Chapter 13

Doped Bioactive Glass Materials in Bone Regeneration

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Additional information is available at the end of the chapter

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Abstract

In the arena of orthopaedic surgery, autograft is considered to be the gold standard for correction of fracture repair or other bone pathologies. But, it has some limitations such as donor site morbidity and shortage of supply, which evolved the use of allograft that also has some disadvantages such as immunogenic response to the host, low osteogenicity as well as possibilities of disease transmission. Despite the benefits of autografts and allografts, the limitations of each have necessitated the pursuit of alternatives biomaterials that has the ability to initiate osteogenesis, and the graft should closely mimic the natural bone along with regeneration of fibroblasts. A variety of artificial materials such as demineralised bone matrix, coralline hydroxyapatite and calcium phosphate-based ceramics such as hydroxyapatite (HA), β-tricalcium phosphate (β-TCP) and bioactive glass have been used over the decades to fill bone defects almost without associated soft tissue development. Most of them were having only the properties of osteointegration and osteoconduction. Only bioactive glass possesses osteogenic property that stimulates proliferation and differentiation of osteoprogenitor cells and in some cases influencing the fibroblastic properties. But, this material has also some disadvantages such as short-term and low mechanical strength along with decreased fracture resistance; but, this was further minimised by ion doping that positively enhanced new bone formation. There are many metal ions such as magnesium (Mg), strontium (Sr), manganese (Mn), iron (Fe), zinc (Zn), silver (Ag) and some rare earths that have been doped successfully into bioactive glass to enhance their mechanical and biological properties. In some of the cases, mesoporous bioactive glass materials with or without such doping have also been employed (with homogeneous distribution of pores in the size ranging between 2 and 50 nm). These biomaterials can be served as scaffold for bone regeneration with adequate mechanical properties to restore bone defects and facilitate healing process by regeneration of soft tissues as well. This chapter encompasses the use of bioactive glass in bulk and mesoporous form with doped therapeutic ions, their role in bone tissue regeneration, use as delivery of growth factors as well as coating material for orthopaedic implants.

1. Introduction

Bone tissue repair and regeneration have made considerable strides in the modern era. An in-depth perceptive of the underlying principles has been achieved, new methods and materials developed and a multidisciplinary approach was used to accomplish successful bone tissue regeneration. Many scaffold systems have been planned for hard tissue engineering. Novelty has been worked out in terms of scaffold design, material selection, inclusion of drugs and growth factors, mechanical stability and bone regeneration competence. Nevertheless, autografts are still considered as 'gold standard' for bone tissue repair; equivalent osteogenic or osteoinductive performance is not obtained by the synthetic bone graft substitutes. Due to limitations of autografts in sufficient quantities to meet the overall medical demand for orthopaedic implants, allografts and xenografts are alternatives sources to overcome such problems, but are having the risks of disease transmission and immune rejection. As a result, synthetic bone graft substitutes are the rational choice to meet the huge demand for orthopaedic implants, even though its inherent limitations in terms of strength, osteoconduction, osteoinduction, osseointegration and biodegradation. Accordingly, modern research area has been focussed on development of new biomaterials, modification of mechanical and structural features, improvement of biocompatibility, osteoinductivity and to incorporate growth factors and stem cells onto scaffolds to encourage bone regeneration.

Bone tissue regeneration strategies intend to use synthetic temporary templates to assist the natural healing of bone defects. Bone extracellular matrix (ECM) containing collagen fibrous structure, with mineralised calcium phosphate, is secreted from osteoblasts [1, 2]. For effective bone regeneration in non-load-bearing defects require a biomaterial scaffold that might have a three-dimensional (3D) fibrous structure mimicking the ECM [3–5] and can be easily placed into position during surgery. The scaffolds are also required to be biocompatible (should not elicit an inflammatory response nor exhibit immunogenicity or cytotoxicity), bioactive (bond with bone), bioresorbable, allow new bone formation at an acceptable rate, be economical to make and allow easy fabrication into the final preforms [6–8]. The scaffolds must be easily sterilisable to prevent infection especially for bulk degradable scaffolds [9]. Additionally, the mechanical properties of the scaffold must be optimal to prevent structural failure during handling and patient's normal activities. Furthermore, controllable interconnected porosity is of paramount necessity for cells to grow into the scaffold and to support angiogenesis. The scaffolds should also have porosity of 90% with pore diameter of at least 100 μm for proper cell penetration and vascularisation of the ingrown tissue [10–12].

A number of inorganic and organic materials are being used as bone substitutes that include calcium phosphate ceramics, phosphates of magnesium, sulphate, carbonate and silicate of calcium and collagen with positive cell-material interactions. Inert inorganic materials, such as alumina, zirconia, titanium alloy and cobalt-chromium alloy, are also used in hard tissue applications, but lack resorbability and absence of osseointegration at the bone-implant interface. Positive interaction with cells was established using synthetic biodegradable polymers, such as polylactic-co-glycolic acid (PLGA), polycaprolactone (PCL) and polyethylene glycol (PEG) [13, 14]. The degradation products of these materials have no detrimental

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effects in body system. Furthermore, degradation rate, hydrophilicity and mechanical strength can be controlled by changing the chemical composition. Many natural biopolymers are also available and are very suitable bone substitutes in terms of cell-material interactions. Large polymers of very high molecular weight such as chitosan, alginate, cellulose, gelatin, collagen, keratin and hyaluronic acid also exhibit favourable cell-material interactions. Additional biocompatibility to a structurally stable scaffold is the selection criteria for bone substitute materials currently in vogue [15, 16].

In bone tissue engineering, commonly used materials are ceramic and glass due to their superior biocompatibility. Poor mechanical strength and stability are the major deficits rendering them unsuitable as porous scaffolds. In addition, processing defects such as irregularly shaped pores, surface defects and residual stress, all reduce the mechanical strength of the scaffold systems. These limitations compelled the researchers to find out the solutions for the improvement of biological performance of these materials by combinations of various strategies to augment cell-material interactions and stimulation of cells to ensure rapid but controlled bone regeneration. One of the alternate strategies is metallic ion doping for improving biological performance enhancement.

The aim of this chapter is to summarise the recent advancement of metallic ion dopants in addition to bioactive glass scaffold and their studies in orthopaedic surgical challenges. Our discussion broadly covers innovations in materials development and fine tuning together with structural and functional improvisations.

2. Bioactive glass materials

"Bioactive" glass can be defined by its name itself, which include "Bioactive", means *One that elicits a specific biological response at the interface of the material which results in the formation of a bond between the tissues and the material*, and "glass", often defined as *solid that possesses a non-crystalline (that is, amorphous) structure at the atomic scale and that exhibits a glass transition when heated towards the liquid state* [17]. In short, bioactive glass has been designed to elicit a particular biological reaction at the interface of the material, which stimulates cell proliferation, gene response and the formation of a bond between living tissues and the material [17–20]. Its surface develops a biologically active apatite layer (HCA), which initiates bonding with bone. The apatite phase formed chemically and structurally mimics the mineral phase of bone [21]. Among other essential qualities of bioactive glass are that they should be non-mutagenic, non-carcinogenic and non-antigenic so that they do not have any adverse effect on the cells [22]. With these typical properties, bioactive glasses are reported to be capable of more bone regeneration than other bioactive ceramics available. However, in the case of bioactive glass there are many areas to improve as it has not yet reached its true potential.

The invention of bioactive glass was not by accident, in contrary it was being invented through a series of curious set of events. The first bioactive glass as an alternative to nearly inert implant materials was invented by Prof. Larry Hench at the University of Florida in 1969. A US army colonel, returned from Vietnam war, asked him if material could be developed that could

survive the aggressive environment of human body. All available materials at that time, such as metals and polymers, were designed to be bio-inert, which were found to trigger fibrous encapsulation after implantation rather than forming a stable interface or bond with the tissues [23]. The melt-derived bioactive glass invented by professor L. Hench was composed of 46.1 mol% SiO_2, 24.4 mol% Na_2O, 26.9 mol% CaO and 2.6 mol% P_2O_5, later termed as 45S5 and Bioglass®, which forms a bond with bone strong enough so that it could not be removed without breaking the bone [24]. It is now almost 50 years since the discovery of bonding of bioactive glass with living bone and over time many advances have been made in this field, understanding the mechanism of bone bonding, and respectively modifying the properties of bioactive glass by adjustment of the composition [25–28]. The most interesting aspect of bioactive glass is the high adaptiveness to the biological environment and the tuneable properties, by which the rate of bonding with bone can be controlled, thus the fabrication of patient-specific implants is possible. Today, new bioactive glasses can be made specifically for different types of clinical applications, in different forms such as fibres, microspheres and to show required bioactivity at when implanted.

2.1. Synthesis

According to process method used, bioactive glasses can be classified into two different categories: (1) melt derived, (2) sol-gel derived. In these fabrication techniques, melting method is traditional [27, 29–32]; however, the latter appealed the scientists in the last two decades [33, 34]. The synthesis route of bioactive glass has eminent effect on the specific surface area as well as degradability of the material.

2.2. Melt derived

The first bioactive glass itself made by Professor Larry Hench in the 1970s was made through melt-quenched method. The idea behind the invention was to make an implant material which can form a hydroxyapatite (HA) layer on its surface when implanted, which can develop a living bond with the host [35]. As the main aim was to mimic bone and bone contains hydroxyapatite [$Ca_5(PO_4)_3OH$], Ca^+ and PO_4^{3-} were taken as a component of glass. The other main components of glass Si^{4+} and Na^{2+} can also be found in human body. Among the compositions Hench and co-workers made, 45S5 were found to bond with rat femur. The selection of the components of this glass, named as Bioglass®, was ideal. The low silica content compared to the previous soda-lime-silicate glasses forms a layer of silica and amorphous calcium phosphate on the surface of the implant. Since then the research on bioactive glass somehow concentrated mostly compositions similar to 45S5 bioactive glass.

Most of those bioactive glasses were produced by melting raw materials at an elevated temperature because it is a simple, low-cost technique and does not take much time to complete. It typically involves raw materials selection, weighing, mixing of components in appropriate proportion and removal of impurities to get a homogeneous melt. The reactivity of a glass in aqueous solutions is strongly dependent on the composition of the glass and thus the choice of composition is very important. Because the limited range of glass composition shows bioactivity, the glass composition should be chosen in a way so that it can be melted

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and formed into required shapes with available methods. The raw materials can be divided into five different categories according to their role: glass former, flux, modifier, colourant and fining agent. Glass formers are the most important components of glass as they form the matrix of the glass structure. Silica (SiO_2), boric acid (B_2O_3) and phosphoric acid (P_2O_5) are the most common type of glass former normally present in oxide glass. In between these silica is widely used; however, the melting temperature of silica is too high (1600–1725°C) and so different types of flux such as Na_2O and PbO can be used to decrease the melting temperature of the mixture. The addition of flux sometime degrades the properties of glass, which can be overcame by introducing different property modifier or intermediates such as boron, sodium, magnesium, titanium and calcium. Colourants are used to control the colour in the final product. Finally, fining agents such as arsenic, antimony oxides, potassium and sodium nitrates are added to raw materials to remove bubbles from the melt. During melting of the raw materials inside the furnace, they react with each other and carbon dioxide and Water-vapour emission takes place, which causes the formation of bubbles. To raise the bubbles up to the upper surface of the melt, low viscosity is maintained. Batch particle size and their mixing in proper proportion are other factors that provide homogeneity in glass structure. Glass forming is an intermediate stage in between glass melting and annealing. The stages of glass synthesis are illustrated schematically in **Figure 1**.

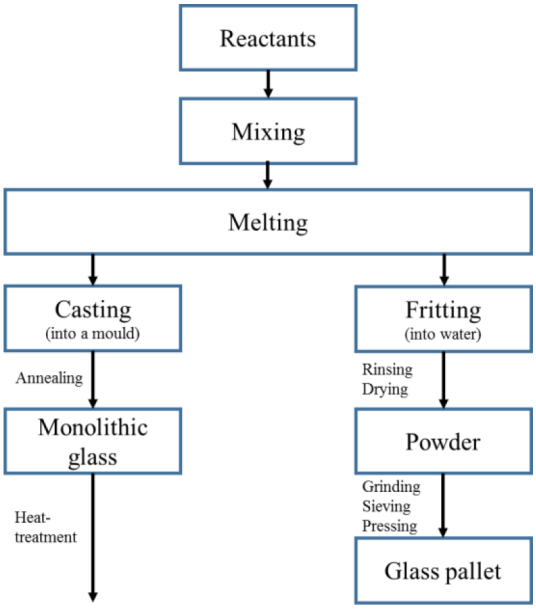

Figure 1. Schematic representation of melt-derived glass synthesis.

Practically appropriate amount (mole/weight fraction) of initial ingredients is mixed, followed by grinding, to break agglomerated particles. In order to obtain more uniform powder, the

mixture of ingredients is ground in ball mill using acetone (water can also be used unless some ingredient is hygroscopic). After drying the mixture in air, the powder can be transferred in platinum crucible and melted in a high-temperature furnace. Generally, around 500°C, the gaseous substances (moisture and gas) come out of the composition. Hence, it is better to calcine the mixture at 500°C for at least 2 h. Before taking out the melt, it must be confirmed that the glass mixture is held at the melting temperature for at least an hour to achieve homogeneous, bubble-free molten materials. Then, the molten glass can be quenched in liquid such as water, liquid nitrogen, etc. Granules of different sizes formed collectively known as frits can be collected and milled to get glass powder. Desirable size and shapes can be made by pouring the molten mixture into moulds of particular shapes. In the case of preparation of glass with particular shape, the poured glass is annealed slightly below the glass transition temperature of the corresponding glass for 12 h in air in pit furnace.

2.2.1. Important factors

Important factors to remember while melting a glass are viscosity, thermal expansion and crystallisation characteristics. Low viscosity helps the melt to be bubble free and homogeneous and also facilitates easy elimination from the platinum pot. It is a crucial factor in determining the best possible procedure for a particular composition. Viscosity values at high temperatures can be linked with melt-forming processes and low-temperature values indicate the suitability of the glass, whether for sintering into porous bodies or coating on metal implants. The approximate viscosity values for a bioactive-glass-forming process are given in **Table 1**.

Processing	**Viscosity (η) (dPa s)**
Melting	$10\text{-}10^2$
Pressing	$10^4\text{-}10^6$
Drawing of continuous fibres	$10^{2.5}\text{-}10^{3.5}$
Sinter glass powder to porous body	$10^8\text{-}10^9$
Annealing	$10^{12}\text{-}10^{13}$

Table 1. Approximate viscosity values (dPa s) for bioactive-glass-forming process.

Bioactive glass coating provides better bone-implant connection when coated on metal prostheses [36–41]. According to the implantation area, lower surface reactivity may be preferred and in such cases glass composition with less bioactivity are favoured. Whatever be the case the thermal expansion of the glass must be compatible with the metal otherwise cracks may appear on the coating leading to peeling off of the coating.

Another important factor is that the melting temperature should be higher than liquidus temperature of the compositions. Recent development of bioactive glasses focuses on the change of chemical composition and different heat treatment condition [42, 43]. Aboud et al. analysed the effect of increasing temperature on the crystallisation behaviour and the phase formation order of different crystals of SiO_2–P_2O_5–Al_2O_3–MgO–Na_2O glasses [44]. The changes

in microstructure, mechanical and chemical properties of this glass with different heat treatment conditions result in an important application in dental restoration [45]. Also, thermal treatments of bioactive glass tend to enable the glass to attain different elastic properties and a range of bioactivity, which could be helpful for making patient-specific implant [46].

2.3. Sol-gel derived

Sol-gel glasses are made by a chemical-based process at much lower temperatures than the traditional processing methods [47–51]. The method has been recently accepted by a number of research groups to make a new generation of bioactive glass and offers assurance for tailoring the composition to match the specific requirements. Recently, scientists have preferred the sol-gel method in order to increase the specific surface area, and thus, the surface reactivity and degradability of the material [52]. It also provides better control over homogeneity and purity [53].

A sol is a colloidal suspension of solid particles (with a diameter of 1–100 nm) in a liquid, where the colloids exhibit *Brownian motion*, a random walk driven by momentum imparted by collisions with molecules of the suspending medium. Gel can be described as a rigid network of covalently bonded silica comprised of interconnected pores [54, 55]. Three methods can be used to make sol-gel materials: gelation of colloidal particles, hypercritical drying or controlled hydrolysis and condensation of metal alkoxide precursors followed by drying at ambient pressure. All the three methods create a three-dimensional, interconnected network. Gels can be categorised into three types, such as alcogels, xerogels and aerogels [53]. Alcogels are generally alcohol based, whereas xerogels are formed from thermal removal of pore liquid. Gels with low density (80 kg m^{-3}) and large pore volumes (up to 98%) are called aerogels, which are the result of removal of pore liquid from the rigid network without collapsing it.

Preparation of gel glasses by a sol-gel method composed of seven steps. First, the alkoxide or organometallic precursors are mixed to form the low-viscosity sol, followed by hydrolysis of liquid alkoxide precursors with de-ionised water [56, 57]. Hydrolysis of silicon alkoxide forms silanol groups [$Si(OH)_4$], eventually interact with each other to make the Si-O-Si bond and increase the viscosity of the sol (**Figure 2**). This is the time where the sol can be applied as a coating, be pulled into fibre, electrospun, impregnated into a composite or formed into powders. During the process of gelation, the viscosity of the solution sharply increases [58]. The gelation time depends upon the concentration of the solvent, nature of the oxide group and the amount of water used for the hydrolysis [59, 60]. While aging of a gel for several hours at 25-80°C, decrease in porosity and increase in the strength can be observed due to polycondensation and reprecipitation of the gel network [61–63]. Aging process also affects the pore volume, surface area and density of the gel. The removal of pore liquid has different effect on arising stress for colloidal gels (pore size > 100 nm) and alkoxide-based gels with pore size 1-10 nm. Colloidal gels can be dried easily; however, in the case of alkoxide-based gels, large capillary stress may arise during drying. Hypercritical drying at elevated temperature and pressure, above the pore-liquid-solid critical point, avoids the solid-liquid interface and eliminates drying stress [17].

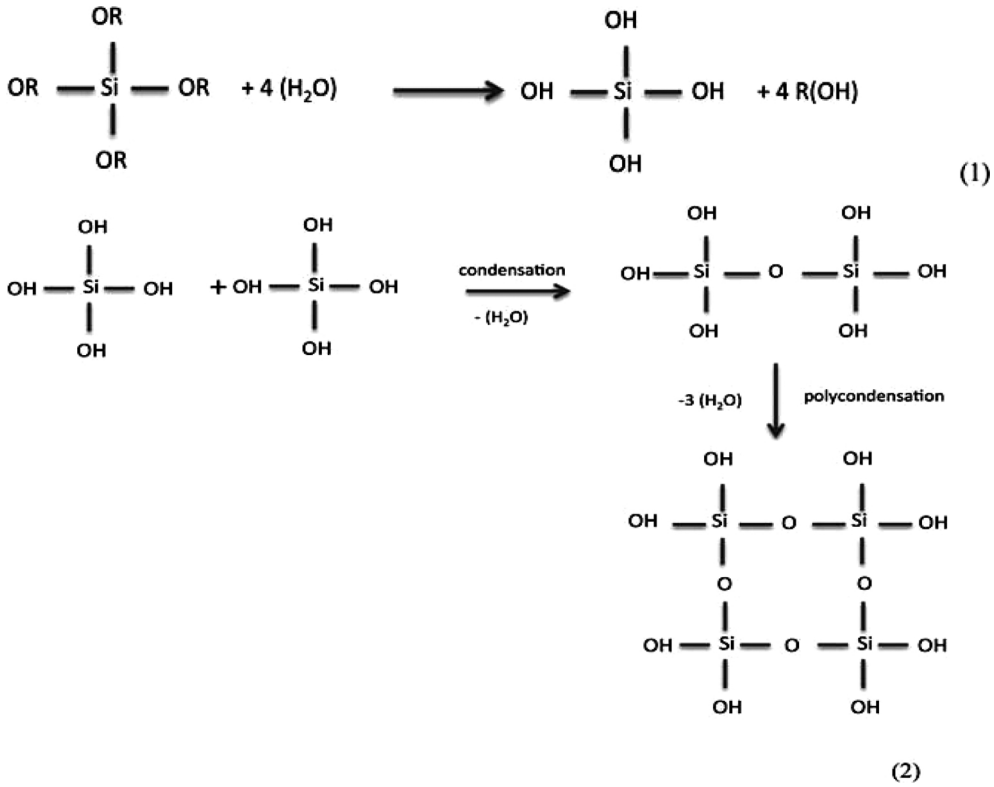

Figure 2. (1) Hydrolysis of $Si(OH)_4$; (2) formation of Si-O-Si bond.

In order to control the stability of the material, chemical stabilisation of the dried gel is required. Sintering of the gel at 500-900°C desorbs silanol groups from the surface and eliminates 3-Si rings from the gel. It also increases the density, strength and hardness of the gel. The sintering temperature of alkoxide-based gels is in the range of 900-1150°C depending upon composition. The schematic diagram of the sol-gel process is provided in **Figure 3**.

The physical differences between the two synthesis routes are that sol-gel glasses tend to have an inherent nanoporosity whereas melt-derived glasses are dense in nature [64]. The surface area of sol-gel glasses is also higher than melt-quenched glass, which results in greater dissolution rate, and hence higher cellular response. The hierarchical pore structure consisting of interconnected macropores (>100 μm) and nanopores is beneficial for interaction and stimulation with cells as it mimics the hierarchical structure of natural tissues. Also bioactive glasses in the form of nanoporous powders or monoliths or as nanoparticles can be made by changing the pH of the sol-gel process [65]. However, the sol-gel made scaffolds have lower strengths than melt-quenched glasses, and thus inappropriate to use in hard tissue engineering (**Figures 4** and **5**).

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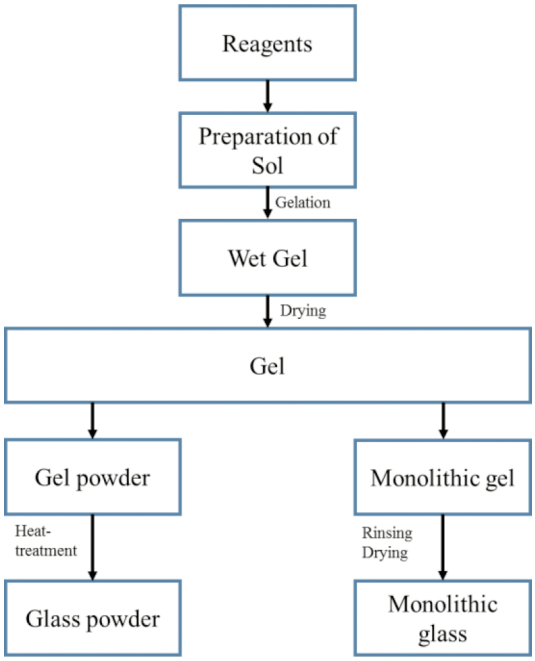

Figure 3. Schematic representation of sol-gel glass synthesis.

2.3.1. Important factor

The physical and chemical properties of sol-gel bioactive glass mainly depend upon silica and so the hydrolysis and condensation of silica plays an important role. The kinetics of hydrolysis and condensation of silica depend upon several factors such as pH, composition, temperature, precursor, catalysis and concentration of ions and the ratio of moles of water/moles of tetraethyl orthosilicate (TEOS). Iler divides the polymerisation of silica in between three pH ranges: <pH 2, pH 2-7 and >pH 7. pH 2 and pH 7 appear to be boundaries because at pH 2 the surface charge (PZC) and the electrical mobility of silica (isoelectric point, IEP) are zero, whereas above pH 7 the solubility and dissolution rates of silica are maximised leading to particle growth without gelation [65].

2.4. Composition of bioactive glasses and their effects on bioactivity

Since the report of bone-bonding properties of bioactive glass, silica has been used as the major component of glass composition and also most widely researched with changing its amount. Silicate glasses comprise an amorphous network structure based on SiO^{4-} tetrahedron, which are linked to each other at the oxygen centres. Silicate glasses have open structure of silica due to the presence of non-bridging oxygen ions attached with silicon. Addition of network modifiers such as Na^{+}, K^{+}, Ca^{+} also causes the opening of silica network structures. These ions

replace bridging oxygens of the network with non-bridging oxygens, hence opening of the glass structure. The number of modifier ion-oxygen bonds and non-bridging oxygen bonds determines several properties of the corresponding glass [66]. Detailed structural features of silicate glasses and their effect on different physical and chemical properties have been reported by various research groups [67–69]. In the case of bioactive silicate (SiO_2 less than 60 wt%) glasses, each silica tetrahedron contains more than 2.6 number of non-bridging oxygen ions, which is necessary in order to be bioactive [70].

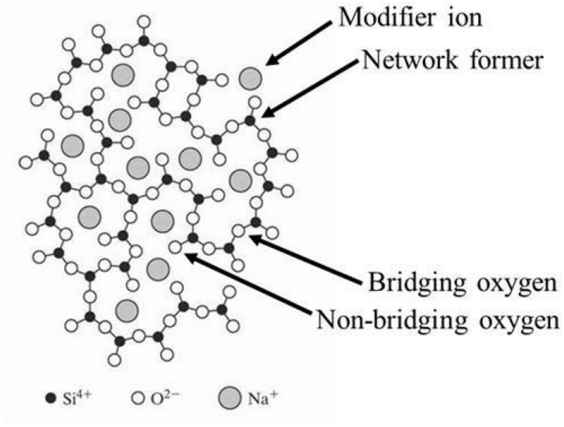

Figure 4. 2D presentation of random glass network modifiers and network formers [70].

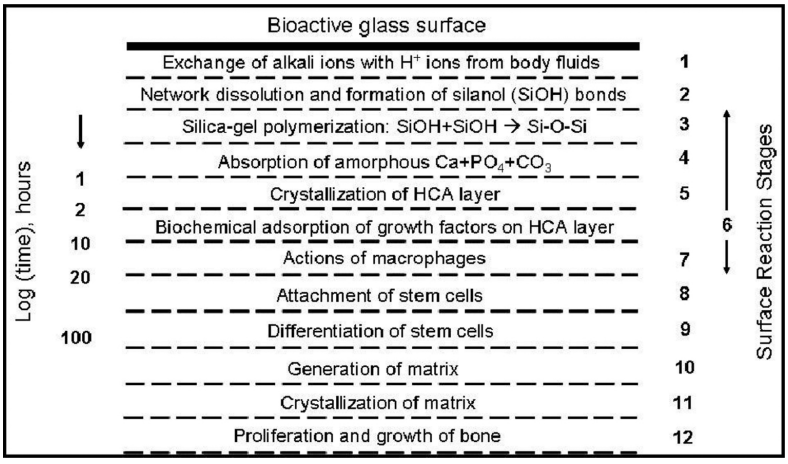

Figure 5. Sequence of interfacial reactions kinetics involved in forming a bond between bone and a bioactive glass [87].

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The composition of bioactive glass is different from the traditional soda-lime-silica gasses that consist more than 65 wt% of silica. Basic components required for a glass to obtain bioactivity are SiO_2, Na_2O, CaO and P_2O_5, which can be distinguished in three main features according to Hench and Anderson [71]; the amount of SiO_2 should be in between 45 and 60 wt%, Na_2O and CaO content must be high and a high CaO/P_2O_5 ratio. Higher content of SiO_2 decrease the dissolution rate of the glass ions from the surface, leading to decrease of bioactivity. Very low content of silica also leads to totally dissolvable monomeric SiO^{4-} units. Silica content also plays an important role to form hydroxyapatite carbonate (HCA) upon contact with physiological fluids, thus leading to the chemical attachment to soft/hard tissues. As a result, the interfacial bonding strength with bone increases, and a stable bond with strength equivalent to or greater then bone forms. High CaO/P_2O_5 ratio tends to enable the release of ions from the surface of the material when soaked in body fluid, forming a surface layer of HCA in a very short time span. It also supports cell proliferation on the surface of the implant by maintaining the ion concentration [35]. Previously, Hench and co-workers assumed that a typical range (2–6 wt%) of P_2O_5 is required for a glass to be bioactive as it aid the formation of calcium phosphate phase on the surface, but later Hench and Andersson observed that bioactivity can be independent of P_2O_5 as phosphate ion is also available in physiological fluids.

In the last two decades, a number of different oxide systems have been studied to understand the effect on glass bioactivity and to increase its mechanical strength, still a complete understanding of the correlation between composition and bioactivity is insufficient but mechanical improvement can be possible. Different partial substitutions in the already approved glass compositions have been made, as CaO by 12.5 wt% CaF_2, SiO_2 by 5-15 wt% B_2O_3, but no significant effects were found. Even fluoride substitution reduced the bone bonding capability of the glass [72]. The substitution of MgO for CaO or K_2O for Na_2O showed slight increase in bioactivity. During 1990s glasses with alumina and boron oxide gained enormous interest. Sadly, the addition of small 3 wt% Al_2O_3 to the 45S5 formula was found to prevent bonding with bone. Andersson proved that substitution by Al_2O_3 (1-1.5 wt%) can reduce the bioactivity of glass because of its carcinogenicity [71]. Osaka et al. and Saranti et al. studied glasses with B_2O_3 content and found that the presence of boron has a positive impact on the bioactivity of the glass [73, 74]. In the case of only B_2O_3-substituted glass, the ratio between B_2O_3 and SiO_2 plays an important role in the rate of formation of calcium phosphate layer on the surface of the implant [75]. Later, de Arenes proposed to control the B_2O_3/Al_2O_3 ratio in B_2O_3 and Al_2O_3 containing glasses in order to show bioactivity [76]. In recent years, researchers tend to play with the composition of glass incorporating the ions that are abundant in human bone, such as Mg, Zn, Cu etc. [77–83]. Xia Li et al. found that by incorporating Mg, Zn or Cu in different amounts in place of Ca^{2+} can affect the bioactivity of the glass to different extent in a sequence of Cu < Mg < Zn [84]. Potassium substitution in place of Na^+ reduces the viscosity of silicate glasses and their susceptibility of crystallisation [85]. Even now, a lot of research is going on to find a relation between the composition of the glasses, which have more than four components and tissue connectivity through phase diagram, but relation between these two factors is yet to come. Some researchers such as Andersson et al. and Brink et al. predicted the *in vivo* reactivity of glasses with six or seven oxides as a function of their composition with phenomenological models suggested by regression analysis [71, 86].

2.5. Surface reaction kinetics

Chemical reactivity of a glass in contact with body fluid holds the key of the bone bonding properties of the glass. Due to the chemical reactions, a layer of hydroxycarbonate apatite forms on the surface to which bone can connect. When immersed in an aqueous solution, such as SBF (simulated body fluid) or PBS (phosphate-buffer solution), three general processes occur: leaching, dissolution and precipitation. Leaching can be characterised as release of ions, generally by exchange of alkali or alkaline earth metals ions with H^+ or H_3O^+ ions of the solution. Glass modifier ions leach very easily from the surface of the glass when immersed in an aqueous solution, as they are not part of the glass network. The ion exchange process leads to increase in the hydroxide ion concentration, i.e., the basicity of the solution increases to pH > 7. Network dissolution occurs simultaneously by breaking of the network forming silica bonds (-Si-O-Si-O-Si-) by the attack of hydroxyl ions (OH^-). It releases silica into the solution in the form of silicic acid ($Si(OH)_4$). In this step, glass composition plays an important role as the rate of silica dissolution depends very much on glass composition. Silica dissolution rate rapidly decreases if the weight percentage of SiO_2 goes beyond 60% because of the increase of bridging oxygen, which can hold the network very strongly. Hydrated silica then undergoes polycondensation with neighbouring silanols to form silica-rich layer. In the precipitation part, calcium and phosphate ions released from the glass together with those from solution to form a calcium-phosphate-rich layer on the glass surface. Slowly, it crystallises to form HCA by incorporating carbonate ions from solution. Generally, there are five reaction stages on the implant side of the interface with a bioactive glass [72].

Stage 1: Leaching and formation of silanols (SiOH).

Stage 2: Loss of soluble silica and formation of silanols.

Stage 3: Polycondensation of silanols to form a hydrated silica gel.

Stage 4: Formation of an amorphous calcium phosphate layer.

Stage 5: Crystallisation of a hydroxycarbonate apatite layer.

Hench et al. have been extensively described the reaction processes [25, 72, 87–89].

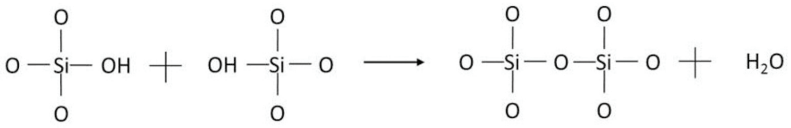
1. Rapid exchange of alkali or alkaline earth metal ions Na^+ or K^+ with H^+ or H_3O^+ from solution

   $Si\text{-}O\text{-}Na^+ + OH \rightarrow Si\text{-}OH^+ + Na^+ (solution) + OH^-$.

2. -Si-O-Si-O-Si- bonds break through the action of hydroxyl ions and form Si-OH (silanols)

   $Si\text{-}O\text{-}Si + H_2O \rightarrow Si\text{-}OH + OH\text{-}Si$.

3. Condensation of Si-OH groups near the glass surface: re-polymerisation of the silica rich layer



4. Migration of Ca^{+} and PO_4^{3-} groups to the surface through the SiO_2-rich layer forming a $CaO\text{-}P_2O_5$-rich film on top of the SiO_2-rich layer, followed by growth of the amorphous $CaO\text{-}P_2O_5$-rich film by incorporation of soluble calcium and phosphate ions from solution.

5. Incorporation of hydrolysis and carbonate from solution and crystallisation of the $CaO\text{-}P_2O_5$ film to HCA.

As these stages were proposed many years ago, they are proved through time by various types of characterisation techniques. ^{17}O nuclear magnetic resonance (NMR) confirmed the increase of bridging oxygen bonds during leaching, which indicates the repolymerisation of Si-OH groups in the silica-rich layer [90]. The formation of crystallise HCA layer on the surface was confirmed by surface-sensitive-small-angle X-ray diffraction (XRD) [91]. Calcium phosphate nucleate on the SI-OH groups as they have negative charge in solution and the separation of the SI-OH groups is thought to dictate the orientation of the apatite crystals, which grow with a preferred orientation in the 001 plane on Bioglass 45S5 [23, 92–95].

2.6. Bioactive glass *in vivo*

The bioactivity of glasses can only be investigated and confirmed after testing with living tissues. If a calcium phosphate layer can be found on a silica gel layer at the surface of the implants, the glass can be called bioactive. The extent of bioactivity of the glass is directly dependent on the ability of the glass to form calcium apatite layer. The above-mentioned five stages on the surface of bioactive glass do not depend on the presence of tissues. The sequence of *in-vivo* reactivity of bioactivity glass with tissues has been investigated by Hench and Andersson [37, 87, 96].

Stage 6: Adsorption of biological moieties in the SiO_2-hydroxycarbonate apatite layer

Stage 7: Action of macrophases

Stage 8: Attachment of stem cells

Stage 9: Differentiation of stem cells

Stage 10: Generation of matrix

Stage 11: Mineralisation of matrix

Through the 11 stages, a bioactive glass bonds with the bone. Gradually, the bioactive glass will be absorbed with increasing bone ingrowth.

45S5 Bioglass® was the first bioactive glass successfully investigated *in vivo* by many researchers [17]. After that another bioactive glass S53P4 was developed by Andersson and Karlsson

and has been successfully used in clinical applications [97–99]. Later, glass 13-93 and glass 1-98 also showed good bioactivity *in vivo* [86, 100–102].

Extensive research in this field in recent years comes out with some limitations of the model of reaction kinetics proposed by Prof. Hench. Hench proposed that in the first stage of the reaction a rapid exchange of Na^+ ions released from the glass with the protons (H^+) of the solution occur, although in the modern era bioactive glass has been synthesised without sodium. Influence of the mole fraction of silica on the bioactivity is still not clear. Also, it was observed that if the implant is broken and the broken surfaces stay in contact with SBF, they tend to self-repair by fusing themselves through their apatite surface layers [103].

In the case of clinical trial, the main problem is to make patient-specific implants because every patient is different. To study the implant specificity and implant site adjustment *in vivo* animal model, studies can be compared if the same models are used. The first *in vivo* study was completed for Bioglass monoliths on the rat femurs, and after 6 weeks the interfacial shear strength of the bond between the glass and the cortical bone was equal or greater than the strength of the host bone [24, 104]. Bioglass 45S5 also degrades more rapidly than hydroxyapatite, and the degeneration was because of solution-mediated dissolution. The model of the study later named as Oonishi model was completed by drilling 6 mm diameter into the femoral condyle of rabbits. Bleeding was stopped before inserting the particles [105–107]. Recently, it was found that initially the bone grew into the particles that were on the outer periphery in contact with the host bone, but within 2 months of implantation bone also formed inside the isolated Bioglass particles. This study indicates that the Bioglass particles can trigger stem cell differentiation and convert it into osteoblasts [108]. Hands-on experience by various surgeons points out the advantage of making a putty-like material by mixing the particles with blood prior to implantation, which later encourages the development of Nova bone [109]. The explanation behind this advantage of putty-like material is either it can separate the particles to allow new bone to grow between them or the pH environment created was more suitable for bone ingrowth. Fujibayashi et al. used the Oonishi model to test phosphate-free glass particles and for one of his compositions almost similar amount of bone ingrowth to Bioglass was found. But with increasing SiO_2 content the bone ingrowth reduce rapidly [110]. Wheeler et al. compared Bioglass 45S5 with sol-gel glasses 77S and 58S using the Oonishi model and observed that up to 8 weeks the bone ingrowth was more in the case of Bioglass, but after 12 weeks the amounts were equivalent. The procedure of bone ingrowth, *viz*. formation of silica layer, apatite formation and finally bone formation via HCA was found to be same as Bioglass [111]. The initial slower rate can be result in the rapid release of calcium in the case of sol-gel glasses causing increase in pH at the site.

2.7. Bioactivity *in vitro*

Before going to *in vivo* trials, a glass material has to be passed *in vitro* tests. The *in vitro* test helps us both ethically and economically as they reduce the number of animals necessary for *in vivo* tests. Earlier *in vitro* test was performed by immersing the glass in either distilled water or tris-buffered solutions, but after development of SBF by Kokubo et al. it has become the most widely used solution for *in vitro* investigation. SBF contains all the essential inorganic

components of human blood, and proportions are also almost similar to human blood plasma [112]. During *in vitro* studies, pH of the solution is buffered between 7.25 and 7.4 at 37°C.

	Na^+	K^+	Mg^{2+}	Ca^{2+}	Cl^-	HCO_3^-	HPO_4^{2-}	SO_4^{2-}
Plasma	142.0	5.0	1.5	2.5	103.0	27.0	1.0	0.5
SBF	142.0	5.0	1.5	2.5	147.8	4.2	1.0	0.5

Table 2. Ion concentrations of SBF and human blood plasma (mM) [112].

SBF is a supersaturated solution and hence precipitation of calcium phosphate can easily take place during preparation, storage and *in vitro* test. Many researchers have tried to correct the difference of ion concentrations of Cl^- and HCO_3^-. Oyane et al. made a revised SBF (r-SBF) in which the concentrations were matched, but the solution shows a strong tendency to precipitate calcium carbonate [113]. In 2004, Takadama proposed a modified SBF (n-SBF) in which only CL^- ion concentration was increased [114]. Several properties of bioactive glasses have been studied in SBF by observing the changes in weight and surface morphology of the glass and also observing the change of pH and ionic concentrations of the solution. Some research groups focussed on the physical and mechanical properties whereas some groups are interested in knowing chemical and bioactive properties of glass [115–118]. It was observed that the extent of bone ingrowth among glass particles increased according to their ability to form apatite in SBF. Thus, it can be said that the *in vivo* bioactivity of a glass can be assumed precisely from its nature in SBF.

Five typical reaction stages, as described in surface reaction kinetics part, occur when *in vitro* bioactivity test is performed. Initially, due to ion exchange of alkali or alkaline earth metal ions with H^+ ions of the SBF solution, pH of the solution increases. By the action of OH^- ions network, dissolution occurs with the formation of $Si(OH)_4$. The dependency of dissolution rate is more or less same as described before. The leaching and dissolution phenomenon is followed by a formation of silica-rich layer on the surface by polycondensation of neighbouring silanols, which ultimately form a calcium-phosphate-rich layer by incorporating Ca^+ and PO_4^{3-} ions. The layer increases by including soluble calcium and phosphates from the SBF, forming an amorphous CaP-rich layer. Finally, the CaP-rich layer crystallises to a hydroxycarbonate apatite structure.

With changes in composition, differences in sample dosage, shape and size, sample porosity and surface morphology also affect the bioactivity of a glass [117, 119–123]. Most studies of bioactive glasses have used samples in the form of discs or plates, however in accordance with their applications other forms are also of interest.

2.8. Mesoporous bioactive glass (MBG)

For treatment of bone defects resulting from trauma, infections, tumours or genetic malformations, bioactive glass scaffolds have been extensively studied. In the case of bone regeneration, combination of osteoconductive, osteostimulative and angiogenic factors with bioactive

glass are proved to be useful [124–126]. This advantage of bioactive glass made it a subject of interest for almost 50 years and day by day according to rise in life expectancy, the field of its application is increasing. Extensive research in this topic comes out with a handful of modifications for the last two to three decades. Recently, it was found that kinetic deposition process of HCA on bioactive glass can be enhanced by increasing the surface area and pore volume [127]. Therefore, control over porosity, pore size and internal pore connectivity of bioactive glasses is essential to understand and design better bone forming biomaterials. A new field of application was started when surgeons found that in the case of bone reconstruction surgery, bacterial infection may cause osteomyelitis. Traditionally, techniques such as systemic antibiotic administration, surgical debridement, wound drainage and implant removal have limitations and may lead to additional surgical interventions for the patients [128]. Conventional drug delivery options, such as injection or taking a pill, increase the concentration of drug in blood up to peaks and then suddenly decline [129]. Hence, to improve drug delivery efficacy, continuous action, reduce toxicity and convenience to patients a lot of work has done. In addition, the procedure was also considered for treating malignant bone disease in which drug will be effectively released at the sites of bone disease from loaded biomaterials [130, 131]. Since the invention of first bioactive glass, in the last 40 years it has shown various attractive properties for bone tissue regeneration application by virtue of their osteoconductivity and degradability [124, 132, 133]. In 2004, Yu et al. for the first time prepared mesoporous bioactive glasses (MBG) by the sol-gel method using surfactants, which opened a new direction in the field of regenerative medicine [134]. The materials were composed of highly ordered mesopore channel structure with a pore size ranging from 5 to 20 nm. MBG has gained the interest of researchers very rapidly for its drug loading and release properties, which depend on the mesoporous structure of the materials. Due to its tuneable pore size, large specific surface area and pore volume, the materials can be used in bone-forming activity and can be loaded with osteogenic or therapeutic agents [125, 126, 128, 131, 135, 136].

2.8.1. Preparation of different types of mesoporous bioactive glasses and their in vitro bioactivity

Mesoporous bioactive glasses were emerged when the supramolecular chemistry of surfactants was incorporated into the bioactive glasses field. These materials have the composition of bioactive glasses but with designed mesoporosity and textural parameters. MBGs are generally prepared by combining non-ionic surfactants (triblock copolymers, CTAB, P123, F127, PEO, PU, etc.) into the reaction system, which are essential for obtaining well-ordered structures [134, 137]. The most well-known and accepted procedure of making mesoporous bioactive glass is evaporation-induced self-assembly (EISA) method [138]. The initial homogeneous mixture is obtained by dissolving precursors in a common medium such as ethanol-water mixed solvent system. The surfactants can act as micelles and are able to link with the hydrolysed precursors (e.g., TEOS and TEP) to form an ordered mesophase, where a constant ratio of network former and precursors and the surfactant was kept [139]. After that, following the process of sol-gel, gelling and drying takes place, and by the removal of surfactant through calcination finally gives MBG with a well-ordered mesoporous structure. The order of porosity of the material depends on surfactant chemistry (ionic, non-ionic, polymeric, etc.), surfactant concentration, organic/inorganic phase volume ratio, temperature and pH of the sol.

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Recent studies on mesoporous bioactive glass show increasing use of MBG in different fields of tissue engineering and drug delivery. The types of MBG used in these fields may be particle, sphere, fibres or 3D scaffolds. The first MBG powders or particles were prepared by using P123 and F127 as a surfactant, with the composition of 80Si-15Ca-5P, 70Si-25Ca-5P and 60Si-35Ca-5P. Calcination at 700°C gives a highly ordered MBG powder. The bioactive characteristics of a scaffold can be assumed from their ability to form apatite layer on their surface *in vitro*. Zhu and Kaskel reported that the rate of apatite formation in the case of MBG is noticeably higher than its contemporary bioactive glass scaffolds [140]. Other than the mesoporous structure, the chemical composition of the mesoporous bioactive glass is the other factor to influence *in vitro* bioactivity. Now a days, scientists are focussing on modifying the basic properties of MBGs, which are high specific surface area, porosity etc. and found that upon changing these properties the apatite-formation ability of MBG could be fine-tuned [49, 141–143]. Lei et al. prepared MBG microspheres through the sol-gel process with uniform diameter range of 2-5 μm and a mesoporous shell [144]. Zhao et al. prepared MBG microspheres with high P_2O_5 contents (up to 15%) and studied the apatite formation *in vitro* [145]. Studies indicate that the diameter of the microspheres has a positive effect on the bioactivity. Moreover, MBG microspheres with higher P_2O_5 content were found to be more bioactive due to their different ion diffusion rates from the glass network. MBG can also be prepared as ultrathin fibres by electro-spinning techniques with high matrix homogeneities. By controlling the parameters of electro-spinning, the properties of the fibres such as pore volume, surface area and diameter of the hollow core can be tuned. These fibres were found to be highly bioactive when tested *in vitro* [146, 147].

2.9. Ion-doped bioactive glass with and without mesoporosity

2.9.1. Introduction

The clinical demand of bioactive glass is increasing rapidly day by day due to its versatile properties *viz*. bioactivity, resorbability, ostioproductive, osteoconductive and osteoinductive nature, depending upon its flexible compositional range. With increasing population, the diversity of required implants is also expanding. The wide range of application of bioactive glasses include implants for bone defects, repairing or replacing damaged diseased tissues, scaffolds for bone grafting, preparing bone cement, as novel drug carrier and coating material for implants [26, 37]. When implanted in human body, a hydroxyapatite carbonate layer forms on the implant-bone interface which is chemically and structurally similar to the mineral phase of human bone. In the last two decades, researchers found that the sites of implantation of different parts of our body require different chemical and physical properties, and hence bioactive glass with different or modified compositions. Bioactivity of a glass is mainly dependent on its surface reactivity and composition and by modifying those, improvement of the system can be possible. Sometimes modification also needed in order to overcome the disadvantages of traditional bioactive glasses such as high solubility and low fracture toughness.

Recent trends in literature suggest that ionic dissolution products from inorganic materials are keys to understand and assume the behaviour of bioactive glasses *in vitro* and *in vivo*. Since many trace elements such as Sr, Cu, Zn, Mg or Co present in the human body are known for their anabolic effects in bone metabolism, in order to mimic the natural system new approaches for enhancing bioactivity, beneficial and appropriate ions are being introduced [148–151]. It is believed that more similar system such as the host body will increase the bioactivity of the implant. The release of these ions after exposure to a physiological environment tends to improve the bioactive activities of the implant related to both osteogenesis and angiogenesis. Thus, recent trend is to incorporate different ions into the composition of bioactive glasses to enhance their physical characteristics and therapeutic benefit.

This incorporation of different ions in the composition of glass is called doping and it is very crucial for production of functional materials. By definition, a doping element is an additional incorporation in the main composition at a very low concentration compared to the main constituents ranging from a few ppm to a few percent. In many cases, it was found that the functionality of the material is directly dependent on the doping elements. In some other cases, doping may improve surface structure of the implant or the physical attributes of it. In particular, the points related to doping can be listed as follows [152]:

1. The functionality is directly associated with doping.
2. Doping provides a structural control over the material.
3. Doping provokes unexpected structural modifications.
4. Doping brings new unexpected functionality to the material.

It is hard to identify the particular time when doping was first started, but around late 1985 the trend of incorporating different ions were started. First, a number of different ions such as Al, Ag, Fe, Ni, Cr, Cu, Co, Ta, Sb, La, etc. were doped and then tested *in vitro* and *in vivo* [153]. Initially, the dopants were chosen according to their similarity in valence with the elements already present, but with time and following the literature about the essential trace elements required in our body, the interest about dopants has been focussed on some specific elements and their affects [149, 150].

2.9.2. Role of inorganic ions present in human body

Human bone is a highly vascularised tissue which can remodel throughout the life by regulated activity of osteoblasts (bone-forming cells) and osteoclasts (bone-resorbing cells) [154]. The process of bone remodelling is dependent on a variety of local regulatory agents such as growth factors, hormones, etc. [155]. Inorganic ions such as calcium [156–158], phosphorous [159], silicon [160, 161], strontium [162–164], zinc [165], boron [166] and magnesium [167] are also affect the bone metabolism. The acts of the inorganic ions in this context are given in **Table 3**.

Ion	Biological activity	Reference
Si	• Metabolic processes, formation of bone tissue	[160, 168]
	• Intake of Si increase bone mineral density	[169] [170]
	• HAP precipitation	[161]
	• Help to stimulate collagen I formation and osteoblastic differentiation	
Ca	• Favours osteoblast proliferation, differentiation and mineralisation	[156]
	• Activates Ca-sensing receptors in osteoblast cells	[155]
P	• Matrix gla protein (MGP) stimulation	[159]
Zn	• Shows anti-inflammatory effect	[171]
	• Bone formation *in vitro* by activation of protein synthesis in osteoblasts	[172]
	• Increase ATP's activity	
Mg	• Help to form new bone	[173]
	• Increase bone-cell adhesion and stability	[174]
Sr	• Beneficial effects on bone formation *in vivo*	[155]
	• For treating osteoporosis	[175]
Cu	• Promote synergic stimulating effects on angiogenesis when associated with angiogenic growth factor FGF-2	[176] [177]
	• Stimulates proliferation of human endothelial cells	
B	• Stimulates RNA synthesis in fibroblast cells	[178]
	• Stimulates bone formation	[179]
Li	• treatment of both bipolar and unipolar depressive disorder	[180, 181]
	• effects on blood and brain	[182]
	• enhance immunological activities of monocytes and lymphocytes	

Table 3. Acts of different inorganic ions in human body.

By acting as an enzyme cofactors, metal ions influence signalling pathways and stimulate tissue formation [150, 183]. These effects make metal ions interesting for use as doping materials in the field of hard and soft tissue engineering. Several ions, such as Sr, Zn, Cu, Mg, B, etc. have been considered to be promising in enhancing the bioactivity of implant materials by controlling the release of specific ions during *in vivo* dissolution.

2.9.3. Ion-doped bioactive silicate-based glasses

In order to improve the bioactivity, stimulating effects on osteogenesis, angiogenesis and antibacterial effects of bioactive glasses in a specific physiological environment, many methods

have been studied incorporating various metal ions in the silicate network. Different substituted silicate glasses exhibit a certain level of acellular bioactivity when tested *in vitro* by standard SBF test, according to Kokubo et al. [21]. The formation of HCA layer on the surface has been the unit of bioactivity measurement as from these results one can assume the bioactivity *in vivo*.

2.9.3.1. Zinc-bioactive glass

Zinc is an essential trace element in our body as it is a cofactor for many enzymes. It also helps to stimulate protein synthesis which is essential for DNA replication and also has an important role in the growth, development and differentiation of bone cell [184–187]. In addition, zinc also has antibacterial properties against *Staphylococcus aureus* [188].

Balamurugan et al. synthesised a bioactive glass in CaO-P_2O_5-SiO_2-ZnO system by the sol-gel method containing 5 mol% ZnO which increased ALP activity and osteoblast proliferation [189]. They also examined that incorporation of zinc does not reduce the bioactivity of the bioactive glass. Higher surface area of Zn-substituted glass can be a better nucleation site when immersed in SBF solution making the calcium phosphate phase more crystalline [190, 191]. Recently, Atkinson et al. found that up to 5 mol% of zinc substitution in a sodium-free bioactive glass composition has the ability to induce apatite formation alongside a calcite phase. Increase in Zn content has a tendency to decrease the calcite phase, however it does not affect the apatite deposition [187]. This calcite phase can also bond with bone without the formation of an appetite layer [192]. Du et al. observed that initially Zn retarded the nucleation of HCA at the early stage of SBF soaking, but did not affect the HCA formation in long-term immersion [193]. Scientists have also reported that more than 10 mol% of Zn has a negative effect on bioactivity and after 20% an excessive drop can be seen [194]. ZnO can act as a network modifier or an intermediate oxide or both in the glass structure. It is found that up to a certain amount ZnO works as a network modifier, but with increasing ZnO content it switched from network modifier to an intermediate oxide [191]. Shahrabi et al. found that 5 mol% ZnO may reduce the number of non-bridging oxygen atoms, resulting in a decrease in glass bioactivity [195]. Zinc has the ability to remove cations from silica network and the new bond formed (Si-O-Zn) have considerably lower bond strength than Si-O-Si bond, which leads to decline in glass transition temperature. As observed, zinc can show very good antibacterial activity for the *Bacillus subtilis* and *Pseudomonas aeruginosa* strains [187].

2.9.3.2. Strontium-bioactive glass

Strontium (Sr) is a naturally occurring mineral found in water and food. It is also an essential trace element of human body. The total amount of Sr in human body of a 70 kg standard man is around 0.32 g. Recently, researchers have found that Sr positively affects bone metabolism to promote bone formation and osteoblast replication while inhibiting bone resorption by osteoclasts [196]. Evidence also showed that strontium not only enhances osteogenic differentiation, but also helps to stabilise the bone structure [197]. However, too much Sr may

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increase the number of osteoclast cells which can inhibit bone regeneration and remodelling, leading to osteonecrosis. Thus, strontium has very good effects up to an optimum level. Among the trace elements human body have, only Sr was correlated with bone compression strength [198]. *In vitro* and *in vivo* studies showed that strontium ions upregulate osteoblasts and downregulate osteoclasts [175, 199]. The presence of Sr on the surface of a biomaterial decreases the rate of ion-release at the defect site, which is therapeutically beneficial [200]. Sr-substituted boron glasses show a good adhesion with osteoblast-like cells, Saos-2, thus enhances the cyto-compatibility of borate glass. Lao et al. confirmed that Sr-doped bioactive glasses are more bioactive *in vitro* than their original counterparts. Sr-doped glasses are also able to increase the rate of bone-like apatite layer formation on their surface. Moreover, it also decreases the Ca/P ratio very rapidly, which leads to faster stability of apatite layer, and hence greater bioactivity [201]. Substitution of 5 wt% strontium in place of calcium shows advantageous effect on foetal mouse calvarial bone cells [202].

Strontium-based bioactive glasses has a tendency to increase metabolic activity in osteoblasts and to decrease osteoclast activity. The decrease of osteoclasts is may be caused by decreasing tartrate resistant acid phosphate activity and inhibiting resorption of calcium phosphate films [203]. In some cases, it was found that substitution of Sr in place of Ca is more effective strategy for building materials suitable for bone regeneration therapies [203]. The substitution of Ca by Sr (in mol%) sometimes increases silica content as Sr is heavier than Ca, which results in reduced solubility and hence bioactivity. Though replacing by wt% sometimes increases the rate of HCA formation [201, 204]. In comparison, Sr is slightly larger than Ca, which expands the silica network and increases ion dissolution rates, leading to significantly increased *in vitro* and *in vivo* reactivity. The *in vivo* bioactivity is greater in the case of Sr-doped bioactive glasses due to the biological effects of Sr on bone-forming cells [205].

In corporation of mesoporosity in bioactive glass was found to enhance bone-forming ability, degradation and drug delivery properties compared with traditional bioactive glasses. Therefore, there has been a growing interest on ion-doped mesoporous bioactive glasses and their properties. Zhang et al. found that Sr-MBG shows very good mechanical stability from the viewpoint of its original counterpart, which is required for bone repair [206]. They also observed good apatite forming ability of the Sr-doped MBG. Further study of Sr-MBG scaffolds showed that substitution of Sr for Ca stimulated the proliferation, ALP activity, osteogenic-related gene expression and ECM mineralisation of MC3T3-E1 cells [206].

Zhao et al. tested Sr-MBG scaffold in restoration of the rat critical-sized calvarial defects model and found that Sr-MBG scaffolds have superior osteoconductive property in course to normal MBG scaffolds. Moreover, it was found that Sr-MBG scaffolds has a tendency to stimulate new blood vessel formation in bone defect areas [207]. Very recently, Sriranganathan et al. reported that with increase of the Sr substitution for Ca in high phosphate bioactive glasses decreases the formation of apatite layer directly. They proposed that the apatite formation proceeds via the formation of an octacalcium phosphate ($Ca_8(PO_4)_6H_2{\cdot}5H_2O$) phase, which then transforms into hydroxyl-carbonate apatite. Above a certain concentration of strontium, the octacalcium phosphate phase is unable to form, which ultimately delays the HCA formation [208].

2.9.3.3. Lithium-bioactive glass

Lithium has a prolonged medical history as it has been used for over 100 years to treat manic depression [180]. Lithium also marked its importance in the treatment of both bipolar and unipolar depressive disorders. Along with that lithium also has several other effects on blood and brain [181]. Clinicians also observed that lithium often increases the white blood cell counts (granulocytosis) and reduces blood lymphosite counts (lymphomenia). Lithium also has a tendency to enhance immunological activities of monocytes and lymphocytes. Researchers have also found evidence of lithium in bone mineral metabolism [182, 209, 210].

In vitro bioactivity test indicates a decrease in bioactivity with increase in lithium-ion concentration. The theory behind it is that lithium forms lithium oxide groups by reacting with the hydroxyl groups present in the pure sol-gel, which limits crystal formation. Recently, Khorami et al. observed the *in vitro* bioactivity of lithium substituted 45S5 glasses and found no certain advantage of lithium in the reactivity of the bioactive glass composition. A theory based on observations state that *in vitro* reactivity increases with increasing glass solubility. In this study, lithium was replaced for sodium (in wt%) and hence a little decrease in the molar concentration of glass network formers (SiO_2 and P_2O_5) takes place, which may result in an increase in glass solubility. However, the ionic radius of Li^+ is lower than Na^+. Thus, lithium has a strong affinity for bonding to oxygen and tends to contract the free spaces in the silicate network. This phenomenon reduces the rate of glass dissolution and improves chemical durability [211].

The release of lithium ions in SBF is higher for sample with higher lithium content, with an initial burst in the first 24 h followed by more sustained release. Lithium also shows ALP activity and mineralisation in a dose-dependent manner from 0.2 to 0.85 ppm when exposed to murine osteoblast cells [212].

2.9.3.4. Magnesium-bioactive glass

Magnesium naturally exists in human body and it is amongst the most important elements in the bone matrix. Enamel, dentin and bone contain 0.44, 1.23 and 0.72 wt% magnesium, respectively [213]. Magnesium is involved in over 300 chemical reactions inside human body. It is also known to activate phagocytosis and regulate active calcium transport. Magnesium also has positive effect in wound healing, bone metabolism, fracture prevention and bone density [214, 215].

When doped, Mg can act as a network former or network modifier. This indicates that an increase in Mg content may lead to more Mg^{2+} ions participating in the silica network by making weaker Si-O-Mg bond rather than stronger Si-O-Si bonds, leading to weakening of overall glass network [216]. With increasing MgO content glass degradation gradually decreases, and the formation of apatite layer is hampered [213, 217].

MgO can affect the surface reactivity of Mg-doped bioactive glasses by indirectly influencing the early stage of mineralisation by favouring the silica atom with non-bridging oxygen speciation [116]. Surface reactivity of Mg-BG increases with increasing MgO/CaO ratio, which can play an important role in glass bioactivity. Based on another study, it was found that the

role of Mg^{2+} in the formation of HCA apatite layer in SiO_2-CaO-Na_2O-P_2O_5 system was insignificant. These contradictory observations created a variety of theories based on ionic potential [218], structural parameter [66] or network connectivity [219]. However, all these theories failed to explain glass bioactivity properly. Varanasi et al. observed significant effect of MgO on the osteoblast differentiation [220]. Other studies also support the increased osteoblast proliferation and differentiation. These findings proved the positive effect of magnesium doping in the bioactivity of bioactive glass.

2.9.3.5. Silver-bioactive glass

In bone reconstruction surgeries, there are two main factors that should be considered: (1) chemical bond with living bone; (2) prevents bacterial infection. As we know that bioactive glasses show well bioactivity and bond with living bone, but a colonisation of bacteria on the surface of the implant can lead to failure of the treatment. The consequences of implant infections are serious and sometimes it leads to second surgery with a lot of suffering [221].

Due to the antimicrobial properties of silver, the recent focus on development of silver-doped implants is increasing. The antibacterial properties of bioactive glasses containing silver have been investigated by several researchers [222, 223]. The main advantage of incorporating silver ions in a gel-glass system is that the porous glass matrix enables a controlled, sustained delivery of antibacterial agent. Some researchers found that high concentration (2 wt%) of silver ions show cytotoxicity, but in the range of 0.75-1 wt% silver has no toxic influence [224]. Due to the higher efficacy of silver, it has gained the interest of scientists, and after extensive research different mechanisms have been proposed for its antimicrobial activities:

1. Interface with electron transport.

2. Binding to DNA.

3. Interaction with the cell components [225, 226].

Silver incorporation has no significant effect over the bioactivity of the glass [222]. However, silver has a tendency to reduce the dissolution of silica when replaced in place of calcium. As silver is monovalent in comparison with bivalent calcium ion, it takes two silver ions to make two non-bridging oxygen groups in place of one calcium ion. Thus, replacement of calcium by silver lessens the number of non-bridging oxygen groups, and reduces the glass dissolution [191]. Due to its highly promising antibacterial and anti-inflammatory properties, silver-doped bioactive glasses are considered to be very useful for wound healing applications alongside tissue engineering.

3. Clinical relevance of doped bioactive glass

Bioactive glasses are that bone substitutes which posses3. Clinical relevance of doped bioactive glasss the unique property of osteoconduction as well as osteoproduction by stimulating proliferation and differentiation of osteoprogenitor cells through a direct genetic control [24,

227]. The discovery of these new materials led Hench and Wilson to propose the concept of osteostimulation or osteopromotion to define this class of bioactive materials and their effects on the genetic activation of bone cells [228]. Bioactive glasses are surface reactive biomaterials that, when in contact with physiological fluids, release soluble ionic products that have been suggested to stimulate *in vitro* osteogenesis [227, 229]. On critical analysis, Young's modulus of bioactive glass being between 30 and 50 GPa, nearly that of natural bone, is a great advantage. One disadvantage is the low mechanical strength and decreased fracture resistance [230]. This can be easily overcome by altering the composition, using it in low load-bearing areas, and using it for the bioactive stage. Furthermore, bioactive glass manufactured via the sol-gel technique permits the synthesis of material with higher purity and homogeneity at low temperatures [52]. Additives can be easily introduced during the sol-gel process to improve the bioactivity of such glasses. Indeed, improvement of the biological properties of bioactive materials can be achieved by the incorporation of ions (doping) that positively affect osteoblast behaviour and consequently enhance new bone formation [202].

In addition, *in vivo* studies have demonstrated beneficial results from their use in various clinical situations [231–234]. After implantation, interaction with surrounding tissues results in a time-dependent alteration of the material's surface and the formation of a hydroxyl carbonate apatite layer that is very similar to the mineral phase of bone [235]. More recently, a new category of sol-gel glasses has been manufactured with enhanced bioactivity and open pores enclosed in a mesoporous matrix [134, 236]. Furthermore, bioactive glass manufactured via the sol-gel technique permits the synthesis of materials with higher purity and homogeneity at low temperatures [52]. Additives can be easily introduced during the sol-gel process to improve the bioactivity of such glasses. Indeed, improvement of the biological properties of bioactive materials can be achieved by the incorporation of ions that positively affect osteoblast behaviour and consequently enhance *de novo* bone formation.

Metallic ions in body play a crucial role as cofactors of enzymes and excite a chain of reactions related to cell signalling pathways [176]. A number of literatures have been cited on the interaction of metallic ions in various diseases and metabolic disorders such as cancer, CNS disorders, infectious diseases and hormonal disorders [237, 238]. Hence, the effectiveness and selectivity of the beneficial effect of metallic ions can be enhanced by controlling the exact level in the body. Additionally, due to unstable ionic states of certain metallic ions, toxic effects may follow while directly ingested. Hence, wide spread research is underway to develop matrices to control the local release of metallic ions with less systemic toxicity as well as availability of relatively high concentrations of metallic-ion-based drugs to target tissues. The degree of metallic ion loading into matrices for local delivery as well as their controlled and sustained release is of paramount importance for optimal therapeutic use. Common strategy is to load metallic ions into matrices such as hydroxyapatite, bioactive glass, silica and carbon fibres to improve ionic stability and to release for a prolong period of time [148, 239–248]. Due to these superior characteristics, metallic ion doping in biomaterials is an alternative, cost-effective, safe strategy than use of recombinant proteins or genetic engineering approaches [249].

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3.1. Doped bioactive glass in bone regeneration

In bone tissue engineering, bioceramics or bioactive glasses and biodegradable polymers [15], often comprise metallic ions as part of the bioceramic or bioactive glass structural composition. The metal ion is generally released during their degradation *in vitro* or *in vivo* [148, 250]. For instance, when bioactive glass (e.g., 45S5 Bioglass) [26, 251] is used as scaffolds to fill a bone defect, critical concentrations of soluble Si, Ca, P and Na ions are released, with the capacity to generate both intracellular and extracellular effects at the interface between the glass and the cellular environment [124, 133, 148, 227, 252–261]. It has also been observed that released ions from bioactive glasses can induce gene expression which in turn helps in bone metabolism by signal transduction as well as enhance cell differentiation and osteogenesis [27, 124, 227, 254]. Furthermore, the ionic dissolution products of bioactive glasses can also encourage angiogenesis [262]. Other metallic ions which may have significant role in bone tissue engineering include copper, magnesium, strontium, manganese, iron, zinc and silver owing to their imminent role as cofactors in metabolic processes in bone, articular tissues and immune system functions [149, 263].

The application of chitosan-doped bioactive glass (BG-CH) was assessed in the guided bone regeneration in ovariectomised rats. The histomorphometric analysis showed increased bone/tissue volume, osteoblast number and osteoblast surface/bone surface and trace elements such as Sr and Fe were detected in the newly formed bone may be responsible for enhanced bone healing and found clinically useful as a therapeutic and implantable material [264].

Zinc being a trace mineral in human body performs a variety of functions in relation to the immune system, cell division, fertility and the body growth and maintenance. Moreover, zinc is also a necessary element for the formation, mineralisation, development and maintenance of healthy bones. These unique properties of zinc evoked the interest of researchers to use it along with silicate-based bioactive glasses for bone tissue engineering and found to have significant ability to enhance antibacterial effects, bioactivity and distinct physical, structural and mechanical properties of bioactive glasses [265]. Zinc also stimulates bone formation and mineralisation by activating aminoacyl-tRNA synthetase in osteoblastic cells, and it stimulates cellular protein synthesis. Zinc plays a role in the preservation of bone mass by inhibiting osteoclast-like cell formation from marrow cells [171]. It also promotes attachment, proliferation of osteoblast and increase ALP expression that is responsible for laying down the bone callus. The doping of Zn into bioactive glasses produces higher chemical stability and densification of glasses matrices. Zinc doping in bioglass for repair of diaphyseal defect creates a good link of interface between bone and Zn-BG during the first speeds, whereas during the last speeds osseoingration, resorption and degradation of bioactive glass and their replacement by bone cells occurs [266].

Strontium (Sr) is a naturally occurring trace element often acts similarly to Ca in the human body; both have strong bone-seeking properties, and Sr can be substituted with Ca in the apatitic phase of bone mineral [267]. Administration of Sr in moderate doses prevented caries in rats [268]. Among the trace metals present in human bone, Sr was the only that was correlated with bone compression strength [198]. Furthermore, over the past few years, Sr has attracted attention through its beneficial effects on bone healing. Indeed, both *in vitro* and *in*

vivo studies have demonstrated stimulatory effects of Sr on osteoblasts and an inhibitory effect on osteoclasts, associated with an increase in bone density and resistance [199, 269–271]. Nowadays, strontium ranelate is used as a commercial antiosteoporotic oral drug that has been proven to reduce the incidence of fractures in osteoporotic patients [196, 272]. Addition of strontium-substituted bioactive glass (SrBG) into PCL and fabricating into 3D bioactive composite scaffolds utilising additive manufacturing technology yield higher compressive Young's modulus [273]. Oxidative stress, a pivotal pathological factor inducing bone osteoporosis, can also be reduced by Zn doping of bioglass in overiectomised Wistar rats as Zn significantly enhances superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx) and the Ca/P ratio whereas decreases thiobarbituric acid-reactive substances and thus improves bone mineralisation [274]. The study on effects of the substitution of calcium oxide with Sr on bioactive glass also shows promotion of osteogenesis in a differentiating bone cell culture model using mesenchymal stromal cells obtained from rat bone marrow and proved to be potential for bone tissue regeneration [275]. Sr-doped bioglass implant enhances bone regeneration in patients suffering from osteoporosis [276]. The growing evidence of the beneficial effects of strontium on bone justifies the increasing interest in Sr incorporation into biomaterials for hard tissue repair. Thus, strontium-doped bioactive glasses have been recently developed via a sol-gel method that enables a better control of the reaction kinetics [201, 277].

A multifunctional bioactive scaffold should combine angiogenesis capacity, and osteostimulation, for regenerating lost bone tissues. To achieve these objectives when copper (Cu)-containing mesoporous bioactive glass (Cu-MBG) scaffolds with interconnective large pores are used *in vitro* both Cu-MBG scaffolds and their ionic extracts stimulates hypoxia-inducible factor (HIF)-1α and vascular endothelial growth factor (VEGF) expression in human bone marrow stromal cells (hBMSCs). Thus, incorporation of Cu^{2+} ions into MBG scaffolds increase hypoxia-like tissue reaction which enhance angiogenesis and osteogenesis and has promising scope for the treatment of large bone defect [278]. Controlled delivery of 3 wt% CuO from borate bioactive glass scaffolds implanted in rat calverial defect shows significantly better capacity to stimulate angiogenesis and regenerate bone when compared to the undoped glass scaffolds [279]. It is also evident that copper-doped bioglass scaffold *in vivo* acts on BMSCs ((bone-marrow derived mesenchymal stem cells) to stimulate secretion of VEGF which in turn enhances the angiogenic growth into the scaffolds [280]. Copper (Cu) has the property to stimulate vascularisation/angiogenesis and silicate bioceramics have also stimulatory effects on vascularisation *in vitro* due to the release of silicon (Si) ions. Hence, when combined in bioceramic implant Cu and Si have synergistic effects [281].

Biomaterial-centred bacterial infection, one of the major reasons for revision surgery [282], led the researchers to explore such material that could control infection as well as promote bone healing. Incorporation of silver oxide (Ag_2O) proved its promising future to combat against microbial infection on biomedical materials and devices [241, 242, 283–285]. The introduction of Ag_2O into the bioactive glass shows promising bactericidal efficacy against *Escherichia coli*, *P. aeruginosa* and *S. aureus in vitro* by leaching of Ag^+ ions from bioglass matrix [223, 286–288]. Doping of Ag^+ ions in 45S5 bioglass based scaffolds even proves to be effective against MRSA (methicillin-resistant *S. aureus*) *in vitro* [289]. Silver-doped bioactive gel-glass Ag-S70C30 has

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beneficial role as antimicrobial wound healing agent in inflammatory response in a local body compartment such as in acne lesions and in non-healing dermal wounds as it has no cytotoxicity against human epidermal keratinocytes [290]. Mesoporous bioactive glasses doped with Ti/Ag have improved hydroxyapatite- (HAP) induced growth and antimicrobial properties and more potency than pure MBGs in bone-tissue regeneration and surgery [291]. Very recently, scaffolds of a borosilicate bioactive glass (composition: $6Na_2O$, $8K_2O$, 8MgO, 22CaO, $36B_2O_3$, $18SiO_2$, $2P_2O_5$; mol%) doped with varying amounts of Ag_2O (0.05, 0.5 and 1.0 wt%) is being used for bone defect repair and as well as to control infection caused by *E. coli* and *S. aureus*. Better adhesion, proliferation and alkaline phosphatase activity of murine osteoblastic MC3T3-E1 cells on the Ag_2O-doped bioactive glass scaffolds is found than on the undoped scaffolds *in vitro* [292].

Wnt pathway has been found to play a central role in controlling embryonic bone development and bone mass [293] during the past decade. In the developing skeletogenesis, Wnt signalling is required for limb bud initiation, early limb patterning, and, finally, late limb morphogenesis events. It has been reported that Wnt-3a and Wnt-7a are expressed in the limb bud and have roles in skeletal pattern determination [294], and that Wnt-14 is involved in joint formation [295]. In addition, Wnt-3a, Wnt-4, Wnt-5a and Wnt-7a all influence cartilage development [295]. Wnt are 39-46 kDa cysteine-rich, secreted glycoproteins that have been identified in organisms ranging from hydra to humans [296]. Recently, it has been suggested that canonical Wnt signalling plays an important role in fracture healing [297]. Lithium (Li) is an element known to mimic the Wnt signalling pathway, which plays a central role in osteoblast proliferation and differentiation [298]. Expression of various Wnts has been reported to be upregulated during fracture repair, and increased β-catenin signalling by lithium administration has been shown to improve fracture healing [299]. Edgington et al. reported that lithium-based dopants to β-TCP induced an effect on the cell-material interaction of osteoblast cells as well as the study exhibited increased proliferative activity at the lower concentration of Li-doping, while the higher concentration showed a decrease in activity, indicating a toxic effect of Li at elevated doses *in vitro* [300]. Lithium activates β-catenin signalling by inhibiting GSK3β [301–303]. It is also reported that lithium enhances bone formation and improves bone mass in mice [304]. Bioactive glasses with Li-containing composition (55% SiO_2-36% CaO-4% P_2O_5-5% Li_2O) synthesised through a quick alkali sol-gel process stimulate apatite formation after immersion in SBF. Furthermore, addition of Li enhances chemical durability and antibacterial activity against *Enterococcus faecalis*. Li-doped bioglass has excellent antibacterial property against tooth infections for the treatment of root canal, other dental applications [305]. Researches reveal that different concentrations of Li_2O (0-12 wt%) substitution for Na_2O in 45S5 bioglass causes *in vitro* more apatite formation and osteoblastic cell responses than non-substituted 45S5 bioglass thus prove its efficacy for bone defect filler [211]. Another study shows that Li doping in therapeutic range (<8.3 ppm) in 45S5 Bioglass causes more HA deposition than non-doped bioglass *in vitro* [306].

There are even some more ions or materials, doping of which positively improve the quality, bioactivity or bone regeneration. Study with boron modified bioactive glass particle shows significantly more thickness of osseointegrated tissue and more area of neoformed bone tissue

than non-doped 45S5 glass along with increase in the Ca:P ratio. Boron modification enhances bone formation more than 45S5 glass when implanted into the intramedullary canal of rat tibiae [307]. Modification of bioactive glass by substitution of Na_2O with doping of fluorides, such as CaF_2 and MgF_2 or B_2O_3 increases its mechanical property [308]. Nickel and cobalt both stimulate the hypoxia-inducible factor-1 (HIF-1a), which significantly improving blood vessel formation in tissue engineering applications. No significant structural differences or dissolution rate occur when nickel and cobalt are doped in bioactive glasses [309]. Magnesium-doped melt-derived glasses in the system SiO_2-CaO-Na_2O-P_2O_5 influences the formation and the evolution of the newly formed layers, promotes the dissolution of the silica network, increases the thickness of the silica gel layer as well as slows down the crystallisation of the apatite layer [310]. Silica- and phosphate-based bioactive glass nanoparticles ($58SiO_2$-33CaO-$9P_2O_5$) doped with neem (*Azadirachta indica*) leaf powder and silver nanoparticles show good antimicrobial activity against *S. aureus* and *E. coli* and less bioactivity compared with silver-doped glass particles [311].

3.2. Doped bioactive glass as coating of orthopaedic implants

Since the discovery of bioglass it had mainly been used for coating of metallic implant which are bioinert in nature, i.e. bonding ability to bone tissue is poor [312]. On the other hand, bioglass being an excellent osteogenic agent it has also some inherent disadvantages such as poor mechanical properties leading to its limited application in load-bearing implants where metallic alloys are still the materials of choice. Hence, coatings have drawn attention of researchers as a method to improve adherence of bone tissue to metallic alloy to be used as load-bearing implant in orthopaedic surgery. For this purpose, coating material should have thermal coefficient similar to that have bioglass, as well as, has some other properties such as firing cycle during preparation of coating should not degrade the quality of metal and optimum adherence should be achieved with hydroxyapatite formation in contact with body fluid.

To achieve the goal researchers embedded bioglass or hydroxyapatite particles on coating of Ti6Al4V by a simple enamelling technique to enhance their bioactivity and found excellent glass/metal adhesion with well-attached bioactive particles on the surface that can withstand substantial chemical and mechanical stresses [313]. Another family of glasses in the SiO_2-Na_2O-K_2O-CaO-MgO-P_2O_5 system has been synthesised for coatings on Ti-based and Co-Cr alloys by the scientists, where desired achievement were observed to alloys by formation of 100–200 nm thick interfacial layers (Ti5Si3 on Ti-based alloys and CrO*x* on Co-Cr) and commercially Ti alloy-based dental implants were fabricated with 100 μm thick glass coatings successfully [314]. Surgical suture materials such as absorbable polyglactin 910 and non-resorbable Mersilk when coated with silver-doped bioactive glass powder (AgBG) and tested *in vitro*, after 3 days of immersion in SBF, hydroxyapatite forms on the coated suture surfaces and thus their bioactive behaviour is enhanced as a result their use in body wall repair and wound healing property is also enhanced [243] it also limits bacterial attachment [315]. *In vivo* histologic and histomorphometric study on osteointegration of gradient coatings composed of bioactive glass and nanohydroxyapatite (BG-nHA) on titanium-alloy orthopaedic implants and surrounding

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bone tissue. Fluorescence micrograph shows better osteointegration of orthopaedic implant in BG-nHA than uncoated implant [316].

Mesoporous bioactive glass coatings immobilised with L-ascorbic acid phosphate magnesium salt *n*-hydrate (AsMg) on stainless steel plate causes osteoblast MC3T3-E1 cells stimulation by the MBG with enhanced cell attachment, proliferation, differentiation and better developed cytoskeleton as well as, enhanced fibroblast NIH3T3 proliferation *in vitro* [317]. To compare the behaviour of hydroxyapatite and the bioactive glass coated titanium dental implants different clinical and radiological parameters were studied for 12 months in 31 patients. The study revealed equal potency of bioglass as hydroxyapatite to achieve osteointegration in dental implants [318]. Similarly, nanoparticulate bioactive glass coating on the porous titanium implants promotes better osteointegration and stimulates the formation of bone within the pores than non-coated implants [319]. Incorporation of nanosized HAP into ZnO containing bioglass coating on Ti-6Al-4V substrate improves mechanical properties of the coating but do not hamper *in vitro* bioactivity [320]. Composite orthopaedic coatings with antibacterial capability containing chitosan, Bioglass particles (9.8 μm) and silver nanoparticles (Ag-np) were coated in stainless steel 316 substrate and studied *in vitro* in SBF. Result showed low released concentration of Ag ions (<2.5 ppm) was efficiently antibacterial against *S. aureus* up to 10 days and coating enhanced proliferation of MG-63 osteoblast-like cells up to 7 days in culture and it was also found that high concentration of Ag-np (342 μg) have cytotoxic effect [321]. 45S5 bioglass-silica coatings on 316L stainless steel also causes good osteointegration as well as prevents the metallic implant from corrosion in presence of body fluid [322].

3.3. Doped bioactive glass for delivery of growth factors in bone healing

Growth factors are proteins secreted by cells, act on the appropriate target cell or cells to carry out specific action and thereby there over expression have also been shown in different stages of fracture healing. This phenomenon has led the researchers to study their role as well as the potential to be used as therapeutic agent to accelerate fracture healing. Hence, growth factors are also incorporated into bioactive glass implant, scaffold or coating materials to enhance osteogenic property. Incorporation of bioactive glass and fibroblasts into alginate beads stimulates VEGF as a result potentially it can be used for therapeutic angiogenesis [323]. Combination of prolonged localised VEGF presentation from a matrix coated with a bioactive glass enhances bone regeneration as VEGF has beneficial role in osteogenesis [324]. The combination of novel MBG/silk fibrin scaffold and BMP7 and/or PDGF-B adenovirus synergistically promotes wound healing in acute buccal periodontal defects and osteoporosis related fracture by recruitment of recruitment of mesenchymal progenitor cells [325, 326]. Borate bioactive glass microfibres doped with 0-3.0 wt% CuO has remarkable ability to stimulate angiogenesis which help to heal full-thickness skin defects in rodents and promotes human umbilical vein endothelial cells (HUVEC) migration, tubule formation and secretion of vascular endothelial growth factor, as well as the expression of angiogenic-related genes of the fibroblasts *in vitro* [327].

4. Conclusion and final remarks

Innovative research on bone tissue engineering has made considerable strides over the few decades in the development of new materials, processing techniques and their evaluation and applications. Bioresorbable scaffolds with controlled porosity and tailored properties are of paramount necessity in the successful outcome of bone healing. Silicate bioactive glasses have been extensively investigated over last 40 years. Borate and borosilicate bioactive glass compositions are promising and currently being used in tissue engineering. Although the ability of bioactive glass to support osteogenesis has been proved, recent work has shown the angiogenic potential which may be utilised for the benefits of bioactive glass to soft tissue repair. Due to its biodegradable properties, it may release ions during the degradation process. Apart from doping the bioactive glass with several metallic ions, the degrading ions of its own are known to have a beneficial effect on osteogenesis and on angiogenesis. Current findings show that they may also have a favourable effect on chondrogenesis. Metallic ion doping with the presently available bioactive glass may further improve the biological performance of the material that may open a new vista in bone tissue engineering. Future research will take benefit of the advantageous properties of doped bioactive glass in bone healing as well as coating of several metallic implants.

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