Chapter 10
Bioactive Composites Reinforced with Inorganic Glasses and Glass–Ceramics for Tissue Engineering Applications

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Abstract Bioactive composites, prepared by the combination of glasses or glass–ceramics with natural or synthetic polymers or blends, have been extensively exploited in bone tissue engineering. Their bioactive character is usually derived from the glass or glass–ceramic phase and is one of the most relevant properties to generate bone bonding. Herein we focus on the development of bioactive composite structures that target tissue engineering applications, with special emphasis on bone regeneration. Some concepts, e.g., bioactivity and biocompatibility, are initially introduced, followed by a description of the synthetic approaches that have been reported for the preparation of bioactive inorganic glasses or glass–ceramics. Different strategies to compound these inorganic particles with polymeric phases are detailed, spanning from conventional methodologies and wet spinning to rapid prototyping. Finally, a series of systems that have been developed for bone tissue engineering are described (including injectable systems, 3D scaffolds, membranes, and biomimetic layer-by-layer structures), as well as their in vitro biological response.

Keywords Bioactive composites • Glass–ceramics • Bioactivity • Biomaterials • Tissue engineering

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10.1 Introduction

Autografts have been considered as the gold standard for bone reconstructive surgeries because of its high performance and no risk for transfer of diseases or rejection (problematic in the allografts) [1]. However, there are several problems related with this procedure, such as the limited amount of available material and donor site morbidity [1–3]. Thus, the development of alternatives has been highly relevant for bone reconstructive surgery. In this context, the tissue engineering concept was introduced during the 1980s [4]. Since then, the bone tissue has been one of the most investigated tissues to regenerate or repair [5].

Current challenges in bone tissue engineering involve the development of scaffold biomaterials which provides local environment that enhances the recruitment of endogenous progenitor cells, improving the healing process and tissue regeneration [6]. In addition, the ideal bone tissue engineering construct should be readily available, easily adaptable to the site in terms of size and shape, mechanically strong, and biodegradable [7, 8]. Moreover, the biomaterial should be biocompatible (i.e., giving an appropriate response under the biological medium) and present bioactivity (i.e., able to generate a calcium phosphate layer onto its surface).

The weak fixation of the biomaterials within the bone structure is one of the major reasons that impair the use of scaffolds in load-bearing sites. The lack of interfacial adhesion between the implant and bone, which results in a fibrous capsule surrounding the implant which allows micromotion to occur, causes pain to the patient and space for wear particles to accumulate, ultimately contributing to the failure of the implant. To overcome this problem, a series of strategies has been developed to generate bioactive fixation. Under this concept, an interfacial bonding between the implant and the bone tissue is promoted through the coating of the biomaterial with a biologically active hydroxyapatite layer. As bone is also highly mineralized (by hydroxyapatite), this bioactive fixation forms a bond at the implant–bone interface with strength equal to or greater than the bone itself.

In this context, the bioactivity of a specific biomaterial gains significant relevance within bone tissue engineering. In fact, a large number of biomaterials are not bioactive, and several methods have been proposed to induce this property [9]. A widely established strategy to promote bioactivity is the incorporation of glass or glass–ceramic inorganic particles. These particles are usually produced with the inclusion of calcium and phosphate groups in order to feed the biomaterial with the main constituents of hydroxyapatite. In the first years of development, bioactive glass coatings onto metals and ceramics were being proposed to avoid the formation of capsules of fibrous tissue surrounding the implants [10]. Nowadays it is common their incorporation into composite systems, where polymers can act as a continuous medium for their immobilization [11]. Additionally, the biodegradation of the polymeric phase has been also considered as critical within a bone tissue engineering perspective, in order to allow new bone to be formed while the composite degrades [12].

In the following sections, we will give an overview on the most important topics related with composites containing bioactive glasses or glass–ceramics, including some concepts that are relevant in this field; synthetic approaches to the production of glass/glass–ceramic particles; their processing in combination with biodegradable polymer to generate composite structures that combine biocompatibility, bioactivity, and biodegradability; and the capacity to promote new bone formation.

10.2 Biocompatibility and Bioactivity in Bone Tissue Engineering

Biocompatibility is defined as the ability of a material to give an appropriate response to a specific biological application [13]. In addition to effectiveness, the materials used in biomedical devices must also be safe. In this sense, biocompatibility may be generally regarded as the ability of a material to interact with living cells/tissues or a living system by not being toxic and injurious or causing immunological reactions while performing or functioning appropriately [14].

The evaluation of the biocompatibility of biomaterials involves a detailed characterization (e.g., bulk and surface chemical composition, density, porosity, mechanical and degradation properties) and extensive biological testing, first at the in vitro level, then in in vivo animal models, and ultimately in human clinical trials [15].

The first biomaterials were based on the concept of bioinertness. Under this approach, the development of a material that would not react with the surrounding tissues was targeted. Nowadays, bioactive materials are preferred, where bioactivity is defined as "the ability of the material to induce the formation of an interfacial bonding between the implant and living tissues, without the formation of a fibrous capsule separating the biomaterial and the tissue." In the case of the bioactive glasses, various in vitro and in vivo studies show that a series of interfacial reactions occur that leads to the formation of an apatite layer on the glass surface responsible for bone bonding [16, 17].

The formation of an apatite layer is governed by a complex set of steps that start on the immobilization of calcium and phosphate ions in the surface of the biomaterial forming a biologically active hydroxycarbonate apatite. This layer evolves to form different calcium phosphate phases until it generates hydroxyapatite or hydroxyapatite-like coatings [18, 19] with their characteristic cauliflower morphology (Fig. 10.1).

In a bone tissue engineering perspective, bioactivity can be categorized into class A, when the biomaterial presents osteoproduction (bone growth in the bulk of the biomaterial) and osteoconduction (stimulation of bone growth at the interface), and class B, when it only presents osteoconduction [18].

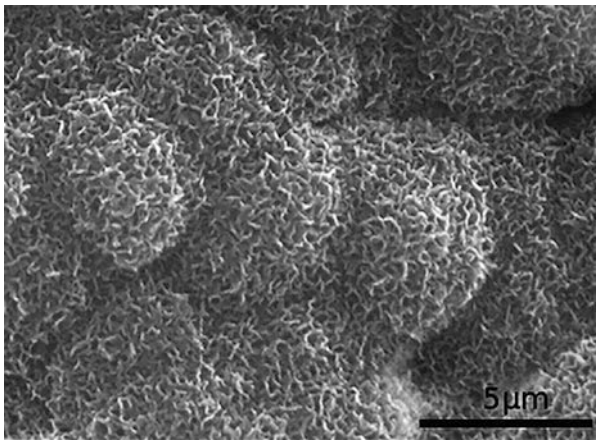

Fig. 10.1 Typical cauliflower morphology of hydroxyapatite when deposited on the surface of a bioactive biomaterial (Reprinted from Ref. [20], Copyright 2007, with permission from Elsevier)

In vitro bioactivity test evaluates the ability of the biomaterial to form an apatite surface layer when in contact with simulated body fluid (SBF), a solution that presents ionic concentrations similar to human blood plasma [21, 22].

After the initial description of this methodology, a series of modifications have been proposed in the composition of SBF. One of the most relevant is mSBF that presents ionic concentrations equal to the human plasma, with the exception of HCO_3^-, whose concentration was reduced to the level of saturation of calcite [23]. This type of approach is also used as a surface modification methodology, generating an apatite coating that enhances the bioactivity of the biomaterial [24]. Other modifications to the SBF solution have been reported, the use of higher ion concentrations (e.g., $1.5 \times$ SBF, $5.0 \times$ SBF, among many others) to reduce the time frame of the apatite deposition are common [24, 25].

The use of SBF or SBF-like solutions is one of most commonly used approaches to test the bioactivity of a biomaterial. However, the different steps of this methodology should be carefully controlled, and the conclusions that one can take from such a characterization are not consensual [19, 26].

10.3 Synthesis and Preparation of Bioactive Composite Systems

10.3.1 Bioactive Inorganic Particles and Their Synthesis

Bioactive inorganic particles (glasses or glass–ceramics) are usually composed of silicates or phosphosilicates (as network formers) combined with different proportions of glass modifiers, e.g., sodium oxide (Na_2O) and calcium oxide (CaO), among others. They have been studied for more than 40 years and are characterized

by their bioactivity and unique bone bonding properties, which are usually related to their surface chemistry. A landmark on the development of these systems was the work of Hench et al. [27]. They showed that the bioglass 45S5 (with a general composition $0.461SiO_2{:}0.026P_2O_5{:}0.269CaO{:}0.244Na_2O$) is able to promote the formation of a calcium phosphate layer on its surface within a time frame of 30 days. This glass composition is one of the most studied ones [27–29]. The concentration of SiO_2 in the glass/glass–ceramic structure seems to be a critical parameter that governs the bonding of the material to living tissue [27]. It has been proven that glass formulations that present fast bonding not only to bone but also to soft tissues are prepared with 45–52 % (by weight) of SiO_2 [30]. Glasses with a SiO_2 content ranging from 55 to 60 % react more slowly and do not bind to soft tissues. Compositions with >60 % of SiO_2 do not bond to bone and are considered as bioinert [27, 31]. Additionally, bioactive glasses have been demonstrated to stimulate the growth and maturation of osteoblasts [31–33]. Most of these materials degrade naturally, similarly to other synthetic substitutes, such as calcium phosphates (CaPs). The degradation products are, usually, metabolized by the body and excreted through the urine [34].

The brittle behavior and weak mechanical properties observed in bioactive glasses are the major difficulties associated with their use in biomedical applications. The bending strength of most bioactive glasses is in the range of 40–60 MPa, which is not enough for load-bearing applications, while their modulus is between 30 and 35 GPa, very close to that of cortical bone. The combination of biodegradable polymers with bioactive glasses has been proposed to produce biodegradable products that show high levels of bioactivity and also improve some of their mechanical properties as compared to conventional glasses [35–37].

Bioactive glasses and glass–ceramics can be obtained by melting at high temperatures followed by casting into a mold or splat quenching. An alternative approach for glass synthesis is the sol–gel route [29, 38, 39]. Both strategies are detailed in the following sections.

10.3.1.1 Melt-Based Approach

Bioactive inorganic particles can be synthesized by different methodologies, and the melt-quenching approach is one of the most traditional and straightforward routes. Under this procedure, the glass precursors (e.g., silica, phosphates, carbonates, etc.) are mixed in a mortar, transferred to a crucible, and fired to temperatures able to melt the whole mixture that can go up to 1,600 °C or higher, depending on the formulations [16, 40–44]. The homogeneous mixture is then splat quenched into ice water or onto a metal plate maintained at room temperature. This process allows the chemical structure to be frozen [13, 45]. Depending on the starting materials, the heating process can be done stepwise, namely, 300 °C if the starting materials contain ammonia in their chemical structure (e.g., sodium ammonium hydrogenophosphate) and/or 650 °C if the starting materials are carbonates

(e.g., calcium carbonate). These thermal treatments allow the release of by-products, such as ammonia or CO_2 that diffuse through the precursors and are released from the mixture [40–42]. Bioactive glasses prepared by melting are usually denser and do not contain any remnants of organic components or water; nevertheless, melt-derived bioactive glasses have a limited surface area, being one of their main disadvantages when in contact with the biological tissues or fluids [46, 47]. The high temperature used in the fabrication of these inorganic particles through the conventional melt-based process does not allow the incorporation of bioactive organic molecules/materials (e.g., proteins, growth factors, genes, hormones, etc.) during the fabrication process.

10.3.1.2 Sol–Gel Approach

Advances in sol–gel processing technology allowed the manufacture of a new generation of bioactive glasses overcoming some of the drawbacks of the melt-based approach (e.g., high processing temperatures) [48]. This low-temperature process dispenses the use of Na_2O in bioactive glass compositions (the main role of this component in the glass compositions is related to the lowering of the glass melting temperature) and allows incorporating polymers and organic molecules to make less brittle hybrid materials [11]. Also, sol–gel route allows a wider range of bioactive compositions for a better response to specific clinical applications, permits the easier control of its morphology and chemical composition, enables an easier design of the material's morphology (powders, monoliths, nanoparticles, gels), and generates materials with high specific surface area, osteoconduction properties, degradability, and nanoporosity [35, 45–47, 49]. In fact, the main physical differences between melt and sol–gel-derived glasses are that the latter ones tend to have inherent nanoporosity, whereas melt-based glasses are denser. The nanoporosity can result in improved cellular response due to the nanotopography that might reach a surface area 100× higher than for similar compositions produced through the melt-based approach [50, 51]. This property increases the solubility of the glass, which is important in a bioactivity perspective [11]. This characteristic is confirmed by in vitro (in SBF) and in vivo studies that report a higher bioactivity and degradability of the sol–gel-derived glasses when compared to the melt-derived ones [51].

The sol–gel route essentially forms and assembles nanoparticles of silica at room temperature. It is a synthetic route where a solution containing the glass compositional precursors (e.g., alkoxides or metal chlorides) undergoes hydrolysis and condensation reactions to form a gel either in water or in an organic solvent. A typical silicate precursor is tetraethyl orthosilicate (TEOS), while common precursors for calcium and phosphate groups are calcium nitrate tetrahydrate and triethyl phosphate, respectively [11, 52]. Adding water or a water/alcohol mixture to TEOS promotes the hydrolysis of the alkoxide functionality generating silicic acid. The addition of an acid or a base as a catalyst allows the silicic acid to condense into a network of silica-based gel through the release of water molecules. Depending on the experimental conditions and compositions, these two steps might

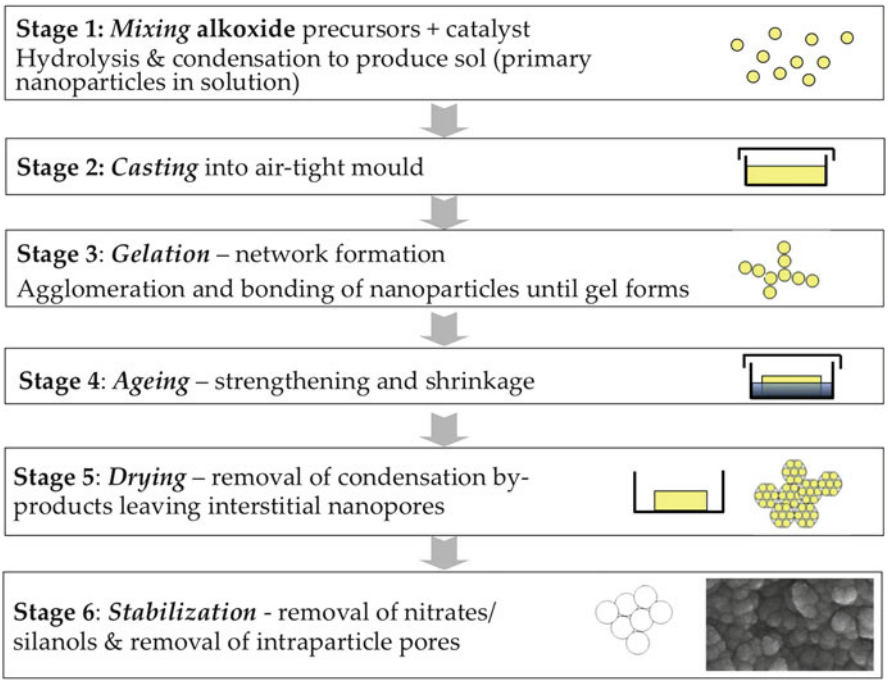

Fig. 10.2 A flow chart of the acid-catalyzed sol–gel process used to synthesize bioactive glasses, including schematics of the evolution of the gel and its nanoporosity (Reprinted from Ref. [11], Copyright 2013, with permission from Elsevier)

occur simultaneously [53]. At the initial time frame of the silicic acid condensation, a sol is generated. The subsequent agglomeration of the particles that constitute the sol forms the gel. This gelation step proceeds slowly. Complete cross-linking of the silicate network is achieved during the ageing step [53, 54]. A procedure can be adapted to generate glass particles and not a 3D glass structure (e.g., Fig. 10.2). In this approach the experimental conditions are manipulated to inhibit the agglomeration of the particles generated in the initial steps of the procedure [55, 56]. Typical bioactive compositions can be binary systems, e.g., SiO_2-CaO; ternary systems, e.g., SiO_2-CaO-P_2O_5; or quaternary systems, e.g., SiO_2-CaO-Na_2O-P_2O_5 [52, 57, 58]. The silanol group is critical for the bioactivity of the glass. In addition, gel-derived bioactive glasses may contain high-energy silicate ring structures, which further activate the material reactivity [12, 14, 15].

Microparticles, monoliths, or foams are usually produced using acidic catalysis (Fig. 10.2) [11]. Using this methodology, the primary nanoparticles (with diameters around 2 nm) present in the sol coalesce and condensation (polymerization) occur, forming Si–O–Si bonds. The nanoparticles coarsen, coalesce, and bond together, forming a gel network of assembled nanoparticles [59]. The gel is dried and is heated at temperatures above 700 °C to produce a nanoporous bioactive glass [11].

As water and alcohol evaporate during drying, they generate an interconnected porous network. The pores are formed in the interstices between the coalesced nanoparticles [59], and their size depends on the precursors used, the glass composition, and the pH of the reaction [60]. Pore diameters are typically in the range of 1–30 nm [52].

If the synthesis is carried out under basic conditions, submicrometer particles are formed [61]. Compared with micron-sized bioactive ceramic particles, nano-sized particles have a higher specific surface area and can form a tighter interface with the polymer matrix in a composite formulation [62–65]. Furthermore, reducing the size of the particles would not only accelerate the formation of a bioactive hydroxyapatite surface layer but also provide more active sites for osteoblast attachment, enhancing their proliferation and differentiation, as well as tissue growth [66, 67]. Also, they exhibit, to some extent, similar nanoarchitecture as the physiological bone [68]. Work on the fabrication of bioactive glass nanoparticles (BG-NPs) by the sol–gel route has been reported [55, 65, 69]. Hong et al. obtained ternary BG-NPs (SiO_2-CaO-P_2O_5) by the combination of two strategies, sol–gel and coprecipitation approaches. The mixture of precursors was hydrolyzed in an acidic environment and condensed in alkaline condition, and the resultant particles were collected by freeze-drying [62]. Briefly, the sol–gel synthesis procedure comprised as follows: mixture of TEOS, $Ca(NO_3)_2$, ethanol, and water; addition of citric acid (catalyst) to adjust solution pH at 1–2; vigorous agitation to promote the hydrolysis of the silica precursor; adding drop by drop the resultant sol into a $(NH_4)_2HPO_4$ solution under vigorous agitation; continuously adding ammonia into the solution to maintain the pH at 10–11; separating the particles by centrifugation; and the collection after freeze-drying. After calcination at 700 °C, it was possible to obtain particles with an average diameter of around 20–40 nm [62]. It was found that both binary and ternary BG-NPs prepared by this method exhibit bioactive features [70]. Formulations, morphologies, and sizes of BG-NPs could be tailored by varying the production conditions and the feeding ratio of the reagents [56, 71]. For instance, Chen et al. investigated the effects of the experimental conditions on the morphology of BG-NPs of the system SiO_2–CaO–P_2O_5, and they found that the use of lactic acid decreased the size of the BG-NPs [72].

The sol–gel versatility allows the incorporation of different ions to the glass structure (e.g., Zn^{2+}, Mg^{2+}, Ag^{+}, etc.) in order to improve the glass functionality and bioactivity [43]. El-Kady et al. produced silver-doped BG-NPs that showed antibacterial activity against different types of bacteria. This type of particles could be used to minimize the occurrence of bacterial infections generated by the implantation of bone tissue engineering scaffolds [73].

The sol–gel technique has been also applied in the synthesis of a variety of bioactive monolithic and particulate glasses (e.g., Fig. 10.3). However, a high-temperature calcination step is required to eliminate organic remnants, the processing is relatively time consuming, and it is difficult to obtain defects-free bioactive glass monoliths with diameters above 1 cm. Defects are mainly due to the shrinkage that occurs during drying. For particles, vapor stresses through the interconnected pore

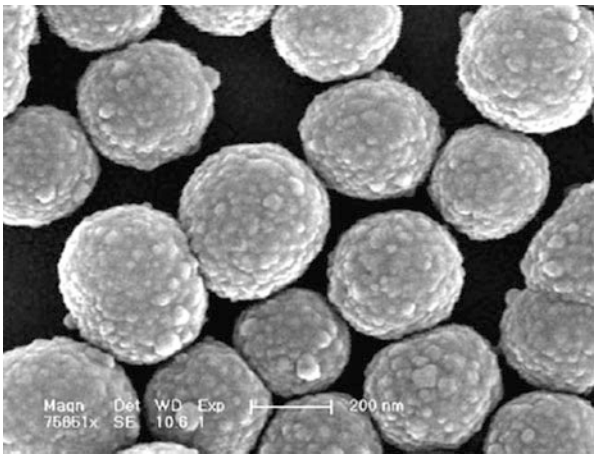

Fig. 10.3 Bioactive silica-based particles prepared through the sol–gel route (Reproduced from Ref. [76] by permission of John Wiley & Sons Ltd.)

network are small, and the path of evaporation is short, although, for monolithic objects, the path from the center of the monolith to the surface is long, and the drying stresses can introduce fracture [11, 74]. The enumerated drawbacks of the sol–gel route, allied with the low cost of the melting procedure makes this latter route dominant in the commercial production of bioactive glasses [75].

10.3.2 Natural-Based Polymeric Phases/Composites and Their Processing

The combination of bioactive inorganic particles with natural-based polymers generated a family of bioactive composites with a wide range of applications, from structural implants to tissue engineering scaffolds [74, 77]. These composites combine the flexibility of polymers with the stiffness, strength, and bioactive character of the inorganic glass fillers. So far, most of the published work on this class of composites has been carried out using conventional (micron-size) inorganic particles as fillers (or coatings) [77, 78]. However, the capacity to produce nano-sized particles/fibers allowed the development of bionanocomposites through the combination of nano-reinforcements and biodegradable polymers of different origins. These bionanocomposites have been explored, for example, as porous scaffolds for bone tissue engineering applications or membranes with potential applications in the dental field [79–81].

These types of bioactive composite structures can be produced using different methodologies, namely, melt-based ones (which requires the use of a thermoplastic polymer), wet chemistry approaches, or rapid prototyping. These three main processing methodologies will be further detailed in the next sections.

10.3.2.1 Conventional Melt-Based Methodologies

Conventional melt-based processing techniques (e.g., extrusion, injection molding, compression molding, fused deposition molding, etc.) are based on the melting of a particular polymer in order to shape it or mix it with different reinforcements [82]. In this perspective, non-thermoplastic materials are not possible to be processed using these methodologies. Most of the natural-based biodegradable polymers are not thermoplastic, limiting their melt-based processing [83, 84]. Researchers have overpassed this limitation through the combination of natural-based polymers with thermoplastic polymers [85]. Under this approach, different polymeric blends, able to be processed using melt-based technologies, were developed (e.g., blends of starch and synthetic polymers and blends of chitosan and aliphatic polyesters) [86–92].

Thermoplastic polymers or blends have been used as matrices for the production of glass-/glass–ceramic-based composite systems [93–98]. Most of the published work targeted their use in bone tissue engineering. The inorganic phases are introduced to enhance the bioactivity of the material, while some of the mechanical properties are improved [96].

Melt-based processing using blowing agents or compression molding followed by particulate leaching has been used to obtain porous inorganic/organic biodegradable composites [99, 100]. It is also worth to highlight that physical blowing agents, such as supercritical CO_2 or water, have been shown to be effective on developing porous glass-/glass–ceramic-based composites for bone tissue engineering applications [101]. In fact, porosity is a critical parameter when complete bone regeneration is targeted. It allows the cell colonization in the bulk of the biomaterial and, subsequently, new bone production.

10.3.2.2 Wet-Spinning Approaches

Wet spinning is a phase inversion technique allowing the production of polymer fibers through an immersion–precipitation process. In the methodology, a solution of polymer is continually spun through a spinneret leading to the formation of a polymeric filament. The spun occurs into a coagulation bath composed of a poor solvent (non-solvent) or a non-solvent–solvent mixture with respect to the polymer being processed. A homogeneous solvated filament, composed of polymer, solvent, and possible additives, solidifies because of polymer desolvation, caused by solvent–non-solvent exchange [102]. The essential feature in wet spinning is the transfer of solvent from the polymer to the coagulating bath. The major drawbacks that limit the rate of wet spinning are usually related with the need for sufficient time for coagulation to occur, the dependence on the rate of solvent diffusion, and the considerable viscous drag of the coagulating baths [102].

Through the assembly of the thin wet-spun fiber meshes, it is possible to fabricate 3D networks of fiber meshes. This methodology imparts flexibility to the manufacturing process, allowing the design of scaffolds with properties that

can be adjusted depending on the targeted site (e.g., thicker scaffolds for the long bones or membrane-like scaffolds for the maxilla regions) [103, 104]. It has been reported that the combination of wet-spun chitosan fibers with bioglass enhances the material bioactivity and promotes osteoblast proliferation and ALP activity [105]. Wet-spinning technologies also enable to create hybrid materials with high levels of organization and avoid the thermal degradation of natural origin polymers compared to other techniques, such as the melt spinning [103]. Wet spinning has been used for the production of wet-spun fibers for drug release [106] and polymeric scaffolds for tissue engineering applications [104, 107].

10.3.2.3 Rapid Prototyping

Rapid prototyping is based on an approach that combines computer science and manufacturing technologies. The main advantage of these techniques is their ability to produce complex structures using a computer-aided design (CAD) model [13]. Currently, different rapid prototyping techniques are used for biomedical applications and can be classified as (1) laser-based, (2) nozzle-based, and (3) printer-based systems [108].

Laser-based systems benefit from the photopolymerization pathway as a basis to fabricate cross-linked polymeric scaffolds. This type of processing methodology has been also employed to develop 3D scaffolds constructed solely with bioactive glasses [109]. In this approach a CO_2 laser is used to soften or melt the glass particles that are injected to the targeted position by means of a nozzle. The processed particles bind to the substrate at the position where they interact with the laser radiation. These 3D constructs exhibit a higher crystallization degree than their parent glass powder precursors. They maintain their bioactive character after processing, although the rate of apatite formation when immersed in SBF is slower [109].

The main limitation of the nozzle-based techniques is that the resolution is determined by the nozzle size, which makes it difficult to design and fabricate scaffolds in the micron- or nano-size. The well-known processing of (pre)-polymers by pressure exerted on extrusion/dispensing units supports the second category of rapid prototyping systems. Under this approach, the production of bioactive glass–polycaprolactone (PCL) composites is achieved by stereolithography, through the printing of PCL–glass mixtures and cross-linking of methacrylated PCL. The resulting 3D constructs are bioactive, being able to generate an apatite layer upon immersion in SBF. Additionally, the constructs revealed to be non-cytotoxic towards fibroblasts, inducing an increase in cell activity when the bioactive glasses were added up to a concentration of 20 % [110].

The printer-based systems combine powder beds and the deposition of a binder that fuses the particles, or directly depositing material using inkjet technology [108, 111, 112]. It has been used to process biodegradable polymeric scaffolds for tissue engineering applications [13]. This process prints a binder onto a surface in a sequential layered way. The processing parameters such as the speed, flow rate, and drop position can be computer controlled to produce complex 3D scaffolds.

Several recent research works have been reported on the fabrication of 3D scaffolds using highly bioactive mesoporous glasses as starting materials. For example, Yun et al. reported the synthesis of ordered bioactive 3D glass scaffolds exhibiting a hierarchical porosity composition using a combination of sol–gel, double polymers templating, and rapid prototyping techniques [113]. The resulting scaffolds presented three lengths of porosity: mesopores (2–50 nm), macropores (>50 nm), and giant pores (30–100 μm) [114]. The in vitro tests revealed that the bioactive behavior of mesoporous bioactive glasses was preserved because a carbonate hydroxyapatite layer was formed onto the material surface after 24 h of immersion in SBF [108, 115].

The processing of alginate–glass composites has also been achieved using 3D printing, with the inclusion of mesoporous bioactive glass particles in a concentration up to 50 % (w/w). The addition of glass particles to the alginate phase induced an increase on its compressive strength and stiffness. Only the compositions with high glass content (30 and 50 %) exhibited apatite formation onto the composite structure upon SBF immersion. The glass particles were also loaded with dexamethasone prior to the processing of the composite. Its release profiles showed that the glass particles have the capacity to exhibit sustained delivery properties within the glass–alginate system. The authors also report the formation of apatite particles when the constructs were cultured with human bone marrow stem cells for 7 days [116]. The use of other bioactive agents, such as growth factors, can also be incorporated into the scaffolds during the printing process, although the maintenance of their stability depends strongly on the experimental conditions [112].

10.4 Applications of Bioactive Composites Within Bone Tissue Engineering

10.4.1 Injectable Systems

Some injectable systems composed by the combination of polymers and bioactive inorganic particles have been proposed for bone tissue engineering [71]. However, one of the main difficulties in the injection of such suspensions of a solid phase is the filter-press effect responsible for a variation of the solid/liquid ratio during the injection that eventually hampers the injection flow through the creation of a backpressure in the syringe [117]. The injectability is related to the viscosity of the suspension, in particular the solid/liquid ratio and the suspension stability [43]. A number of additives have been used to improve the injectability.

Another drawback is the washout phenomenon, which corresponds to the disaggregation or dispersion of the paste during or after its placement. This event is not intrinsic to the material and is related to the viscosity of the medium in which the

paste is injected. It has been shown that the viscosity of the paste has to be higher than that of the target medium to avoid dispersion from the administration zone [7, 43, 118]. Calcium phosphate-based cements have been extensively explored in this form; they generate apatite following the setting and hardening reactions. The key advantages of these cements are their self-setting properties and the possibility to apply them within a minimum invasive surgery framework.

Injectable systems have been proposed as new tissue engineering strategies to deliver cells and bioactive agents (encapsulated in a biodegradable matrix) through minimally invasive procedures. Couto et al. combined a chitosan–β-glycerophosphate salt formulation with sol–gel-derived BG-NPs to prepare bioactive thermo-responsive hydrogels for orthopedic reconstruction and regenerative medicine applications [71]. The size and spherical shape of the particles guarantee the efficient injection of these systems through small-gauge needles into the bone defects. The system presented a gelation point around 36.8 °C, being suitable for intracorporal application. It was also observed that the gelation temperature could be manipulated through the increase of the chitosan deacetylation degree. Upon immersion in SBF, the composites containing BG-NPs induced the formation of bone-like apatite clusters that were well integrated in the organic structure. The density of the apatite precipitates increased with increasing BG-NP content and soaking time in SBF. Despite more research is required to fully validate the use of such systems in the bone regeneration context, this osteoconductive injectable biodegradable system has a large unexplored potential in bone tissue engineering [71].

10.4.2 3D Scaffolds

3D porous composite scaffolds can induce the ingrowth of cells to the desired shape and may facilitate the vascularization of new tissue [119]. Composites found in nature contain an inorganic phase embedded in an organic matrix, usually assembled in a complex and hierarchical structure [120]. Biomimetic osteoconductive nanocomposites may be obtained by combining bioactive glass/ceramics with a polymeric matrix [74].

One approach to combine scaffolds with bioactive glasses is the use of highly porous glass scaffolds coated with polymer, such as poly(DL-lactide) (PDLLA) or poly(3-hydroxybutyrate) (PHB) [121, 122]. Chen et al. used PDLLA to coat a bioglass foam with 90 % porosity with pore diameter between 500 and 700 μm [122]. The thin coating resulted in an improved work of fracture [122]. However, these types of coatings can mask the surface of bioactive glass scaffolds generating a non-bioactive surface. This drawback significantly decreases the effectiveness of polymer-coated scaffolds in terms of osteoconductivity and osteoinductivity. The long-term effectiveness of the coating is also under concern since its degradation lead to a brittle glass–ceramic scaffold [96].

Foams produced by thermally induced phase separation are promising bioglass containing composites for bone regeneration [123]. In this case, the biodegradable polymer is dissolved in dimethyl carbonate, and the particulate glass fraction is added. The mixture is quenched in liquid nitrogen and lyophilized. PDLLA foams containing 40 % (w/w) of bioactive glass have been produced with tubular pores (with 100 μm of diameter) with interconnectivity between 10 and 50 μm and porosities up to 97 %. The thin pore walls allow the bioactive particles to be exposed. Despite the high porosity, the pore and interconnectivity sizes are too low to allow the cells to colonize the interior of the scaffold, while the high percentage of porosity (and thin pore walls) contributes to low mechanical resistance [124].

The addition of bioglass particles to a biodegradable polymeric matrix is a typical approach to generate conventional composite systems. Polymers, such as polylactic acid (PLA), polyglycolic acid (PGA), and their copolymers (PLGA), have been utilized as matrices. These systems have been used clinically for the past years [77]. The incorporation of bioglass in these polymeric matrices (e.g., PLGA) can increase the stiffness and compressive strength of the composite. However, in some cases, the addition of a glass phase can be detrimental. For example, composite systems based on the compounding of PDLLA with bioactive glass particles (with particle size between 50 and 125 μm) were produced using twin-screw extrusion, and, as the glass content was increased, the bending, torsional, and shear strengths of the composite decreased [96]. Moreover, it is still difficult to match the degradation rate of the polymer with that of the glass.

The development of nanocomposites using nanoparticles dispersed in a polymeric matrix has the potential to improve the interaction with the host tissue/cells [125, 126]. Sol–gel-derived BG-NPs have also been introduced into freeze-cast gelatin–chitosan foams resulting in pore sizes in the range of 150–300 μm [127]. Their mechanical strength was augmented with the reduction of the composite porosity. Bioactive glass–collagen–phosphatidylserine scaffolds (including 65 wt% of the sol–gel-derived 58S glass) were produced with 75 % of porosity and with pore sizes up to 300 μm. They exhibited a compressive strength of 1.5 MPa. However, pore interconnectivity was low [128].

Hong et al. combined PLLA and sol–gel-derived BG-NPs [65]. The porous PLLA/BG-NP scaffolds were prepared by a thermal-induced phase separation method [55]. They found that increasing the amount of BG-NPs into the PLLA matrix altered the morphology and porosity of the scaffolds. The addition of BG-NPs to the PLLA matrix induced an increase of both compressive modulus and strength of the scaffolds. In vitro tests revealed that these composites (prepared with different proportions of BG-NPs) are bioactive. The addition of BG-NPs increased the water uptake of the scaffolds, especially at a low BG-NP loading, and also greatly affected the degradation rate of the PLLA matrix [55].

The correct dispersion of bioactive glasses in a polymeric matrix is dependent on the interfacial bonding and the compatibility between both phases. The BG-NP/PLLA composite system was studied by Liu et al. [129] to evaluate strategies to overcome these dispersion and compatibility drawbacks. The authors prepared BG-NPs via the sol–gel route and grafted low-molecular-weight PLLA in the

particle surface using a diisocyanate. They were able to homogeneously disperse the modified particles within the PLLA matrix, improving both the nucleating rate and degree of crystallization of the composite. The grafting modification induced an increase on the tensile strength, tensile modulus, and impact energy of the composites by increasing the phase compatibility. Thus, this strategy can be used to prepare composite structures able to be employed in bone tissue engineering at load-bearing sites. In vitro bioactivity tests compared pure PLLA scaffolds and BG-NP/PLLA nanocomposite [129]. The results demonstrated that BG-NP containing composites had a higher capacity to induce the formation of an apatite layer on the scaffold surface. The culture of bone marrow stromal cell onto these composites revealed that BG-NP particles facilitate the attachment and proliferation of stem cells onto the surface of the scaffolds [129].

Nonetheless, it is important to notice that when bioactive glass particles are combined with a polymer matrix, most of the cells are only in contact with the polymeric phase. Additionally, some particles might protrude from the surface through a process difficult to control [96].

10.4.3 Membranes and Layer-by-Layer Structures

Composite membranes have been also developed for dental applications and bone tissue engineering. Specifically, in guided tissue/bone regeneration, membranes are used as barriers to prevent the invasion of the faster-growing soft tissue cells into the defect site and to regenerate periodontal ligament, cementum, and bone.

Membranes generated by the combination of chitosan and bioglass [130, 131] or BG-NPs [81] (the ternary SiO_2-CaO-P_2O_5 and quaternary SiO_2-CaO-P_2O_5-MgO systems) have been prepared by solvent casting. Under this approach a polymeric solution containing the inorganic filler is poured onto a vessel and left to dry at room temperature to generate the membranes [81]. The membranes prepared with the composite formulations presented an improved mechanical performance and excellent apatite forming ability compared to pure chitosan membranes [81, 130–132]. The combination of BG-NPs and chitosan increased the composite stiffness [132], while the metabolic activity of periodontal ligament cells (hPDL) and stem cells cultured on these membranes was enhanced with the addition of BG-NPs. In addition, an increase in the hPDL proliferation when they were cultured onto the composite membranes was observed [132]. The addition of BG-NPs promoted greater cell matrix mineralization by both types of cells. Thus, the chitosan/BG-NP membranes could have potential to be used as a temporary guided tissue regeneration membrane in periodontal regeneration. They could act as barrier membranes to prevent the invasion of the periodontal defects by soft tissues since these membranes were non-cytotoxic and did not present early degradation [132].

Nonetheless, the application of these materials for periodontal regeneration requires the contact of the two sides of the membranes with distinct biological environments in which only one of the sides is in contact with a region where

osteointegration is targeted. Thus, composite membranes were developed to present distinct properties in both sides. One of the sides was designed to be a barrier to soft tissue invasion by blocking the migration of epithelial cells [130, 131, 133]. The other side of the membrane was formulated to promote bone growth. In this way, biocompatible and biodegradable composite membranes were produced through the combination of PDLLA and bioglass particles, featuring an asymmetric bioactivity and a good integration between the polymeric and inorganic phases. These membranes were prepared using an adjusted solvent casting method, which promoted the nonuniform distribution of the inorganic component along the membrane thickness. Only the bioglass-rich side of the membrane induced the precipitation of bone-like apatite in SBF, indicating that this biomaterial exhibit asymmetric osteoconductive properties [80, 133]. Their mechanical properties were also evaluated, and a clear plasticization effect of water was detected as well as an increase in stiffness. SaOs-2 cells attached on both sides and proliferated during 7 days of culture [80]. Results with both human bone marrow stromal cells and hPDL cells revealed an improved cell adhesion and proliferation and stimulated cell differentiation, mineralization, and production of extracellular matrix and calcium nodules, suggesting the positive effect of adding the bioactive microparticles into the PDLLA matrix [133]. The results indicate that the proposed asymmetric PDLLA/bioglass membranes have potential to be used in guided bone tissue regeneration therapies.

Luz et al. used micro-contact printing to pattern mineralizable elements onto a membrane [134]. Freestanding chitosan membranes were used, and circular motifs containing BG-NPs were printed on them using previously inked PDMS stamps. The bioactive character of the BG-NP spots allowed the nucleation and growth of apatite, highly localized in the patterned regions of the chitosan membranes. Results showed that L929 cells replicated the initial inorganic pattern preferring the environment created by the BG-NPs rather than migrating to chitosan. With this simple micro-contact printing approach, it was proved that it is possible to control the cellular interactions with a bioactive substrate at the microscale level. Successful patterning of fibroblasts is an indication of the versatility of the developed system. This approach allows the spatial control of the biomaterials' properties at the microscale and could be potentially used for skin, vascular, articular, and bone tissue engineering. Additionally, these systems can also be used under cocultures systems or to develop substrates able to confine cells in specific regions [134].

Finally, Couto et al. developed a biodegradable multilayer coating, through the sequential deposition of a polycation (chitosan) and an anionic element (BG-NPs) [135]. Quartz crystal microbalance (QCM-D) study revealed that this methodology could be used to produce nanostructured multilayers upon increasing the number of layer-by-layer cycles [135]. The hypothesis of this concept was that such robust coatings could also induce the formation of apatite upon immersion in SBF [135]. The proposed method could be also employed in the coatings of substrates with complex geometries, including scaffolds for bone tissue engineering, and, thus, constitutes a new technological solution to improve osteoconductivity of a variety of implants for orthopedic applications [135].

10.5 Future Trends

During the past years the development of bioactive composite systems has been mainly focused on the incorporation of different inorganic mechanical reinforcements, which are also able to improve the bioactivity of the composites. The inorganic particles can be also used for the controlled delivery of bioactive agents, such as anti-inflammatory drug or proteins that promote different biological events (e.g., growth factors).

The decoration of the composite surface with bioactive molecules is being extensively studied in tissue engineering. A strong investment in the exploitation of grafting of different peptides, antibodies, or proteins that are able to induce stem cell recruitment, proliferation, and differentiation both in the surface and in the bulk of the 3D constructs is expected.

At the initial years of development, it was given a strong effort on materials science and engineering, although the science in this field is evolving into a more multidisciplinary approach, where materials science and biology are combined to create methodologies and biomaterials able to promote complete bone regeneration.

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