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# The unexplored role of alkali and alkaline earth elements (ALAEs) on the structure, processing, and biological effects of bioactive glasses

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Bioactive glass has been employed in several medical applications since its inception in 1969. The compositions of these materials have been investigated extensively with emphasis on glass network formers, therapeutic transition metals, and glass network modifiers. Through these experiments, several commercial and experimental compositions have been developed with varying chemical durability, induced physiological responses, and hydroxyapatite forming abilities. In many of these studies, the concentrations of each alkali and alkaline earth element have been altered to monitor changes in structure and biological response. This review aims to discuss the impact of each alkali and alkaline earth element on the structure, processing, and biological effects of bioactive glass. We explore critical questions regarding these elements from both a glass science and biological perspective. Should elements with little biological impact be included? Are alkali free bioactive glasses more promising for greater biological responses? Does this mixed alkali effect show increased degradation rates and should it be employed for optimized dissolution? Each of these questions along with others are evaluated comprehensively and discussed in the final section where guidance for compositional design is provided.

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## 1. Introduction

Glass-based materials have been developed for medical applications since the discovery of their bone bonding capabilities by Larry Hench in 1969.[1] Bioactive glasses (BGs) have then been expanded to several applications in medicine outside of hard tissue. These new applications required a new definition of BGs to be proposed: "a non-equilibrium, non-crystalline material that has been designed to induce specific biological activity".[2]

BGs have been developed from network formers such as $SiO_2$, $B_2O_3$, and $P_2O_5$ and have been modified with nearly every biocompatible element on the periodic table. Varying chemical compositions of BGs has been the best method to tailor bioactivity, dissolution rates, and other properties to the desired application. 45S5 Bioglass®, the original parent composition, was designed as belonging to the quaternary $24.5Na_2O$–$24.5CaO$–$45SiO_2$–$6P_2O_5$ (wt%) oxide system. Invented by Larry Hench, 45S5 Bioglass® has been employed as the most used composition so far and has been implemented in several commercially available medical devices mainly addressed to bone repair. Other glass systems such as 13-93B3, a borate-based composition of $5.5Na_2O$–$11.2K_2O$–$4.6MgO$–$18.5CaO$–$56.5B_2O3$–$3.7P_2O_5$ (wt%), have been shown effective in soft tissue repair with accelerated dissolution rates.[3,4] Several other compositions have been developed modifying biological capabilities with transition metals and using glass science phenomena such as the mixed alkali effect and mixed former effect to tailor properties.[5–7]

In order to produce a wide array of BG devices, several processing methods have been developed. Melting route and sol–gel process are currently the only methods used to create BGs but there are many ways to process the "raw" glass materials obtained thereof. Thermal processing of BGs can aid in terms of the mechanical properties of final products and provide better control of the dissolution rate.[8] Crystallization of a secondary biocompatible phase to create a glass-ceramic material or sintering of BG powder are both common thermal processing techniques.[9–14] Additive manufacturing techniques to fabricate porous scaffolds as well as advanced deposition methods to create thin films have also been studied to further add to the diverse potential of BG medical devices.[15–18]

Ions released from BGs have been shown to play several roles in increasing glass bioactivity and potential in tissue engineering. The first role is promoting the precipitation of

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bone-like mineral phases such as hydroxyapatite and fluorapatite. The bone bonding ability of BGs relies on the ability to slowly dissolve and induce the formation of nanocrystalline apatite at the bone/implant interface.[19] Regeneration of hard tissues is further stimulated through BG-driven genetic expression and stimulation of osteogenic markers and control of cell cycle regulators *via* the release of key therapeutic ions.[20] Soft tissue growth is also stimulated through genetic expression with proteins such as vascularization epithelial growth factor (VEGF) and different forms of matrix metalloproteinase (MMO).[21] The angiogenic nature of some BGs also helps reform broken blood vessels and promote neo-vascularization, further accelerating the soft tissue repair timeline.[22,23] Several other studies have shown that including other elements in the BG matrix can aid in antibacterial effects, anti-inflammatory stimulation, antioxidant and other beneficial biological processes.[24–26] Fig. 1 provides a visualization of the periodic table highlighting the role of each element in bioactive glasses.[2]

Applications of BGs have dramatically grown over the last 15 years to include all aspects of medicine including both hard tissue and soft tissue regeneration, drug delivery, and cancer therapy.[27,28] The ability to customize the composition and tailor the processing to target the specific needs of a medical device suits BGs well for most applications in tissue engineering.[29] Increasing evidence is reported each year displaying the unique ability of BG-based devices to deliver key therapeutic ions while providing a bioresorbable platform for tissue engineering or as a drug delivery vehicle. The regeneration of hard tissue through BG-based devices has been shown effective in many forms such as glass monoliths, cements, putties, and 3D-printed scaffolds.[30–35] Soft tissue applications have implemented "cotton candy-like" glass fibers, putties, and dozens of synthetic and natural polymer–matrix composites.[36–40] Mesoporous BGs produced through sol–gel synthesis have provided a high surface area platform for high-capacity drug delivery.[41–43] The applications of BGs are continually expanding as the level of interest in the field has grown exponentially as illustrated in Fig. 2.

Decades of research have explored the impact of bioactive glasses through *in vitro* and *in vivo* studies focusing on the glass structure, processing, and release of inorganic ions. The objective of this review is to guide the design of BG compositions by focusing on the different effects of ALAEs. Key properties, such as dissolution behavior, phase precipitation from solution, and mixed alkali effects, are briefly introduced in relation to their influence on glass structure. Additionally, the review emphasizes the influence of ALAEs on processing methods and related properties of bioactive glasses, providing

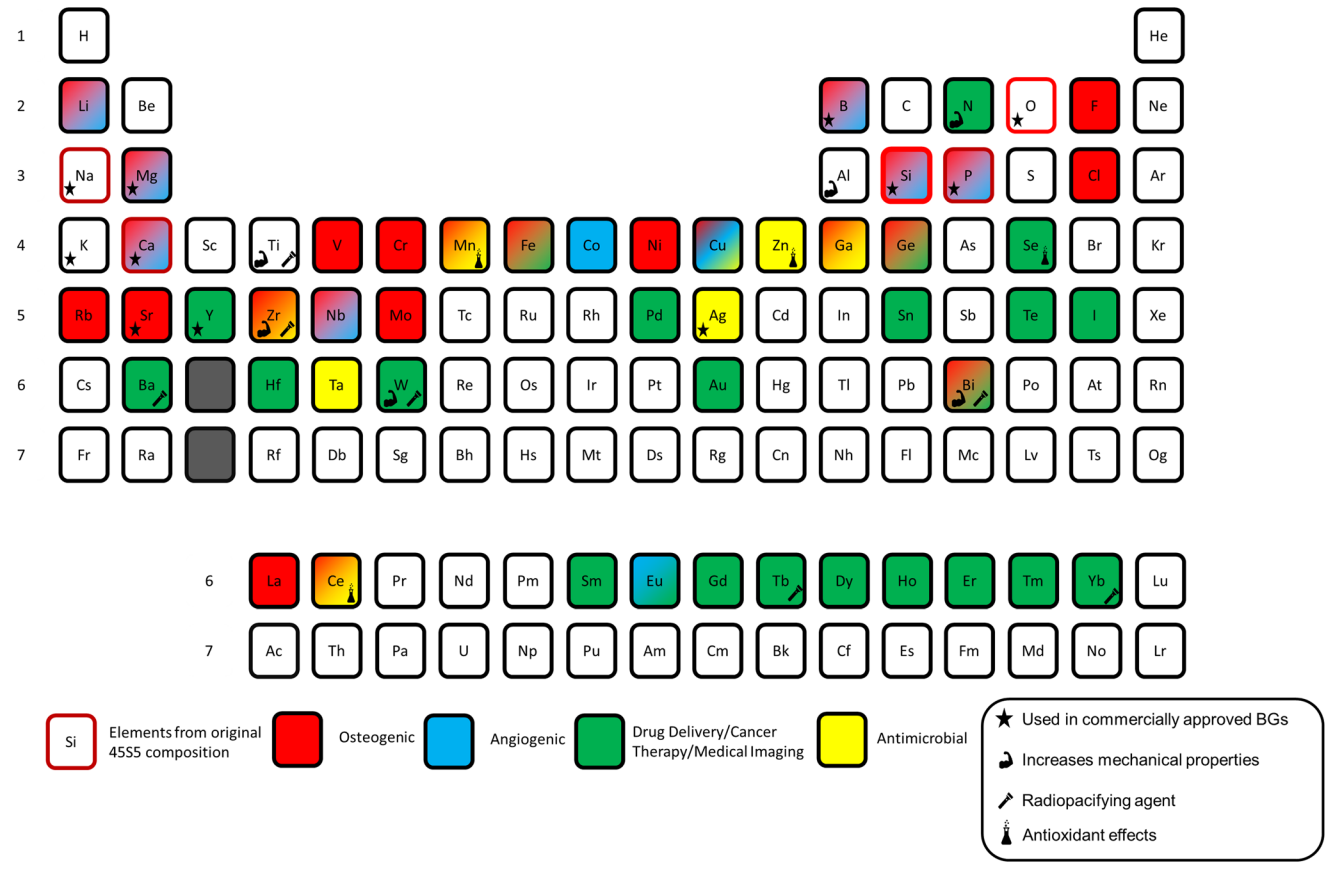


**Fig. 1** Periodic table of elements used in bioactive glasses with different therapeutic applications highlighted. Modified and reproduced from ref. 2 with permission from Elsevier, 2023.

 

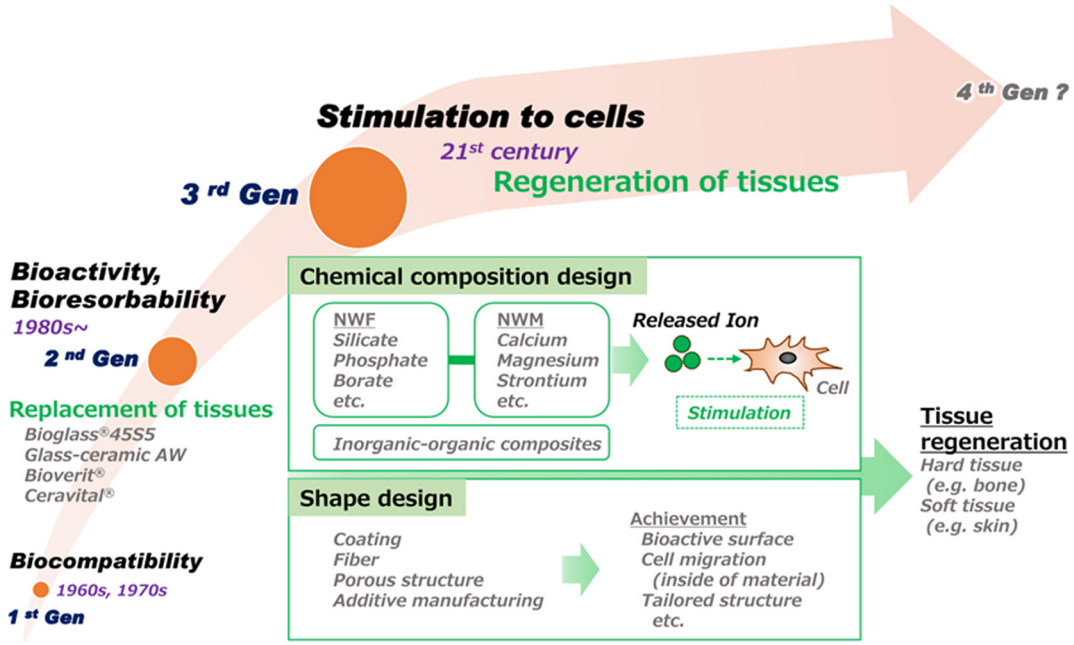


Fig. 2 Progress in the development of bioactive glasses for clinical applications. This illustration highlights the advancement of research in the field from the discovery of BG biocompatibility to specific biological action due to release of inorganic ions.[44]

valuable insights into how these ions affect the manufacturing and characteristics of these materials. This review places a primary focus on exploring the physiological consequences of leached alkali and alkaline earth ions, shedding light on their role in the human body and potential implications for medical applications. Understanding these effects is vital for the development and optimization of bioactive glasses for various biomedical purposes.

## 2. Role of ALAEs on structure and properties

The structure of bioactive glasses is critical to control its properties and processibility. Compositional design factors such as total network modifier content, elemental selection, and alkali to alkaline earth ratio, which primarily include $Li_2O$, $Na_2O$, and $K_2O$, and MgO, CaO, SrO, and BaO, can affect structure and subsequently key properties such as apatite forming ability, dissolution kinetics, and cell biology. As depicted in Fig. 3, Na and Ca, as the primary representatives of ALAEs, have the capability to modify the glass network in silicate glass. This modification renders the glass suitable for a variety of applications in dental, bone/soft tissue engineering, and drug delivery. Researchers have diligently explored additional ALAEs to expand and provide further justification for the applications of BGs.

### 2.1 Structure

Wallace *et al.*[45] produced various melt-quenched bioactive glass formulations and investigated the influence of $Na_2O$ on the glass properties while keeping the glass network connectivity (NC) constant by systematically replacing CaO with $Na_2O$. Linear decrease in the glass transition temperature and peak crystallization was detected as the content of $Na_2O$ increased. This was why the addition of $Na_2O$ caused glass network disruption because the glass network expanded with increasing $Na_2O$ content. Accordingly, decrement of glass transition temperature was observed with increasing $Na_2O$ content because this is an effect of glass network disruption.[27] In fact, increasing the $Na_2O$ content in bioactive glasses as a replacement for CaO leads to the widening of the silicate network: in other words, the packing density decreases due to the substitution of one bivalent $Ca^{2+}$ ion with two monovalent $Na^+$ ions. As a macroscopic result, Farooq *et al.*[46] showed that the glass density and hardness decrease because the glass network becomes less compact as $Na_2O$ increases.

Glass formation and stability are critical to understanding the durability and dissolution of a glass.[47] In bioactive glass design, these are parameters of key interest because the primary mechanism for apatite forming ability and therapeutic ion release are through dissolution.[48–50] The connectivity of a glass network and cross-linking density are used to understand solubility and surface reactivity of bioactive glasses.[51] Furthermore, ALAEs with the same valency but different sizes can be substituted for each other resulting in the glass network to expand or compact.[52] This has been shown to affect solubility and bioactivity of bioactive glass, both of which are important to increasing efficacy.[52] The size of the cation can change the packing density of the glass which directly affects the dissolution behavior. An example of this would be replacing CaO with SrO will compact the silicate rings of the glass and slow dissolution.

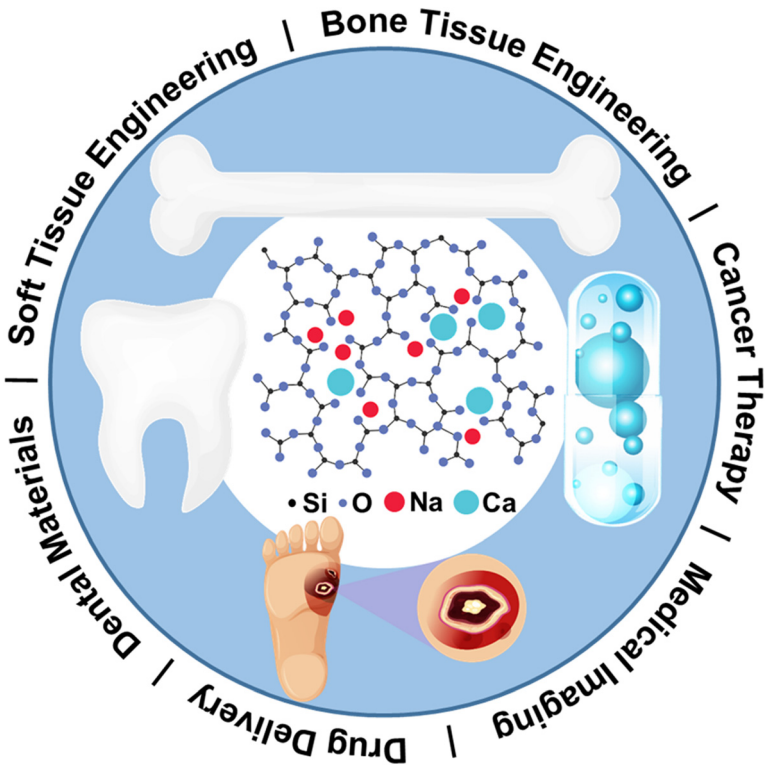


Fig. 3 Sodium and calcium as primary representatives of ALAEs on glass network modification that render BGs for a variety of applications in dental, bone/soft tissue engineering, and drug delivery. From ref. 2 with permission from Elsevier, 2023.

Some ALAEs behave differently, such as MgO that acts as a network intermediate instead of a modifier in a highly disrupted silicate network.[53] In bioactive glasses, a modifier to intermediate transition is expected to inhibit bioactivity because the dissolution and release of therapeutic ions is restricted compared to glasses which are less stable. However, for the case of MgO, it was observed that the likelihood for crystallization was also decreased when MgO acted as an intermediate.[53] This would be a benefit for processing bioactive glasses. When new bioactive glasses are designed it is important to fully characterize the structure of the glass to ensure each component is acting as expected. The specific properties which are tailored by optimization of added ALAEs will be determined by the application and processing specifications.

### 2.2 Apatite forming ability

*In vitro* studies show BGs to form multiple phases including hydroxyapatite (HAp), hydroxy-carbonate apatite (HCA), amorphous calcium phosphate (ACP), fluorapatite, and chlorapatite.[54,55] The phases that form are dependent on the glass composition, duration of exposure to a biologic environment, temperature, and pH. Understanding the factors which govern phase formation mechanisms and *in vitro* mineralization will guide the future of bioactive glass science. Apatite forming ability has been shown to depend on the level of polymerization of the glass network. Bioactive glasses with high network connectivity show decreased ion release rates which in turn slows apatite formation.[56] A network connectivity of 2.4 has been established as the cut-off point for a BG to form apatite at its surface.[51]

Hydroxyapatite, $Ca_{10}(PO_4)(OH)_2$, naturally requires free calcium and phosphate ions to be released at the surface of BGs. Both of these ions occur naturally in physiological fluids, the formation of HAp is highly compositional dependent.[57] Although replacing calcium with other alkaline earth elements may not negatively affect dissolution rates, it can reduce the rate and amount of HAp formed.[58,59] Magnesium has been well studied and described to delay the formation of an apatite layer.[60,61] On the other hand, strontium has been found to assist in apatite formation through strontium-substituted apatite.[62] Finally, alkali free BGs have also shown equal performance in apatite formation as alkali containing glasses. This suggests that free alkali elements in solution do not contribute to the formation of an apatite surface.[63,64]

### 2.3 Solubility

Solubility control of bioactive glasses is critical to improving bioactivity and performance. For bone formation, too much solubility can hinder the rate of HAp formation because the ions may be transported away from the healing site.[65] In soft tissue healing applications, high solubility is important to ensuring no glass remains once the healing reaction is complete.[66,67] Finding the right solubility rate for each application will help to optimize the effectiveness of bioactive

 

glasses. Compositional dependence and processing dependence on the solubility of bioactive glasses is discussed further here.

Glass network disruption, through formation of non-bridging oxygens (NBOs), means higher reactivity of the glass in aqueous solution, for example, like biological fluids. As a matter of fact, the major added value of $Na_2O$ in melt-derived bioactive silicate glasses is related to surface reactivity and, hence, apatite-forming ability, which is key for bone-bonding. The bioactive role played by $Na_2O$ and other ALAEs in biomedical glasses is discussed elsewhere in this paper. As mentioned earlier here that although Na-containing bioactive glasses generally show good bioactivity and biocompatibility, high contents of $Na_2O$ can elicit a cytotoxic response.[45] A similar disrupting effect on the glass network is associated to the introduction of MgO, leading to the creation of NBOs, decreasing the glass transition temperature and also – albeit indirectly – increasing the rate of bioactive glass dissolution,[68] with an obvious impact on apatite-forming kinetics.

The solubility of glasses is directly tied to the structure of the glass which results from the manufacturing process and composition. A study on glasses containing $SiO_2$, $P_2O_5$, CaO, and $Na_2O$ showed that the reactivity and bioactivity will decrease with increased density of the structure.[69] The authors characterized the densification of glass by calculating the activation energy for silicon release in a series of glasses and found that 45S5 was very bioactive. Furthermore, an increase in silica and decrease in CaO and $Na_2O$ content respectively increased the activation energy, decreasing bioactivity. The sol–gel glasses in the study were found to be in the middle range of the melt-quench glasses studied.[69] This indicates that the compositional effect on solubility is larger than the processing effect. However, it is not clear whether the observed decrease in solubility is primarily attributable to modifications in silicon, calcium, or sodium content. The mixed alkali effect (MAE), which means multiple alkali cations in the network can be observed to have non-linear property changes, should be further evaluated in these systems to understand the structure role.[70] For example, Tylkowski and Brauer when substituted sodium ions for potassium or lithium ions in bioactive glass compositions observed that in compositions with a mixture of sodium and potassium, the crystallization temperature increased compared to 45S5. This means that the glass processing window was larger.[71] Further evidence shows the MAE to decrease the probability of crystallization and promote glass stability.[72] Therefore, the MAE should be considered for processing bioactive glasses containing more than one modifier.

When more than one alkali oxide is present in a glass composition and the MAE is observed, it is also common for structural changes to take place. For example, if sodium is replaced by lithium, a silicate network becomes more compact due to the variation in ionic radii between the interchanged ions.[73] This is a good example of the structural characteristics of a glass network having direct influence on mechanical and thermal properties. Current bioactive glass literature focuses on sodium, lithium, and potassium.[72–74] For application to bioactive glasses, it is important to tailor the combination of the alkali elements present to achieve optimal properties while limiting toxicity. Furthermore, understanding how the MAE may influence the solubility of the glass network is of current importance. By connecting the ion release rate and ion mobility to the MAE, a deeper understanding of diffusion in bioactive glass networks would emerge.[75] If the solubility can be directly correlated to the mixed alkali effect, the MAE will be essential to understand the inclusion of multiple modifiers in bioactive glasses.

### 2.4 Alkali free bioactive glasses

Alkali free BGs have similar structures to alkali containing compositions as alkaline earth elements also act as network modifier. However, there are a variety of reasons researchers are exploring alkali free bioactive glasses. Improving cytotoxicity, decreasing the tendency for crystallization, and optimizing the dissolution rate are a few good examples.[76–79] Limiting the alkali content can reduce cytotoxicity as elements like sodium rapidly increase the pH of the surrounding environment upon dissolution. Some glasses including alkali oxides have also been shown to absorb water through osmosis which limits their mechanical properties for certain applications like coatings on implants.[76] These alkali free BGs have been studied primarily through sol–gel derived glasses as the compositional workspace is broadened through this method. The following sections of processing and physiology will discuss the potential advantages of alkali free BGs in greater detail. Alkali free BGs have delayed dissolution rates compared to alkali containing analogue compositions. Dissolution is driven by the ion exchange at the glass surface with hydroxyl groups causing the formation of silanol groups.[80] Both static and dynamic dissolution studies show that alkali elements are the fastest to be released allowing for increased overall dissolution rates and formation of crystalline phases at the glass surface.[81–83] Furthermore, the concentration and ratio of different alkaline earth elements in the glass matrix can further affect dissolution rate.[84]

## 3. Effect of ALAEs on processing and crystallization

### 3.1 Melt-derived glasses

While there is abundant literature on the biological effects elicited by ALAEs released from bioactive glasses, there is a much more limited amount of systematic studies specifically focusing on the role of these elements on sintering and processing. The reason is that processing of bioactive glasses per se is somewhat similar to that of common glasses with similar composition; hence, the consolidated knowledge of the latter group can be applied to the former.

**3.1.1 Effects on viscosity.** In general, it is well known that modifiers – including ALAEs – disrupt the glass network and, therefore, are useful to allow glass production at lower temp-

eratures because of their effect in decreasing the viscosity of the glass melt. In this regard, the viscosity-temperature relationship is key in determining the easiness of glass formation of any melt. Glass formation is promoted when crystallization is discouraged by the kinetic barrier to atomic arrangement, provided that (i) the viscosity is sufficiently high at the melting temperature of the crystalline phase that would form from the melt and/or (ii) the viscosity decreases very rapidly as temperature increases.[85] Just to provide some quantifications, melting of silicate commercial glasses typically takes place at a viscosity below 10 Pa s and the viscosity at the working point (*i.e.*, when a glass product is ready for being shaped) is about $10^3$ Pa s. Modifiers generally tend to open up the 3D structural network of glass, thereby increasing the fluidity and decreasing the working temperature.[85]

**3.1.2 Effects on thermal behavior, sintering and processing.** Bioactive glass products deriving from melts are typically obtained through three approaches: (i) direct casting of the melt into a mold (in this case a key role is played by the viscosity of the melt), (ii) fiber drawing (in this case a key role is played by glass transition temperature), and (iii) sintering of previously-prepared glass powders to fabricate, for example, porous scaffolds.

Therefore, glass stability against crystallization must be considered not only during the initial melting procedure (case (i)) or drawing (case (ii)) of the material, but also when glass-based products are obtained by applying secondary high-temperature treatments above $T_g$ (case (iii)). It is well known that viscous flow sintering of glass particles is highly effective when the surface tension is high, the viscosity is low and crystallization does not take place.[86,87] Lara *et al.*[88] introduced a parameter, $S_c$, that quantifies the sinterability of glass, defined as:

$$S_c = T_x - T_{MS}$$

where $T_x$ is the crystallization onset determined by DTA/DSC and $T_{MS}$ is the temperature of maximum shrinkage determined from hot-stage microscopy (HSM) or dilatometer.

This parameter measures the competition between glass sintering and crystallization that may concurrently occur during heating (sinter-crystallization): the larger $S_c$, the more independent the kinetics of the two processes. A general rule can be proposed for the interpretation of $S_c$: if $S_c < 0$, only partial densification is achieved before crystallization begins (like in the case of 45S5 Bioglass®[89]); otherwise, if $S_c \geq 0$, full densification occurs prior to crystallization (*e.g.* in bioactive glasses/glass-ceramics belonging to the CaO–MgO–$SiO_2$–$P_2O_5$–$Na_2O$–$CaF_2$ system[90]). Therefore, higher values of $S_c$ are related to higher final densities due to better sintering behavior and, ultimately, higher mechanical properties of the final product.

Another parameter describing glass stability is the difference between onset of crystallization and glass transition temperature ($T_x - T_g$). In this regard, the presence of MgO in the glass can produce a broadening of this range.[91] In fact, it is known that resistance to crystallization is improved when CaO is partially substituted by MgO in $SiO_2$–$Na_2O$–CaO glasses.[92] Verné *et al.*[93] even revealed that replacement of CaO with MgO in a silicate $SiO_2$–$Na_2O$–$K_2O$–MgO–CaO–$P_2O_5$ system, keeping all the other oxide amounts fixed, led to the inhibition of a crystalline phase.

The level of viscous flow during sintering is directly affected by the ALAEs content in the BGs. Nommeots-Nomm *et al.* studied the viscous flow sintering of BGs in a 3D-printed scaffold using synchrotron X-ray tomography to visualize the changes in inter-particle spacing (Fig. 4).

To date, perhaps the most systematic, comprehensive and specific study on the influence of ALAEs on thermal processing of bioactive glasses was reported by Brink,[95] who analyzed the thermal behavior of 40 silicate glasses in the $Na_2O$–$K_2O$–MgO–CaO–$B_2O_3$–$P_2O_5$–$SiO_2$ system by primarily using hot-stage microscopy. The silica content in the glasses was in the range of 39 to 70 wt% and all glasses containing below 54 mol% $SiO_2$ were shown to devitrify during viscosity measurements. Generally, glasses that devitrified contained more alkaline modifiers but less alkaline-earth modifiers than glasses with a large working range (or alternatively, a high $T_x - T_g$ range). This study suggested that such temperature interval can be enlarged in bioactive glasses by decreasing the amount of alkaline modifiers (especially $Na_2O$) and/or increasing the amount of alkaline-earth modifiers (especially MgO). These conclusions, coming from the analysis of a relatively large number of glass systems, are consistent with those arisen in the previously-cited reports.

It is worth underlining that, if the complexity of glass composition increases – which is the typical case of most bioactive glasses –, it is impossible to precisely recognize the effect of each single alkaline/alkaline-earth modifier on thermal behavior. On one hand, Mancuso *et al.*[96] prepared a set of multicomponent bioactive silicate glasses containing various amounts and types of alkaline ($Na_2O$, $K_2O$)/alkaline-earth modifiers (CaO, MgO) and reported that they showed similar thermal profiles upon heating according to hot-stage microscopy, which were characterized by an increase in sample dimensions after the maximum shrinkage temperature and before the melting onset took place. These results are consistent with the findings reported by Baino *et al.*[89] who found that a bioactive glass (CEL2) with composition $45SiO_2$–$3P_2O_5$–26CaO–7MgO–$15Na_2O$–$4K_2O$ (mol%) exhibited a significant volumetric expansion after the first densification step. Hence, these studies suggest that the sintering profiles of multicomponent silicate glasses are relatively insensitive to the presence of different network modifiers in the glass structure. On the other hand, hot-stage microscopy analysis reported by Fiume *et al.*[97] on a bioactive glass with similar composition to the previously-cited CEL2 ($47.5SiO_2$–$2.5P_2O_5$–20CaO–10MgO–$10Na_2O$–$10K_2O$ (mol%)) revealed an elbow-shaped sintering profile without any volumetric expansion before melting. This was also the same profile exhibited by the simpler 45S5 quaternary composition.[98]

Perhaps, a clearer, more conclusive and general picture about the role of modifiers on glass sintering could be obtained by systematically analyzing the existing data from the literature by machine learning/artificial intelligence approaches.

 

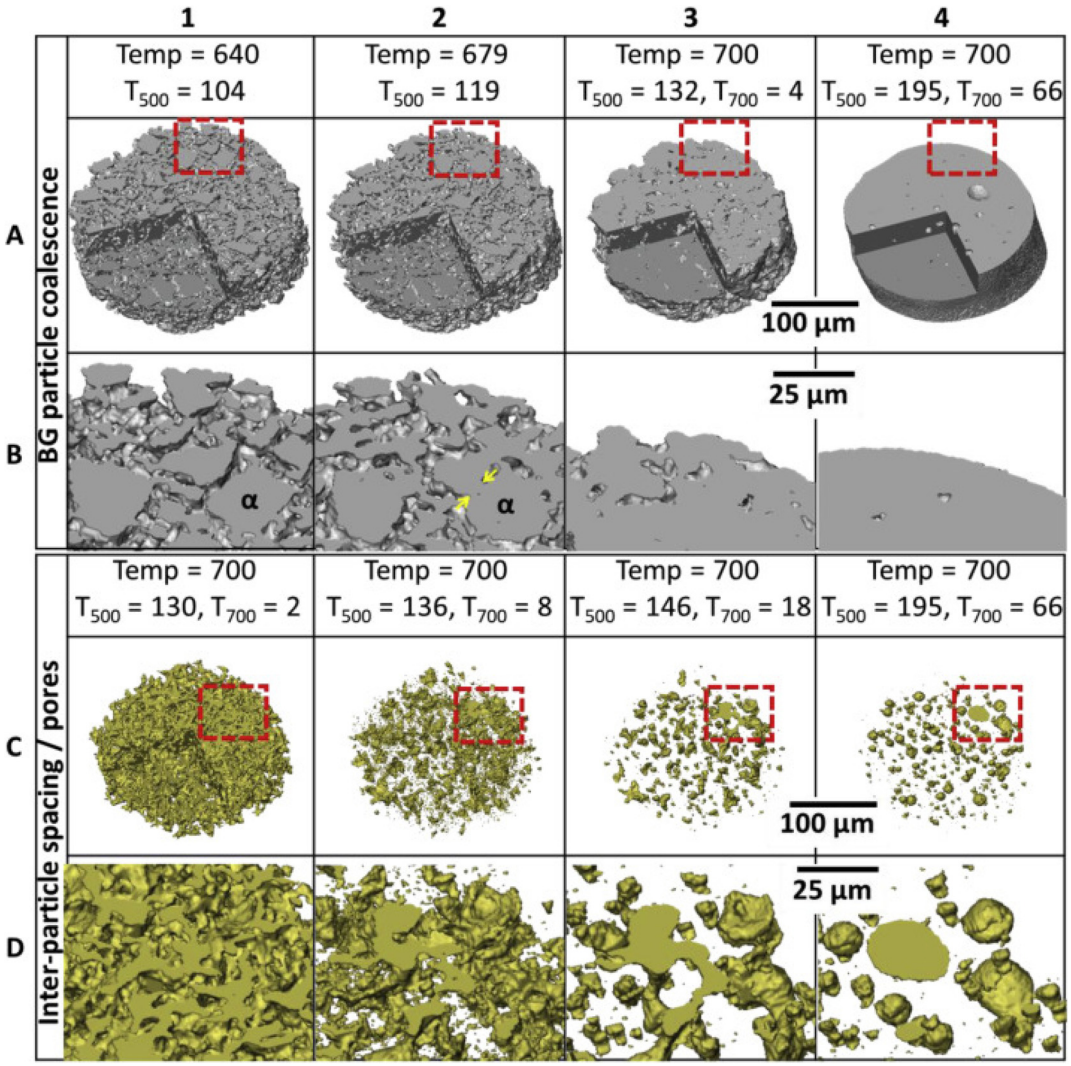


**Fig. 4** A 3D reconstructed volume of a small section of a scaffold strut showing the bioactive glass (BG) (A, B) and inter-particle spacing (pores) (C, D) phases sequentially with time and temperature. The lower images (C and D) are magnified areas of the red boxes in A and D. Yellow arrows indicate neck formation between particle α and one of its neighbors. From ref. 94 with permission from Elsevier, 2019.

Modifiers also affect the thermal expansion coefficient (TEC) of glasses; when a glass coating has to be produced, the TEC of glass should match that of the substrate to prevent the glass pulling away from the base implant upon processing.[17] The TEC of 45S5 Bioglass® ($15 \times 10^{-6}$ °C$^{-1}$) is significantly higher than that of titanium alloys (about $9 \times 10^{-6}$ °C$^{-1}$) and alumina (about $8 \times 10^{-6}$ °C$^{-1}$), which are commonly used to fabricate orthopedic and dental implants: therefore, there has been the need for developing new glass formulations with a more suitable TEC for use as coating materials. In this regard, bioactive glasses belonging to the $SiO_2$–CaO–MgO–$Na_2O$–$K_2O$–$P_2O_5$ system have been widely investigated to match the TEC of the Ti6Al4 V alloy and alumina.[99,100] Partial replacement of $Na_2O$ with $K_2O$ and CaO with MgO was the most common strategy to design and adjust the TEC of the glass in a controlled way.[101,102]

Gonzalo-Juan *et al.*[103] also investigated the role played by alkaline/alkaline-earth modifiers on the injectability of bioactive pastes produced using a melt-derived CaO–MgO–$SiO_2$–$Na_2O$–$P_2O_5$–$CaF_2$ glass and two organic carriers (polyethylene glycol (PEG) and glycerol). While pure physical interactions were detected between PEG and the surface of the bioactive glass particles, chemical bonding was observed between glycerol and glass, enhancing the network cross-linking degree. Accordingly, the network of glycerol-glass paste was more condensed in comparison to that of PEG-glass and as-such glass because the fraction of $Q^2$ sites significantly decreased while the fraction of $Q^3$ units increased due to the covalent interaction between glass particles and the –OH groups of glycerol. Such chemical interactions between organic carrier and glass particles negatively affected the viscoelastic behavior of the pastes yielding to lower flowability of glycerol-glass composites as compared to the PEG-based counterpart.

**3.1.3 Effects on crystallization and mechanical properties.** As already mentioned, many bioactive glass products are

obtained by sintering of "greens", which may be associated to concurrent devitrification leading to glass-ceramic implants. In general, crystallization can have benefits and disadvantages. The motivation behind developing bioactive glass-ceramics is typically dictated by the mechanical limitations of bioactive glasses.[9] Kokubo *et al.*[104] developed the bioactive glass-ceramic A/W Cerabone, which is obtained from the crystallization of a glass in the $SiO_2$–$P_2O_5$–CaO–MgO–$CaF_2$ system and contains fluorapatite and wollastonite crystals. This glass-ceramic is mechanically stronger than other bioactive glasses (*e.g.* 45S5 Bioglass®) due to the inherently higher mechanical properties of crystals compared to the amorphous matrix, is able to bond to bone *in vivo* and can be machined to match anatomical sites.[104]

The presence of specific crystalline phases can be deliberately induced by properly modulating glass composition (including the content of ALAEs) and/or applying subsequent heat treatment. Phase diagrams are useful to predict if a melt of given formulation will originate either a fully amorphous glass or a partially-crystalline solid (glass-ceramic). For example, a proper amount of CaO was introduced in the A/W Cerabone in order to obtain wollastonite crystals in the final product. On the other hand, devitrification may compromise the material bioactivity: in this regard, 45S5-derived glass-ceramic is less bioactive than the parent glass since crystallization of a calcium-sodium silicate phase lead to a decrease of apatite-forming ability because of a decrement in the overall glass-ceramic reactivity, which is mainly associated to the amorphous phase.[105] Therefore, the achievement of a balance between bioactivity and mechanical strength must is a challenge.

The thermal properties of a glass determine the processing regime for bioactive glasses and glass-ceramics, while thermal properties are in turn dictated by glass composition. Controlled crystallization is key for tailoring the properties of glass-ceramics. Spontaneous crystallization, by contrast, caused by a pronounced tendency to devitrify during cooling of the melt, is undesirable because it prevents obtaining a bioactive glass in an amorphous state and makes it challenging – if not impossible – to control crystal phases, size and number *via* heat treatment. Bioactive glasses tend to crystallize easily during both cooling of the melt and heat treatment of a glass, and one of the main reasons is their low NC compared to conventional silicate glasses.[5,106] While many bioactive glasses show surface crystallization, the famous 45S5 Bioglass® shows both surface and internal crystallization, which does not allow viscous flow to take place for both bulk and powder sample, thereby impeding full densification.[107–109]

The sinterability window of 45S5 Bioglass® is extremely narrow and, thus, it cannot be sintered without undergoing devitrification.[110] This issue pushed scientists to develop glass compositions with appropriate dosage of modifiers and, thus, a larger sintering window allowing the fabrication of mechanically stronger product. For example, Fu *et al.*[111] prepared foam-replicated 13–93 glass scaffolds having a compressive strength of 18 MPa, which was significantly higher than that of 45S5 Bioglass®-derived glass-ceramic scaffolds (0.2–0.4 MPa (ref. 105)) with analogous porosity (85 vol%) and 3D trabecular architecture.

Studies on Biosilicate[112] showed that the glass-ceramic with 34% of crystalline volume exhibited much better mechanical properties than the parent glass, while the crystal size seemed to have a lower influence on mechanical performance. Fig. 5 highlights this change in flexural strength while also providing microstructural images of the glass-ceramics.[112] The type of crystal phases can also have direct influence on the mechanical properties of a glass-ceramic. This is particularly noticeable in apatite-containing glass-ceramics such as the apatite-wollastonite, apatite-mica or apatite-mullite systems,[113] where the function of the apatite phase was to provide bioactivity, while the other phase(s) provided mechanical strength. In the case of A/W Cerabone, wollastonite ($CaSiO_3$) strongly improves the compressive strength (up to 1080 MPa), flexural strength (up to 215 MPa), Young's modulus (118 GPa) and fracture toughness (up to 2 MPa $m^{1/2}$) in comparison to bioactive glass-ceramics without wollastonite crystals.

Machinability of glass-ceramics is also important in the production of orthopedic and dental implant. In the Bioverit glass-ceramics, machinability originates from the presence of mica crystals. While both Bioverit I and Bioverit II contain a mica crystalline phase, the mica crystals in the former show a morphology resembling flat flakes, while those in the latter have the shape of spherical lamellae, giving the crystals a cabbage-like appearance.[114,115] As a result, Bioverit II can be machined more easily than Bioverit I.

Finally, alkali-free bioactive glass-ceramics have also been developed within the CaO–MgO–$SiO_2$–$P_2O_5$–$CaF_2$ glasses and glass-ceramics composition, in a diopside ($CaMgSi_2O_6$) – fluorapatite ($Ca_5(PO_4)_3F$) – tricalcium phosphate ($3CaO{\cdot}P_2O_5$) join. Favorable results by sintering the glasses to appropriate density, leading to good bending strength have been reported. By opting for alkali-free compositions, it is possible to avoid the undesirable effects of alkali ions on the sintering and crystallization behaviors of the glasses. Moreover, the choice of a chain silicate mineral like diopside, exhibiting an elongated and interlocking morphology, contributed to the improvement of mechanical properties.[76,116,117]

### 3.2 Gel-derived glasses

Sol–gel technique is attracting an increasing attention due to the lower temperature required for the process compared to melting as well as the possibility to synthesize glasses with different shapes (bulk, powders, coatings...) and tune the composition, porosity and surface area.[118–120] In particular, the control and modulation of the composition of sol–gel glasses have been the subject of several works.[118,121] In fact, the introduction of specific elements can confer peculiar properties to the material.[24] However, several parameters, such as the type of used precursors, their amounts and ratio, have to be taken into consideration because they could affect the gel formation, the gelling time, or induce the precipitation of crystalline phases, which in turn can influence glass porosity and bioac-

 

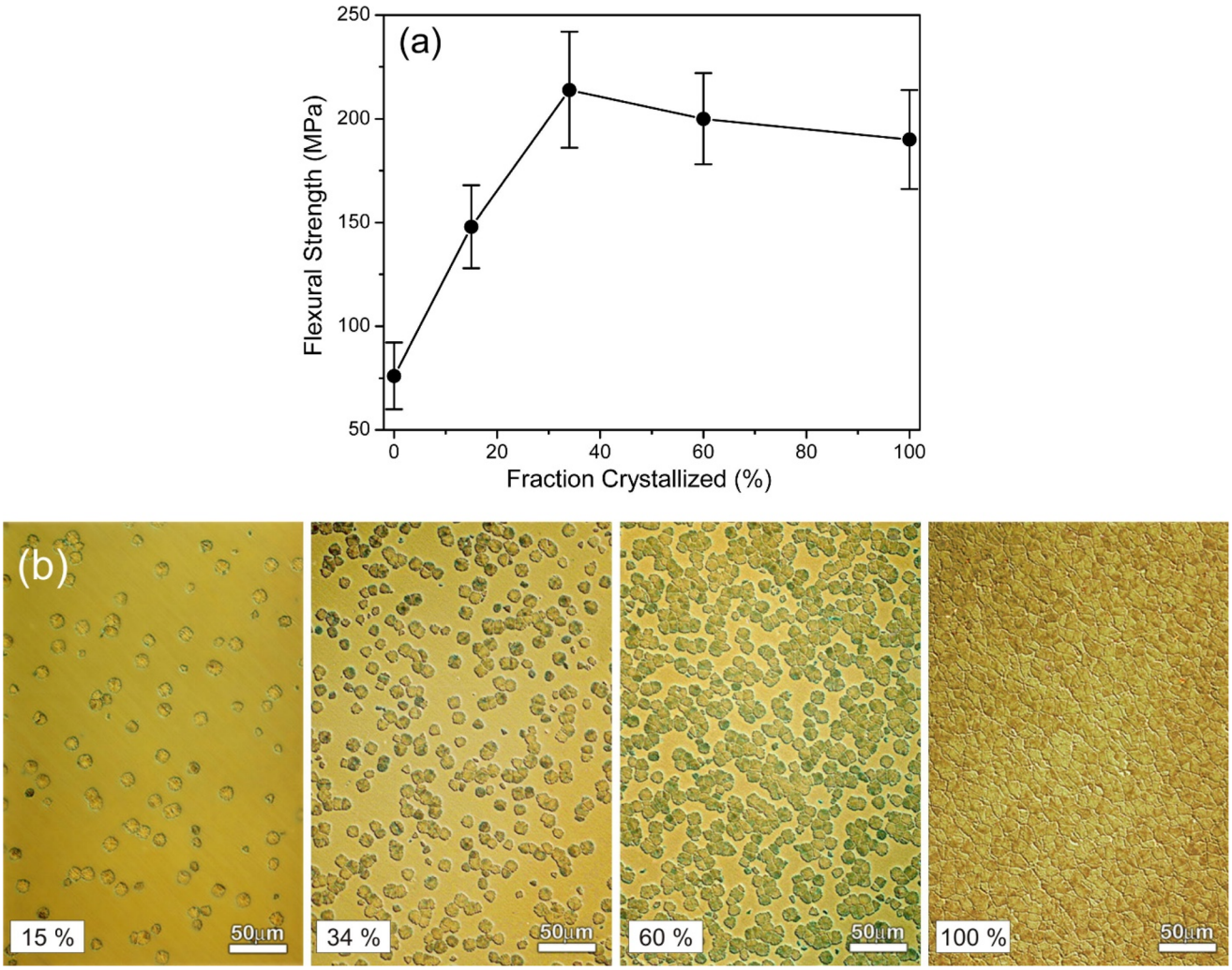


**Fig. 5** Effect of crystallization percentage on mechanical properties of a phospho-silicate combeite glass-ceramic. (a) Flexural strength *vs.* fraction crystallized during a multistage heat treatment schedule. (b) Optical microscope images showing microstructure examples of increasingly crystalized glass-ceramic samples. From ref. 112 with permission from Elsevier, 2001.

tivity. Among the elements present or introduced in the compositions of bioactive glasses obtained *via* sol–gel, some ALAEs have been reported to play a key role.

Calcium, for example, has always been present in bioactive sol–gel glass compositions, since the first $SiO_2$–CaO binary or $SiO_2$–CaO–$P_2O_5$ ternary systems[122–124] because of its intrinsic role in the bioactivity mechanism. Ca is conventionally introduced into sol–gel bioactive glass compositions as $Ca(NO_3)_2{\cdot}4H_2O$. However, some works highlighted a non-homogeneous distribution of Ca, particularly in large monoliths.[125,126] The cause of this inhomogeneity was attributed to the solubility of calcium nitrate in the pore liquor, which is a by-product of the condensation reaction during gelation and ageing steps. In fact, this liquid is eliminated during the shrinkage of the gel and evaporates during the drying phase. Therefore, calcium nitrate remains as a deposit on the external surfaces of the monolith and manages to enter and diffuse in a limited way only after the stabilization heat treatment.[126,127]

In order to improve Ca introduction in the glass network, several authors investigated different calcium sources, such as calcium chloride, calcium acetate, calcium hydroxide, calcium ethoxide or calcium methoxyethoxide (CME).[126,128–131] highlighting advantages and disadvantages in sol–gel glass processing. For example, some authors showed that the use of calcium alkoxides causes a rapid gelation of the sol.[131,132]

Bossard *et al.*[131] investigated in detail the mechanisms and precursors that allow the Ca incorporation into sol–gel silica-based bioactive glasses by means of low-temperature synthesis. They evaluated the Ca introduction in a bioactive glass composition ($75SiO_2$–25CaO wt%) employing different precursors, including $CaCl_2$, tricalcium dicitrate tetrahydrate, calcium acetate monohydrate, calcium ethoxide (CE – $Ca(OC_2H_5)_2$), or calcium hydroxide (CH – $Ca(OH)_2$). First of all, they estimated the influence of the pH on Ca incorporation, since previous studies have observed a good Ca introduction for samples using calcium alkoxides precursor (at pH > 2), whereas Ca was

not incorporated employing $CaCl_2$ or $Ca(NO_3)_2$ (at pH < 2).[126] However, the obtained results evidenced that the pH alone does not explain the Ca introduction: even if the pH was raised to 5 in all the syntheses, Ca was not incorporated using $CaCl_2$ or calcium citrate. The authors highlighted the significant importance of the basic property of the Ca precursor in the incorporation mechanisms. In fact, even if the pH of the sol was raised above the isoelectric point of the silicic acid, allowing the formation of $SiO^-$ and therefore the possible ionic-covalent bond with $Ca^{2+}$ ions, the nature of the $Ca^{2+}$ counterion – especially that of the neutral salts (*e.g.* $Cl^-$) – prevents the formation of a $SiO^-$–$Ca^{2+}$ bond. Therefore, Ca incorporation at low temperatures is allowed when strongly basic Ca precursors are used.

Among alkali metals, the successful incorporation of Na in sol–gel bioactive glass composition was achieved only recently. Indeed, all the sol–gel bioactive compositions belonging to 49-86S family contain $SiO_2$, CaO and $P_2O_5$.[122] The issue of introducing $Na_2O$ into the sol–gel composition is due to the high hydrolytic reactivity of sodium alkoxide in water. A first attempt to introduce Na in a phosphate-based sol–gel bioresorbable glass composition was performed by Carta *et al.*,[133] employing alkoxide precursors in an ethylene glycol solution and nitrogen atmosphere. Despite the good results obtained, this synthesis was discontinued, probably due to the technical difficulties and cost related to the highly-controlled environment required for the process.

Subsequently, other authors developed a new sol–gel-based protocol for the synthesis of $Na_2O$-containing bioactive glass-ceramics in aqueous solution under ambient conditions using $NaNO_3$ as Na precursor.[134] The adopted process allowed obtaining the complete decomposition of $NaNO_3$ for samples sintered at 1000 °C, with the consequent formation of $Na_2Ca_2Si_3O_9$ as a crystalline phase. $NaNO_3$ was adopted as Na precursor in different works[135,136] but some concerns recently arose about its use. In this regard, Chitra and co-authors[136] compared the role of sodium nitrate and sodium hydroxide in the synthesis and properties of $SiO_2$–$P_2O_5$–CaO–$Na_2O$ glass. Concerning the synthesis process, the authors evidenced a greater Na introduction when $NaNO_3$ was employed as Na source and an increased tendency to crystallize. According to the authors, this result, together with that obtained from the evaluation of the bioactivity and biological testing, suggested that sodium nitrate was a preferable precursor to develop bioactive and stable bioactive glass *via* sol–gel.

As regards mesoporous sol–gel glasses, Kumar *et al.*[137] in 2017 developed for the first time a mesostructured 45S5 bioactive glass by acid-assisted sol–gel method followed by evaporation-induced self-assembly process, using sodium acetate and calcium acetate hydrate as sodium oxide and calcium oxide sources, respectively. They obtained a fully amorphous glass with a mesoporous structure; however, the amount of alkali/alkaline earth elements was significantly lower (especially Na) than the theoretical composition.

Magnesium[138–141] and potassium[142,143] have also been introduced as nitrates in sol–gel compositions. However, no particular effects on the glass synthesis process were found in the analyzed studies, which mainly concerned the investigation of structure, bioactivity and biological behavior. For example, Fiume *et al.*[141] synthesized a complex composition *via* sol–gel ($SiO_2$–$P_2O_5$–CaO–MgO–$Na_2O$–$K_2O$) and highlighted that the introduction of several alkali/alkaline earth elements in the system, together with the need to perform a calcination treatment at high temperature to remove the nitrates used as precursors, produced the partial crystallization of the material. The formation of crystalline phases affected the specific surface area (SSA), which decreased by increasing the calcination temperature and was significantly lower than the SSA of simpler sol–gel compositions.

The formation of crystalline phases during heat treatments in sol–gel processes can affect both the SSA and the formation of an ordered mesoporosity. As previously noted, very complex compositions and the use of reagents, such as nitrates, which require high temperatures to be removed, often induce the formation of crystalline phases. However, some authors[143] proposed a new sol–gel method to synthesize a 45S5-derived glass with high amount of CaO (45.6 mol%), partial/complete substitution of sodium oxide with potassium oxide, and crystallization temperature pushed to 900 °C. These authors observed that by varying the content of alkaline elements, and in particular by using the highest potassium amount, it was possible to avoid crystallization phenomena. This allowed producing amorphous materials even with a calcination temperature of 850 °C.

Recently, the introduction of lithium in sol–gel glasses has also been investigated[144–147] due to its role in bone regeneration and osteochondral tissue. Different Li precursors were used, including lithium citrate, lithium chloride and lithium nitrate; however, the influence of lithium in the sol–gel process was little explored. Moghanian and co-authors evidenced a lower cross-link density and lower crystallization temperature for the Li-containing bioactive glass, and consequent higher stability of the glass structure. These results can be ascribed to the smaller ionic radius of lithium and the different coordination number in comparison with calcium. Maçon *et al.*[145] investigated the role of lithium nitrate and lithium citrate as precursors in the synthesis of binary sol–gel glasses ($90SiO_2$–$10Li_2O$ mol%) by adding the precursors in an acidic solution of hydrolyzed tetraethylorthosilicate (TEOS). They observed a different gelation time using the two precursors: the sol containing lithium citrate gelled in 1 h, while the sol containing lithium nitrate gelled in 3 days. These differences were ascribed to the pH increase (up to 5.3, above the isoelectric point of silicic acid) for the solution containing the lithium citrate (due to the release of citric acid) and the consequent change of the kinetics of the silica network condensation. The authors also underlined that that the use of lithium citrate allowed obtaining mesoporous glass containing lithium as a network modifier, due to the lower decomposition temperature of citrate; on the contrary, a dense glass-ceramic was obtained using lithium nitrate since the complete nitrate decomposition generated lithium metasilicate. As already

 

observed for Ca, the use of nitrates involves high calcination temperatures which can induce the formation of crystalline phases.

Finally, several articles can also be found in the literature concerning Sr-doped sol–gel glasses.[148–154] Usually, Sr substitutes Ca because they have the same valence and similar ionic radius; also, both elements should act as network modifiers. Sr is introduced in sol–gel glass composition again mostly as nitrate; however, some studies reported the use of Sr chloride as a precursor.[153] The composition of the obtained glasses often differs from the theoretical one and has a lower Sr content.[148] This is due – like for Ca-containing glasses – to the use of nitrates, which allow the Sr incorporation only by ion diffusion during the heat treatment; furthermore, some of the strontium nitrate is removed during washing steps performed before the heat treatment. The introduction of Sr has no effect on the morphology of the obtained glass (in the case of particles), but a decrease in the oxygen density and an increase in the size of the particles were observed due to a slightly larger ionic radius of Sr compared to Ca, which produces an expansion of the glass network.[148,149]

In another work, Taherkhani *et al.* investigated the effect of the substitution of Sr for Ca in a mesoporous sol–gel bioactive composition.[154] They evidenced a tendency to crystallize with increasing Sr amounts and the consequent formation of $Sr_2SiO_4$. The performed BET analyses showed mesoporous texture with a high specific surface area; however, the influence of Sr and the resulting crystallization on the specific surface area was not investigated in detail.

Recently, other alkali and alkaline earth metals have been introduced in sol–gel bioactive glass compositions. For example, Ouyang and co-workers evaluated the effect of rubidium addition (0, 1, 3 and 5 at%) into a bioactive $SiO_2$–CaO sol–gel glass.[155] The incorporation of Rb did not affect the synthesis process as well as the glass size, shape and structure. However, its amount incorporated in the glass was lower than the theoretical one; the authors explained this difference highlighting that, also in this case, the metallic ions diffuse into the silicate matrix only after the heat treatment and the washing step performed before the treatment could remove the Rb precursor. Moreover, the larger radius of Rb as compared to Ca decreased its affinity with Si and the repulsion interaction between Ca and Rb ions (both positively charged) could further decrease its adsorption.

Majumdar *et al.* investigated the introduction of barium on 45S5 sol–gel glass nanoparticles ($44.85SiO_2$–$2.6P_2O_5$–$24.3Na_2O$–26.9CaO–1.35BaO mol%). Doping with Ba had no influence on the synthesis process: the obtained glass had a mesoporosity similar to 45S5 and a slightly lower specific surface area.[156]

### 3.3 Processing of composites and hybrids

The introduction of alkali or alkaline earth ions into glass compositions and their effect on the synthesis process can also affect the design of composite and hybrid materials. In particular, hybrid organic/inorganic systems consist of interpenetrating nanoscale networks of silica and biodegradable polymers and can be produced by introducing a polymer in the sol–gel process. The presence of alkali or alkaline earth elements in sol–gel glasses and, above all, the use of specific precursors of these elements can significantly influence the synthesis process of the hybrid material.

Ca, for example, has been introduced in the hybrids as calcium nitrate[157] but, as previously discussed, high temperatures for removing nitrates must be reached, which are not compatible with the synthesis of hybrids. For this reason, a different Ca source is needed for hybrids.

In this context, different authors[126,132] investigated the role of calcium precursors in the sol–gel synthesis of hybrids. The comparison was performed among calcium methoxyethoxide (CME), calcium chloride and calcium nitrate, investigating also the temperature at which Ca is incorporated into the 70S30C ($70SiO_2$–30CaO mol%) network. These studies evidenced that, by using calcium nitrate, Ca enters the glass network only above 400 °C, while calcium was not incorporated at any temperature if calcium chloride was adopted. Instead, the use of CME permitted the incorporation of Ca at very low temperature (about 60 °C), which is useful for the synthesis of hybrids.[126] In fact, CME as Ca precursor allowed a fast gelation in the mixed organic/inorganic mixing system without using hazardous catalysts, such as hydrofluoric acid, and a better Ca incorporation.[132] Moreover, the Ca source strongly influenced the dissolution rate of hybrid system, since CME allows effective cross-linking of the polymer and a consequent steady release, while a sudden release was observed by adopting $CaCl_2$ as a precursor. Also, Lao *et al.*[158] have recently developed a simple process at room temperature for synthesizing bioactive glass/gelatin hybrid scaffolds. In this work, Ca $(OEt)_2$ was used as the calcium source and the gelling of the hybrid solution was delayed to 2 hours using a water/TEOS molar ratio reduced by two under diluted conditions. These studies represented an advancement in the synthesis of organic–inorganic hybrid scaffolds at room temperature.

The problem of the heat treatment necessary to incorporate ions starting from nitrates into the sol–gel glasses does not arise if composite materials are produced. However, some studies have highlighted the effects of alkali/alkaline earth ions on the properties of the obtained composites. For example, in a recent study[150] some authors evaluated the influence of Sr incorporation in sol–gel bioactive glasses in chitosan/alginate/strontium-doped glass composite scaffolds. They highlighted how the presence of Sr in the glass improved the mechanical properties of the scaffolds. In fact, it was observed that Sr, after substituting Ca, increased the number of interactions in the glass bonding network due to the higher ionic radius. On the other hand, no effects of the replacement of Ca with Sr were found in terms of degradation profile and scaffold swelling. On the contrary, Jalise *et al.*,[159] evidenced an accelerated degradation rate for Sr-modified bioactive glass nanoparticle/gelatin scaffolds, and they ascribe this behavior to the larger ionic radius of Sr that favors the disorder of the glass lattice.

# 4. Physiological role of ALAEs

An understanding of the natural function of ALAEs in biological systems is critical in unlocking the breath of applications in bioactive glasses. Elements such as sodium, potassium, and calcium exist at high concentrations in the body, while lithium, strontium, and barium exist at very trace amounts. Table 1 provides the concentration of the many inorganic including some ALAEs and ions in the body. This following section discusses the physiological role of each alkali and alkaline earth element as well as reviews *in vitro* and *in vivo* studies showing the biological impact of BGs.

Compared to transition metals, ALAEs do not have as versatile physiological effects, but are almost always more biocompatible. As shown in Table 1, most of the ALAE elements are abundant in the body while the transition metals or rare earth elements are not. However, in order to achieve expression of certain proteins, exhibit strong antibacterial effects, or create reactive oxygen species, the addition of transition metals may be required. Table 2 provides a comparison of the ALAE elements with several other species most commonly studied in BGs.

## 4.1 Alkali Elements

### 4.1.1 Lithium

*4.1.1.1 Biological activity.* Lithium is the smallest of the alkali ions yielding the highest charge density and lowest coordination numbers for complex binding.[296] Although there is no known natural biological role of lithium, this element has been implicated in a variety of biomedical processes ranging from pharmacological treatment of neuropsychiatric disease and cytopenia to regenerative medicine applications of angiogenesis and neuron regeneration.[160,297]

Oral lithium carbonate is approved for the treatment of bipolar disorder.[298] The mechanism of action has still to be definitely elucidated; however, lithium was revealed to produce a myriad of both excitatory and inhibitory effects at the cellular level.[299–302] The glycogen kinase synthase 3 beta (GSK-3B) pathway has been implicated with bipolar disorder and as a possible therapeutic target of lithium. It has been proposed that lithium inactivates apoptotic activities in the GSK-3B pathway serving as a neuroprotective agent. Other therapeutic targets investigated include mitochondrial proteins, glutamatergic neurons, and circadian rhythm proteins.[300,301]

The therapeutic potential of lithium does not end with neurosynaptic disorders, but this small alkali metal has been heavily investigated in the treatment for granulocytopenia.[303] Granulocytes, such as neutrophils, basophils, and eosinophils can become depleted as a result of a variety of insults including strong chemotherapeutics.[304] Lithium has been shown to boost the production of such cells.[305–307]

Lithium salts have been investigated in angiogenesis to determine a possible role in tissue engineering applications. Guo *et al.* investigated the effects of millimolar concentrations of lithium chloride on human brain endothelial cells and rat astrocytes. They illustrated that lithium ions, when compared with sodium ions, promoted higher phosphorylation of GSK-3B and increased expression of Vascular Endothelial Growth Factor (VEGF).[308] VEGF is a key protein in angiogenesis, *i.e.* the growth of new blood vessels.[309] Furthermore, lithium has been explored for a possible role in axonal regeneration. Lithium initiates all key cellular processes in axonal regeneration including inhibiting GSK-3, expression of c-JUN, expression of BCL-2, and induction of angiogenesis.[308,310–312] The combined effects of these cellular processes have been illustrated in a variety of animal models.[313,314]

Another key aspect related to biological effects of lithium lies in the interplay between similar chemical species, mainly sodium and magnesium. Sodium is a monovalent alkali ion with the most similar size to lithium while magnesium, although being divalent, has a size comparable to lithium. It has been shown that lithium can compete with sodium and magnesium for various substrate binding sites and ion channels.[299,300] For example, lithium can inhibit several magnesium-dependent proteins including G proteins, inositol monophosphates, and bisphosphate 3-prime-nucleotidase.[299,315–317]

Administration of lithium can carry a set of negative consequences. By mimicking alkali and alkali earth metals, lithium can cause damage to a variety of organ systems. Chiefly, lithium will utilize sodium transporters in the kidney to increase its own reabsorption. Therefore, in states of dehydration and increased expression of sodium transporters, excessive lithium will be reabsorbed creating higher serum levels of lithium that perpetuate lithium toxicity.[318,319] Lithium is also known to induce nephropathy causing kidney failure; however, the mechanism of action has still to be clearly elucidated.[320] Through competing with calcium, lithium can block calcium receptors in the parathyroid leading to hyperparathyroidism.[321] Lastly, lithium can displace potassium in myocardial tissue, possibly leading to arrhythmia.[322] Nonspecifically, lithium has been associated with psoriasis, hair loss, tremor, nausea, and diarrhea.[298]

*4.1.1.2 BG evidence.* Khan *et al.* studied the *in vivo* biological properties of binary doping of lithium and strontium into

**Table 1** The main elements that compose the human body molecules (including water). Na, K, Ca, Mg and P which are the main components of BGs are among these elements

| Element | Symbol | Percent mass | Percent atoms |
|---|---|---|---|
| Oxygen | O | 65.0 | 24.0 |
| Carbon | C | 18.5 | 12.0 |
| Hydrogen | H | 10 | 62.0 |
| Nitrogen | N | 3.2 | 1.1 |
| Calcium | Ca | 1.5 | 0.22 |
| Phosphorus | P | 1.0 | 0.22 |
| Potassium | K | 0.4 | 0.03 |
| Sulfur | S | 0.3 | 0.038 |
| Sodium | Na | 0.2 | 0.037 |
| Chlorine | Cl | 0.2 | 0.024 |
| Magnesium | Mg | 0.1 | 0.015 |
| All others | | <0.1 | <0.3 |

 

**Table 2** Comparison of alkali and alkaline earth elements to other relevant species when added to bioactive glasses

| Class | Species | Positive interaction | Negative interaction | Ref. |
|---|---|---|---|---|
| ALAEs | Li | - Increases osteoblast cell activity<br>- Increases angiogenesis through Wnt/β-catenin pathway and TGFβ | - Toxic in high concentrations | 145, 146, 160 and 161 |
| | Na | - Encourages dissolution through NBO creating | - Drastically increases pH during dissolution | 45 and 46 |
| | K | - Encourages dissolution<br>- Improve glass forming ability and cause mixed alkali effect | - Increases pH during dissolution | 162–165 |
| | Mg | - Highly cyto-compatible<br>- Increases cell attachment<br>- Increases osteogenic differentiation<br>- Increases expression of collagen type 1, alkaline phosphatase, and osteocalcin | - Decreases solubility<br>- Slows dissolution kinetics<br>- Decreases rate of calcium-phosphate layer precipitation | 93, 116 and 166–168 |
| | Ca | - Stimulates expression of angiogenic genes: VEGF and bFGF<br>- Encourages HAp layer precipitation<br>- Regulates homeostasis; specifically factors II, VII, IX, X, and XI | | 10 and 169 |
| | Sr | - Improves osteogenesis<br>- Inhibits osteoclastogenic action<br>- Anti-bacterial | | 156 and 170–181 |
| | Ba | - Anti-inflammatory through reduction of LPS-induced elevation of interleukin-6 (IL-6)<br>- Potential for magnetic hyperthermia cancer treatment applications | - Toxic in high concentrations | 156, 182 and 183 |
| Transition metals | Ti | - Minimal effect on pH during dissolution<br>- Increases mechanical properties of glass<br>- Increased ALP activity<br>- Increases dentin remineralization in dental applications | - Decreases dissolution rate of glass through strengthening glass network<br>- High $TiO_2$ concentrations required for antibacterial activity | 184–187 |
| | V | - Luminescent on 450–800 nm range<br>- $V^{5+}$ initiates early stages of wound healing<br>- *In vivo* studies show significant increase in wound vascularization and bone mineralization | - Extensive studies on cytocompatibility still required<br>- Oxidation state of dissolution product may have different effects<br>- Only small compositions studied (up to 3 mol%) | 188–192 |
| | Mn | - Stimulates expression of osteogenic genes: ALP, type 1 collagen, osteocalcin, bone morphogenetic proteins, and sICAM-1<br>- Magnetic properties with potential for magnetic hyperthermia<br>- Antibacterial against several bacterial strains | - Concentrations limited to below 5 mol% (less than 0.1 mg $ml^{-1}$) for long term biocompatibility | 193–199 |
| | Fe | - Magnetic properties with potential for magnetic hyperthermia<br>- Potential for Fenton reaction therapy<br>- Improved HAp formation | - Cytocompatibility requires more study | 200–204 |
| | Co | - Stimulates expression of angiogenic genes: VEGF, HIF-1α, and ALP<br>- Increases ESM deposition<br>- Radiopaque | - Cytotoxicity at moderate concentrations (above 10 ppm)<br>- Can be carcinogenic and genotoxic through various pathways<br>- Hinders osteogenic actions | 205–210 |
| | Ni | - Increase mechanical strength | - Limited bioactive role<br>- No effect on mineralization | 211–213 |
| | Cu | - Stimulates expression of angiogenic genes: VEGF, HIF-1α, ALP. bFGF, and PDGF<br>- Antibacterial against a wide variety of strains<br>- Anti-inflammatory through over-expression of interleukins<br>- Promising for applications in photothermal therapy | - Some studies show toxicity at low concentrations (1 μm $mL^{-1}$) | 38, 201, 209 and 214–220 |
| | Zn | - Increases osteopontin expression to facilitate osteogenesis<br>- Antibacterial against several bacterial strains<br>- Encourages enamel remineralization<br>- Antimicrobial | - Large pH changes upon dissolution (composition dependent) | 215 and 221–227 |
| | Zr | - Increases mechanical properties<br>- Radiopaque | - Decreases dissolution rate of the glass | 228–231 |
| | Ag | - Antibacterial against several bacterial strains | - Can show cytotoxic properties with concentrations varying from 1–20 mg/mL | 232–240 |
| | Au | - Antibacterial against Gram positive and Gram negative bacteria<br>- Does not hinder hydroxyapatite formation | - Studies limited to Au-nanoparticle composites<br>- Au nanoparticle concentrations studied are very low (0.15–0.2 mol%) | 241 and 242 |

**Table 2** *(Contd.)*

| Class | Species | Positive interaction | Negative interaction | Ref. |
|---|---|---|---|---|
| Metalloids/ non-metals | B | - Encourages precipitation of HAp<br>- Stimulates expression of angiogenic gene VEGF<br>- Encourages increased secretion of ECM<br>- Early stage research shows promise for axon regeneration | - Inhibits the proliferation of bone marrow stromal cells if B concentration is >0.65 mM | 4, 78, 189 and 243–248 |
| | Si | - Primary component of most BGs<br>- Stimulates expression of osteogenic genes: ALP, BSP, BMP, collagen type 1<br>- Stimulates expression of osteogenic genes VEGF, ECM | | 249–253 |
| | P | - Stimulates expression of osteogenic genes matrix Gla protein (MGP) for bone<br>- Stimulates expression of angiogenic genes: VEGF and FOXC2<br>- Enhances precipitation of HAp | | 63, 63, 106, 245 and 254–257 |
| | Ge | - Encourages precipitation of Hap | - Limited studies available with *in vitro* or *in vivo* specific results | 258–260 |
| | Te | - Strong antibacterial effects against Gram positive and Gram negative strains<br>- Alkali-tellurite glasses able to form CaP layer at surface during dissolution | - In depth *in vitro* and *in vivo* studies required to ensure biocompatibility and long term efficacy | 261 and 262 |
| Post-transition metals | Al | - Increases mechanical properties of glass | - No major biological properties<br>- Some compositions show cytotoxic effects | 263–267 |
| | Ga | - Excellent antibacterial properties<br>- Produces reactive oxygen species | | 191 and 268–273 |
| | Bi | - Radiopaque<br>- Photothermal effect for cancer therapy<br>- Antibacterial effect<br>- Antifungal properties | - Hinders HAp formation at higher concentrations<br>- Higher concentrations less effective than un-doped compositions in cell proliferations (<5 wt%) | 274–277 |
| Rare earth elements | Ce | - Antioxidant activity through reduced oxygen speies<br>- Can be antibacterial at higher concentrations<br>- Either no effect or positive effect on cell viability in most studies | | 191, 269, 273 and 278–285 |
| | Eu | - Strong luminescence under ultraviolet radiation<br>- Stimulates expression of osteogenic genes: ALP, OCN, OPN and COL-I in BMSC cells<br>- Stimulates expression of angiogenic genes: CD31, MMP9, VEGFR1/2 and PDGFRα/β of HUVEC cells | | 286–288 |
| | Gd | - Activates Wnt/β-catenin signaling pathway<br>- Stimulates expression of osteogenic genes: OCN and BSP | - Decreases dissolution kinetics of the glass<br>- Slightly lower cell viability than glasses containing no rare earth elements | 287 and 289–291 |
| | Sm | - Increases dissolution rate of glass<br>- Makes local pH during dissolution more stable<br>- Strong luminescent properties in visible wavelengths | - Biocompatibility as function of Sm concentration not thoroughly studied | 292–295 |

boro-silicate BG composition ($10.9Na_2O$–$23.9CaO$–$1.5TiO_2$–$1.4B_2O_3$–$1.7P_2O_5$–$56.3SiO_2$, by mol%).[323] Lithium-containing BG (L-BG) was doped with 0.25 mol% $Li_2O$ and the binary doped strontium and lithium-containing BG (LS-BG) was doped with 0.3 mol% $Li_2O$ and 1 mol% SrO. The BG powders were isostatically cold-pressed in the form of implantable scaffolds. Preliminary bioactivity testing and *in vitro* characterization showed promising results for both doped samples. Crystallization of HAp was decreased through lithium doping in the bioactivity studies. However, both L-BG and LS-BG showed greater concentrations of bi-carbonates and bi-phosphates in the supernatant from static dissolution studies. This was argued to increase cell proliferation through the synthesis of extracellular matrix and collagen *in vitro*. All doped BGs showed accelerated cell proliferation rates as compared to the base glass composition with L-BG showing the highest performance. BG scaffolds were then implanted into sixteen New Zealand White rabbits. Chronological radiographs were taken immediately after implantation and once a month up to four months. Intermuscular injections of fluorochrome were administered prior to sacrificing in order to quantify new bone growth along with micro-computed tomography to assess bone-in growth into the scaffolds. L-BG showed increased bone growth as compared to the base glass with enhanced defect healing. LS-BG radiographs show narrowing of the bony defects and shrinking of the implant at one month post operation. Fig. 6 shows images of the radiographs taken monthly post operation for each of the implanted compositions. Histological evaluations after 4 months showed that doped samples had significantly increased levels of angiogenesis. Specifically, lithium-doped BG samples had higher proliferation of osteoblasts and angiogenic proliferation. Finally, the researchers concluded that lithium, even at small doping levels, had a profound effect on bone healing, better implant

 

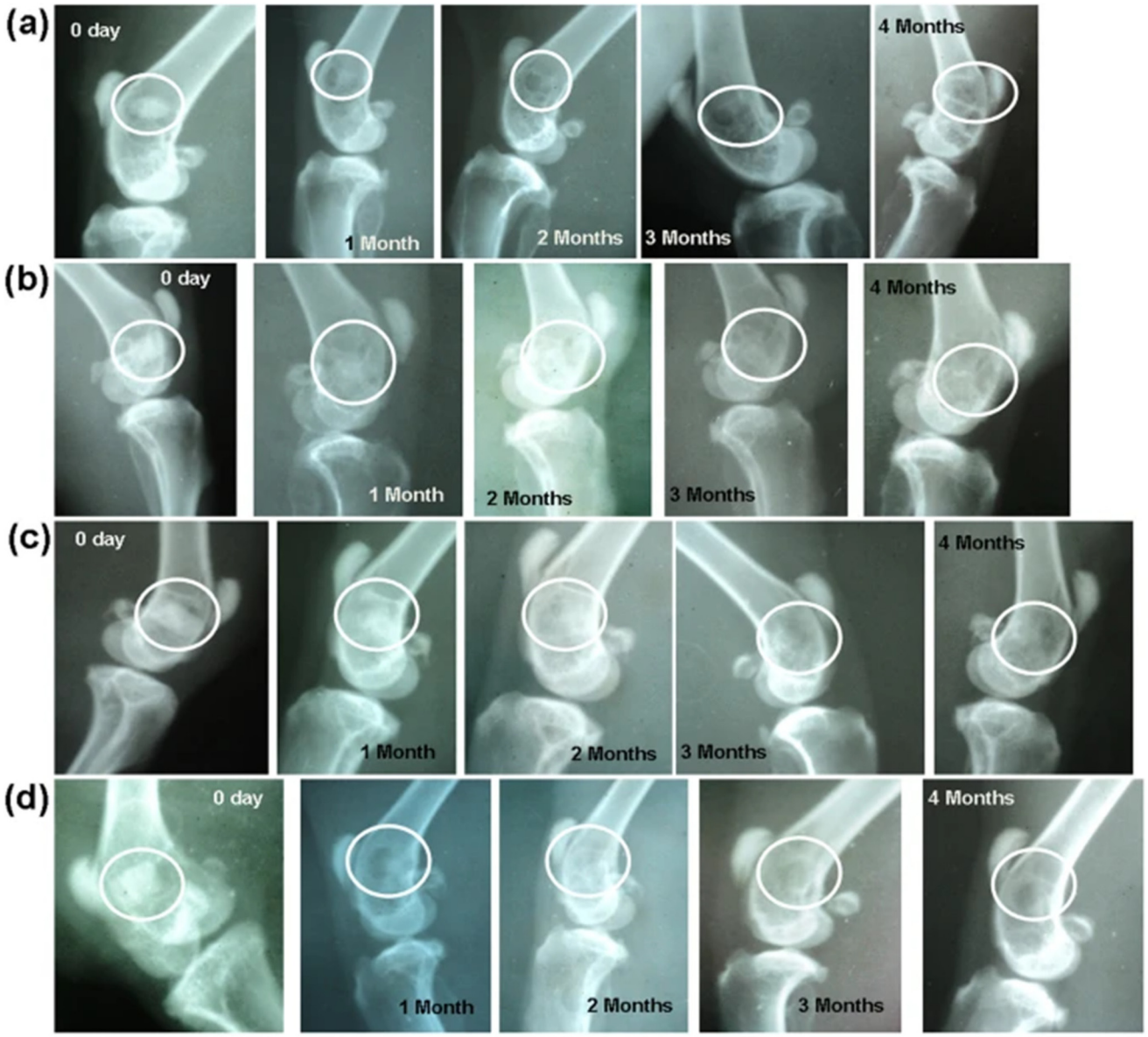


**Fig. 6** Radiographs taken after implantation and after 1, 2, 3 and 4 months post-operation. BG compositions implanted are (a) BG, (B) L-BG, (c) S-BG, and D (LS-BG). From Khan *et al.*[323] with permission from Springer Nature, 2016.

anchorage, and formation of new blood vessels. However, further studies are recommended to determine the exact mechanisms that $Li^+$ has in the early stages of dissolution.

A study published in 2017 specifically investigated the angiogenic nature of lithium-containing BGs.[324] The 45S5 composition was doped with 5 mol% $Li_2O$ directly replacing $Na_2O$. The effect of the dissolution products from both 45S5 and 45S5.5Li were evaluated *in vitro* through a series of tests including cell proliferation assay, cell migration assay, cell transmigration assay, enzyme linked immunosorbent assay, and determination of β-Catenin by Western Blot. Greater cell proliferation was shown with the dissolution products containing $Li^+$, however both BG compositions showed increased performance due to proangiogenic ions such as silicon. Migration studies showed a significantly increased performance of lithium containing 45S5 with 28 ± 2% gap closure after 8 hours as compared to roughly 20% closure for 45S5. The tubulogenesis assay demonstrated $Li^+$ role in angiogenesis with nearly twice the number of endothelial tubules formed after 8 hours. Western blot analysis confirmed that lithium-containing 45S5 expressed β-catenin, a canonical pathway aiding in the accelerated activation of transcription factors. Ultimately, lithium doping of BGs was determined to provide therapeutic effects with an emphasis on accelerating angiogenic factors.

Above report indicated that Li-incorporated 45S5 BG could directly stimulate HUVEC behavior *in vitro* by activating the canonical Wnt/-catenin pathway,[28] however, the detailed intra-

cellular mechanisms involved in Li-mediated angiogenesis and its stimulatory effect on *in vivo* blood formation were studied later by Liu *et al.*[325] They tried to delve deeper into the mechanisms behind lithium-mediated angiogenesis and investigated the pro-angiogenic potential of 3D printed Li-incorporated glass-ceramic scaffolds (based on the 45S5 composition) using an *in vivo* ectopic angiogenesis model. The angiogenic nature of lithium-containing BGs was evaluated through the specific monitoring of cell derived exosomal miR-130a.[325] A comprehensive *in vitro* study was used to deeply understand the performance and genetic activation of a lithium doped bioactive glass ceramic. *In vitro* experimentation shows lithium provides increased activation of Wnt/β-catenin, AKT, and NF-κB signaling pathways. $Li^+$ dissolution also was shown to increase expression of proangiogenic factors secreted from bone marrow stromal cells. When incorporated into 3D printed scaffolds, Li-containing BGs were shown to accelerate vascularization in bone regeneration for large-sized defect repair.

### 4.1.2 Sodium

*4.1.2.1 Biological role.* Sodium, the most abundant alkali ion in extracellular fluids, has a multitude of physiological roles at various system levels.[326] It controls blood volume and pressure in the circulator system, osmotic gradient, electrical activity, and nutrient absorption on the cellular level, and mediates protein function on the subcellular.[327]

Sodium exerts a massive influence on osmotic pressure in the vascular system. As the most plentiful ionic species in blood plasma, tight regulation of sodium is critical for control of blood volume and blood pressure.[326–328] For example, activation of the Renin-Angiotensin-Aldosterone-System (RAAS) can induce reabsorption of sodium in the nephrons of the kidney. As a result, the increased osmotic pressure in the nephron pulls water back in the vascular space, thereby decreasing urinary excretion and maintaining blood volume.[329]

On the cellular level, sodium aids the creation and maintenance of cellular membrane electrical potentials. Membrane potential arises from the Nernst potential that balances the diffusion force from the concentration gradient across the ion semi-impermeable phospholipid membrane.[330] To generate such concentration gradients, active transport through the sodium–potassium pump is required. Utilizing adenosine triphosphate (ATP) as an energy source, the cell membrane protein will take 3 sodium ions from the intracellular space to the extracellular space, against the concentration graduation. Simultaneously, 2 potassium ions will be taken from the extracellular space into the intracellular space. The net exchange will yield a charge differential between the internal and external side of the cell membrane, thereby creating a membrane potential.[330,331] An illustration of this process is provided in Fig. 7 visualizing the ion movement across the membrane. The membrane potential is a key aspect of neuron excitability and has been found to play a role in other biological phenomena such as fertilization, kidney function, cell growth, and wound healing processes.[332,333]

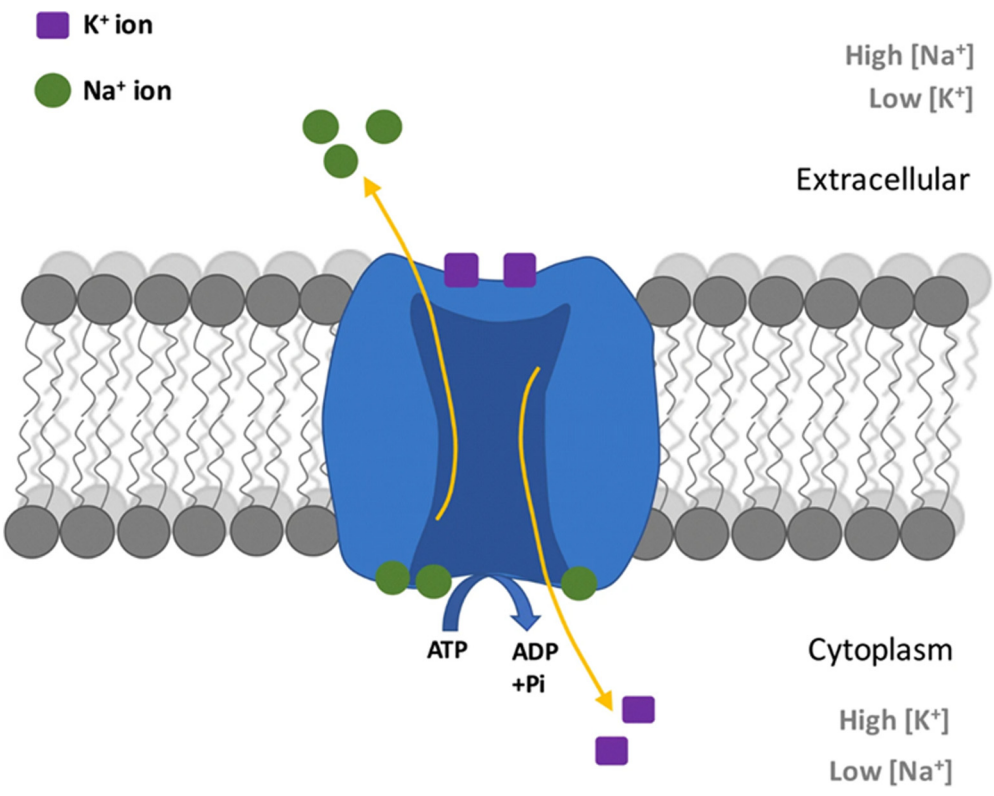


Fig. 7 Diagram showing process in a $Na^+/K^+$ pump in neurons caused by action potential. From ref. 334 with permission from Springer, 2019.

 

Another function of sodium is in the nutrient absorption. Cotransport occurs when two separate species are required for passage through a cell membrane transporter. In the digestive tract, sodium is required for absorption of key nutrients such as glucose and amino acids.[332,335] Furthermore, in cells throughout the body, sodium is required for not only nutrient uptake but also mineral transport. Sodium-dependent transport of chloride, calcium, and magnesium are all critical for maintaining homeostasis.[335]

Sodium forms a small, mobile ion that typically complexes with a coordination number of 6. The ion interactions with biomolecules have been shown to be critical for biological activity.[296] For example, the protein thrombin was found to be most active in the presence of sodium chloride solutions when compared to lithium chloride, potassium chloride, and rubidium chloride.[336] These findings imply a high level of specificity between the substrate protein and sodium ions. Although integrations of sodium ions and biomolecules exist and influence biology, it is important to note that such interactions are up to 4 orders of magnitude lower than those of divalent alkali earth ions.[296,337]

As illustrated above, sodium is essential for a large variety of physiological functions; however, too much sodium can be deleterious at the whole organism level. High amount of sodium in the blood (or hypernatremia) can lead to increase blood volumes causing high blood pressure. High blood pressure can cause heart failure or arterial damage in the heart, eyes, or kidney.[338] Transiently, hypernatremia can cause intense thirst, lethargy, and decreases reflexes.[339]

*4.1.2.2 BG evidence.* Sodium is a major component of many biomaterials formulations as well as most of experimental and commercial BG compositions. Sodium plays important roles in wound dressings such as sodium alginate and sodium carboxymethyl cellulose.[340,341] However, the specific biological role of sodium in BG has not been studied extensively yet. 45S5 Bioglass® has been characterized comprehensively for its influence on cell proliferation and gene expression with no direct evaluation of the role of sodium ions.

Wallace *et al.* studied the biological response of BGs with varying sodium content to establish sodium role in gene expression and cell proliferation.[45] The base BG composition used a general soda-lime phosphosilicate system ($3SiO_2$–$0.07P_2O_5$–$(3–x)CaO$–$xNa_2O$, by mol%) with constant network connectivity of 2.04 with varying $Na_2O$ content from 0–30 mol%. The glasses were pressed and sintered into disks at appropriate firing temperatures. Bioactivity testing showed that increased sodium content yielded rapid dissolution along with rapid pH increases. The result of the rapid pH increase inhibited cell proliferation as well as significantly lowered protein generation. Cell viability test and total protein assay suggested that the optimal concentration of sodium in the above-studied BG composition was roughly 7.5 mol% from a biological viewpoint. This study brought awareness to the potential cytotoxic effect of high sodium content and challenged the mainstream development of sodium-rich BG compositions, which were considered favorable from the viewpoints of processing (*e.g.*, need for lower sintering temperature) and apatite-forming ability.

### 4.1.3 Potassium

*4.1.3.1 Biological role.* As a relatively small monovalent cation, potassium acts similarly to sodium; however there are distinct differences in their physiological roles. While sodium is maintained in high levels in the extracellular space, potassium is maintained in high levels in the intracellular space. The relative gradients help maintain neutral overall osmotic gradients between cells and extracellular fluid, thereby avoiding excessive influx and efflux of water into/from cells.[332,337] These gradients are maintained by the ATP hydrolyzing sodium–potassium pump as mentioned previously. Potassium is also critical to electrochemical tissue properties and protein function.

One of the differences between the roles of sodium and potassium arises in their relative activity in excitability tissues. The resting membrane potential is created by the sodium–potassium pump, but specific ion channels can perturb that potential. When sodium channels open, sodium will flow down the concentration gradient and rush into cells causing the once negative resting potential to shoot up or depolarize, thus becoming slightly positive. This is the first step in an action potential, or the electrochemical manner in which neurons transmit signals. The next step is the closure of sodium channels and opening of potassium channels. Similar to sodium, the positively charged potassium ions will follow their concentration gradient; however, they will flow out of the cell causing the potential at the cell membrane interior to become negative once again or repolarize.[333,342] Although being chemically similar in terms of relative mobility and charge, potassium and sodium have opposite effects on membrane potential. Similar activity can be seen in other excitable tissue such as cardiac muscle and pacemaker cells.[343]

Potassium ions can also form complexes with biological molecules just as sodium does. However, potassium ions are larger and thereby facilitate large coordination numbers spanning from 6 to 8.[296] Due to these differences, potassium will interact with different substrates. For example, 70 kDa heat shock cognate protein is more active in solutions of potassium chloride when compared to sodium chloride.[344]

Excessive potassium can lead to hyperkalemia and impacting heart, skeletal muscle, and kidney function. High potassium content in the serum will disrupt normal cardiac electrical function leading to decreased cardiac output.[345] In muscles, high extracellular potassium disrupts impulse conduction, thus leading to global muscle weakness.[346] In the kidneys, high potassium content will lead to retention of hydronium ions, therefore lowering blood pH.[347]

*4.1.3.2 BG evidence.* Several studies have been published investigating the replacement of $Na_2O$ with $K_2O$ in different BG systems.[163,164] The main objective, as indicated in the preceding section, was to increase the glass stability of BGs by substituting Na with K and producing fiber. Mixed alkali effects have also been researched in order to improve mechanical properties and sinterability. However, there is a lack of sys-

tematic studies observing the biological impact of the incremental replacement of $Na_2O$ with $K_2O$.

A mesoporous K-doped BG has been developed to determine the biomedical properties and drug delivery capabilities.[142] This mesoporous BG was produced through sol–gel processing with the composition $49SiO_2{\cdot}20CaO{\cdot}20Na_2O{\cdot}7K_2O{\cdot}4P_2O_5$ (mol%). The sol–gel derived product showed an average particle size of 1 μm, an interconnected mesoporous network, and an average pore size of 21 nm. Due to the high surface area, the BG particles degraded rapidly in physiological fluid showing accelerated HAp formation. *In vitro* results demonstrated biocompatibility of the product with encouraging results from cell proliferation and cell cycle assays. As compared to 45S5 glass, the K-doped mesoporous BG showed increased osteoblastic activity by roughly 50% and slightly higher levels of cell proliferation.

Potassium-containing BGs have shown increased performance in dental applications due to the desensitization properties of $K^+$.[165] For example, $Na_2O$ was fully replaced with $K_2O$, on a weight basis, in the BioMinF composition (36–40) $SiO_2$–(28–30)CaO–(22–24)$Na_2O$–(4–6)$P_2O_5$–(1.5–3)$CaF_2$ to determine the improvement on dissolution rates, apatite forming ability, and the eventual suitability for dentin hypersensitivity treatment. Static dissolution studies showed nearly equivalent performance of both parent BioMinF and K-substituted BioMinF with similar dissolution rate, pH change, and precipitation of fluorapatite. Although both BG compositions have shown nearly identical performance for *in vitro* bioactivity, the K-containing BG was selected for future dentin sensitivity treatments. Several further studies have been published showing that potassium-based compositions provide therapeutic effects through blocking nerves in dentin tubules while also aiding in dental restoration.[165,348]

#### 4.1.4 Rubidium

*4.1.4.1 Biological activity.* Rubidium has no known natural biological role. The properties of rubidium are similar to those of potassium and, as a result, both of them behave similarly in cells. Radioactive rubidium has been utilized as a potassium ion tracer in biological research.[349] Similarly, radioactive rubidium has been used as a diagnostic contrast agent in positron emission tomography.[349,350] High metabolic activity and, therefore, increased expression of sodium–potassium pumps cause the preferential uptake of the radioactive rubidium in cancers cell over normal tissues.[331,350] Therapeutically, rubidium has been investigated in the fields of neuropsychiatric diseases as well as osteogenesis. Rubidium has been studied in preclinical and clinical trials as a possible treatment for depression.[351–353] To date, no consensus has been achieved about the mechanism for anti-depressive effects. As for osteogenesis, rubidium chloride was shown to activate osteoblast activity and inhibit osteoclast activity. Specifically, rubidium ions target Jnk/p-38-mediated NF-κB activation.[354] NF-κB encompasses a family of transcription factors. In the case of bone, it acts as key mediator in osteoclastogenesis, a critical part of healthy bone formation.[355] Rubidium levels tend to collect in the body due to the elements long half-life of over 50 days.[356]

*4.1.4.2 BG evidence.* Although rubidium is not abundant biologically, it has been applied for use in several biomaterial platforms. Rubidium has shown antibacterial properties and modulatory cell responses when used in biomaterials such as hydrogels, implant coatings, and other ceramic materials.[357,358] When used as a dopant in BGs, rubidium shows biocompatibility through *in vitro* cytotoxicity screenings, as well as promising results from cell adhesion testing.[155,359]

Rubidium has been successfully added to bioactive glass microspheres for transient embolic medical devices.[360] Glass composition BRS2 ($70B_2O_3$–$28Rb_2O$–2SrO, by mol%) has been reported to be degradable and radiopaque making them suitable for transarterial embolization. The borate based composition shows degradation times of around 24 hours without circulating to unwanted locations in the body such as the brain or lung. Thus, these BGs may be suitable for cancer treatment through blocking blood flow to tumors or other abnormal areas.

**4.1.5 Cesium.** Cesium, lying below rubidium in the periodic table, is fairly unremarkable in terms of biomedical applications. Radioactive cesium has been utilized in brachytherapy for treatment of cancers.[361,362] Furthermore, cesium chloride was explored in high pH cancer therapy.[363,364] However, the mechanism and efficacy have yet to be validated and cesium is associated with a variety of toxicities such as cardiac arrhythmia.[363,365,366] No examples of cesium-containing BGs have been reported so far in the literature.

**4.1.6 Francium.** Francium is the largest alkali element. Due to its nuclear instability, it has no known biological function. This highly radioactive element has no biomedical applications. No examples of francium-containing BGs have been reported so far in the literature.

### 4.2 Alkaline earth elements

**4.2.1 Beryllium.** Beryllium, the smallest of the alkali earth elements, has no known biological role. Aside from device hardware, beryllium has no biomedical applications either. Occupational exposure to beryllium has been associated with a variety of maladies including allergic reactions as well as intestinal and lung disease.[367]

#### 4.2.2 Magnesium

*4.2.2.1 Biological role.* Magnesium, the second smallest alkali earth ion, has a divalent ionic state with high charge density.[368] The small divalent ion forms strong ligand complexes with typical coordination numbers of 2 or 4 in solution.[296,369] As a result, magnesium can interact strongly with both nucleotides and proteins and influence a plethora of biological processes.[370]

The majority of intracellular magnesium is complexed with nucleotide triphosphates, chiefly ATP. Positively charged magnesium interacts strongly with negatively charged phosphate groups.[371] This complex is critical for facilitating the hydrolysis of ATP to adenosine diphosphate (ADP). The energy released in this reaction is essential to many biological functions including muscle contraction, cellular signaling, active transport, and nucleotide synthesis among many others.[372]

 

Nuclei acids such as deoxyribose nuclei acid (DNA) and ribose nucleic acid (RNA) require magnesium cations to confer stability. Similar to ATP, negatively charged phosphate groups attract magnesium ions. In aqueous solutions, a magnesium ion maintains a shell of water molecules around it. As magnesium and its associated water shell approach nuclei acids, the water molecules will form hydrogen bonds. The hydrogen bonds reduce charge density on the DNA or RNA and stabilize optimal configurations.[373] It is also possible for magnesium to directly interact with nuclei acids. For example, Mg can from a complex ligand bond with DNA and, instead of stabilizing DNA, it will cause a tight distortion and render the macromolecule unusable.[374] Therefore, a balance of magnesium is critical for DNA and RNA function.

Many enzymes utilize magnesium as either a cofactor or an activator. For example, many enzymes involved in glucose metabolism require magnesium to catalyze reactions that leverage the transfer of high energy inorganic phosphate. For example, hexokinase catalyzes the first step of glycolysis by extracting a phosphate from ATP and attaching it onto a molecule of glucose.[315,369,375] Similarly, key cell signaling enzymes, such as the insulin receptor, require magnesium to transduce endocrine signals into cellular responses. There are over 800 known enzymes that somehow use magnesium in the reactions where they are involved.[368]

The bulk of magnesium in the body can be found in bone. Magnesium can coat HAp crystals in the matrix of cortical bone as well as be doped into mineral component of bone. Magnesium ions control the solubility of calcium and potassium, therefore greatly influencing the size of HAp crystals and, as a result, their stability.[368] Magnesium deficiency has been associated with osteoporosis and increased bone fractures due to large HAp crystals decreasing overall stiffness and strength of bone.[376] On a cellular level, it was found that control of magnesium levels in bone tissue is critical for proper enzyme activity and protein expression associated with osteogenesis.[377]

Apart from the role in hard tissues, magnesium and calcium interactions influence cardiac physiology, too.[368,370,375] Calcium plays a critical role in the cardiac action potential (details in section 4.2.1). However, magnesium can block L-type calcium through either direct inhibition of ion permeability or perturbation of voltage gate properties. Magnesium functions in a protective manner regulating myocardial contractions.[378] Clinically, magnesium has been proven to be life-saving for the deadly cardiac arrhythmia Torsade's De Pointes.[379] However, the mechanism has yet to be definitively determined.

Transmission of glutamate, an excitatory neurotransmitter, through *N*-methyl-D-aspartate (NMDA) receptor is regulated by magnesium ions.[370] At normal resting membrane potential, Mg ions will block glutamate bound ion channels on the postsynaptic neuron. When the membrane potential rises, magnesium will release from NMDA receptors allowing for an influx of calcium and transmission of the neural signal.[380] During states of hypomagnesemia, these neural pathways will be unregulated and hyperexcitability ensues.[381]

Excessively high levels of magnesium, though rare outside the context of kidney failure and excessive intake, can be deadly. Moderately high amounts of magnesium will yield an altered mental state, decreased reflexes, constipation, and headache.[382] However, excessively high serum magnesium can lead to decreased breathing, high blood pressure, muscle paralysis, cardiac arrhythmia, and even coma.[382,383]

*4.2.2.2 BG evidence.* Magnesium is perhaps the most commonly used element in BGs outside of those used in the original 45S5 composition (Si, Ca, Na, P). The incorporation of magnesium into BGs has been studied extensively for structural, processing, and biological impacts.[166] As magnesium is among the most abundant metals in the body, it is natural to consider it in biomaterial applications. Magnesium has been shown to not only tailor the degradation rates of BGs, but also stimulate increased cell proliferation and antibacterial activity. Further discussion of magnesium in BGs is reported by Cacciotti.[227]

Moghanian *et al.* studied the *in vitro* effects of incremental calcium replacement with MgO on cell proliferation and antibacterial activity.[384] The 58S compositional system was implemented using the formula $60SiO_2$–$4P_2O_5$–$(36–x)$CaO–$x$MgO (where $x$ = 0, 1, 3, 5, 8, 10) on a molar basis. All glasses were synthesized using the sol–gel process. The results of the cell proliferation study revealed that 5 mol% MgO had the highest stimulation of cell proliferation. Concentrations over 5 mol% showed decreasing returns, which was thought to be due to higher pH values of the dissolution product solution. Mg-containing BGs were then tested for antibacterial properties against methicillin-resistant Staphylococcus aureus (MRSA) bacteria. Again, 58S glass containing 5 mol% MgO showed the most promising results with a bactericidal percentage of nearly 50% in just 24 hours.

A glass using the composition $45SiO_2$–$3P_2O_5$–26CaO–$15Na_2O$–7MgO–$4K_2O$ mol% was then studied *in vivo* for osteogenic potential.[385] This melt-derived glass was delivered in the form of foam-like 3D scaffolds. 20 Wistar rats received scaffolds in induced 6 mm cranial defects and were monitored for up to 12 weeks. Rats from each sample group were sacrificed at 4 and 12 weeks post-implantation for histology and immunohistochemistry evaluations. Compared to a standardized reference, the Mg-doped BG clearly demonstrated increased levels of bone regeneration after both time periods. Immunohistochemistry also demonstrated the ability of Mg-doped BGs to express osteocalcin and osteonectin after 12 weeks. This study concluded that magnesium is suitable and recommended for use in BGs applied to hard tissue regeneration.

Magnesium-containing bioactive glass-ceramics have been reviewed comprehensively for attractive biomedical applications.[167] Several crystalline phases containing magnesium were listed including diopside, fluorophlogopite, potassium fluorrichterite, akermanite, merwinite, monticellite, whitlockite, and enstatite. Magnesium was also discussed to be used as a stabilization agent to control crystallization of high-calcium-content phases. Magnesium content in the discussed glass

ceramic families were shown to negatively impact the apatite-forming ability. However, the *in vitro* and *in vivo* studies included in the review showed high biocompatibility with cells and animal models. Furthermore, good osteointegration of magnesium-doped implants was shown with rabbit models.

The combinatorial effects of $Si^{4+}$, $Ca^{2+}$ and $Mg^{2+}$ ions released from BGs were studied for their effects on osteoblast osteocalcin expression and biomineralization.[249] Silicate and calcium ions were found to up-regulate osteocalcin expression while magnesium was found to down-regulate it. Osteocalcin is discussed to be critical in early stage osteogenesis suggesting that magnesium may not be ideal for applications requiring fast results. However, delayed release of magnesium ions can be used to develop materials with controlled bone formation rates. Magnesium is also recognized to aid in the remodeling process which will strengthen the newly formed bone.

Finally, magnesium has been employed as a dopant in BG nanofiber membranes created *via* electrospinning.[386] Anti-bacterial, anti-inflammatory, angiogenic, and wound healing capabilities of these fibers were evaluated. *In vitro* assessment of cytocompatibility showed strong cell proliferation and angiogenesis. Furthermore, cellular response from murine macrophages showed decreased cellular inflammation as a response to the dissolution products. Proliferation of both Gram-positive and Gram-negative bacterial strains were diminished by the BG fibers. Lastly, gene expression assays showed down-regulated pro-inflammatory factors, up-regulated anti-inflammatory factors, and up-regulated angiogenic factors. Overall, magnesium doped silicate based electrospun fibers show promising properties in the treatment of infected wounds as a wound dressing material.

#### 4.2.3 Calcium

*4.2.3.1 Biological role.* Calcium is key to many biological processes. Unlike other electrolytes, free calcium ion concentrations are over 4 orders of magnitude higher in extracellular fluids than in the intracellular environment.[387] The high level of control is critical to calcium cell signaling, cofactor action, neuronal and cardiac properties. Calcium not only functions in physiology but plays a structural role as a key element in bone.

Secondary messengers are molecules in cells than can disseminate a single extracellular signal throughout a cell.[388] For example, an extracellular ligand can bind G-protein coupled receptors triggering the activation of intracellular g-proteins. The g-protein can activate a variety of targets such as phospholipase C. From the cell membrane, phospholipase C can trigger the release of 1,4,5-trisphosphate (IP3) into the cytoplasm. At this point, soluble IP3 can bind to IP3 receptors on the endoplasmic reticulum, leading to the release of many calcium ions into the cytoplasm ultimately spreading the initial signal throughout the entire cell.[389] The calcium-dependent signal transduction is crucial to a myriad of processes including fertilization, proliferation, metabolism, and glandular secretion.[389–391] A specialized version of calcium signaling exists for muscle contractions. As motor neurons excite muscle tissues, changes in membrane potential will trigger the intracellular sarcoplasmic reticulum to release calcium ions. As calcium ions bind troponin C on muscle fibers, a confirmational change occurs activating muscle fibers for contraction.[392]

Similar to magnesium, calcium plays the role of cofactor in many enzymatic activities. The small, highly charged ion interacts with negatively charged groups on both substrates and enzymes. Calcium cofactor abilities are strongly exemplified in the blood coagulation cascade. Triggered by a blood vessel injury, a series of serine protease are activated to ultimately produce a fibrin clot to cease bleeding.[393,394] Calcium is required by factors XI, IX, X, VII, and II as well as tissue factor. Specifically, calcium functions as a cofactor for serine protease activity. Serine proteases facilitate the activation of clotting factors and therefore calcium is a strict requirement for the clotting cascade.[395] Fig. 8 provides an illustration of the coagulation process emphasizing each factor. Transglutaminases represent another class of enzymes requiring calcium[396] for catalyzing the formation of a covalent bond between glutamine side groups and primary amines, transglutaminases are critical for formation of crosslinked structures that offer protection and stability for tissues including skin, nerves, lung, and bone.[396–399]

Calcium dynamics are essential to functioning to a variety of electrically excitable tissues. Cardiac pacemaker cells, such as those in the sinoatrial node of the heart, control the rate and rhythm of human heart beats. Hyperpolarization-activated cyclic nucleotide–gated (HCN) calcium channels allow for a slow, controlled depolarization of pacemaker cells. Once a threshold membrane potential is reached, L-type calcium channels open, leading to full depolarization and initiation of a heart beat signal.[401] These calcium channels are common therapeutic targets for arrhythmia.[402] Furthermore, calcium is responsible for the plateau phenomena seen in cardiomyocytes action potentials.[403] This is key in facilitating complete contraction of the heart muscle.

In neurons, calcium is essential in signal transmission across synapses. As an electrochemical signal reaches the terminal aspect of a nerve, voltage gated calcium channels open. The rapid influx of calcium triggers the release of neurotransmitter into the synaptic cleft. Neurotransmitters then diffuse across the cleft and interact with the postsynaptic neuron to transmit the signal.[404]

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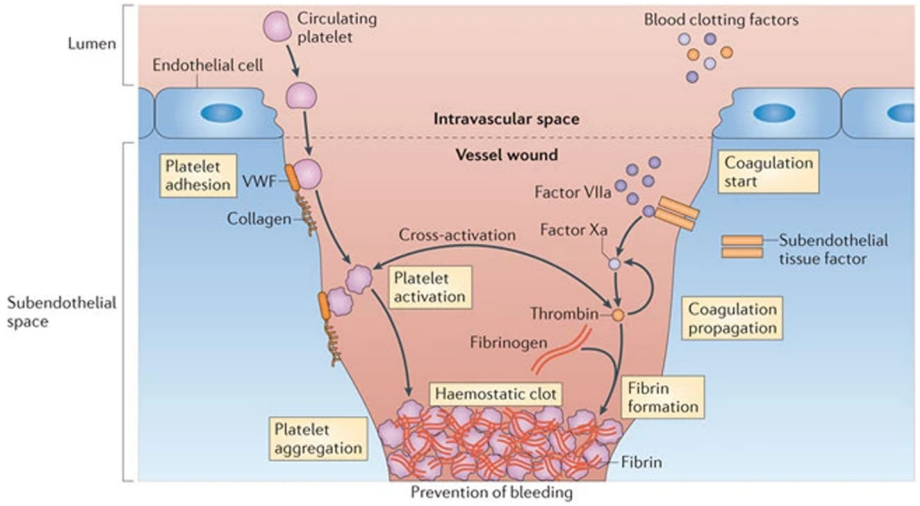


**Fig. 8** Visualization of hemostasis showing all processes involved in clot formation. From ref. 400 with permission from Springer Nature, 2012.

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Calcium is a vital element playing various roles in biochemical/biological reactions but is also dramatically important in maintaining the structure of the skeletal system. In bone, calcium is nearly entirely present in calcium phosphate mineral phases. Hydroxyapatite is the major mineral phase of bone which provides strength for the skeletal system while also acting as a metabolic reservoir for cellular fluids.[405] The skeletal system is dominated by this crystalline inorganic phase in cortical bone, showing 80%–90% calcification, which provides the majority of structural function.[406] Trabecular bone provides metabolic roles with 15%–25% calcification.

*4.2.3.2 BG evidence.* Calcium has been implemented in nearly every current BG composition due to its well-known structural role in both glass science and physiology. It has been used as a baseline in most studies replacing it incrementally with other modifiers to determine the biological implications. A study published in 2020 reverses the stereotypical approach and investigates the biological impacts of calcium content on a binary $CaO–SiO_2$ system with CaO concentration up to 15%.[407] Glasses were produced through a modified Stöber sol–gel process with spherical morphology and a monodispersed sized. *In vitro* bioactivity and *in vitro* cytotoxicity were used to evaluate the impact of calcium concentration. The effects of calcium content on bioactivity were relatively small over the investigated compositional range, suggesting that dissolution rates were not compositionally dependent at this range. Rate of HAp formation was directly related to the calcium content of the glass. Increased calcium content did not show any *in vitro* cytotoxicity.

Another study by Catauro *et al.* investigated the effect of a $SiO_2–CaO–P_2O_5$ system on cell proliferation.[408] A wider compositional range of BGs with molar Ca/P ratios of 3, 6, 9, 12 was prepared through sol–gel synthesis. *In vitro* testing included cell attachment, cell morphology observation, and cell proliferation. Increasing calcium concentration was found to have the largest effect on cell proliferation. Calcium content increased the biological performance of the compositional workspace suggesting that different ratios of Ca/P can be applied to multiple clinical applications.

Calcium has proven paramount in formation of new blood vessels through production of angiogenic growth factors. Simulation of the up-regulation and production of critical growth factors like vascularization growth factor (VEGF) or basic fibroblast growth factor (bFGF) among others.[409,410] Several *in vitro* and *in vivo* trials illustrate the role of calcium and bioactive glasses as whole in stimulating angiogenesis in different tissue engineering environments.[23] These effects are essential wound healing applications in order to accelerate the tissue healing process.

### 4.2.4 Strontium

*4.2.4.1 Biological role.* Strontium exhibits similar properties to calcium in biological systems. As a result, it is readily absorbed and has found clinical relevance in bone health. Strontium has also been investigated for their roles in dental health, radiotherapy, and sensory irritation suppression.[411–414]

Calcium sensing receptor (CaSR) can be found on the parathyroid gland function in calcium homeostasis as well as the renal tubules controlling urine calcium level.[415] Due to chemical similarities, strontium ions can bind to the ligand binding domain of CaSR. Furthermore, CaSR is found on osteoblasts and osteoclasts, and thus allows strontium to influence bone metabolism.[416] Specifically, strontium activates osteoblast activity and inhibits osteoclasts. Through NFATc1/Wnt, Runx2, and Cox-2 signaling strontium enhances the differentiations of preosteoblasts into mature osteoblasts.[417,418] Furthermore,

strontium activates ERK1/2, p38, and AKT to enhance bone formation.[419,420] As for osteoclasts, strontium will block cellular maturations and induce apoptosis.[421] Strontium has been investigated in the treatment for osteoporosis. Strontium ranelate has been studied in several clinical trials showing the ability to both limit fractures and increase bone mass.[422–425]

Similar to bone, strontium can play a protective effect on teeth. The presence of strontium ions in an acidic solution reduces enamel erosion. It is proposed that strontium forms a protective layer around the enamel.[426] Additionally, strontium in drinking water and toothpaste has been shown to both stave dental caries as well as mineralize bone tissue.[427] Interestingly, strontium has an additional benefit of treating dentin hypersensitivity.[428]

Radioactive strontium-89 has been leveraged for local radiotherapy. Due to chemical similarities to calcium, this therapeutic ion is preferentially taken up by malignancy in bones. Clinically, strontium-89 has been successful in treating painful bony metastasis for a variety of cancers.[429–431]

Strontium biological influence expands into sensory applications as well. Zhai *et al.* illustrated in humans that topical strontium nitrate can lower both the magnitude and duration of a histamine induced itching sensation.[432] Several similar studies have corroborated these results.[433–435] This effect is likely due to strontium blocking of calcium channels in sensory neurons.[436]

*4.2.4.2 BG evidence.* The effects of strontium in hard tissue applications has been previously reviewed extensively.[170] The biological impacts of strontium have been studied outside of BG field in biomaterial platforms such as strontium chloride solutions, strontium ranelate, and in other implantable bioceramics such as calcium orthophosphates.[169,171,177] Strontium has been studied as a dopant in BGs in many different forms including powders, granules, fibers, scaffolds, and pastes while also being added to all families of BGs. Primary *in vitro* studies have shown that strontium improves osteogenesis induction through various mechanisms which include upregulation of critical genes, activation of signaling pathways, and regulating differentiation of stem cells.[172–176,178] Moreover, strontium inhibits osteoclastogenic action as shown by Gentleman *et al.*[179] Finally, strontium shows antibacterial properties through mechanisms that are not well understood yet. Dissolution products of strontium-containing BGs have been shown effective against certain strains of bacteria including Escherichia Coli (*E. coli*) and Porphyromonas gingivalis (P, gingivalis).[180,437]

Bone regeneration of strontium-containing BGs was studied *in vivo* by Esfahanizadeh *et al.*[181] Critical size calvarial defects in 12 New Zealand White rabbits were treated with 45S5, magnesium-doped 45S5 (Mg-45S5), and strontium-doped 45S5 (Sr-45S5) glasses. The rabbits were allowed 4 and 8 weeks for tissue regeneration with half the populations being sacrificed at 4 weeks. Both Mg-45S5 and Sr-45S5 glasses showed increased new bone regeneration as compared to undoped 45S5 group. However, after 8 weeks the Sr-45S5 group showed significantly higher rates of new bone regeneration as compared to the other test groups.

### 4.2.5 Barium

*4.2.5.1 Biological role.* Barium has no known natural biological role; however, it finds great use as an X-ray contrast agent. This high-atomic-number alkali earth ion can absorb X-rays much better than human tissues. Administered orally or transrectally, barium sulfate can be used to probe maladies of the upper and lower digestive tract, respectively. Due to toxicity, no intravenous applications of barium exist.[438]

*4.2.5.6 BG evidence.* Barium has been incorporated in BGs showing several therapeutic effects including antibacterial and anti-inflammatory properties. The radiopaque performance of barium has been well studied for applications involving medical imaging as it allows for easy differentiation between the implant and host tissue. Experimental evaluation on the effects of barium *in vitro* have included cell proliferation studies, hemolysis, and genetic stimulation assays to screen the Ba effects before continuing to *in vivo* studies.

Majumdar *et al.* comprehensively probed the biological effects of barium on the 45S5 system. 1.35 mol% BaO replaced $Na_2O$ and $SiO_2$ and was produced through sol–gel synthesis.[156] This composition was compared against a sol–gel derived 45S5 product. Both glasses showed comparable particle size and specific surface area through dynamic light scattering and nitrogen adsorption BET. A hemolysis assay proved that the barium-containing glass was biocompatible without disrupting red blood cells and causing hemoglobin to leave the cells. Comparable results were generated from a cell proliferation study showing that dissolution products from both glasses increased cell count from the original count. Wound healing assays again showed comparable results to 45S5 with the 62.36 ± 4.73% closure after 24 hours for the barium-containing glass. Increased performance of the barium-containing BG was seen in the genetic upregulation of anti-inflammation genes. As compared to 45S5, barium-containing BG had an increase in anti-inflammatory marker IL-10 of 19.91 pg $mL^{-1}$. Inflammatory markers were decreased by the barium containing BG as compared to 45S5 for marker IL-6 by 55.87 pg $mL^{-1}$ and for TNF-α they were decreased by 39.98 pg $mL^{-1}$.

The same research group recently published an *in vivo* toxicological evaluation of barium-doped 45S5 BG in adult albino Wistar rats.[182] Fig. 9 highlights the experimental procedures and toxicological assays used in this study. Acute oral toxicity, subacute oral toxicity, organ coefficients, histological, and biochemical indices were used to critically evaluate the role of barium ions in bone and soft tissues. Oral doses of 300 and 2000 mg $kg^{-1}$ of both barium-containing BG and Ba-free 45S5 were administered with no treatment-related mortality observed. Organ coefficient studies showed no statistical changes in organ coefficient of the brain, heart, kidney, lungs, liver, or spleen for both BG compositions. Furthermore, no changes in muscle coordination, spontaneous locomotion, or observed anxiety was shown in any of the treated rats. The study concluded that barium was safe and therapeutic in its use in BGs.

An *in vivo* study published in 2018 showed the potential for barium-containing BGs in the application of gastro-duodenal ulcers.[183] 1.3 mol% BaO was added to the 45S5 Bioglass®

 

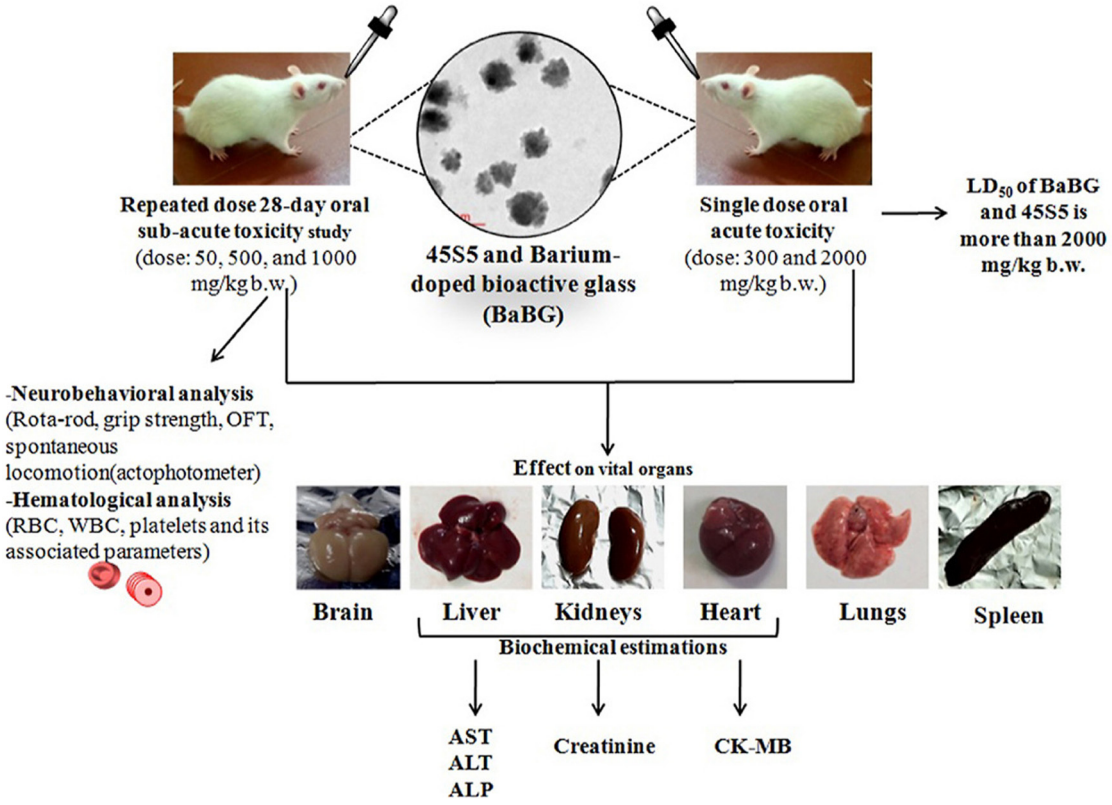


**Fig. 9** Illustration showing the procedures and assays used by Majumdar and Krishnamurthy. Wistar rats received single or repeated dosage of 45S5 or barium doped 45S5 to comprehensively determine acute and chronic toxicity in several organs. Two characterization techniques were used to analyze the brain, liver, kidneys, heart, lungs, and spleen of sacrificed rats. No organ toxicity was found in male or female rats. From ref. 182 with permission from Elsevier, 2022.

system and synthesized *via* melt quenching. The particle size of 45S5 and barium-containing BG were comparable at around 2 μm. BGs suspended in a 0.5% carboxy-methyl-cellulose mixture were administered orally to the rats. Several types of ulcers were studied including ethanol-induced, aspirin-induced, pylorus litigation-induced, acetic acid-induced, and cysteamine-induced. Dissolution products from barium-containing BGs aided in neutralizing gastric acid showing normal architecture, less hemorrhages, and less necrosis in several ulcer models. The barium-containing glasses also showed the creation of a physical protective barrier providing antiulcer effects.

**4.2.6 Radium.** Radium, the heaviest of the alkali earth metals, has no known biological function and is severely limited in applications due to its radioactive nature. Similar to strontinum-89, radium-223 in its chloride salt form has been approved for radiation therapy in bony metastatic cancers.[439]

## 5. Conclusion and guidance for compositional design

ALAEs play a vital, multifunctional role in bioactive glasses that affect the glass structure, processing, and physiological impact. By modifying the glass structure, phase precipitation and dissolution rates can be tailored to a specific medical application. ALAEs play a direct role in certain processing methods that can enhance medical device performance *in vitro* and *in vivo*. Due to the complexity of the structure-property relationships and biological responses of bioactive glasses, many questions are raised on the topic of compositional design. Below, several questions regarding the design of bioactive glass compositions are raised and discussed thoroughly.

*What is the objective of designing bioactive glasses in modern medicine surpassing the traditional notion of bone bonding ability?*

• First, the desired application needs to be considered. Soft tissue or bone tissue engineering, antibacterial deficiency (*e.g.*, osteomyelitis treatment), preventive dentistry, cancer treatment, *etc.* all need the proper adjustment of dissolution. Hard tissue applications, such as bone regeneration, do not require rapid dissolution rates. Rather, bone bonding ability, HAp formation, and some therapeutic effects are more important. For soft tissue wound healing, delivery therapeutic ions and production of growth factors are needed quickly to initiate the wound healing process. Complete dissolution is also required in order to ensure that no immune response or negative effects occur in the long-term timeframe.

*What structural features govern dissolution rates and how ALAEs help?*

• Network connectivity of the glass structure directly affects the dissolution of the BG composition. For example, the creation of NBOs related to introduction of NWMs improves the degradation of silicate-based glasses. On the contrary, for borate glasses, the addition of NWM creates a unique anomaly. The inclusion of the ALAEs shifts the structural units from the three coordinate $BO_3$ to the $BO_4$ unit. This enhances the connectivity of the glass and therefore increases its durability. The structural units of the glass need to be considered intently when designing a BG composition.

*Can we control congruent vs. incongruent dissolution through compositional design and ALAEs addition?*

• To start, congruent dissolution refers to a material that dissolves completely into the constituent ions rather than leaving a residual solid behind. This is vital when considering applications that require the BG particle to be removed before the healing process is complete. Congruent dissolution can be designed as a function of the composition. Certain compounds are less likely to dissolve in solution as they strengthen the glass network and durability. Potential examples of these compounds include $Al_2O_3$, $ZrO_2$, and $TiO_2$.

*How can we optimize liquidus temperature through composition?*

• Minimizing the liquidus temperature allows for optimization of processing costs as well as glass forming ability. Optimizing the ratio of ALAEs with NWFs can lower overall melting temperature while still maintaining the desired level of network connectivity. Designing compositions with lower processing temperatures will require much less energy to manufacture, driving costs down. Also, by minimizing the temperature difference between the melt temp and solid minimizes tendency to crystallize. Designer glasses around thermodynamic eutectics create lower temperature compositions with higher glass forming ability.

*What role do ALAEs in the thermodynamics and kinetics of crystallization? Can they be used to influence or prevent crystallization?*

• The thermodynamics of crystallization can be brought back to the fundamental thermodynamics of mixing. If Gibbs free energy of mixing is positive, the system will become separated. The temperature at which nucleation occurs is usually found somewhere between the glass transition temperature and the crystallization peak temperature, which can be determined through differential scanning calorimetry. By adding ALAEs to the glass network, these temperatures decrease, and sometimes the temperature difference between them does as well. The atomic radius also plays an important role here. As previously mentioned, larger ALAEs will create a denser ring structure. The kinetics of crystallization is heavily dependent on the viscosity of the glass composition. ALAEs play an important role in lowering the viscosity of the melt. In lower viscosity melts, it is easier for atoms to diffuse across the liquidus-nucleus barrier which grows the crystallized phase.

*How do BGs containing ALAEs provide therapeutic effects instead of harmful reactions?*

• BGs excel for local therapy such that high levels of therapeutic species are only experienced at the site of administration, while whole body levels remain below toxic limits. For example, lithium is known to be toxic in humans at serum levels in excess of 1.5 mM.[440] However, *in vitro* studies suggest lithium levels in excess of 5 mM may be required for induction of desired therapeutic response.[308] This precludes systemic administration from clinical relevance. The key with BGs is twofold: local delivery and controlled dissolution. Implanted lithium doped BG biomaterials have been utilized for the growth of soft and hard tissues alike, yet evidence of lithium induced toxicity was not noted in *in vivo* studies.[323–325] This is due to the fact that high lithium levels are restricted to the area directly surrounding the implant. Lithium is slowly released from the implant and will initially diffuse through the local environment mediating its therapeutic action. The stray ions that do diffuse out of the treated area and into the blood stream are heavily diluted and therefore induce no adverse effects. The key is that the amount of lithium is sufficient for local treatment but not large enough that escaping ions induce systemic toxicity. This idea can be generalized to all ALAEs.

• Furthermore, the incorporation of ALAEs in bioactive glass compositions to elicit specific therapeutic effect could carry an additional advantage. The existing literature has demonstrated that many elements from the transition metal class can elicit potent therapeutic responses (*e.g.* pro-angiogenic effect by copper and cobalt, antibacterial properties by silver) but, on the other hand, they are also known to induce important toxic effects in the body at relatively low dosages. In order to overcome this drawback, using "safer" ALAEs at proper concentration can be considered as an alternative to transition metal incorporation in the attempt to achieve the same beneficial effect while avoiding toxic side effects.

*Can machine learning/artificial intelligence be used to further the advancement of bioactive glasses especially more rational addition of ALAEs?*

• Recently, there has been an effort to "decode the glass genome" in order to accelerate our understanding of the structure–property relationships in glass. With an emphasis on modeling, vast datasets can be generated on properties such as dissolution behavior, fracture toughness, viscosity, and glass forming ability, among many others. However, computation techniques cannot be relied upon for modeling the biological response between a biomaterial and a cellular interface. Combining the datasets created through modeling techniques and experimental tests can yield powerful information for designing the optimized BG composition. Machine learning has been employed to predict the properties of biomaterials (including glasses). Developing larger datasets will in turn generate more accurate results from machine learning models allowing for the creation of the next generation of bioactive glasses.

*How does the introduction of lesser studied ALAE elements or even other therapeutic inorganic ions effect the commercialization process of novel bioactive glass compositions?*

 

• Novel bioactive glass compositions, morphologies, and products have been approved for commercial use by different international regulatory agencies through different methods. In the United States, the 510k process allows an application to forego comprehensive clinical trials is deemed sufficiently similar to a product that is existent on the market.[441] This allows similar compositions to the original 45S5 Bioglass® composition to be passed without the scrutiny of the many studies needed in clinical trials. This "sufficiently similar" rhetoric has been used for commonly studied compositions such as 45S5 or 53SP4. Compositions studied in academia containing more exotic elements that have not been introduced in the clinic may receive more curiosity and hesitation for regulatory agencies forcing a more demanding road to approval.

## Conflicts of interest

There are no conflicts to declare.

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