A Novel Unidirectional Porous Hydroxyapatite Cylinder Implanted in the Dorsal Muscles of Dogs Promotes Fibrous Tissue Vascularization and Invasion

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Abstract. We recently synthesized a novel unidirectional porous hydroxyapatite (UDPHAp) material with a microstructure consisting of cross-sectional oval pores (diameter, 100-300 μm). The unidirectional pores of UDPHAp are expected to facilitate the ingrowth of new tissues at sites of implantation. Here, we estimated the osteoinductive capacity of UDPHAp following its implantation in the dorsal muscles of dogs, and also investigated the affinity of UDPHAp for muscle and connective tissues. As a reference material, the HAp porous ceramic product Apaceram® (HOYA, Tokyo, Japan), which is commercially available in Japan and has a different microstructure from UDPHAp, was also used. A cylinder-shaped UDPHAp block was implanted in the dorsal muscles of two beagle dogs. At 1 and 2 years post-implantation, muscle and connective tissues had directly attached to UDPHAp at the upper and lower perforated surfaces. Histological assessment, revealed the direct invasion of fibrous tissues and small capillaries into the unidirectional pores of UDPHAp. Notably, no osseous tissue had formed within UDPHAp. Our findings suggest that the unidirectional pores of UDPHAp are advantageous for the vascularization and invasion of fibrous tissues. However, this unique structure does not contribute to osteoinductive capacity.

Introduction

We recently developed a new type of hydroxyapatite (HAp) ceramic, termed unidirectional porous HAp (UDPHAp), which represents a promising material for promoting bone ingrowth and capillary formation due to its unidirectional pore structure [1-3]. UDPHAp, known commercially as REGENOS® (Kuraray Co., Ltd., Okayama, Japan), was developed in a collaborative project involving Kuraray Co., Ltd., the University of Tsukuba, and the National Institute of Material Science, Japan, and has been in clinical use since 2009. To fabricate UDPHAp, ice columns are first formed in a solution of HAp granules by unidirectionally cooling the slurry using a cold plate and liquid nitrogen. Following the removal of the ice columns by repeated freeze drying and thaw, unidirectional and interconnected pores are formed with sizes ranging from 100-300 μm . The unidirectional porous structure, which has an average porosity of 75%, can be clearly seen in the micro-computed tomography (CT) image of a cross-sectioned UDPHAp cylinder (Fig. 1).

Due to its unique structure, UDPHAp can mimic the orientational structure of collagen and hydroxyapatite of long bone, thereby increasing bone compressive strength in the longitudinal direction. The initial compression strength (prior to implantation) of UDPHAp is approximately >8 MPa parallel to the direction of pores and >1 MPa perpendicular to the pore direction.

In addition, the unidirectional orientation and suitable pore size of UDPHAp facilitates the deep penetration of osteogenic cells into the material, as demonstrated in animal models [1-3]. However, the ectopic bone formation of UDPHAp and the invasion of soft tissues after intramuscular implantation remain unclear.

Fig .1 SEM (A) and 3-D micro-CT images (B) of UDPHAp show unidirectional pores in the vertical plane and interconnections in the horizontal direction. White: HAp, black: pores

We anticipated that the unidirectional pores of UDPHAp would facilitate the ingrowth of new tissues in the material upon its implantation into bone. Here, we estimated the osteoinductive capacity of UDPHAp following its implantation in the dorsal muscle of dogs, and investigated the affinity of UDPHAp for muscle and connective tissues.

Materials & Methods

Implant materials

UDPHAp with an average pore size of 100-300 μm and porosity of 75% was used as an implant material. As a reference material, the HAp porous ceramic product Apaceram[®] (HOYA, Tokyo, Japan), which is commercially available in Japan and has a different microstructure from UDPHAp, was also used. This material has 50%-55% porosity and contains interconnected spherical macropores of >100 μm in diameter, and micropores ranging from several hundred nanometers to several micrometers in diameter that create a rough surface on the macropores. Cylinder-shaped UDPHAp and Apaceram[®] ceramics (5 mm in diameter and 10 mm in height) were used as implant materials in the following animal experiment.

Animal experiment

Two female one-year old beagle dogs (approximately 10 kg body weight) were generally anesthetized with an intramuscular injection consisting of midazolam (0.4 mg/kg) and medetomidine (0.04 mg/kg). The surgical site was shaved and a vertical incision was made in the skin of the back. An incision was then made in the muscles of the right and left dorsal areas. The cylinder-shaped UDPHAp and Apaceram[®] materials were then inserted within the right and left dorsal muscles pouches, respectively, of each beagle. Both HAp ceramics were placed parallel to the muscle fibers. After 1 (n=1) and 2 years (n=1), the implanted cylinders and surrounding tissues were harvested and evaluated.

For histology analyses, the four specimens were fixed in 10% neutral formalin and then coronally sectioned into halves. One half of the specimen was embedded in methylmethacrylate resin, while the other was embedded in paraffin after demineralization in a 10% EDTA solution. Longitudinal sections (5- μm thickness) of the undecalcified and decalcified samples were then prepared. The resin sections were stained with Villanueva-Goldner (VG), and the paraffin sections were stained with hematoxylin and eosin (HE).

Results

At one and two years after the intramuscular implantation of UDPHAp and Apaceram® cylindrical blocks into beagles, the implant sites were examined macroscopically (Fig. 2). Muscle and connective tissues were found to have directly attached to UDPHAp at the upper and lower perforated surfaces of the implanted material. In addition, fibrous capsular formation was observed surrounding UDPHAp, except at the perforated implant surface. In contrast, the Apaceram® implant was completely encapsulated in fibrous tissue (Fig. 2).

Fig. 2 Photographs of the implantation sites of the UDPHAp and Apaceram® materials at one year after the intramuscular implantation. Muscle and connective tissues directly attached to UDPHAp at the upper and lower perforated surfaces (A&C). The encapsulation of Apaceram® by thin fibrous tissues was observed over all surfaces of the material (B&D).

Histological assessment of the implanted HAp materials revealed the direct invasion of fibrous tissues and small capillaries into the unidirectional pores of UDPHAp. Notably, no osseous tissue was detected in the UDPHAp implant. In contrast, new bone and small capillaries were found to have penetrated the spherical pores of the Apaceram® block, even within the central area of the implant (Fig.3). The UDPHAp and Apaceram® specimens appeared similar after 1 and 2 years of implantation.

Fig. 3 Histological findings for UDPHAp (A&B&E) and Apaceram® (C&D&F) at one year after intramuscular implantation.

A&C: Low magnification, decalcified slices with HE staining. B&D: Low magnification, undecalcified slices with VG staining. E&F: High magnification, decalcified slices with HE staining. Fibrous tissue and small capillaries were formed in the unidirectional pores of UDPHAp (E), while newly formed bone and small capillaries were found in the spherical pores of Apaceram® (F).

Discussion

We confirmed that ectopic bone was not formed after the intramuscular implantation of UDPHAp into beagles during two years of follow-up. In our previous studies, UDPHAp demonstrated good osteoconductivity and promoted osteogenesis, with respect to mesenchymal stem cells and blood flow, when implanted into the intramedullary cavity of rabbit and canine models[1-3]. However, the unique structure of UDPHAp did not contribute to a ectopic bone formation in the present study. In contrast to the histological findings for UDPHAp, ectopic bone formation was observed after the intramuscular implantation of Apaceram®. Although osteoinduction by biomaterials has been widely observed, the underlying mechanism remains unknown.

In the present study, UDPHAp formed direct connections to the surrounding muscle tissues without encapsulization of the implanted material. In contrast, Apaceram® was completely encapsulated after intramuscular implantation, and the direct osseointegration between Apaceram® and the surrounding tissue was not detected macroscopically. It is possible that differences in the porosity of the two materials may have affected fibrous capsular formation. In addition, the pore structure also seems to have affected the invasion of fibrous tissue and small capillaries into the two HAp ceramic materials. Our findings suggest that the unidirectional pores of UDPHAp are advantageous for vascularization and invasion of soft tissues, but limit fibrous capsular formation.

Conclusions

We confirmed that ectopic bone was not formed after the intramuscular implantation of cylindrical UDPHAp blocks during one and two years of follow-up. The unidirectional pores of UDPHAp facilitate the invasion of fibrous tissues and small capillaries, without promoting fibrous capsular formation.

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