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# The effect of composition on ion release from Ca–Sr–Na–Zn–Si glass bone grafts

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**Abstract** Controlled delivery of active ions from biomaterials has become critical in bone regeneration. Some silica-based materials, in particular bioactive glasses, have received much attention due to the ability of their dissolution products to promote cell proliferation, cell differentiation and activate gene expression. However, many of these materials offer little therapeutic potential for diseased tissue. Incorporating trace elements, such as zinc and strontium, known to have beneficial and therapeutic effects on bone may provide a more viable bone graft option for those suffering from metabolic bone diseases such as osteoporosis. Rational compositional design may also allow for controlled release of these active ions at desirable dose levels in order to enhance therapeutic efficacy. In this study, six differing compositions of calcium–strontium–sodium–zinc–silicate (Ca–Sr–Na–Zn–Si) glass bone grafts were immersed in pH 7.4 and pH 3 solutions to study the effect of glass composition on zinc and strontium release in a normal and extreme physiological environment. The zinc release levels over 30 days for all zinc-containing glasses in the pH 7.4 solution were 3.0–7.65 ppm. In the more acidic pH 3 environment, the zinc levels were higher (89–750 ppm) than those reported to be beneficial and may produce cytotoxic or negative effects on bone tissue. Strontium levels released from all examined glasses in both pH environments similarly fell within apparent beneficial ranges—7.5–3500 ppm. Glass compositions with identical SrO content but lower ZnO:$Na_2O$ ratios, showed higher levels of $Sr^{2+}$ release. Whereas, zinc release from zinc-containing glasses appeared related to ZnO compositional content. Sustainable strontium and zinc release was seen in the pH 7.4 environment up to day 7. These results indicate that the examined Ca–Sr–Na–Zn–Si glass compositions show good potential as therapeutic bone grafts, and that the graft composition can be tailored to allow therapeutic levels of ions to be released.

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## 1 Introduction

Research into bioactive materials for skeletal tissue regeneration has increased due to the need to address the shortcomings of traditional bone grafts. Autografts show intrinsic immunologic- and histo-compatibility, but require a second surgery often leading to major complications including donor site morbidity, blood loss and deep infection [1]. Allograft bone eliminates the need for a second surgery but shows no osteogenecity [2], poses an increased disease transmission risk [3] and, due to an ever aging population, is in limited supply. Bioactivity refers to the ability of materials to interact with cells and tissues stimulating repair and regeneration [4]. Bioactive glasses in particular are a key focus due to the fact that a specific compositional range of soda lime phosphosilicate glasses [5] intimately bonds to bone with out evoking a foreign body response. Subsequently trademarked as Bioglass 45S5 (45% $SiO_2$, 24.5% $Na_2O$, 24.5% CaO and 6% $P_2O_5$), its bone bonding ability has been attributed to cation release from the glass when exposed to physiological

solution [6]. This ultimately leads to the formation of a silica-rich layer in which dissolved $CO_2$, $Ca^{2+}$ and $PO_4^{2-}$ ions combine to form HCA (Hydroxy-carbonate apatite) [7]. Though useful, recent evidence has indicated that the formation of this HCA layer is not the critical mechanism for bone regeneration [8]. The ionic dissolution products from Bioglass appear to stimulate the growth and differentiation of osteoblasts at the genetic level [9], an effect which has been found to be dose dependent [10]. Though addressing some of the classical bone grafting problems such as disease transmission and limited supply, Bioglass does have some limited disadvantages especially in relation to the provision of therapeutic effect in vivo for patients suffering from metabolic bone diseases such as osteoporosis. Its high solubility may limit the ionic effect on bone formation due to released ions being transported away too fast by body fluid [11]. Also, Bioglass has been shown to exert an antibacterial effect on certain oral bacteria [12], but limited research has been carried out with regard to its effect on clinically important bacteria at orthopaedic surgery sites [13, 14]. There is thus a pressing need for a material which is not only biocompatible and osteoconductive, but one which is also inherently antibacterial and through controlled ion release offers therapeutic effects to aid the regeneration of diseased or damaged tissue.

Calcium–Strontium–Sodium–Zinc–Silicate (Ca–Sr–Na–Zn–Si) glass may address this need. $Zn^{2+}$ has been shown to stimulate fracture healing by enhancing osteoblast differentiation [15] and increasing osteoblast DNA content [16]. $Zn^{2+}$ ion release from biomaterials has also been shown to have antibacterial efficacy killing many bacterial strains commonly associated with infection after orthopaedic surgery [17]. Synergistically, $Sr^{2+}$ has been shown to have a dual mode of action increasing bone formation by osteoblasts while simultaneously decreasing bone resorption by osteoclasts [18] and is currently in use as an osteoporosis treatment in the form of Strontium Ranelate [19]. Na has been shown to impart degradability on $SiO_2$ based glass networks [20] so incrementally increasing Na content may allow for controlled dissolution and ion release. In order to assess the ion release profiles for such glasses and their clinical applicability as therapeutic bone grafts, it is essential to assess their degradability, post-sterilization, under normal and extreme physiological conditions. Therefore, this study aims to evaluate the $Zn^{2+}$ and $Sr^{2+}$ ion release profiles for six different $\gamma$ irradiated Ca–Sr–Na–Zn–Si glass compositions under simulated physiological conditions; pH 7.4 representative of the physiological environment [21] and pH 3.0, representative of extreme physiological conditions and the acidic environment produced by osteoclasts [22].

## 2 Materials and methods

### 2.1 Glass synthesis

Six glass formulations (Table 1) were synthesised. Glasses were prepared by weighing out the appropriate amounts of analytical grade reagents (Sigma Aldrich, Wicklow, Ireland); silicon dioxide, zinc oxide, calcium carbonate, strontium carbonate and sodium carbonate into a plastic container. Each formulation was thoroughly mixed in the closed container for 30 min. Compositions were then fired (1480°C for 1 h) in platinum crucibles and the glass melts shock quenched into water. The resulting frit was dried in an oven at 100°C for 1 day, ground and then sieved to retrieve a glass powder with a particle size in the range 90–710 μm for subsequent analysis.

### 2.2 Glass sterilization

Each glass was sterilised using $\gamma$ irradiation by Isotron (Westport, Co. Mayo, Ireland) in accordance with 'ISO11137: 2006; Sterilisation of healthcare products' [23]. The minimum and maximum doses recorded during sterilisation were 30.5 and 30.8 kGy, respectively. Sterilised glass was used subsequently for all characterisation and experimentation.

### 2.3 Thermal characterization

The glass transition temperature ($T_g$) onset of each glass powder was determined using a combined thermal gravimetric-differential scanning calorimetry analyzer (TG-DSC) (LABSYSTM, Setaram, France). Between 60 and 70 mg of each ground glass was heated in a platinum crucible in a nitrogen atmosphere alongside an empty reference crucible. A standard reference material is already pre-selected for a given temperature range. A heating rate of 10°C $min^{-1}$ was used and sample measurements were carries out every 6 s up to 1000°C.

**Table 1** Glass composition (mol. fraction) and ZnO:$Na_2O$ ratio

| Glass designation | $SiO_2$ | ZnO | CaO | SrO | $Na_2O$ | ZnO:$Na_2O$ ratio |
|---|---|---|---|---|---|---|
| BT110 | 0.4 | 0.2 | 0.1 | 0.2 | 0.1 | 2 |
| BT111 | 0.4 | 0.1 | 0.1 | 0.2 | 0.2 | 0.5 |
| BT112 | 0.4 | 0 | 0.1 | 0.2 | 0.3 | 0 |
| BT113 | 0.4 | 0.2 | 0 | 0.3 | 0.1 | 2 |
| BT114 | 0.4 | 0.1 | 0 | 0.3 | 0.2 | 0.5 |
| BT115 | 0.4 | 0 | 0 | 0.3 | 0.3 | 0 |

### 2.4 Structural characterization

The network connectivity (NC) of the glasses was calculated with Eq 1 using the molar compositions of the glass. ZnO was assumed to adopt a network modifying role in accordance with the literature [24].

$$\mathrm{NC} = \frac{\text{No. BOs} - \text{No. NBOs}}{\text{Total no. bridging species}} \quad (1)$$

where NC is the network connectivity, BO bridging oxygens, NBO non-bridging oxygens.

### 2.5 X-ray Diffraction

X-ray diffraction (XRD) analysis was performed using a Philips Xpert MPD Pro 3040/60 X-ray Diffraction (XRD) Unit (Philips, Netherlands). Zero background nickel coated sample holders were used for analysis of the 90-710 μm glass particles with Cu kα radiation (at 40 kV and 35 mA). Diffractograms were collected in the range $5° < 2\theta < 80°$, at a scan step size 0.0083° and a step time of 10 s.

### 2.6 Surface area determination

The specific surface areas of the glasses were determined using the advanced surface area and porosimetry, ASAP 2010 System analyzer (Micrometrics Instrument Corporation, Norcross, USA). 100–300 mg of each glass powder was used in a nitrogen atmosphere to calculate the specific surface areas using the Brunauer–Emmett–Teller (BET) method.

### 2.7 Dissolution experiments

In order to simulate normal and extreme environments TRIS-HCL buffer and Citric acid buffer solutions were prepared to have a pH of $7.4 \pm 0.1$ and $3.0 \pm 0.2$, respectively at a temperature of 37°C ± 1°C, according to ISO 10993-14 [25] An equivalent surface area of 1 $m^2$ of each glass powder was immersed in 10 ml of TRIS buffer ($n = 3$) and citric acid buffer ($n = 3$) and maintained at 37°C in a shaking waterbath (Haake SWB25, Fisher scientific, UK) agitated at 2 Hz. Specimens were stored for 1, 7 and 30 days. After each storage period, specimens were removed via filtration and filtrates retained for ionic content analysis. The $Zn^{2+}$ and $Sr^{2+}$ content of each filtrate was analysed using a Perkin Elmer AA Analyst 100, using flame atomic absorption spectroscopy in an acetylene-air flame. Zn and Sr hollow cathode lamps were used at wavelengths 213.9 and 460.7 nm, respectively. In order to eliminate interferences when measuring strontium levels 0.5 g KCL was added to each filtrate.

### 2.8 Statistical analysis

Each experiment was performed in triplicate and analysed using Graphpad prism 4 software (Graphpad software Inc.) Results are expressed as mean ± standard error of the mean of triplicate determinations. Analysis of the results was carried out using Students's *t*-test, with a significance level of $P < 0.05$ as previously used [21].

## 3 Results

### 3.1 Glass characterization

The glasses synthesized in this work are divided into two groups. Group 1 comprises BT110, BT111, and BT112 all having a fixed SrO content of 0.2 mole fraction with incremental additions of $Na_2O$ in place of ZnO across the series. To maintain the Network Connectivity (NC) of these glasses at 1, 0.1 mole fraction of CaO was added. Group 2 comprises BT113, BT114 and BT115 all having a fixed SrO content of 0.3 mole fraction and identical incremental additions of $Na_2O$ for ZnO as group 1. All group 2 glasses were free from CaO. Table 2 presents the results of the DTA analysis, NC, and specific surface area determinations carried out for each composition. Glasses from each separate group with the same ZnO:$Na_2O$ ratio showed similar $T_g$ onset values.

Figure 1 is the X-ray diffractogram collected from BT110 and is representative of X-ray diffractograms collected from glasses BT111, BT112, BT113, BT114, and BT115 confirming the amorphous nature of each glass.

### 3.2 Dissolution experiments

#### *3.2.1 Zinc release*

The $Zn^{2+}$ release profiles at pH 7.4 for Zn containing glasses BT110, BT111, BT113 and BT114 over 1, 7 and 30 days are shown in Fig. 2.

**Table 2** Glass transition temperature ($T_g$) onset, network connectivity (NC), and specific surface area for each glass

| Glass designation | Tg onset (°C) | NC | BET surface area ($m^2/g$) |
|---|---|---|---|
| BT110 | 603 | 1 | 15.32 |
| BT111 | 546 | 1 | 9.68 |
| BT112 | 522 | 1 | 10.23 |
| BT113 | 598 | 1 | 10.39 |
| BT114 | 542 | 1 | 15.49 |
| BT115 | 524 | 1 | 4.39 |

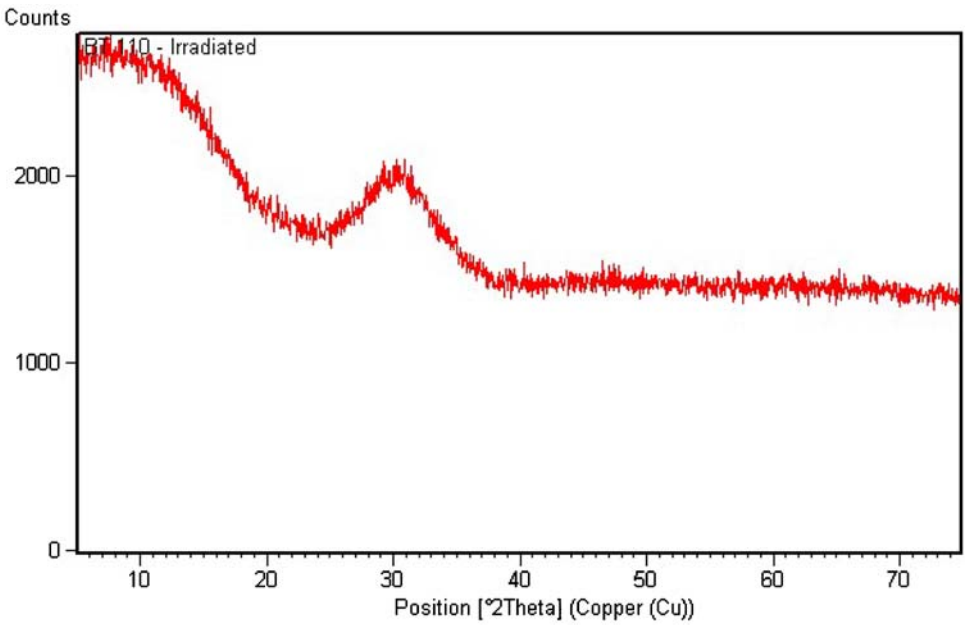


**Fig. 1** X-ray diffractogram for BT110

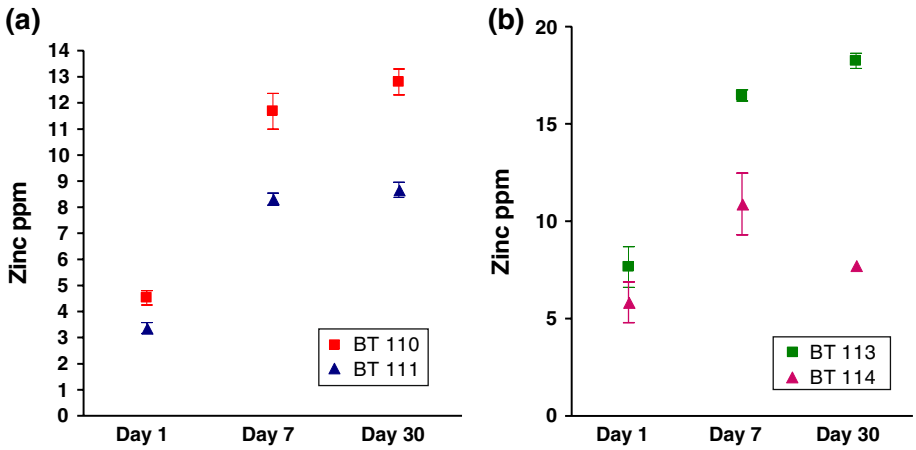


**Fig. 2** Zinc release at normal pH 7.4 over 1, 7 and 30 days (cumulative) for **a** BT110 and BT 111, and **b** BT113 and BT114

Glasses BT110 and BT113, (comparable compositions from each group) showed a similar trend with a statistically higher release of $Zn^{2+}$ at every time point when compared to the other glasses in their respective group, except on day 1 when comparing BT113 and BT114. On comparing BT110 and BT113, BT113 shows a statistically higher level of $Zn^{2+}$ release at every time point (Day 1 $P = 0.0353$, Day 7 $P = 0.0029$, and Day 30 $P = 0.0010$).

The $Zn^{2+}$ release profiles at pH 3.0 for Zn containing glasses BT110, BT111, BT113 and BT114 over 1, 7 and 30 days are shown in Fig. 3.

No statistically significant difference in $Zn^{2+}$ release was recorded at day 1 and 7 when comparing BT110 and BT111. However, large error bars are associated with the mean $Zn^{2+}$ concentration recorded for BT110 at day 7. At day 30, $Zn^{2+}$ release from BT110 was significantly higher ($P = 0.0003$) than BT111. When comparing BT113 and BT114, statistical significance was achieved at day 7 and day 30 ($P < 0.0001$ and $P = 0.0059$, respectively) with BT113 releasing higher $Zn^{2+}$ levels. $Zn^{2+}$ release was significantly higher for all glasses at all time points when immersed in the pH 3 compared to the pH 7.4 solution.

### *3.2.2 Strontium release*

$Sr^{2+}$ release profiles at pH 7.4 for glasses BT110, BT111, BT112, BT113, BT114 and BT115 over 1, 7 and 30 days are shown in Fig. 4.

$Sr^{2+}$ release showed a similar trend in both groups. As the ZnO:$Na_2O$ ratio decreased across the series (refer to Table 1), $Sr^{2+}$ release increased. This increase was statistically significant at every time point when comparing all glasses in group 2, and similarly in group 1 with the exception of Day 1 when comparing BT110 and BT111.

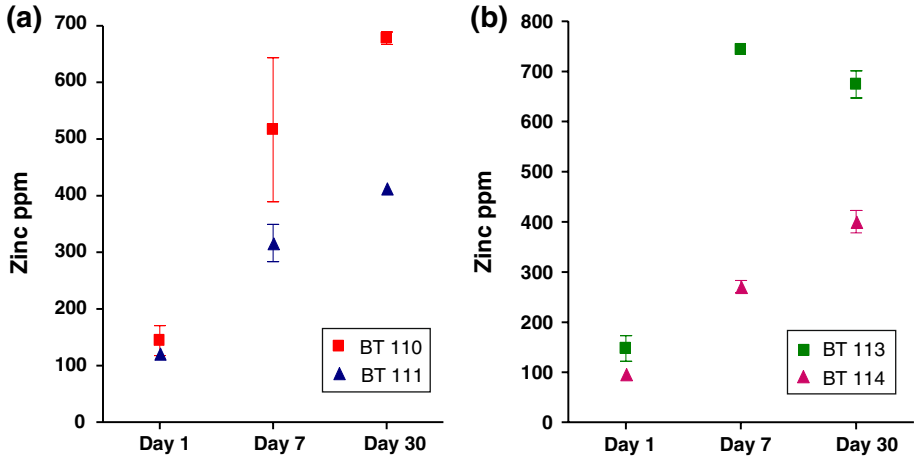


**Fig. 3** Zinc release at extreme pH 3 over 1, 7 and 30 days (cumulative) for **a** BT110 and BT111, and **b** BT113 and BT114

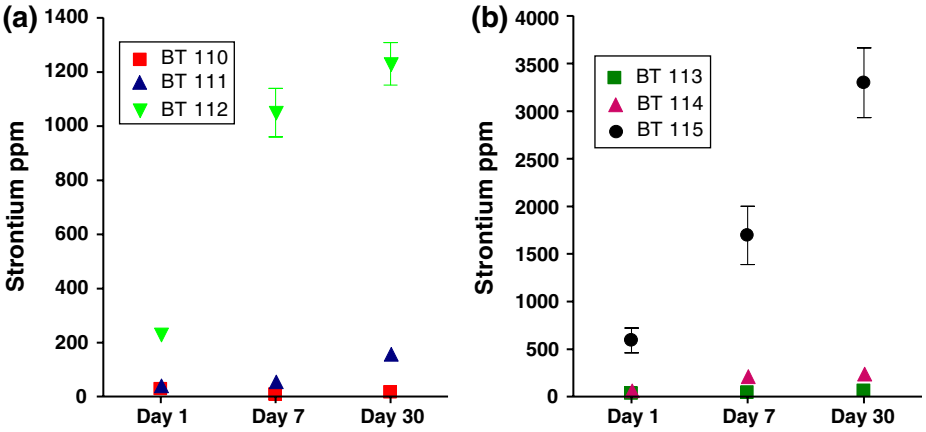


**Fig. 4** Strontium release at normal pH 7.4 over 1, 7 and 30 days (cumulative) for **a** BT110, BT111, BT112, and **b** BT113, BT114 and BT115

$Sr^{2+}$ release profiles at pH 3.0 for glasses BT110, BT111, BT112, BT113, BT114 and BT115 over 1, 7, and 30 days are shown in Fig. 5.

There was a statistically significant difference between $Sr^{2+}$ release from BT110 and BT112 at Day 1 and 7 ($P = 0.0059$ and 0.0130, respectively) with BT112 showing higher $Sr^{2+}$ release. By Day 30 there was no significant difference in $Sr^{2+}$ release between any of the glasses in group 1.

BT115 released a statistically higher amount of $Sr^{2+}$ at Day 1 when compared to BT113 ($P = 0.0488$) but the same was not seen on Day 7 and 30. Similarly to group 1, group 2 by Day 30 showed no significant difference in $Sr^{2+}$ release between glasses. Of interest is that BT112 and BT115, glasses with the highest $Na_2O$ content in their series, showed no significant difference in $Sr^{2+}$ release over 1, 7 and 30 Days. $Sr^{2+}$ release, when comparing pH 3

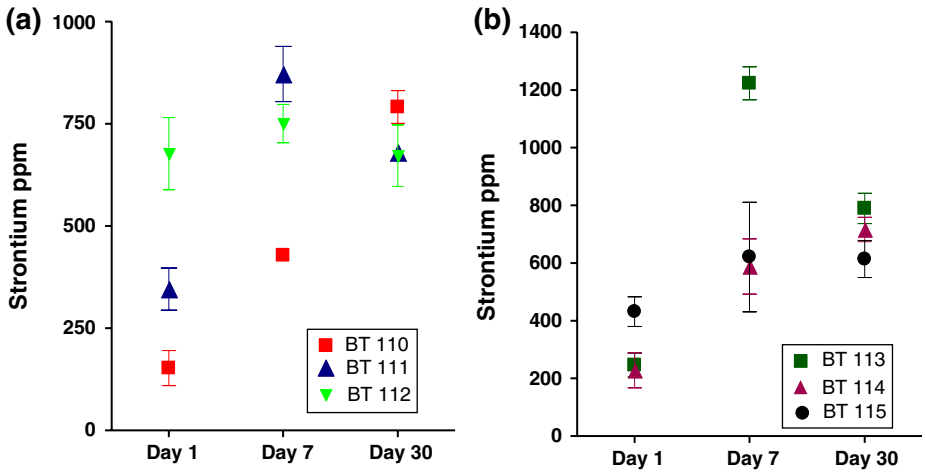


**Fig. 5** Strontium release at extreme pH3 over 1, 7 and 30 days (cumulative) for **a** BT110, BT111 and BT112,and **b** BT113, BT114 and BT115

and pH 7.4, was consistently higher in pH 3 for glasses BT110, BT111 and BT113. Interestingly, BT112 showed statistically higher $Sr^{2+}$ release at pH 7.4 on Day 7 and 30 ($P = 0.0413$ and 0.0171, respectively). BT114 and BT115 showed no significant difference in $Sr^{2+}$ release between pH solutions at Day 1 and 7. By Day 30, BT115 showed a higher $Sr^{2+}$ release at pH 7.4 ($P = 0.0109$).

### 3.2.3 *Composition versus $Sr^{2+}$ and $Zn^{2+}$ release profiles*

The effect of ZnO:$Na_2O$ compositional ratio (Table 1) on $Sr^{2+}$ and $Zn^{2+}$ release profiles at Day 30 and pH 7.4 is shown in Fig. 6.

Glasses with a ZnO:$Na_2O$ ratio of 0 released much higher levels of $Sr^{2+}$ than other glasses in their respective groups. Group 2 glasses consistently released statistically higher levels of $Sr^{2+}$ than their group 1 ZnO:$Na_2O$ counterpart (ZnO:$Na_2O = 0$ $P = 0.0052$ BT115 higher, ZnO:-Na2O $= 0.5$ $P = .0096$ BT114 higher, ZnO:Na2O $= 2$ $P = 0.0234$ BT113 higher). As ZnO:$Na_2O$ ratio increased, $Zn^{2+}$ release increased. At ZnO:Na2O of 2, $Zn^{2+}$ release is statistically higher for group 2 glass BT113 ($P = 0.001$).

## 4 Discussion

The aim of this work was to evaluate the $Zn^{2+}$ and $Sr^{2+}$ release profiles of Ca–Sr–Na–Zn–Si bioactive glasses, in order to assess the ability of various compositions to control degradation and therapeutic ion release. The authors have previously demonstrated, during a preliminary investigation, the biocompatible nature of Ca–Sr–Na–Zn–Si glasses [26]. However, the preliminary study did not quantify the ion release profiles of those glasses which were evaluated. Moreover, the glass compositions in the preliminary paper were synthesized without the addition of Na to control degradation and optimize ion release in the physiological environment. The glass systems studied in this work are designed with incremental additions of $Na_2O$, at the expense of ZnO in order to evaluate the effects of this substitution on the ion release profiles for $Sr^{2+}$ and $Zn^{2+}$. $Na_2O$, through its role as a network modifier and its high diffusivity has been known to reduce the durability and hence increase the degradation of various glass compositions [27]. From a surgical perspective, $\gamma$ sterilisation of such glasses will be a likely requirement prior to clinical use. Thus, the ion release profiles for each glass was conducted post 30 kGy $\gamma$ –irradiation, in order to eliminate any variables that may arise in the glass structure as a result of ionizing radiation. In order for ion release profiles to be evaluated fully both extreme and normal physiological environments are required to be considered.

Zinc containing glasses in this study demonstrated $Zn^{2+}$ release in the range of 3 ppm up to 750 ppm (Figs. 2 and 3 respectively). $Zn^{2+}$ levels previously reported to have clinically beneficial effects on bone formation range from 2.45 to 6.5 ppm in vitro and in vivo [28, 29], higher levels appear to induce cytotoxicity [30]. Similarly human $Zn^{2+}$ blood plasma levels have been shown to be approximately 6.4 ppm [31], and $Zn^{2+}$ release from biomaterials in the range 3–7 ppm has shown antibacterial efficacy [17]. It can be perceived that any glasses releasing $Zn^{2+}$ levels within these ranges will have similar beneficial effects. Thus all zinc-containing glasses, BT110, BT111, BT113 and BT114, at pH 7.4 may have therapeutic potential. After 1 Day $Zn^{2+}$ release for BT113 had reached 7.65 ± 1.04 ppm which according to the literature borders on the upper limit tolerable by cells in vitro [30]. Literature on the release of $Zn^{2+}$ from biomaterials in vivo, in particular bioactive glasses are still few. So further investigation into whether high $Zn^{2+}$ levels cause in vivo cytotoxicity is warranted. $Zn^{2+}$ release from these glasses appears to be dependant on the Zn content—as Zn content increases (Table 1 and Fig. 6) so does $Zn^{2+}$ release, an effect seen in other Zn containing glasses [11, 30]. An anomalous result occurred when comparing BT113 with BT110 (comparable in respect of their ZnO:$Na_2O$ concentrations). At pH 7.4 BT113 exhibited a statistically higher release of $Zn^{2+}$ at

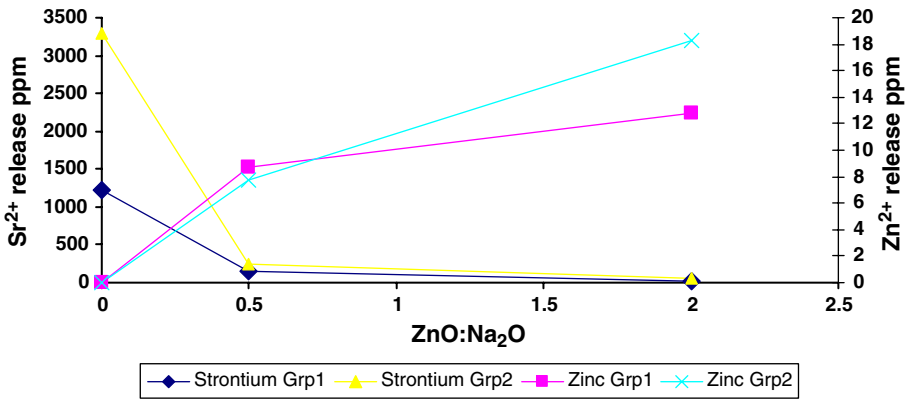


**Fig. 6** ZnO:$Na_2O$ compositional ratio for group 1 and group 2 glasses versus strontium and zinc release at day 30 pH 7.4

each time point, an effect difficult to explain and one not seen in the pH 3 environment.

Strontium containing glasses demonstrated $Sr^{2+}$ release in the range 7.5–3500 ppm (Figs. 4 and 5, respectively). $Sr^{2+}$ levels which have been reported to induce stimulatory effects on osteoblasts range from 8.7 to 87.6 ppm, and inhibitory effects on osteoclast action from 8.7 to 2102.8 ppm in vitro [19, 32]. The blood active $Sr^{2+}$ concentration in postmenopausal osteoporotic patients treated with Strontium Ranelate (Protelos®, Servier laboratories, Dun Laoighaire, Ireland) has been measured at 10.5 ppm [19]. Some evidence has also indicated that very high doses of $Sr^{2+}$ may induce mineralization defects [33]. It can similarly be perceived that any glasses releasing $Sr^{2+}$ within the above ranges will show similar effects. Thus all glasses may have therapeutic potential with respect to the $Sr^{2+}$ release profiles measured herein. The effect of Sr on osteoclastic activity in vivo appears to be dose-dependent [19]. The higher the Sr level the more pronounced the inhibitory effect on osteoclast differentiation and resorption up to levels as high as 2102.8 ppm [19]. The effect of Sr on osteoblastic activity in vitro shows stimulatory effects up to 87.6 ppm [19], but at present no study has examined this osteoblastic effect at much higher Sr levels. Caution should be used in presuming that higher levels of Sr will cause similar stimulatory effects. So as to assess the bioactive glass with the optimum $Sr^{2+}$ release profile, relevant in vitro and subsequent in vivo studies with higher $Sr^{2+}$ levels are warranted. $Sr^{2+}$ release at pH 7.4 showed a similar trend in both groups. As ZnO:$Na_2O$ ratio decreased across the series (Table 1 and Fig. 6), $Sr^{2+}$ release increased. Decreasing $Zn^{2+}$, a metal ion which has been known to retard degradation of bioactive glasses [31], and increasing $Na_2O$ content, which has been known to impart degradability, appears to have the synergistic effect of increasing $Sr^{2+}$ release. Thus this substitution appears to induce increased degradability on the glass network and may be a useful tool in controlling dissolution rate and specific ion release.

Sustainability of ion release over time is important to prolong and enhance therapeutic effect. For all glasses at pH 7.4, $Sr^{2+}$ and $Zn^{2+}$ ion release was sustained up to Day 7. But by Day 30, for most glasses ion release levels had plateaued, showing no statistical difference between Day 7 levels. Saturation of silica at the surface and condensation of surface silanol groups are generally considered to be responsible for this slow down in ion release [34]. The pH of the immersion solution also played an important role on the levels of $Sr^{2+}$ and $Zn^{2+}$ ion release from the glasses. The introduction of network-modifying cations to a glass increases the polarizability of the oxygen ions and, therefore, its vulnerability to acid attack [35]. This is greatest when the oxygen atoms are associated with cations of low electrostatic field strength, e.g., $Na^+$, $Ca^{2+}$ and $Sr^{2+}$ and least when the cations have a high electrostatic field strength e.g., $Si^{4+}$ and $Zn^{2+}$ [35]. This is reflected in the results. At pH 3.0, glasses BT112 and BT115 which contain a high percentage of $Na_2O$ and SrO and no ZnO, show high levels of $Sr^{2+}$ release at Day 1 (mean 431 and 677 ppm respectively). $Zn^{2+}$ release for all glasses at every timepoint was statistically higher at pH 3 than at pH 7.4. Whereas at pH 3 $Sr^{2+}$ release for the glasses with the highest $Na_2O$ content, BT112 and BT115, showed either no statistical difference or was significantly lower than its pH 7.4 counterpart. This result may be related to a possible interaction of $Sr^{2+}$ with the citric acid in the pH 3 buffer, as citric acid has previously been shown to complex with metal ions such as calcium [36].

## 5 Conclusion

The objective of this work was to evaluate $Zn^{2+}$ and $Sr^{2+}$ ion release from varying compositions of Ca–Sr–Na–Zn–Si glass bone grafts under normal and extreme physiological environments. The $Zn^{2+}$ release results indicate that at pH 7.4 all Zn-containing glass compositions may have potential as therapeutic bone grafts, but at pH 3 high $Zn^{2+}$ levels may produce cytotoxic effects. $Sr^{2+}$ release results indicate that all glass compositions may have therapeutic potential in both pH environments. Increasing the $Na_2O$:ZnO compositional ratio increased the levels of $Sr^{2+}$ release, where as $Zn^{2+}$ release was related to the Zn content of the glass. Similarly, $Zn^{2+}$ and $Sr^{2+}$ release for all compositions in the pH 7.4 environment was sustainable up to 7 days. One limitation to the study is the use of citric acid as a buffer in the pH 3 solution. It appears to complex with $Sr^{2+}$ thus giving lower than expected $Sr^{2+}$ levels in solution. On the basis of these results, further in vitro and in vivo investigation is warranted in order to assess the composition and corresponding ion release profile offering optimum therapeutic efficacy.

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