PREPARATION OF POROUS β-TRICALCICUM PHOSPHATE BY FOAMING METHOD USING CELLULOSE NANOFIBERS WITH DIFFERENT MANUFACTURING METHODS AS FOAM STABILIZER

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Abstract: In this study, we found that the use of phosphite esterified cellulose nanofibers (CNF-P) in combination with the foaming method can produce porous bodies due to the interaction between nonionic surfactants and hydrophobic/hydrophilic CNF-P. When the foaming method was applied to the water-based slurry, the hydrophilic-lipophilic balance of the nonionic surfactant used was about 11-14.5, indicating that the foam was stable during preparation. Furthermore, it was shown that the addition of CNF-P promoted the formation of both micron-sized pores and macro-sized pores (100-400 µm).  

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INTRODUCTION

In today’s aging society, bone diseases such as osteoporosis and arthropathy, as well as fractures resulting from accidents, are the most common causes of bedridden elderly people. In order to improve the quality of life of such elderly people, orthopedic surgeons require materials that can compensate for bone defects.

One such material is β-tricalcium phosphate (β-Ca₃(PO₄)₂; β-TCP), which has attracted attention because of its high biocompatibility and excellent osteoconductivity.¹ When it is implanted in vivo, it serves as a scaffold for bone formation and promotes autogenous ossification. The material itself dissolves to leave behind a bone-like apatite layer, which chemically binds to the calcified matrix of the bone, resulting in excellent bone affinity.²,³

There are three types of bone graft materials: dense sintered materials, porous materials, and pastes. In the present study, we focused on highly porous bone-filler materials that have a mixture of interconnecting micro-sized pores (0.1-10 µm) and macro-sized pores (100-600 µm). Such materials are known to serve as scaffolds for angiogenesis and cell growth.³ Angiogenesis in bone not only provides nutrients essential for the survival of living tissue, but also plays an important role in osteocyte activity and bone remodeling. Therefore, a material with a high pore density and moderate permeability is conducive to tissue regeneration. However, since highly porous materials with large pore sizes exhibit low compressive strength, it is important to control the porosity and pore size.

Commonly known methods for preparing porous bone fillers include direct foaming,⁵ calcination of organic materials,⁶ the use of a foaming agent,⁷ and pore formation using a mold made of polyurethane foam.⁸ In the foaming method using a foaming agent, atmospheric air is trapped by mechanical stirring to produce a porous material. This method is relatively simple, and it is known that the addition of a surfactant prevents bubble breakage. We have already reported that cellulose nanofibers (CNFs) can be used as a bubble stabilizer and thickener during the foaming process to produce biomaterials.⁹  

We have shown that CNFs can act as pore formers during calcination, where burnt areas are opened by pyrolysis. However, it was difficult to disperse the CNFs obtained by mechanical treatment, even using dispersants, and the pores formed had an irregular shape. In the present study, we fabricated porous β-TCP using the foaming method with chemically treated CNFs that had a short fiber width and exhibited high dispersibility, and evaluated the physical properties of the resulting material.

EXPERIMENTAL METHODS

The CNFs used in this study  
Two types of CNFs were used: mechanically milled and fine-grained water-dispersed CNFs (CNF-S; Daio Paper Co., Ltd.) used in a previous study⁴, and milled water-dispersed CNFs (CNF-P; Daio Paper Co., Ltd.) treated with phosphite ester to produce a fiber width of a few nanometers.
Preparation of raw powder

To obtain a Ca/P molar ratio of 1.50, a mixture of 11.32 g of calcium carbonate (CaCO₃, purity 99.5%, Wako special grade), 39.51 g of calcium hydrogen phosphate dihydrate (CaHPO₄·2H₂O, purity 98.0%, genuine special grade) and 450 ml of pure water heated to 80°C was ground and mixed for 24 h in a zirconia pot (Nikkato) with an inner diameter of 105 mm and a height of 115 mm. Zirconia balls (Nikkato, 600 g of 5 mm diameter balls and 900 g of 10 mm diameter balls) were used in the grinding and mixing process. The mixed samples were dried at 70°C for 24 h and then initially calcined at 750°C for 10 h. The powder composition was evaluated by powder X-ray diffraction (XRD; Miniflex 600, Rigaku) and Fourier-transform infrared absorption spectroscopy (FT-IR; FT-IR-4200, JASCO) using KBr method. Scanning electron microscopy (SEM; VE-7800, KEYENCE) was used to observe the particle size obtained by temporary burning.

Preparation of porous materials

30 g of calcined powder and 10 g of CNF-P and CNF-S aqueous dispersions (Daio Paper Co., Ltd.) were mixed, and 30 ml of aqueous ammonium polyacrylate solution (Fujifilm Wako Pure Chemical Co., Ltd.) diluted to 10 vol% was added. The mixture was dispersed in an ultrasonic bath (AS ONE) using a multi-hand blender (HB-502WJ, Cuisinart) for 5 min. Then, 4 ml of the nonionic surfactant polyoxyethylene lauryl ether (CH₃(CH₂)₇OH-(CH₂CH₂O)nH (n = 2, 4, 9, POELE-n) was added as a foaming agent, and foaming by mechanical agitation was performed under the same conditions as in the dispersion process. The hydrophilic-lipophilic balance (HLB) for the nonionic surfactants was 9.5 for POELE-2, 11.0 for POELE-4, and 14.5 for POELE-9. The obtained foams were poured into square pots with dimensions of 60 mm (length) × 60 mm (width) lined with paraffin paper, tapped to adjust the height to 25 mm, and dried at 40°C. After drying, the pots were fired at 1000°C for 40 min. Table 1 shows the preparation conditions for each sample in this study.

| Sample | Foaming Agent | HLB value | CNF Additive amount of CNF/g | Drying temperature °C | Sintering temperature °C |
|--------|---------------|-----------|-----------------------------|-----------------------|-------------------------|
| a      | POELE-4       | 11.0      | 10.0                        | 40                    | 1120                    |
| b      | POELE-4       | 11.0      | CNF-S 10.0                  | 40                    | 1120                    |
| c      | POELE-4       | 11.0      | CNF-P 10.0                  | 40                    | 1120                    |
| d      | POELE-2       | 9.5       | CNF-P 10.0                  | 40                    | 1120                    |
| e      | POELE-9       | 14.5      | CNF-P 10.0                  | 40                    | 1120                    |
| f      | POELE-4       | 11.0      | CNF-P 10.0                  | 55                    | 1120                    |
| g      | POELE-4       | 11.0      | CNF-P 10.0                  | 75                    | 1120                    |
| h      | POELE-4       | 11.0      | CNF-P 10.0                  | 40                    | 1090                    |
| i      | POELE-4       | 11.0      | CNF-P 10.0                  | 40                    | 1060                    |

Evaluation of porous materials

The sample powder obtained by grinding a part of the obtained porous material was evaluated by XRD and FT-IR measurements. The porous materials were cut into two sections, one at the top and the other at the bottom, and the pore structure was observed by SEM. The porosity and bulk density were measured by the Archimedes method (JIS R 1634), and the compressive strength (JIS R 1680, Autograph AGE-X 10kN, Shimadzu Corporation, Kyoto, Japan) was evaluated. In addition, the pore size distribution and the pore volume were measured by the mercury intrusion method (Mercury porosimeter, AutoporeIV 9520 Shimadzu Corporation, Kyoto, Japan).

RESULTS AND DISCUSSIONS

Freeze-dried samples of CNF-P and CNF-S were analyzed by FT-IR spectroscopy. Figure 1 shows FT-IR spectra of the CNFs produced by the two different methods. For both types of CNF, peaks associated with C=O (1681 cm⁻¹) and C-H (2896 cm⁻¹) bonds are observed. The CNF-P spectrum shows additional peaks due to P-O-C (1090 cm⁻¹), P=O (1238 cm⁻¹), P-H (2391 cm⁻¹), and C=O (1714 cm⁻¹), which are attributed to phosphite esters.

![Figure 1](image-url)
were dispersed in the water phase, without being present in the oil phase. These results suggest that in the case of CNF-P, the OH groups of cellulose become hydrophobic by phosphite esterification, and some OH groups remain.

Figure 3 shows XRD patterns for samples obtained by foaming without CNFs (a), with 10 g of CNF-S (b), and with 10 g of CNF-P (c) heated at 1120°C. All of the diffraction peaks correspond to the β-TCP structure, with no evidence of other impurity phases.

Figure 4 shows FT-IR spectra of the porous samples shown in Figure 3. All of the spectra are seen to be similar. PO₄ bending vibrations attributed to β-TCP are observed around 420 cm⁻¹ (ν₂) and 560-600 cm⁻¹ (ν₂), in addition to stretching vibrations at 960-1120 cm⁻¹ (ν₁, ν₃). However, no absorption bands due to impurities such as calcium pyrophosphate or hydroxyapatite are observed, indicating that the obtained samples are single-phase β-TCP.

Figure 5 shows SEM images of the pore structure in the same samples, which were cut into upper and lower parts. In the case of the sample prepared without CNFs, no macro-sized pores are observed in the upper or lower part of the sample. This may be due to the fact that the foam obtained by the foaming method disappeared during the drying process. In the case of the porous sample prepared by adding 10 g of CNF-S, some large pores with sizes of about 400 μm are observed in the upper part, but not in the lower part. This is because the bubbles in the lower part coalesced and became large during the drying process, and then migrated to the upper part. On the other hand, for the sample prepared by adding 10 g of CNF-P, many pores larger than 200 μm are observed in both the upper and lower parts, indicating that the addition of CNF-P stabilized the formation of bubbles during the drying process. As we previously reported⁹, we believe that this is due to the presence of CNFs at the bubble interface with the foam, which increases foam stability. The results show that hydrophobic CNF (CNF-P) is better than hydrophilic CNF (CNF-S) as a stabilizer for bubbles forming in the porous body.

Table 2 shows the results of the evaluation of the physical properties of samples (a), (b) and (c). The porous material produced using CNF-P with stable pores had a high degree of porosity and a uniform pore distribution, leading to a high compressive strength.
TABLE 2 Physical properties of porous $\beta$-Ca$_3$(PO$_4$)$_2$ obtained in this work (samples a, b, and c)*.

| Sample | Total porosity / % | Open porosity / % | Bulk density / g $\cdot$ cm$^{-3}$ | Compressive strength / MPa |
|--------|--------------------|--------------------|-------------------------------------|-----------------------------|
| a      | 58.3 $\pm$ 0.49    | 57.4 $\pm$ 0.47    | 1.28 $\pm$ 0.02                    | 6.48 $\pm$ 0.70             |
| b      | 47.7 $\pm$ 2.12    | 43.8 $\pm$ 23.0    | 1.61 $\pm$ 0.05                    | 4.93 $\pm$ 4.20             |
| c      | 62.8 $\pm$ 6.80    | 54.3 $\pm$ 5.10    | 1.14 $\pm$ 0.21                    | 4.10 $\pm$ 1.48             |

*: See TABLE 1 for sample symbols

Figure 6 shows SEM images of porous bodies prepared using bubble agents with different HLB values. The results indicate that sample (d) prepared with hydrophobic POEIE-2 with a HLB of 9.5 has a nonuniform pore structure, with a mixture of many macro-sized and micro-sized pores. On the other hand, the pore size distribution for sample (e) prepared with POEIE-9, which has high hydrophilicity, is more uniform than that for the other samples. This is considered to be because the nonionic surfactant had a strong effect on reducing the surface tension of the water, and thus bubbles of a certain size were uniformly formed. It is therefore better to use a highly hydrophilic bubble agent to form the porous body.

Table 3 shows the results of the evaluation of the physical properties of samples (d) and (e). Changing the HLB for the nonionic surfactant used as a foaming agent had little effect on the total porosity, open porosity or bulk density, but the compressive strength of the sample with higher pore size distribution, size and shape uniformity was higher.

Figure 7 shows SEM images of porous bodies prepared at different drying temperatures. Compared with the material dried at 40°C (c), the materials dried at 55°C (f) and 70°C (g) had a larger pore size, which increased with increasing drying temperature. This was attributed to the progressive coalescence of bubbles during the drying process.

Table 4 shows the evaluation results for the physical properties of porous bodies obtained for different drying and sintering temperatures. Again, the total porosity and the open porosity increased with increasing drying temperature, while the bulk density and compressive strength decreased. The total porosity and the open porosity for the samples sintered at 1000°C (h) and 1060°C (i) both increased, while the bulk density and compressive strength decreased, compared to the sample sintered at 1120°C (c). However, the optimum balance between porosity and compressive strength was obtained for the sample sintered at 1060°C. For the sample sintered at 1120°C, SEM observations indicated that the number of micro-sized pores (less than 1 μm) decreased, which led to an increase in the number of closed pores, while for the sample sintered at 1060°C, the number of such pores remained constant, which increased the number of open pores.

**SUMMARY**

In this study, it was found that in the foaming process with ambient air by mechanical stirring, the hydrophobic/hydrophilic interaction between the
nonionic surfactant and CNF-P enabled the preparation of porous materials with uniform distribution of pores throughout the sample and spherical pore shape. It was also found that the formed porous body did not show foam stability with the addition of nonionic surfactant only, but the addition of CNF-P stabilized the foam and promoted the formation of both micro-sized (less than 1 μm) and macro-sized (100-400 μm) pore sizes. In particular, the micro-sized pores were considered to be formed by the combustion of CNF-P at sintering temperature. It was also found that the porosity and compressive strength could be controlled by changing the HLB value of nonionic surfactant, drying temperature and sintering temperature.

These results indicate that the addition of CNF-P is effective for the preparation of porous β-TCP ceramics for biological hard tissues by a simple foaming method.

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