Research Article **Open Access**

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Zinc-containing bioactive glasses for bone regeneration, dental and orthopedic applications

DOI 10.1515/bglass-2015-0006
Received Mar 01, 2015; accepted May 25, 2015

Abstract: Zinc is a vital and beneficial trace element found in the human body. Though found in small proportions, zinc performs a variety of functions in relation to the immune system, cell division, fertility and the body growth and maintenance. In particular, zinc is proven to be a necessary element for the formation, mineralization, development and maintenance of healthy bones. Considering this attractive attributes of zinc, recent research has widely focused on using zinc along with silicate-based bioactive glasses for bone tissue engineering applications. This paper reviews relevant literature discussing the significance of zinc in the human body, along with its ability to enhance antibacterial effects, bioactivity and distinct physical, structural and mechanical properties of bioactive glasses. In this context, even if the present analysis is not meant to be exhaustive and only representative studies are discussed, literature results confirm that it is essential to understand the properties of zinc-containing bioactive glasses with respect to their *in vitro* biological behavior, possible cytotoxic effects and degradation characteristics to be able to effectively apply these glasses in bone regeneration strategies. Topics attracting increasing research efforts in this field are elaborated in detail in this review, including a summary of the structural, physical, biological and mechanical properties of zinc-containing bioactive glasses. This paper also presents an overview of the various applications in which zinc-containing bioactive glasses are considered for use as bone tissue scaffolds, bone filling granules, bioactive coatings and bone cements, and advances and remaining challenges are highlighted.

Keywords: Bioactive glasses, Zinc, Degradation, Dissolution, Bone Tissue Engineering

1 Introduction

Bone tissue engineering (BTE) applies the basic principle of combining biomaterial-based scaffolds, functional cells and signaling molecules or growth factors to restore the function and structure of damaged or diseased bone and to promote the regeneration of new bone [1]. Over the recent years, a wide variety of synthetic and natural biomaterials along with various combinations of cells and signaling molecules have been investigated for this purpose [2, 3].

Silicate bioactive glasses (BGs) have attracted the attention of researchers for BTE due to their excellent bone-bonding ability through the formation of hydroxyapatite (HA) surface layers and their osteoconductive and osteoinductive properties [4]. 45S5 Bioglass® is the name given to the soda-lime-phosphosilicate (Na_2O-CaO-P_2O_5-SiO_2) glass which was developed in the late 1960s by Hench *et al.* [5] as an effort to find a material to fill voids in damaged bone and which would not be rejected by the human body. This was the first synthetic material that could bond to bone and thereby it opened the broad research area of bioactive ceramics for orthopedic and bone regeneration applications. 45S5 Bioglass® has the highest bioactivity index (Class A) of all bioceramics and it belongs to the so-called third generation biomaterials [6]. *In vitro* results have confirmed the formation of a hydroxycarbonate apatite (HCA) layer on Bioglass® surfaces when in contact with simulated body fluid which can chemically bond to collagen fibrils and induces the development of a strong interface between Bioglass® and bone [4]. In addition, bioactive glasses possess attractive properties for BTE such as being osteoinductive, as their dissolution products (for example, Si, Ca, P and Na ions in Bioglass®) activate genes associated with the differentiation of osteoblasts [7]. The angiogenic effects of Bioglass® have also been inves-

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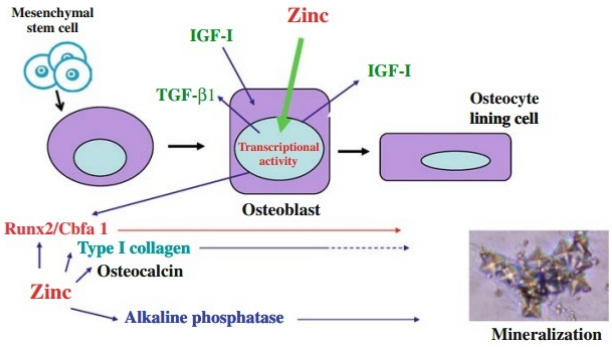

Figure 1: The mechanism of zinc action in stimulating osteoblastic bone formation and mineralization. (Reproduced from re. [17] with permission from Springer)

tigated [8]. Over the years, changes have been made to the original Bioglass® composition and several types of bioactive glass products have been developed for orthopedic bone defect repair, osteomyelitis treatment, periodontal reconstruction and small bone implants [9] as well as bioactive coatings [10] and bone tissue scaffolds [11, 12]. Though bioactive glasses hold great promise for stimulating bone growth, they are very brittle exhibiting limited structural resistance which is required for replacement of bone tissue. To tackle this problem and also for biological considerations, combinations of biodegradable polymers and bioactive glasses are being developed [13]. Also, the original silicate glass matrix can be suitably modified by the incorporation of low amounts of bioinorganics, *i.e.* biologically active elements, such as Cerium, Gallium, Zinc, Magnesium, Cobalt, Copper, Strontium, Lithium and Boron, to impart new biological functionalities, enhancing the therapeutic behavior of the glasses and to serve as micronutrients in critical concentrations to enhance bone growth [14].

One metallic ion which is expected to have significant impact on the healing of bone is zinc. The human body contains about 1.5 – 2.5 g of zinc, with 85% of the whole body zinc in bones and muscle, 11% in the skin and liver and the rest in the other tissues [15, 16]. Zinc is also involved in the process of bone resorption, preservation of bone mass and it can be considered an option for the treatment of osteoporosis (Figure 1) [17]. Apart from being an effective antimicrobial agent, zinc has an active part in normal physiological functions which are related to bone metabolism [18] and also has a stimulatory effect on bone formation [19, 20].

Zinc takes up major biological roles as a cofactor, and as a structural and regulatory ion being also involved in homeostasis, immune responses, oxidative stress and apoptosis [21]. In addition, zinc is a component of almost 300 enzymes and a large number of proteins and it has the outstanding ability to be involved in readily exchangeable ligand binding and other processes such as optimal nucleic acid and protein metabolism, cell growth, division and function [22]. It serves as a cofactor in numerous transcription factors and enzyme systems including zinc-dependent matrix metalloproteinases that augment autodebridement and keratinocyte migration during wound repair [23]. The functional role of zinc in repair systems can be found from the demonstrated evidences of zinc metalloenzymes like alkaline phosphatase, RNA and DNA polymerases and matrix metalloproteinases [23]. Of relevance to BTE, zinc plays a physiologically important role in bone metabolism, formation and resorption. It has been shown to be concentrated in the layer of the osteoid prior to bone calcification [24] and it is also found to be present in the bone mineral component [25]. Zinc deficiency is mostly associated with bone growth retardation [26, 27] and in humans, the vertebral calcium/zinc ratio is inversely related to age indicating that skeletal zinc is conserved better than calcium in later life [28]. Appropriate amounts of zinc can improve the alkaline phosphatase (ALP) activity and DNA content in bone tissue [29]. Yamaguchi [30] reviewed in detail the positive role of zinc in bone metabolism, bone formation, protein and DNA syntheses in osteoblastic cells, bone resorption, and osteoclast-like cell formation.

The huge impact of zinc in relation to bone growth and remodeling, in addition to its antibacterial effects has prompted the possibility of using zinc ions in combination with bioactive glasses to synthesize a new family of bioactive materials for bone regeneration applications. Other applications of zinc-containing BG are being explored, for example in nerve regeneration [31, 32]. Recently, zinc-silicate glass microspheres were produced which were found to be non-mutagenic thereby supporting the material's potential as a suitable embolic agent [33]. The increasing number of publications in the field reflects the high interest in the biomedical use of Zn-containing bioactive glasses. This fact has motivated the preparation of the present review paper which captures the essential research carried out to date in the field. Further sections in this review describe the structural, physical, biological and mechanical properties of Zinc-containing bioactive glasses, the *in vitro* bioactivity and degradation of these materials and their applications as BTE scaffolds, granules, bone filling granules, in bone cements and in coatings. Possible avenues for future research in this field are highlighted based on the identified gaps in knowledge and considering non-addressed challenges.

Table 1: Zinc-containing bioactive glasses and their applications

Type	Glass composition (mol%)						Investigated properties	Ref
	ZnO	SiO_2	CaO	P_2O_5	Na_2O	Rest		
Nanoparticles (wt%)	1, 3, 5	58	32, 30, 28	9	-	-	Glass characterization *In vitro* bioactivity study in SBF	[34]
Sol-gel glass samples	6, 8, 10	46.1	26.9	2.6	12.4, 14.4, 16.4	MgO	Glass characterization *In vitro* bioactivity study in 27-Tris SBF Brunauer-Emmett-Teller studies *In vitro* drug release using gentamicin	[35]
Sol-gel glasses	10	60	5 - 20	5 - 20	-	B_2O_3	Thermal analysis, particle size, zeta potential, hardness and density *In vitro* study in SBF Cytotoxicity and live-dead cell viability using J774A.1 cells	[36]
Particles/slabs (wt%)	5.0, 20.2	42.6, 37.3	23.4, 18.9	5.7, 4.8	23.3, 18.8	-	Surface area measurements after immersion in Tris and DMEM solution Surface reactivity analysis by in situ IR spectroscopy Ion release studies Endothelial cell adhesion and proliferation	[37]
Glass and glass-ceramics (wt%)	1	45	24.5	6	24.5	-	Glass nucleation and crystallization temperature *In vitro* bioactivity test in SBF and ion release studies Density and mechanical properties	[38]
Scaffolds	0-5	50-56	10-18	1-4	10-12	K_2O, MgO, B_2O_3, TiO_2	Glass structure Ion release *In vitro* bioactivity in SBF hASC proliferation and differentiation	[39]
Nanoparticles	0, 5.0, 10.0	52.0, 51.0, 47.0, 42.0	45.0, 41.0, 36.0	3.0	-	-	Surface morphology Chemical composition Phase composition and crystallization *In vitro* bioactivity in SBF	[40]
Glass powder	0,	46.2,	26.9,	2.6,	24.3,		Surface characterization – Infrared spectra	[41, 42]

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Table 1: ... continued

Type	Glass composition (mol%)						Investigated properties	Ref
	ZnO	SiO_2	CaO	P_2O_5	Na_2O	Rest		
	3.8, 7.8, 15.9	44.4, 42.5, 38.8	25.9, 24.8, 22.6	2.5, 2.4, 2.2	23.4, 22.5, 20.5	-	Surface area measurements – BET model, N_2 adsorption *In vitro* bioactivity study in SBF Leaching tests by ICP Cytocompatibility tests *In vivo* studies in rats	
Nuclear waste glass frits	4.20, 3.60, 3.90	57.1, 48.9, 52.1	1.90, 1.60, 1.80	-	11.3, 9.60, 10.3	B_2O_3, Li_2O, Al_2O_3	Structural role of zinc – X-ray absorption near edge and extended x-ray absorption fine structure spectroscopy	[43]
Glass powder	0 – 10	38.49	36.07	5.61	-	MgO, CaF_2, SrO	Structural role of zinc in biodegradation – molecular dynamics simulations, IR spectroscopy, Magic angle spinning-nuclear magnetic resonance (MAS-NMR) spectroscopy. *In vitro* bioactivity study in SBF Chemical degradation in Tris-HCl *In vitro* proliferation of MG63 cells Mesenchymal stem cells proliferation and ALP activity	[44, 45]
Coatings	14 – 25	44 – 55	7	-	19	K_2O	Glass dissolution behavior at physiological (7.35) and acidic pH	[46]
Glass-ceramics	0 – 14.2	35.4	42.8 – 57	7.2	-	CaF_2	Glass transition and crystallization temperatures *In vitro* bioactivity	[47]
Mesoporous BG	7	80	15	5	-	Ce_2O_3, Ga_2O_3	Characterization of mesoporous BG *In vitro* bioactivity	[48]
Sol-gel glasses	5	64	26	5	-	-	Glass characterization *In vitro* studies in SBF *In vitro* studies in hFOB 1.19 cells	[49, 50]
Sol-gel glasses	0.3 – 3.8	59.7	33.7 – 36.4	2.8 – 3.9	-	-	Physicochemical characterization Surface roughness and topographical characterization	[51, 52]

Continued on next page

Table 1: ... continued

Type	Glass composition (mol%)						Investigated properties	Ref
	ZnO	SiO_2	CaO	P_2O_5	Na_2O	Rest		
							In vitro bioactivity *In vitro* studies in human osteosarcoma cell line SAOS-2	
Sol-gel glasses	6 – 21	44 – 59	20	20	15	-	*In vitro* bioactivity	[53]
Sol-gel glasses	5	64	26	5	-	MgO	Glass transition and crystallization temperatures *In vitro* bioactivity Ion release measurements	[54]
Glass ceramics and sol-gel glasses (wt%)	0, 0.5, 4	58	33, 32.5, 29	9	-	-	Glass characterization *In vitro* bioactivity Effect of ionc products from glass dissolution on osteoblasts	[55, 56]
Scaffolds	3, 7, 10	60	32, 28, 25	5	- -	- -	Scaffold characterization *In vitro* bioactivity	[57]
Macroporous and mesoporous scaffolds	0.075, 0.15, 0.225	80	14.925, 14.85, 14.775	5	-	-	Scaffold characterization Bone MSCs proliferation	[58]
Glass-ceramics (mol fraction)	0 – 0.4	0.4	0.1, 0.2	-	0.1 – 0.3	SrO_2	Particle size analyser Scaffold characterization – X-ray tomography Dissolution and ion release studies	[59]
Scaffolds	1.24, 2.4	55.9, 58	16.14, 18	2.5, 7	11.8, 12	MgO, K_2O	Scaffold characterization Cytotoxicity and wound healing assay using MG-63 cells *In vitro* drug release studies using gatifloxacin	[60]
Mesoporous scaffolds	0.4, 2, 4, 7	80	15	5	-	-	Scaffold characterization *In vitro* bioactivity Anitbacterial capacity using Gram-positive	[61, 62]

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Table 1: ... continued

Type	Glass composition (mol%)						Investigated properties	Ref
	ZnO	SiO_2	CaO	P_2O_5	Na_2O	Rest		
							Stapilococcus aureus bacteria *In vitro* cytocompatibility using HOS cells	
Granules	2, 5	70	28	-	-	-	Characterization of glass granules Growth and osteogenic differentiation of MSCs	[63]
Bone graft	0.32	0.40	0, 0.14, 0.28	-	-	-	Thermal and structural characterization of glasses *In vitro* assessment using L-929 cell line *In vivo* assessment in healthy and osteoporotic rats	[64]
Bone grafts	0, 0.1, 0.2	0.4	0, 0.1	-	0.1, 0.2, 0.3	SrO	Structural characterization Dissolution experiments and ion release	[65]
Bone cements (ratio)	35	12	-	-	-	HA, SrF_2	Characterization of synthesized monomers and polymers Exotherm of cements Shrinkage of cements	[66]
Bone cements	0.36	0.48	0.08, 0.12, 0.16	-	-	SrO	Determination of working and setting times Biaxial flexural and compressive strengths	[67]
Bone cements (mol fractions)	0.53	0.42	0, 0.05	-	-	SrO	Zinc release measurements Investigation of bone-cement interface Antibacterial capability against *Streptococcus mutans* and *Actinomyces viscosus*	[68]
Bone cements	0, 19.12	47.32, 47.80	10.41, 5.26	-	-	CaF_2, SrO, SrF_2, MgO	Compressive and adhesive bond strength Cytotoxicity assessment using MC3T3-E1 cell line	[69]
Bone cements	0, 2.5, 5	85	15, 13, 10	-	-	-	Odontogenic differentiation and angiogenesis of human dental pulp cells	[70]

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Table 1: ... continued

Type	Glass composition (mol%)						Investigated properties	Ref
	ZnO	SiO_2	CaO	P_2O_5	Na_2O	Rest		
Bone cements (mol fraction)	0.11 – 0.53	0.54 – 0.42	0.35 – 0.05	-	-	-	Thermal and structural characterization Cement preparation and evaluation	[71]
Bone cements	0.210 – 0.360	-	-	-	-	B_2O_3, GeO_2, $CaCO_3$, ZrO_2	Glass synthesis Structural and thermal characterization Cement preparation and characterization Cement handling properties Cement mechanical properties	[72]
Dental tubules	0.67 – 1	29.61 – 44	10.09 – 15	3.36 – 5	6.73 – 10	CaF_2, SrO, K_2O, CaF_2 +SrF_2	Glass synthesis and characterization Dissolution study and ion release measurements in Tris-buffer solution Occlusion of dentinal tubules	[73]
Coatings	12.8, 31.6	29.4, 23.1	-	-	8.2, 6.4	B_2O_3, Al_2O_3, K_2O	Coating characterization Antibacterial properties versus *Escherichia coli*	[74]
Coatings	3	49.96	0 – 32.62	1.07	3.30	MgO, K_2O, SrO	Glass characterization Characterization of coatings	[75]

Concluded

2 Zinc-containing bioactive glasses

2.1 Structure, physical and mechanical properties

A variety of compositions of zinc-containing bioactive glasses have been tailored and investigated and some key findings on the effect of the addition of ZnO to the characteristics of the investigated BGs are discussed in this section. Table 1 shows the compositions, investigated properties and applications of a significant number of zinc-containing BG produced and reported in the literature.

Dietzel formulated a theory in which the field strength is given by Z/a^2, where Z is the ion charge and a is the bond distance [76]. Network modifier ions have field strengths in the range of 0.1 to 0.4 and intermediate ions in the range of 0.4 ≤ field strength ≥ 1.3. Zinc (Z/a^2 = 0.53 [77]) falls thus close to the boundary between being a network modifier and an intermediate oxide. ZnO has been reported to modify the silicate network structure as the oxide tends to decrease the degree of network connectivity by replacing the binding oxygen (BO) which forms the link between two SiO_4 tetrahedra by non-bridging oxygen (NBO) [34]. Several studies have investigated the network modifier character of ZnO. Anand *et al.* [35] reported that the increase of zinc content in BG of the system x ZnO(22.4 – x)Na_2O-46.1 SiO_2-26.9 CaO-2.6 P_2O_5-2 MgO leads to the decrease in surface area and pore size of the bioactive glass samples which can be correlated to the network modifier behavior of zinc. Kaur *et al.* [36] prepared BG of the system CaO-P_2O_5-SiO_2-B_2O_3 containing 10 wt% ZnO. They observed that zinc acted as a network modifier and boron, phosphorus and silicon interacted with the network modifiers CaO and ZnO via non-bridging oxygens. Apart from its role as a modifier of the network structure, ZnO also acts as an intermediate oxide when present in higher amounts [78, 79]. When ZnO is present in higher concentrations, it acts as an intermediate oxide creating a more stable glass structure by forming covalent links between adjacent SiO_4 tetrahedral and building bridging species rather than forming NBO [34].

The presence of zinc does not seem to influence the crystal phase behavior of BG which is highly dependent on the base composition of the BG. It has been observed that zinc was not present in a separate crystalline phase in ZnO (4 wt%) substituted 45S5 BG which was sintered to a temperature of approximately 650°C. Sodium calcium silicate was present as the main crystalline phase and this might be due to the relatively low content of zinc in the BG composition [38]. It has been also reported that despite the introduction of 5 mol% of ZnO, in the BG of the composition (mol%) 10 - 12% Na_2O, 10 - 12% K_2O, 4 - 6% MgO, 10 - 18% CaO, 1 - 4% P_2O_5, 1 - 2% B_2O_3, 0 - 1% TiO_2, 50 - 56% SiO_2 no crystallization was found and completely amorphous scaffolds could be produced [38]. In general, no distinct difference in structure has been observed between Zn-free BG of composition 52 mol% SiO_2, 45 mol% CaO, 3 mol% P_2O_5 and the zinc-containing counterpart (Zn concentration = 5 mol% and 10 mol%) [40]. In some cases, the XRD patterns of Zinc-containing BG did not contain any diffraction maxima, indicative of the internal disorder and the glassy nature of the zinc-containing BG [50, 53]. The addition of ZnO has been shown to have an effect on degradation behavior of BGs (see also next section). For example, higher concentrations of zinc are responsible for a significant reduction in the overall leaching activity of zinc-containing BG of composition 1 Na_2O-1.1 CaO-0.1 P_2O_5-1.9 SiO_2-x ZnO where x = 0.16, 0.32 and 0.78 [42]. This effect might be because zinc is tightly trapped into the silicate network and the first step of glass degradation which involves quick exchange of Na^+ with H_3O^+ ions is hindered as a function of zinc concentration. Also, the electrostatic attraction of the excess of negative charge in the $(ZnO_4)^{2-}$ tetrahedral should restrain further the mobility of Na^+ ions [41]. In general, zinc has been shown to exhibit a marked preference for square planar complexes in simple alkali silicate glasses [43] and therefore, in most cases of zinc-containing 45S5 BG, Zn^{2+} can be a four-coordinated weak network former [80]. Kapoor *et al.* [44] studied the effect of zinc on the structure of melt-quenched alkali-free CaO-MgO-SiO_2-P_2O_5-CaF_2 based glass system. A series of glass compositions with varying ZnO content (0, 2, 4, 6, 8 and 10 mol%) in place of MgO was prepared and it was found that the ZnO substitution for MgO did not affect the silicate network connectivity of these glasses. The global reduction in the overall leaching activity with higher zinc concentration can also be explained by the fact that amorphous phases are usually more prone to ion leaching phenomena than crystalline phases [81]. Chen *et al.* [46] incorporated ZnO (14 mol% - 25 mol%) in silicate glasses of the composition (mol%) 44 – 55% SiO_2, 7% CaO, 5% K_2O, 19% Na_2O, 14 – 25% ZnO and reported that glasses with low ZnO content (< 19 mol%) were amorphous while the glasses with higher ZnO content (> 21 mol%) contained small amounts of crystalline sodium zinc silicate. The results are related to the findings of Kamitakahara *et al.* [46] who showed that the glass transition and crystallization temperatures of CaO-SiO_2-P_2O_5-CaF_2 glass-ceramics decreased with increasing ZnO content up to 14.2 mol%. These observations were similar to a recent study by Kapoor *et al.* [45] wherein the addi-

tion of zinc to a system of CaO-MgO-ZnO-P_2O_5-SiO_2-CaF_2 glasses gradually decreased the glass transition temperature from 752 to 718°C. This, in turn, translates in lowering the viscosity of the glasses and thereby, decreasing their crystallization temperatures as well. In another study, DTA curves revealed that the crystallization temperature of the 80% SiO_2-15% CaO-5% P_2O_5 (mol%) system, including up to 7 mol% ZnO, was zinc-content dependent [48]. Zinc substituted mesoporous BG scaffolds have also been fabricated which exhibit increasing stability with increase in zinc content [60]. The textural properties such as the surface area, pore volume and pore size diameter of the scaffolds progressively decreased with increasing concentration of zinc in the (80 – x)% SiO_2-15% CaO-5% P_2O_5-x% ZnO (mol%) system where x = 0.4 and 2.0 [61]. Aina *et al.*[37] showed that zinc-doped 45S5 silicate glass systems develop a much smaller surface area in contrast to the BG without zinc which shows a very high increase in surface area even in the first few hours of reaction in Tris buffer solution. Similar results were obtained with higher specific surface area for zinc-free 45S5 BG after reaction in Minimum Essential Medium Eagle (MEM) solution [82]. In contrast, Lao *et al.* [83] found that a homogenous porosity in the range of 50 – 100 nm was obtained for low zinc content (1 wt%) while higher zinc concentrations (5 wt%) led to larger pore size distribution in the range of 50 – 400 nm. It was also shown that low Zn-doping increases the specific surface area in mesoporous glasses of the composition 75 wt% SiO_2-25 wt% CaO which is more advantageous for interaction with the biological medium by providing more nucleation sites for the formation of phosphocalcic precipitates [83]. These zinc-containing BGs are thus attractive for BTE as they display higher porosity than the normal SiO_2-CaO sol-gel BG, with, however, the disadvantage of exhibiting a significantly lower bending strength.

2.2 *In vitro* bioactivity and degradation

As it is well known [84], the formation of HA on the surface of BGs immersed in relevant physiological fluids, *e.g.* simulated body fluid (SBF), is considered a marker of the bioactive character of BGs. Zinc has been found to have a great influence on the growth kinetics of HA in pseudo-physiological solutions [85]. For example, it has been recognized that ZnO retards the crystal nucleation of HA during the initial periods of *in vitro* bioactivity study in SBF but it does not affect the growth of HA after long periods of immersion. It was considered that during the initial nucleation stage of apatite, zinc ions are adsorbed at the active growth sites of HA preventing the growth of the HA (001) crystal face. Thus, there are fewer HA nuclei sites on zinc-containing BGs and for every site, the chance to absorb more Ca^{2+} and PO_4^{3-} in SBF increases and this results in the larger size of the HA granules observed [85]. As expected, with increased immersion time in SBF, HA crystallites cover the surface of the BG gradually and it becomes more difficult for the Zn ions in the BG to diffuse to the solution than for Ca ions as the bond energy of ZnO is higher than the bond energy of CaO. As such, the influence of zinc on the growth of the HA layer is reduced. Du *et al.* [55] observed a similar result on BGs of composition 58 wt% SiO_2, 33% CaO and 9% P_2O_5 with 4% ZnO substituted for CaO showing that the HA layer grew steadily with prolonged soaking time in SBF. It was also noted that the deposition rate of HA decreased with increase in zinc content in 58S BG and an elevated concentration of zinc was seen to prevent the growth of HA mainly in the *c*-face. When immersed in SBF, Ca^{2+} and Zn^{2+} ions are released and PO_4^{3-} ions combine with Zn^{2+} ions and form hopeite, $Zn_3(PO_4)_2 \cdot 4\,H_2O$, and HA, $Ca_5(PO_4)_3(OH)$. This is due to the fact that the solubility constant of $Zn_3(PO_4)_2$ is 9.1×10^{-33} and thus lower than the solubility constant of $Ca_3(PO_4)_2$ which is 2×10^{-29} [55]. Therefore, in aqueous solution PO_4^{3-} ions combine more readily with Zn^{2+} than with Ca^{2+}. The combination of Zn^{2+} with PO_4^{3-} results in the lower bioactivity of zinc-containing BG in comparison with zinc-free BG. It is well known that the bioactivity of bioactive glasses depends on the glass network connectivity [86], which, as mentioned above, is affected by the presence of ZnO.

Indeed when zinc is added as a doping element to silicate BGs, it delays the breakdown of the silicate network by biological fluids [83]. In zinc doped sol-gel derived glasses of base 75 wt% SiO_2-25 wt% CaO composition, for example, the incorporation of phosphorus from DMEM at the glass surface begins early. However, the diffusion of Ca ions from the core of the material to its periphery is delayed. This is because zinc adopts a tetrahedra structure in the glassy network and copolymerizes with SiO_4^- tetrahedra resulting in an overall complexation of the glass network which increases its chemical durability. For low zinc content, a Ca-P rich layer was seen to form after 1 day whereas higher zinc doping was seen to delay the migration of Ca from the inner regions of the material to the periphery. Lao *et al.* [83] found that for zinc doped glasses, a composition of 24.5% Ca, 5.5% P and 19.7% Si (wt%) was present whereas for undoped glasses 42.3 wt% silicon-rich grains with only 3.8 wt% calcium and no phosphorus were present. This observation confirmed that the Ca-P peripheral layer is dissolved after a few days in the case of undoped 75 wt% SiO_2-25 wt% CaO glasses. The Ca/P atomic ratio of zinc doped

samples was in the range 1.2 – 2.5 (which is close to the theoretical Ca/P ratio in HA of 1.67) suggesting that a stable apatite layer has formed. It has been also found that higher zinc content leads to the addition of more phosphate ions at the glass surface during the early stages of reaction in biological fluids. Goh *et al.* [40] observed that the Ca/P ratio for pure BG of composition 52 mol% SiO_2, 45 mol% CaO, 3 mol% P_2O_5 and for BG substituted with 5 mol% Zn was close to 1.67. Although ZnO does not affect adversely the Ca/P ratio, its value is lower than the value found for pure 52S BG (1.8), which confirms the retardation of initial HA formation by ZnO.

In related research, it has also been shown that the addition of zinc causes a global reduction in ion leaching in the 45S5 BG composition [42]. Zinc influences the insertion of phosphorus into the 3D glass network and therefore the subsequent addition of phosphorus into the silicate network results a function of zinc content. As mentioned above, glass degradation is retarded by the presence of high zinc content and only a low degree of surface modification is evident in such case. For phosphosilicate glasses with about 3.8 mol% Zn, immersion in SBF for 15 days was found to be accompanied by the formation of a second phosphate-containing crystalline phase, $Zn_3(PO_4)_2{\cdot}4\ H_2O$. This is the reason for the presence of phosphate-rich particles with Ca/P ratio of approximately 1 [42]. It was reported that zinc phosphate is difficult to dissolve which explains the absence of zinc in the solution. The phosphosilicate glass with 3.8 mol% Zn exhibits in SBF, in terms of formation and degree of crystallinity of HA surface layer, a similar behaviour to that of 45S5 Bioglass® and therefore, it falls under the category of highly bioactive glasses. Moreover XRD spectra of glasses with increased zinc content have not shown the presence of any new crystalline phases (Lusvardi *et al.*, 2009). Shruti *et al.* [61] added 2 mol% ZnO to mesoporous BG based on the 80% SiO_2-15% CaO-5% P_2O_5 (mol%) system and they found a drastic decrease in the *in vitro* bioactivity in SBF. FTIR analyses showed a band at 559 cm^{-1} corresponding to phosphate after 8 h of immersion in SBF. The double peaks, corresponding to crystalline phosphate, at 561 and 600 cm^{-1}, appeared only after 7 days. Also the level of Ca and P showed very slow increase up to day 1 and then, however, the Ca/P ratio was found to reach 1.54 suggesting the nucleation and growth of HA. The low *in vitro* response of zinc-containing mesoporous BG is consistent with the studies described above and it is due to the fact that zinc ions are involved in the formation of zinc phosphate which was also confirmed by ICP results. In these conditions the concentration of Ca continuously increased up to 168 h and the concentration of P drastically decreased during the same period. The absence of zinc ions in the SBF was also noted. A relatively low *in vitro* bioactivity has been also found in 4 mol% ZnO-substituted mesoporous glasses of the composition 80% SiO_2-15% CaO-5% P_2O_5 (mol%) [48].

In related studies, Oki *et al.* [50] have suggested that when zinc is present in BG in the SiO_2-P_2O_5-CaO system, it does not reduce significantly the bioactivity of the material and also helps to maintain the pH of the SBF within physiological values. The authors performed an SBF study for zinc-containing phosphosilicate glasses and noticed that zinc controls the hydroxide ion concentration by the formation of insoluble zinc hydroxide. This result was also confirmed by Balamurugan *et al.* [49] who used a highly bioactive glass system with 64% SiO_2, 26% CaO, 5% P_2O_5, 5% ZnO (mol%) for their study. As expected, due to the interactions between the glass surface and the SBF solution, a decrease of solution pH was measured. The precipitation of zinc ions as $Zn(OH)_2$ re-establishes the pH of the medium and helps in maintaining it. The involvement of zinc in the nucleation of HA was asserted by considering the changes in zinc concentration with increasing immersion time. In a related study, Saino *et al.* [52] showed that addition of 5 wt% Zn to 58S BG increased the HA formation rate and more than 90% crystalline HA phase was formed after one day of immersion in SBF. However, the continuous addition of zinc to the BG composition SiO_2-P_2O_5-CaO makes the glasses more resistant to crystallization of the HA phase [51]. Interestingly, incorporation of ZnO in place of both CaO and P_2O_5 leads to an increase in the formation rate and content of the HA layer. In this case, ZnO plays the role of glass former and helps to maintain P_2O_5 in the glass matrix [50]. Singh *et al.* [53] prepared the BG system x (ZnO, Fe_2O_3)-(65–x) SiO_2-20 (CaO, P_2O_5)-15 Na_2O ($6 \leq x \geq 21$ mol%) by melt-quenching technique. It was shown that the addition of ZnO did not affect the dissolution of Ca^{2+} and Si^{4+} from the surface and also, it did not influence the super-saturation of the fluid with ions required for nucleating apatite crystallites on the glass surface. Zinc ions therefore contribute to HA formation by providing more OH^- ions necessary for the crystallization of the amorphous Ca-P to form HA by migrating into the fluid as $Zn(OH)_2$. In the study of Singh *et al.* [53] it was shown that with increasing amount of zinc and iron oxides, an overall increase in the average size of HA crystals occurred and also the HA peaks in XRD spectra were seen to sharpen [53].

The formation of a HA layer on the surface of zinc-containing CaO-SiO_2-P_2O_5 BG system has been also ascertained by evaluating porosity values [54]. The porosity increased from 14.5% to 16.6% after 7 days of immersion in

SBF and then, continuously decreased to 14.5%. This result is due to the fact that Ca^{2+} and Si^{4+} ions were released from the glass surface to SBF increasing the porosity. After seven days, the Ca^{2+} and Si^{4+} ion concentrations in SBF decreased according to the formation of the HA layer. This HA layer covered the pores and decreased the overall porosity. On the other hand it was confirmed that the density of the zinc-containing BG did not change after immersion in SBF [54].

Du *et al.* [56] evaluated the influence of zinc on the formation of HA by adding 4 wt% of ZnO in place of CaO in 58S bioactive glass and by soaking pure 58S bioactive glass in zinc-containing SBF. It was shown that both zinc in bioactive glass and zinc in SBF tend to retard the deposition of HA. This was found to be due to the preferential reaction of Zn^{2+} with PO_4^{3-} which resulted in the decrease of the PO_4^{3-} concentration in the soaking fluid leading to reduced nucleation of HA. In the case of zinc containing 58S bioactive glass, due to the lower CaO content, there was a decrease in the release of Ca^{2+} during the initial soaking period, which resulted in lower nucleation rate of HA. Although there are contradictory results and opinions on whether the presence of zinc enhances or inhibits the *in vitro* bioactivity of the BG, it is clear that the degradation of glass is retarded with increase in zinc content. Nevertheless, further experiments are necessary to validate the role of zinc in glass dissolution and HA deposition.

2.3 The biological significance of Zinc in bone tissue

2.3.1 Influence of zinc intake on bone

Zinc is known as an essential mineral for bone development. Therefore, the sensitive influence of zinc intake on bone metabolism has been subject of a large number of experimental studies. In 1984, studies in monkeys showed that zinc deprivation during gestation leads to disturbed skeletal development that was similar to rachitis [87]. Another group investigated zinc deficiency in gestating rats that caused impaired bone matrix formation and bone mineralization in their neonates [88]. These results suggest that zinc plays a key role in endochondral bone formation during embryonic development.

Moreover dietary zinc enhances osteoblast differentiation and simultaneously inhibits osteoclast bone resorption in growing rats [89]. In adult mice, high dietary zinc is reported to enhance the expression of ALP, a marker of osteoblast differentiation, and to reduce tartrate resistant acid phosphatase (TRAP), an osteoclastic marker [90]. These findings highlight the reactions of growing and adult organisms to zinc supply, which is necessary for bone development and plays a significant role in bone metabolism [91]. In contrast to these findings it has been shown that healing of calvarial defects in adult rats is not influenced by alimentary zinc supplementation or depletion [92].

Dietary zinc supply has also been examined in clinical trials naturally. Zinc intake and plasma zinc level were measured in 396 men (aged 45 – 92 years) while men with low plasma zinc concentration had a significantly reduced bone mineral density compared to men with high plasma zinc concentration independent of age or body mass index [93]. The same result was found in a French study on 139 pre-menarcheal adolescent girls (aged 12.4 +/– 1.0 years) showing zinc being an essential trace element for normal bone growth [94].

2.3.2 Effects of zinc on bone cell cultures *in vitro*

Direct effects of zinc on bone cells and skeletal metabolism are still not completely understood. Zinc supplementation in cell culture medium of MC3T3-E1 murine osteoblasts has been shown to enhance mineralization and osteocalcin mRNA expression levels of these cells [93]. Moreover, it is suggested that zinc increases osteogenic function by stimulating PKC/MAPK signalling pathways in osteoblastic cells [95]. Yamaguchi *et al.* [96] demonstrated that zinc antagonised NF-κB activation, a pathway that is necessary for osteoclastogenesis and suppression of osteoblast differentiation [96]. NF-κB is used as a major pathway by both RANKL (receptor activator of NF-κB ligand) and TNFα (tumor necrosis factor α) to stimulate osteoclastogenesis [97]. In agreement with these findings, Kwun *et al.* [98] showed that zinc deficiency in cell culture medium attenuates osteogenic activity by reducing Runx2 expression and consequently leads to a decreasing transcription of numerous bone-specific genes [98]. Beyond that zinc and magnesium are required cofactors for ALP. This important enzyme for bone formation incorporates 4 zinc atoms per molecule. An active ALP-enzyme needs minimum 2 atoms of zinc [99]. Thus the absence of zinc leads to a downregulation of extracellular matrix mineralization through inhibition of ALP activity in osteoblasts [98]. Besides dietary zinc intake and the direct effect of zinc on *in vitro* cell culture, local release of zinc from bone substitute materials has been the subject of many experimental studies. The essential findings regarding zinc release from bioactive glasses are discussed in the following sections focussing on typical applications such as bone tissue scaffolds, gran-

Table 2: Summary of zinc-containing BG scaffolds reported in the literature indicating also fabrication methods used

Zinc content (mol%)	Fabrication technique	Reference
0 – 5	Melt spinning and sintering of fibers	[39]
3 – 10	Sol-gel and sacrificial template	[57]
0.075, 0.15, 0.225	Sol-gel and sacrificial template	[58]
0 – 0.2 (mol fraction)	Sacrificial template	[59]
1.24, 2.4	Cold-isostatic pressing and sintering	[60]
0.4, 2	Rapid prototyping	[61]
4, 7	Rapid prototyping	[62]

ules for bone filling, bone cements and orthopaedic coatings.

3 Applications of zinc-containing bioactive glasses

3.1 Scaffolds

Scaffolds, in bone tissue engineering, act as templates for cells to attach, proliferate, differentiate and develop into healthy, functioning bone [100]. It is considered that the degradation kinetics of the scaffold should match the growth of new bone. In addition, design of scaffolds for bone tissue engineering requires the fulfillment of certain criteria such as biodegradability, high porosity with interconnected pores, suitable mechanical properties, osteoconductivity and commercialization potential [100]. As mentioned above, BGs are widely considered as scaffold materials for bone tissue engineering because of their high bioactivity and ion releasing ability leading to osteoconductive and osteroinductive behavior [4]. Considering the significant role of zinc in bone formation and mineralization, zinc-containing BG-based scaffolds are receiving increasing attention. Table 2 shows the fabrication techniques used to produce zinc-containing BG scaffolds.

Haimi *et al.* [39] prepared BG scaffolds based on the system Na_2O-K_2O-MgO-CaO-B_2O_3-TiO_2-P_2O_5-SiO_2 using fibres made by melt spinning. Different compositions of ZnO (between 0 – 5 mol%) were substituted for CaO. No crystallite phase formation was observed even in the scaffold with the highest percentage of ZnO (5 mol%). The fabricated zinc-containing BG fibers were collected and sintered together to form 3-D scaffolds of nominal dimensions $14 \times 14 \times 5$ mm^3 with a porosity of 70%. These scaffolds were used for cell culture studies. Human adipose stem cells (hASCs) spread well across all the scaffolds and showed elongated phenotype. The hASCs were found to elongate along the fibers and to form bridges from one glass fiber to another on the surface of the scaffolds. Qualitative live/dead staining showed that the addition of zinc prevents cell spreading and proliferation. However, no significant effect on DNA content, ALP activity and osteopontin concentration of hASCs was found with the addition of zinc when measured quantitatively.

Veres *et al.* [57] have recently produced 3D interconnected macroporous glass-ceramic scaffolds with different amounts of zinc using sol-gel technique. The bioactive glass system contained 60 mol% SiO_2, (35 – X) CaO, X ZnO, 5 P_2O_5 with x = 3, 7, 10 mol%. The scaffolds were obtained using sol-gel method with and without the addition of polyethylene glycol (PEG) combined with sacrificial template method. The microstructure of the obtained scaffolds was found to be dependent on the chemical composition of the glass-ceramic and a denser microstructure was observed for the highest concentration of zinc. From FTIR analysis it was found that zinc tends to act as a network modifier in a stronger fashion than the calcium ions that they replace, as discussed also in section 2.1. *In vitro* SBF studies indicated that the increase of zinc content prevents HA formation. On the other hand, HA phase occurred in samples prepared with PEG even before SBF immersion and it was observed to be better developed with the increase of zinc concentration. In the PEG-free scaffolds, HA was observed only for the lowest ZnO content (3 mol%) before immersion in SBF. In the scaffolds containing 3 mol% and 7 mol% ZnO, HA formation was found to occur after immersion in SBF. The PEG-free samples with the highest

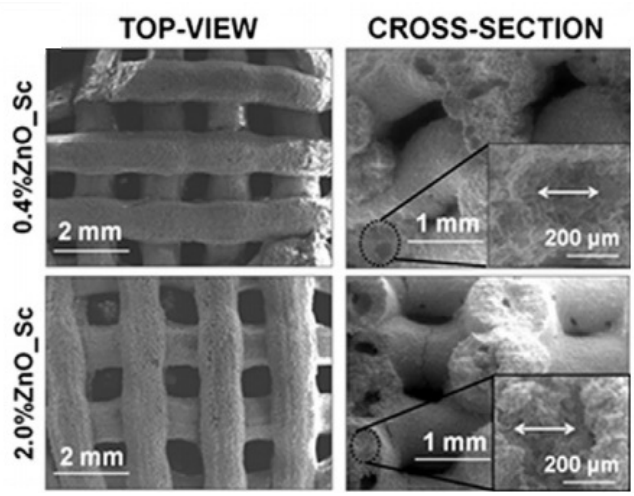

Figure 2: SEM images of Zn-substituted mesoporous BG scaffolds produced using rapid prototyping technique. (Image is reproduced from ref. [61] with permission from Elsevier)

ZnO content did not show any formation of HA which was confirmed by SEM, XRD and FTIR studies.

In a related study, zinc-doped (0.075, 0.15, 0.225 mol%) hierarchical macroporous and mesoporous CaO-SiO_2-P_2O_5 bioactive glass scaffolds were synthesized by Wang et al.[58] using the sol-gel technique. The green bodies were sintered at 700°C at a heating rate of $1°C{\cdot}min^{-1}$ to obtain porous scaffolds. Open pores with diameters in the range of 100 – 400 μm and a pore wall thickness in the range 10 – 50 μm were observed. The cellular response of the zinc-containing scaffolds was evaluated using mesenchymal stem cells (MSC). The zinc-doped scaffolds showed no cytotoxicity and the gradual release of zinc into the culture medium led to the enhancement of cell proliferation and ALP activity of MSCs. In this study, the zinc concentration range in the culture medium was from 0 to $0.59{\pm}0.03\ mg^{-1}$ which was confirmed to be non-toxic and expected to promote bone growth *in vivo*. Looney *et al.* [59] prepared a series of strontium-doped zinc silicate (Ca-Sr-Na-Zn-Si) glass ceramic scaffolds with a porosity of 93% – 96%. After dissolution in Tris-HCl, the levels of Zn^{2+} detected as a result of the degradation of the crystalline phases were found to be 1.4 – 600 parts per million. The detected levels are comparable to the levels of Zn^{2+} associated with clinical benefits including osteoblastic differentiation and impaired osteoclastic resorption. The authors reported that this will be advantageous in terms of augmenting bone turnover and antibacterial efficacy.

More recently, Soundrapandian *et al.* [60] developed two glass compositions based on the SiO_2-Na_2O-ZnO-CaO-MgO-P_2O_5 system containing 1.24 and 2.4 mol% ZnO (BGZ and MBG, respectively). Scaffolds fabricated from these two glasses were found to exhibit open porosity of 66% and 63%, respectively, and a closed porosity of 4% and 2%, respectively. The MBG scaffolds (2.4% Zn) were found to be more bioactive than the BGZ scaffolds (1.4% Zn) with respect to apatite formation after 28 days of immersion in SBF. In addition, the MBG scaffolds were non-cytotoxic and exhibited excellent wound-healing potential. Both types of scaffolds could successfully release gatifloxacin for 43 days following the Peppas-Korsmeyer release pattern based on Fickian diffusion [101] to treat bone infections.

Indeed the development of mesoporous BGs with in situ drug delivery capability (drugs and bioactive molecules are loaded in the mesoporous network of the materials) is receiving increasing attention [102]. In a BG system incorporating ZnO, Shruti *et al.* [61] prepared mesoporous bioactive glass scaffolds using a combination of evaporation-induced self-assembly and rapid-prototyping techniques based on the SiO_2-CaO-P_2O_5 system with 0.4 mol% and 2.0 mol% ZnO. The fabricated scaffolds exhibited a well interconnected porosity with pores in the range of 400 μm. Figure 2 shows the SEM images of Zn-substituted mesoporous BG scaffolds produced using rapid prototyping technique [61]. The mesopore order was maintained in the lowest substituted zinc-containing BG scaffolds. In addition, the mesoporous BG scaffold with 2 mol% ZnO showed lower *in vitro* response (lower bioactivity) than the 0.4 mol% zinc-doped scaffold. This result was due to the involvement of the zinc ion in the formation of zinc phosphate leading to lower bioactivity during *in vitro* assay (as discussed also above). In a related study [62], the *in vitro* antibacterial capacity and cytocompatibility of the same hierarchical meso-macroporous glass scaffolds doped with 4 mol% (4.0 Zn) and 7 mol% (7.0 Zn) ZnO was studied. After 2 days of culture, both zinc-containing scaffolds showed viable and well spread osteoblasts in close contact with the surfaces. Cell proliferation was measured in terms of mitochondrial activity and the 4.0 zinc scaffolds showed an increase in the levels of cell proliferation as a function of incubation time. The 4.0 Zn scaffolds also led to higher cell proliferation than the 7.0 Zn and Zn-free scaffolds and it was also shown that 4.0 Zn scaffolds were non-cytotoxic whereas the low proliferation on the 7.0 Zn scaffold indicated a cytotoxic effect after 6 days. During this study, it was also observed that the antibacterial capacity against *Staphylococcus aureus* increased with the increase in the percentage of zinc present in the scaffold.

The discussed studies thus show that zinc-containing BG scaffolds possess suitable characteristics such as bioactivity, antibacterial properties, drug delivery capabil-

ity (in case of mesoporous BGs) and cytocompatibility under specific Zn concentrations, which are all essential for applications in bone regeneration.

3.2 Bone filling materials and bone grafts

Zinc-containing BG granules have been prepared using sol-gel technique and the effects of addition of small concentrations of zinc on the growth and osteogenic potential of MSCs were investigated by Oh *et al.* [63]. They prepared three different BG granules of composition (in mol%) 70 SiO_2-30 CaO, 70 SiO_2-28 CaO-2 Zn and 70 SiO_2-25 CaO-5 ZnO. Granules with sizes in the range of 500 to 1000 μm were chosen for further biological studies. *In vitro* mineralization behavior in SBF was confirmed, observing that the surface of the granules was fully covered by a thick mineral layer after 7 days in SBF. The influence of the zinc-containing bioactive glass granules on the osteogenic differentiation of MSCs was analyzed based on the increase of the ALP activity. It was demonstrated that the presence of zinc in the bioactive glass granules induced cells to differentiate to a higher level than on the zinc-free granules. Hence, zinc-added BG granules preserved the growth of adult stem cells and stimulated further osteogenic differentiation.

Boyd *et al.* [64] developed a series of calcium-strontium-zinc-silicate glass based bone grafts and studied their structural and physical properties, *in vitro* cytotoxicity and *in vivo* biocompatibility. Among the range of glass systems prepared, a graft with the formulation 0.28 SrO/0.32 ZnO/0.40 SiO_2 (mol fraction) exhibited the best behavior *in vitro* by inducing extremely mild cytotoxic effects on L929 mouse fibroblast cells with a cell viability of up to 95%. The commercially available bone graft Novabone® exhibited a cell viability of 72%. Also, the developed grafts performed equally well in osteoporotic tissue and in healthy tissue when tested in rats. As a sequel to this study, the authors analyzed the effect of glass composition on zinc and strontium release in normal and extreme physiological environments for six different compositions of Ca-Sr-Na-Zn-Si glasses. The zinc release levels were found to be 3.0 – 7.65 ppm over 30 days for all the zinc-containing glasses in pH 7.4 solution. On the other hand, the zinc release levels in acidic environment (pH 3) were 89 – 750 ppm. These are found to be higher and may produce cytotoxic or negative effects on bone tissue [65]. The number of studies on zinc-containing BG as granules for bone fillings is very limited. However, from the few studies available, it has been observed that zinc enhances cell viability, osteogenic differentiation and performs well in healthy as well as diseased tissue. These findings show the potential for further analysis on zinc-containing BG for application in bone grafts.

3.3 Bone cements

Zinc and glass polyalkenoate cements were first introduced as dental cements in the late 1960s [103, 104]. During *in vivo* studies, it was noticed that the zinc component resulted in the formation of a fibrous collagen capsule around the zinc polycarboxylate cement which led to the decrease in the strength of the intermediate region between the bone and the cement [105]. It is well known that bioactive glasses support the formation of a mechanically strong chemical bond at tissue/implant interfaces which is called "bioactive fixation" [5]. This type of implants has the ability to tolerate more complex stress states than dense inert implants which have only "morphological fixation". Darling *et al.* [106] reported that any zinc-silicate glass with a low silica mole fraction (below 0.5) and high zinc oxide content is capable of forming glass polyalkenoate cements suitable for dental use. Also, hydrolytically stable glass polyalkenoate cements (GPCs) could not be produced from alkali metal-zinc-silicate glasses or calcium-zinc-silicate glasses, except at very low calcium oxide mole fractions. An alternative bone cement containing sintered zinc-calcium-silicate phosphate glass as a reactive filler and hybrid polyalkenoate as a resin matrix was developed by Xie *et al.* [66]. The prepared cement showed significantly higher mechanical properties (which is due to the sintering of the glass as it improved the compressive strength and extended the curing time of the cement), lower exotherm and shrinkage compared to conventional PMMA bone cement. Boyd *et al.* [68] synthesized and characterized Zn-containing GPCs to analyze their potential use in Total Hip Arthroplasty (THA). They prepared cements based on two glass compositions, namely, (in mole fractions) glass A (0.05 Cao, 0.42 SiO_2 and 0.53 ZnO) and glass B (0.05 SrO, 0.42 SiO_2 and 0.53 ZnO). In both cements, the rate of zinc release was seen to decrease with time and no detectable zinc was released after 7 days. However, the absolute levels of zinc release were lower in cement B than in cement A which might have been due to the formation of a more integral network in the case of strontium containing BG, thereby inhibiting the release of zinc. The released zinc ions could penetrate into contiguous bone by up to 40 μm and it was reported that the growth of two characteristic bacterial species, *Streptococcus mutans* and *Actinomyces viscosus* was significantly inhibited in *in vitro* studies. The antibacterial activity ob-

served with cement A was greater than that of cement B corresponding well with the higher zinc release rates of the former cement. In another relevant study, Boyd *et al.* [67] prepared glass ionomer cements (GIC) based on two glass compositions (mol%) – glass A (0.05 CaO, 0.53 ZnO and 0.42 SiO_2) and glass B (0.14 CaO, 0.29 ZnO and 0.57 SiO_2). They found that a GIC based on the glass B formed an amorphous calcium phosphate layer on the surface of the cement indicating that they are bioactive in nature. In a later study, Boyd *et al.* [67] incorporated Zn^{2+} ion into an aluminium-free GPCs based on silicate glass. They found that 1g of the calcium-zinc silicate glass of composition (mol%) 0.16 CaO, 0.36 ZnO and 0.48 SiO_2 mixed with 50 wt% aqueous solution on polyacrylic acid at a powder:liquid ratio of 2:1.5 exhibited the best combination of working time, setting time, compressive strength and biaxial flexural strength. Boyd *et al.* [71] characterized the structural role of zinc in aluminium-free GPCs and related the glass structure to the reactivity. Their results showed that glasses capable of forming Zn-GPCs have network crosslink densities greater than 2. From MAS-NMR studies, it was found that the primary role of zinc in these glass networks is as a network modifier and not as an intermediate oxide. In a more recent study, Brauer *et al.* [69] prepared zinc-containing GPCs based on the glass composition (mol%): 47.80 SiO_2, 5.26 CaO, 5.52 CaF_2, 5.26 SrO, 4.55 SrF_2, 12.43 MgO and 19.12 ZnO. The zinc-containing cements showed adhesion to bone close to 1 MPa which was reported to be significantly greater than that of zinc-free cements (< 0.05 MPa) and other currently approved biological adhesives. However, the cements failed basic biocompatibility tests and produced an acute cytotoxic response *in vitro* demonstrating the importance of investigating ion releasing GPC in their *in vivo* compatibility. Lewis *et al.* [107] prepared a zinc-based GPC of the composition (mol%) 0.12 CaO, 0.04 SrO, 0.36 ZnO and 0.48 SiO_2. The authors found that the injectability, radiopacity, uniaxial compressive strength and biaxial flexural modulus of the prepared zinc-based GPC were comparable to those of a PMMA bone cement used in vertebroplasty and balloon kyphoplasty. In a related study, bioactive calcium phosphate cements incorporating zinc-BG of the system SiO_2-CaO with 2.5 and 5 mol% ZnO were prepared by Zhang *et al.* [70]. No cytotoxicity was found in any of the systems. Zinc-containing cements showed increased ALP activity, enhanced formation of mineralized nodules and upregulated mRNA expression of DMP-1, DSPP, Runx2, and osterix in a time- and dose-dependent manner, relative to cements without zinc. The authors showed that Zn-BG incorporated within cements activates odontogenic differentiation and promotes angiogenesis *in vitro*.

Zhang *et al.* [72] designed a unique system of zinc-boron-germanium based glasses using a Design of Mixtures methodology. The glass-ionomer cements provided working time, setting time and compression strength in the range of 48 – 132 s, 206 – 602 s and 16 – 35 MPa respectively. By varying the ZnO/GeO_2 mol fraction in the glass phase, the working time, setting time and compression strength were found to be extended. The authors hypothesize that this provides an alternative formulation of glass ionomer cements for applications beyond the dental clinic. A system of BG containing SiO_2-P_2O_5-CaO-CaF_2-SrO-SrF_2-ZnO-Na_2O-K_2O was produced and investigated for apatite formation, ion release and occlusion dentinal tubules *in vitro* by Lynch *et al.* [73]. The authors incorporated zinc mainly for its antibacterial and anti-gingivitis properties. However the concentration of zinc in the Tris-buffer solution was very low which is attributed to the low zinc content (1 mol%) in the glasses. Nevertheless the formation of fluorapatite and the combined action of the release of therapeutic ions such as fluoride, strontium, potassium and zinc occluded exposed dentinal tubules.

Zinc-doped BG based cements have thus proven to be versatile with varied characteristics and potential applications in different areas such as skeletal augmentation, dental repair, hip arthroplasty and vertebroplasty, however studies on the cytotoxicity of different zinc-containing formulations are continuously being conducted. Zinc-doped BG based bone cements have been introduced a few decades ago. However, the importance/role of zinc in these cements has not been clearly established. There are still varying opinions regarding the cytotoxicity of these cements which necessitates further analysis considering the other advantages of the presence of zinc, for ex. antibacterial effects.

3.4 Biomedical Coatings

As mentioned above, zinc acts as a local regulator of bone and stimulates bone metabolism *in vitro* and *in vivo* [28]. Thus zinc has been considered as an active elemental addition to coatings for orthopedic applications. For example, zinc has been introduced into the sub-surface of TiO_2 coatings by plasma immersion ion implantation and deposition [108]. Rat bone marrow cells showed higher ALP activity and up-regulated osteogenic-related genes in zinc-incorporated coatings in comparison with zinc-free coatings. In addition, zinc is antimicrobial and plays a major role in wound healing [46]. Antimicrobial coatings for metallic implants can thus be developed based on this property of zinc. Chen *et al.* [46] incorporated zinc in sil-

icate glasses to be applied as coatings on stainless steel. The authors hypothesized that zinc present in the silicate glass system influences the ion release behavior of the glass ion response to the pH environment, wherein glasses are comparatively stable in neutral pH and they dissolve readily under acidic conditions. The release of zinc can be therefore controlled and optimized to achieve therapeutic doses. The results of the investigation showed that zinc-containing BGs are promising coating materials in orthopedic and dental applications where the degradation of the glass is required under acidic conditions, such as in the presence of bacterial infections, and inflammations, but it is not desired under normal physiological environments. An important study was reported recently by Esteban-Tejeda *et al.* [73] who developed bactericidal coatings based on the glass system B_2O_3-SiO_2-Na_2O-ZnO. Crack-free, single layer coatings on different biomedical metallic substrates such as Ti-alloy, Nb, Ta and stainless steel were fabricated and characterized. All the coatings obtained were found to be strongly antibacterial versus *Escherichia coli* (> 4 log). Lotfibakhshaiesh *et al.* [75] incorporated 3 mol% of ZnO in a SiO_2-MgO-Na_2O-K_2O-ZnO-P_2O_5-CaO-SrO glass system to restrict the tendency towards crystallization and to provide a small amount of Zn^{2+} release from the glass, mainly for its bactericidal properties and significance in wound healing and bone formation [23]. All BG compositions investigated favored sintering without crystallization and provided a larger processing window, except in the case where calcium was completely replaced with strontium. Interestingly, BG coatings with a thermal expansion coefficient matching that of Ti6Al4V alloy were produced. From the literature, it is noted that there were very few studies reporting on coatings based on zinc-containing BGs. It is essential that the potential of zinc as an antibacterial agent is considered in future research by developing novel zinc-doped BG-based coatings.

4 Conclusions and Outlook

As reviewed in this paper the addition of zinc to bioactive glasses has a great influence in modifying the structural, physical, mechanical, degradation and biological properties of the bioactive glass. Depending on the concentration in which ZnO is present in specific bioactive glass 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 behaviour of the silicate matrix having consequently an effect on the glass surface reactivity in contact with physiological fluids. The influence of zinc on the properties of bioactive glasses not only depends on the zinc content but also on the relative content of the other oxides. Various biomedical applications of zinc-containing bioactive glasses in the context of bone regeneration, dental and orthopedic applications were proposed in the literature and they are discussed in this review, highlighting the potential of zinc incorporation for the development of different products such as scaffolds for bone TE, bone filling granules, bone cements and coatings for implants. One important function of zinc in these applications is its antibacterial activity. From the analysis of the literature, it is clear that despite the demonstrated effect of the zinc ion in bone metabolism and as antibacterial agent, *in vitro* and *in vivo* biological studies on zinc-containing BGs are still very limited. Thus, future research efforts should include systematic approaches with a combination of ion release studies on different BG compositions considering the effect of zinc release on cells *in vitro* and *in vivo* for comprehensive characterization of zinc release effects on osteogenesis and to assess potential cytotoxicity in specific situations. Also, it is necessary to investigate the angiogenesis stimulation capability of zinc-containing bioactive glasses for application in bone TE. This specific field of research is in its early stages and there is scope for understanding and improvement, considering the increasing interest in developing BG with angiogenic potential [8]. Clearly, as it is the case for other biologically active metallic ions, the incorporation of zinc in bioactive glasses opens the opportunity to expand and improve the family of ion-doped BGs to develop new materials with enhanced performance for bone regeneration, dental and orthopedic applications.

Acknowledgement: The authors would like to acknowledge the European Commission funding under the 7th Framework Programme (Marie Curie Initial Training Networks; grant number: 289958, Bioceramics for bone repair).

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