

Evaluation of strontium introduced apatite cement as the injectable bone substitute developments

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Abstract— We have developed bone cement introducing Strontium (Sr) to promote early bone regeneration. To prolong the release duration of Sr, we applied inorganic Sr filler for containing into the cement powder. The purpose of this study is to evaluate the mechanical properties, crystalline properties, and ion release activities, especially Sr anion, of this cement. Alpha-TCP powder was mixed with Sr filler, with 0.1wt%, 0.5wt%, 1.0wt%, and 5.0wt%. These were mixed with mixing liquid and formed for each test. They were incubated and crystallized in 95% moisture for 1 week. The mechanical properties were studied by the compression, the diametral tensile strength and 4-point vending. Tested specimens were evaluated by X-ray diffraction(XRD) and scanning electron microscopic(SEM) imaging. The ion release behaviors were measured by inductively coupled plasma mass spectrometry(ICP-MS). The mechanical properties were increased in consistency of filler, but decreased in some samples because of declining the apatite matrix. And the Sr release showed interesting results as the sequential resource of Sr. By adjusting the mixing ratio or considering the application of these Sr releasable cements, this material would show good performance by its strength and longer Sr release for bone regeneration.

I. INTRODUCTION

One of the problems of HAp cement for bone substitute is the trade-off balance of solubility and strength. We have developed calcium phosphate (CP) cement introducing Strontium (Sr) in liquid phase, to promote the distortion on the apatite lattice for their early degradation and bone regeneration, in apatite crystallinity aspect [1]. Sr has known as the factor to prevent the bone resorption, such as therapeutic medication in strontium ranelate for treatment of osteoporosis [2-4]. From our preliminary study, we revealed the important effect of Sr existence inside the apatite bone substitute that might be depending on the satisfactory absorption speed of apatite, and the earlier facilitation of the osteogenesis. From another point of view, we have applied inorganic glass-ionomer fillers that could release mineral ions, as the release source of Sr ions. The purpose of this study is to evaluate several material properties, crystallinity, and anion release behavior, by comparing with normally transformed hydroxyl apatite (HAp) and our Sr-apatite.

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II. MATERIALS AND METHODS

The main material of alpha-tricalcium phosphate (α -TCP, Taiho Chemical, Japan) powder was prepared for 2 μ m of mean particle size. And the surface pre-reacted glass ionomer (S-PRG; Shofu, Japan) filler was prepared for 3 μ m of mean particle size. The fluoroaluminosilicate glass, the base material of S-PRG filler, was reacted polyacrylic acid solution and silane coating treatment, and that could be the release source of Sr^{2+} , Na^+ , Al^{3+} , F^- , and several ions in the acidic surroundings *in vivo*[5,6]. S-PRG was mixed with α -TCP at the ratio of 0wt% (HAp; as a control), 0.1wt%, 0.5wt%, 1.0wt%, 5.0 wt%. Mixed each powders were combined and mixed with mixing liquid. The mixing liquid was prepared for two-step mixing for making the cement pastes, the 1st liquid is CaCl_2 with 1.0 mol/L solution and 2nd one is $\text{NaH}_2\text{PO}_4 \cdot 2\text{H}_2\text{O}$ solutions with 0.6 mol/L. The mixing ratio of these powders and liquid was as follows; powder:1st liquid:2nd liquid = 4:1:1. And our formally studied Sr-apatite cement (Sr-Ap) was prepared with SrCl_2 and $\text{NaH}_2\text{PO}_4 \cdot 2\text{H}_2\text{O}$ solutions, similarly (Table 1).

Table 1 Consistent variation of tested cement specimens

Specimen name	CP powder	Volume of S-PRG	Mixing liquid
HAp	α -TCP	—	$\text{CaCl}_2 + \text{NaH}_2\text{PO}_4$
Sr-Ap	α -TCP	—	$\text{SrCl}_2 + \text{NaH}_2\text{PO}_4$
0.1% S-PRG	α -TCP	0.1 wt%	$\text{CaCl}_2 + \text{NaH}_2\text{PO}_4$
0.5% S-PRG	α -TCP	0.5 wt%	$\text{CaCl}_2 + \text{NaH}_2\text{PO}_4$
1.0% S-PRG	α -TCP	1.0 wt%	$\text{CaCl}_2 + \text{NaH}_2\text{PO}_4$
5.0% S-PRG	α -TCP	5.0 wt%	$\text{CaCl}_2 + \text{NaH}_2\text{PO}_4$

Each cement pastes were formed several shapes for mechanical test and crystallinity observation. The cement paste was injected in the cylindrical mold with ϕ 6 mm x 12 mm for the compression strength testing, and ϕ 6 mm x 4 mm for the diametral tensile strength testing. For the 4-point vending, the cement paste was formed in the rectangular mold at 20 mm x 70 mm x 5 mm. These specimens were pre-incubated with the mold for 7 days with 37 degrees Celsius and 95% humidity, for transforming cement to the apatite crystals. After pre-incubation, crystallized specimens were taken, and dehydrated overnight in vacuum desiccator.

Cylindrical specimens were evaluated by the compress strength test and the diametral tensile strength test, using

precision universal testing machine (AG-1kNX, Shimadzu, Japan). Both tests were performed at a speed of 10mm/min. The rectangular specimens were tested by 4-point vending test with the ratio of the upper length against the below length was one third. The test speed was set 1mm/min in the whole tests.

After these mechanical evaluations, broken specimens of diametral tensile test were prepared by the dehydration treatment for scanning electron microscopy (SEM). The broken surfaces of specimens were coated around by osmium on the ion-coater (Neoc-ST, MeiwaFosis, Japan) approximately at a 10 nm of thickness. Specimens' surfaces were observed under SEM (JCM-5700, Jeol, Japan) at an accelerating voltage of 20 keV.

A part of non-tested each specimens were studied by X-ray diffraction (XRD; MiniFlex, Rigaku, Japan) for evaluating the existence of apatite crystal and the rest of α -TCP. The radiation source was Cu K α ($\lambda= 1.54178 \text{ \AA}$) and the measurement condition was set at a step size of 0.05° , and at a scan speed of $1^\circ/\text{min}$.

Normally, the CP cement group shows bio-degradable and bio-absorbable performance in vivo, so the material themselves would be dissolved into the body fluid or surroundings. Furthermore, the S-PRG behaves the positive ion release in water-soluble medium, and that would be promoted in acidic condition that was depending on the CP cement existence and minor solubility. To study such release and soluble performance of chemical elements and compound of cement matrix, several ion release behavior was studied by inductively coupled plasma mass spectrometry(ICP-MS; ELAN DRCII, Perkin Elmer, US) in solvent. The each crystallized CP cement at a size of $\phi 3 \text{ mm} \times 5 \text{ mm}$ in cylinder shape was doped into the 1 mL of sterilized distilled water as the solvent. They were stayed at 37 degrees Celsius and 95% humidity for 1, 2, 4, 8 and 12 weeks. After the periods, the cement specimens were taken out and a part of each solvent were diluted to 500 times volume to adjust on the measurable level of the ion density for ICP-MS. The measurements of ICP-MS have had high reliable and quantitative method for detecting much element in stable liquid sample, so this study was evaluated in single measurement.

III. RESULTS

The result of the compression strength testing was shown in Fig.1. This result indicated some mechanical increase by containing the S-PRG filler, especially in 0.1% S-PRG group. The others in 0.5% and 1.0% were similar with normal HAP group, or extremely decreased in 5.0%. The result of diametral tensile testing was shown in Fig.2, this result could show the high strengthen of Sr-Ap and 1.0% S-PRG. From the results of 4-point vending test, the specimens containing S-PRG group were shown the brittle for vending stress compared to HAP control group and Sr-Ap group. Especially, 5.0% S-PRG couldn't maintain the forms of molded specimen because of their feasibility, and couldn't test, thus excluded from the results. These results indicated a disadvantage of these composite material and non-sintering apatite.

By using the broken specimens that were tested diametral tensile test, the crystallinity analyses were conducted by SEM observation (Fig.3) and XRD. In the HAP and Sr-Ap groups, there could be observed the elaborate pure apatite crystals and

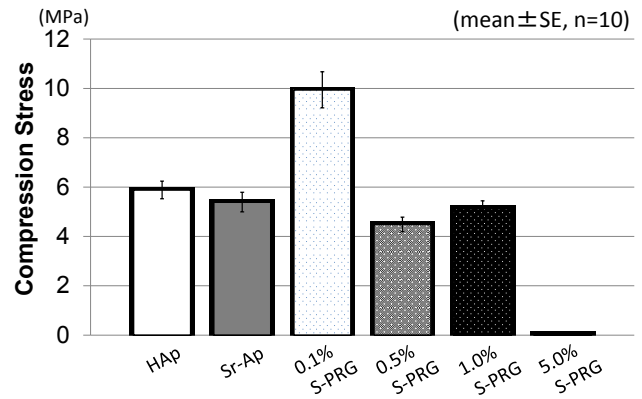


Fig.1 Results of compression strength test; Normal HAP, Sr-AP, and 0.1 wt%, 0.5 wt%, 1.0 wt%, and 5.0 wt% of S-PRG container.

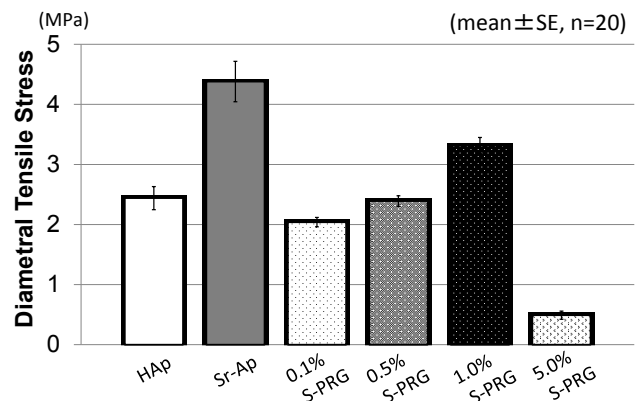


Fig.2 Results of diametral tensile test ; Normal HAP, Sr-AP, and 0.1 wt%, 0.5 wt%, and 1.0 wt% of S-PRG container.

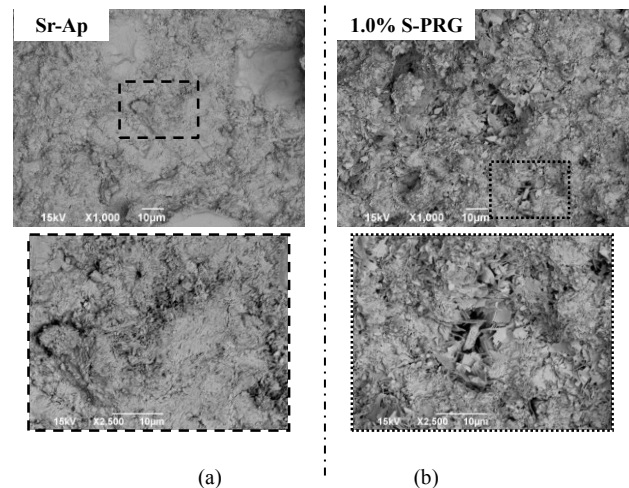


Fig.3 SEM images of specific specimens (a) Sr-Ap group in x1,000(upper), x2,500(below), (b) 1.0% S-PRG in x1,000(upper), x2,500(below)

matrix in 1 week incubation, and Sr-AP group was widely fine compared to HAP group. On S-PRG groups, there were

exactly fine apatite, but there existed some loss part of matrix around the S-PRG filler that could be specified by the particles size of filler.

Results of spectrum for X-ray diffraction of all cements were shown in Fig.4. From the results of XRD peak analyses, apatite crystals and residual α -TCP were identified in the whole tested groups. These results have indicated the qualitative existence of apatite layer, and the residue of untransformed TCP in 1 week incubation. Their consistency in the mechanical tests would be quietly similar, that were deposition period of apatite crystals.

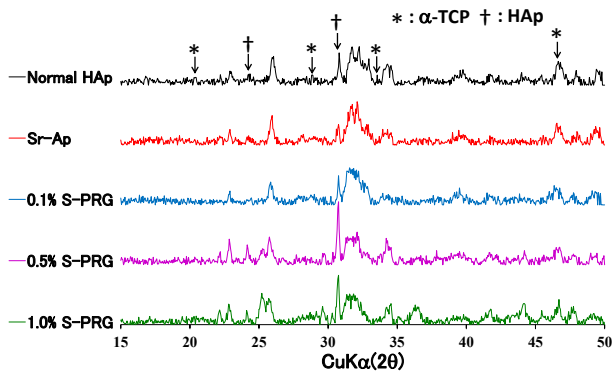


Fig.4 X-ray diffraction pattern of several CP cement crystallized for 1 week

; Normal HAp, Sr-Ap, and 0.1 wt%, 0.5 wt%, 1.0 wt% of S-PRG container

In the ICP-MS for detecting the ion release behavior in water, we could detect the quantitative density of the main consistence of the apatite and releaser of S-PRG ions, that is Na, Al, P, Ca, and Sr. The results of specific ion release from 1week to 3 months by ICP-MS were shown Fig.5. All of these results were calibrated by the measured data of distilled water as the background. The densities of ^{23}Na , ^{31}P , and ^{40}Ca derived from the basis of CP cement, those were TCP powder and the mixing liquid, showed similar values in each group and every duration. The S-PRG was the resource of ^{27}Al and ^{88}Sr in this test, but the density of ^{27}Al showed quiet low value in whole groups and didn't varied in all duration. The density of ^{43}Ca and ^{88}Sr was shown in Fig. 4. In the results of ^{43}Ca -ion quantification, the ion density varied in a certain degree and similar value in the whole group. The 5.0% S-PRG group showed the maximum value, and the HA, control group, showed secondary counts of ^{43}Ca anion. The density of Sr-ion, the remarkable element, was gradually increased according to the doped duration, except for the HAp because they didn't contain the Sr compound. And the 5.0 wt% of S-PRG group shows the highest value of ^{88}Sr ion release and that is the twice of Sr-Ap. And the ion densities of other S-PRG containers depended on the containing ratio of S-PRG. These results were similar in the ion release of ^{84}Sr , ^{86}Sr , and ^{87}Sr .

IV. DISCUSSION

The high performance of Sr-Ap was already revealed by our preliminary studies with several physical and mechanical

testing and *in vivo* animal experiments [1]. Normally, the pH condition of CP cement was acidic around 3 or 4 in the mixing paste, and that would be toward to the neutral. In this CP cement, the characteristics would be depending on the pH condition via the Sr existence that was containing inside the mixing liquid. The fact is that Sr has the neutralization ability such that the acidic condition to the neutral, just like the buffer. In such situations on our Sr-Ap componets, the acidification tendency by mixing condition of CP cement would promote the earlier transformation of TCP toward octa-calcium phosphate (OCP). And this reaction also derives earlier and much transformation of OCP to HAp. So in this

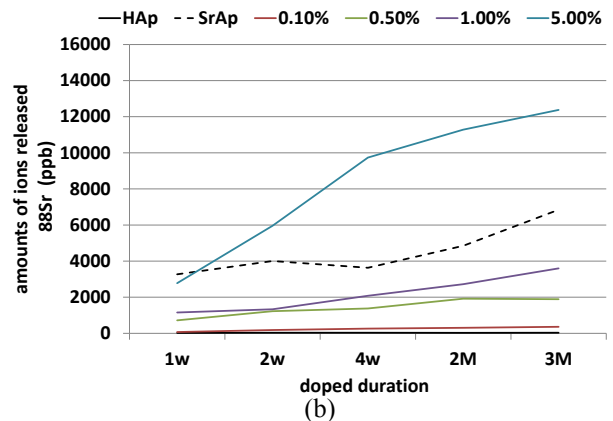
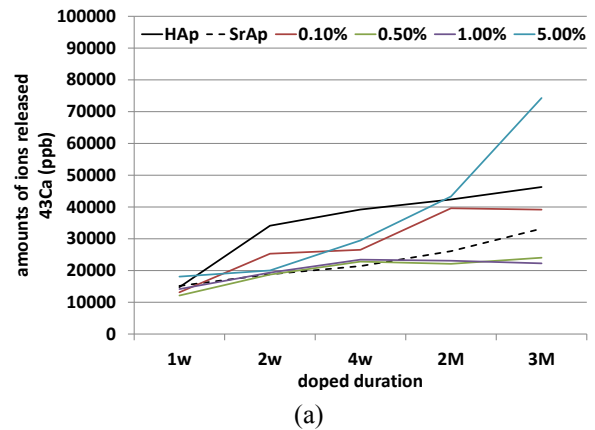


Fig.5 Results of released ion density into distilled water by ICP-MS (a) ^{43}Ca amounts, (b) ^{88}Sr amounts

study, the Sr-Ap showed the good performance on several mechanical testing. In the S-PRG group, 0.1 wt% and 1.0 wt% showed the individual results on compression test and diametral test, respectively. In the compression test, 0.1 wt% showed the peculiar value, and our hypothesis is that the S-PRG filler would behave like the composite compound in the apatite crystal and would have shown the high strength. And then in the diametral test, 1.0 wt% of S-PRG group showed the high stress and that is the second value in the diametral testing. The reason of these results was simply hypothesized that the buffer effect of acidification of CP cement would be the key. As I mentioned above about the buffer effect of Sr contains, the S-PRG would be made the pH condition of apatite transformable CP cement the acidic to

the neutral. The 1.0% S-PRG group would be the similar reaction that could disperse the Sr anion inside the cement paste in pre-incubation phase, and such action would be toward to their hardness higher. This character was the potential and individual performance of S-PRG filler itself. This buffer effect was already shown in our preliminary studies those were incubated specimens of CP cement including S-PRG with some cell line, those were often made the medium condition the neutral to the acidic, though those results were not mentioned about in this paper. From these reasons, the CP cement on 1.0 wt% S-PRG was promoted its crystallinity. And the results of SEM observation shown in Fig.3 followed such promotion inside the CP cement. Figures showed exactly fine and minute apatite transformation, and these were similar crystalline formation to the figure of Sr-Ap. Additionally, we could observed another kind of crystals around the S-PRG filler in 0.5 wt% and 1.0 wt of S-PRG specimens as shown in Fig.3(b). That might be the key components of apatite mixed with S-PRG filler to promote this S-PRG containing material as the characteristic bone substitute. This subject should be investigated after this.

In the results of crystallization analyses by XRD shown in Fig.4, the residue of α -TCP was certificated in the all group because these CP cement were crystalized on hydrolysis method depending on the surroundings of satisfied existence of OH⁻, and that is low crystallization method for apatite transformation. In our crystallization method for 1 week, the α -TCP couldn't be fully crystalized. So the mixing liquid components of CP cement would also be existed as the residual in the specimens, and one of the components, mainly the PO⁴⁻, made the acute acidic conditions of the medium in cell testing or the surrounding tissues for *in vivo* testing. Similarly, Sr²⁺ that couldn't be incorporated to apatite lattice might be exist as the anion residue or some subtracts that could easily soluble into the water. On the contrary, the Sr anion of S-PRG is contained the particle surface of silane coating just like "chemically charged" in thin surface of glass fillers. That was the difference point of ion dispersion into the water solvent of Sr-Ap group and S-PRG groups. From results of ICP-MS, several ions were measured that were dispersed into distilled water. Especially in Fig.5(a), the ⁴³Ca were measured with similar value and trend in all of specimens. Though the 5.0 wt% S-PRG group was so high, that would be due to its fragility. These results indicated that the five-kind of low crystallized apatite, except for 5.0 wt% group, were similarly solved into the solvent in adequate and certain ratio in the whole durations. However, ⁸⁸Sr were highly eluted into the solvent according to the weight percentage of S-PRG containing. But the maximum density of ⁸⁸Sr is the Sr-Ap group by calculating the number of ⁸⁸Sr against the ⁴³Ca. Consequently, the Sr-Ap group showed the good results in the mechanical and the chemical point of views.

V. CONCLUSION

For evaluating the effect of inorganic filler that could disperse Sr and several ions, we've studied the mechanical properties, crystalline and chemically solvable characteristics. From the results of mechanical properties, there would have some differences among with and without the S-PRG fillers and containing ratio of S-PRG. The results indicated that there would have some optimum volume of

S-PRG or adequate incubation condition for making the best matter of apatite transformation. On the other hand considering about the CP cement as the releasable source of Sr anion, the S-PRG had the good potential to long-term and sequential release. And the S-PRG was consisted of inorganic glass filler so that wouldn't be absorbed *in vivo* and would be remained in the tissue. These characteristics might be the advantages for considering the quiet long time release of Sr anion, though the further long-term release of ion detection and *in vivo* study for evaluating the bio-absorption should be conducted.

And in the sight of the clinical use as the bone substitute, the Sr-Ap group and S-PRG group also showed the good and beneficial performance as the Sr anion resource for assisting the treatment of the bone resorption and for applying bone regenerative medicines. And the S-PRG containing CP cement would be expected the further long-term release, i.e. over the several years, of anions because of its unabsorbable and biocompatible characteristics of S-PRG filler. To testify that, following studies should be conducted.

In our conclusion, Sr-Ap and S-PRG containing CP cement showed the interesting results in mechanical, crystalline and ion detective studies suggested in this paper. The Sr-Ap showed the good performance in all of examination considering about the development of bone substitutional cement. And, the S-PRG filler has much potential for improving the remineralization of hard tissues via the slow and long-term release of Sr ion for *in vivo* acidic surroundings. So the HAp cement containing S-PRG filler might be the useful for earlier but long-term sequential bone substitute by our following improvement.

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