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Porous calcite for bone substitute

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Abstract: Porous calcite is expected to be useful as an artificial bone substitute that could be fabricated by using calcium hydroxide ($Ca(OH)_2$) as precursor and wax spheres as porogen. Different weight percentages of wax spheres were used to produce calcite structure with various degree of porosity. Sintering at 850°C under mixed atmosphere CO_2/O_2 was carried out to eliminate embedded-wax spheres and transform to calcite. X-ray diffraction (XRD) patterns assigned to calcite single phase could be detected from the obtained porous blocks. Porosities of the porous calcite were in the range of 60-70% approximately. Diameter of pores was about 200- to 400-µm is accepted for cell activities and bone ingrowth. Porous calcite blocks also showed the suitable mechanical strength for handling properties if it would be applied in the repair of clinical bone defect.

Keywords: *Calcite, Interconnecting Porous Structure, Wax Spheres*

Introduction:

Calcium carbonate ($CaCO_3$) has been have achieved considerable success as artificial bone substitute due to its good biocompatibility and osteoconductivity. Calcium carbonate has three polymorphs, which are vaterite, aragonite and calcite. Calcite has ability to be bonded directly to host bone. Additionally, it started to be resorbed after 8 weeks of implantation [1,2]. Structural properties affect significantly biological performance of the artificial bone graft. The rate of bone remodeling is known to be depending on bone structure. Bone remodeling in cortical bone is considerably slower than in cancellous bone [3,4]. Fast bone remodeling of cancellous bone is a result of its interconnecting porous structure. Interconnecting porous structure is necessary for bone tissue formation to facilitate bone cell activities. It can maximize bio-fluid exchange, vascularization and bone ingrowth. Consequently, mechanical interlocking at the interface between implant and natural bone can be achieved as bone tissue developed well through the interconecting pores [5-7].

Lee *et al* (2006) reported the method of preparation porous calcite block from calcium hydroxide/sodium chloride composite. Porous structure was produced using water-soluble porogen. However, salt leaching method has two key disadvantages. The first one is long time for eliminating salt crystals and the second one is difficult controlling of pore size and morphology [8].

In this study, porous calcite block was fabricated by setting method using $Ca(OH)_2$/wax spheres paste as starting materials. $Ca(OH)_2$/wax spheres paste with suitable viscosity can be flexible to form any shape, hence, this setting method is advantageous on producing more complex structure than normal uniaxial pressing method. Interconnecting porous structure of calcite block is created due to removing of wax spheres during heating. Duration for burning of pore-creating volatile particles and carbonation is shorter than that of the previous method. Moreover, as wax sphere is in round shape, it is convenient to decide pore size and morphology by selecting diameter of individual wax sphere. However, there might be a possibility for remaining wax residues due to insufficient burning. Therefore, a controlled atmosphere of CO_2/O_2 gaseous mixture was applied during heat treatment to increase the efficiency of wax burning. The experimental results showed a successful improvement in complete elimination of embedded-wax spheres and a stable mechanical strength of porous calcite blocks for handling properties.

Materials and Methods:

Calcium hydroxide ($Ca(OH)_2$; Wako Chemicals, Osaka, Japan) and wax spheres (200 to 400 µm diameter, YETI Dentalprodukte GmbH, Engen, Germany) were mixed to prepare powder mixture (30% - 60% wt of wax spheres). Mixture was added with PVA solution (5%wt) as a binder to make paste. Paste was packed into split stainless steel mold (6mm x 3mm). Mold was then placed inside oven to dry at 40°C for 24 h. After setting, mold was unpacked to obtain composite block. Composite block was sintered under stream of CO_2/O_2 atmosphere at 850°C in a ceramic electric tubular furnace (ARF-30MC, Asahi Rika Seisakusho Co., Ltd., Chiba, Japan) for carbonation and removing of wax spheres.

Composition of the heat-treated composite block under stream of CO_2/O_2 was evaluated by means of powder X-ray diffraction (XRD) analysis. After heat treatment, specimens were ground to fine powder, the XRD patterns were recorded using a diffractometer system (D8 Advance, Bruker AXS GmbH, Karlsruhe, Germany) using Vario1 Johansson focusing monochromator and high flux K□-1 radiation generated at 40 kV and 40 mA. The

specimens were scanned from 10º to 60º 2θ (where θ is the Bragg angle) in a continuous mode. Morphology of the sintered specimen was observed by a field emission scanning electron microscope (SEM: S-3400N, Hitachi High-Technologies Co., Tokyo, Japan) at 10 kV of accelerating voltage after gold sputter coating. Diametral tensile strength (DTS) of calcite specimens was measured at a crosshead speed of 1mm/min by using a universal testing machine (AGS-J; Shimadzu Corporation, Kyoto, Japan).

Results and Discussion:

Figure 1 shows the XRD patterns of composite blocks with 30-60 weight percent of the wax spheres subjected to heating and carbonation under CO_2/O_2 atmosphere at 850°C. It is shown that pure calcite was obtained as all main peaks of calcite could be seen whereas $Ca(OH)_2$ peaks were no longer detectable.

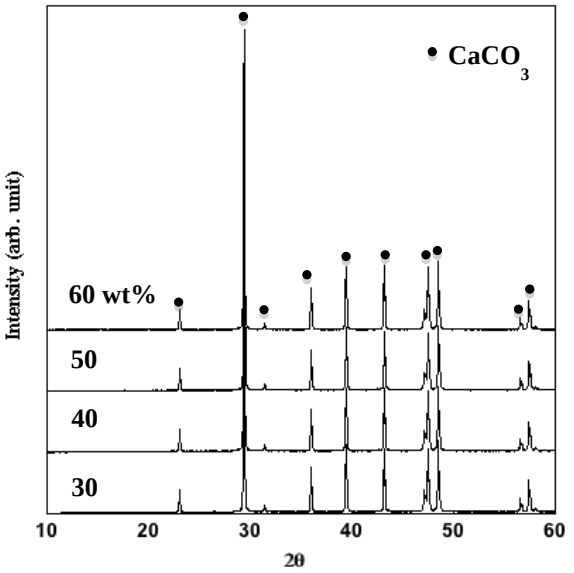

Fig 1. *XRD patterns of composite blocks with 30-60 weight percent of the wax spheres subjected to heating and carbonation under CO_2/O_2 atmosphere at 850°C.*

The structure of composite block after burning out wax and carbonation was observed by scanning electron microscopy (SEM). The SEM image of its fracture surface is shown in Fig. 2. Calcite block was highly porous with evenly distributed and interconnected pore structures. Besides, the wax residues were no longer presented. It proved that mixed CO_2/O_2 sintering atmosphere supported effectively for complete burning of wax spheres.

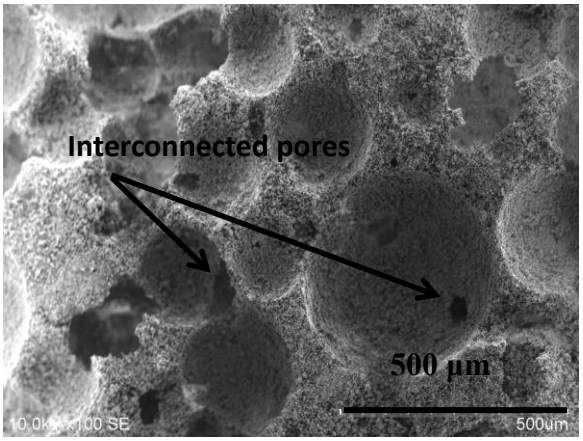

Fig 2. *Fracture surface of composite block (60 wt% wax spheres) after burning out wax and carbonation.*

Figure 3 and 4 show the porosity and diametral tensile strength (DTS) of specimens after heat treatment. The DTS values decreased with increasing wax content. In contrast, the increase of porogen loading resulted in more porosity. Porosity is thought to be one of the most important key factors in designing artificial bone graft. Highly porous structure plays an important role in manipulating cell functions however it leads to low mechanical strength generally restricts material usages to low load-bearing applications [9-12]. Balance of porosity and mechanical strength should be established in order to maximize the performance of bone substitute material when it is applied to reconstruct bone defect.

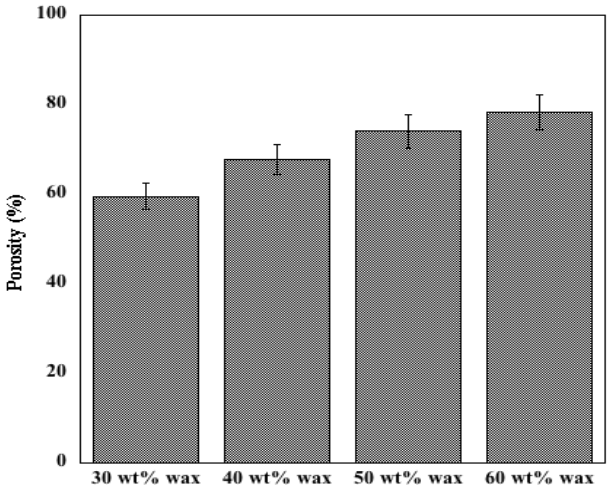

Fig 3. *Porosity of porous calcite blocks (n=12, p <0.05)*

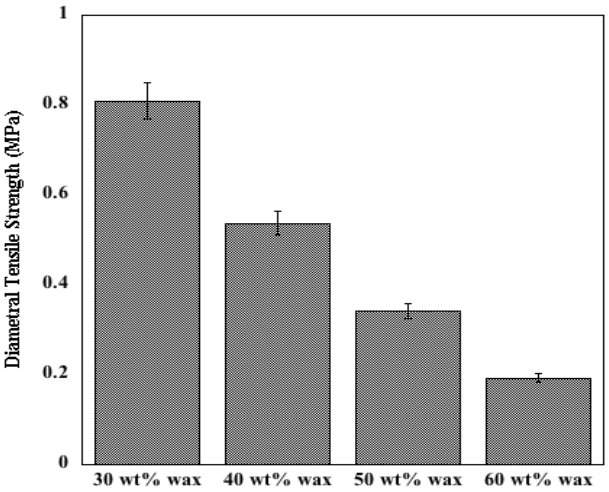

Fig 4. *Diametral tensile strength (DTS) of porous calcite blocks (n=12, p <0.05)*

Conclusion:

On the basis of the above-mentioned experimental results, it was concluded that porous calcite block with interconnecting porous structure was successfully prepared in this study. Heat treatment under stream of CO_2/O_2 was advantageous on burning out wax spheres and carbonation completely. It indicated that porous calcite block was useful in the bone reconstruction field since it could achieve both interconnecting porous structure and adequate mechanical strength. *In vitro* and *in vivo* experiments are necessary to evaluate the biological bone cell activities as well as interactions between host bone tissue and biomaterials.

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