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Zirconia–hydroxyapatite composite material with micro porous structure

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ABSTRACT

Objectives. Titanium plates and apatite blocks are commonly used for restoring large osseous defects in dental and orthopedic surgery. However, several cases of allergies against titanium have been recently reported. Also, sintered apatite block does not possess sufficient mechanical strength. In this study, we attempted to fabricate a composite material that has mechanical properties similar to biocortical bone and high bioaffinity by compounding hydroxyapatite (HAp) with the base material zirconia (ZrO_2), which possesses high mechanical properties and low toxicity toward living organisms.

Methods. After mixing the raw material powders at several different ZrO_2/HAp mixing ratios, the material was compressed in a metal mold (8 mm in diameter) at 5 MPa. Subsequently, it was sintered for 5 h at 1500 °C to obtain the ZrO_2/HAp composite. The mechanical property and biocompatibility of materials were investigated. Furthermore, osteoconductivity of materials was investigated by animal studies.

Results. A composite material with a minute porous structure was successfully created using ZrO_2/HAp powders, having different particle sizes, as the starting material. The material also showed high protein adsorption and a favorable cellular affinity. When the mixing ratio was ZrO_2/HAp = 70/30, the strength was equal to cortical bone. Furthermore, *in vivo* experiments confirmed its high osteoconductivity.

Significance. The composite material had strength similar to biocortical bones with high cell and tissue affinities by compounding ZrO_2 and HAp. The ZrO_2/HAp composite material having micro porous structure would be a promising bone restorative material.

1. Introduction

Bone tissue is an important locomotive tissue that supports the living body. Furthermore, it possesses diverse functions such as protection of important organs like brain, heart, and lungs [1,2]. Bone tissue is greatly involved in the retention of teeth, mastication, and face structure particularly in the maxillofacial region, and is an important tissue in terms of QOL. There are various causes of osseous defects and reduction in bone volume, such as absorption of the alveolar bone due to a loss of teeth or periodontal diseases as well as tumors and injuries [3,4]. In general, the bone defect size is an important factor when deciding the treatment direction. For

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example, for rather small osseous defects, the guided tissue regeneration (GTR) method has been introduced in addition to autogeneous bone grafting and biomaterial grafting [5]. Tissue regeneration methods using soluble factors such as basic fibroblast growth factor (bFGF) and bone morphogenetic protein (BMP) are expected to be effective methods in recent years [6]. For larger osseous defects caused by bone fracturing or bone segmentectomy for tumors, reconstruction with artificial materials is commonly performed [7,8]. This is because a sufficient strength is required to compliment a large region with an osseous defect and there is a limit in the amount of usable autograft bones required for the restoration.

Although titanium is widely used for this purpose for its high bioaffinity and favorable mechanical properties [9,10], higher biosafety is still demanded due to recent reports of allergies caused by the elution of Ti ions [11,12]. While hydroxyapatite (HAp) that is the source material of apatite blocks, the principal component of teeth and bones, and is known for its high bioaffinity [13], apatite blocks, which are sintered compacts, are fragile, thus higher strength is demanded [14,15]. Due to these limitations, development of bone restorative materials with both higher biocompatibility and sufficient mechanical strength is strongly desired.

An inorganic material zirconia (ZrO_2) has high mechanical properties and low toxicity [16,17]. Therefore, it is used as a biomaterial for hip prosthesis [18,19], tooth crown [20], and dental implants [21], and is expected to be a new bone restorative material. However, when considering the use of ZrO_2 as a new bone restorative material, mechanical properties of sintered ZrO_2 are far greater than a cortical bone. Therefore, when bonding to a host bone, uneven stress concentrations and easy bone fracture may occur due to difference in strength. Moreover, ZrO_2 having poor affinity to cells and tissues is also a critical issue [22,23]. Researchers have fabricated ZrO_2/HAp composite materials to solve these problems; however, the reported materials still showed mechanical properties that are far greater than cortical bone. We hypothesized that sintered ZrO_2/HAp composite material with microporous structure would improve these properties. To obtain such material, we fabricated the composite material by using source material with different particle size distributions and by compressing with relatively low pressure. The material characteristics including the cellular and tissue affinity of the fabricated material were investigated in this study.

2. Materials and methods

2.1. Synthesis of HAp-L particle

HAp particles were synthesized in wet condition. A hundred milimolar/liter $Ca(CH_3COO)_2{\cdot}H_2O$ solution (500 ml) and 60 mmol/l $NH_4H_2PO_4$ solution (500 ml) were added to 1000 ml of mechanically stirred 1.3 mol/l acetate buffer solution. Temperature and pH were maintained at $80 \pm 1\,^{\circ}C$ and 7.4 ± 0.1, respectively. The obtained particles (HAp-L) were washed with distilled water and phosphate-buffered saline (PBS), and then dried for 72 h at 80 °C.

2.2. Fabrication of ZrO_2/HAp composite

Yttria-stabilized ZrO_2 powder (containing 3% Y_2O_3, particle size: 70–100 nm, Tosoh, Japan,) was used as the ZrO_2 raw material. Commercially available HAp powders (HAp-S, particle size: 30–100 nm, Taihei Chemical) and above-mentioned HAp-L (particle size: 500–1000 nm) were used as the HAp raw material. The morphology of each particle was observed using a transmission electron microscope (TEM, 200 kV, H-800, Hitachi, Japan, Fig. 1). The raw material HAp powders differed in particle size, respectively. After stirring and mixing the raw material powders at different ZrO_2/HAp mixing ratios (100/0, 80/20, 70/30, 60/40, 0/50, and 0/100), the material was compressed in a metal mold (8 mm in diameter) at 5 MPa. Finally, the compressed material was sintered for 5 h at 1500 °C to obtain the ZrO_2/HAp composite.

2.3. Evaluation of properties of the ZrO_2/HAp composite

Surface morphology of the obtained ZrO_2/HAp composite specimen was examined using a scanning electron microscope (SEM, acceleration voltage: 20 kV, JSM-6390 BU, JEOL, Japan). Also, changes in the crystal structure of the ZrO_2/HAp composite were investigated using an X-ray diffractometer (XRD, CuKα, 40 kV, 30 mA, Rint 2000, Rigaku, Japan). A compression test was performed using mechanical tester (AGS-500D, crosshead speed = 1 mm/s, Shimadzu, Japan). The size of the fabricated composite material was measured before and after sintering, and the shrinkage rate of the material was calculated.

2.4. Protein adsorption study

The ZrO_2/HAp composite was soaked in 1.5 ml of 100 μg/ml bovine serum albumin (BSA: Nacalai Tesque, Japan) for 1 h ($n = 4$). The amount of protein in the supernatant was measured by the BCA protein assay kit (Pierce, Rockford, IL), followed by the calculation of adsorbed protein on the specimens.

2.5. Cell adhesion study

Bone marrow stromal cells (BMSC) derived from BALB/c mouse were cultured in Minimum Essential Medium Alpha (MEMα: Wako Pure Chemical, Japan) containing 10% fetal bovine serum (FBS: Sigma–Aldrich, MO). The cells were seeded on the specimen in a manner such that BMSC would have a density of 5.0×10^3 cells/pellet. After 24 h culture, the specimen was fixed in 4% paraformaldehyde and post-fixation was performed using 1% OsO_4. After graded dehydration with ethanol and isoamyl acetate, the specimen was dried using a critical point dryer (HCP-2, Hitachi, Japan), and observed by SEM (acceleration voltage: 20 kV).

2.6. Animal experiment

ZrO_2/HAp composites each having a diameter of 4 mm and different compounding ratios (100/0, 70/30, and 0/100) were implanted in the artificially created osseous defects in