Received Date : 29-Sep-2016

Revised Date : 22-Dec-2016

Accepted Date : 24-Dec-2016

Article type : Review Article

Bioactive glasses as delivery systems for antimicrobial agents

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Summary

Most biomaterial-associated infections are caused by opportunistic pathogens and bacteria that are regularly found within the microflora of the implant site. In addition, a biomaterial implant or device remains at risk of infection by hematogenous spread of bacteria disseminated from infections elsewhere in the body or from infected peri-implant tissue in revision surgery. The resulting infections are frequently accompanied by patient morbidity and discomfort and can lead to surgical replacement of the implant after lengthy,

unsuccessful attempts to mitigate infections with antibiotic treatments. Therefore, extensive is aiming to find new infection-resistant antimicrobial biomaterials and coatings for implants and devices to effectively reduce the incidence of biomaterial-associated infections. An overview of the *in vitro* and *in vivo* antimicrobial efficacies of the numerous biomaterials currently available is beyond the scope of this review. Herein, we provide a comprehensive review of bioactive glasses as biomaterial delivery systems for antimicrobial agents.

Keywords: Bioactive glasses, biomaterials, antimicrobial agents, delivery systems, antibacterial activity, infection.

I. Bioactive glasses

The most widely used bioactive glasses (BGs) are amorphous solids based on a silica matrix, whose structure can be described as a three-dimensional covalent and interconnected network formed by SiO_4 tetrahedra, linked together by bridge oxygens, where each bridge oxygen is bound to two Si^{4+} atoms (Cormack 2012; Brauer 2015; Kaur *et al*. 2016). The addition of cations (Na^+, K^+, Li^+, Ca^{+2}, Mg^{+2}, Sr^{+2}) or anion modifiers (Cl^-, F^-) plays a very important role in the physical, chemical and biological properties of these materials (Brauer 2015; Kaur *et al*. 2016), because it allows reducing the glass transition temperature, decreasing both the density and the local symmetry of the network, reducing the viscosity of the material, increasing the solubility of the glass in physiological medium and releasing ions with therapeutic potential, as for example with antibacterial (Ag, Cu, Au, Mg, Ti, Ga), osteogenic and/or pro-angiogenic (B, Cu, Co, Sr, Li) effects (Gorustovich *et al*. 2010; Hoppe *et al*. 2011).

BGs can be obtained mainly by two methods: melt-quenching and sol-gel (Jones 2012; Brauer 2015; Kaur *et al*. 2016). The former is the most conventional and widely used, although the need for increased temperatures can be a disadvantage. In addition, the process of cooling of the melt-quenching method can lead to the appearance of defects such as bubbles, cracks, etc., which may affect the properties of the glass. On the other hand, the sol-gel technique (Jones 2012; Kaur *et al*. 2016) allows working at low temperatures and manufacturing both amorphous and polycrystalline materials with special characteristics such as high purity and homogeneity. The disadvantages of the sol-gel method include: the drastic shrinkage during the drying stage, which can lead to the appearance of microcracks, the permanence of carbon traces in the final oxide, the long duration of the hydrolysis stage, the difficult drying of structures of complex shape, the difference in the kinetics of the hydrolysis of the different alkoxides, the possible existence of reactive and volatile intermediaries, the large amount of solvent used, and the release of water formed during the polycondensation (Jones 2012; Kaur *et al.* 2016).

The presence of interconnected pores with a reduced diameter (2-50 nm) in the matrix of sol-gel BGs allows the material to trap and immobilize water molecules, so that the structure of these BGs presents a certain concentration of OH groups. This property of BGs also promotes cell migration, cell penetration and vascularization. This interesting property has recently promoted the use of sol-gel processes to obtain hybrid, nanostructured and mesoporous materials with very different applications (Wu and Chang 2014; Izquierdo-Barba and Vallet-Regí 2015). Mesoporous BGs present textural and bioactive characteristics far superior than those of BGs obtained by melt-quenching or sol-gel (Jones 2013; Wu and Chang 2014; Izquierdo-Barba and Vallet-Regí 2015). Mesoporous BGs are obtained by the incorporation of structure-directing agents (in general surfactants) to its process of synthesis, and can present values of specific surface and porosity up to five times higher than those of BGs

obtained by other methods. Their pores have a homogeneous distribution of size and an orderly disposition, which makes them excellent matrices to incorporate compounds such as antibiotic, osteogenic, or anti-osteoporotic agents (Wu and Chang 2014; Izquierdo-Barba and Vallet-Regí 2015).

The discovery of melt-derived BGs in the SiO_2-CaO-Na_2O-P_2O_5 system as biocompatible materials established a new family of biomaterials highly relevant to regenerative medicine (Hench 2015). For example, the BG 45S5 (% in weight: 45% SiO_2, 24.5% Na_2O, 24.5% CaO, 6% P_2O_5 (Bioglass®)) and related compositions (S53P4: 53% SiO_2, 23% Na_2O, 20% CaO, 4% P_2O_5) have a long history of clinical use (Hench 2015). Marketed under different brands (NovaBone™, PerioGlas™, BonAlive®, etc.), these biomaterials are currently used in the form of particles of different size as bone fillers in orthopedic and maxillofacial surgery (Peltola *et al.* 2008; Lindorfs 2010b; Rantakokko *et al*. 2012). It has been shown that the mechanisms by which BG particles promote bone repair are associated with the release of ions (Si, Ca, F, B, Sr, Co), which exert control over the cell cycle that leads to the differentiation and proliferation of bone cells, as well as over the expression of genes that regulate osteogenesis (Jell and Stevens 2006; Hench 2009).

Finally, from the point of view of tissue engineering, BGs can be used for the manufacture of three-dimensional porous matrices, known generically as scaffolds. The functions of these scaffolds include providing a temporary biocompatible mechanical support and promoting the biological processes of repair and/or regeneration of vascularized tissues, serving as scaffolding for cells and growth factors involved in the repair process and as a base to add antimicrobial agents (Lee and Atala 2013; Johnson and García 2015; Baino *et al*. 2016).

II. Bioactive glasses as biomaterial delivery systems for antimicrobial agents

The potential of BGs as biomaterial delivery systems for antimicrobial agents has been investigated mainly using three strategies (Fig. 1). One strategy has based on the release of their ionic compounds once BGs are suspended in an aqueous solution. A second strategy has implied the doping of BGs during its synthesis with biocide metals. The third strategy has involved the delivery of antibiotics from BGs surfaces or from a polymer phase.

Bioactive glasses without any special bactericidal ion

When suspended in an aqueous solution, BG systems show antimicrobial activity via the release of their ionic compounds over time. Through *in vitro* experiments, Stoor *et al*. (1998) were the first to demonstrate that the BG powder S53P4 had a broad antibacterial effect on oral bacteria. Thereafter, many other experiments have shown that both sol-gel- and melt-quenching-derived particulate BGs have a broad and specific antibacterial effect *in vitro*. One of the most studied BG systems for this purpose is the 45S5 Bioglass® formula (Waltimo *et al*. 2009; Hu *et al*. 2009; Misrna *et al*. 2010). Also, particulate Bioglass® reduces the viability of *Streptococcus sanguis* biofilms formed on its surface (Allan *et al.* 2002). Bioglass® particles incorporated into natural or synthetic polymers also show antibacterial properties. Pratten *et al*. (2004) found that bacterial colonization on surgical sutures coated with 45S5 decreases significantly as compared to non-coated ones. In another study, Misra *et al*. (2010) showed that biocomposites made of Poly(3-hydroxybutyrate) and nanoparticles of 45S5 BG (P(3HB)/n-BG 10 wt%) inhibited the cell growth of *Streptococcus aureus* NCTC6571. These authors also found that the inhibitory strength correlates with the incubation periods as well as with an increase in the pH of the culture medium. Our group showed that the coating of 45S5 BG on agar–gelatin films promotes biocomposites with strong anti-staphylococcal activity *in vitro* (Rivadeneira *et al*. 2013). In agreement with the results of Misrna *et al*., our results showed that the antibacterial effect was correlated with an

alkalinization of the medium. In contrast, in an *in vivo* study, Xie *et al*. (2008) found that particulate Bioglass® failed to prevent experimental staphylococcal infection of open tibial fractures in rabbits.

In another study, Munukka *et al*. (2008) found that the sol-gel-derived glass $CaPSiO_2$ showed antibacterial effect on 29 clinically important bacterial species. This antibacterial effect was similar to that of the BG S53P4, although the increase in pH was lower for $CaPSiO_2$.

Although it is known that the antibacterial effects of BGs vary with the bacterial type, glass composition, and sample concentration (Munukka *et al*. 2008; Hu *et al*. 2009; Zhang *et al*. 2010), the exact mechanisms of the antibacterial action of these materials is still under debate. One of the mechanisms believed to be responsible for their antibacterial effects is the high-pH environment resulting from the rapid ion exchange between the alkaline ions (Na^+, Ca^{+2}) from the glass surface and the hydrogen ions from the solution (Zehnder *et al.* 2006; Rivadeneira *et al.* 2013). Another consequence of the high concentrations of calcium and alkalis released from BGs is the perturbation of the membrane potential of bacteria (Munukka *et al.* 2008). Another mechanism that has been associated with the antibacterial effects of BGs is the release of silica from these materials (Zehnder *et al.* 2006). The antibacterial effects of BGs have also been related to the destruction of the cell walls by BG debris (Hu *et al.* 2009). Finally, the particle size is also involved. Waltimo *et al*. (2009) reported that nanometric BGs release more alkaline species because of the large surface area, and consequently display stronger antimicrobial effect than micron-sized glasses.

The BG S53P4 (BonAlive®) has been in clinical use for over 20 years and has been used in frontal sinus, mastoid, and benign bone tumor, as well as in trauma and spine surgery, with no observable signs of toxic reactions (Rahaman *et al*. 2014). It is well known that Staphylococci are the most prevalent etiological agents of orthopedic infections. Also, after an initial colonization, *S. aureus* produce biofilm which is difficult to eradicate (Arciola *et al.*

2015; ter Boo *et al.* 2015; Inzana *et al.* 2016). Drago *et al.* (2014) have recently shown that different formulations of S53P4 interfere with the bacterial biofilm produced on prosthetic material by methicillin-resistant *S. aureus* (MRSA) and multi-drug-resistant *Pseudomonas aeruginosa*. S53P4 has also shown significant potential in the treatment of chronic osteomyelitis. In a multicenter study carried out by Lindfors *et al*. (2010), 11 patients with verified chronic osteomyelitis in the lower extremity and the spine were treated with S53P4 as a bone substitute. The cavitary bone defect and the surroundings of a spinal implant were filled with S53P4. The authors found that S53P4 was well tolerated and that nine of the patients healed without complications. More recently, Romanò *et al*. (2014) carried out a comparative study between the use of S53P4 and antibiotic-loaded calcium-based bone substitutes in the treatment of chronic osteomyelitis. They studied three groups: Group A, consisting of 27 patients affected by chronic osteomyelitis of the long bones, who were treated with S53P4; Group B, consisting of 27 patients who were treated with an antibiotic-loaded hydroxyapatite and calcium sulfate compound; and Group C, consisting of 22 patients who were treated with a mixture of tricalcium phosphate and an antibiotic-loaded demineralized bone matrix. The authors showed that patients treated with the BG without local antibiotics achieved similar eradication of infection (up 85%) and less drainage than those treated with the different antibiotic-loaded calcium-based bone substitutes.

Bioactive glasses doped with biocide metals ions

Biocide metal ions (silver, copper, zinc) are of special interest because of their known antibacterial properties (Palza *et al.* 2015). BGs can be used as carriers of these agents by following different methods. Basically, there are three common ways to dope BGs: 1) by adding a compound during the glass-melting route, 2) by adding a compound during the sol-gel synthesis process, or 3) by incorporating certain ions into the glass structure via an ion-exchange process. Less common strategies include coatings on the glass structure or the

compound as the disperse phase within glass matrices (Diba and Boccaccini 2014). Once incorporated, generally, the metal-doped glass gradually dissolves (in aqueous medium) depending on its dissolution rate. During its dissolution, the ions incorporated into its structure are released into the medium and inhibit the growth of bacteria. Different BGs with different composition such as silicate, phosphate, borate in different forms and architecture have been doped with different metals. Herein, an overview of the most relevant BGs doped with antibacterial agents will be presented and also resumed en Table 1.

Bioactive glasses doped with silver

Silver (Ag) ions have been the most studied metals to dope BGs. The suggested mechanism considers that Ag^+ reacts with and disrupts the function of bacterial cell membranes and crucial metabolic proteins and enzymes by binding to DNA and thiol groups in proteins. In addition, Ag^+ does not develop bacterial resistance (Simchi *et al.* 2011). These strategies have been used to synthesize Ag-doped BGs (Diba and Boccaccini 2014). Ag_2O-doped BGs from sol-gel or melt-derived synthesis origin (Ag-BGs) present bacteriostatic and bactericidal properties against various pathogen bacteria like *Escherichia coli*, *P. aeruginosa* and *S. aureus* (Bellantone *et al*. 2001, 2002), *S. epidermidis* (Prattern *et al.* 2004), *Enterococcus faecalis*, (Chatzistavrou *et al.* 2014) or *Streptococcus mutans* (Wang *et al.* 2015) among others. Several authors have attributed the antibacterial action of Ag-BGs exclusively to the leaching of Ag^+ ions from the glass matrix (Bellantone *et al.* 2002; Chatzistavrou *et al.* 2014; Wang *et al.* 2015).

Ahmed *et al*. (2011) found that the antibacterial effect of Ag-doped phosphate glasses prepared by the melting method and tested against *S. aureus*, *P. aeruginosa* and *E. coli*, depends on the glass composition, Ag_2O content and type of microorganism tested. These authors observed an increase in the antibacterial activity as the Ag_2O content in the glass increased. Other authors have found similar results for some sol-gel Ag-doped BGs (Ciolek *et*

al. 2011; Hu *et al.* 2012; Catauro *et al.* 2015) and Ag-calcium borosilicate systems tested on *S. aureus* and *E. coli* (Lucacel *et al.* 2014). However, in calcium-free borophosphate glasses, Magyari *et al.* (2014) found that the glasses containing Ag_2O content between 0.2 and 1 mol% exhibited a relatively increased antibacterial activity against *Listeria monocytogenes* ATCC19115, while the samples with 1.5 and 2 mol% Ag_2O exhibited a reduced antibacterial effect. The authors associated this behavior with the appearance of small Ag nanoparticle clusters in the glass system, which prevented the penetration into the cell membrane. Ag shows biocidal activity at critical concentrations. Balamurugan *et al.* (2008) reported that Ag-BG samples exhibited antibacterial activity on *E. coli* at a concentration of 0.02-20 mg mL^{-1}. Bellantone *et al.* (2002) also showed antibacterial properties of a 3% (w/v) Ag-BG sample at concentrations ranging from 0.05 to 0.20 mg mL^{-1}.

Despite the great number of reports on Ag-doped BGs, the *in vivo* effects of these systems have been poorly investigated. Xiao *et al.* (2012) used titanium plates coated with borate BGs containing 0.75 and 1.0 wt% Ag_2O and investigated them *in vitro* and *in vivo* as devices for fracture fixation and implants to eradicate MRSA-induced osteomyelitis in a rabbit tibial model. After implantation, the BG coating doped with 1.0 wt% Ag_2O was the most effective for the simultaneous eradication of the infection and the fracture fixation. The titanium plates coated with Ag-doped BGs eradicated the infection in all 10 animals within 6 weeks.

Although some reports have shown that BGs containing Ag (in different architectures) release Ag ions at a rate high enough to be bactericidal but not cytotoxic to bone cells (Catauro *et al.* 2015), the cytotoxicity of Ag is still a controversial issue (Mozafari and Moztarzadeh 2013; Diba and Boccaccini 2014). Nevertheless, other antibacterial doping agents, such as copper, have also been investigated (see below).

Bioactive glasses doped with copper

Copper (Cu) can also be used in antimicrobial applications since it is one of the well-studied antimicrobial agents against some gram-negative and gram-positive bacteria (Jaiswal *et al.* 2012; Chatterjee *et al*. 2014). In addition, Cu presents low toxicity to humans (Borkow and Gabbay 2005) and plays an essential role in bone formation and healing (Palza *et al.* 2013; Lin *et al.* 2016). In that sense, Cu-containing BGs have been studied for wound healing and bone regeneration (Wang *et al*. 2014; Zhao *et al*. 2015; Lin *et al*. 2016). In the frame of biomaterials with antibacterial activity, Cu-BGs have been evaluated against *E. coli* (Goh *et al.* 2014, Lin *et al.* 2016), *Staphylococcus epidermidis*, a known bacterium found in many biomaterial-associated infections (Neel *et al.* 2005), and *Streptococcus mutans* and *Streptococcus sanguis*, human oral pathogens associated with diverse oral infections (Palza *et al.* 2013).

Similar to that described for Ag-BGs, the antibacterial action of Cu-BGs has been attributed exclusively to the leaching of Cu^{+2} ions from the glass matrix (Neel *et al.* 2005, Goh *et al.* 2014). Also, the increase in Cu content increases the antibacterial effectiveness (Neel *et al.* 2005; Goh *et al.* 2014; Lin *et al.* 2016).

In general, Cu-BGs present weaker antibacterial properties than Ag-BGs. Some authors have suggested that the much lower release of Cu ions from BGs during the first 24 h is responsible for its inferior antibacterial properties compared with Ag-doped BGs, which have shown a burn release of the ion (Goh *et al.* 2014). However, Palza *et al.* (2013) showed that Cu-BG samples release more biocide ions than Ag-BG samples during the first h, suggesting than Ag ions are much more effective in eliminating bacteria than Cu ions. The same authors showed that BGs doped with these metal ions present different biocide-bacterium specificity.

Ag-doped BGs present a Minimum Bactericidal Concentration toward *E. coli* around 30 times lower than Cu-based materials, whereas the difference toward *S. mutans* is reduced to around 8 times.

Cu-doped BGs exhibit a more progressive release than Ag-doped BGs (Palza *et al*. 2013; Goh *et al.* 2014). The rapid *plateau* found in the curves of Ag ion release against either time or concentration has not been explained in detail but it has been suggested that the dynamics of Ag ion release changes when bacteria are present (Palza *et al.* 2013). In fact, some authors have reported depletion of Ag from the medium in some cases, suggesting Ag accumulation in or binding to the bacteria (Bellantone *et al.* 2001, 2002; Balamurugan *et al.* 2008).

Bioactive glasses doped with gallium

The role of gallium (Ga^{+3}) as an antibacterial agent is attributed to the chemical similarity of its $^{+3}$ oxidation state to Fe^{3+} (Kelson *et al.* 2013; Chitambar 2016). The reduction of Fe^{+2} is a key step in many intracellular processes and since many proteins require Fe^{+3} as a key cofactor, Ga^{+3} can also function as an Fe^{3+} analog. However, unlike Fe^{+3}, under physiological conditions, Ga^{+3} cannot be reduced to Ga^{+2} (Chitambar 2016). Hence, since most microbes require iron to survive, gallium has the potential to function as an antibacterial agent with very broad spectrum activity by targeting bacterial Fe^{+3} metabolisms. Also, gallium does not appear to be susceptible to classical resistance mechanisms commonly associated with antibiotics (Kelson *et al.* 2013).

Gallium-doped BGs with antibacterial effects have been evaluated on melt-quenched and sol-gel phosphate (Valappil *et al.* 2008, 2009; 2012; 2014; Pickup *et al*. 2009; Sahdev *et al.* 2015), borate based, (Deliormanli 2015; Deliormanlı *et al*. 2016), silicate (Franchini *et al.* 2012; Zeimaran *et al.* 2016; Deliormanlı *et al.* 2016) and sol-gel (Wren *et al.* 2014) glass compositions. Different kinds of bacterial strains have been evaluated, mainly those mentioned in biomaterial-associated infections like *S. aureus*, *MRSA*, *S. epidermidis*, *P.*

aeruginosa, *E. coli* and human oral pathogens associated with diverse oral infections like *Porphyromonas gingivalis*, *Streptococcus gordonii* and *S. mutant*s.

In general, gallium has been tested at concentrations between 1 and 8% mol of Ga_2O_3. Phosphate-based glasses have shown good antibacterial effect against a broad-spectrum of planktonic bacterial pathogens and some biofilms (Valappil and Higham 2014; Valappil *et al*. 2014). It has been found that gallium ions are delivered in a controlled way and it has been suggested that the net bactericidal effect is due to the presence of gallium (Valappil *et al*. 2014). One advantage of phosphate-based glasses is that some systems have predictable dissolution rates that can be manipulated via chemical composition to give materials that can either degrade over a few hours or remain stable for over one year (Valappil *et al.* 2008). Also, the degradation rate of the glasses predicts the antibacterial efficacy. It has been found that increasing the gallium content of the glasses decreases the rate of degradation and subsequent release of gallium ions. As a consequence, BGs doped with 1% mol gallium produce a zone of inhibition larger in size than that produced by BGs doped with 3 or 5% mol gallium (Valappil *et al.* 2008). Increasing the calcium content (14, 15 and 16 mol.% CaO) in the glass composition also causes a decrease in its degradation rate, affecting the gallium ion release and the antibacterial activity against planktonic *P. aeruginosa* (Valappil *et al*. 2009). Also, decreasing the calcium content in gallium-Ag-phosphate-based glasses increases their antibacterial properties (Valappil and Highman 2014). These glasses have been found to deliver gallium and Ag in a controlled way and to exert cumulative antibacterial action on planktonic and biofilm growth of *P. aeruginosa* (Valappil and Higham 2014) and biofilm of oral human pathogens (Valappil *et al.* 2012). A study carried out by Sahdev *et al*. (2015) on the *in vivo* compatibility of gallium-containing phosphate-based glasses in rats showed a non-toxic and no foreign body response after 2 weeks of subcutaneous implantation to rats. The

study also revealed that gallium-containing phosphate-based glasses had an antibacterial effect against *Porphyromonas gingivalis*.

In contrast to the results found in phosphate-based glasses, Deliormanlı (2016) and Deliormanli *et al.* (2016) have recently found that 13-93-based BG fibers and borate 13-93 BG scaffolds containing gallium show no antibacterial effects. The authors suggested that this lack of antimicrobial activity can be explained by the concentration present in the samples and their slow release behavior. The authors also suggested that the architecture of the BGs may have a control on microbial toxicity.

Bioactive glasses doped with zinc

Zinc (Zn) is a cofactor for many enzymes, stimulating protein synthesis, which is essential in DNA replication as well as in bone cell growth, development and differentiation (Atkinson *et al.* 2016). Zn-doped BGs have been investigated in terms of their biocompatibility and bioactivity (Miola *et al.* 2015; Balasubramanian *et al.* 2015; Theodorou *et al.* 2016). In general, depending on the concentration in which ZnO is present in specific BG compositions, ZnO acts either as a network modifier or as an intermediate oxide. The presence of ZnO in the glass structure controls the overall leaching behavior of the silicate matrix, thus having an effect on the glass surface reactivity in contact with physiological fluids. The influence of Zn on the properties of BGs depends not only on the Zn content but also on the relative content of other oxides (Balasubramanian *et al.* 2015).

The antibacterial effects of Zn-doped BGs have not been extensively evaluated. Baghbani *et al.* (2013) reported BGs in the systems SiO_2–CaO–P_2O_5–MgO with (BGZn 0 mol%) and SiO_2–CaO–P_2O_5–MgO–ZnO (BGZn 5 mol%), prepared by the sol–gel method, with antibacterial activity against *P. aeruginosa* according to the halo zone test. Esteban-Tejeda *et al.* (2014) developed antibacterial coatings based on the glass system B_2O_3-SiO_2-Na_2O-ZnO on different biomedical metallic substrates and found that all the coatings had strong

antibacterial effect on *E. coli* (>4 log). In calcium phosphate glasses, Liu *et al.*(2014) found that the presence of Zn (1.2 mol%) enhanced the antibacterial effects of the glasses against *S. mutants.* Other authors also found that some mesoporous BGs, Zn-doped BGs based cements, and ZnO-based glass-ceramics have antibacterial activity against *S. aureus* (Sánchez-Salcedo *et al.* 2014; Riaz *et al.* 2015), *S. epidermidis*, *Klebsiella pneumoniae* (Riaz *et al.* 2015), *P. aeruginosa*, *Bacillus subtilis* (Riaz *et al.* 2015; Atkinson *et al*. 2016), *S. mutans*, and *Actinomyces viscosus* (Boyd *et al*. 2006).

In general, similar to that observed in Ag-doped or Cu-doped BGs, the antimicrobial effect of Zn-doped BGs increases as the Zn concentration increases (Esteban-Tejeda *et al.* 2014; Riaz *et al*. 2015; Atkinson *et al.* 2016). The antibacterial mechanism of ZnO includes oxidative stress and the release of high Zn ion concentrations, which can damage the cell membrane and interact with intracellular contents (Yousef *et al.* 2012; Esteban-Tejeda *et al.* 2014).

Bioactive glasses doped with fluoride

Fluoride (F), another known antibacterial agent (Wiegand *et al.* 2007), has also been used to dope BGs. Liu *et al.* (2014) also combined F with Zn. These authors showed that F can enhance the antibacterial effects of calcium phosphate glass against *S. mutants*, and that F has lower capacity than Zn to inhibit the bacterium but better antibacterial capacity than when combined with Zn. By means of the disk susceptibility test, Rostami *et al.* (2015) evaluated sol-gel-derived BG particles doped with F (5, 10 and 20% mol) against *E. coli*, *S. aureus* and *P. aeruginosa*. The results showed that all the F-BG samples had antibacterial activity against the mentioned bacteria, while F-free BG samples had no antibacterial activity. The antibacterial activity of the samples against the bacteria increased with the increase in the fluoride molar ratio. In addition, the synthesized F-BG samples were found to be cytobiocompatible when tested *in vitro* and *in vivo*.

Bioactive glasses doped with other antibacterial agents

BGs doped with other antibacterial agents such as strontium (Liu *et al.* 2016) and cerium (Goh *et al.* 2016) have also proved to have antibacterial activity against oral pathogenic bacteria. Prabhu *et al.* (2014) prepared and characterized silicate and phosphate BGs by sol-gel and doped them with neem (*Azadirachta indica*) leaf powder and silver nanoparticles. These authors found that neem leaf powder-doped BG nanoparticles showed good antimicrobial activity against *S. aureus* and *E. coli* and were less bioactive than Ag-doped glass particles. In addition, the biocompatibility of the prepared nanocomposites revealed better results for neem-doped and Ag-doped glasses at lower concentrations. More recently, Shih *et al.* (2016) showed that graphene oxide-doped BGs have better anti-staphylococcal activity than un-doped BG powder.

Bioactive glass-based materials loaded with antibiotics

BGs have been studied as antibiotic delivery systems both directly as carriers and in association with some polymer loaded with an antibiotic. BG-based carriers can be prepared in different forms (Table 2). The method to prepare BGs influences the forms of the carriers and the methodology of loading the drug.

Bioactive glasses as carriers loaded with antibiotic

The simplest method to prepare BG carriers is that represented by particles. Based on the fact that sol-gel-derived BGs are intrinsically nanoporous and have a high surface area, which allows loading biomolecules (Zheng *et al.* 2015), the loading of Tetracycline hydrochloride (THC) to sol-gel particles has been extensively studied (Domingues *et al.* 2004; Andrade *et al.* 2009; Cavalu *et al*. 2013; Zheng *et al.* 2015). The surface area and porosity determine the drug-loading capacity (Cavalu *et al.* 2013). The release pattern is presumably controlled by diffusion through the nanoporous structure (Zheng *et al.* 2015). Although the release behavior

shows a sustained release of the drug, the systems present a limited ability to control the release rate of the antibiotic (Domingues *et al*. 2004; Andrade *et al.* 2009; Cavalu *et al*. 2013; Zheng *et al.* 2015).

Melt-derived borate BGs have been prepared in forms of microparticle pellets as carriers of teicoplanin (Jia *et al.* 2010, Zhang *et al.* 2010), vancomycin (Xie *et al.* 2009) and gentamicin (Xie *et al.* 2013). In these cases, the loading of the drug was limited because of fracturing problems of the pellets after immersion for several days in simulated body fluid. In some cases, the pattern of drug release was improved by the presence of a biopolymer (Jia *et al.* 2010).

Conventional melt-derived BG particles are known to be typically dense, which makes it difficult to load biomolecules (Zheng *et al*. 2015). Nevertheless, diverse melt-derived BGs scaffolds have been proposed as drug delivery systems. The vacuum infiltration method is usually used. By this method, the scaffolds are loaded with the antibiotic by immersing them in a drug solution and then applying a negative pressure (Soundrapandian *et al.* 2014). Nandi *et al*. (2009) and Soundrapandian *et al.* (2014) used this method to explore the release of gatifloxacin and cefuroxime axetil. They demonstrated that the drug release is mainly controlled by a Fickian diffusion mechanism. Besides, the loading efficiency or release kinetics was not influenced by the composition of the BG-scaffolds but was influenced by the initial drug concentration in the loading solution. Thus, Soundrapandian *et al.* (2014) suggested that when a scaffold is immersed in a dilute drug solution, fewer drug molecules enter the pores, and hence, the pore channels of the scaffolds contain fewer drug molecules.

Bioactive glasses in association with a polymer

When a BG scaffold is associated with a biopolymer, the incorporated polymer phases coat the scaffolds and act as the vehicle for local delivery of antibiotics. Different authors have studied Bioglass®-based scaffold with layers of poly(D,L-lactide-co-glycolide) (PLGA) and

poly(n-isopropylacrylamide-co-acrylic acid) (Olalde *et al.* 2013), polycaprolactone and chitosan (Yao *et al.* 2013), or coatings of poly(3- hydroxybutyrate-co-3-hydroxyvalerate) (PHBV) microspheres (Li *et al.* 2014). Yao *et al.* (2013) found that the presence of the biopolymers allowed a sustained release of the drugs compared with the uncoated scaffolds and that the mechanical properties of the coated scaffolds were significantly improved compared with those of uncoated scaffolds.

Another alternative developed for BG-based materials as drug delivery systems is based on antibiotic loaded-biopolymer films in which BG particles are dispersed (see below).

Antibacterial effects of loaded bioactive glasses

Although there are numerous studies on the release of drugs by BGs, there are few studies on the antibacterial activity of BGs loaded with antibiotics. Most are related to bone regeneration or osteomyelitis treatments and some have been done in the context of skin repair and wound dressing. In general, the *in vitro* antibacterial efficacy of BGs loaded with antibiotics is related to the concentration of the drug released and the MIC of the bacterium studied (Koort *et al.* 2008; Zheng *et al.* 2015). Our group loaded THC to collagen bovine type I membranes coated with nanosized Bioglass® (Rivadeneira *et al.* 2013) or a SiO_2-CaO-P_2O_5 system (Rivadeneira *et al*. 2015b) and found that the cell growth inhibition of three staphylococcus strains was not dependent on the THC concentration released. Both BG systems showed similar antibacterial performance. In that way, changing the BG system or the THC concentration did not reflect any improvement in terms of antibacterial effects. In another study, our group showed that 45S5 microparticles dispersed in agar-gelatin matrices increased the concentration of vancomycin released compared with bare matrices, but these modifications did not reflect an anti-staphylococcal performance (Rivadeneira *et al.* 2015a).

In vivo studies carried out by Xie *et al.* (2009) and Zhang *et al.* (2010) have shown that a borate glass loaded with vancomycin or teicoplanin was successful at eradicating osteomyelitis induced by MRSA in a rabbit model, showing excellent biocompatibility and compressive strength, supporting full osteointegration with direct apposition of the newly formed bone, and stimulating bone regeneration as it degraded (Fig. 2). The composites were able to eradicate the infections after 8-12 weeks of implantation in more than 80% of the cases and also showed a superior performance than other alternatives studied like pure borate glass, vancomycin-loaded calcium sulfate or teicoplanin injected intravenously. Xie *et al.* (2013) also used the same pellets for the release of gentamicin and found positive results in a rabbit tibia model of osteomyelitis induced by *E. coli*. The results showed that the implantation of the gentamicin-loaded pellets into the osteomyelitis region of the tibia resulted in the eradication of 81.82% of infections, and radiographic evaluation supported the ingrowth of new bone into the tibia defects after 6 weeks of implantation. Melt BG-based scaffolds have also been found to be able to eradicate infection cases induced by *S. aureus* in experimental osteomyelitis (Nandi *et al.* 2009; Soundrapandian *et al.* 2010). In this case, the release of the drug was influenced by the size of the scaffold, the concentration of the drug solution, the polymer coat, and the dissolution medium. The larger the size and the higher the drug concentration, the better the release profile.

III. Conclusions and future scope

The overwhelming evidence in the literature, part of which we attempted to describe in this review, that confirms the antibacterial potential of bioactive glasses anticipates the need for more research efforts focusing on *in vivo* studies which will help to develop optimized BG-based biomaterials for various different applications in regenerative medicine in the near future.

Conflict of interest

The authors declare that they have no competing interests.

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Table 1. Different forms of BGs-based carriers loaded with antibiotics

Table 2. BGs doped with different elements

Figure 1 Antibacterial activity of BGs as biomaterial delivery systems for antimicrobial agents: a) Mechanism of action of BGs particles once in dissolution; b) Mechanism of action of biocide metals released from doped BGs; c) mechanism of action of BG-based materials loaded with antibiotics.

Figure 2. Radiographic images showing (a) experimental osteomyelitis in rabbit tibia induced by methicillin-resistant *S. aureus* ; (b) teicoplanin-loaded borate BG (TBDC) pellets implanted into rabbit tibia osteomyelitis model after debridement (Group 1); (c) completely degraded TBDC pellets in rabbit tibia 12 weeks post implantation; (d) the cleared cavity and bone window after debridement in Group 2 animals (injected intravenously with teicoplanin); (e) evidence of deteriorated infection in Group 2. Reproduced with permission of Elsevier

Table 1.

Carrier System	Composition	Antibiotic released	References
Particles	$80SiO_2$,$4P_2O_5$,16CaO	THC	Domingues *et al.* 2004 Andrade *et al.* 2009
	$55SiO_2$,$4P_2O_5$,41CaO	THC	Cavalu *et al.* 2013
	$70SiO_2$,30CaO (70S)	THC	Zheng *et al.* 2015
Cylinders	$58SiO_2$,36CaO,$6P_2O_5$	Gentamicin	Meseguer-Olmo *et al.* 2006
Pellets	$6Na_2O$, $8K_2O$, 8MgO,22CaO, $54B_2O_3$,$2P_2O_5$ (Borate glass)	Vancomycin	Xie *et al*. 2009 Jia *et al.* 2010
	$6Na_2O$, $8K_2O$, 8MgO,22CaO, $54B_2O_3$, $2P_2O_5$	Teicoplanin	Zhang et al. 2010
	$6Na_2O$, $8K_2O$,8MgO,22CaO,$54B_2O_3$, $2P_2O_5$	Gentamicin	Xie *et al*.2013
Pellets + polymers	$53SiO_2$,$6Na_2O$,$12K_2O$,5MgO,20CaO, $4P_2O_5$ 13-93/PDLLA	Ciprofloxacin	Koort *et al.* 2008
	13-93/PLGA	Ciprofloxacin	Mäkinen et al. 2005
Scaffolds	$43.70SiO_2$,19.20CaO,$5.46P_2O_5$, $9.40B_2O_3$,$22.24Na_2O$	Cefuroxime axetil	Nandi *et al.* 2009
	$55.9SiO_2$,$11.8Na_2O$,16.14CaO, $2.5P_2O_5$,1.24ZnO,9.93MgO,$2.5K_2O$ (BGZ) $58SiO_2$,$12Na_2O$,18CaO,$7P_2O_5$, 2.24ZnO,2.6MgO(MBG)	Gatifloxacin	Soundrapandian *et al.* 2014
	$45SiO_2$,21.2CaO,$26Na_2O$,$7.8P_2O_5$ (SSS)	Gatifloxacin	Soundrapandian *et al.* 2010
Scaffolds +Polymers	$45SiO_2$,$24.5Na_2O$,24.5CaO,$6P_2O_5$ (45S5)/ PLGA/ poly(N-isopropylacrylamide-co-acrylic acid)	Vancomycin	Olalde *et al.* 2013
	$46SiO_2$,24CaO,$24Na_2O$,$6P_2O_5$ (46S6)/PVA (polyvinyl alcohol)	Ciprofloxacin	Mabrouk *et al.* 2014

	45S5/PHBV	Vancomycin	Li *et al.* 2014
	45S5/ PCL (polycaprolactone)/ chitosan	Vancomycin	Yao *et al.* 2013
	45S5/alginate or gelatin	TCH	Nooeaid *et al*. 2014
Films	45S5 nanosized/Collagen	THC	Rivadeneira et al. 2013
	45S5/agar-gelatin	Vancomycin	Rivadeneira et al. 2015a
	$55SiO_2$,40CaO,$5P_2O_5$ nanosized /Collagen	THC	Rivadeneira et al. 2015

Table 2

BG composition and doping method	Doped ion concentration	Bacterium type	Major results related with antibacterial effects	Ref.
76SiO2, (22-x)CaO, $2P_2O_5$ wt% Sol-gel (SG)	x=0, 3 wt% Ag_2O	*E coli* MG1655, *P aeruginosa* PAO6049, *S. aureus* NCIMB 11852	Antibacterial effects depend exclusively to the leaching of Ag^+ from the glass matrix and of the glass concentration. Bacteriostatic and bactericidal properties at concentration ranging from 0.05 to 0.20 $mgmL^{-1}$ of AgBG. Complete bactericidal effect at concentrations of 10 mg ml^{-1}.	Bellantone *et al.* 2002
$65P_2O_5$,10CaO,(25–x) Na_2O, $70P_2O_5$,20CaO,(10–x) Na_2O and (70–x) P_2O_5,30CaO mol% Melting (M)	x = 0, 0.5, 1 and 2 mol% Ag_2O	*S. aureus* ATCC 25923, *E. coli*(ATCC 25922, and *P. aeruginosa* ATCC 27853	Antibacterial effects depend to the glass composition and the type of bacteria. Antibacterial effects increased	Ahmed *et al.* 2011

			while the Ag_2O content in the glass increased. G^+ bacterium was more susceptible than G^- ones.	
$6Na_2O,8K_2O,8MgO,16CaO,2P_2O_5,$ $6SrO,36B_2O_3,18SiO_2$ mol%. M	0, 0.75, or 1.0 wt% Ag_2O	Methicillin-resistant *S. aureus*	Titanium plates coated with Ag-doped 1.0 wt% BGs eradicated the infection in all animals within 6 weeks	Xiao *et al.* 2012
$62.3SiO_2,28.9P_2O_5,8.6CaO$ wt% (pure BG) SG	4.7 and 9.0 CuO wt%. 4.0 and 8.0 Ag_2O wt%.	*E. coli* DH5α ampicillin-resistant and *S. mutans*	The antimicrobial behavior depends on the bacteria and the biocide ion used. Ag-doped BGs present a MBC toward *E. coli* around 30 times lower than Cu-based materials, whereas the difference toward *S. mutans* is reduced to around 8 times.	Palza *et al.* 2013
$50SiO_2,(45-x)CaO,5P_2O_5$ mol% SG	x=1, 5, 10 mol% CuO or Ag_2O	*E. coli* ATCC25922	Antibacterial action of Cu-BGs depends exclusively to the leaching of Cu^{+2} from the glass matrix. Increase in Cu content increases the antibacterial effectiveness. Ag-doped BG is a better rapid bacteria-killing agent than Cu-doped BG, but Cu-doped BG exhibits a prolonged release of ions.	Goh *et al.* 2014

$xCaO,(52-x)Na_2O,45P_2O_5$ mol% M	3 mol% Ga_2O_3 x=14,15,16	*P. aeruginosa*	All glasses showed antibacterial effects against planktonic bacteria. C14 reduced the biofilm growth of *P. aeruginosa*	Valappil *et al.* 2009
$65SiO_2,30CaO$ $70SiO_2,25CaO$ mol% SG	5 Ga_2O_3 mol%	*E. coli* ATCC8739; *S. epidermidis* ATCC14990	Bacteriostatic and bactericidal effects against both kind of bacteria. Bacteriostatic properties were found predominantly against *E. coli.*	Wren *et al.* 2014
$70SiO_2,(26-x)CaO, 4P_2O_5, xZnO$ mol%. SG	x=0,3,5 mol% ZnO	*P. aeruginosa*, *Bacillus subtilis* 1016	Antibacterial action depends to ZnO presence. Antibacterial effects increased while ZnO content in the glass increased.	Atkinson *et al.* 2016
$58SiO_2,33P_2O_5,9CaO,xCaF_2$ mol% SG	x=0,5,10,20 CaF_2 mol%	*E. coli* ATCC25922, *S. aureus* 25923, *P. aeruginosa* ATCC27833	Antibacterial action depends to fl presence. Antibacterial effects increased while fluoride content in the glass increased.	Rostami *et al.* 2015