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Analysis of γ -irradiated synthetic bone grafts by ^{29}Si MAS-NMR spectroscopy, calorimetry and XRD

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ABSTRACT

Ca–Sr–Zn–Si glasses have demonstrated excellent biocompatibility both *in vitro* using the MTT assay with L929 mouse fibroblast cells, and *in vivo* using healthy and ovariectomized female Wistar rats. However, the biological evaluation of the materials was performed on glass granules that were autoclaved, rather than γ -irradiated; the sterilisation procedure required prior to implantation of these materials in the human body. Given the fact that when a glass is exposed to ionizing radiation changes in its physical properties can take place, it is imperative to determine whether the structure of such glasses will be altered as a result of exposure to the typical amounts of γ -irradiation required to sterilise such materials prior to implantation. This paper examines the structure of Na–Ca–Sr–Zn–Si glasses using ^{29}Si MAS-NMR, XRD and DTA and to evaluate the effect of 30 kGy γ -irradiation on their structure. The ^{29}Si MAS-NMR results indicate that the peak maxima for each glass remains between -74 ppm and -79 ppm; a chemical shift for ^{29}Si associated with Q^1 units in silicate glasses, and that the local environment around the ^{29}Si isotope remains unaltered as a result of exposure. Additional analysis (DTA and XRD) showed that the onset of the glass transition temperature, T_g (in the range 553 °C– 619 °C depending on composition) typically remains unchanged, as a result of exposure to the ionizing radiation, as do the XRD diffractograms for each glass. Therefore it can be concluded that the use of 30 kGy γ -irradiation does not effect the local environment of the ^{29}Si isotope in the glasses, nor does it significantly alter the XRD diffraction patterns or the T_g values for CaO–SrO–NaO–ZnO–SiO₂ described in this work.

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1. Introduction

Calcium–Strontium–Zinc–Silicate glasses have potential as both constituents of bone cements [1–7] and, independently, as bone grafts [1,2].

Low doses of the strontium ion (Sr^{2+}) are effective in the treatment of osteoporosis [3]. Sr^{2+} demonstrates a dual action mechanism in the bone metabolic cycle [4], wherein it:

- (1) enhances the replication of preosteoblast cells [5], increasing alkaline phosphatase activity, a marker for osteoblast differentiation [6] and
- (2) decreases osteoclast markers leading to a decrease in bone resorption activity [7].

Large randomized controlled trials [8,9] have demonstrated that Sr^{2+} release increases bone density and decreases incidence of vertebral fracture by 41%. Bone biopsy data (>5 year) confirms the beneficial effects of Sr^{2+} on the three dimensional architecture of bone [3]. As such, the localized release of Sr^{2+} from a graft is likely to have a beneficial effect *in vivo*.

Glasses have been formulated to incorporate both strontium and zinc (Zn). Publications by the authors have highlighted the antibacterial efficacy of zinc ion (Zn^{2+}) release from hard tissue biomaterials [10]. Additionally, zinc plays a crucial role in increasing DNA in osteoblasts [11] and has been shown to be capable of increasing osteoblast proliferation, biomineralization and bone formation [12,13], indicates that Zn^{2+} release from the grafts will elicit a therapeutic effect. Succinctly, one can expect synergistic effects from the localized release of Sr^{2+} and Zn^{2+} from Ca–Sr–Zn–Si based glass grafts.

The authors have previously reported the synthesis and preliminary biological evaluation of grafts based on the Ca–Sr–Zn–Si system [1]. The biological evaluation examined both *in vitro* response using the MTT assay with L929 mouse fibroblast cells, and *in vivo* response using both healthy and ovariectomized female

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Wistar rats [1,2]. In these studies, the grafts demonstrated superior *in vitro* cell viability over the commercial control Novabone (Novabone products, FL, USA) and integrated well into host bone tissue with bone in close apposition to implanted graft granules [1]. However, the biological evaluation of the materials was performed on glass granules that were autoclaved, rather than γ -irradiated; the sterilisation procedure required prior to implantation of these materials in the human body.

When a glass is exposed to ionizing radiation, changes in the physical properties of the material can take place. The interaction between radiation and glasses causes a variety of changes which are dependant on the composition and structure of the materials, as well as the irradiation parameters [14]. Consequently, it is imperative to determine whether the structure of Ca–Sr–Na–Zn–Si glasses will be altered as a result of exposure to the typical amounts of γ -irradiation required to sterilise such materials prior to implantation. Thus, the objective of this paper is to examine the structure of Na–Ca–Sr–Zn–Si glasses using ^{29}Si MAS-NMR, XRD and DTA and to evaluate the effect of 30 kGy γ -irradiation on their structure.

2. Experimental

2.1. Glass preparation

Five glass compositions were prepared (Table 1). Appropriate amounts of analytical grade sodium carbonate, silica, zinc oxide, strontium carbonate and calcium carbonate were weighed out and mixed in a ball mill (1 h), then dried in an oven (100 °C, 1 h). Each glass batch was then transferred to a platinum crucible for firing (1480 °C, 1 h). Glass melts were shock quenched into water, and the resulting frit was dried, then ground and sieved to retrieve a glass powder (90–710 μm). Table 1 shows the chemical compositions (mol. fraction) of the glasses used.

2.2. Thermal characterisation of glasses

A combined differential thermal analyser–thermal gravimetric analyser (DTA–TGA, Setaram Labsys, Setaram Instrumentation, UK) was used to determine the onset of glass transition temperature (T_g) of each glass. A heating rate of 10 °C min^{-1} (up to 1000 °C) was used in an air atmosphere with alumina as a reference in a matched platinum crucible. T_g onset was determined graphically based on the onset point of the glass transition endotherm, the tolerance of the specific DTA used in this work is 2%.

2.3. Structural characterisation of glasses

2.3.1. Network connectivity

The network connectivity (NC) of the glasses was calculated with Eq. (1) using the molar compositions of the glass [15]

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

where:

- NC = network connectivity
- BO = bridging oxygen's
- NBO = non-bridging oxygen's

2.4. X-ray diffraction

Powdered samples of each glass were pressed to form discs (32 mm \varnothing \times 3 mm). Samples were analysed using Cu K α 1 radiation emitted from an Xpert MPD Pro 3040/60 X-ray diffraction unit

Table 1
Glass compositions (mol. fraction).

Glass designation	SiO ₂	ZnO	CaO	SrO	Na ₂ O
BT 110	0.40	0.20	0.10	0.20	0.10
BT 111	0.40	0.10	0.10	0.20	0.20
BT 112	0.40	0	0.10	0.20	0.30
BT 113	0.40	0.20	0	0.30	0.10
BT 114	0.40	0.10	0	0.30	0.20

(Philips, Eindhoven, Netherlands) with ethyl cellulose as a backing material.

2.5. MAS-NMR spectroscopy

^{29}Si MAS-NMR spectra were recorded at 7.05T on a Varian UNITY INOVA300 FT-NMR spectrometer (Palo Alto, CA, USA), equipped with a CP-MAS probe. Powdered glass samples were placed in a zirconia sample tube (7 mm \varnothing). The sample spinning speed at the magic angle to the external magnetic field was 6 kHz. ^{29}Si MAS-NMR spectra were taken at 59.59 MHz with 5.0- μs pulse length (pulse angle, $\pi/4$), 90-s recycle delays, where the signals from about 895 to 1695 pulses were accumulated. ^{29}Si NMR chemical shifts are reported in ppm, with PDMS (polydimethyl silane) as the external reference (–34 ppm vs. TMS 0 ppm). All NMR spectra were recorded in a room for exclusive use of NMR, where the room temperature was kept at 300 K by means of an air-conditioner. The estimated error of chemical shift for data is ca. 0.1 ppm for an ideal material, small variations above this value were observed.

2.6. Sterilisation

Each glass was sterilised using γ -irradiation by Isotron (Westport, Co. Mayo, Ireland) in accordance with 'ISO11137: 2006; sterilisation of healthcare products' [16]. The minimum and maximum doses recorded during sterilisation were 30.5 kGy and 30.8 kGy, respectively.

3. Results

Table 2 presents the results of the DTA analysis carried out on each glass composition pre- and post- 30 kGy γ -irradiation. The change in T_g onset (ΔT_g) between each glass as a result of sterilisation is also noted. No appreciable ΔT_g was evident for the glasses within the 2% tolerance of the DTA employed in this study. A representative DTA trace (Fig. 1) has also been included to illustrate the effect of irradiation on T_g .

Fig. 2 is the X-ray diffractogram collected for BT 114 pre- and post-irradiation and is representative of X-ray diffractograms collected from all glasses, pre- and post-irradiation, bar BT 113, where some partial crystallinity is evident; the peaks corresponding with those of Silicon oxide or sodium oxide. All materials were amorphous prior to irradiation, and all except BT 113 remained amorphous post-irradiation.

Fig. 3 illustrates the ^{29}Si MAS-NMR spectra collected from each glass pre- and post-irradiation, with chemical shift data collated in Table 3. The predominant Q^n structure of each glass was identified as Q^1 , as predicted from the network connectivity calculations (also Table 3). The local environment around the ^{29}Si isotope in each glass remained unchanged as a result of irradiation. However, it must be noted, though not quantified in this study, that a darkening of the glass occurred in all glass compositions.

Table 2

Glass transition temperatures onset (T_g) pre and post 30 kGy γ -irradiation. Error based on 2% capability index of DTA in parenthesis.

Class	T_g (°C) (pre-irradiation)	T_g (°C) (post-irradiation)	ΔT_g (°C)
BT 110	619 (12.38)	618 (12.36)	-1
BT 111	556 (11.12)	554 (11.08)	-2
BT 112	553 (11.06)	543 (10.86)	-10
BT 113	618 (12.36)	617 (12.34)	-1
BT 114	555 (11.1)	559 (11.18)	4

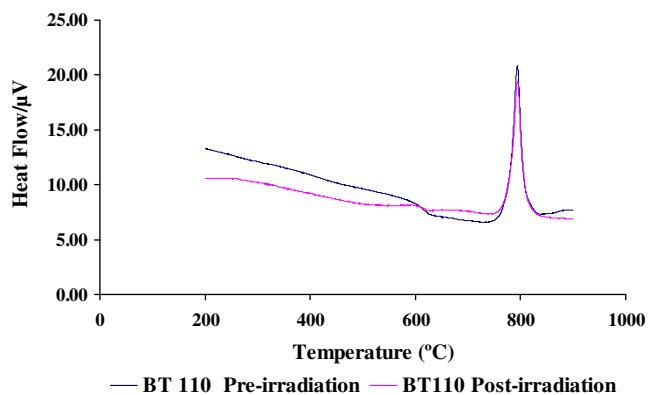


Fig. 1. DTA traces for BT 110 pre (blue trace) and post (pink trace) 30 kGy γ -irradiation. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

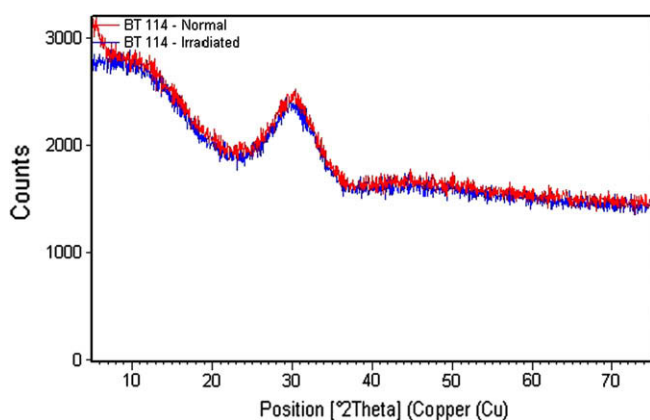


Fig. 2. X-ray diffractogram for BT 114 pre (red trace) and post (blue trace) 30 kGy γ -irradiation. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

4. Discussion

The ability of bioactive glasses to perform with the appropriate response in a specific application is driven by their composition–structure property relationships. However, it is known that exposure to γ -irradiation, at the levels (typically 25 kGy–30 kGy) required to sterilise such biomaterials can lead to significant changes in the structure of silicate glasses by breaking bonds in the SiO_2 structural units [17]. The authors have previously demonstrated the potential of Ca–Sr–Zn–Si glasses and synthetic bone grafts but this potential will be affected by the stability of these glasses when γ -irradiated.

The ^{29}Si MAS-NMR spectra (Fig. 3) for pre- and post-sterilised glasses exhibit large broad peaks between -60 ppm and -90 ppm for each material and remain unchanged as a result of

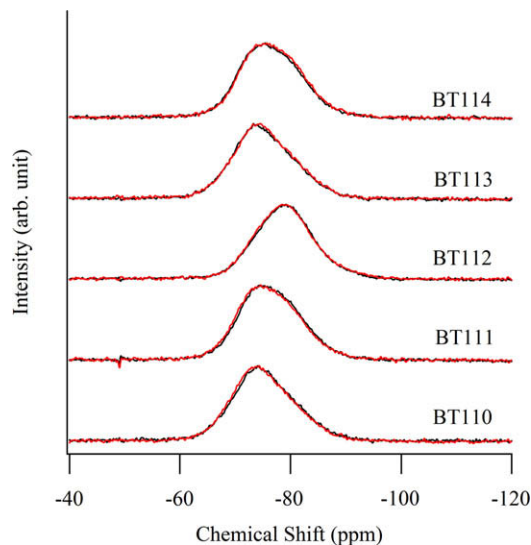


Fig. 3. ^{29}Si MAS-NMR spectra for BT 110–BT 114, pre (black line) and post (red line) 30 kGy γ -irradiation. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Table 3

^{29}Si MAS-NMR chemical shift data (peak maxima), associated Q^n structures and network connectivity (NC) values (Typical error in chemical shift for ideal material, based on equipment described is 0.1 ppm).

Glass designation	Pre- γ -irradiation chemical shift (ppm)	Post- γ -irradiation chemical shift (ppm)	Δ chemical shift (ppm)	NC	Predominant Q^n structure
BT 110	-74.1	-74.3	-0.2	1	Q^1
BT 111	-74.5	-74.6	-0.1	1	Q^1
BT 112	-78.6	-78.4	+0.2	1	Q^1
BT 113	-73.3	-74.5	-1.2	1	Q^1
BT 114	-75.4	-75.7	-0.3	1	Q^1

exposure to 30 kGy γ -irradiation. The broad chemical shifts recorded for each glass are typical of glasses containing the presence of a range of Q^n units based on ^{29}Si in a four co-ordinate state [18,19]. However, the peak maxima for each glass remains between -74 ppm and -79 ppm; a chemical shift for ^{29}Si associated with Q^1 units in silicate glasses [20], it is reasonable to conclude that such structures are pervasive in the networks of BT 110–BT 114 both before and after 30 kGy γ -irradiation. Whilst the estimated error of chemical shift for data is ca. 0.1 ppm for an ideal material, small variations above this value were observed (Table 3). However, the variations recorded are likely to have arisen as a result of the signal to noise ratio which affects small differences in the recorded peak maxima. Notwithstanding these slight variations, such data demonstrates that the structure dependant properties of such glass compositions in bone graft applications as previously demonstrated [1] will not be affected by γ -irradiation.

An additional finding arising from the ^{29}Si MAS-NMR spectra relates to the role of ZnO in the glass network of such glasses. Previous studies have demonstrated that ZnO acts as a network modifier in Ca–Sr–Zn–Si glasses whose network connectivity (NC) is between 1.83 and 2.3, *i.e.* glasses with predominant Q^2 structures [15,21]. However, Ca–Sr–Zn–Si compositions which have been considered as synthetic grafts in previous publications have a theoretical NC of 1, and it is unclear from the literature if decreasing the theoretical network connectivity, by controlling the composition of the glass (Eq. (1)) will result in a concurrent positive chemical shift on the ^{29}Si MAS-NMR spectra. Fig. 3 indicates that

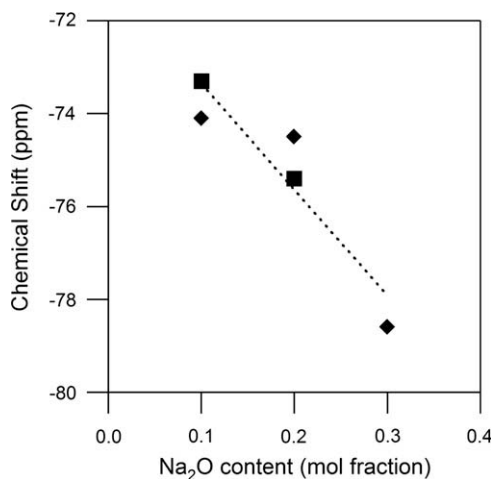


Fig. 4. Variation in chemical shift with respect to Na₂O content (line drawn as guide to reader).

previous assumptions in the literature that Zn acts as a network modifier in CaO–SrO–ZnO–SiO₂ glass compositions hold true as the theoretical value of NC is reduced to 1. To further validate the network modifying role of Zn in such glasses as those discussed herein, it is also clear from the results (Fig. 4) that as Na is substituted for Zn the glass the chemical shift for the glass series moves in a negative fashion from –74.1 ppm for BT 110 to –78.6 ppm for BT 112, indicating a shift in the network towards a Q² predominant glass structure [18].

Further to the ²⁹Si MAS-NMR spectroscopy the effect of γ -irradiation on the glasses was analysed using XRD and DTA (Figs. 1 and 2 and Table 2). The results of this analysis show that γ -irradiation does not alter the nature of the glasses nor does it lead to a ΔT_g for the majority of the compositions. Table 2 highlights the T_g onset values pre- and post-sterilisation for each glass composition. The associated error in measurement is 2%. This being the case the maximum deviation noted is for glass BT 112 where ΔT_g onset was 10 °C which is within the error of the machine, highlighting the stability of the glass compositions with respect to required exposures for clinical sterilisation procedures.

In addition to the quantified data presented and discussed above, it was noted that darkening of each glass was also observed. It has been demonstrated on numerous occasions that, upon exposure to ionizing radiation, silicate glasses experience changes in their physical properties, and that such changes depend on numerous variables including the composition and structure of the materials, as well as the irradiation parameters, energy dose and dose rate [19,20]. One of the processes which occur during γ -irradiation of silicate glasses is the formation of electron–hole pairs. Such free carriers can move and recombine, so that the photoelectrons are trapped at structural defects or impurities such as oxygen vacancies and multivalent impurities, while the holes are self-trapped at bridging or non-bridging oxygen's. The resultant electronic con-

figurations lead to high absorption levels called ‘color centers’ which cause the darkening of the glass as observed, but not quantified, in this work.

5. Conclusions

The use of 30 kGy γ -irradiation does not effect the local environment of the ²⁹Si isotope in the glasses, nor does it significantly alter the XRD diffraction patterns or the T_g onset values for CaO–SrO–NaO–ZnO–SiO₂ described in this work. It was noted however that the T_g of the zinc free glass (BT 112) did drop by 10 °C post sterilisation. In addition to these findings the ²⁹Si MAS-NMR results validate the assumption in the literature that Zn²⁺ predominantly acts as a network modifier in glasses of the generic composition SrO–CaO–NaO–ZnO–SiO₂ irrespective of the NC being as low as 1.

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