Available online at www.sciencedirect.com

ScienceDirect

journal homepage: www.intl.elsevierhealth.com/journals/dema

Fabrication of strontium-releasable inorganic cement by incorporation of bioactive glass

Jun-Ichi Sasaki[a], *Wakako Kiba*[a], *Gabriela L. Abe*[a], *Chihiro Katata*[a,b], *Masanori Hashimoto*[c], *Haruaki Kitagawa*[a,*], *Satoshi Imazato*[a,d]

[a] *Department of Biomaterials Science, Osaka University Graduate School of Dentistry, 1-8 Yamadaoka, Suita, Osaka 565-0871, Japan*

[b] *Department of Restorative Dentistry and Endodontology, Osaka University Graduate School of Dentistry, 1-8 Yamadaoka, Suita, Osaka 565-0871, Japan*

[c] *Institute of Dental Research, Osaka Dental University, 8-1 Kuzuhahanazonocho, Hirakata, Osaka 573-1121, Japan*

[d] *Department of Advanced Functional Materials Science, Osaka University Graduate School of Dentistry, 1-8 Yamadaoka, Suita, Osaka 565-0871, Japan*

ARTICLE INFO

Article history:
Received 25 December 2018
Accepted 13 February 2019

Keywords:
Bioactive glass
Strontium
Glass ionomer cement
Osteoblast
Bone cement
Pulp capping material

ABSTRACT

Objectives. Bioactive glass (BG) is widely used as a bioactive material for various clinical applications, and effective and efficient elemental release and an increase in mechanical strength are expected with further development. The purpose of this study is to clarify the physicochemical and biological characteristics of Sr-doped BG-incorporated glass ionomer cements.

Methods. Sr-doped BGs ($45SiO_2$–$6P_2O_5$–$24.5Na_2O$–$(24.5-x)CaO$–$xSrO$) (wt%), where $x = 0, 6, 12$, were prepared, and the particle size, crystallinity, and elemental release profiles were evaluated. The Sr-doped BGs were then incorporated into a glass ionomer cement at a weight ratio of 1:4, and the physicochemical properties (compressive strength, bending strength, hardness, and elemental release profile) were investigated. Cell attachment, cell proliferation, and osteoblastic differentiation were used to evaluate the biological characteristics.

Results. The Sr-doped BGs were amorphous phases with a homogeneous particle size and exhibited sustained release of Ca, Si, and Sr. The BG-incorporated cements were able to release these elements while retaining the same mechanical properties as those of the pure glass ionomer cement. In addition, no cytotoxicity of osteoblasts or differences in the cell attachment or proliferation were observed for the BG-incorporated cements. In contrast, the Sr-doped BG-incorporated cements promoted the alkaline phosphatase activities of the osteoblasts without the need for any media supplements for osteoblastic differentiation.

Significance. Sr-releasable inorganic cements with high mechanical properties were successfully fabricated by incorporating Sr-doped BGs in glass ionomer cement. These bioactive materials are promising candidates for bone grafting materials, bone cements, and pulp capping materials.

1. Introduction

Bioactive glass (BG) is a widely known biomaterial that is capable of releasing various ions [1]. There has been consid-

* *Corresponding author.*
E-mail address: h-kita@dent.osaka-u.ac.jp (H. Kitagawa).

https://doi.org/10.1016/j.dental.2019.02.019

erable research on BGs over the past 50 years, and BGs are currently applied in hard tissue restoration in various craniofacial, maxillofacial, and periodontal regions [2,3]. BG has a bioactive function in the body because of the elemental release of sodium (Na), silicon (Si), and calcium (Ca) [4] and shows high compatibility with hard tissue [5]. BGs have been reported to form the hydroxyapatite layer on the bone surface and connect the bone tissue, regarded as osteoconductivity [6,7]. In addition, angiogenesis is promoted by the multiple ionic releases *in vivo*; therefore, BGs are considered useful for soft tissue regeneration [8,9]. Ag-doped BGs were fabricated experimentally, and they were shown to possess antibacterial property and were harmless to the human body [10]. Furthermore, some studies have been performed on the use of BGs as a drug-delivery system [11]. Therefore, BGs could potentially be used for a broad range of applications in the medical and dental fields.

Strontium (Sr), an alkaline earth metal, exhibits similar chemical characteristics as Ca; therefore, Sr can be used as a substitute for Ca in bone and tooth enamel [12,13]. Sr is known to promote the osteogenic differentiation of mesenchymal stem cells and suppresses the activity of osteoclasts [14]. In clinical settings, administration of Sr has been shown to increase bone density and results in a decrease of fracture in vertebral and peripheral bones; therefore, the Sr salt of ranelic acid has been applied in the treatment of osteoporosis [15,16]. Strontium ranelate has been reported to stimulate the calcium-sensing receptor of osteoblasts and to promote the secretion of osteoprotegerin (OPG) from the osteoblasts; the OPG inhibits the osteoclast differentiation that is processed via the receptor activator of the NF-κB ligand [17,18]. These interactions affect bone metabolism and drive bone formation.

Sr has been combined with bone grafting materials, and Sr-releasing scaffolds have been reported to exhibit fair cytocompatibility with osteoblasts [19]. In addition, a method adopting Sr in a titanium alloy using hydrothermal treatment was applied in an implant fixture, and these composite materials promoted osteoblastic differentiation and the migration of mesenchymal stem cells [20]. Molino et al. [21]. reported that Sr-containing BGs could be coated onto porous glass scaffolds using electrophoretic deposition and revealed its bioactive behavior with increased mineral deposition in simulated body fluid.

Thus, Sr-releasable BGs are promising bioactive materials that can be applied as bone grafting materials, bone cements, inorganic dental cements for fillings, and pulp capping materials; however, it is difficult to form BGs into arbitrary shapes while achieving high mechanical properties [22,23]. Therefore, it was hypothesized that novel inorganic bioactive materials with high mechanical properties and osteoconductivity could be fabricated by combining Sr-doped BGs with glass ionomer cements. Previous studies have reported on the combination of BGs and dental cements [24,25]; however, the physicochemical and biological characteristics of Sr-doped BG-incorporated glass ionomer cements have not yet been investigated.

The purpose of this study was to clarify the physicochemical characteristics, including the elemental release and mechanical properties, of Sr-doped BG-incorporated cements and to further evaluate the effects of the BG incorporation on the cytotoxicity and osteoblast activity.

Table 1 – Raw materials and weight ratios (wt%) used for fabrication of bioactive glasses.

	Na_2O	CaO	SrO	SiO_2	P_2O_5
Sr0	24.5	24.5	0	45	6
Sr6	24.5	18.5	6	45	6
Sr12	24.5	12.5	12	45	6

2. Materials and methods

2.1. Preparation of Sr-doped BGs

The Sr-doped BGs were designed using 45S5 bioglass, which consists of 45% silica (SiO_2), 24.5% calcium oxide (CaO), 24.5% sodium oxide (Na_2O), and 6% phosphorous pentoxide (P_2O_5) in wt% [26,27]. Strontium oxide (SrO) was used as a supplemental material to fabricate the Sr-doped BGs with the compositions shown in Table 1. Briefly, BGs ($45SiO_2$–$6P_2O_5$–$24.5Na_2O$–$(24.5-x)$CaO–xSrO) (wt%), where x = 0 (Sr0), 6 (Sr6), 12 (Sr12), were prepared by melting the raw materials in a platinum crucible at 1200 °C for 2 h followed by immediate quenching in deionized water. The BG was then grounded and sieved with a planetary ball mill to obtain <10 μm powder. To verify the size and morphology of the BG particles, the obtained glass powders were coated with gold and examined using scanning electron microscopy (SEM; JSM-6390, JEOL, Tokyo, Japan).

2.2. Elemental compositions and release profiles of Sr-doped BGs

The crystallinity of the BG was evaluated using X-ray diffraction (XRD; Ultima+, Rigaku, Tokyo, Japan). XRD analysis was performed using Cu-Kα radiation, and diffraction patterns were recorded from 10° to 70° 2θ with a step size of 0.02°. X-ray fluorescence (XRF) spectrometry (Supermini, Rigaku) was used to quantify the elemental composition (Na, Ca, Sr, Si, and P) of the samples. The data were acquired using an axial wavelength-dispersive XRF unit (n = 5).

The elemental releases of Sr, Ca, and Si were determined using inductively coupled plasma optical emission spectroscopy (ICP-OES) [28]. Briefly, 0.32 g of glass powder was immersed in 10 mL of distilled water at 37 °C. After 1, 3, 7, 14, and 21 days, the concentrations of Sr, Ca, and Si were measured using an ICP-OES instrument (ICPS-8000; Shimadzu, Kyoto, Japan) with external calibration at wavelengths of 407.771 nm (Sr), 393.366 nm (Ca), and 251.612 nm (Si) (n = 4). The specimens were transferred to fresh water at each time point.

2.3. Preparation of Sr-doped BG-incorporated cement

The experimental cement was prepared by mixing the BG at a ratio of 1:4 with fluoroaluminosilicate glass powder of commercial glass ionomer cement (Base cement; Shofu, Kyoto, Japan). Next, the compound powder was mixed with poly-

acrylic/tricarboxylic acids (powder/liquid = 10/3 in weight) and poured into a rubber-based mold (diameter: 10 mm, thickness: 2.0 mm). Disc specimens were obtained after 24 h of incubation at room temperature. Glass ionomer cement without BG was prepared following the protocol established by the manufacturer and employed as a control specimen (Cont.).

To investigate the elemental release from the BG-incorporated cements, ICP-OES measurements were performed using the previously described procedure. Briefly, a disc specimen was immersed into 500 μL of distilled water at 37 °C; then, the eluate was collected after 1, 3, 7, 14, and 21 days of incubation. The releases of Sr, Ca, and Si were determined using the ICP-OES instrument (ICPS-8000) (n = 6).

2.4. Mechanical strength of Sr-doped BG-incorporated cements

The compressive strength, flexural strength, and surface hardness of the Sr-doped BG-incorporated cements were determined. The specimens were prepared into cylindrical shapes ($\varphi = 4$ mm, h = 6 mm) for the compressive strength measurements, bar shapes (2 × 2 × 25 mm) for the flexural strength measurements, and disc shapes ($\varphi = 5$ mm, h = 2 mm) for the surface hardness measurements. Each specimen was immersed into distilled water and stored for 24 h at 37 ± 1 °C before the measurements.

The compressive load was applied along the long axis of the cylindrical specimens at a cross-head speed of 1.0 mm/min using a universal testing machine (Autograph AGS-500D, Shimadzu) (n = 6). The compressive fracture strength was determined in accordance with ISO 9917-1:2007 [29].

The bar specimens were subjected to three-point flexural tests using a 500-N load cell (EZ-Test, Shimadzu) at a loading rate of 1.0 mm/min (n = 8). A span length of 20 mm was used. The flexural strength was calculated using the following formula: flexural strength = $3FL/2bh^2$, where F (N) is the maximum load; L (mm) is the distance between the two supports; and b (mm) and h (mm) are the width and depth of the specimen, respectively [30].

The Vickers method was used to evaluate the surface hardness of each disc specimen using a micro hardness tester (MicroWiZhard, Mitsutoyo, Kawasaki, Japan) [31]. A load of 200 g was applied by the Vickers indenter for an indentation time of 15 s. The dimensions of the indentations were evaluated using the optical microscope of the hardness tester, and the data are reported in Vickers hardness numbers (Hv) (n = 6). Hv is defined by the *F*/*S* ratio, where *F* is the force applied to the diamond indenter in kilograms-force and *S* is the surface area of the indentation in square millimeters.

2.5. Osteoblast behavior of Sr-doped BG-incorporated cements

2.5.1. Cell culture and cytotoxicity

MC3T3-E1 osteoblast-like cells were provided by the Riken cell bank (Riken, Saitama, Japan) and were cultured in Dulbecco's modified Eagle's medium (Wako, Osaka, Japan) containing 10% fetal bovine serum (Japan Bioserum, Hiroshima, Japan) and 1% penicillin/streptomycin (Sigma Aldrich, St. Louis, MO, USA). The cells were stored in a humidified incubator at 37 °C and 5% CO_2.

Cytotoxicity tests were performed in accordance with ISO 10993-5 [32]. Briefly, the disc-shape specimens ($\varphi = 5$ mm, h = 2 mm) were sterilized in ethylene oxide gas for 8 h at 40 °C. The samples were then immersed into 2 mL of culture media at 37 °C, and the extracts were collected after 24 h. Next, the MC3T3-E1 cells (1×10^4 cells/well) were cultured in a 96-well plate for 24 h. After being washed with phosphate buffered saline (PBS), the cells were cultured for another 24 h with the extract (100%) or serially diluted extract in culturing media (50%, 25%, 12.5%, and 6.25%). The cell viability was measured using the WST-8 assay (CCK-8; Dojindo, Kumamoto, Japan) [33]. The medium was removed, and the cells were washed twice in PBS. Then, fresh medium (180 μL) and the WST-8 test solution (20 μL) were added to each well, and the plate was incubated for 1 h. The culture supernatant (100 μL) was then placed in new 96-well plates, and the colorimetric absorbance of each well at 450 nm was measured using a microplate reader (iMark; Bio-Rad, Hercules, CA, USA) (n = 8). The blank and medium controls were treated identically. The results were compared with those of cells cultured using fresh medium without extract (0%).

2.5.2. Cell proliferation

The effects of the elemental release on the MC3T3-E1 proliferation were evaluated using indirect co-culture with the Sr-doped BG-incorporated cements. Briefly, 2×10^3 cells/well were seeded on the 12-well plate. Each cement specimen was placed in the cell culture insert (Corning, NY, USA), and 1 mL of medium was added to the insert. After 1, 3, and 7 days of culture, the cell number was assessed using the WST-8 assay (CCK-8) (n = 6). Fresh medium (360 μL) and the WST-8 test solution (40 μL) were added to each well, and the plate was incubated for 1 h; then, the absorbance of each sample was measured using the same procedures described above.

2.5.3. Alkaline phosphatase activity

The effect of release from the Sr-doped BG-incorporated cement on the alkaline phosphatase (ALP) activity of MC3T3-E1 was evaluated using an ALP staining kit (Takara Bio, Shiga, Japan). Briefly, the cells were cultured under the same conditions as those used in the proliferation assay. After 14 days of culture, the cells were washed with PBS, fixed with an acetone/ethanol mixture for 5 min, and then washed with distilled water twice. Next, a mixed solution of bromo-chloro-indolyl phosphatase and nitro blue tetrazolium chloride was applied to the fixed cells for 30 min at 37 °C; then, blue-colored formazan dye was deposited on the cells. The stained area was semi-quantitatively determined from the captured images using image analysis software (ImageJ, NIH, MD, USA) (n = 4).

2.6. Statistical analysis

One-way analysis of variance (ANOVA) with a Tukey or Dunnett post-hoc test was used for comparison of more than two groups, and Student's t-test was used for comparison of two groups. A significant difference was reported for *p*-values < 0.05.

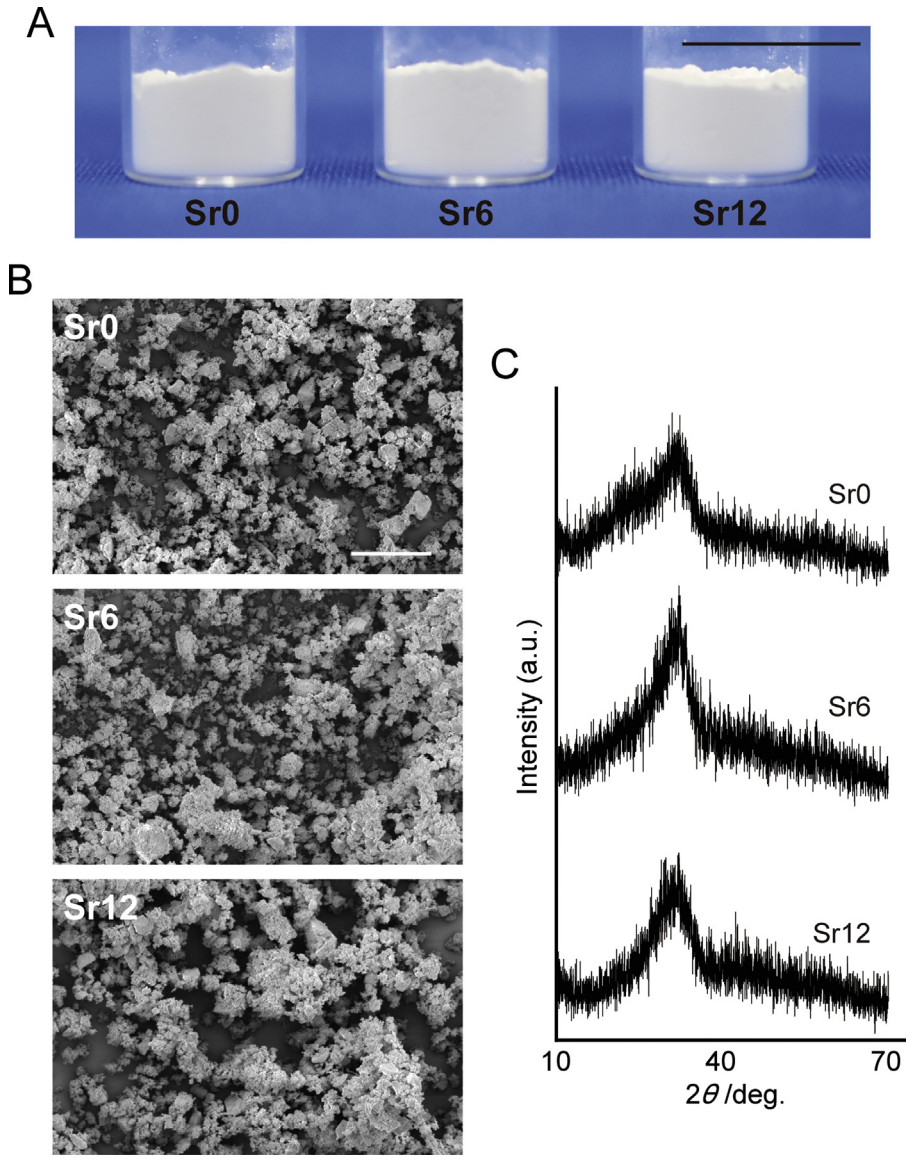

Fig. 1 – Particle characteristics of Sr-doped BG: (A) photographs, (B) SEM images, and (B) XRD patterns of BG. Scale bars: (A) 10 mm, (B) 50 μm.

Table 2 – Elements and weight ratios (mean ± SD wt%) of fabricated bioactive glasses analyzed by XRF spectrometry.

	Na_2O	CaO	SrO	SiO_2	P_2O_5
Sr0	24.32 ± 1.25	28.50 ± 0.73	0	39.86 ± 0.77	7.29 ± 0.78
Sr6	21.62 ± 1.49	21.56 ± 1.22	13.80 ± 1.01	36.30 ± 0.49	6.73 ± 0.87
Sr12	19.60 ± 1.32	14.08 ± 0.92	26.61 ± 1.24	33.22 ± 0.50	6.47 ± 0.89

3. Results

3.1. *Characteristics of Sr-doped BGs*

All the fabricated BGs were white refined powders composed of amorphous particles with sizes of approximately 5.0–8.0 μm (Fig. 1A,B). The XRD profiles of the BGs revealed a broad band, indicating a halo pattern with an amorphous phase (Fig. 1B). XRF spectrometry indicated that Sr12 contained twice the amount of Sr as Sr6, which is consistent with the as-prepared compositions of the powders (Table 2). The majority of the values were within the uncertainty of the as-prepared compositions, with the notable exceptions of Na_2O and SiO_2, the ratios of which decreased continuously with increasing SrO addition.

The results of the elemental release of the bioactive glass are shown in Fig. 2. The Sr component was continuously released from Sr6 and Sr12 for over 21 days but was not detected in Sr0, as expected (Fig. 2A). For the Ca concentration, Sr0 exhibited significantly higher release than Sr6 and Sr12 at day 1; however, the Ca release of Sr0 gradually decreased

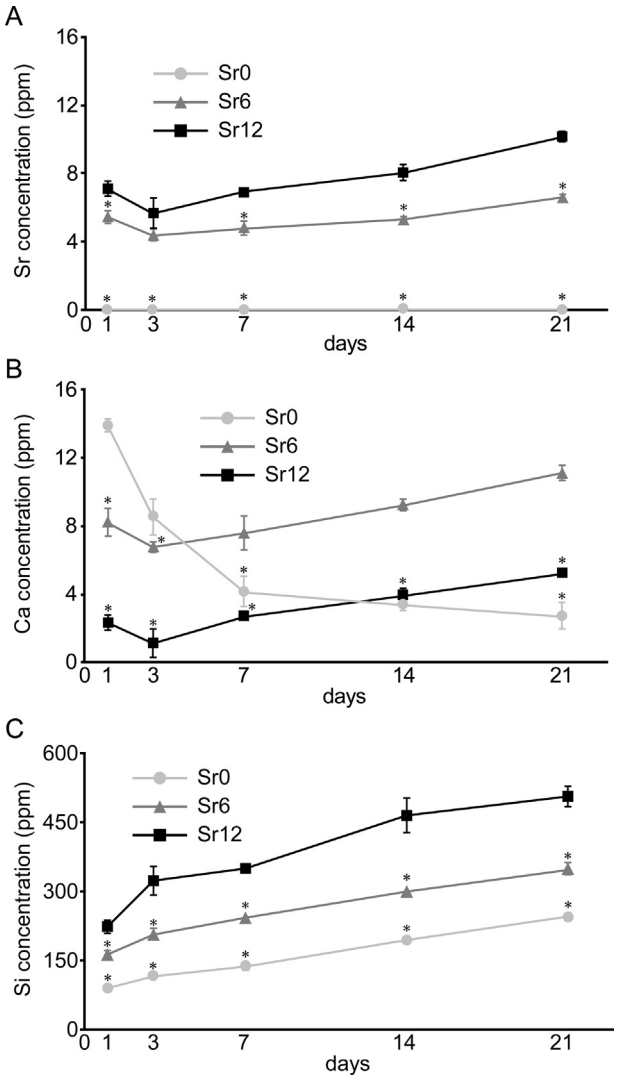

Fig. 2 – Elemental release from BG. Concentrations of (A) strontium (Sr), (B) calcium (Ca), and (C) silicon (Si) released from BGs measured using ICP-OES (*: $p < 0.05$, mean ± SD, n = 4).

thereafter. In contrast, the Ca release for Sr6 and Sr12 gradually increased throughout the observation period (Fig. 2B). For the Si release, the specimens containing higher Sr contents exhibited significantly increased Si concentrations. The Si release of all the specimens gradually increased over the observation period (Fig. 2C).

3.2. *Mechanical properties of Sr-doped BG-incorporated cements*

The compressive test results indicated that the control specimen, which did not contain bioactive glass, possessed slightly higher strength than specimens Sr0, Sr6, and Sr12; however, there were no significant differences in compressive strength among the tested specimens (Fig. 3A). Similar results were observed for the three-point bending tests; the control specimens exhibited a flexural strength of 14.9 ± 1.3 MPa, which did not differ significantly from that of 13.6 ± 2.3 MPa for the Sr12 specimens (Fig. 3B). In addition, all the tested specimens exhibited similar Vickers hardness (Fig. 3C).

3.3. *Elemental release of Sr-doped BG-incorporated cements*

The glass ionomer cements containing Sr-doped BGs released a high amount of Sr at 1 day after water immersion; thereafter, the Sr concentration gradually decreased until 21 days (Fig. 4A). There was no significant difference in the released Sr concentration for Sr6 and Sr12. As expected, the glass ionomer cements containing Sr-undoped bioactive glass (Sr0) did not show any Sr release. The Sr6-incorporated cements exhibited significantly higher Ca release than the Sr12 cement; however, there was no significant difference in the Ca release of the Sr6 and Sr0 cements (Fig. 4B). The highest concentration of released Ca was also observed at day 1 for all the specimens, and the Ca release then decreased over 21 days. The control cements did not show evidence of Ca release throughout the experimental period. In contrast, the Si release profiles were approximately the same for all the BG-incorporated cements with a certain concentration during the observation period (Fig. 4C). In the control samples, the Si release concentration

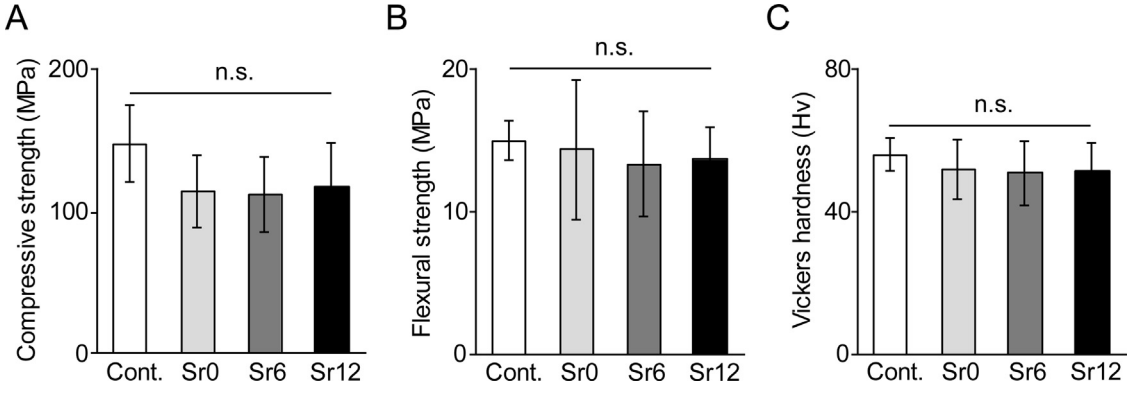

Fig. 3 – Mechanical properties of BG-incorporated cements: (A) compressive strength (n = 6), (B) flexural strength (n = 8), and (C) Vickers hardness (n = 6) evaluated for pure glass ionomer cement (Cont.) and that containing BGs (Sr0, Sr6, and Sr12) (n.s.: not significant, mean ± SD).

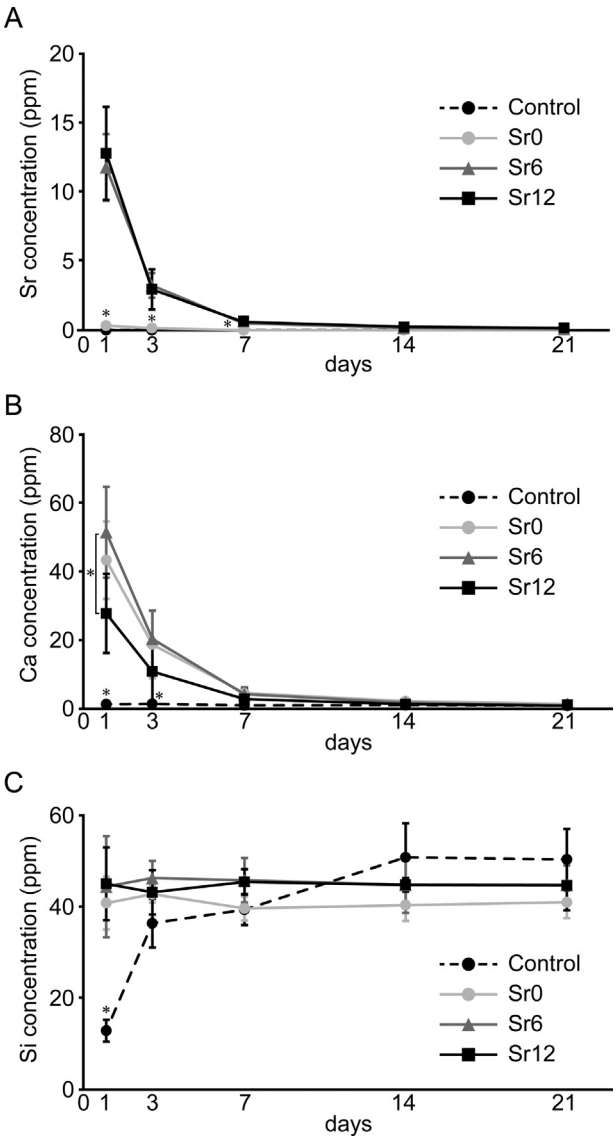

Fig. 4 – Elemental release of BG-incorporated cements. Concentrations of (A) strontium (Sr), (B) calcium (Ca), and (C) silicon (Si) released from BG-incorporated glass ionomer cements measured by ICP-OES (*: $p < 0.05$, mean ± SD, n = 6).

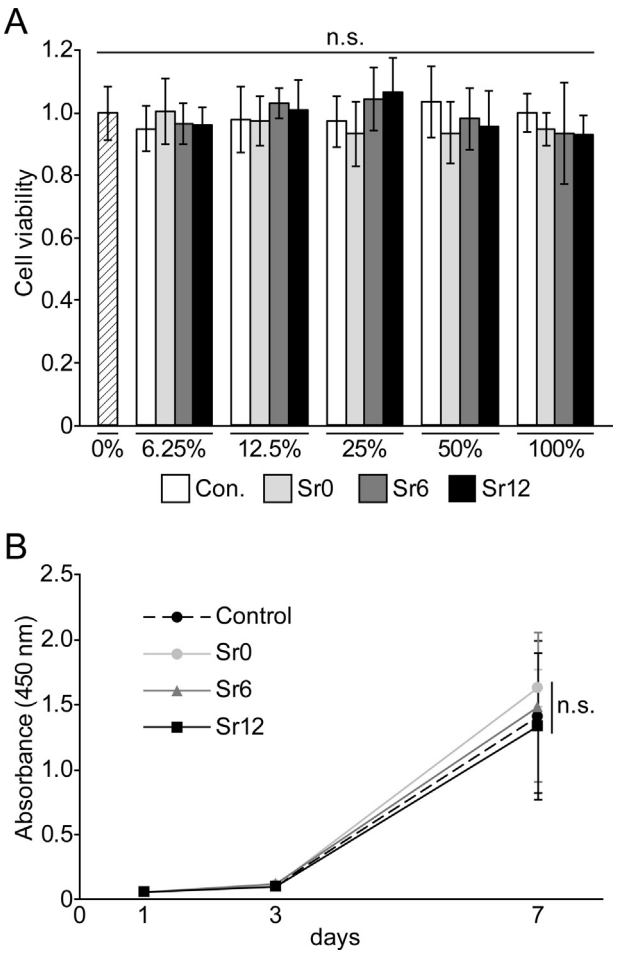

Fig. 5 – Cytocompatibility of BG-incorporated cements. (A) Cell viability and (B) proliferation of osteoblast-like MC3T3-E1 cells cultured with BG-incorporated cements. (A) Cells cultured with pure eluates (100%), serially diluted eluates (6.25%–50%) from the BG-incorporated cements (Sr0, Sr6, Sr12), fresh medium (0%), or pure glass ionomer cement (Cont.). (n.s.: not significant, mean ± SD, n = 6).

was relatively lower at day 1 and then increased to a similar extent as that of the Sr-doped BG-incorporated cements.

3.4. *Biological effect of Sr-doped BG-incorporated cements*

The cell viabilities were assessed using extracts from the BG-incorporated cements and pure glass ionomer cement (Fig. 5A). The cell viabilities were approximately 100% after 24 h of culture with extract (100%) and serially diluted extract (50%–6.25%) compared with those of the cells cultured with fresh medium (0%). There were no significant differences among the different types of dilutions and specimens, including the pure glass ionomer cements (Cont.). After 7 days of cell culture with the BG-incorporated cements, there were no significant differences in the cell proliferation among the tested groups (Fig. 5B).

In contrast to the cell viability and proliferation, the osteoblasts cultured with the Sr6- and Sr12-incorporated cements exhibited higher ALP activities than the cells cultured with glass ionomer cement without Sr-doped BGs (Cont. and Sr0) (Fig. 6). Semi-quantitative evaluation revealed that the cells cultured with the Sr6- and Sr12-incorporated cements showed significantly higher ALP activities than the cell cultured with Sr0 cement. The cells cultured with the Sr6-incorporated cement exhibited higher ALP activity than the cells cultured with Sr12; however, there was no difference between the cells cultured with the Sr6 and Sr12 cements.

4. Discussion

Bioactive Sr-releasable inorganic cements containing Sr-doped BGs were successfully fabricated, and the mechanical properties, elemental release profiles, and cytocompatibility

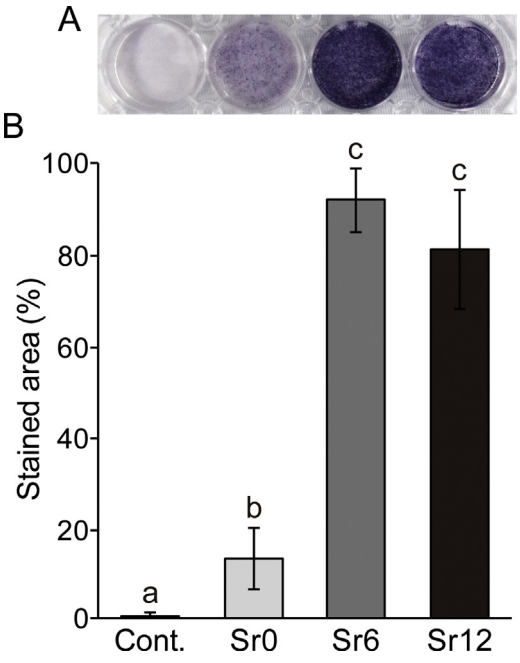

Fig. 6 – ALP activity of osteoblast-like cells cultured with BG-incorporated cements. (A) ALP staining after 14 days of culture of MC3T3-E1 cells cultured with BG-incorporated cements (Sr0, Sr6, Sr12) or pure glass ionomer cement (Cont.). (B) Semi-quantitative evaluation of ALP staining images. The different letters indicate significant differences among the groups (*: $p < 0.05$, mean ± SD, n = 4).

for osteoblasts were evaluated. The Sr-doped BGs were fabricated with the exact composition expected, and Sr12 released more Sr than Sr6 over 21 days. The results suggest that the Sr release from the BGs occurred in a dose-dependent manner in distilled water. Contrary to expectations, the Ca release from the Sr-undoped BG (Sr0) gradually decreased over 21 days. It is possible that less soluble components in Sr0 were exposed after the initial burst of Ca at 1 day of water immersion. The methods for fabricating the BGs can be divided into wet (sol–gel) and dry (melt-quenched) systems [34–36]. In wet systems, it is easy to obtain nanoparticles that are homogeneous in terms of size and components. In contrast, dry systems allow production on a large scale; therefore, the dry systems employed in this study are more suitable for clinical application requiring industrial mass production.

There have been some reports on BGs being incorporated into glass ionomer cement to compensate for the low mechanical properties of BGs and low bioactivity of glass ionomer cement [24,25]. Yli-Urpo et al. [25] reported that 10 wt% incorporation of BGs into inorganic cement resulted in a slight decrease in compressive strength but without any significant difference. In addition, a previous study revealed that the incorporation of BGs in cement did not affect the Young's modulus. These results motivated the current study, in which 25 wt% BGs was incorporated into glass ionomer cement. However, there have been no reports on the physicochemical and biological characteristics of Sr-doped BG-incorporated glass ionomer cements.

The release of Sr and Ca from the Sr-doped BG-incorporated cements was observed for 7 days; however, there was no release of these ions from the glass ionomer cement alone. In addition, the release profiles of Sr and Ca were different for the BG powder and BG-incorporated cements. It was considered that these elemental releases were developed only from the BGs existing in the surface layer, and a few elements were released from the BG-incorporated cements after 14 days of immersion. The release of Si occurred at a certain concentration with a similar profile over the experimental periods for the BG-incorporated cements, and a similar concentration of Si was reached for the control cement after 3 days of water immersion. It was considered that Si was dissolved in a saturated amount in all the samples. It is known that Si also promotes extracellular matrix production from osteoblasts [37,38]. In this study, Sr-doped BG-incorporated inorganic cements that could release multiple ions, thereby promoting bone formation, were successful fabricated.

The cytocompatibility test results with indirect co-culture with osteoblasts revealed that the Sr-doped BG-incorporated cements exhibited no cytotoxicity with no effects of the BG incorporation on the cell adhesion and proliferation. In contrast, elemental release from the Sr-doped BG-incorporated cements promoted osteogenic differentiation without the need for medium supplements such as ascorbic acid, β-glycerophosphate, or dexamethasone [39]. Kim et al. [40] reported an increase in mechanical strength with the incorporation of nanosized BGs prepared using a wet system to inorganic cements. In that study, dental pulp stems cells were cultured with the composite cements and exhibited no cytotoxicity and promotion of odontogenic differentiation in the differentiation medium. Our results support and extend these previous observations, with the bioactive function of cell differentiation provided solely with the Sr-doped BG-incorporated inorganic cement.

In addition, Sr and Ca are known to work synergistically for osteogenesis *in vitro* and *in vivo* [41,42]; therefore, the Sr6- and Sr12-incorporated cements could induce higher ALP activity than the Sr0-incorporated cement, which enabled the release of Ca. Aimaiti et al. [43] reported that the addition of 25–500 μM of Sr promoted osteogenic differentiation of mesenchymal stem cells; however, a dose of Sr greater than 1000 μM promoted apoptosis of cells. This apoptosis is thought to result from activation of the extracellular signal-related kinase, which is known as an ERK signaling pathway, and subsequent Bcl-2-family-mediated apoptosis. In our study, the Sr-6- and Sr-12-incorporated cements released up to approximately 350 μM Sr after 1 day of water immersion. Therefore, the Sr-doped BG incorporated cements developed in this study would be suitable for use as bioactive materials, especially for hard tissue regeneration.

5. Conclusion

In this study, Sr-releasable inorganic biomaterials with high mechanical properties were successfully fabricated by incorporating BGs into glass ionomer cement. These bioactive materials could be applied as bone grafting materials and bone cements. In addition, because dental pulp stem cells have a

similar phenotype as mesenchymal stem cells, the novel biomaterial developed in this study also shows potential for use as a pulp capping material.

Acknowledgements

This work was supported in part by Grants-in-Aid for Scientific Research (Nos. 16K11623, 18K17044, 17K11778, and 17H04383) from the Japan Society for the Promotion of Science. The funding sources were not involved in our study or our decision to publish the manuscript. The authors would also like to thank Tiffany Jain, M.S., from Edanz Group (www.edanzediting.com/ac) for editing a draft of this manuscript.

REFERENCES

[1] Rahaman MN, Day DE, Bal BS, Fu Q, Jung SB, Bonewald LF, et al. Bioactive glass in tissue engineering. Acta Biomater 2011;7:2355–73.
[2] Hench LL. The story of bioglass. J Mater Sci Mater Med 2006;17:967–78.
[3] Baino F, Hamzehlou S, Kargozar S. Bioactive glasses: where are we and where are we going? J Funct Biomater 2018;9:E25.
[4] Mistry S, Roy R, Kundu B, Datta S, Kumar M, Chanda A, et al. Clinical outcome of hydroxyapatite coated, bioactive glass coated, and machined Ti6Al4V threaded dental implant in human jaws: A short-term comparative study. Implant Dent 2016;25:252–60.
[5] Hoppe A, Guldal NS, Boccaccini AR. A review of the biological response to ionic dissolution products from bioactive glasses and glass-ceramics. Biomaterials 2011;32:2757–74.
[6] Hench LL, Polak JM. Third-generation biomedical materials. Science 2002;295:1014–7.
[7] El-Rashidy AA, Roether JA, Harhaus L, Kneser U, Boccaccini AR. Regenerating bone with bioactive glass scaffolds: a review of in vivo studies in bone defect models. Acta Biomater 2017;62:1–28.
[8] Miguez-Pacheco V, Hench LL, Boccaccini AR. Bioactive glasses beyond bone and teeth: emerging applications in contact with soft tissues. Acta Biomater 2015;13:1–15.
[9] Kargozar S, Hamzehlou S, Baino F. Potential of bioactive glasses for cardiac and pulmonary tissue engineering. Materials 2017;10:E1429.
[10] Mijnendonckx K, Leys N, Mahillon J, Silver S, Van Houdt R. Antimicrobial silver: uses, toxicity and potential for resistance. Biometals 2013;26:609–21.
[11] Soundrapandian C, Mahato A, Kundu B, Datta S, Sa B, Basu D. Development and effect of different bioactive silicate glass scaffolds: in vitro evaluation for use as a bone drug delivery system. J Mech Behav Biomed Mater 2014;40:1–12.
[12] Li C, Paris O, Siegel S, Roschger P, Paschalis EP, Klaushofer K, et al. Strontium is incorporated into mineral crystals only in newly formed bone during strontium ranelate treatment. J Bone Miner Res 2010;25:968–75.
[13] Mantzourani M, Sharma D. Dentine sensitivity: past, present and future. J Dent 2013;41:S3–17.
[14] Gentleman E, Fredholm YC, Jell G, Lotfibakhshaiesh N, O'Donnell MD, Hill RG, et al. The effects of strontium-substituted bioactive glasses on osteoblasts and osteoclasts in vitro. Biomaterials 2010;31:3949–56.
[15] Reginster JY, Brandi ML, Cannata-Andia J, Cooper C, Cortet B, Feron JM, et al. The position of strontium ranelate in today's management of osteoporosis. Osteoporos Int 2015;26:1667–71.
[16] Pilmane M, Salma-Ancane K, Loca D, Locs J, Berzina-Cimdina L. Strontium and strontium ranelate: historical review of some of their functions. Mater Sci Eng C Mater Biol Appl 2017;78:1222–30.
[17] Alrabeah GO, Brett P, Knowles JC, Petridis H. The effect of metal ions released from different dental implant-abutment couples on osteoblast function and secretion of bone resorbing mediators. J Dent 2017;66:91–101.
[18] Mi B, Xiong W, Xu N, Guan H, Fang Z, Liao H, et al. Strontium-loaded titania nanotube arrays repress osteoclast differentiation through multiple signalling pathways: in vitro and in vivo studies. Sci Rep 2017;7:2328.
[19] Koc Demir A, Elcin AE, Elcin YM. Strontium-modified chitosan/montmorillonite composites as bone tissue engineering scaffold. Mater Sci Eng C Mater Biol Appl 2018;89:8–14.
[20] Zhou C, Xu AT, Wang DD, Lin GF, Liu T, He FM. The effects of Sr-incorporated micro/nano rough titanium surface on rBMSC migration and osteogenic differentiation for rapid osteointegration. Biomater Sci 2018;6:1946–61.
[21] Molino G, Bari A, Baino F, Fiorilli S, Vitale-Brovarone C. Electrophoretic deposition of spray-dried Sr-containing mesoporous bioactive glass spheres on glass–ceramic scaffolds for bone tissue regeneration. J Mater Sci 2017;52:9103–14.
[22] Chen QZ, Thompson ID, Boccaccini AR. 45S5 bioglass-derived glass-ceramic scaffolds for bone tissue engineering. Biomaterials 2006;27:2414–25.
[23] Massera J, Hupa L. Influence of SrO substitution for CaO on the properties of bioactive glass S53P4. J Mater Sci Mater Med 2014;25:657–68.
[24] Mousavinasab SM, Khoroushi M, Keshani F, Hashemi S. Flexural strength and morphological characteristics of resin-modified glass-ionomer containing bioactive glass. J Contemp Dent Pract 2011;12:41–6.
[25] Yli-Urpo H, Lassila LV, Narhi T, Vallittu PK. Compressive strength and surface characterization of glass ionomer cements modified by particles of bioactive glass. Dent Mater 2005;21:201–9.
[26] Hench LL, Splinter RJ, Allen WC, Greenlee TK. Bonding mechanisms at the interface of ceramic prosthetic materials. J Biomed Mater Res 1971;5:117–41.
[27] Fiume E, Barberi J, Verne E, Baino F. Bioactive glasses: from parent 45S5 composition to scaffold-assisted tissue-healing therapies. J Funct Biomater 2018;9:E24.
[28] Miki S, Kitagawa H, Kitagawa R, Kiba W, Hayashi M, Imazato S. Antibacterial activity of resin composites containing surface pre-reacted glass-ionomer (S-PRG) filler. Dent Mater 2016;32:1095–102.
[29] ISO 9917-1. Dentistry – water-based cements – part 1: powder/liquid acid–base cements. Switzerland: International Standardization Organization; 2007.
[30] Yamaguchi S, Mehdawi IM, Sakai T, Abe T, Inoue S, Imazato S. In vitro/in silico investigation of failure criteria to predict flexural strength of composite resins. Dent Mater J 2018;37:152–6.
[31] Sasaki J, Hashimoto M, Yamaguchi S, Itoh Y, Yoshimoto I, Matsumoto T, et al. Fabrication of biomimetic bone tissue using mesenchymal stem cell-derived three-dimensional constructs incorporating endothelial cells. PLoS One 2015;10:e0129266.
[32] ISO 10993-5. Biological evaluation of medical devices-part 5: tests for in vitro cytotoxicity. Geneva, Switzerland: International Standardization Organization; 2009.
[33] Yoshimoto I, Sasaki JI, Tsuboi R, Yamaguchi S, Kitagawa H, Imazato S. Development of layered PLGA membranes for periodontal tissue regeneration. Dent Mater 2018;34:538–50.

[34] Montazeri M, Karbasi S, Foroughi MR, Monshi A, Ebrahimi-Kahrizsangi R. Evaluation of mechanical property and bioactivity of nano-bioglass 45S5 scaffold coated with poly-3-hydroxybutyrate. J Mater Sci Mater Med 2015;26:62.
[35] Kaur G, Pickrell G, Sriranganathan N, Kumar V, Homa D. Review and the state of the art: sol-gel and melt quenched bioactive glasses for tissue engineering. J Biomed Mater Res B Appl Biomater 2016;104:1248–75.
[36] Zheng K, Boccaccini AR. Sol-gel processing of bioactive glass nanoparticles: a review. Adv Colloid Interface Sci 2017;249:363–73.
[37] Zhai D, Xu M, Liu L, Chang J, Wu C. Silicate-based bioceramics regulating osteoblast differentiation through a BMP2 signalling pathway. J Mater Chem B 2017;5:7297–306.
[38] Juraski AC, Rodas ACD, Elsayed H, Bernardo E, Soares VO, Daguano J. The in vitro bioactivity, degradation, and cytotoxicity of polymer-derived wollastonite-diopside glass-ceramics. Materials 2017;10:E425.
[39] Sasaki J, Matsumoto T, Imazato S. Oriented bone formation using biomimetic fibrin hydrogels with three-dimensional patterned bone matrices. J Biomed Mater Res A 2015;103:622–7.
[40] Kim DA, Lee JH, Jun SK, Kim HW, Eltohamy M, Lee HH. Sol-gel-derived bioactive glass nanoparticle-incorporated glass ionomer cement with or without chitosan for enhanced mechanical and biomineralization properties. Dent Mater 2017;33:805–17.
[41] Yuan X, Cao H, Wang J, Tang K, Li B, Zhao Y, et al. Immunomodulatory effects of calcium and strontium co-doped titanium oxides on osteogenesis. Front Immunol 2017;8:1196.
[42] Oryan A, Baghaban Eslaminejad M, Kamali A, Hosseini S, Sayahpour FA, Baharvand H. Synergistic effect of strontium, bioactive glass and nano-hydroxyapatite promotes bone regeneration of critical-sized radial bone defects. J Biomed Mater Res B Appl Biomater 2019;107:50–64.
[43] Aimaiti A, Maimaitiyiming A, Boyong X, Aji K, Li C, Cui L. Low-dose strontium stimulates osteogenesis but high-dose doses cause apoptosis in human adipose-derived stem cells via regulation of the ERK1/2 signaling pathway. Stem Cell Res Ther 2017;8:282.