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A study of the fabrication of cylindrical implants using hydroxyapatites

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Bioceramic hydroxyapatite (HAp) has excellent biocompatibility with bone tissue, and it exhibits good mechanical properties. We studied its mechanical properties and the effect on bone integration in a rabbit tibial defect model. HAp powder was prepared by spray drying and compression forming. The HAp powder sintered at 1350 °C under a load of 1 tonne(9.8 KN) exhibited outstanding mechanical properties. In this test, the rabbit tibial defect was regenerated by using an HAp cylindrical implant.

Key words: Hydroxyapatite, Spray dryer, Cylinder, Sintering, Implant.

Introduction

Calcium phosphate (CaP) ceramics, such as hydroxyapatite, β-tricalcium phosphate, and biphasic calcium phosphate, have been widely used as grafts for bone repair, augmentation, or substitution. The wide usage of CaPs can be attributed to the similarity between their composition and that of bone mineral, bioactivity (the formation of bone apatite-like material on their surfaces and a strong bone-CaP biomaterial interface), and osteoconductivity (an ability to provide the appropriate scaffold for bone formation) [1-7].

Synthetic hydroxyapatite (HAp) has been recognized as a suitable material for the fabrication of inorganic scaffolds due to its close relationship with the mineral component ofbone and because of its excellent osteophilic properties [8-12]. It has also attracted considerable attention in the field of material science and engineering because of its unique biomimetic properties and osteoconductivity that is similar to the osteoconductivity of natural biomaterials [13-15].

In this study, a slurry of HAp nanoparticles was prepared using an attrition mill to change the status of HAp powders. HAp granules were prepared by a spray-drying method. The HAp ceramic samples were prepared at different temperatures and molding pressures. The mechanical properties of these samples were evaluated to determine the optimum conditions for their preparation. The bioactivities of the HAp ceramic samples with varying compositions were tested using a Simulated Body Fluid solution (hereafter referred to as SBF). In the animal testing, a histological analysis was conducted by implanting sintered HAp cylinders into rabbit bones.

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Experimental

HAp powder (≥ 98% purity), which was filtered through a 200-mesh (≥ 75 μm) sieve, was considerately supplied by the Bone Tech Inc. (Korea) and used without further purification. After this filtering process, HAp powder with particle sizes of less than 45 μm were obtained. The properties of the HAp powder were characterized by powder X-ray diffraction (XRD, Miniflex II, Rigaku, Co. Ltd., Tokyo, Japan), inductively coupled plasma-optical emission spectroscopy (ICP-OES, Optima 3300 DV, Perkin-Elmer, Norwalk, CT), and energy-dispersive X-ray spectroscopy (EDS, JEM-2011, Jeol Ltd., Tokyo, Japan). Fig. 1 shows a schematic flowchart of the experimental procedure for preparing a sintered HAp body.

Preparation of the HAp granular powder

The HAp slurry was prepared using HAp powder, distilled water, and zirconia balls in an attrition mill. A dispersing agent for 4 h disperse and then a leaving agent, antifoaming agent, plasticizer, binder, and for 10 minute intervals into the order of 12 h gives nano grinding and homogeneous mixing. The HAp slurry was separated from the zirconia ball using a 200-mesh sieve.

Manufacture of HAp cylinder samples

The HAp granular powder was prepared by a spray-drying method. The HAp cylindrical samples were prepared by pressing the powder sample using compression weights of 0.5, 1, and 1.5 tonne(9.8 KN). The HAp cylindrical samples prepared from this powder had a diameter of 3.5 mm and a thickness of 4 mm. In order to test the bending strength of the HAp samples, cylinders with a length, width, and thickness of 21 mm, 4 mm, and 1.2 mm, respectively, are fabricated using the granular powder. The samples

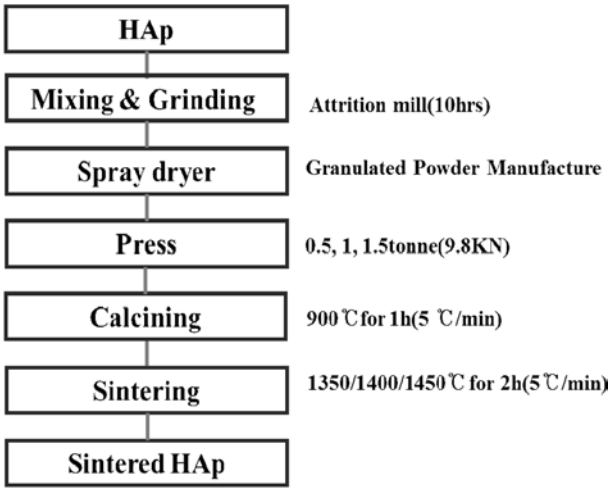

Fig. 1. Flow chart of the experimental procedure.

were sintered at temperatures of 1350 °C, 1400 °C, and 1450 °C for 2 h at the same heating rate of 55 °C/min. The structures of the sintered HAp samples were determined by powder X-ray diffraction (XRD, Miniflex II, Rigaku, Co. Ltd., Tokyo, Japan). The mechanical properties of the samples were determinedusing a universal testing machine (SMTEST, SMB-001-5T, Korea), and the hardness was measured using a micro Vickers Hardness tester (FM-700, Future Tech, Japan).

Bioactivity test

The samples were in immersed an SBF solution to test the bioactive property over 4 weeks. The temperature of the samples in the SBF solution was similar to that of human beings (37 °C). After 4 weeks, we observed the HAp sample surface using a field-emission scanning electron microscope (FE-SEM, HITACHI-S4700, HITACHI, Japan).

In vivo test

In this study, we used New Zealand white rabbits that were approximately 15 weeks old at the beginning of the experiment and weighed 2-2.3 kg each. The protocol for the in vivo test was approved by the Institutional Animal Care and Use Committee of Dong-A University, Busan, Korea. All experimental procedures conformed to the life science and humanitarian ethics code. The rabbits were given ad libitum access to both sterile water and soft food.

The surgical procedures were performed after an intramuscular administration of 1.5 ml ketamine and 0.5 ml Rompun (standard, 2.5-3 kg). Prior to the surgery, the skin was shaved and then cleaned using a mixture of iodine and 70% ethanol. The upper one-third of the tibia was perforated by making a 2-cm midline incision through the skin, fascia, and periosteum. One hole each was drilled on the left and right side of the incision, bilaterally across the near cortex; each hole had a diameter of 3.5 mm, and the rabbits with four perforations were divided into four treatment groups. The skin was sutured layer by layer after the HAp cylinders were implanted into their respective perforations. The rabbits were subcutaneously administered 0.05 mg/kg of buprenorphine every 12 h for the first 48 h after the surgery. Within 2-3 days, the rabbits resumed normal ambulation and did not show signs of pain or distress. Radiologic analysis was performed every 2 weeks, and all the rabbits were sacrificed 8 weeks after implantation for a histological analysis. For each treatment group, the volume of bone ingrowth and change in the bone mineral density were statistically calculated five times 0, 2, 4, 6, and 8 weeks after the implantation by computed tomography or CT (FLEXTM for platform X-OTM, GMI, Northridge, CA, USA). Histological analysis was performed 8 weeks after implantation. The blocks were sectioned along a plane parallel to the major axis of the HAp cylinder using a micro-grinding machine (MG4000, EXAKT Apparatebau GmbH, Norderstedt, Germany). The sections were stained with Hematoxylin-Eosin. Routine histology and histomorphometric analyses were performed by transmission light microscopy (Axioskop Carl Zeiss GmbH, Jena, Germany) and image-analysis software (KS 300; Kontron Electronic GmbH, Munchen, Germany), respectively.

Results and Discussion

Characterization of HAp

The structural properties of the HAp powder used in this study were determined using the XRD data, as shown in Fig. 2. The XRD peaks of the HAp sample are found at 2θ angles of 31.8, 32.25, and 32.91. The peak positions and corresponding peak intensities were in good agreement with the reference values provided by the JCPDS card (#9-432). The EDS data given in Table 1 shows that the atomic percentages of Ca and P were 19.25% and 11.50%, respectively, which leads to a Ca/P ratio of 1.674. This ratio was the same as the reference value of

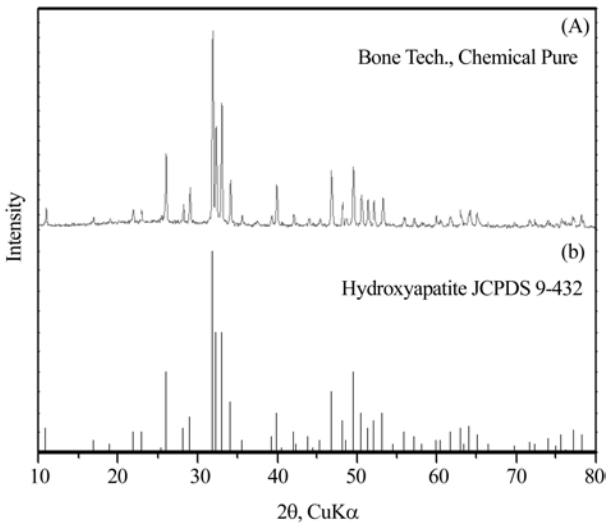

Fig. 2. XRD patterns of the HAp powder: (A) prepared HAp and (B) standard peaks of HAp provided by JCPDS.

Table 1. EDS profile of HAp powder

Element	Atomic%
O	69.25
P	11.50
Ca	19.25
Totals	100.00
Ca/P	1.674

Table 2. Heavy metal analysis of HAp powder by ICP-OES

Analysis Elements	Analysis Date
As (ppm)	0.00
Cd (ppm)	0.00
Co (ppm)	0.00
Cr (ppm)	0.00
Hg (ppm)	0.00
Mn (ppm)	0.02
Ni (ppm)	0.00
Sb (ppm)	0.00
Se (ppm)	0.00
V (ppm)	0.00
Zn (ppm)	0.00
Total	0.02

1.67. Table 2 shows the ICP-OES heavy metal analysis results, and it shows that the total amount of heavy metal is 0.02 ppm. The overall quantitative results indicate that the HAp powder prepared was identical to natural HAp.

Measurement results of sample properties

Fig. 3 shows the results of XRD analysis of the HAp body sintered at temperatures around 1350 °C to 1450 °C for 2 h. When sintered at 1350 °C, only HAp crystalline peaks were observed. However, β-TCP crystalline phase peaks appeared when the sample was sintered at 1400 °C. At 1450 °C, the number of β-TCP crystalline phase peaks were found to increase with the temperature.

Figs. 4 and 5 show results of the compressive and bending strengths of sintered HAp body at temperatures ranging from 1350 °C to 1450 °C over a period of 2 h and under formation loads ranging from 0.5 to 1.5 tonne(9.8 KN). When the sample was fabricated under a pressure of 1 tonne (9.8 KN) and subjected to heat treatment at 1350 °C, the compressive strength and the bending strength were found to be higher than those of the samples treated under the other conditions. Therefore, the best conditions for HAp sample preparation were found to be a pressure of 1 tonne (9.8 KN) and a temperature of 1350 °C.

Fig. 6 shows the results of micro Vickers Hardness tests. The samples were prepared by heat treatment at temperatures around 1350 °C to 1450 °C and under a formation pressure of 1 tonne(9.8 KN). The hardness of the sintered sample at 1350 °C was higher than that of the samples prepared under the other conditions.

According to the above results, the best mechanical properties of the sintered HAp were obtained when the sample was prepared under a formation pressure of 1 tonne (9.8KN) and was sintered at a temperature of 1350 °C for 2 h. The mechanical properties of HAp also depend on the Ca/P ratio. HAp samples containing tricalcium phosphate (TCP) showed poor mechanical properties [16]. The HAp samples sintered at 1350 °C showed a high

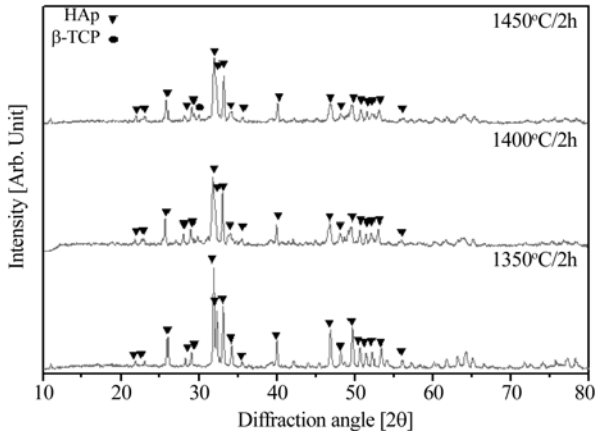

Fig. 3. XRD patterns of HAp sintered at 1350 °C, 1400 °C, and 1450 °C for 2 h.

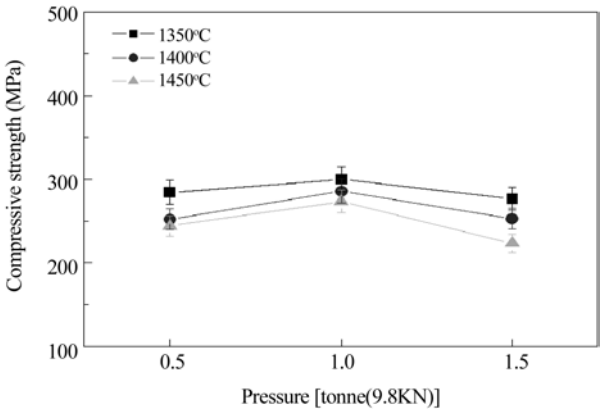

Fig. 4. Compressive strength of HAp sintered at 1350 °C, 1400 °C, and 1450 °C for 2 h under different loads.

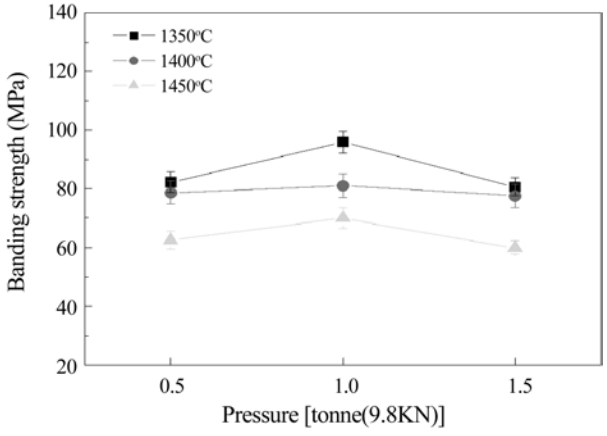

Fig. 5. Binding strength of HAp sintered at 1350 °C, 1400 °C, and 1450 °C for 2 h under different loads.

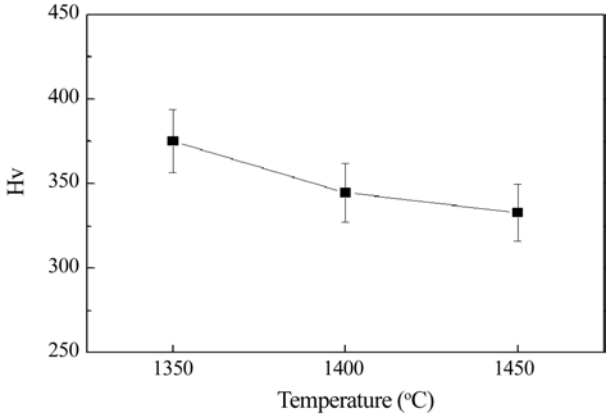

Fig. 6. Micro Vickers hardness of HAp sintered at 1350 °C, 1400 °C, and 1450 °C for 2 h under a formation pressure of 1tonne(9.8 KN).

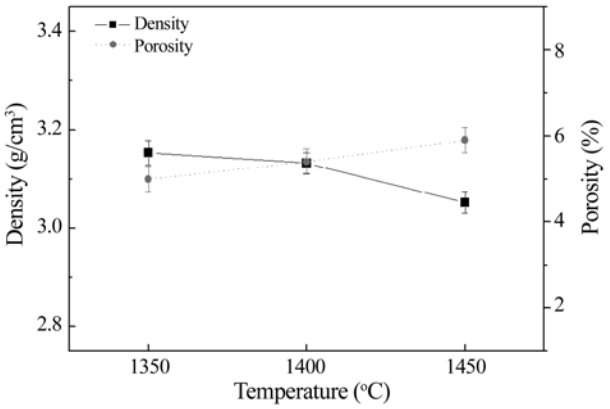

Fig. 7. Density and porosity of HAp sintered at 1350 °C, 1400 °C, and 1450 °C for 2 h under a formation pressure of 1 ton.

density and low porosity.(Fig. 7) Because the HAp was sintered at 1400 °C, at 1450 °C, β-TCP crystal phase was obtained.

Theoretical densities of HAp and β-TCP were 3.16 g/cm^3, and 2.65 g/cm^3 respectively; i.e., the density of β-TCP was significantly lower than that of HAp. β-TCP has been created in the HAp sintered density decreased and porosity affect the mechanical properties of the increase should be considered. Even in vivo β-TCP is present in the HAp in vivo due to preferential melting of the mechanical properties of implants can cause degradation and micro-structural disassembly of the implant will lose its function [17].

HAp cylinder SBF activation test

We tested the HAp sample in the SBF solution by heating it at 1350 °C under a pressure of 1 tonne(9.8 KN) to examine its binding activity in human bones at 37 °C. Fig. 8 shows the FE-SEM image of the sample other 4 weeks of immersion. As shown in Fig. 8, the surface of HAp samples exhibits changes. This result indirectly proves that the HAp samples are bioactive in the human body. We assumed on the basis of the concentration of Ca^{2+} ions that reactions between the Ca^{2+} ions and HAp occurred at the sample surfaces.

Results of the in vivo test

HAp cylinders were used in this study as a bone graft material in order to determine the possibility of using the right and left sides of the rabbit tibial defect that was artificially generated in situ and in which the HAp cylinder was transplanted.

Rabbits were kept in individual cages during the experiments. No histopathological features of a graft-versus-host disease or immune rejection were observed in any of the treatments. Approximately one week after surgery, the amount of feeding was maintained or slightly reduced (200 g per feed). However, the rabbits began to gain weight soon after the food was reduced to 150 g 2-

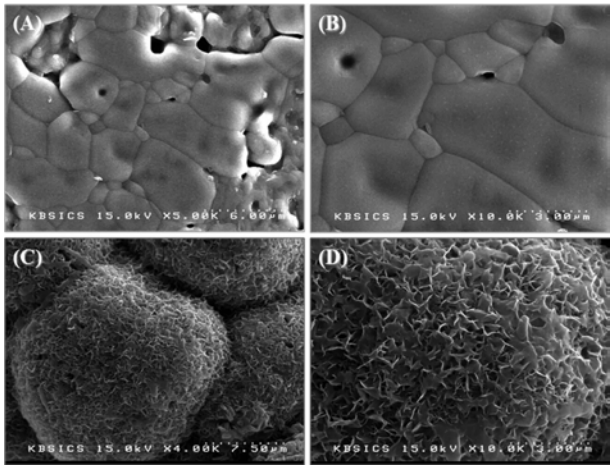

Fig. 8. FE-SEM micrographs of HAp cylinder sintered at 1350 °C for 2 h under a formation pressure of 1 tonne(9.8 KN). (A) and (B): non-immersed in SBF solution; and (C) and (D): after 4 weeks.

3 days after surgery, and the limp in their legs disappeared. Moreover, the following was observed through the naked eye: the shape of the adjacent tissue did not change and edema or inflammation of tissues was not observed. Exactly 2, 4, 6, and 8 weeks after surgery, CT scans were conducted on the tibial defect areas in the rabbits because after 2 weeks, the formation of a new cortical bone was observed at the site of the bone defect where the HAp cylinder was implanted. The close contact between the HAp cylinder and the bone for 4 weeks resulted in the formation of new cortical bone, and a complete check up was carried out periodically up to 8 weeks (Fig. 9). After 8 weeks, the sliced tissue photographs show the rabbit tibia evenly covered by the periosteum and no signs of erythema or edema (Fig. 10). The experimental results showed that an HAp cylinder can replace damaged bone and the observed bone defects may suggest excellent restoration.

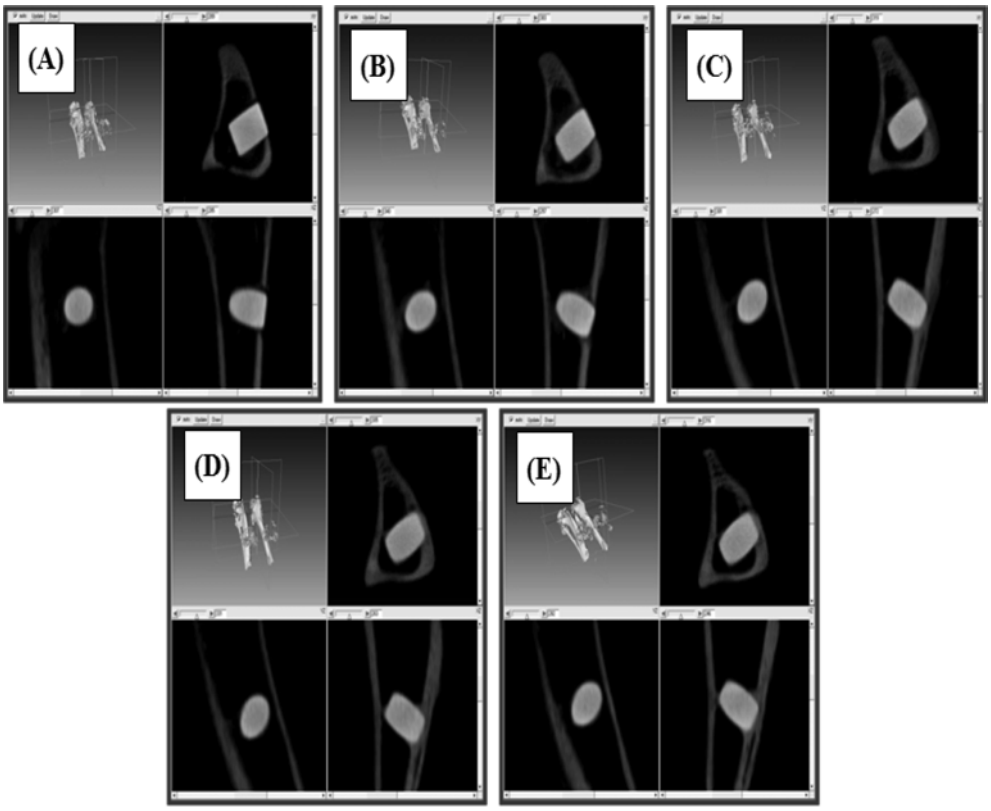

Fig. 9. CT images showing tibia of a rabbit inserted with an HAp cylinder. (A) normal, after (B) 2 weeks, (C) 4 weeks, (D) 6 weeks, and (E) 8 weeks.

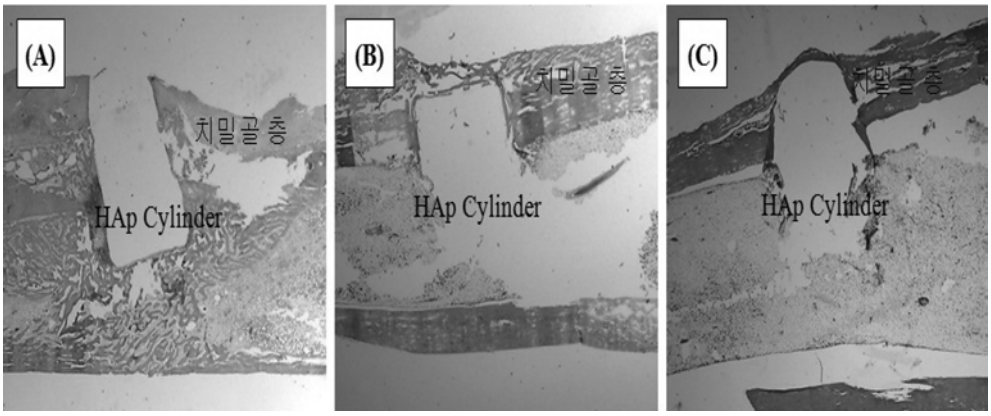

Fig. 10. Photomicrograph of tibia that was regenerated using an HAp cylinder. After (A) 2 weeks, (B) 4 weeks, and (C) 8 weeks.

Conclusions

The temperature and pressure changes for the sintering HAp the powder were noted; a granular HAp powder was prepared using the spray-drying method. The best mechanical properties were obtained for a HAp prepared under a formation load of 1 tonne(9.8 KN) and sintered at a temperature of 1350 °C for 2 h. The results of the binding activity were used to study the bioactivity of the various HAp samples in an SBF solution over four weeks so that the activity over the complete surface of the samples could be estimated.

Animal test results on erythema or edema in the bone marrow without osseointegration was a quick check on the repair of damaged bone tissue which could be induced and determined. The HAp cylinder used in this study can thus be used as an excellent bone substitute, and HAp also shows potential for use in artificial bones, artificial teeth, and ceramic implants, e.g., of various human organs to replace the use of other materials and thus be

possible, for the use of unfavorable materials for these purposes to be avoided.

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