Effect of macro to nanostructure and porosity on regeneration of dogs' segmental large bone defect

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Introduction

Hydroxyapatite (HAp: $Ca_{10}(PO_4)_6(OH)_2$) is well known as a bioactive ceramics and the main inorganic component of vertebrates' hard tissues, i.e., bone and teeth. Accordingly, HAp ceramics both in dense and porous ones have been being investigated by many researchers and practically used in both medical and dental fields. These "sintered" HAps show excellent biocompatibility to bond directly to bone. However, they also have high elastic modulus, brittleness and low solubility in comparison to HAp in bone tissue; thus, HAp ceramics remain for patients' lifetime and sometimes cause bone fractures after healing. To solve the problems, many researchers have been trying to prepare composites of bioactive ceramics and organic polymers, *e.g.*, HAp/polyethylene such as the HAPEX® [1], calcium phosphate/biodegradable synthetic polymers [2, 3] and HAp/collagen [4-7] and so on. In these composites, HAp/collagen composites are composed of both the main inorganic and organic components of bone, and expected to be most favorable materials as a bone substitute. The authors synthesized HAp/collagen (HAp/Col) nanocomposite having bone-like nanostructure and chemical composition via self-organization of HAp and collagen based on their interfacial interaction. Further, the authors demonstrated that the HAp/Col is incorporated into bone remodeling metabolism as the same as transplanted autogenous bone. We already reported that high-porous (~95%) HAp/Col artificial bone was beneficial for large segmental bone defect in comparison to conventional artificial bone, i.e., commercially available hydroxyapatite and β-tricalcium phosphate porous ceramics; however, stable bone formation in the center of the defect had to be still improved. In the present study, a novel core-shell artificial bone material was fabricated from the HAp/Col bone-like nanocomposite membrane, and its bone tissue reaction was evaluated.

Experimental

Materials

The HAp/Col nanocomposite (HAp:Col=4:1 in mass ratio) long and short fibers were synthesized according to the previous report [6], and mixed well at 1:4 mass ratio in pure water The HAp/Col filtered sheet obtained was frozen at -20 °C, vacuum dried at -20 °C, pressed at 30 MPa to be a membrane. The membrane, cut into 22 × 33 mm^2, were moisturized by dipping in water for less than 1 sec. and rolled around ϕ11-mm cylindrical ceramic filter which was covered with Teflon® membrane filter. The rolled-up HAp/Col with filter was packed in thin rubber bag. The bag, sealed with adhesive and thread, was cold-isostatic pressed at 100 MPa. Then, the pressed HAp/Col was removed from cylindrical filter. The height was adjusted to approximately 20 mm by surgical knife. The HAp/Col membrane was also cut into 50 × 33 mm^2, moisturized with H_2O, placed on the wavy mold (wave height is 250 μm) and pressed at 140 kPa for 1 second. The wavy membrane obtained was cut into 20 mm in width, rolled up around Teflon® rod and filled in the central hole of the HAp/Col cylinder. The cylindrical artificial bones obtained were vacuum dried at -20 °C and crosslinked by vacuum heating at 140 °C for 12 hours.

Procedures

Compressive strength and Young's modulus of the core-shell artificial bone obtained were measured with a universal testing machine under dry and wet, soaking in phosphate buffered saline for 1 day, conditions. Cross section of the specimen was determined from micro X-ray computer tomographic (CT) image (SMX-90CT, Shimadzu Co., Japan) using CT image analysis software (VGStudio Max ver 2.0, Volume Graphics GmbH, Germany).

The animal tests were accepted by Tokyo Medical and Dental University Institutional Animal Care and Use Committee (Approval No. 0090072). Experimental animals were three male mongrel dogs of 1 year old and weighting 19.8 to 23.3 kg. Right tibia was set in a ring external fixator. The bone defects of 20 mm in length were made with a power saw (Osada Surgery Success V®, Osada Medical) and the HAp/Col was implanted into the defects after dipping in self-blood. The experimental period was 12 weeks, and the bone formations within the bone defects were examined with digital radiograph (FCR pico V system XG-1V®, Fuji Film) in every 2 weeks.

Results and Discussion

The HAp/Col cylinder obtained after cold isostatic press had inner diameter of 11 mm, outer diameter of approximately 15 mm with several crinkles and height approximately 20 mm. The cylinder obtained had an expected size from calculation before cold isostatic

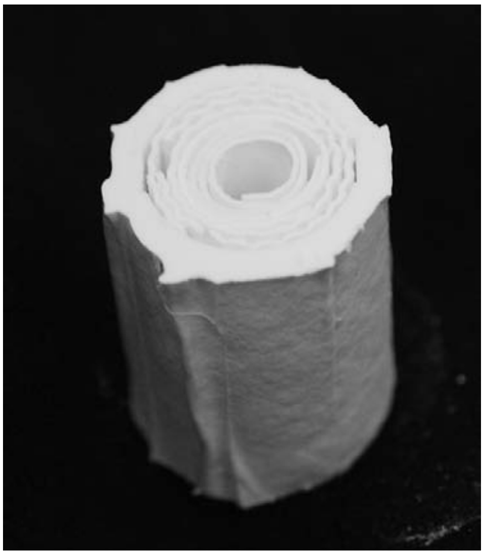

Fig. 1. Core-shell type HAp/Col artificial bone.

pressing. Fig. 1 shows the novel artificial bone after filling HAp/Col porous core composed of its membrane. Compressive strength of dry and wet specimen indicated that mechanical strength of the artificial bone shown in Fig. 2 was drastically decreased; however, as

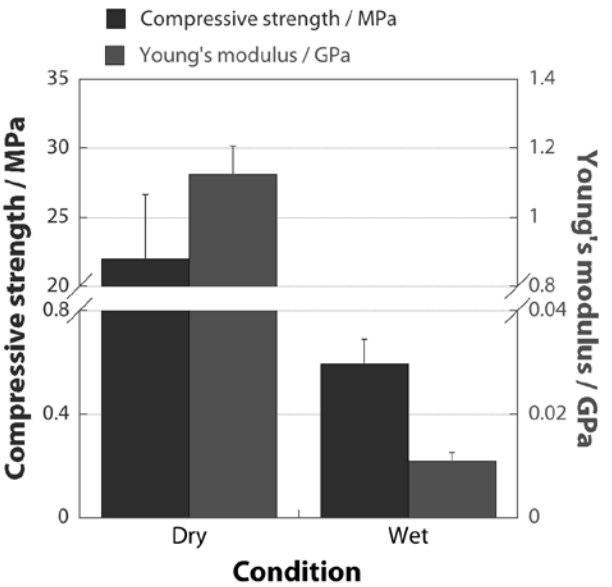

Fig. 2. Mechanical properties of core-shell type HAp/Col artificial bone.

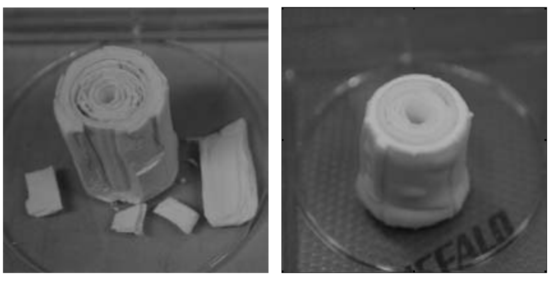

Fig. 3. Core-shell type HAp/Col artificial bone in dry (left) and wet (right) condition after compressive strength test.

shown in Fig. 3, wet specimen showed slight viscoelastic property as the same as the HAp/Col porous body that was reported previously.

In the dogs' experiment, a periodic X-ray photographs demonstrated that gradually filling of all segmental defects with newly formed bone. Further, calcified bridging between host bone and the shell of artificial bone was observed in 4 weeks. The shell part was obviously remained at least 8 weeks.

Conclusion

The novel core-shell type artificial bone with unidirectional interconnected pore composed of hydroxyapatite/collagen bone-like self-organized nanocomposite was successfully prepared. The artificial bone revealed good mechanical strength for operation and excellent bone formation activity both *in vivo*. The novel artificial bone is expected to be better bone filling materials for large segmental bone defect as well as tissue engineering scaffold.

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