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Microstructure control of TCP/TCP-(t-ZrO_2)/t-ZrO_2 composites for artificial cortical bone

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ABSTRACT

In this study, bone like continuously porous TCP/TCP-(t-ZrO_2)/t-ZrO_2 composites with a central channel were fabricated using a multi-pass extrusion process and their mechanical properties and microstructure at different sintering temperatures were investigated. Hydroxyapatite (HAp) powder was used as the raw powder which undergoes a phase transformation into the α-tricalcium phosphate phase (α-TCP) at a sintering temperature of 1500 °C. The external diameter and inside cylindrical hollow core were approximately 10.3 mm and 4.8 mm, respectively. The frame region contained numerous microchannels that extended from one side of the fabricated body to the other. The channeled frame region had a multi-layer microstructure with a TCP/TCP-(t-ZrO_2)/t-ZrO_2 layer configuration. The inner layer consisted of TCP, which make the wall of the microchannel. The material properties were characterized and microstructural analysis was carried out. The maximum pore size, compressive strength, and relative density of the fabricated system were approximately 86 μm, 53 MPa, and 77% when sintered at 1500 °C. The composites exhibited excellent biocompatibility and cell proliferation behavior resulted in the MTT assay and cell adhesion test using osteoblast-like MG-63 cells.

1. Introduction

In orthopedic surgery, three approaches are used to treat and replace a bone defect: auto grafting, allo grafting and artificial grafting. Among these treatments, auto grafting is regarded as the gold standard even though this approach has limitations including the need to harvest normal bone from the patient. When the injured bone site is large, the bone graft and defect area must be compatible [1]. In contrast, allo grafting usually utilizes bone banks, which harvests the bone from cadavers. Thus, this approach does not have the same issues associated with harvesting the bone as well as shows the possibility of disease transmission from the donor to recipient [2]. Therefore, the development of artificial bone as a substitute of natural bone is needed to solve the problems of conventional methods. To apply for load bearing part, the artificial bone must be mechanically strong and biocompatible during the healing process for osteo-integration [3].

Calcium phosphate ceramics such as hydroxyapatite (HAp, $Ca_{10}(PO_4)_6(OH)_2$) and tricalcium phosphate (TCP, $Ca_3(PO_4)_2$) have similar mineral constituents as natural bone, and as such they have been in development for use in bone substitution and reconstruction for the last few decades [4–6]. However, natural bone has superior mechanical strength that is unmatched by any synthetic material of similar composition. Especially, the fracture toughness of the bone is higher by an order of magnitude than the HAp counterpart. This is because of the unique lamellar hybrid composite microstructure of bone, which consists of collagen protein and HAp nanocrystals [7]. One of the key points to consider in these approaches is the need to retain the superior biocompatibility of the calcium phosphate ceramics, which limits the number of prospective materials that can be used for this application. Bioceramic composites were developed to improve the mechanical properties of the bone substitute [8]. Optimum fabrication processes and conditions have been explored with the goal of increasing the mechanical properties such as bending strength, compressive strength, and fracture toughness of bone substitutes [9,10].

ZrO_2 is a bioinert material that has been used in hard tissue related applications. Especially, the most of its applications are in the area where high wear is encountered, such as the knee joint and hip joint [11,12]. The strength and fracture toughness of ZrO_2 are much higher than calcium phosphate ceramics, which can be exploited to significantly improve the material properties of fabricated bio-composites. However, the addition of this compound will also decrease the biocompatibility of the fabricated materials since ZrO_2 is not biodegradable and complete ossification of the implant material would not be possible.

There are several important characteristics of natural bone including the following [13]: (1) the Haversian lamella which consists of a columnar osteon, (2) the osteon distribution in the shape of concentric circle around a central axis randomly, and (3) connection of the osteons. Many studies have been conducted with the goal of realizing the artificial bone that was similar to natural bone using bioceramics. Moreover, they have tried to increase the mechanical properties, especially the compressive strength of bioceramics based

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on the calcium phosphates [14,15] and to resemble the characteristics of human bone in the fabricated artificial implant [16,17]. Further, it appears that other researchers have produced HAp scaffolds with compressive strengths as high as 145 MPa, albeit with pore sizes only it the range of 20 μm [18]. However, it was demonstrated that the porous microstructure was essential for fast osteo-integration and proper bone cell ingrowth into the implant. So the optimal design would be one that can incorporate the porosity which is necessary for biocompatibility while still maintaining strong mechanical properties.

The fibrous monolithic process has been used to improve mechanical properties such as bending strength and fracture toughness [19,20] because of its ability to incorporate multi toughening mechanisms as well as favorable microstructure control. Using this process both layered and channeled structure can be incorporated into a single microstructure, which is a key feature of the natural bone microstructure.

In this study, a novel artificial bone structure similar to natural bone was fabricated using multi-layers of TCP, TCP-(t-ZrO_2), and (t-ZrO_2) phase, and the relationship between the material properties and microstructure of the continuously porous multi-layer bodies was investigated in detail using X-ray diffraction (XRD) and backscattered electron scanning electron microscope (BSE-SEM) techniques. In addition, the biocompatibility of the artificial bone was evaluated using human osteoblast like MG-63 cells, which were cultured on the cross-sectional surface of the composite where all three layers came in contact with the cultured cells.

2. Experimental procedure

The starting powders were t-ZrO_2 (TZ-3Y, Tosoh, Japan) with a particle size of 70 nm and hydroxyapatite (HAp) nanopowders(about 100 nm) formed by the ultrasonic-assisted process. Carbon powder (<15 μm, Aldrich USA) and ethylene vinyl acetate (EVA) (ELVAX 210 and 250, Dupont, USA) were used as a pore-forming agent and binder, respectively. Stearic acid (Daejung Chemicals & Metals Co. Korea) was used as a lubricant in order to increase fine mixing. The Haversian canal like channel was wrapped up by three layers which consisted of TCP as the inner layer followed by a t-ZrO_2-TCP intermediate layer and a t-ZrO_2 outer layer. The first layer (48vol.%HAp/42vol.%EVA/10vol.%stearic acid) was fabricated by mixing in a shear mixer (Shina Platec, Korea), at 120 °C. The second layer material (48vol.% HAp-(t-ZrO_2)/42vol.%EVA/10vol.%stearic acid) and the third layer material (43 vol.%/t-ZrO_2 45 vol.%/EVA 12vol.%stearic acid) were made using the same process. HAp-(t-ZrO_2) mixture powders were 75 vol.%/25 vol.% in composition and were mixed by wet ball milling using ethanol. The carbon mixture (50 vol.%carbon/40 vol.%EVA/10 vol.%stearic acid) was mixed using a shear mixer at 120 °C, and extruded in a cylindrical die with a feed end diameter of 30 mm and extrusion out let end diameter of 22 mm so that after extrusion the carbon rod had a diameter of 22 mm. The three layer material was compacted to make a concentric tube with an external diameter of 30 mm where the thickness of the individual layer was 3 mm. This process was used to form the feed roll for Haversian canal. It was extruded in a 30 mm diameter extrusion die to obtain filaments with a diameter of 3.5 mm. The 61 first pass filaments obtained from the first pass extrusion were arranged to the same steel extrusion die with 30 diameter mm and with the same condition to obtain second pass filaments with a diameter of 3.5 mm.

For the final extrusion, the five layers were arranged in the same cylindrical die with the following layering sequence: the first layer contained the tube type shell (1 mm in thickness) (48vol.%HAp/42vol.%EVA/10vol.%stearic acid), the second and the third layers contained 34 second pass filaments (3.5 mm in diameter) and the fourth and the fifth layers contained 10 carbon filaments (3.5 mm in diameter). The schematic diagram of arrangement was depicted clearly in Fig. 1 and was extruded to obtain a final green extruded body that was 13 mm in diameter. The green preform of the artificial bone constructs was subsequently drilled orthogonal to their long axis using a 0.5 mm diameter drill bit in attempts to provide connectivity between the pores and the surface of the parts. In order to remove the EVA binder, the 1st burnout process was carried out at 700 °C in a flowing nitrogen atmosphere, because the flowing gas expelled the vaporized EVA and stearic acid and made the sample free of organic compounds. Next, the pore-forming carbon filaments were burnt out at 1000 °C in an air atmosphere during the 2nd burnout in a sintering furnace. Finally, the samples were sintered in an air atmosphere using a sintering furnace from 1350 °C and 1500 °C for 2 h. The biocompatibility of the porous TCP/TCP-(t-ZrO_2)/t-ZrO_2 composites was determined using the MTT assay in accordance with ISO 10993-5 standard. These experiments were carried out on 96well plates using osteoblast-like MG 63 cells, which were obtained from the Korea Cell Line Bank (KCLB no. 10001). MG-63 cells were seeded on the longitudinal section of porous TCP/TCP-(t-ZrO_2)/t-ZrO_2 composites at a density of 1×10^4cells/well and were then incubated at 37 °C for 24 h. To prepare the extraction solution, the samples were immersed in Dulbecco's modification of Eagel's Medium (DMED: Hyclone, Logan, USA) for 1 day. The extraction media were added to the 96 well plates at different extraction media concentrations (12.5%, 25%, 50% and 100%). The cells were incubated for 72 h and then 20 μl of the MTT (Sigma, USA) solution in PBS (5 mg/ml) was added to each well. After 4 h of incubation, the media were discarded carefully and 200 μl of dimethylsulfoxide (DMSO; Samchun, D0458, Korea) was added to each well to extract the formazan crystals under shaking. The absorbance was measured at 595 nm using an ELISA plate reader (EL 312e, Bio-Tek). To observe cell morphologies, the MG-63 osteoblast-like cells were seeded on cross sections of porous TCP/TCP-(t-ZrO_2)/t-ZrO_2 composites at a density of 1×10^4 cells/ml. The cells were fixed with 2% glutaraldehyde. After incubation, the specimens were dehydrated in an ethanol solution of varying concentrations (50, 60, 70, 80, 90, 95 and 100%, respectively) followed by immersion in hexamethyldislazane (Sigma, USA) for dry. After removal of hexamethyldislazane, the specimens were coated by platinum sputtering for SEM observation.

The microstructure of the composites was observed by scanning electron microscopy (SEM, JSM 6401F, JEOL, Tokyo, Japan). X-ray diffractometer (XRD, D/MAX-250, Rigaku, Tokyo, Japan) was used to confirm the crystal structure and phase. The average compressive strength was measured by a universal testing machine (UnitechTM, R&B, Korea), using 5 samples. The samples were cut 3 mm in length and the cut surface was polished on diamond plate, the cylindrical surface was not treated as it was smooth as received.

3. Results

Fig. 2(a) shows an image of the final arrangement of the second pass filament, carbon filaments for the central hollow space and the outer HAp boundary layer prior to extrusion. The second and third layers of the arrangement contained 34 previously extruded second pass filaments, which were fabricated to incorporate Haversian canals in the microstructure. Next to the Haversian canals was the central space, which contained 10 carbon filaments with a diameter of 3.5 mm. The polymer and stearic acids were removed after the binder burn out stage and only the ceramic body remained as shown in Fig. 2(b). The central hollow space was used for housing the bone marrow. During the 2nd burnout process, all of the carbon materials in the 2nd pass filaments and in the central space were removed resulting in the formation of channels. The central channel was approximately 4.8 mm in diameter and the finer channels distributed inside the frame were around 80 μm as shown in Fig. 2(b). Fig. 2(c) shows an enlarged SEM image of the fabricated composites. In the enlarged cross-sectional image, each filament was clearly shown to be attached to each other and the interface was continuous without any signs of delamination or cracks and the pore channels were homogeneously distributed.