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Multifunctional Bioactive Glass and Glass-Ceramic Biomaterials with Antibacterial Properties for Repair and Regeneration of Bone Tissue

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Abstract

Bioactive glass and related glass-ceramics have been used in bone tissue repair for over 30 years, and many of the features that relate to their bone bonding characteristics are relatively well understood. More recently, attention has focused on the development of advanced compositions to not only enhance this osteogenic behaviour but also impart other characteristics such as antimicrobial activity. The aim of this review is therefore to consider how inorganic modifications to bioactive glasses and glass-ceramics may be used to introduce greater biofunctionality towards the creation of a new generation of versatile, multifunctional materials for health.

1. Introduction

Multiple degenerative and inflammatory joint and bone diseases affect millions of people worldwide. In fact, in 2007 the Bone and Joint Decade's association predicted that the percentage of people over 50 years of age affected by bone diseases will double by 2020 [1]. The huge increase in joint and bone implant surgeries parallels that of medical-device associated infections [2, 3]. Bacterial infections associated with contamination of implanted medical-devices are a critical complication that often leads to the failure of the implant with significant impact concerning public health in developed countries [4, 5]. Moreover, the management of medical-device associated infections often requires the need for surgical intervention or/and prolonged usage of intravenous or oral antibiotic therapies leading to bone loss and significant morbidity resulting in severe limitations to the patients regarding normal life and wellbeing [6, 7].

Furthermore, there is a desire to limit the use of antibiotics in a hospital setting and reduce the risk of encouraging the growth of drug-resistant microorganisms. Several pathogenic microorganisms (predominantly Staphylococcus aureus, Staphylococcus epidermidis, Escherichia coli and Pseudomonas aeruginosa) have been identified at the site of approximately 90% of all implants, and many of these organisms showed drug-resistance [5, 8, 9]. Moreover, the critical complications of bacterial contamination are mostly related with the adhesion of bacteria to the medical device, which aggregates in a hydrated polymeric matrix of their own synthesis to form biofilms [10-12]. These multifaceted structure made from microorganism and extracellular matrix is capable of resisting antibiotics and antibacterial agents being at the root of many persistent and chronic bacterial infections [13].

Therefore, conventional therapy with systemic antibiotics is expensive and often unsuccessful. It presents poor antimicrobial dispersal at the site of infection due to limited blood circulation to infected skeletal tissue [14]. Several glass and glass-ceramic biomaterials where successfully tested against bacterial biofilm. Valappil et al. [15] used phosphate-based glasses combined with gallium and silver for controlled delivery against oral biofilm models with success. While, Mulligan et al. [16] studied the effect of $Na_2O–CaO–P_2O_5$ system doped with increasing amounts of copper on in vitro biofilm of Streptococcus sanguis for potential application as antibacterial agents for oral infections.

While there is undoubtedly a growing clinical need for antimicrobial devices, the regulatory environment makes it increasingly difficult to bring these to market. Major pharmaceutical companies with research and development potential to make progress are also losing interest in the antibiotics market. The Food and Drug Administration (FDA) is approving increasingly fewer antibiotics. Statistical analysis performed by the Center for Disease Dynamics, Economics & Policy (CDDEP) highlighted that only six antibiotics were approved in the period between 2010 and 2014, 10 fewer than in the four-year period between 1983 and 1987. Actually, many of the drugs approved by FDA in the 1980s and 1990s have since been taken off the market for a variety of reasons, including: safety, efficacy or lack of profit [17]. Additionally, antibiotics are not an ideal solution due to challenges in reaching the target organisms, especially when these become associated with a medical device [18]. Local and/or preventive treatments may therefore be a superior approach to combat bacterial infections. Allan et al. [19] tested with success the use of 45S5 bioglass® to inhibit certain oral bacteria (including Streptococcus sanguis, Streptococcus mutans and Actinomyces viscosus) while repairing periodontal defects. Whereas, Brauer et al. [20]

developed a strontium-releasing injectable bone cement with antibacterial activity against S. aureus and Streptococcus faecalis for the treatment of osteoporosis-related vertebral compression fracture.

Therefore, after several years of improving surgical procedures, implementing strict and efficient antiseptic pro-operative and intra-operative procedures [18]. There is a growing interest in the investigation and development of smart and suitable biomaterials for bone and joint replacement with bioresorbable, biocompatible and bone bonding properties that are simultaneously effective treating implant-related bone infections. Different methods of loading implantable materials are been applied to for local antibiotic application [21-24]. But, manufactures are focusing their efforts to improve existing active ingredients for new applications instead of developing new compounds. Among them, multifunctional glass and glass-ceramic biomaterials have found extensive application as an orthopaedic and dental graft material as well as tissue engineering scaffolds [25-28]. Rahaman et al. have summarised part of the field, but they limited their review to a narrow range of papers and did not consider more detailed or complex aspects of glass design and structure-properties relationships [29].

Since 1969, Hence [30] and their co-workers were largely responsible for the development of bioactive glasses and study their bone bonding properties. More recently, work in this field was comprehensively renewed by Rees Rawlings [31] in 1993, which included a description of the key features and properties of bioactive glasses and their glass-ceramics derivatives. Silicate glasses, the most used bioactive glasses, are well studied to form of a bone-like hydroxyapatite (HA) layer that is fundamental for a strong interfacial bond between implants and bone [30, 32].

Though, a slow degradation rate is their major drawback, making it difficult to match their degradation rate with the rate of new tissue formation, also presenting an incomplete HA conversion [33-35]. Outstandingly, the addition of borate to the glass network for the formation of borosilicate bioactive glasses has the potential to increase bio-degradation and conversion to HA [35-37]. Borosilicate bioactive glasses offer a more controlled dissolution rate that triggers a range of biological responses required for the final implantable biomaterial [38]. A number of parameters might influence the design of antibacterial bioactive glass. Undoubtedly, addition of specific ions (e.g. Ag^{+}, Ce^{3+}, Cu^{+}) had demonstrated antimicrobial properties [16, 39-42]. Balamurugan et al. [40] reported the antibacterial properties of silver-incorporated bioactive glass system against E. coli attributed to the leaching of Ag^{+} ions from the glass matrix. Although there are other properties related with the network disruption that originate antimicrobial properties (e.g. pH, osmolarity, particle size and morphology) [19, 43-45]. For instance, Hu et al. [45] described antibacterial activity of 45S5 bioglass® against S. aureus, S. epidermidis and E. coli and correlated it with high pH and morphology of the glass. Glass and glass-ceramic biomaterials can either possess intrinsic antibacterial properties or/and be designed to have enhanced activity against specific bacteria and be used according to their final application form, including scaffolds, fibres, hydrogels or injectable materials.

The aim of this review is to consider recent advances in the development of antibacterial strategies for glass and glass-ceramic based biomaterials and identify those that appear to offer most promise for use in orthopaedics medical devices and related technologies. Particularly, to consider how inorganic modifications to glass and glass-ceramics can be used to introduce greater biofunctionality to create a new generation of versatile, multifunctional materials for health.

2. Bioactive glass and glass-ceramic biomaterials

Glass and glass-ceramic biomaterials are unique ion-containing matrixes that recently are being investigated for the prevention and treatment of bone infections. Glass and glass-ceramic biomaterials are very well known for their bioactive properties, the ability of bonding bone tissue through complex reactions forming strong and harmonious interfaces between biomaterials and tissue [30] and also for having good biocompatibility with great inductive and conductive properties [30, 32, 46, 47]. They have been used in the form of particles, porous or dense scaffolds for orthopaedic surgery and dentistry for bone repairing [48]. Glass biomaterials can predominantly be fabricated either by the traditional melt-quench or sol-gel processes, where a number of simple compounds are able to solidify as a glass [40, 49]. The glass structure is composed of network formers (e.g. Si^{4+}, B^{3+} and P^{3+}), usually silica, which contributes to the network formation containing either intermediate oxides (e.g. Al^{3+}, Zn^{2+}, Mg^{2+}) and/or network modifiers (e.g. Sr^{2+}, Ca^{2+}, Na^{+}). Intermediate oxides, depending on the composition of the glass, may play a network or disrupting function, while network modifiers disrupt the network and produce non-bridging oxygen ions.

A second step of controlled heat treatment is necessary to obtain glass crystallisation forming glass-ceramic biomaterials [50] (REFP2). This second heat treatment that leads to crystallisation involves two stages, first a nucleation and then a crystal growth stage, which promote the re-arrangement of the glass structures generating a well-ordered and crystalline structure. However, not all glasses are able to undergo a controlled heat treatment and form glass-ceramics either because they are already too stable or too unstable and difficult to have a controlled heat treatment. Therefore,

glasses and glass-ceramic biomaterials possess the same building units just arranged in many different patterns, which leads to different final properties. The work in this field was extensively revised by Hench et al. [30] and Rawlings et al. [31].

The mechanism of bioactivity and bone bonding has been extensively studied in vitro (immersion in SBF) and in vivo, mainly for 45S5 bioglass® and was discussed elsewhere [34, 46]. Thus, the bonding ability of glass and glass-ceramic biomaterials relies in the degradation process of the biomaterials and subsequent formation of a HA layer on their surface, which mimics that mineral bone composition, bonding firmly with living bone tissue. Briefly the process follows the succeeding steps, (1) dissolution of ions from the glass into the medium, (2) reaction of Ca^{2+} dissolved and $(PO_4)^{3-}$ from the media and consequent precipitation of amorphous calcium phosphate (ACP) layer, (3) the pH unbalance and increased dissolution of ions supports the growth of ACP, and (4) ACP layer incorporates $(OH)^-$ and $(CO3)^{2-}$ from the media and crystallises as HA layer.

The silicate-based glasses and glass ceramic biomaterials are commonly associated with slow degradation rates and incomplete conversion to HA. This might result in a mismatch of the degradation rate with the rate of new tissue formation and the presence of long-term unconverted glass and glass-ceramic biomaterials in human body [33-35, 51]. More recently, borate- and borosilicate-based glasses have been used with great potential to overcome silicate-based glasses [35-37]. Due to their lower chemical durability, borate- and borosilicate-based glasses present increased bio-degradation and more complete conversion to HA. Furthermore, boron is associated with bone healing, stimulating bone formation and maintenance and with the increase in bone resistance to fractures [52-54]. Thereby, the compositional

flexibility is at most importance while designing a glass or glass-ceramic biomaterials. As already has been shown a controlled release of ions promotes HA formation leading a perfect osteointegration, while stimulating osteogenic functions of the surrounding cells [52, 55]. Specific trace amount of component ions (e.g. Ag^{+}, Cu^{+}, Sr^{2+}, Zn^{2+} and Ce^{3+}) incorporated and released in a controlled manner can trigger a range of different biological responses, particularly antimicrobial activity [39, 55, 56].

As matter of fact, different inorganic modifications have been introduced by several researchers in order to achieve glass and glass-ceramic biomaterials (Figure 1) endowed with antibacterial properties, resulting either in intrinsic and/or enhanced antibacterial glass and glass-ceramic biomaterials. Figure 1 displays a wide variety approaches used to develop glass and glass-ceramic biomaterials. Those biomaterials can be applied through a diversity of final forms and materials depending on their application in the body. As shown by in Figure 1a, glasses can either be designed with inorganic species into the bulk glass network or surface modified after glass formation. Moreover, different temperature schedules can be used to induce a phase separation. This phase separation can create groups of specific ion components to be released at different rates, increasing biological properties. On the other hand, glasses can be submitted to controlled heat treatments, resulting in to a glass-ceramic biomaterial with different properties (Figure 1b). Different properties can be obtained either by inducing crystalline phases formation or by the formation of a residual glass in to the glass-ceramic structure providing different releasing profiles.

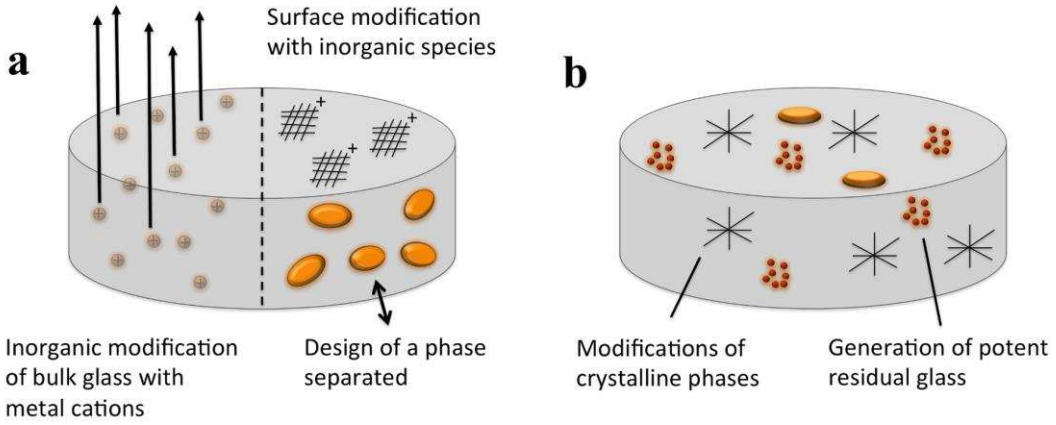

Figure 1 - Potential routes to enhance the antimicrobial properties of a) bioactive glasse and b) glass-ceramic biomaterials via inorganic modification at different sites.

The following section will review the different glass and glass-ceramic biomaterials currently proposed to diminish the susceptibility of joint and bone implant surgeries to the development of infections.

3. Composition and modifications

Glass and glass-ceramic biomaterials that are design to have suitable properties for bone integration have been demonstrating antibacterial activity when specifically assessed (REF paper3) [19, 57-59]. This antibacterial activity has been generally attributed to release of ions to the reaction media and their effect in the local physiological environment (e.g. pH, osmolarity). Zhang et al. [57] have demonstrated that bioactive glasses without any special bactericidal ions exhibited antibacterial effects for a large selection of bacteria with a concentration-dependent manner. The authors correlated the antibacterial effects essentially with the increase of pH and also the concentration of alkali ions, in which the glass S53P4 inhibited all the bacteria tested with (e.g. E. coli, P. aeruginosa, Moraxella Catarrhalis, E. faecalis, S. epidermidis). Moya et al. [60, 61] studied the borosilicate glasses (SiO_2–Na_2O–CaO–

B_2O_3 system) with a high content of calcium oxide and found that Ca^{2+} concentration is related with biocide activity against Gram positive, negative bacteria. Several other authors also related the antibacterial effect of glass biomaterial with pH and ion concentrations [43, 58, 62]. This kind of activity based on intrinsic antibacterial properties mainly relies on the degradation of the network and their consequent effects on the surrounding environment. Therefore, it is of most importance to fully understand the mechanisms of glass structure formation and their effect on degradation (Figure 1) to specifically design glasses towards the final application.

Another important issue regarding the final glass applications are the extreme local environment condition that can harm the host tissues. Often, glasses are associated to a certain degree of cytotoxicity of glass biomaterials, which can potentially affect host cell viability in cells surrounding the implant. For instance, large increases of pH can induce adverse tissue responses as well as the high local osmolarity variations can unbalance the perfect behaviour of cells. Bakry et al. [63] showed that some cytotoxic effects of 45S5 bioglass® were associated with its initially acidic. In these cases it might be beneficial to use of heat treatments in order to induce crystallisation of glass biomaterial forming a glass-ceramic biomaterial with different physico-chemical properties [50, 64]. Hurrell-Gillingham et al. [64] investigated the effects of devitrification of glass-ionomer cements from SiO_2-Al_2O_3-P_2O_5-CaO-CaF_2 system on glass-ceramic formation and in vitro biocompatibility and could improve theirs biological properties.

Other intrinsic glasses and glass-ceramic biomaterials have been found to be effective against bacteria sessile communities that are at the root of many persistent and chronic bacterial infections. Allan et al. [65] showed that 45S5 bioglass® significantly

lowered the viability of biofilms of S. sanguinis grown in respect to inert glass control. While, Batalu et al. [62] reported that although MgB2 nano or micropowders did not affected S. aureus biofilm formation, it strongly inhibited E. coli adhesion and viability. The authors related the activity mainly with pH and boron derivatives released.

Although, intrinsic glass and glass-ceramic biomaterials activity is rarely highly specific and uniquely oriented towards prokaryotic cells. Then, antibacterial glass and glass-ceramic biomaterials can also be developed by the simple incorporation of specific metal ions with known antibacterial properties into inorganic materials. These specific metal ions (e.g. Ag^{+}, Ce^{3+}, Cu^{+}, Zn^{2+}, Sr^{2+}) can either be incorporated into the bulk network of the glasses or at the surface (Figure 1). Within the last few years, a number of glass and glass-ceramic biomaterials have been developed specially designed to have antibacterial properties [16, 39, 41, 43]. The majority of the studies were carried out with silver doped glass biomaterials. For instance, Bellantone et al. [41] and Ahmed et al. [43] demonstrated that silver doped glass biomaterials presented not only bacteriostatic, but they also caused a rapid bactericidal action against E. coli, P. aeruginosa, and S. aureus. Bellantone et al. prepared their silica-based Ag^{+} glass via an acid-catalysed sol-gel route and observed that the dissolution profiles of Ag^{+} from glasses were consistent with silver accumulation by the bacteria. While, Ahmed et al. prepared their phosphate-based Ag^{+} glass by melt quench technique verifying the increase of antibacterial activity with increasing Ag_2O contents.

Other metal ions referred as antibacterial where also studied. Mulligan et al. [16] used cooper doped glass biomaterials to combat S. sanguis biofilm found in oral cavity.

They prepared phosphate-based glasses system doped with increasing amounts of copper by melt quench technique with capacity to decrease viability of S. sanguis biofilm. However after a time period it returned to levels similar to those of controls. Nell et al. [42] also prepared phosphate-based glasses containing copper in the final form of fibres. Those fibres were capable to reduce the number of viable S. epidermidis attached to the fibres and in the surrounding environment. Another well know metal, Zinc, was incorporated into sol–gel silica-nanoparticles showing well-defined antimicrobial activity. Halevas et al. [66] tested different concentration of incorporated zinc against S. aureus, Bacillus subtilis, Bacillus cereus, E. coli, P. aeruginosa, Xanthomonas campestris bacteria exhibiting higher activity for higher concentrations. There are other rare metals, such as cerium and galium that were also tested for antibacterial properties. Goh et al. [39] have tested cerium doped glasses for their antibacterial properties. They reported significant improvements regarding the antibacterial properties against E. coli of silica based glasses with 5 mol% of Ce or higher. While Valappil et al. [15] tested phosphate-based glasses doped with gallium and silver to test their combined action. They showed that the simultaneous release of Ag^{+} and Ga^{3+} from the glass reduced Porphyromonas gingivalis biofilm growth with a maximum effect after 168 h.

Different strategies other then specific ions incorporation and related with bone tissue engineering were also reviewed on this revision paper. Although the composition of the glass is the essence of the antibacterial properties modulating their rate of ions release and consequently osmolarity and pH at the reaction site, there are other features such as particle size and morphology that can alter the potency of those biomaterials. Mortazavi et al. [67] assessed the antibacterial effect of bioactive glass nanoparticles obtained by sol-gel technique reporting that the antibacterial activity

was caused by a synergetic effect of high calcium concentration and alkaline pH level, which might have been improved by the particle size reduction. Compositions 58S showed antibacterial activity against E. coli, P. aeruginosa, and S. aureus while 63S exhibit activity only against E. coli, S. aureus and 72S didn't show any activity. Some other studies reported the influence of particle size and morphology of glass and glass-ceramic biomaterials [44, 57]. For instance, Waltimo et al. [44] studied SiO_2-Na_2O-CaO-P_2O_5 nano bioactive glasses and the influence of more than ten-fold higher specific surface area in ionic release and antibacterial effects. They reported that the increase of surface area might induce a faster dissolution of alkaline species to the medium and therefore, increasing the pH of the medium.

Enhanced glass and glass-ceramic biomaterials differ from intrinsic because they have one or more ions intentionally incorporated to add bactericidal properties. Bulk materials that exert an antibacterial action in the absence of modifications, such as loaded with bactericidal substances or coated with active functional molecules, can generally be described as intrinsically antibacterial. Table 1 summarises substituted bioactive glasses that reported antibacterial activity correlating them with their active factor.

Table 1 - Example of ions substituted bioactive glasses that reported antibacterial activity.

Active factor	**Glass system**	**Organisms**		**Ref**
		Gram (-)	**Gram (+)**	
Ag^+	SiO_2-CaO-P_2O_5-Ag_2O	E. coli	-	[40]
		P. aeruginosa	S. aureus	[41]

		E. coli	S. aureus	[68]
	P_2O_5-CaO-Na_2O-Ag_2O	E. coli, P. aeruginosa	S. aureus	[43]
	B_2O_3-Na_2O-P2O_5-Ag_2O	-	Listeria monocytogenes	[69]
	SiO_2-Ag (ceramic)	E. coli	S. aureus	[70]
	Ag_2O-B_2O_3-SiO_2-CaO	E. coli	S. aureus	[71]
	SiO_2-CaO-P_2O_5-Al_2O_3-Na_2O-K_2O-Ag_2O	E. coli	E. faecalis	[56]
Ag^+ and pH	CaO-SiO_2-Ag_2O	E. coli	S. aureus	[72]
Ag^+ and Ga^{3+}	CaO-Na_2O-P_2O_5-Ga_2O-Ag_2O	biofilm (Streptococcus gordonii and P. gingivalis)		[15]
Ag^+ and Zn^{2+}	Ceramic doped with Ag-Zn	E. coli	-	[73]
Ce^+ and pH	SiO_2-CaO-P_2O_5-Ce	E. coli	-	[39]
Cu^+	Na_2O-CaO-P_2O_5-Cu	-	biofilm (S. sanguis)	[16]
	Na_2O-CaO-P_2O_5-Cu	-	S. epidermidis	[42]
Si^{4+} and pH	S53P4	E. coli	-	[74]
Zn^{2+}	SiO_2-Zn NPs	E. coli	S. aureus	[75]
	SiO_2-Zn NPs	E. coli, P. aeruginosa, X. campestris	S. aureus, B. subtilis, B. cereus	[66]
[ions] and pH	45S5 bioglass®	E. coli, P. aeruginosa, Actinobacillus actinomycetemcomitans, P. gingivalis, Fusobacterium	S. sanguis, S. mutans, A. viscosus and E. faecalis	[19, 44, 45]

		nucleatum		
		biofilms (S. sanguis)		[65]
	58S and 63S bioglass®	E. coli, P. aeruginosa, Salmonella typhi	S. aureus	[67]
	S53P4	Acinetobacter spp, Haemophilus influenza, Enterobacter aerogenes, M. catarrhalis, E. coli, P. aeruginosa	S. epidermidis, E. faecalis	[57, 58, 76]
	MgB_2	E. coli	S. aureus	[62]
	Na_2O-MgO-CaO-B_2O_3-P_2O_3-SiO_2/K_2O/Al_2O_3	Acinetobacter spp, H. influenza, E. coli, P. aeruginosa	E. faecalis	[57]
	Na_2O-K_2O-MgO-CaO-P_2O_3-SiO_2	Acinetobacter spp, H. influenza, E. aerogenes, E. coli, P. aeruginosa	S. epidermidis, E. faecalis	[57]
	Na_2O-K_2O-MgO-CaO-B_2O_3-P_2O_3-SiO_2	-	S. epidermidis	[58]
	P_2O_5-CaO-Na_2O	E. coli, P. aeruginosa	S. aureus	[43]
	SiO_2-B_2O_3-Na_2O-MgO/SrO	P. aeruginosa	S. epidermidis	Paper3
$[Ca^{2+}]$	SiO_2-B_2O_3-Na_2O-CaO-K_2O-Al_2O_3	E. coli, P. aeruginosa	S. aureus, S. epidermidis and Micrococcus	[77]

			luteus	
	SiO_2-Na_2O-CaO-P_2O_5-Al_2O_3-Fe_2O/B_2O_3/K_2O/MgO	E. coli	M. luteus, Candida kruse	[61]
	SiO_2-Na_2O-CaO-B_2O_3/K_2O-Al_2O_3	E. coli	-	[60]
$[Ca^{2+}]$ and pH	SiO_2-CaO-Na_2O-K_2O-P_2O_5/MgO	-	S. aureus	[59]
$[Sr^{2+}]$	SiO-SrO-CaF_2-MgO	-	S. aureus, E. faecalis	[20]
pH	CaO-SiO_2	E. coli	S. aureus	[72]

4. Mechanisms of action

Composition is the basis of glass and glass-ceramic biomaterial properties. It can modulate the rate of ions release and consequently osmolarity and pH at the reaction site, influencing the physiological conditions at the implant surrounding. Therefore, glass and glass-ceramic biomaterials antibacterial activity is often engaged by their composition and dissolution properties [19, 65].

Recently, Echezarreta-López et al. [78] compiled from literature a large database on glass biomaterial production bacterial properties and experiments using an artificial intelligence tool, neurofuzzy logic technology. They verified that the antibacterial properties of glass and glass-ceramic biomaterials could be caused by the alkaline ions released, particularly calcium ions, and the increase of the pH of the medium. Briefly, the mechanisms of action of antibacterial glass and glass-ceramic biomaterials are by the: (i) release of ions that increases their (ii) osmolarity and (iii) pH at the reaction site, unbalancing the intracellular Ca^{2+}, which results in to cell

membrane depolarisation and their subsequent death. Cabal et al. [77] reported that borosilicate glass-ceramic biomaterials were able to inhibit bacterial growth, minimise bacterial adhesion and prevent biofilm formation by the perturbation of intracellular Ca^{2+} compartmentalisation, causing cytotoxicity and result in either apoptotic or necrotic bacteria cell death. This work tested the borosilicate glasses against five ATCC strains (S. aureus, S. epidermidis, P. aeruginosa, E. coli and Micrococcus lutea) with high percentages of bacterial cells reduction.

However, there are several enhanced glass and glass-ceramic biomaterials that have their antibacterial activity based in the use of stable noble metals, such as silver (between many other: Ce^{3+}, Cu^{+}, Zn^{2+}, Sr^{2+}), which are acknowledged to have antibacterial activity [79]. Their antibacterial activity is oriented towards prokaryotic cells, often with specific activity. However, occasionally they are associated to a certain degree of cytotoxicity to animal cells [80]. Regarding antibacterial metals use, which is frequently active due to their corrosion in the physiological environment or the leaching to reaction medium, the high releasing concentration of ions might cause local toxicity.

Even though the exact mechanism of metal ions regarding antibacterial action is still unknown it is recognised that it relies on a series of actions. Silver has been one of the earliest materials to be intentionally used in surgery for its bactericidal properties and the most studied. It acts by inactivating critical enzymes of the respiratory chain by biding to thiol groups and inducing hydroxyl radicals formation, creating oxidative stress [81]. Although other cellular components, like hydrogen bonding may also be involved that might implicate inhibition of bacterial cell wall synthesis, inhibition of protein synthesis, inhibition of synthesis of bacterial RNA and DNA, as well as

inhibition of a metabolic pathway [81, 82]. Therefore, the activity is generally associated to the ionic form rather than to the elemental metal. Moreover, Jung el al. [81] studies demonstrated a higher antibacterial activity against gram-negative (E. coli) in respect to the gram-positive (S. aureus). This suggests that metal ions antibacterial activity might be related to the thickness of the peptidoglycan layer of gram-positive, which may difficult the action of the silver ions at the bacterial cell membrane. An overview of the hypothesised mechanisms associated with the antibacterial activity of metal particles is displayed in Figure 2.

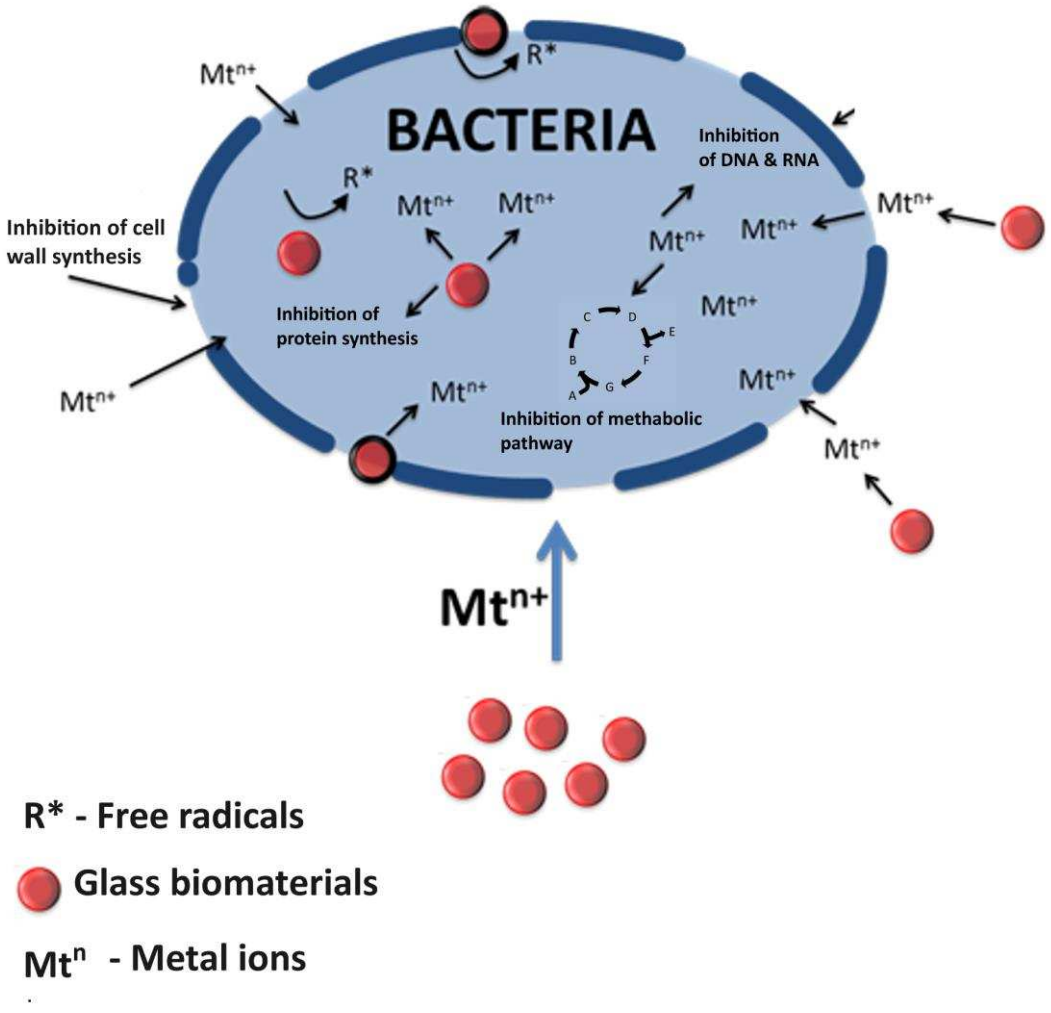

Figure 2 – Overview of the hypothesised mechanisms associated with the antibacterial activity of metal particles. The most pronounced effects of silver ions is related with

cellular metabolic activity (respiratory chain inhibition and cell pathways) as well as generation of ROS and damage DNA and RNA of bacteria. Diagram was modified from [83].

5. Conclusions

The growing impact of medical-device associated infections along with the efficacy loss of antibiotic common therapies are urging to find new preventive and treating strategies that costly and effectively combat this matter of concern. Bioactive glass and glass-ceramic biomaterials represent a powerful candidate to develop a biocompatible, osteointegrative biomaterial able to effectively treat implant-related bone infections. This review aimed to contribute to the development of the next-generation of glass and glass-ceramic biomaterials that couples bone regenerative properties with intrinsic antibacterial activity relevant in the bone tissue engineering context.

Herein was demonstrated that antibacterial glasse and glass-ceramic biomaterials are capable of supressing the growth of pathogenic organisms. While a number of classical compositions such as 45S5 bioglass® glass appear to have some antimicrobial activity, there is no doubt that enhanced composition are far more potent. For example, the addition of Ag^{+}, Zn^{2+}, Cu^{+}, Ce^{3+} and Sr^{2+}; all increased antimicrobial activity. While the presence of these specific ions had a direct effect on bacteria, it is important to note that other glass properties related to network disruption are also influenced by small compositional changes (i.e. pH, osmolarity, particle size). Having in consideration that these effects were frequently neglected by authors who often focused solely on the effects of specific ions. It is therefore recommended that glass and glass-ceramics scientists then pay more attention to the design of their biomaterials to aim an ideal system that provide a controlled local delivery of high concentrated antimicrobial compounds to the site of infection and

simultaneously minimise risk of toxic effects while granting a structure that supports bone regeneration.

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