

Article

Fabrication of Dicarboxylic-Acid- and Silica-Substituted Octacalcium Phosphate Blocks with Stronger Mechanical Strength

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Abstract: Octacalcium phosphate (OCP) is an attractive base material to combine into components developed for medical purposes, especially those used in bone replacement procedures, not only because of its excellent biocompatibility but also because of its ability to intercalate with multiple types of molecular layers such as silica, dicarboxylic acid, and various cations. On the other hand, there are no examples of simultaneous substituting for several different compounds on OCPs. Therefore, in this study, the physical and mechanical strength (DTS: diametral tensile strength) of OCPs substituted with both silica and dicarboxylic acids (thiomalate: SH-malate) were evaluated. By optimizing the amount of SH-malate, we were able to prepare a block consisting of OCPs with both silica and SH-malate supported in the interlayer. The composition of the OCP-based compound comprising this block was $\text{Ca}_8\text{Na}_{1.07}\text{H}_{6.33}(\text{PO}_4)_{4.44}(\text{SiO}_4)_{1.32}(\text{SH-malate})_{2.40} \cdot n\text{H}_2\text{O}$. Interestingly, the low mechanical strength, a drawback of silica-substituted OCP blocks, could be improved by dicarboxylic acid substituting. The dicarboxylic acid addition increased the mechanical strength of silica-substituted OCP blocks, and the acid successfully incorporated into the interlayer, even with the presence of silica. These results are expected to advance the creation of better silica-substituted OCPs and improved bone replacement materials.

Keywords: octacalcium phosphate (OCP); bioceramics; silica; dicarboxylate; co-substitute



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1. Introduction

A prevalent health issue among the elderly is hard tissue syndrome [1,2]. This is because as individuals age, they develop hard tissues that have relatively low healing abilities. As typified by bone fracture and periodontal diseases, hard tissue syndrome leads to a greatly decreased quality of life. In particular, alveolar bone recovery as well as the creation of effective bone regeneration materials are required to restore oral functions. Therefore, medical infrastructures that help with the rapid healing from diseases related to hard tissue syndrome are essential for the maintenance of a vibrant society, especially among elderly populations.

Octacalcium phosphate [OCP: $\text{Ca}_8(\text{PO}_4)_4(\text{HPO}_4)_2 \cdot 5\text{H}_2\text{O}$], the main inorganic component of immature bone, exhibits excellent biocompatibility and can be medically replaced in the bone of a patient through the bone remodeling process [3–8]. In addition, the OCP crystal structure can be incorporated within various ions and molecules such as transition metal ions and dicarboxylic acids [5,9–14]. Thus, OCP has been an attractive base material to combine with components for the development of medical products, especially those

utilized in bone regeneration. Furthermore, OCP is a precursor of other calcium phosphates that aid in bone healing and regeneration such as hydroxyapatite [HAp: $\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2$] and carbonate apatite [CO_3Ap : $\text{Ca}_{10-a}(\text{PO}_4)_{6-b}(\text{CO}_3)_c(\text{OH})_{2-d}$] [15–18]. Depending on the molecule, the OCP unit lattice-substituted materials interlayer might be maintained or released through the phase conversion process [19–22].

Silica enhanced the viabilities of osteoblasts and increases their bone production [23–25]. In fact, there have been attempts to demonstrate high bone regeneration ability by combining silica with vaterite, a polymorph of calcium carbonate, in bone replacement materials [26–29]. In addition, bioceramics containing silica nanoparticles were also introduced for bone regeneration materials [30–33]. We have investigated the usefulness of this material as a bone replacement material by substituting silica onto OCP (OCP-silica) without using organic silica such as tetraethyl silicate (TEOS), which possess the harmful problem of residual organic matter material feasibility tests for application in bone regeneration because silica enhances osteoblastic activities [34–36]. It was found that OCP-silica exhibited several times higher bone regeneration ability compared to CO_3Ap , which is presently used in commercial bone substitutes for dental implant [35]. On the other hand, the low formability and mechanical strength of OCP-silica blocks are serious drawbacks for bone substitute applications and further equipment. Since silica acts as an inhibitor of OCP crystal growth, the interlocking process results in low mechanical strength [34,35]. Moreover, when OCP blocks were converted into apatite blocks through a solution mediated phase conversion process, the mechanical strength of treated blocks further decreased [36].

In a previous related study, the results showed that dicarboxylic-acid-substituted OCP blocks fabricated by the OCP cementing process displayed a mechanical strength that tended to be higher than that of normal OCP blocks [37,38]. This knowledge can be applied in the preparation of OCP-silica blocks that exhibit high strength and serves as a precursor for other silica-substituted calcium phosphates. To further investigate the capabilities of molecular-substituted OCP blocks for practical application, this study prepared silica-dicarboxylic acid molecule-supported OCP blocks using thiomalate acid [SH-malate: $\text{C}_4\text{H}_6\text{O}_4\text{S}$] as the dicarboxylic acid molecule.

2. Materials and Methods

2.1. Preparation of SH-Malate and Silica-Substituted Blocks through the Cement-Setting Reaction

The reagents used for this study were obtained as follows: all reagents (except SH-malate) were purchased as reagent grade from FUJI Film Wako Pure Chemical Inc., Co., Osaka, Japan; SH-malate was purchased from Tokyo Chemical Industrial Co., Tokyo, Japan; H_3PO_4 was diluted by distilled water and then prepared in 4.0 mol/L H_3PO_4 . In addition, the SH-malate and silica-substituted blocks were prepared through the cement-setting reaction [35,37,38].

Next, the OCP component fabrication process is detailed. First, 0.0–0.6 g SH-malate was dissolved into 2.0 mL 4.0 mol/L H_3PO_4 at an agate motor. Then, 1.2 g of CaCO_3 powder was gradually mixed into the solution with mixing by agate pestle. Afterwards, 1.08 mL, 38 percentage by weight (wt%) Na_2SiO_3 solution was subsequently added by stirring with a pestle for several ten-second intervals until the bubbling process was stabilized. Using a stainless spatula, the mixture was placed in a silicon rubber mold ($\phi 6 \times 3$ mm) that was tightly sealed by a 0.3 mm thick polypropylene sheet to avoid evaporation and induce the interlocking of formed crystals during incubation. The packed molds with samples were placed at 60 °C for 1 d. Finally, the treated materials were removed from the mold; then, they were thoroughly washed with distilled water three times and finally placed in a 40 °C drying oven for 1 d.

2.2. Evaluation Process

To evaluate the experimental results, crystallographic information from the samples was obtained through X-ray diffraction (XRD; MiniFlex600, Rigaku Co., Tokyo, Japan) at an

acceleration voltage and amplitude of 40 kV and 15 mA, respectively. For characterization, the diffraction angle was continuously scanned over 2θ values ranging from 3° to 70° at a scanning rate of $5^\circ/\text{min}$. Meanwhile, for the crystallographic parameter analysis, it was scanned from 2° to 12° at a rate of $1^\circ/\text{min}$.

Next, the chemical bonding of the samples was analyzed with Fourier transform infrared spectroscopy (FT-IR) (IRTracer-100, Shimadzu Co., Kyoto, Japan) and a triglycine sulfate detector (30 scans, resolution of 2 cm^{-1}) that has an attenuated total reflection diamond prism. Also, the atmosphere provided the measurement background.

After Os sputtering, the fine structures of the samples were examined by field emission scanning electron microscopy (FE-SEM; JSM-6700F, JEOL Co., Tokyo, Japan) at 3 kV acceleration voltage.

The ^{31}P chemical shifts of the samples were determined by using solid state nuclear magnetic resonance (NMR) spectroscopy (Varian FT-NMR, 400 MHz, Agilent Technologies Co., Santa Clara, CA, USA) at a 161.8 MHz resonance frequency. Next, cross-polarization magic angle spinning (CP-MAS) ^{31}P NMR spectroscopy was performed at 10 kHz. In addition, an Agilent 4 mm T3 CP-MAS HXY solid probe and zirconia rotors were used. Each sample weighed $\approx 0.03\text{ g}$. Meanwhile, the contact time for the ^{31}P CP-MAS measurements was 3 ms, with an acquisition time of 50 ms and relaxation delay of 40 s for each measurement interval. Each measurement was repeated 400 times. The ^{31}P chemical shift of $(\text{NH}_4)\text{H}_2\text{PO}_4$ was used as the external reference (1.0 ppm).

The Si and S content of the samples were measured using energy-dispersive X-ray fluorescence spectroscopy (EDX-8100, Shimadzu Co., Japan) at 15 kV acceleration voltage under a vacuum. Next, the Si/Ca ratios of the samples were calibrated using inner standard samples, which consisted of a hydroxyapatite– SiO_2 mixture in disk-shaped sintered blocks (Ca/Si = 100:0, 90:10, 75:25, 50:50, and 0:100) measured in the same manner as the experimental samples. The S/Ca ratio was also calibrated using a disk-shaped mixture of CaCO_3 – $\text{CaSO}_4 \cdot 2\text{H}_2\text{O}$ set blocks (Ca/S = 100:0, 90:10, 75:25 and 50:50).

Afterwards, the mechanical strengths of five cylindrical specimens were evaluated. Their respective diameter and thickness were measured using a micrometer (MDC-25MU, Mitsutoyo Co., Ltd., Kawasaki, Japan). These specimens were then submitted under compression testing by crushing at a constant crosshead speed of 1 mm/min using a universal testing machine (AGS-J, Shimadzu, Kyoto, Japan). Next, the mean DTS values of the specimens were estimated and reported as the mean standard deviation. The results were then assessed by *t*-test analysis (with a significance level of $p < 0.05$).

3. Results and Discussion

Figure 1 shows photographs of the set samples with various SH-malate amounts. Note that in the study of dicarboxylic acid molecule substituting, the amount of CaCO_3 is increased compared to the conventional OCP-silica preparation. This is because dicarboxylic acid is acidic, and it is necessary to increase the amount of CaCO_3 to neutralize it. The mud mixture foamed more strongly because of this. Through the cement-setting reaction, all the samples revealed block shapes. It should be noted that in low SH-malate amounts (SH-malate: 0.00–0.15 g), the bubbling process was continued during setting reactions.

Next, the XRD evaluation of the obtained blocks was performed. Figure 2 shows the XRD patterns of the set blocks with different SH-malate amounts. As the SH-malate concentration increased, the block composition became from apatite to OCP, and monophasic OCP peaks were observed around 0.45 g and 0.60 g of SH-malate content. The peak corresponding to *d*100 of OCP shifted slightly to the low angle, indicating that SH-malate intercalated with the OCP interlayer. Therefore, the sample with 0.60 g of SH-malate was found to be OCP monophasic and thereby can be referred to as an OCP-silica:SH-malate block.

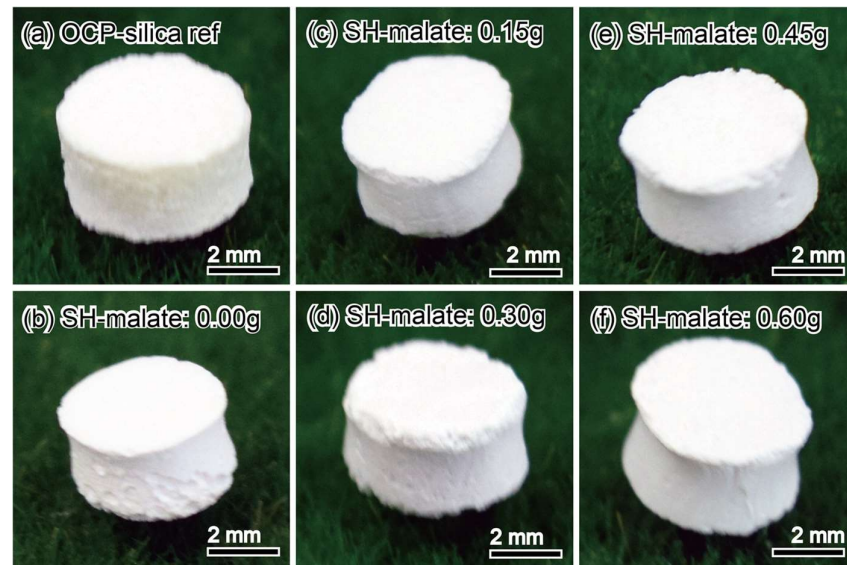


Figure 1. Photographs of the set blocks with different SH-malate amounts. (a) OCP-silica block as reference. (b) SH-malate: 0.00 g. (c) SH-malate: 0.15 g. (d) SH-malate: 0.30 g. (e) SH-malate: 0.45 g. (f) SH-malate: 0.60 g.

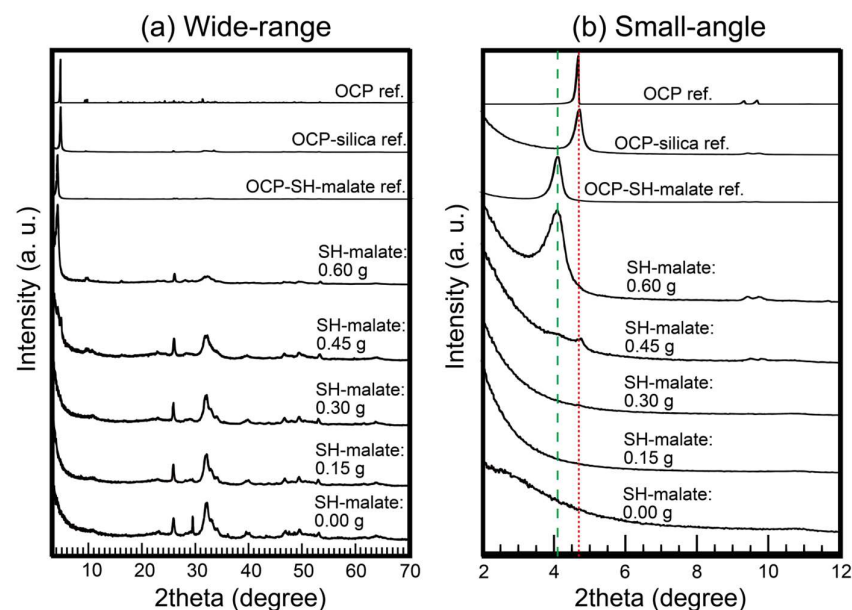


Figure 2. XRD patterns of the set blocks. (a) Wide range. (b) Small angle. Green broken line: OCP-SH-malate $d(100)'$ peak. Red dotted line: OCP $d(100)$ peak.

The conditions for substituted silica and SH-malate in OCP were further evaluated. Figures 3 and 4 show the OCP-silica:SH-malate block FT-IR and NMR spectra, respectively. In FT-IR spectra, the OCP-silica:SH-malate block clearly exhibited -COOH band ranging from 1400 to 1600 cm^{-1} , which is the same as in OCP-SH-malate. In addition, a weak band ranging from 1100 to 1200 cm^{-1} , which corresponds to the silanol band, was observed. Then, the OCP-silica:SH-malate block revealed both OCP-silica and OCP-SH-malate. In NMR spectra, the $P2/P4$ peaks were clearly attenuated, while $P5/P6$ corresponded to the hydrous layer and shifted slightly toward the higher wavenumber. Meanwhile, bands corresponding to P_A/P_B were observed at lower wavenumbers. These results were attributed to the inclusion of dicarboxylic acid molecules [35,36], as well as indicating that both silica and SH-malate are contained in the OCP crystals.

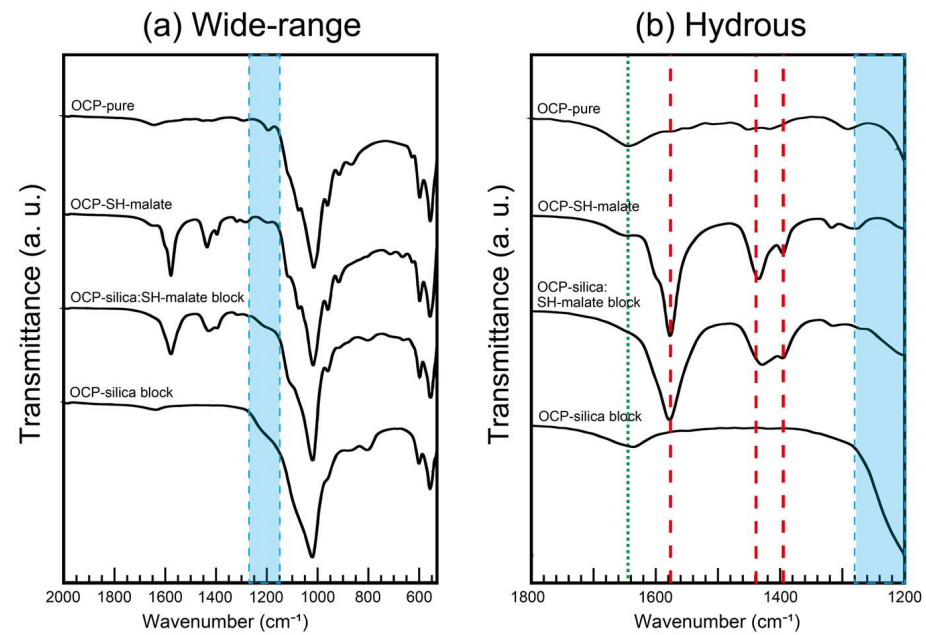


Figure 3. FT-IR spectra of OCP-silica:SH-malate block with OCP-silica and OCP-SH-malate reference for facilitate comparison. (a) Wide range. (b) Hydrus. Blue band: silanol vibration. Red broken lines: -COOH vibration. Green dotted line: HPO₄ vibration [39].

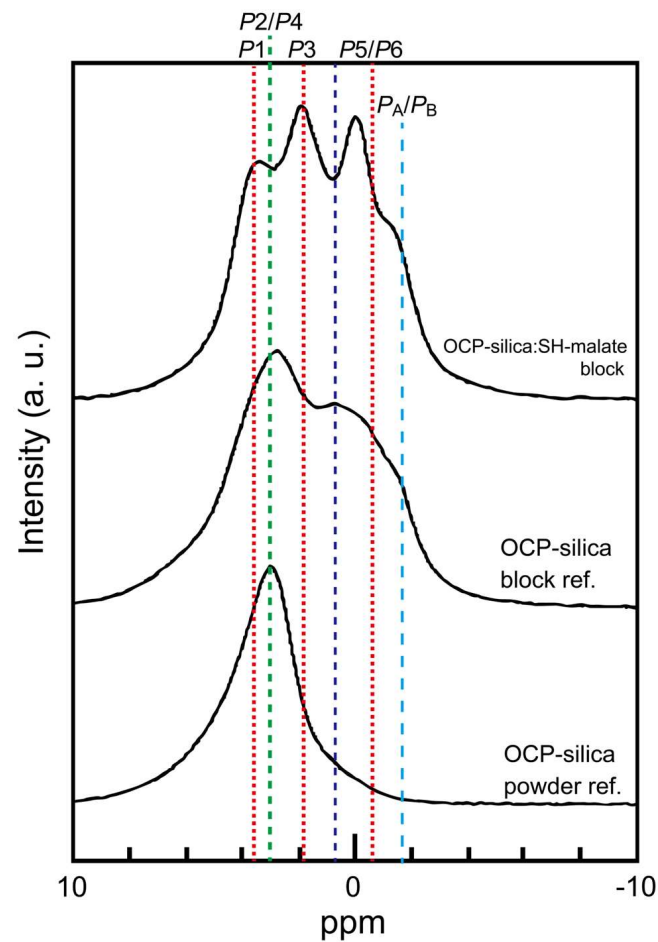


Figure 4. ³¹P solid-state NMR spectra of OCP-silica:SH-malate block with conventional OCP, OCP-silica block, and OCP-silica powder for facilitate comparison. Broken lines corresponded each PO₄ state. Dark blue broken line: PO₄ of OCP-silica block state [35,40].

The OCP-silica:SH-malate block (SH-malate: 0.60 g) contains of Si (8.35 ± 0.68 at%) and S (15.20 ± 3.38 at%). However, the OCP-silica:SH-malate block Si content was significantly lower than that of OCP-silica powder and block. On the other hand, the OCP-silica:SH-malate block S content was significantly higher than that of OCP-SH-malate powder. Then, the possibly chemical composition of OCP-silica:SH-malate block was described as $\text{Ca}_8\text{Na}_{1.07}\text{H}_{6.33}(\text{PO}_4)_{4.44}(\text{SiO}_4)_{1.32}(\text{SH-malate})_{2.40} \cdot n\text{H}_2\text{O}$.

Next, the mechanical strength of the samples was evaluated. Figure 5 shows the DTS values of the set samples. As SH-malate was increased, the block mechanical strength was attenuated, but increased again. For the OCP-silica:SH-malate block, the mechanical strength of samples was significantly higher than that of blocks without SH-malate and OCP-silica blocks.

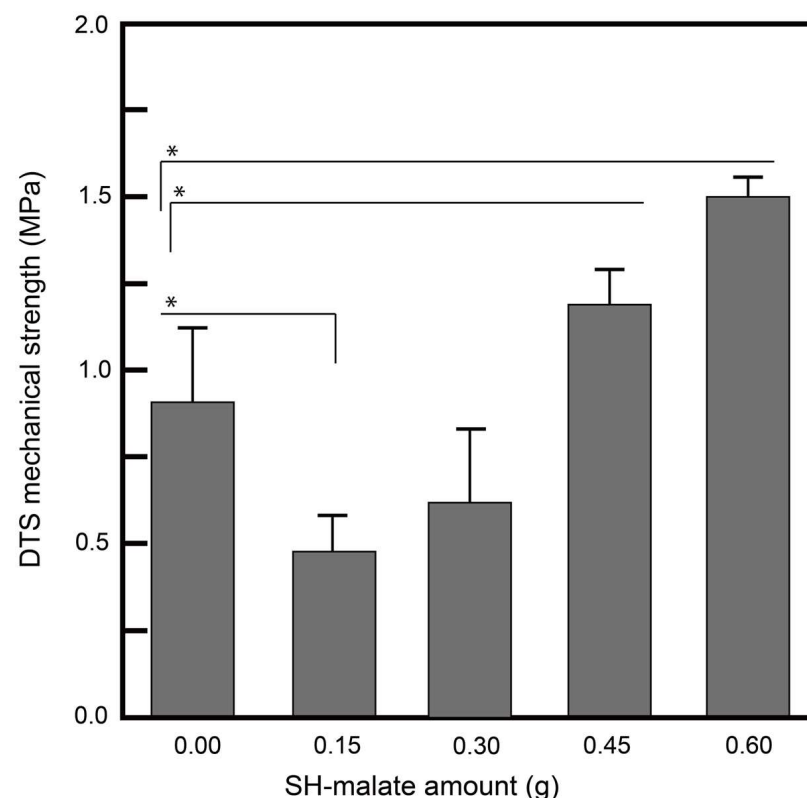


Figure 5. DTS values of the set blocks with different SH-malate amounts. *: $p < 0.05$.

Meanwhile, the fine structure of the samples indicated that they possessed higher mechanical strength. Figure 6 shows SEM micrographs of the OCP-silica:SH-malate block and two types of OCP-silica block ($\text{CaCO}_3\text{-H}_3\text{PO}_4\text{-Na}_2\text{SiO}_3$ and DCPD-MCPM- Na_2SiO_3 systems) for facilitate comparison. In the OCP-silica:SH-malate block, much denser and well-developed crystals were observed compared to both OCP-silica blocks. This suggests that a high degree of entanglement results in higher strength. When dicarboxylic acid molecules and other molecules are substituted on OCP, developing in the a -axis direction is suppressed [40–43]. As a result, the aspect ratio of OCP crystals increases. This is assumed to increase the entanglement and form a denser and stronger structure. Furthermore, since the dicarboxylic acid molecules have carboxy groups, an adhesive effect of free dicarboxylic acid molecules binding to Ca ions on the OCP surface is also expected [44,45]. Since the same phenomenon is observed even when silica is not supported, it is considered that dicarboxylic acid contributes to both aspect ratio and adhesive strength [34,35].

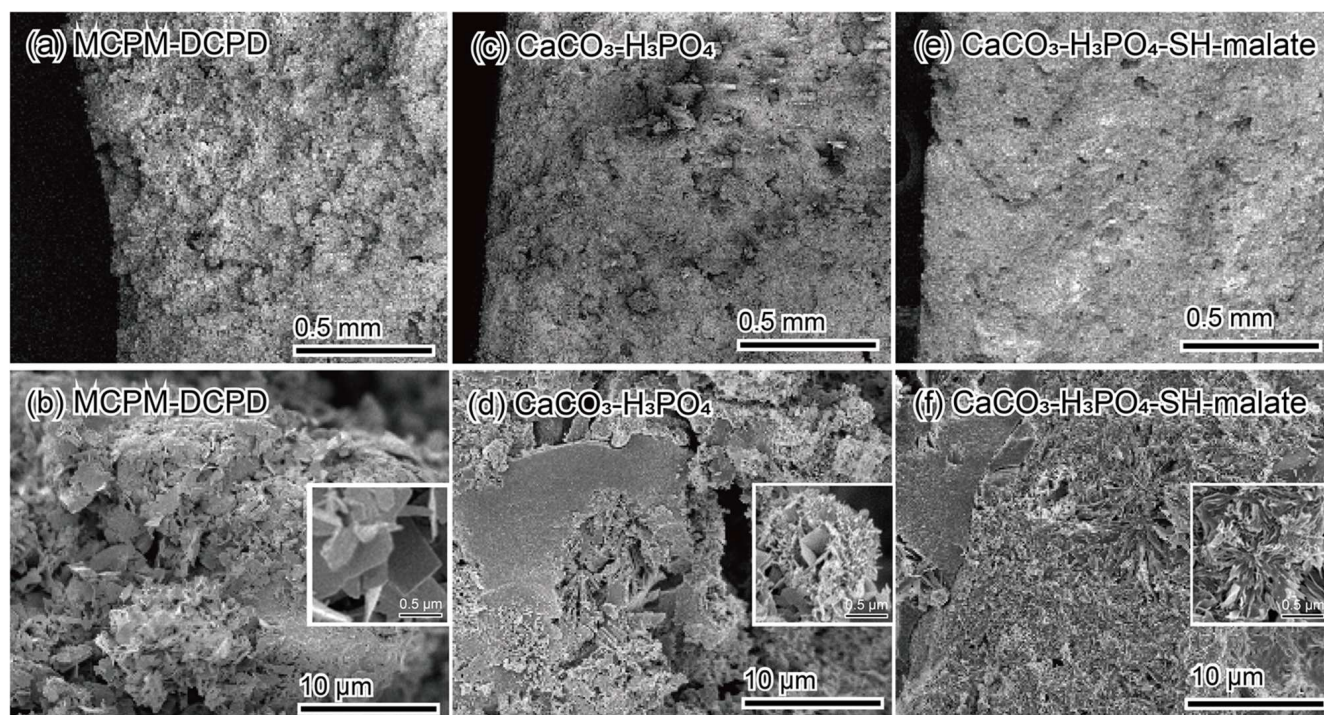


Figure 6. SEM micrographs of OCP-silica:SH-malate block and reference materials in 0.5 mm and 10 μm , respectively. (a,b) OCP-silica fabricated from MCPM-DCPD- Na_2SiO_3 system. (c,d) OCP-silica fabricated from $\text{CaCO}_3\text{-H}_3\text{PO}_4\text{-Na}_2\text{SiO}_3$. (e,f) OCP-silica:SH-malate block.

The results clearly show that both silica and SH-malate can be simultaneously substituted in the OCP crystal structure. Furthermore, we think that the prepared OCP-silica:SH-malate block is a material in which both silica and SH-malate are substituted between the layers of OCP. This is shown by FT-IR and NMR spectral measurements. In addition, compared to conventional OCP-silica, the strength of the prepared blocks is improved. One of the possible reasons for this is the morphological change of OCP crystals due to the incorporation of SH-malate into the OCP interlayer: OCP crystals generally assume a ribbon-like crystal form, while dicarboxylic acid molecules, including SH-malate, are distributed along the *a*-axis direction, that is, the orientation in which the crystal is thinnest [46,47]. Therefore, the *a*-axis direction growth is inhibited, while growth in the *b*- and *c*-axis directions is relatively less inhibited, resulting in the formation of crystals with a large aspect ratio, i.e., crystals that are easily entangled.

For further investigation, it was also tested as to whether OCP is useful for dicarboxylic acid molecules other than SH-malate. As shown in Figure 7, succinic acid ($\text{C}_4\text{H}_6\text{O}_4$) substituting was considered. It was found that any dicarboxylic acid molecule, such as succinic acid, can be incorporated into OCP crystals and blocked. Therefore, these would have the same effect as that observed in SH-malate. Note that the mechanical strength of succinic-acid-substituted OCP-silica blocks was found to be significantly higher than that of SH-malate. This is likely due to the higher acidity of succinic acid and how it might compare to SH-malate, as well as the stronger adhesion of the carboxy groups of the dicarboxylic acid molecules, resulting in a stronger block.

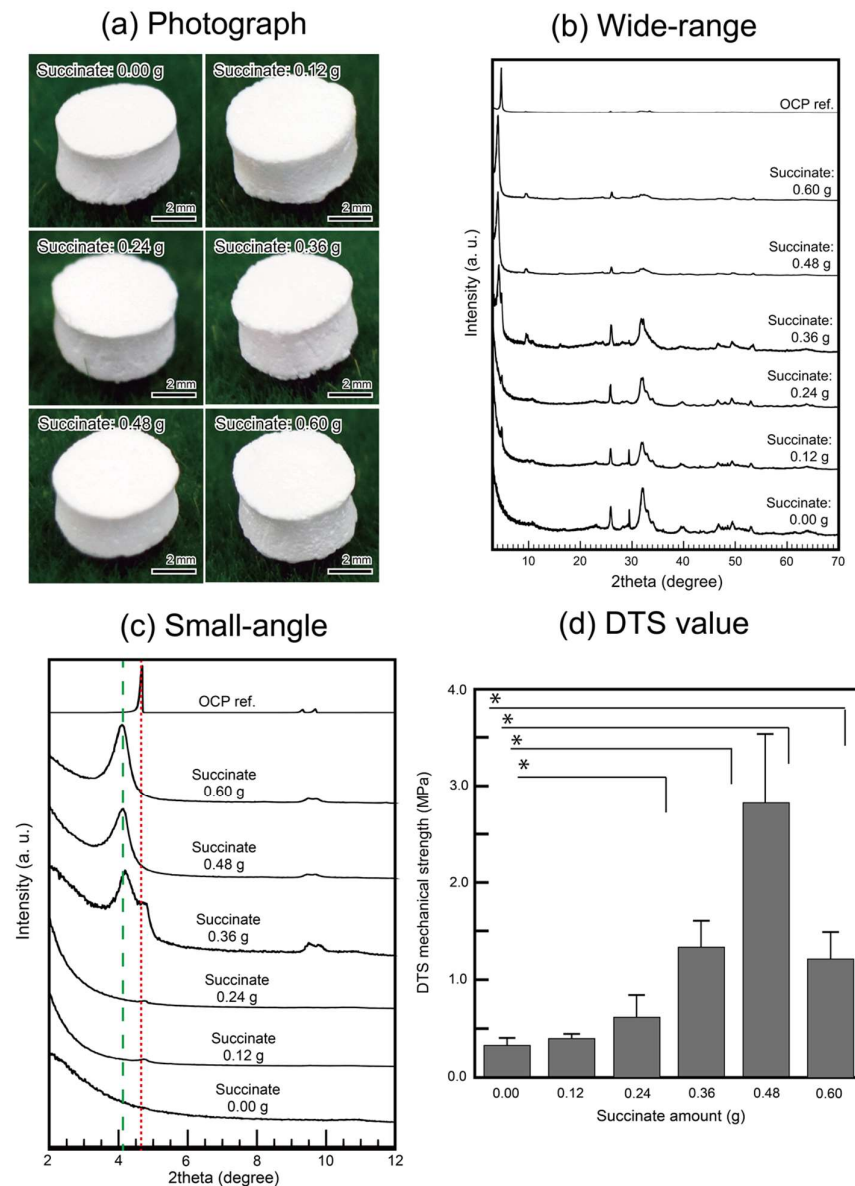


Figure 7. Evaluation results of OCP-silica:succinate block fabrication. (a) Photographs of the set blocks with different succinate amounts. (b) Wide-range XRD patterns of the set blocks with different succinate amounts. (c) Small-angle XRD patterns of the set blocks with different succinate amounts. Green broken line: OCP-SH-malate *d*(100)' peak. Red dotted line: OCP *d*(100) peak. (d) DTS values of the set blocks with different succinate amounts. *: *p* < 0.05.

4. Conclusions

Because of OCP's ability to intercalate with multiple types of molecular layers such as silica, dicarboxylic acid, and various cations, OCP is an attractive base material to combine into components developed for medical purposes. On the other hand, there are no examples of simultaneous substituting for several different compounds on OCPs. Of particular interest is whether low mechanical strength, a drawback of OCP-silica blocks, could be improved by dicarboxylic acid substituting. Therefore, the preparation of silica-substituted OCP blocks with the addition of SH-malate, a dicarboxylic acid molecule, was investigated. If the formulation ratio is appropriate, it is possible to prepare monophasic OCP blocks substituted with dicarboxylic acid molecules and silica. The mechanical strength of the resulting blocks tends to be higher than that of the system without dicarboxylic acid molecules substituted. Based on these results, the findings of this study have promising ap-

plications for the development of improved medical components used in bone replacement that aid in more efficient as well as effective bone recovery and regeneration.

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