific surface area, which works as a stimulant factor for cell response (Lei et al., 2010). This nanoporous architecture mimics more closely the hierarchical structure of natural tissues (Martin et al., 2012). The high surface area stimulates sol–gel-derived scaffolds to degrade and to create faster a HCA layer than melt-derived scaffolds. However, they can only be used in defects where low stress demands are applied since they have low resistance strength (Jones et al., 2006).

Aside from sol–gel, which is the most common method, there are other techniques used in the production of BG scaffolds. For instance, scaffolds can be prepared by thermal bonding of particles or fibers in a mold with the desired size and shape. A porogen, that is, sodium chloride, is mixed with the BG particles to increase the pore size and porosity of the scaffolds. Once the scaffolds are formed, the porogen is removed by leaching or decomposition (Kaur, 2017). This is a simple and straightforward method to produce scaffolds of regular porosity, however with low pore interconnectivity (Deliormanli and Rahaman, 2012).

Another possibility is to use the polymer foam replication method. Here, a synthetic or natural foam is immersed in a BG suspension to obtain a uniform coating. After drying, the polymer template and organic binders are burned at 300°C–600°C, and the glass struts are densified by sintering at 600°C–1000°C, depending on the glass composition and particle size (Fu et al., 2011a). The resulting scaffold displays a microstructure similar to dry human trabecular bone, with a highly porous architecture and interconnected porosity, however with low resistance strength (Xia and Chang, 2010).

12.2.4.1 Mechanical Properties

Most reports have established the BG scaffolds mechanical response to compression loading or elastic modulus for selected deformation rates to be very low. Yet, at specific compositions and microstructures, the BG porous scaffolds may be prepared with compressive strengths similar to the human cortical bone. Fu et al. have shown that by emulating nature's design by direct-ink-write assembling of glass scaffolds with a periodic pattern, and controlled sintering of the filaments into anisotropic constructs similar to biological materials, porous BG scaffolds with a compressive strength (136 MPa) comparable to that of cortical bone (100–150 MPa) and a porosity (60%) comparable to that of trabecular bone can be produced (Fu et al., 2011b; Keaveny and Hayes, 1993). The microstructure of the scaffold has a strong effect on its resistance strength. Indeed, for the same porosity, scaffolds with an oriented pore architecture show far higher compressive strength than scaffolds with a random or isotropic pore architecture. Lui et al. produced 13–93 BG scaffolds by unidirectional freezing of camphene-based suspensions on a cold substrate, followed by thermal annealing to increase the pore diameter and orient their location. They found that the compressive strength along the pore orientation direction was 2–3 times the value obtained in the direction perpendicular to the pore orientation direction and, thus, revealed the potential of 13–93 BG scaffolds for the repair of large defects in load-bearing bones (Liu et al., 2011).

In addition to high strength and elastic modulus, scaffolds that are implanted in load-bearing bone defects and are subjected to cyclic stress should also possess good fracture toughness and reliability. The intrinsic brittleness or low resistance to crack propagation that is characteristic of ceramics and glasses are major limitations to the use of BG scaffolds in bone repair. Because of their low fracture toughness, ceramics and glass are very sensitive to the presence of small defects or cracks particularly under compressive loads. Once again, the organization and distribution of the porous along the scaffold architecture may overcome these limitations (Fu et al., 2011b; Liu et al., 2011). Very little research has however been dedicated to the study of the reliability or probability of failure of brittle materials. This is likely that due to the amount of samples necessary for this kind of studies and the precision in sample dimension, geometry, and testing conditions that are required may discourage researchers from pursuing further this line of investigation (Fu et al., 2011a).

12.3 NATURAL AND SYNTHETIC POLYMER–BIOACTIVE GLASS COMPOSITES

Despite the impressive bioactivity revealed by the many BG, the low fracture toughness and mechanical strength displayed, especially in a porous form, limits the BG clinical application. BG cannot be used alone in applications where significant stress or cyclic load-bearing demands are applied. Most porous BG scaffolds exhibit very low compressive and tensile strength, fracture toughness, and elastic modulus, when compared with cortical and cancellous bone (Fu et al., 2011a). Thus, the development of composites that incorporate BG in biopolymer matrices, represents an interesting approach, not only to improve the scaffold mechanical properties, while maintaining the polymer flexibility and capacity to deform under load, but also to create a better environment for cell attachment and growth (Ding et al., 2016; Rezwan et al., 2006).

The combination of biopolymers and inorganic fillers to develop tissue engineering scaffolds has been investigated for the last 20 years. Inorganic fillers are commonly added to the polymer matrices in the form of particles or fibers. Its size determines the effective mechanical properties of the composite, since depending on the BG microstructure different interactions between filler and matrix may be promoted (Koo, 2006). It has been shown that nanoscaled degradable fillers, like BG, aside from improving the implant biological performance can also increase its alkalinity, which can protect to a great extent the acidic degradation of some polymers, for example, polylactic acid (PLA) (Vollenweider et al., 2007). The nanoparticles' high specific surface area-to-volume ratio contributes to the scaffold superior protein and cell adhesion, since it increases the general bioactivity, in addition to mimicking more closely the structure of natural bone, which contains nanoscaled hydroxyapatite crystals combined with collagen (Webster et al., 1999; Boccaccini et al., 2010).

Natural and synthetic polymers have been used to produce BG composites with desirable properties for bone substitution. Natural polymers, which derive from renewable resources, are widely used in regenerative medicine because of their intrinsically bioactive and biodegradable properties and similarity to the extracellular matrix (Huang and Fu, 2010; Zhong et al., 2010). Among the many polysaccharides, polymeric carbohydrated molecules composed of long chains of monosaccharide units bound together by glycosidic bonds, like chitin, chitosan, hyaluronic acid, alginates, etc., possess the most desirable properties. It has been reported that scaffolds synthesized of chitin-BG by lyophilization technique exhibit adequate swelling and degradation as well as improved bioactivity, revealed by the increased deposition of apatite on the surface of the composite (Peter et al., 2010; Sowmya et al., 2011). Also, nanocomposite films based on chitosan blends with BG nanoparticles have been established as appropriate to develop guided tissue regeneration, as they stimulate osteoblasts' response towards cell differentiation and mineralization (Luz, 2012). Using the layer-by-layer approach, a chitosan-BG

composite has been produced with a homogeneously distribution of the BG nanoparticles along the multilayered surfaces. Chitosan provided the viscoelastic properties, while the BG provided bioactivity for the organic–inorganic structure. In vitro studies indicated that the multilayers induced the formation of apatite, a marker of bioactive behavior (Couto et al., 2009).

Synthetic polymers can be produced under controlled conditions and therefore exhibit predictable and reproducible mechanical and physical properties. They can also be synthesized without impurities reducing the risks of toxicity, immunogenicity, and infection, as they are formed of monomeric units of well-known and simple structure (Tian et al., 2012). Table 12.3 summarizes the properties of the synthetic polymers most commonly used as polymeric matrices for BG composite production: PLA, poly(glycolic acid) (PGA), poly(lactic-co-glycolic acid) (PLGA), poly(ε-caprolactone) (PCL), and poly(3-hydroxybutyrate) (P3HB).

The biodegradable poly-α-hydroxy esters, members of the aliphatic polyester family, PLA, PGA, and PLGA, have been used clinically for many years. Their inherent chemical properties allow fast hydrolytic degradation, being the resultant monomers easily removed by natural pathways (Mano et al., 2004). The degradation rate is affected by the polymers molecular weight, polydispersity, chemical composition and structure, processing parameters, environmental conditions, size, morphology (i.e., porosity), chain orientation, additives, hydrophilicity, etc. (Okamoto and John, 2013). Because, PLA, PGA, and PLGA degrade so quickly scaffolds may fail prematurely and their ionic products may not be removed at the same rate, resulting in strong inflammatory responses. The combination of these polymers with BG has proven to be very successful. Porous scaffolds of PLA and BG nanoparticles have been prepared by thermally induced phase-separation process and the results showed an improvement of the scaffold's

Table 12.3 Physical Properties of Some of the Synthetic Polymers Used as Polymer Matrices in Scaffold Production

Polymer	Melting Point T_m (°C)	Glass Transition Point T_g (°C)	Biodegradation Time (months)	Tensile Strength (MPa)
PLA	180–220	60–65	>24	Film/disk: 8–50 Fiber: 870–2300
PGA	225–230	35–40	6–12	Fiber: 340–920
PLGA	Amorphous	45–55	Adjustable 1–12	41–55
PCL	58	−72	>24	–
P3HB	175	2	–	35–45

Adapted from Rezwan, K., Chen, Q., Blaker, J., Boccaccini, A.R., 2006. Biodegradable and bioactive porous polymer/inorganic composite scaffolds for bone tissue engineering. Biomaterials 27, 3413–3431.

mechanical properties (Hong et al., 2008). Similar observations were made by Liu et al. by using solvent evaporation technique to combine low molecular weight PLA with sol–gel-derived BG nanoparticles. They reported that the mechanical properties were enhanced and the roughness of fractured surfaces decreased with the addition of BG. Besides, the composites were shown to be bioactive by forming a HAC layer once in contact with physiological fluids, and to instigate cell proliferation above nonmodified scaffolds (Liu et al., 2008, 2009).

PCL is bioresorbable and biocompatible, and has been applied in tissue regeneration for many years. Because PCL is degraded by hydrolysis of its ester linkages in physiological conditions, like PLA, PGA, and PLGA, and its biocompatibility efficacy has been extensively proved, the FDA has approved a number of medical devices made of PCL (Zahedi et al., 2012; Yoshimoto et al., 2003). PCL, also a member of the aliphatic polyester family, has a great advantage over the previously numbered polymers as it can take several years to degrade in vivo. Composite scaffolds of mesoporous BG and PCL have been produced by solvent casting–particulate leaching (SCPL) method, and their structure and properties characterized. By incorporating BG, the composite's hydrophilicity was improved and the formation of a dense and continuous layer of apatite was instigated (Li et al., 2008). The biological and mechanical properties of a BG–PCL composite scaffold generated using sol–gel precursors via the electrospinning method revealed great levels of alkaline phosphatase activity and enhanced biocompatibility and bioactivity. The results from in vivo animal experiments established the potential of BG–PCL composite scaffolds as bone regenerative materials (Fig. 12.2) (Jo et al., 2009).

P3HB belongs to the biodegradable polyhydroxyalkanoate family and, because of its biocompatibility (Zhijiang et al., 2012), has been applied in the manufacture of many biomedical devices (Hazer et al., 2012). P3HB has demonstrated remarkable abilities to stimulate consistently favorable bone tissue responses, including instigating the formation of highly organized new bone structures, without inducing undesirable chronic inflammatory reactions, even after long periods of implantation (Misra et al., 2010b). Studies have been conducted to determine the relevance of BG in the P3HB scaffolds. Misra et al. compared the effects of introducing micro- and nanoscale BG particles on the thermal, mechanical, and microstructural properties of BG–P3HB composites produced by solvent casting. Composites with nanoscale BG were determined to be more stiff and its nanoscale topography was altered. As a result, protein adsorption increased and so did its bioactivity and water adsorption (Misra et al., 2008). Cytocompatibility studies (cell proliferation, cell attachment, alkaline phosphatase activity, and osteocalcin production) using human MG63 osteoblast-like cells showed these composite scaffolds to be suitable for cell attachment, proliferation, and differentiation (Misra et al., 2009). Following this line of investigation, in vivo testing were later performed using highly porous P3HB foams supplemented with nanoscale and microscale BG. Foams were implanted in rats as subcutaneous implants. After 1 week of implantation neither toxic nor foreign body responses

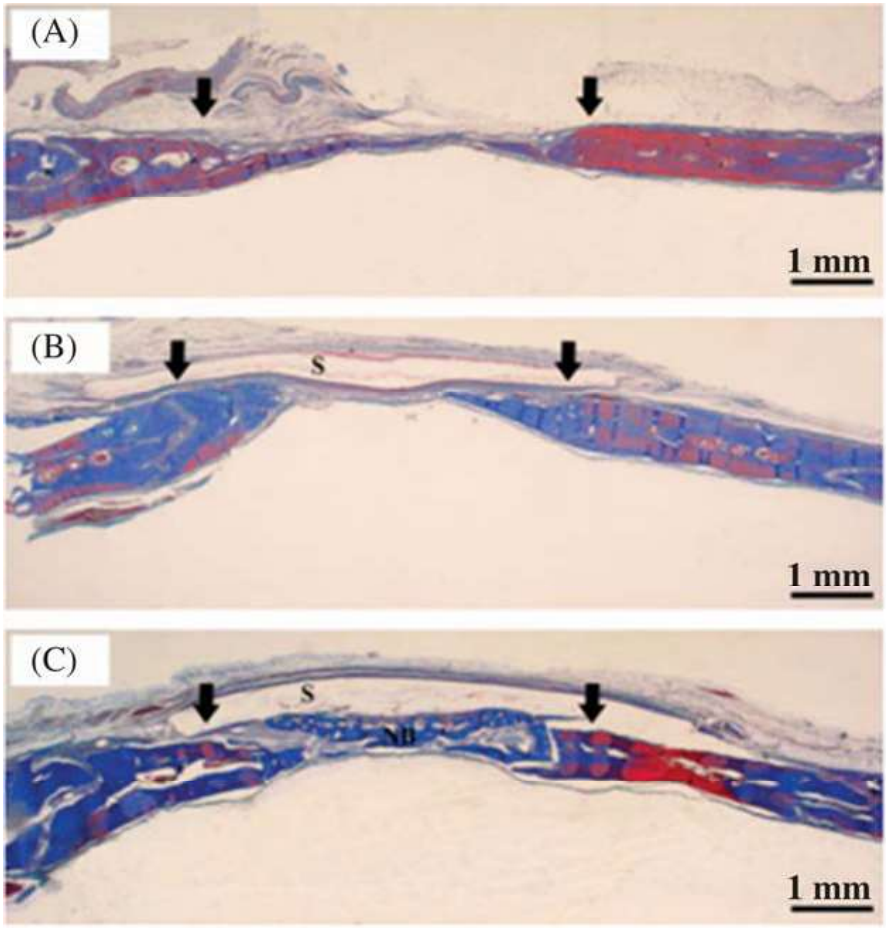

FIGURE 12.2

Optical micrographs of the stained bone tissues 3 weeks after membrane implantation: (A) empty defect, (B) pure PCL, (C) PCL-BG composite (arrows, defect margins; NB, new bone; S, sample) (Jo et al., 2009).

were observed. In addition to showing bioactivity and biocompatibility, the composite foams also displayed bactericidal properties, which were tested on the growth of *Staphylococcus aureus* (Fig. 12.3) (Misra et al., 2010a).

12.4 COMPOSITE PRODUCTION TECHNIQUES

Sol–gel technique has been defined has most suitable to produce BG with increased bioactivity (Jones, 2013). Yet, combining BG with biopolymers to improve both the mechanical and biological properties of the implantable materials can be accomplished with other techniques or combination

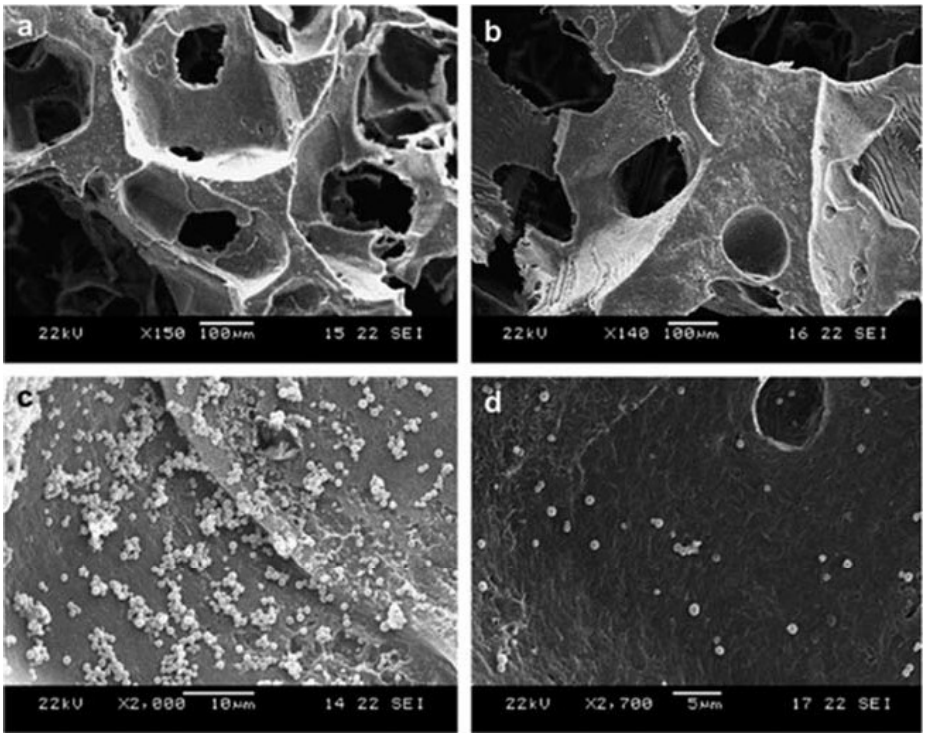

FIGURE 12.3

SEM micrographs of *S. aureus* attachment after 48 h on (A, C) P3HB foams and (B, D) P3HB-BG composite foams. A decrease in *S. aureus* bacteria on the surface of the P3HB-BG composite is evident (D) in comparison to single P3HB (C) (Misra et al., 2010a).

of techniques. Electrospinning, emulsification-solvent evaporation process for microsphere production, solvent casting, freeze-drying, and 3D printing are some of the most commonly used techniques, with the greatest potential for the biomedical field. Within these techniques we can find examples of composites in which both organic and inorganic phases interact, generating strong covalent bonds, or cases where multilayer structures are developed. Depending on the technique, scaffolds with different architectures and mechanical properties can be attained.

12.4.1 ELECTROSPINNING

Electrospinning is an old technique, dating back almost 120 years (Zeleny, 1914). However, it was only after the 1980s that the electrospinning technique regained interest. It is capable of consistently producing fibers in the submicron range with extremely high surface-to-volume ratio, tunable porosity, and malleability to conform over a wide variety of sizes and shapes, and allows to control the fibers

composition to achieve desired properties and functionality (Abrigo et al., 2014; Liang et al., 2007; Huang and Chang, 2003; Hunley and Long, 2008; Lannutti et al., 2007; Coelho et al., 2017). It is a simple and straightforward method, in which a polymer solution is pumped at constant rate by a syringe connected to a high DC voltage source. Electrospinning is based on the principle that strong mutual electrical repulsive forces overcome weaker forces of surface tension in the charged polymer liquid (Agarwal et al., 2013; Felgueiras et al., 2017a,b). The resultant electrospun fibers' diameter, morphology, and fiber orientation in a scaffold are defined by the equipment operating conditions, solution properties, and surrounding environment (Bhardwaj and Kundu, 2010).

The simplicity of the electrospinning technique allows for natural and synthetic polymers to be used in a single or multipolymer blend manner. Indeed, organic–inorganic composites and hybrid fibers are being developed combining the physical and mechanical performance with the material's biological properties. Biopolymer–BG suspensions have also been tested with the goal of integrating the brittle and inorganic phase of BG with the elastic and bioinert organic phase of the biopolymers. At the moment, there are two strategies to accomplish this. The first is to associate BG particles with the polymer solution with and without surfactants in an ultrasonic bath. Electrospun scaffolds of BG–PCL composite have been produced using this approach. It was seen that the bioactivity and the cells' alkaline phosphatase activity is significantly enhanced with the addition of the BG nanoparticles (Kouhi et al., 2013). Also the tensile strength of the fibrous scaffold can be improved (Lin et al., 2012a). The other approach combines the BG with the polymer in a hybrid solution. Here, the organic and inorganic phases interact on a molecular level. Single phase electrospun organic–inorganic scaffolds are thus produced. Chemical characterization revealed that only natural origin polymers, which possess multiple functional groups, can covalently bind to BG, while the synthetic could only accomplish this through weak hydrogen bonding (Allo et al., 2010). To overcome this, Gao et al. have proposed the use of a coupling agent, the 3-glycidoxypropyltrimethoxysilane (GPTMS), to prepare BG-gelatin hybrid scaffolds. The GPTMS agent provided the basis that led to the formation of a covalent bond between the organic and inorganic elements, which resulted in a significant enhancement of the tensile strength and elongation properties of the scaffold (Gao et al., 2013). It should be highlighted though that coupling agents are not always required for processing of synthetic polymers and BG in the form of hybrids, as there are ways to overcome the limitations of this combination. For instance, Kim et al. prepared a BG–PLA composite in which the electrospun fibers were sintered at 700°C, cut, and immersed in a PLA/THF solution followed by thermal compression at 130°C, to obtain a homogeneous dense scaffold. The resultant composite revealed great bioactivity, promoted the osteoblast-like cell attachment and growth, and increased the secretion of collagen proteins and the alkaline phosphatase activity (Kim et al., 2008).

12.4.2 MICROSPHERES

Nowadays, a variety of techniques can be used to prepared microspheres. Yet, the most common remains the emulsification-solvent evaporation process. Here, a polymer solution made in a volatile organic solvent is emulsified by agitation to obtain an oil/water emulsion that can be stabilized as droplets. Once the emulsion is stabilized, agitation is maintained and the solvent evaporates after diffusing through the continuous phase, resulting in solid microspheres. In the end, the microspheres are collected by filtration or centrifugation, washed, and dried (Watts et al., 1989; Hwisa et al., 2013). Depending on the nature of the polymer, application of the final product, and required degradation rate other techniques may be used, for example, coacervation, spray drying, milling, and supercritical fluid techniques (Kulshreshtha et al., 2010). During microsphere fabrication, BG particles can be incorporated or dispersed along the surface to increase the bioactivity of the polymer, accelerate the healing process by enhancing osteogenic and antiogenic phenomena, and to control the degradation rate.

Mesoporous and nonmesoporous BG were incorporated into alginate microspheres, a biocompatible, natural origin polymer. Dexamethasone-loading and release ability was tested in phosphate buffered saline solution, which was found to be enhanced in the presence of BG (Wu et al., 2010a). Alendronate sodium has been incorporated into poly(lactide-co-caprolactone)-BG composite microspheres produced by oil-in-water emulsion solvent evaporation method with successful results. Data determined these microspheres to be bioactive, noncytotoxic, and capable of promoting cell adhesion. Also, this releasing strategy was established as more efficient than oral administration (Mondal et al., 2012). The mechanical performance and capacity to produce a HAC layer on the surface of spherically shaped and small-sized microspheres, prepared of PCL and different concentrations of BG, have also been tested. It was seen that with increasing amounts of BG the microspheres' elastic modulus also increased. BG enhanced the hydrophilicity of PCL, which led to a higher water adsorption and faster degradation rate. The composite microspheres with the highest amount of BG stimulated vigorously the growth of apatite and with it its bioactivity (Lei et al., 2012).

12.4.3 SOLVENT CASTING–PARTICULATE LEACHING

SCPL is a simple, straightforward, and cost-effective method to produce composite polymeric scaffolds. Briefly, a polymer is dissolved in an organic solvent and particles with specific sizes and shapes are added to the solution. The mixture is shaped according to the recipient where the solution is poured or prepared. Once the solvent evaporates, a structure of composite material is attained. The composite material is then placed in a bath to dissolve the particles, leaving behind a porous structure (Farè, 2012; Lin et al., 2012b).

The simplicity of the method and the ability to control the porosity of the resulting structure allows its use in different research fields. However, since the

porous morphology is usually cubic-like, equiaxed, or spherical, and full interconnectivity is unlikely to be achieved, there are some limitations to its actual application (Bencherif et al., 2013). Single and multipolymer blends with and without inorganic fillers or particles like BG have been explored for porous scaffold fabrication in tissue engineering. Composite scaffolds of mesoporous and nonmesoporous BG and PCL were prepared using SCPL and their structure and properties characterized. Incorporation of BG increased the composite hydrophilicity and the ability to form a dense and continuous layer of apatite on the scaffolds' surface. The highly enhanced bioactivity of the PCL-BG composites was attributed to the ordered channels and BG particles high surface-to-volume ratio, which accelerated the ion exchange during incubation (Li et al., 2008). In another study, 45S5 powders were incorporated into a poly(3-hydroxybutyrate-co-3-hydroxyhexanoate) (PHBV) matrix to produce scaffolds by SCPL and in vitro and in vivo testing were conducted. Results showed that aside from increasing the hydrophilicity and compressive strength of the composite, the BG supplement also promoted chondrocyte's penetration length, thickness of cartilage-like tissue of in vivo constructs, and the mechanical strength of the formed cartilage tissue (Wu et al., 2013).

12.4.4 FREEZE-DRYING

Freeze-drying belongs to the family of the thermally induced phase-separation techniques and was first described in the 1980s. In recent years, this technique has been used to produce highly porous scaffolds with interconnected and tailored architecture. The overall scaffold porosity and mechanical properties can be tailored by adjusting the conditions of processing and solution (Kane and Roeder, 2012).

This technique is based on the principle of dehydration. Briefly, a solution consisting of a solvent, polymer, and inorganic particles is subjected to rapid gelation causing the solids to be displaced into the interstitial spaces between ice crystals. Once the solution is fully frozen, the freeze-drying process begins and the materials are subjected to a cycle of temperatures ranging between 20ºC and 80ºC, that are applied for different periods of time. At the end, the suspension is sublimated under vacuum conditions (Qian and Zhang, 2011).

BG nanoparticles can be incorporated into the solution to reinforce the biological properties of the scaffolds (Maquet et al., 2004). Although this technique can be used as a single method, it usually occurs in combination with other techniques (Wu et al., 2010b). Gelatin-BG hybrid scaffolds produced by a combination of sol–gel, freeze-drying and particulate leaching processes are a successful example of that. By using sol–gel the amorphous BG were uniformly distributed into the gelatin matrix, acting as a reinforcing phase. A hierarchical pore structure with round micropores and nanopores was generated from the particulate leaching and freeze-drying processes, respectively. The hybrid scaffolds were established as more favorable for cell adhesion, proliferation, and osteogenic differentiation

than the pure gelatin scaffolds. Moreover, gene expression was highly promoted by BG addition (Qu and Liu, 2013).

12.4.5 3D PRINTING

3D printing technique is one of the most recent innovations introduced to the biomedical field to produce scaffolds of controllable architecture. This technique incorporates ink-jet technology to precisely place successive layers of powder or sheet material to build a 3D model from a series of cross-sectional layers. Once the model is complete, the unfused excess material is removed by compressed air or manually brushed (Rengier et al., 2010; Lipson and Kurman, 2013). Zhao et al.'s work gives us an excellent example of the use of polymer–BG composite scaffolds produced by 3D printing. Mesoporous BG and PHBV were combined at different concentrations, and their biological performance followed. Reports described these composite scaffolds as highly biocompatible, with enhanced bioactive and osteogenic properties, including fast apatite-forming ability. They were also seen to promote mesenchymal stem cells adhesion, proliferation, alkaline phosphatase activity, and bone-related gene expression. Data from in vivo testing revealed these composite scaffolds to exhibit a controlled degradation rate and their potential to stabilize the pH environment with increasing PHBV ratios. The capacity of PHBV-BG scaffolds to stimulate bone regeneration was also established (Zhao et al., 2014).

12.5 CONCLUSIONS

Many in vitro, in vivo, and clinical studies have shown BGs to perform above other bioceramic particles but not as well as autograft bone. The main reasons behind this are the fact that BG particles cannot be made into porous scaffolds without crystallizing during sintering, and the low mechanical performance of BG, which includes low fracture toughness and mechanical strength especially in a porous form. The first issue is being successfully overcome by using new compositions that can be sintered without crystallizing, and by applying new techniques to produce micro- and nanostructured BG scaffolds. Yet, these porous scaffolds can only be used in sites where there is little to no stress/load-bearing demand. It is here that the combination of biopolymers with BG comes as an advantage.

In the past 20 years various combinations of biopolymers and inorganic fillers were developed in the form of tissue engineering scaffolds for biomedical applications. BG, aside from improving the polymeric implant biological performance, due to its high specific surface area-to-volume ratio that contributes to the scaffold superior protein and cell adhesion, can also increase its alkalinity, which can protect at great extent the acidic degradation of some polymers. Conventional

composites do not seem to be able to mimic the hierarchical structure of bone. The greatest advantage of the polymer–BG composites are their ability to mimic closely the structure of natural bone, which contains nanoscale hydroxyapatite crystals (similar chemical composition of BG) combined with collagen, a natural polymer. There are many polymers that have been successfully combined with BG and their results have inspired researchers to investigate new applications of BG in biomedical engineering. Yet, their clinical effectiveness still requires further testing and proper validation. Nonetheless, the great potential of these micro- and nanostructures processed with biopolymers and inorganic fillers should be highlighted. In the future, it is expected these new composites will become more sophisticated, widening their areas of application.

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