INTRODUCTION

The proportion of elderly citizens in the total population is increasing in many countries. An important aspect of ensuring the quality of life of the elderly is research and development of bone replacement materials. Such materials can be used for the treatment of bone fractures or osteoporosis.

Calcium phosphate ceramics are well known as bone replacement materials.1) Our own group has focused on the study of β-type tricalcium phosphate (β-TCP) with continuous pores by applying a β-TCP suspension onto a porous polyurethane foam by spray coating followed by pyrolysis of the foam. In the work reported herein, the three-dimensional (3D) pore structure of a polyurethane foam specimen was scanned using X-ray computed tomography after which a porous acrylic foam with an optimized pore structure was fabricated using a 3D printer based on this scan. The pore size and pillar diameter of the porous printed material could be modified simply by changing the brightness value of the image data in the processing software. Specifically, the pillar diameter was increased while the pore size became smaller as the brightness was increased. β-TCP with interconnecting pores could be easily fabricated by spray coating these printed molds. The physical properties and porous structure of the product could also be tuned by modifying the pore structure of the foam and the amount of spray coating. The porous β-TCP obtained in this study had sufficient physical properties to be used as a scaffold material, suggesting the possibility of applying this scanning process to other porous materials, including living bones.

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properties of these materials were investigated. For comparison purposes, a porous β-TCP scaffold was also fabricated using polyurethane foam.

**MATERIALS AND METHODS**

2.1 Preparation of raw spray powder

The raw spray powder was prepared using a complex polymerization method. In this process, 19.68 g of calcium nitrate tetrahydrate (Ca(NO$_3$)$_2$·4H$_2$O, 99.0% purity, Sigma-Aldrich, Japan) was dissolved in 20 ml of de-ionized water. A 30.32 g quantity of phosphonobutane tricarboxylic acid (C$_7$H$_7$O$_7$P, 50% purity, KI Chemical Industry Co., Ltd., Japan) was added to this solution to obtain a Ca/P molar ratio of 1.50. After mixing, the sample was heated and stirred at 150 °C for 4 h and then dried at 180 °C for 24 h. The dried specimen was heated to 1000 °C at 5 °C/min and calcined at this temperature for 6 h under ambient air. Each calcined powder specimen was evaluated using powder X-ray diffraction (XRD, Miniflex 600, Rigaku Corporation, Japan) and Fourier transform infrared absorption spectroscopy (FT-IR, FT-IR-4200, JASCO, Japan).

2.2 Preparation of polymer templates

A rectangular section of polyurethane foam (MF-13, Inoac Co., Japan) having dimensions of 22×22×11 mm with a continuous pore structure and without a wall was used as the original material to determine the network structure of the template. This foam specimen was placed in an X-ray CT instrument (Rigaku Corporation, Japan) to collect 3D data regarding its morphology. In this process, two dimensional (2D) images (each 512x512 pixels with a 72 μm pitch) were obtained at 512 different depths spaced at intervals of 72 μm to construct a 3D dataset of 512x512x512 voxels. These 3D data (in the DICOM format) were imported into the medical image processing software package Materialise Mimics (Materialise). This software transforms X-ray CT data into a solid/air construction (that is, STL data) based on setting threshold values for gray-scaled CT images. Regions having brightness values (C) above the threshold are regarded as solid while all other areas are regarded as made of air. By setting the threshold to different values, we were able to create duplicate STL datasets having different solid/air ratios but identical network structures. Solid 3D specimens each having a size of 20×20×10 mm were printed from these data (TOKYO Lithmatic Corporation, Tokyo, Japan) using a ProJet3000 printer (3D Systems) together with a UV-curable acrylic resin. Two types of acrylic resin objects were fabricated using this method, employing C values of -940 or -980. These are hereinafter referred to as UV-940 and UV-980, respectively. The original polyurethane foam was also used as a template for comparison.

2.3 Preparation of porous bodies by spray coating

In each trial, a 15.0 g sample of the calcined powder, 150 g of alumina balls and 250 ml of ethanol were placed in a 350 ml plastic bottle, after which the bottle was rotated for 72 h. This ball milling process was used to obtain a uniform particle size, as determined by scanning electron microscopy (SEM). The material was found to comprise elongated granular particles with short axes of approximately 0.7 μm and long axes of approximately 2.5 μm. After milling, a rotary evaporator was used to remove the ethanol from the slurry. Following this step, 2.0 g of the milled β-TCP was mixed with 8.0 g of de-ionized water and 0.10 g of an ammonium polycrylate solution 70-110 (44w/w%, FUJIFILM Wako Pure Chemical Co., Ltd., Japan) and this mixture was stirred at room temperature for 10 min. A 12.5 g quantity of a water-borne acrylic resin (Mizutani Paint Co. Ltd., Japan) was subsequently mixed with the β-TCP dispersion and the new mixture was stirred at room temperature for 30 min to prepare a slurry. This slurry containing the β-TCP was spray-coated onto various template foams at a spray pressure of 0.08-0.13 MPa using an airbrush (nozzle diameter: 0.3 mm; model PS-289, GSI Creos, Japan) and a linear drive free piston compressor (air flow rate: 5 L/min; model PS-251, GSI Creos, Japan). This process was repeated until a coating concentration of 0.375 g/cm³ had been applied. In this paper, the sample preparation was controlled by the amount of coating (g) per apparent volume (cm³) as a standard of application. On the other hand, the actual foam volume in each sample equaled the mass of the foam divided by its true density, which was 0.52 cm³ for urethane, 0.82 cm³ for UV-940 and 1.13 cm³ for UV-980. After the desired amount of coating was applied to a foam specimen, the foam was placed on a sheet of black paper along with a scale, and an image was captured from above the sample using a digital camera (EOS Kiss X2 with a macro lens, Canon, Japan) to determine the shape of the foam after coating.

The dimensions and mass of the foam after coating were measured, after which the coated foam was sintered at 1120 °C for 48 h in air. The temperature profile used for sintering is presented in Figure 1. This heating profile was determined based on thermogravimetric-differential thermal analysis data acquired from dried samples of polyurethane foam, UV-curable acrylic resin and the β-TCP slurry solution. A hold time was added and the heating rate was slowed down at the temperature at which combustion of the organic materials was considered to occur.
2.4 Evaluation of porous materials

The sample powder obtained by grinding a part of the porous raw material was evaluated by XRD and FT-IR spectroscopy. The dimensions and mass of the porous material were ascertained before and after sintering, and the density and volume shrinkage were calculated from these data. The pore structure was evaluated by placing a sample along with a scale on a sheet of black paper and photographing it with a digital camera from directly above. The microstructure of the material was observed by SEM (VE-7800, Keyence Corporation, Japan) and a compressive strength test (JIS-R-1068) was performed at a crosshead rate of 1.0 mm/min using an AG-I instrument at a force of 10 kN (Shimadzu Co., Japan). All data are represented herein as the mean ± one standard deviation. A one-way analysis of variance was performed on the volume shrinkage data with the significance level set at $p < 0.05$. The number of test samples was $n = 20$. However, only the number of samples for volume shrinkage and compressive strength was $n=5$.

RESULTS AND DISCUSSION

3.1 Identification and evaluation of powder samples and porous samples

Figure 2 presents XRD patterns for the powder sample calcined at 1000 °C and the porous sample made of urethane foam obtained by coating the powder sample with a slurry solution and then sintering at 1120 °C. It is evident that the powder sample and the porous material after calcination produced diffraction peaks consistent with the crystal structure of $\beta$-TCP (ICDD 055-0898) and that no peaks related to hydroxyapatite or other by-products were obtained.

Figure 3 shows FT-IR spectra of these same samples; it can be seen that each material generated peaks attributed to PO$_4$ groups in the ranges of 420-610 cm$^{-1}$ and 940-1120 cm$^{-1}$ but no peaks related to the by-products calcium pyrophosphate and hydroxyapatite. These results confirmed that all the prepared samples comprised single phase $\beta$-TCP.

3.2 Effect of foam structure

Photographic and SEM images of the foams used in this work are shown in Figure 4. As noted, the three types of foams were the original urethane foam and two UV-curable acrylic resin foams (UV-940 and UV-980). The brightness values used during processing by the 3D software were adjusted to $C = -940$ and -980. This figure demonstrates that the urethane foam had an average pillar diameter of 409 μm and an average pore size of 2070 μm with continuous pores. In the case of the acrylic foams, as the brightness value was decreased, the diameters of the pillars in the printed foam became larger and the pore sizes became smaller.

Figure 5 presents photographic and SEM images of the various foams after coating and the porous materials obtained by sintering these foam samples. Table 1 summarizes the pore size and pillar diameter for the foams before coating (as determined from SEM observations) along with the pore size, pillar diameter, density, porosity and volume shrinkage data for the porous samples, calculated by the same method. The pore size and pillar diameter varied depending on the pore size and pillar diameter in the original foam. When using the urethane foam, the
average pore size and average pillar diameter in the porous material were 1630 and 260 μm, respectively. In the case of the acrylic foam, as the brightness value used in the image analysis was decreased, the average pillar diameter of the porous material increased from 320 to 360 μm while the average pore diameter decreased from 1130 to 1060 μm. As the pore size of the foam decreased, it became more difficult to coat the inside of the foam without closing the surface pores during the spray coating. The volume shrinkage after sintering was found to be approximately 73% and there was no significant difference (p < 0.05) among the types of foams. In contrast, the apparent density and porosity varied along with the pore structure of the foam, with the apparent density increasing and porosity decreasing as the diameter of the columns increased. It should be noted that compression tests were not performed because the strength of the samples was insufficient to allow for the required handling. Figure 6 shows a cross-sectional SEM image of porous β-TCP made from the urethane foam. As can be seen, the inside of the pillars was relatively densified. However, the area where the mold was originally located was less dense and more porous than the surface of the pillar. These results suggest that the mechanical strength of the sample would be expected to be poor.

Figure 4 Photographic (i) and SEM (ii) images of (a) urethane foam, (b) UV-940, and (c) UV-980.

Figure 5 Photographic images and SEM images of (a) urethane foam, (b) UV-940 and (c) UV-980. Legend: (i) coated foam, (ii) porous β-TCP and (iii) porous β-TCP.
Table 1 Physical properties of foams with different pore structures and porous materials fabricated using the foams.

| Mold            | Treatment*1 | Pore size/µm | Avg. pillar diameter/µm | Apparent density /g·cm⁻³ | Porosity*3 /% | Volume shrinkage/% |
|-----------------|-------------|--------------|--------------------------|--------------------------|---------------|--------------------|
| Urethane foam   | Before coating | 2071±441    | 409±8                    | 0.31⁻²                   | —             | —                  |
|                 | After sintering | 1628±383    | 260±13                   | 0.35±0.01                | 88.6±0.5      | 70.2±1.7           |
| Acrylic foam UV-940 | Before coating | 1650±369    | 461±16                   | 1.18⁻²                   | —             | —                  |
|                 | After sintering | 1128±274    | 320±19                   | 0.41±0.02                | 86.6±0.5      | 69.4±3.1           |
| Acrylic foam UV-980 | Before coating | 1503±366    | 502±13                   | 1.18⁻²                   | —             | —                  |
|                 | After sintering | 1055±255    | 356±17                   | 0.43±0.01                | 86.0±0.5      | 69.1±3.2           |

*1: Spray weight (g) per apparent foam volume (cm³); 0.375g/cm³.  *2: True density of foam.  *3: Porosity=((β-TCP calculation density – β-TCP Apparent density)/β-TCP calculation density) × 100

Table 2 Physical properties of porous materials fabricated with different spray amounts.

| Spray amount*1, *2 / g·cm⁻³ | Pore size/µm | Avg. pillar diameter/µm | Apparent density /g·cm⁻³ | Porosity /% | Volume shrinkage/% | Compressive strength/MPa |
|------------------------------|--------------|-------------------------|--------------------------|-------------|---------------------|--------------------------|
| 0.50                         | 1072±314     | 543±50                  | 0.49±0.01                | 84.0±0.4    | 69.6±2.7            | 0.65                     |
| 0.75                         | 771±243      | 679±42                  | 0.52±0.01                | 83.0±0.5    | 63.4±1.9            | 0.86                     |
| 1.00                         | 566±190      | 789±45                  | 0.56±0.02                | 81.8±0.5    | 60.0±1.8            | 1.17                     |

*1: Spray weight (g) per apparent mold volume (cm³).  *2: The mold used was UV-940 foam.

Figure 6 Cross-sectional SEM image of porous β-TCP.
3.3 Effect of the coating amount

Figure 6 provides photographic and SEM images of the coated foams and the porous materials after sintering. These observations showed that the pore size of the porous material became smaller as the amount of coating sprayed onto the UV-940 foam was increased. The density, porosity, volume shrinkage and compressive strength data for the porous materials are summarized in Table 2. These data confirm that both the porosity and volume shrinkage were reduced with increasing amount of coating. Conversely, the density and compressive strength were increased. It appears that the mechanical strength of the porous materials increased along with the density when increasing the extent of spray coating. In particular, the compressive strength of the β-TCP porous material made with a 1.00 g/cm³ coating was approximately 1.2 MPa.

In a study of the pore size of this porous material, the minimum requirement for pore size was considered to be about 100 μm due to the size of the cells, the need for migration, and the need for transport. However, a pore size of 300 μm or more is recommended due to the accelerated formation of new bone and the formation of new blood capillaries. Pandey et al. investigated in vitro cell cultures of four porous calcium phosphate samples with average pore diameters of 106 μm, 235 μm, 460 μm and 1123 μm. The results showed that osteoblast survival and adhesion were enhanced in osteoblast (MC3T3-E1) culture in the 235 μm and 460 μm samples, while adhesion was hardly enhanced in the 106 μm and 1123 μm average pore size samples. On the other hand, Schek et al. evaluated 300 and 800 μm polypropylene fumaric acid/β-TCP composite scaffolds in vivo and found that both scaffolds showed excellent bone regeneration. Walsh et al. also compared 100-1000 μm porous β-TCP scaffolds with 100-400 μm porous β-TCP scaffolds in vivo and concluded that all samples showed similar osteoconductivity. These results suggest that 200-500 μm scaffolds are effective against osteocytes in in vitro cell culture experiments, but in vivo experiments show that larger diameters (>600 μm) are effective, but there are no specific results due to differences in the animals used in the experiments and the size of the bone defect model. However, due to the differences in the animals used in the experiments and the size of the bone defect model, we have not yet been able to provide specific values. However, even for the maximum requirement of pore size in vivo, pore size is expected to affect the progression of osteogenesis because of vascularization. Smaller pores prefer hypoxic conditions and thus tend to induce osteochondral formation before osteogenesis, while larger pores with sufficient vascularization directly induce osteogenesis before chondrogenesis. It is assumed that a relatively large pore diameter is necessary for this reason.

Figure 7 Photographic and SEM images of specimens with coatings of (a) 0.50, (b) 0.75 and (c) 1.00 g/cm³.
Legend: (i) coated foam, (ii) porous β-TCP and (iii) porous β-TCP.
We have yet to find a paper that mentions the ideal pore size. The ideal pore size cannot be determined because it depends on various conditions such as the chemical composition of the porous material, the shape of the pores, the pore size distribution, the pore diameter, the pillar diameter, and the mechanical properties. Therefore, we believe that it is important to be able to freely change the composition of the material, the shape of the pores, the distribution of the pore size, the pore diameter, the pillar diameter, and other conditions. In this paper, by changing the pore size and strut diameter of the mold, we were able to achieve a pore size of about 500-1600 μm for sintered porous β-TCP. We hope to evaluate these samples in vitro and in vivo in the future.

Inputting the image data for the original foam structure into the 3D software allowed the pore size and pillar diameter of the foam fabricated by the 3D printer to be tuned. These results suggest the possibility of applying this technique to other porous materials and even to live bone.

CONCLUSION

β-TCP with a continuous pore structure was successfully fabricated by applying β-TCP to porous polyurethane foam molds using a spray coating method and then pyrolyzing the molds by heating. The resulting materials retained the pore structure of the original molds and their compressive strength increased along with the spray coating amount. The pore sizes and pillar diameters of the 3D printed molds could be controlled by adjusting the brightness values using 3D medical image processing software together with CT image data for the urethane molds. Using this method, we were able to easily fabricate β-TCP specimens having different pore structures. In addition, the physical properties of these porous materials could be modified by changing the pore structure of the mold or the amount of spray coating. The present porous β-TCP had sufficient physical properties to be used as a scaffold material.

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