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Strontium oxide doped quaternary glasses: effect on structure, degradation and cytocompatibility

N. J. Lakhkar · E. A. Abou Neel · V. Salih ·
J. C. Knowles

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Abstract This preliminary study focuses on the effect of adding SrO to a Ti-containing quaternary phosphate glass system denoted by P_2O_5–Na_2O–CaO–TiO_2. The following four different glass compositions were manufactured: $0.5P_2O_5$–$0.17Na_2O$–$0.03TiO_2$–$(0.3-x)$CaO–xSrO where $x = 0$, 0.01, 0.03 and 0.05. Structural characterisation revealed glass transition temperatures in the range 427–437°C and the presence of sodium calcium phosphate as the dominant phase in all the glasses. Degradation and ion release studies conducted over a 15-day period revealed that the Sr-containing glasses showed significantly higher degradation and ion release rates than the Sr-free glass. Cytocompatibility studies performed over a 7-day period using MG63 cells showed that the addition of 5 mol% SrO yielded glasses with cell viability nearly equivalent to that observed for quaternary TiO_2 glasses.

1 Introduction

Ternary P_2O_5–Na_2O–CaO phosphate glasses have recently generated considerable interest as attractive materials for a variety of clinical applications—dental, maxillofacial and orthopaedic implants; scaffolds for bone tissue engineering; and carriers for antimicrobial ion delivery—on account of their highly controllable degradation properties and compositional affinity to the mineral phase of bone [1]. In particular, many studies have focussed on metal oxide addition to the ternary phosphate glass system for two reasons: (a) the incorporated metal oxides facilitate the manufacture of glasses with degradation rates that can vary over several orders of magnitude from 2 to 3 h to over 1 year and (b) the metal ions released by these glasses have demonstrable antimicrobial and therapeutic effects [1, 2]. To date, phosphate glasses have been doped with MgO [2], Fe_2O_3 [3–6], CuO [7], Al_2O_3 [8], TiO_2 [9–13], ZnO [14, 15], Ag_2O [16–18] and Ga_2O_3 [19]. Out of these, TiO_2 incorporation is of particular interest for developing therapies to combat bone tissue loss caused by injury, disease or congenital defects.

Synthetic bone substitutes are desirable for the treatment of large bone defects which cannot be treated effectively by prevailing bone transplantation techniques. However, it is not possible to use ternary P_2O_5–Na_2O–CaO glasses as bone substitutes because they degrade at rates too high for bone cells to properly attach onto the glass surface and proliferate. The addition of TiO_2 to the ternary glass system has been demonstrated to cause a considerable decrease in glass degradation rate, as explained by Navarro et al. [9]. Moreover, several studies have shown that phosphate glasses doped with TiO_2 elicit a significantly more favourable cell response in vitro than ternary phosphate glasses due to the decreased solubility of Ti-containing phosphate glasses and associated improvements in gene up-regulation of cultured cells [9, 11–13].

Considering the favourable properties of Ti-containing phosphate glasses, it is desirable to produce glasses that degrade at higher rates than currently possible using TiO_2 doping alone, while simultaneously retaining or even enhancing glass cytocompatibility. This would be particularly useful for extending the utility of TiO_2-doped phosphate glasses from orthopaedic applications to treatment of dental and periodontal diseases, where higher

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N. J. Lakhkar · E. A. Abou Neel · V. Salih · J. C. Knowles (✉)
Division of Biomaterials and Tissue Engineering,
Eastman Dental Institute, University College London,
256 Gray's Inn Road, London WC1X 8LD, UK
e-mail: j.knowles@eastman.ucl.ac.uk

degradation and ion release rates would be more beneficial. The addition of SrO as a second dopant in addition to TiO_2 offers interesting possibilities in this regard.

A number of studies have documented the therapeutic properties of Sr and its compounds. For example, Guida et al. have incorporated SrO into glass ionomer cements in order to confer antibacterial properties on them for use in dental caries treatment [20]. While it has generally been considered that fluoride release from the GICs is responsible for their bactericidal action, Guida et al. have observed that in the strontium-containing GICs, the inhibition of caries growth is achieved predominantly by Sr release rather than fluoride release. Researchers are also studying a novel compound named strontium ranelate that stimulates an increase in bone mass and could therefore be used to combat disorders related to bone loss such as osteoporosis [21–23]. Strontium incorporation into $CaSiO_3$ ceramics has revealed that Sr addition reduces the pH changes, degradation rate and ion release rate of $CaSiO_3$ ceramics while simultaneously providing improved biological properties in cell culture studies involving human bone cells [24].

A number of researchers have also attempted to dope bioactive glasses with SrO and have achieved encouraging results. For instance, Lao et al. have incorporated SrO into bioactive glasses to form a SiO_2–CaO–SrO system and have found that controlled dissolution and release of SrO from the glasses is possible and that SrO exerts a positive role in bone remodelling [25]. In addition, they also report that the release of Sr^{2+} ions into the medium increases with the Sr content of the glass. Towler et al. have recently prepared SrO–CaO–ZnO–SiO_2 glasses and carried out cytocompatibility tests, both in vitro using a simulated body fluid (SBF) model and in vivo using a rat femur model [26]. They have found that although the glasses cannot form a hydroxyapatite layer in SBF, they clearly exhibit bioactive behaviour in vivo, with bone formation in close proximity of the implantation site and absence of any inflammatory response.

A previous study by Abou Neel et al. focussed on the structure and properties of P_2O_5–Na_2O–CaO–SrO glasses [27]. They found that quaternary Sr-containing glasses do not possess favourable biocompatibility on account of their excessively high degradation rates. In this context, the present study proposed a first-time investigation of the addition of SrO to a Ti-containing phosphate glass to form a five-member P_2O_5–Na_2O–CaO–TiO_2–SrO system and the resultant effects on the physical properties and cytocompatibility of the glasses. The thermal and structural properties of the investigated glass compositions were investigated by means of differential thermal analysis (DTA) and X-ray diffraction (XRD). Weight loss, pH change and ion release were studied over a 15-day time period using a simple aqueous model; ion chromatography and inductively coupled plasma mass spectroscopy (ICP-MS) were used to measure the release of various ions from the glasses at each time point. Finally, in vitro cell proliferation and attachment were studied over a 7-day period using MG63 human osteosarcoma cells, and the results were compared with standard glass cover slips used as controls.

2 Materials and methods

2.1 Preparation of glass samples

Glass rods with an average diameter of 15 mm were manufactured according to a previously described melt quenching method [12]. The following precursors, all of which had 98% or greater purity, were used without further purification: phosphorus pentoxide (P_2O_5) (Fisher Scientific, Loughborough, UK), calcium carbonate ($CaCO_3$), sodium dihydrogen orthophosphate (NaH_2PO_4), titanium oxide (TiO_2) and strontium carbonate ($SrCO_3$) (BDH, Poole, UK). A total of four compositions were prepared (Table 1). The precursor mixture was initially preheated at 700°C for 10 min and then melted at 1300°C for 3 h in a Carbolite RHF 1500 furnace (Carbolite, Sheffield, UK). For the Sr-free glass, the precursors were melted in a Pt/10% Rh type 71040 crucible (Johnson Matthey, Royston, UK), while for the Sr-containing glasses, they were melted in a vitreous silica crucible (Saint-Gobain Quartz, Tyne & Wear, UK) to avoid the possibility of Sr-containing alloys forming on the platinum crucible surface. Melting was followed by annealing in a preheated graphite mould at 425°C for 2 h (to remove residual stresses) and cooling overnight at room temperature. The glass rods formed were cut into discs of approximately 2 mm thickness using a Testbourne diamond saw (Testbourne, Basingstoke, UK) with methanol as the coolant and lubricant.

2.2 Structural characterisation

The thermal and crystallisation properties of the glass were characterised by means of DTA and XRD studies,

Table 1 Glass compositions and codes used

Glass code	Glass composition (mol%)				
	P_2O_5	CaO	Na_2O	TiO_2	SrO
P50C30N17Ti3Sr0 (Sr0)	50	30	17	3	0
P50C29N17Ti3Sr1 (Sr1)	50	29	17	3	1
P50C27N17Ti3Sr3 (Sr3)	50	27	17	3	3
P50C25N17Ti3Sr5 (Sr5)	50	25	17	3	5

respectively; the methods used for both analyses have been described elsewhere [13]. The DTA experiments were carried out using a Setaram Differential Thermal Analyser (Setaram, France). The DTA samples were heated from room temperature to 1000°C at a heating rate of 20°C min^{-1}, and the following parameters were measured: glass transition temperature (T_g), crystallisation temperature (T_c) and melting temperature (T_m). Further, XRD analyses were performed using a Brüker D8 Advance Diffractometer (Brüker, Coventry, UK) and a LynxEye detector (Brüker, Coventry, UK) in flat plane geometry with Ni-filtered Cu Kα radiation.

2.3 Degradation and ion release studies

The following three parameters were considered: weight loss, pH change and ion release. Degradation studies were carried out on the basis of a weight loss method using a simple aqueous model which has been described elsewhere [12]. The following time points were used: 25, 46, 96, 170, 263 and 356 h (corresponding to 1, 2, 4, 7, 11 and 15 days, respectively). The glass disks were immersed in plastic containers holding 25 ml of high-purity deionised water (resistivity = 18.2 MΩ cm^{-1}) and incubated at 37°C. After every time point, the solution from each container was removed and its pH and ion release were analysed. The discs were dried and weighed, after which they were placed in fresh deionised water and reincubated. The pH of the solution removed at each time point was measured using an Orion pH meter (Thermo Scientific, UK) fitted with a pH glass electrode. The release of polyphosphate anions from the glass discs was analysed using a Dionex ICS-2500 ion chromatography system (Dionex, Surrey, UK), while Na^+ and Ca^{2+} cation release were measured using a Dionex ICS-1000 ion chromatography system [12]. In addition, Ti^{4+} and Sr^{2+} ion release measurements were carried out by means of ICP-MS using a Spectromass 2000 ICP mass spectrometer (Spectro, Germany) [12]. For anion chromatography and ICP-MS, no further purification of the analyte was required, whereas for cation chromatography, the solution was filtered using a Dionex OnGuard IIA cartridge to eliminate anions which could hinder cation detection.

2.4 Cell culture

For the cell culture studies, the methods described by Chen et al. were used with some modifications [28]. MG63 osteoblast-type cells were seeded on to polished and sterilised glass discs at a seeding density of 5,000 cells per disc, followed by incubation at 37°C in an atmosphere of 5% CO_2 for 7 days. All cell culture experiments were carried out in triplicate, and standard glass cover slips were used as the control. Cell proliferation experiments were conducted at time points of 1, 4 and 7 days. The growth in cell population at each time point was determined using the AlamarBlue™ assay (AbD Serotec, UK) [28]. For the scanning electron microscopy (SEM) images, the time points used were 1 and 7 days, and images were obtained with a scanning electron microscope (model JSM 5410LV, JEOL, USA [28].

3 Results and discussion

3.1 Structural characteristics

3.1.1 Differential thermal analysis

The T_g values of the glasses (Table 2) were in the range 427–437°C, and a clear trend in the T_g value was not discernible as the Sr content of the Ti-containing glass was increased. In the DTA traces of the investigated glasses (Fig. 1), the Sr0 glass showed evidence of two distinct crystallisation peaks at T_c values of 602°C and 689°C; this is as expected and in agreement with earlier works on Ti-containing glasses [12, 13]. The addition of Sr to the glass led to the occurrence of a single crystallisation peak which was considerably broader than the sharply defined peaks in the Sr0 glass. The T_c values of the Sr-containing glasses were in the range 618–626°C; thus, the T_c values of the Sr-containing glasses were in between the T_{c1} and T_{c2} values of the Sr-free glass.

Table 2 Glass transition temperatures for the investigated phosphate glasses

Glass code	Sr0	Sr1	Sr3	Sr5
T_g (°C)	431	431	437	427

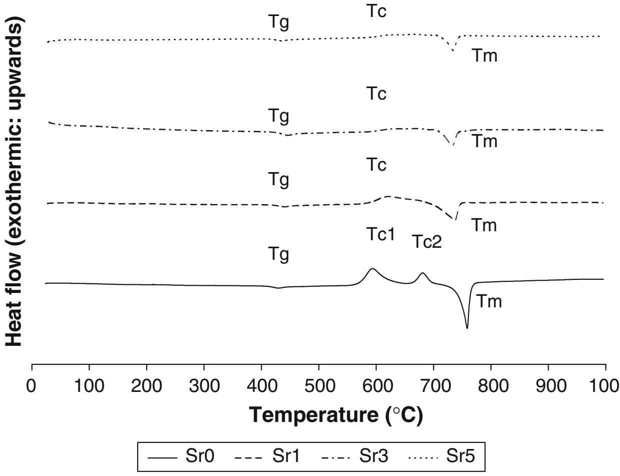

Fig. 1 DTA traces of the investigated phosphate glasses

Melting peaks sharper than the crystallisation peaks were observed in all the investigated compositions. The T_m values decreased from 757°C in the Sr-free glass to 733–736°C in the Sr-containing glasses. The addition of 1 mol% SrO to the Sr-free glass evidently resulted in a significant decrease in the T_m value, but further increases in SrO content did not affect the T_m value to the same extent. The presence of a single crystallisation peak in the Sr-containing glasses which broadens as the Sr content is increased and the significant decrease in T_m from the Sr0 glass to the Sr1 glass indicated that the Sr ions significantly disrupt the polyhedral titanate structures present in the Sr-free glass.

3.1.2 X-ray diffraction

The XRD studies revealed that all the investigated glass compositions exhibit a main phase comprising a sodium calcium phosphate ($NaCa(PO_3)_3$; ICDD No. 23-669), as identified from the ICDD database. Sodium calcium phosphate remained the main phase even with the addition of Sr to the glasses. This is to be expected since the difference between the Sr contents of the Sr-free and Sr-containing glasses is no more than 5 mol%. Moreover, the doping process involved a replacement of Ca^{2+} ions with equivalently charged Sr^{2+} ions. Thus, at such low levels of metal oxide addition involving replacement of equivalently charged ions, the phases within the glasses would not be particularly affected.

3.2 Degradation and ion release studies

3.2.1 Weight loss

Figure 2 shows the plot of degradation—represented in the form of percent cumulative weight loss per unit surface area to take into account the effect of surface area on degradation—as a function of time for the investigated glass compositions. The following equation was used to calculate the percent weight loss per unit surface area:

$$(M_0 - M_t)/M_0 A * 100$$

where M_0 is the original weight of the glass disc (mg); M_t, the weight of the glass disc at time t (mg); and A, the surface area of the glass disc (mm^2).

In all the glass compositions, it was observed that the degradation process increases with time. The Sr-containing glasses degraded to a greater extent than the Sr-free glass, thereby indicating that the Sr-free glass is less soluble than the Sr-containing glasses, possibly due to disruption of the titanate polyhedra in the glass structure by Sr^{2+} ions. This trend is also in agreement with the DTA data, where the Sr-containing glasses showed lower T_m values than the Sr-free glass.

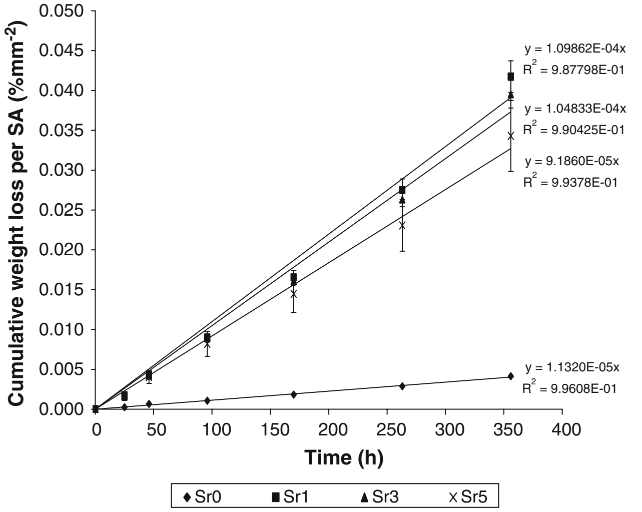

Fig. 2 Degradation (presented as percent cumulative weight loss per unit surface area ($\%mm^{-2}$)) as a function of time for Ti-containing glasses with different Sr contents

The degradation rate can be measured in terms of the slope of the trend line plotted for each composition (Table 3). The Sr0 glass showed a degradation rate of 1.1×10^{-5} $\%mm^{-2}$ h^{-1} which increased substantially by one order of magnitude to approximately 11.0×10^{-5} $\%mm^{-2}$ h^{-1} when 1 mol% SrO was added. However, a further increase in the Sr content of the glass resulted in a slight decrease in the degradation rate to 10.5×10^{-5} $\%mm^{-2}$ h^{-1} for the Sr3 glass and 9.2×10^{-5} $\%mm^{-2}$ h^{-1} for the Sr5 glass. Interestingly, this trend (Sr0 < Sr5 < Sr3 < Sr1) was observed in some results of the ion release studies as well, notably those for the cations Na^+ and Ca^{2+}.

The degradation plot data exhibited R^2 values in excess of 0.98, suggesting a near-linear degradation profile and thereby raising interesting possibilities for the application of these glasses as vehicles for sustained and controlled delivery of ions and biological entities. Similarly, many of the ion release profiles of the glasses showed R^2 values exceeding 0.9. However, visual inspection of some of the ion release plots, notably Ti^{4+} and Sr^{2+}, revealed that the ion release may not necessarily be linear in all cases, in spite of the significantly high R^2 values; a nonlinear function relating ion release and time would possibly be a more appropriate fit in such cases. Nevertheless, linear fits were used for all the degradation and ion release data as a means to rank the various compositions.

Table 3 Cumulative degradation rate ($\%mm^{-2}$ h^{-1}) determined from the slopes of the linear fit against time for the investigated glass compositions

Glass code	Sr0	Sr1	Sr3	Sr5
Degradation rate ($\%mm^{-2}$ h^{-1} $\times 10^{-5}$)	1.1	11.0	10.5	9.2

3.2.2 pH change

The Sr-free glass showed pH values in the range 5.6–6.1, while the Sr-containing glasses showed significantly lower pH values in the range 4.0–4.7 (Fig. 3). Thus, the deionised water solutions of the Sr-containing glasses were more acidic than the solution of the Sr-free glass. In addition, all the glasses exhibited a net decrease in pH from day 0 to day 15. The trend of net decrease in pH shown by the investigated glasses has been observed previously for phosphate glasses containing oxides of Fe, Ti and Zn among others [5, 6, 13, 14].

The significantly lower pH shown by the Sr-containing glasses as compared to the Sr-free glass clearly indicates that the Sr-containing glasses were more soluble than the Sr-free glass so that they released more phosphate ions than the Sr-free glass at all time points, leading to the formation of phosphoric acid in the medium. At each time point, the Sr-containing glasses showed pH values quite close to each other, whereas the Sr-free glass showed a considerably higher pH. The results of the pH study showed the opposite trend as those of the degradation study, and from day 7 to day 15, the pH values were clearly in the order Sr0 > Sr5 > Sr3 > Sr1.

3.2.3 Ion release

Similar to the degradation study, the results of the study on cumulative ion release of Na^+ and Ca^{2+} cations (Fig. 4a, b) and metallic Ti^{4+} and Sr^{2+} ions (Fig. 5a, b) reveal that ion release from the all the studied compositions increased with time. For almost all the species, the Sr-free glass showed markedly lower ion release as compared to the Sr-containing glasses, all of which exhibited ion release values very close to each other. The slope of the linear fit of ion release over time provides the ion release rates of the cations and metallic ions (Table 4); the polyphosphate ion release rates have been determined by the same method. It is worth noting that the Sr0 < Sr5 < Sr3 < Sr1 trend of the degradation study is evident for both Na^+ and Ca^{2+} species and that Sr^{2+} is the only species for which the release rate increases with the Sr content.

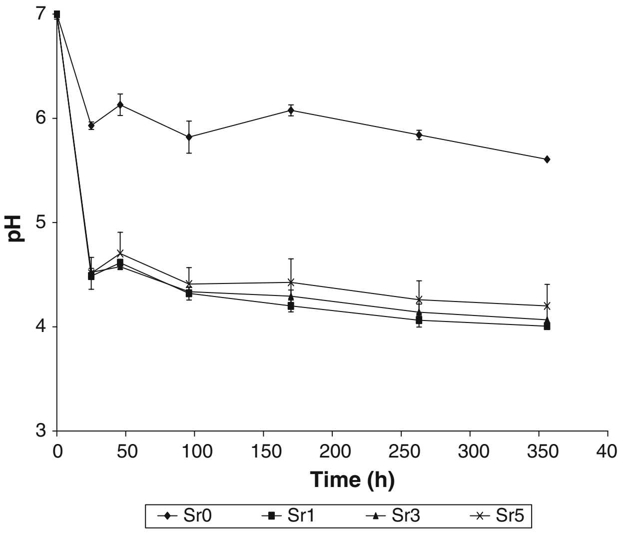

Fig. 3 pH change in deionised water as a function of time for the investigated phosphate glasses

3.3 Cell culture studies

3.3.1 Cell proliferation

The cell proliferation data (Fig. 6) show that by day 7, proliferation on the Sr5 sample was similar to that on the

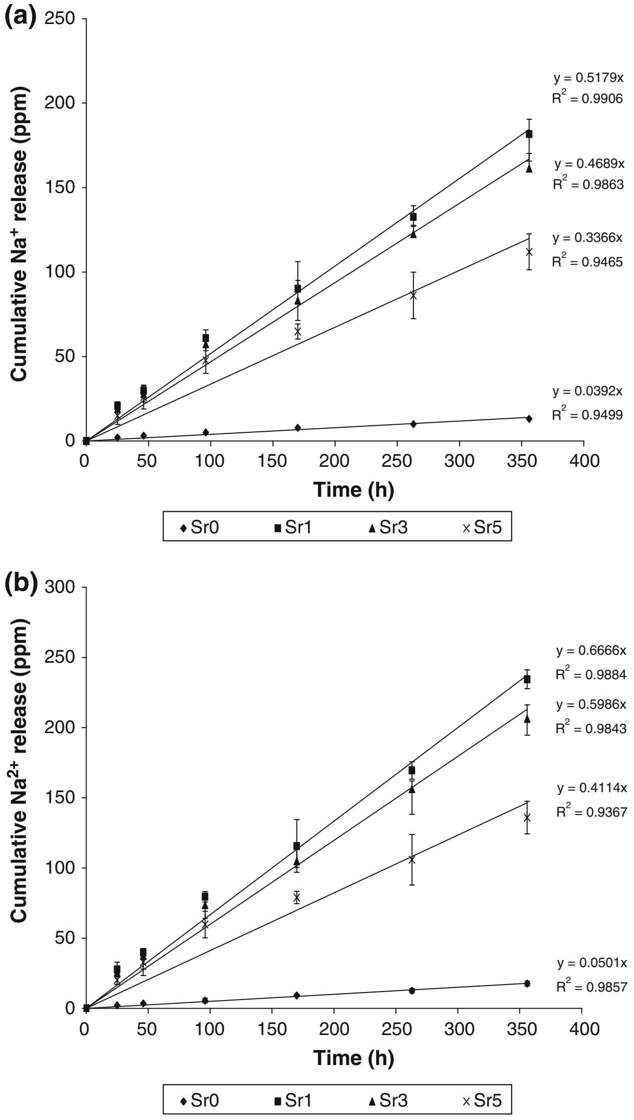

Fig. 4 Cumulative release (ppm) of **a** Na^+ and **b** Ca^{2+} ions as a function of time for Ti-containing glasses with different Sr contents

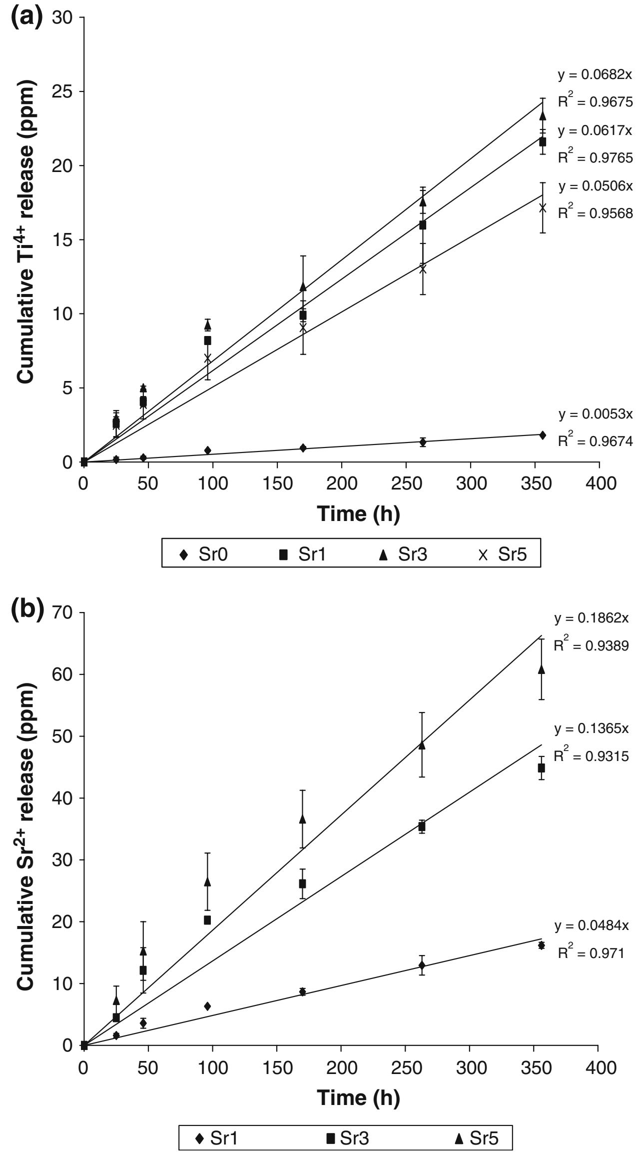

Fig. 5 Cumulative release (ppm) of **a** Ti^{4+} and **b** Sr^{2+} ions as a function of time for the investigated glasses

Sr0 sample, while the Sr3 and Sr1 samples showed a slightly reduced capacity for cell proliferation activity on their surface; thus, in the Sr-containing glasses, the cell proliferation ability increased with the Sr content. Within each glass composition, cell proliferation from day 1 through day 4 increased marginally if at all. However, from day 4 through day 7, there was a marked increase in cell proliferation. This was true for both Sr-free and Sr-containing glasses, indicating that the addition of Sr to the Ti-containing glasses was beneficial for allowing cells to proliferate on the glass surface. Interestingly, the cell proliferation assay reveals that by day 7, cell viability on the glass surface increased in the order Sr1 < Sr3 < Sr5 < Sr0, a trend which was observed in the pH studies as well. This is in agreement with the fact that glasses that degrade slowly tend to exhibit higher cell viability.

Table 4 Cumulative anion, cation (Na^+ and Ca^{2+}), Ti^{4+} and Sr^{2+} release rates (ppm h^{-1}) determined from the slopes of the linear fit against time for Ti-containing phosphate glasses with different SrO contents

Glass code		Sr0	Sr1	Sr3	Sr5
Anion (ppm h^{-1})	PO_4^{3-}	0.0512	0.3434	0.4089	0.3100
	$P_3O_9^{3-}$	0.0567	0.3622	0.3174	0.2725
	$P_2O_7^{4-}$	0.0079	0.3132	0.3745	0.2687
	$P_3O_{10}^{5-}$	0.0182	0.4915	0.5876	0.4282
Cation (ppm h^{-1})	Ca^{2+}	0.0501	0.6666	0.5896	0.4114
	Na^+	0.0392	0.5179	0.4689	0.3366
Ti^{4+} (ppm h^{-1})		0.0053	0.0617	0.0682	0.0506
Sr^{2+} (ppm h^{-1})		N.A.	0.0484	0.1365	0.1862

3.3.2 Scanning electron microscopy

From the SEM images in Fig. 7, the proliferation of cells at day 7 on the Sr0 and Sr5 glasses (Fig. 7d, j, respectively) was clearly visible. The morphology of the cells cultured on the glass specimens is particularly interesting; on day 1 itself, the cells appeared to be reasonably spread out, and by day 7, especially in the Sr0 and Sr5 glasses, the cells had spread out considerably over the glass surface. By day 7, the Sr1 and Sr3 glasses (Fig. 7f, h, respectively) glasses had degraded, leading to the formation of cracks on the glass surface. It is difficult to accurately image groups of cells on such highly degraded glasses. Therefore, only individual cells were imaged with some detail; these cells are indicated by arrows where required. Although the glass

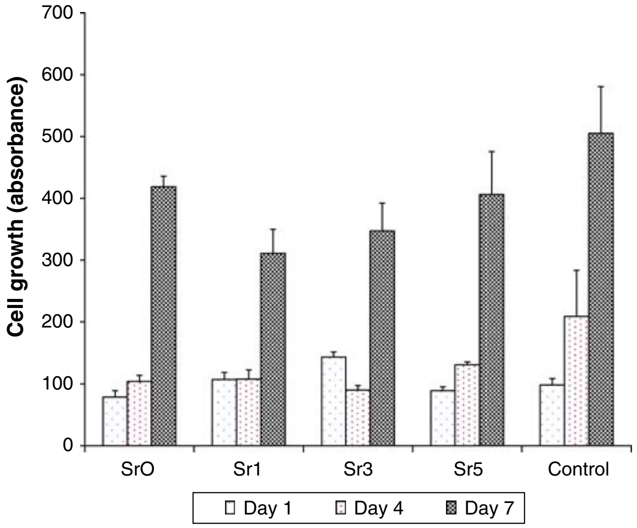

Fig. 6 Cell proliferation data obtained for glasses over days 1, 4 and 7 in culture (error bars = ±sd; $n = 3$)

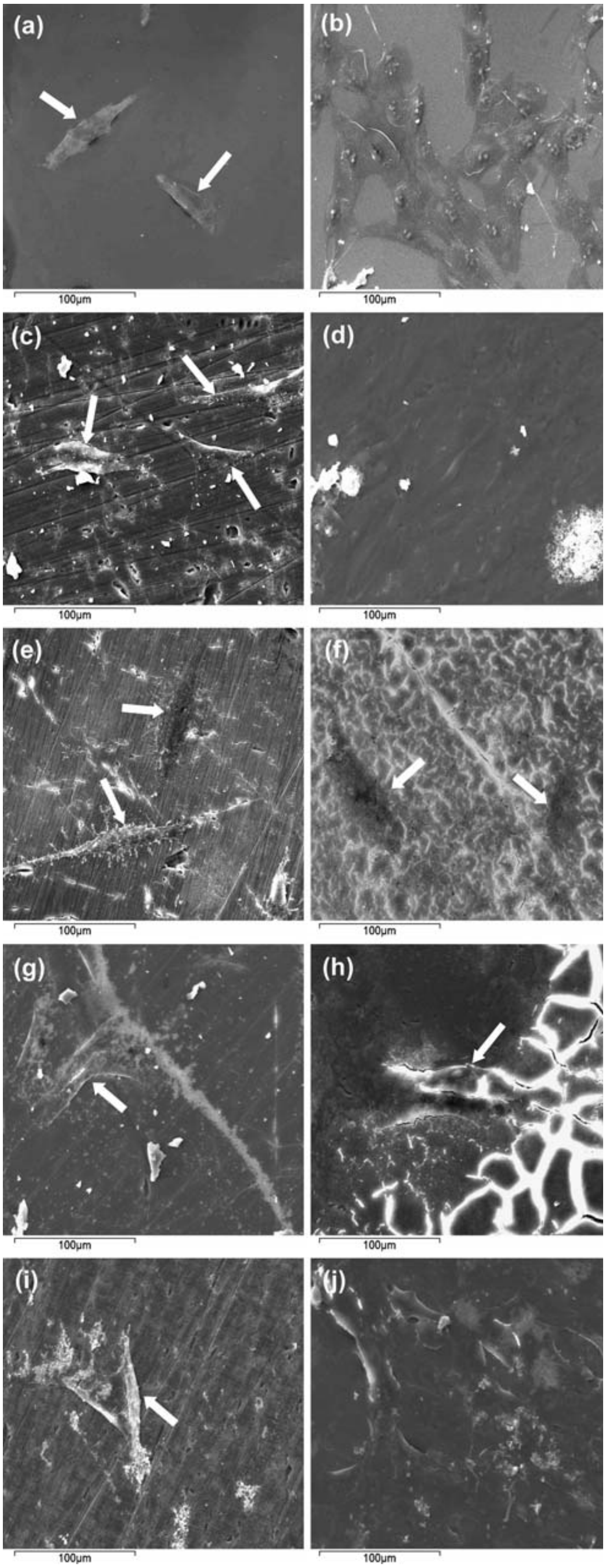

Fig. 7 SEM images showing viability of MG-63 cells on days 1 and 7, respectively, when seeded on **a**, **b** glass cover slip controls; **c**, **d** Sr0 glass; **e**, **f** Sr1 glass; **g**, **h** Sr3 glass and **i**, **j** Sr5 glass. *Arrows* indicate presence of individual cells or groups of cells. White shapes in some of the images correspond to glass fragments formed due to glass break-up when the specimens were removed from the sample wells

cover slip controls showed greater cell growth than the test specimens and the Sr-free glasses exhibited greater cell growth than those containing Sr, it is worth noting that the Sr5 glass provided almost the same viability as the Sr0 glass, even as it degrades at a significantly faster rate than the Sr0 glass. It is possible that a further increase in the Sr content of Ti-containing glasses may yield glasses that are both more soluble and more cytocompatible than those that contain Ti alone.

To our knowledge, the effects of doping ternary P_2O_5–Na_2O–CaO glasses with oxides of both Ti and Sr on the physical properties and cytocompatibility of the glasses have hitherto not been examined. It is worth mentioning that prior to experimentation, doping Ti-containing glasses with Sr was considered to cause random changes in the glass structure and properties. However, the results are clearly indicative of a more systematic alteration. It is possible that the increase in Sr content with a corresponding decrease in Ca content causes the Sr^{2+} ions to migrate towards and preferentially attach to sites previously occupied by Ca^{2+} ions in the glass structure. The difference in atomic radii of Ca^{2+} and Sr^{2+} (Ca^{2+}: 1 A.U.; Sr^{2+}: 1.18 A.U.) may also play a role in this process. As such, it is difficult to accurately model the changes in glass structure and properties that accompany the doping of phosphate glasses with oxides of two different metals, simply because of the sheer number of variables introduced by such a doping scheme. Nonetheless, the systematic change brought about by the addition of TiO_2 and SrO to form a five-component phosphate glass system is particularly interesting and deserves to be studied further.

4 Conclusion

In conclusion, the effect of SrO addition (0, 1, 3 and 5 mol%) on the structural characteristics, degradation behaviour and cytocompatibility of phosphate glasses containing 3 mol% TiO_2 were studied for the first time. All the investigated compositions showed evidence of calcium sodium phosphate as the dominant phase. The degradation and ion release data showed that the Sr-containing glasses degrade to a much greater extent than the Sr-free glass over the same time period. Crucially, the degradation of all the studied glass compositions was highly linear, suggesting interesting possibilities for use of these glasses in applications involving sustained delivery of ions and possibly biological macromolecules. Cell proliferation assays revealed that the capacity to foster cells over a 7-day period increases with the Sr content in the glass. The SEM images of the glass specimens provided visual evidence of cell proliferation and growth over a 7-day time period. The addition of Sr to Ti-containing glasses provided some very interesting trends that were visible across the entire spectrum of obtained data from structural characterisation to degradation/ion release to cell proliferation, and these trends are definitely worth researching in greater detail in future studies. The data obtained from all the experiments suggest that the investigated five-component Sr–Ti glasses degrade at a quicker rate than quaternary TiO_2 phosphate glasses without adversely affecting cytocompatibility. Combined with evidence from other studies that Sr and its compounds can be used to combat dental and periodontal diseases and bone loss, exciting possibilities exist for extending the orthopaedic applications of Ti-containing phosphate glasses to dental and periodontal applications as well.

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