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SUBSTITUTION OF STRONTIUM FOR CALCIUM IN GLASS IONOMER CEMENTS (PART 2): EFFECTS ON THE CEMENT MECHANICAL AND ION RELEASING PROPERTIES

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SUBSTITUTION OF STRONTIUM FOR CALCIUM IN GLASS IONOMER CEMENTS (PART 2): EFFECTS ON THE CEMENT MECHANICAL AND ION RELEASING PROPERTIES

O. A. OSIRO, R. G. HILL and A. J. BUSHBY

ABSTRACT

***Objectives*: To investigate the effects of substituting strontium for calcium in fluoro-aluminosilicate glass on the mechanical and ion-releasing properties of high-viscosity glass ionomer cements.**

***Design*: An exploratory, laboratory-based study.**

***Setting*: Dental biomaterials research laboratory, Dental Physical Sciences Unit, Barts and the London School of Medicine and Dentistry, Queen Mary, University of London.**

***Subjects*: A series of five glasses in which strontium substitutes for calcium and based on the general formula: $4.5SiO_2$ - $3Al_2O_3$ – $1.25P_2O_5$ – xSrO - $ySrF_2$ – zCaO - $yCaF_2$, where x = 0, 0.5, 1.5 or 3; y = 0, 1 or 2; and, z = 0, 1.5, 2.5 or 3 were synthesized, ground, sieved and the powders then characterised by X-Ray Diffraction (XRD), particle size analysis and thermal analysis, as previously described (Part 1). Thereafter, they were mixed with poly (acrylic acid) (PAA) and aqueous tartaric acid to form glass ionomer cements, whose properties were investigated at different time points: the compressive and bi-axial flexure strengths were tested; and, the ion release profile was studied by fluoride Ion Selective Electrode (ISE) and Inductively Coupled Plasma/Optical Emission Spectroscopy (ICP/OES).**

***Results*: While compressive and biaxial flexural strength values did not vary significantly with strontium content, the strontium ion release from the cements increased proportionally with the increase in strontium amounts in the glass.**

***Conclusion*: The results suggest that substitution of strontium for calcium has insignificant effects on the mechanical properties of the cement; however, strontium substitution for calcium enables the controlled release of strontium which may have an important synergistic anti-cariogenic role with fluoride.**

INTRODUCTION

The concept of minimally invasive dentistry advocates for preservation of natural dental tissues by employing less destructive cavity preparation techniques and adhesive restorative materials (1). High viscosity glass ionomer cements are an example of such materials. Developed for use in atraumatic restorative treatment, these materials have several advantages which include chemical adhesion to dental hard tissues as well as anti-cariogenic properties through leaching of fluoride (2).

Strontium is another element that has generated considerable interest in dentistry; while its role in bone metabolism is well established as prevention of resorption and promotion of deposition (3), Strontium is also thought to be anti-cariogenic (4). Furthermore, there is evidence that there is a synergistic remineralising property of strontium in combination with fluoride (5). The anti-bacterial capabilities of strontium have also been demonstrated (6).

Strontium has been substituted for calcium in several commercial glass ionomers in order to confer radiopacity; however, its effects on the material properties as well as its release from these materials have hardly been studied. Substitution of strontium for calcium is possible due to the similarity in size between the two ions: the ionic radius of calcium is 0.94nm while that of strontium is 1.16nm. Therefore, replacing calcium with strontium in a glass results in a relative network expansion but insignificant structural effects (7,8).

This study was motivated by the potential remineralising, anti-bacterial and anti-cariogenic properties of strontium and how these properties may be harnessed by optimising the design of glasses used in high viscosity glass ionomer cements.

MATERIALS AND METHODS

Glass synthesis and characterisation: The glasses studied were based on the following molar ratios: $4.5SiO_2$ - $3Al_2O_3 - 1.25P_2O_5 - xSrO$ - $ySrF_2 - zCaO$- $yCaF_2$, where $x = 0, 0.5, 1.5$ or 3; $y = 0, 1$ or 2; and, $z = 0, 1.5, 2.5$ or 3 (Table 1). The glass synthesis and characterization have previously been described (Part 1 of this paper).

Preparation and characterization of the cements

Cement preparation: The cements were prepared in a similar manner as described in Part 1 of this paper. Briefly, 50gms of each glass powder was pre-mixed with an appropriately adjusted amount of high molecular weight (80KDa) freeze-dried poly (acrylic acid) (S & C Polymer GmbH, Elmshorn, Germany) to correspond to 50% molar mass of poly (acrylic acid). A spatula was then used to obtain cement pastes by further mixing the powders with an aqueous solution of 10% tartaric acid (Sigma Aldrich). A resultant increase in density with increasing amounts of strontium warranted progressively greater amounts of glass to be used for the strontium containing glasses.

Compressive strength tests: The compressive strength tests were performed on cement cylinders 4.0mm in diameter by 6.0mm in height (ISO 9917, 1981). Eight cylindrical specimens for each glass sample (n=8) were fabricated by condensing freshly mixed cement pastes into two-part steel moulds containing four bores of the above dimensions. Thereafter, the moulds were clamped between two steel plates and placed in a preheated oven at 37°C and 100% humidity for one hour. They were then retrieved from the moulds, placed in 10 ml of de-ionised water and incubated at 37°C for 24 hours, seven days and 28 days. The specimen dimensions as well as weight before incubation and prior to testing were recorded. A 30KN load cell was used to load the samples using a compression cage on an Instron 5567 Universal Testing machine (Instron Ltd, High Wycombe, Bucks, UK) at a crosshead displacement speed of 1 mm min-1. Compressive strength was calculated using the formula:

$$\sigma_C = F \div \pi r^2$$

Where: σ_C is compressive strength (MPa); F is applied load at failure (N); and, r is the sample radius as measured prior to testing (mm). Student t-test was used to analyse the results.

Bi-axial flexure tests: Typically, compressive strength testing is conducted on brittle dental materials because it is a simple test that gives a general strength value; however, the clinical relevance is questionable because these materials tend to fail in tension.

Bi-axial flexure strength (BFS) test was selected for this study on the basis of conclusions of similar studies which compared the influence of the test method on the failure strength of brittle dental materials and recommended the ball-on-ring set-up as most suitable for dental cements (9,10). This is because the tensile stresses that eventually cause failure are assumed to concentrate within the radius of the support circle hence best simulating the practical situation. Moreover, it has been proposed that the Hertzian indentation is a simple bi-axial test that can be used to investigate the tensile strength of brittle dental materials (11).

Bi-axial flexure strength test was conducted on cement discs measuring 14.0mm in diameter by 2.0mm thick. Eight cement discs were fabricated for each glass sample (n=8). Teflon moulds of the above dimensions were filled with freshly mixed cement pastes. The moulds were then clamped with steel plates and placed in a preheated forced air oven at 37ºC and 100% humidity for one hour, following which they were retrieved and immersed in 50 ml centrifugal tubes containing 40ml of de-ionised water. The discs were then stored at 37ºC for 24 hrs, 7days and 28 days. Prior to testing, the height of the discs was measured at three points and the average obtained. A 1KN load cell was used to load the samples using a compression cage with a detachable ball-on-ring jig on an Instron 5567 Universal Testing machine (Instron Ltd, High Wycombe, Bucks, UK) at a crosshead displacement speed of 1mm min-1. The Bi-axial flexure strength was calculated using the Timoshenko and Woinowsky-Krieger equation:

$$\sigma_{max} = \frac{P}{h}^{2} \{(1+v)[0.485 \ln(\frac{a}{h})+0.52]+0.48\}$$

Where: σ_{max} is the minimum tensile strength (MPa); P is the measured load at fracture (N); h is the average specimen thickness of three points measured prior to testing (mm);
v is Poisson's ratio [0.33 for GICs12; and, a is the radius of the knife-edge support (5mm).

Ion release measurements in Tris buffer: Tris buffer was selected as the elution medium because it has a neutral pH as well as few elements which may form complexes hence interfere with the ion release processes of the cements. The solution was prepared by dissolving 15.090gm of tris-(hydroxymethyl) aminomethane (Sigma Aldrich, Gillingham, UK) in 800ml of de-ionised water while stirring, followed by addition of 44.2ml of 1M hydrochloric acid (HCl) (Sigma Aldrich). The mixture was stored in an incubator at 37°C and, 24 hours later, the pH was checked using a pH meter (Oakton Instruments, Nijkerk, Netherlands) and adjusted to 7.35 using 1M HCl. The solution was then filled up to 2 litres by addition of de-ionised water, and stored at 37°C.

Ion release profile was conducted for the 0%, 50% and 100% strontium substituted cements. Cement cylinders were fabricated for ion release measurements; three cylinders were prepared for each group. On retrieval from the incubator, they were stored in a 15ml centrifuge bottle containing 10ml of Tris solution. Ion release was measured at specified time points of 1, 3, 7, 10, 14, 21 and 28 days; at the given time point, the cement cylinder was retrieved and transferred to a fresh solution of Tris; the elution media was stored at -10°C and tested at a later date.

Fluoride Ion Selective Electrode (F-ISE) (Orion 9609BNWP, ThermoScientific, Waltham, MA, USA) was used to measure the free fluoride in the elution media. There were three elution specimens for each cement sample; therefore, the stable reading was recorded for each elution, and the average value taken as the amount of fluoride released by the material.

Inductively Coupled Plasma / Optical Emission Spectroscopy (ICP-OES) (Varian Vista-PRO, Varian Ltd, Oxford, UK) was used to measure the amount of strontium, calcium, silicon, phosphorus and aluminium released by the 0%, 50% and 100% strontium substituted cements. On completion of the fluoride ion measurements, the three elution specimens for each cement sample were bulked in a 50ml centrifuge bottle. Thereafter, 1ml of the elution medium was placed into an ICP tube, acidified with 0.2ml of nitric acid and topped up to 10ml by adding 8.8ml of de-ionised water. A series of various concentrations of ICP standard solutions of the elements to be studied were prepared by diluting 1000mg/L stock solutions in 1ml of nitric acid, 10ml of Tris buffer and topping up to 50ml by addition of appropriate amounts of de-ionised water.

RESULTS

Glass synthesis and characterization, and cement handling and setting reaction: The results of the glass synthesis and characterisation as well as those of the cement handling variables and setting reaction were presented and discussed in Part 1 of this paper.

Table 1

Molar ratios of the constituents of the glasses studied

SiO_2	Al_2O_3	P_2O_5	SrF_2	SrO	CaO	CaF_2	Sr to Ca	% Sr substitution
4.5	3	1.25	0	0	3	2	0Sr 5Ca	0
4.5	3	1.25	1	0	3	1	1Sr 4Ca	20
4.5	3	1.25	2	0.5	2.5	0	2.5Sr 2.5Ca	50
4.5	3	1.25	2	1.5	1.5	0	3.5Sr 1.5Ca	70
4.5	3	1.25	2	3	0	0	5Sr 0Ca	100

Figure 1

Compressive (a) and Bi-axial flexure (b) strengths after 24v hours, seven days and 28 days (error bars denote standard deviation)

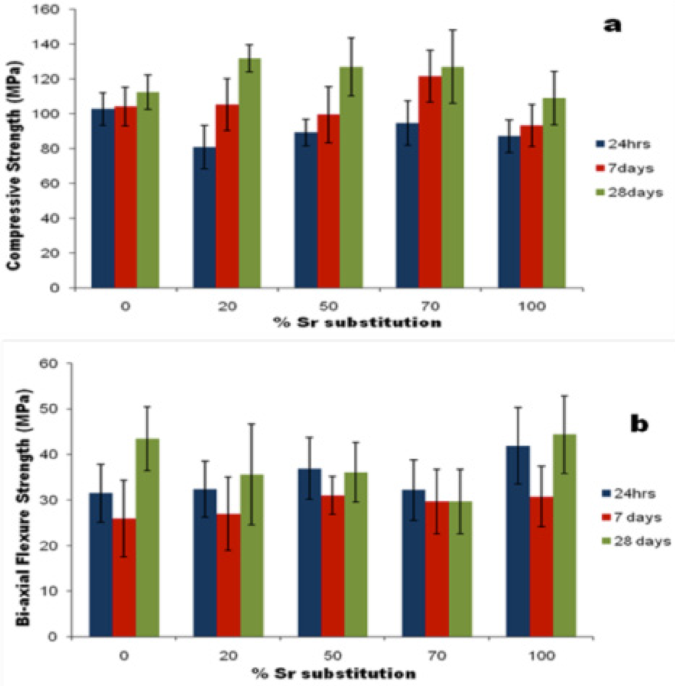


Figure 2

Cumulative fluoride ion (a), Si (b) and P (c) release profiles of cements containing 0%, 50% and 100% strontium

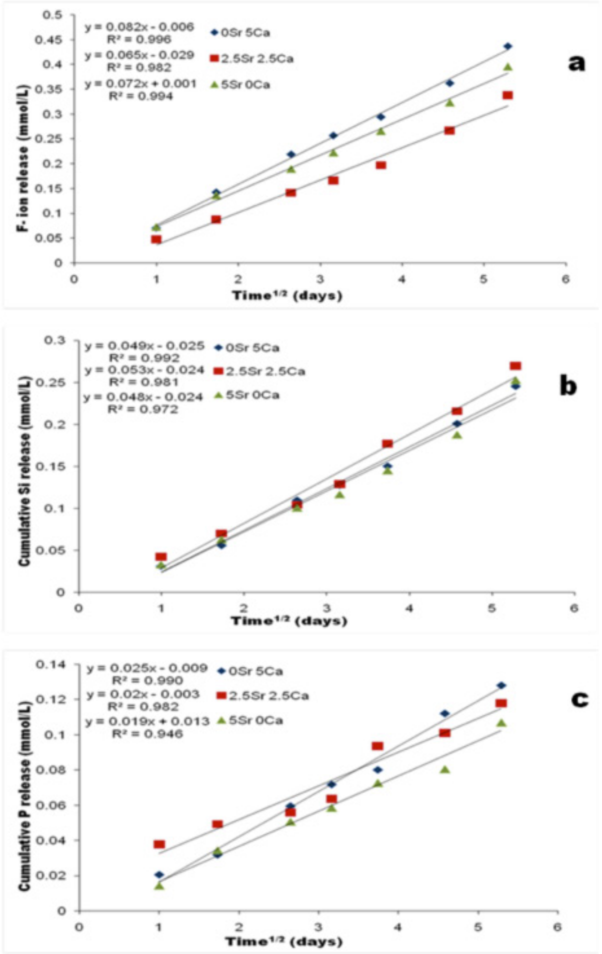
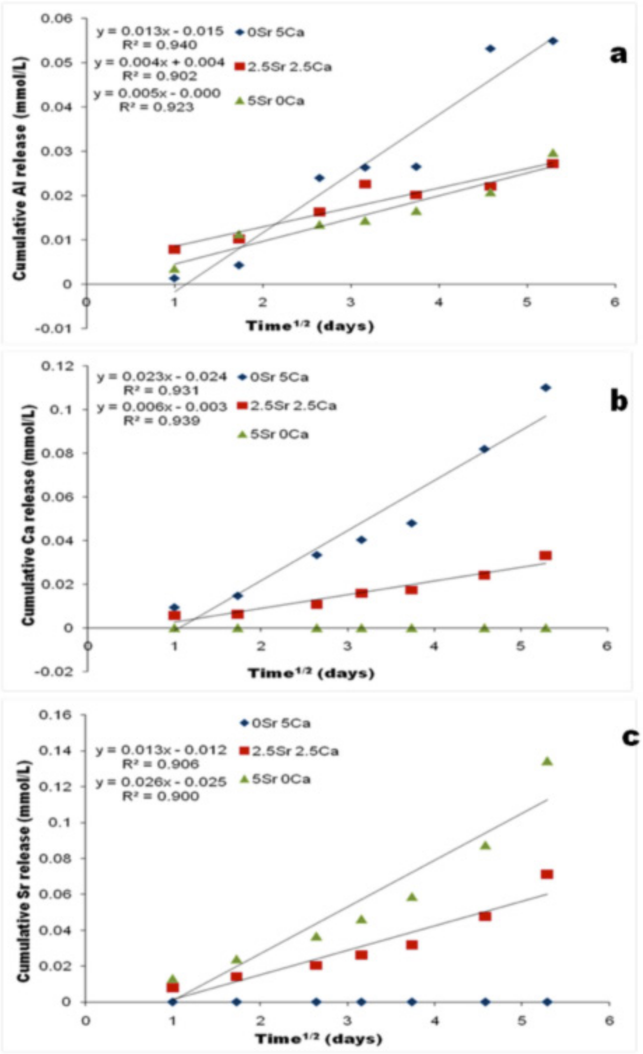


Figure 3

Cumulative Al (a), Ca (b) and Sr (c) release profile of cements containing 0%, 50% and 100% strontium



Compressive strength values: Figure 1(a) is a comparative summary of the compressive strength of the cements which depicts a general increase in strength as the cements aged. It also shows generally higher 7-day and 28-day compressive strength for the strontium containing cements when compared to the strontium free cement. There was no apparent correlation between the glass particle size and the 24-hour compressive strengths.

Unpaired t-test comparing the compressive strength of the cement without strontium to the strontium containing cements was statistically significant for the cement with 20% strontium substitution at 24 hours ($p=0.001$) and 28 days ($p=0.003$). Paired t-test comparing the same cement formulation at different time points was statistically significant: for the cement with 20% strontium substitution between 24 hours and 7 days ($p=0.0004$), 7 and 28 days ($p=0.0004$) and, 24 hours and 28 days ($p=0.00001$); for the cement with 50% strontium substitution between 24 hours and 28 days ($p=0.001$); and, for the cement with 70% strontium substitution between 24 hours and seven days ($p=0.003$).

Bi-axial strength values: Figure 1 (b) is a comparative summary of the bi-axial flexure strength of the cements which depicts a general drop in the bi-axial flexure strength at 7 days as compared to 24 hours, followed by a rise at 28 days except for the cement containing 70% strontium. There is no obvious trend in the bi-axial flexure strength values when comparing the strontium free cement with the strontium containing cements.

Unpaired t-test comparing bi-axial flexure strength of the cement without strontium to the strontium containing cements was statistically significant for the cement with 100% strontium substitution at 24 hours ($p=0.02$) and the cement with 70% strontium substitution at 28 days ($p=0.002$). Paired t-test comparing bi-axial flexure strength of the same cement at different time points was statistically significant: for the cement with 100% strontium substitution between 24 hours and seven days (p=0.04) and seven days and 28 days (p=0.02); and, for the cement without strontium between 24 hours and 28 days (p=0.008) and seven days and 28 days (p=0.008).

Ion release measurements: Figure 2 (a) shows that the cumulative fluoride release profile of the three cements had a linear relationship with square root of time ($t\frac{1}{2}$) with the R^2 values >98%; however, they had low intercept values. While the amount of fluoride released by the three cements followed a similar trend, the strontium free cement showed the highest release (0.44 mmol/L), followed by the cement containing 100% strontium (0.4 mmol/L) and finally, the cement containing 50% strontium (0.34 mmol/L).

A comparison of the cumulative Si release by the three cements (Figure 2 (b)) showed a similar trend for all three cements with nearly the same amount of Si released by the cements containing 0% (0.25 mmol/L) and 100% strontium (0.252 mmol/L); however, the cement containing 50% strontium released a slightly higher amount of Si (0.27 mmol/L).

A comparison of the cumulative P release by the three cements (Figure 2 (c)) showed a similar trend for all three cements with nearly the same amount of P released by the cements containing 0% (0.13mmol/L) and 50% strontium (0.12mmol/L); however, the cement containing 100% strontium released a slightly lower amount of P (0.11mmol/L).

A comparison of the cumulative Al release by the three cements (Figure 3 (a)) showed a similar trend for the cements containing 50% (0.027 mmol/L) and 100% (0.03 mmol/L) strontium with nearly the same amount of Al released; however, the cement containing 0% strontium released a markedly higher amount of Al (0.06 mmol/L).

A comparison of the cumulative Ca release by the three cements (Figure 3 (b)) showed markedly higher amounts of Ca released by the cement containing 0% strontium (0.11 mmol/L), nearly thrice the amount released by the cement containing 50% strontium (0.033 mmol/L).

A comparison of the cumulative Sr release by the three cements (Figure 3 (c)) showed higher amounts of Sr released by the cement containing 100% strontium (0.134 mmol/L), nearly twice the amount released by the cement containing 50% strontium (0.07 mmol/L).

DISCUSSION

This paper is a continuation of the description of preliminary in-vitro testing performed on a nouvelle dental biomaterial. A series of fluoro-aluminosilicate glasses in which strontium substitutes for calcium on a molar basis from 0% to 100% was synthesised. Glass ionomer cements were prepared by mixing the glass powders with freeze-dried poly (acrylic acid) and aqueous tartaric acid and then tested according to ISO standards for dental cements, to determine the effect of the substitution on the mechanical and ion releasing properties.

The results showed that the compressive strength of all the cements increased with age. This is an expected outcome of the maturation of glass ionomer cements (13). Although the results also showed overall higher compressive strength values for the strontium containing cements when compared to the strontium free cement, the results were only statistically significant between the strontium free cement and the cement containing 20% strontium at 24 hours (p=0.001) and 28 days (p=0.003). Therefore, there was no direct evidence as to how the presence of strontium might have influenced compressive strength.

The bi-axial flexure strength values are considered to be more meaningful and practical, given the tensile mode of failure of these materials in the clinical environment (9-11). While there was no definite trend in the results when comparing the strontium containing cements to the strontium free cement, they were statistically significant at 24 hours for the cement containing 100% strontium (p=0.02) and at 28 days for the cement containing 70% strontium (p=0.002). Nevertheless, there was still no pointer towards the influence of strontium; therefore, these results suggest that there is no obvious effect of substituting strontium for calcium on the mechanical properties of the cements.

The results of the cation and anion release studies suggest a diffusion controlled process, based on the observation of a linear relationship with the square root of time ($t\frac{1}{2}$) rather than with time, all of which showed a linear correlation with R^2 values >0.914. However, the low intercept values suggest lack of 'initial burst' release although the cumulative ion release data suggest a sustained release within the period of observation. Both of these findings differ from those observed in G-338 based cements in lactic acid as well as water in which a linear correlation to time suggested a dissolution rather than diffusion controlled process, and also an initial burst of ion release that declined with time (15). However, the different formulation of the glass G-338 to those in the current study as well as the difference in elution media between the two studies is noteworthy; moreover, the lack of 'initial burst' release of fluoride is not surprising as it is a phenomenon associated with monovalent Na+ and which is absent in these cements (16).

The key aspect in this project was increasing substitution of strontium for calcium and its effects on the cement properties. Studies using similar glasses found that there was no effect on fluoride ion release in distilled water by cements in which strontium substituted calcium (17). The results from this project suggest the same as shown by the similarity in fluoride ion release pattern observed among the three cements studied. However, while the former study (17) observed a slightly higher fluoride release from the 100% strontium glass, a finding they attributed to the higher amount of glass used in order to compensate for the density of strontium, the opposite was observed in the current study. In fact, the fluoride released by the 100% strontium glass in the current study was lower than that from the 0% strontium glass despite more glass being used for the former, for the same reason as was done in the earlier study.

Furthermore, the results also suggest no effect of the substitution on silicon and phosphorus release, evidenced by the similarity of the trends among the three cements. However, aluminium and calcium release was significantly higher for the strontium free cement as compared to that observed for the cements containing 50% and 100% strontium, whose aluminium release profiles were similar. It is noteworthy that this finding for the strontium free cement also differs from that on G-338 based cements (15) where it was observed that there was hardly any release of calcium and aluminium, a finding attributed to the formation of insoluble salts by these cations as well as the effect of charge balancing of Al3+ by Ca2+; therefore, the results from the current study suggest that this theory may not apply to glasses with different formulations. Conversely, it is suggested that cation release may be dependent on the molar ratios in the glass, as evidenced by the results of the strontium and calcium ion release. A similar suggestion was made following likewise observations on strontium containing bioactive glasses (18) and may be attributed to the expansion of the glass network by the larger strontium ion.

In conclusion, the results suggest that substitution of strontium for calcium in the glass does not affect the cement mechanical properties significantly, a finding that is attributed to the similarity between the ionic sizes of the two elements; however, the ion releasing properties of the cements appear to be dependent on the molar ratios of the constituents. Therefore, strontium can replace calcium in optimized glass design in order to harness its potential remineralising ability, its antibacterial properties that are thought to be synergistic to fluoride and its radiopaque properties that are useful in a restorative material.

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