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Three-dimensional Observation of Reconstruction Course of Rabbit Experimental Mandibular Defect with rhBMP-2 and Atelocollagen Gel

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Abstract
For the experimental animals, eight rabbits were chosen. A bone defect was made and was filled with 1% atelocollagen gel including rhBMP-2 10μg. The reconstruction course was observed using micro-computed tomography (μCT) in vivo. In μCT observation, the density was slightly elevated at the bone marrow side at day 7, and the phenomenon gradually expanded during the course of this experiment which lasted for 28 days. By utilized μCT, we could construct 3D images, and that process enabled us to visualize bone formation more closely. These data suggest that the experimental animal model μCT and 3D image are extremely useful for follow-up of reconstruction of animal bone defects and that the atelocollagen gel is effective as a carrier of rhBMP-2.

Key words: Micro CT; 3D image; rhBMP-2; atelocollagen gel; bone reconstruction; rabbit

Introduction

It is well known that bone morphogenetic protein (BMP) takes an important role in growth and regeneration of bone, especially in healing of bone fractures. BMP also plays a main role in bone defect remodeling or reconstruction. However, it is essential to develop more suitable carriers for each subject.

Several morphological studies have been conducted on BMP for clinical use with mice, rats and other smaller experimental animals, and have obtained satisfactory results[1, 2]. In experiments using different sized animals such as rabbits[3], dogs [4] and monkeys[5, 6], the following factors have been found: the larger animal, the less bone morphogenetic levels were indicated, and they required much larger quantities of rhBMP. Therefore, it is necessary to develop a more suitable carrier for each subject to induce new bone.

When observing the bone formation process, radiographic study is more effective[1]. Although radiography enables vertical observation from the passage of time, radiography itself cannot reveal the entire morphogenetic process in detail since images are taken only from one direction.

In this examination, we used a rabbit experimental mandibular bone defect model. The bone defect was reconstructed with rhBMP-2 and atelocollagen gel, and examination was carried out using experimental animal model μCT.

Materials and Methods

For this experiment, eight Japanese male white rabbits (JW/CSK, Japan SLC Inc, Hamamatsu, Japan) were chosen. They were eight weeks old and weighed about 1.4kg. After administrating lidocaine nebula on rabbits' ear skin for local anesthetic purpose, 0.2% sodium pentobarbital 25 mg/kg was injected intravenously to parotid vein for general anesthetic purpose. The rabbits were then injected with 1/80,000 epinephrine 2% additive lidocaine 2 ml on several locations after shaving around the inferior border of mandible and disinfection of the operative site. The mandible inferior border was opened, and an incision was made through the masseter muscle and musculus pterygoideus medialis to the periosteum. The mandible inferior border periosteum was abraded, and a quadrate bone defect (4 x 6 mm) was made in the mandibular inferior border which directly continued to the cancellous bone with bone marrow (Fig. 1A). The defect was filled with 1% atelocollagen gel (ACG) including rhBMP-2-10μg (Astellas Pharma Inc, Tokyo, Japan), and covered with a poly (lactic-co-glycolic acid) copolymer (PLGA) membrane to fit with the contour of the mandible (Fig. 1B).

Two different control groups were prepared: a group was filled with ACG without rhBMP then covered with PLGA and observed for 14 days; the other was filled with neither rhBMP nor ACG and covered with PLGA and observed for 28 days.

Under anesthesia, μCT (R_mCT; Rigaku Mechatronics, Tokyo, Japan) was taken immediately after the operation and in the course of the reconstruction period (0, 7, 14, 21 and 28 days) (Fig. 2A). As for the exposure parameters, 60kV and 60μA to 90μA were set.

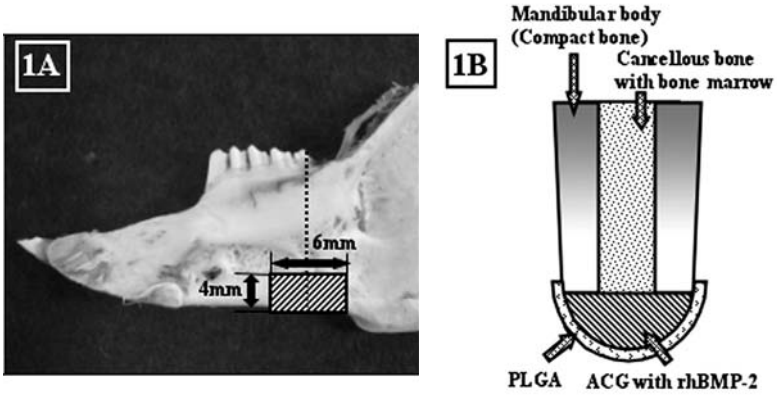

Fig. 1. The mandibular bone defect (A) and schematic illustration (B) of the bone defect of the plane of dotted line of (A).

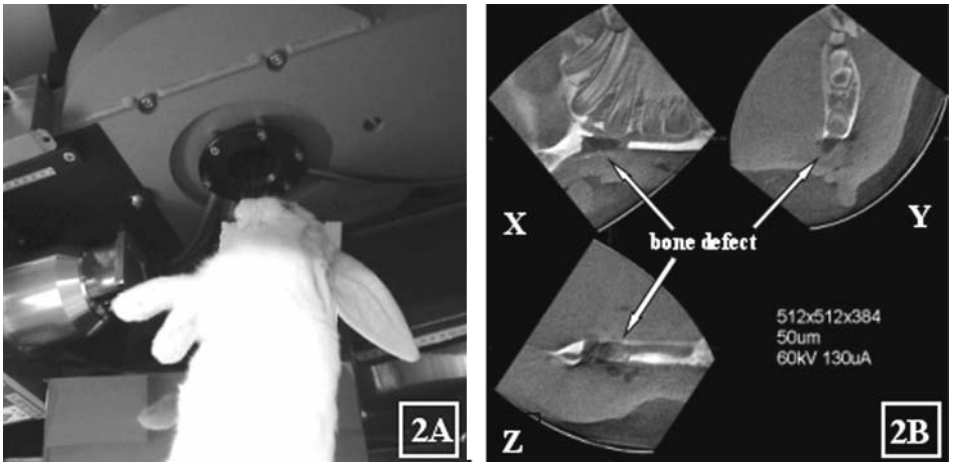

Fig. 2. Overview of μCT and an anesthetized rabbit (A), and cross-sectional images from three directions which were para-sagittal plane (X), frontal plane (Y) and horizontal plane (Z) (B).

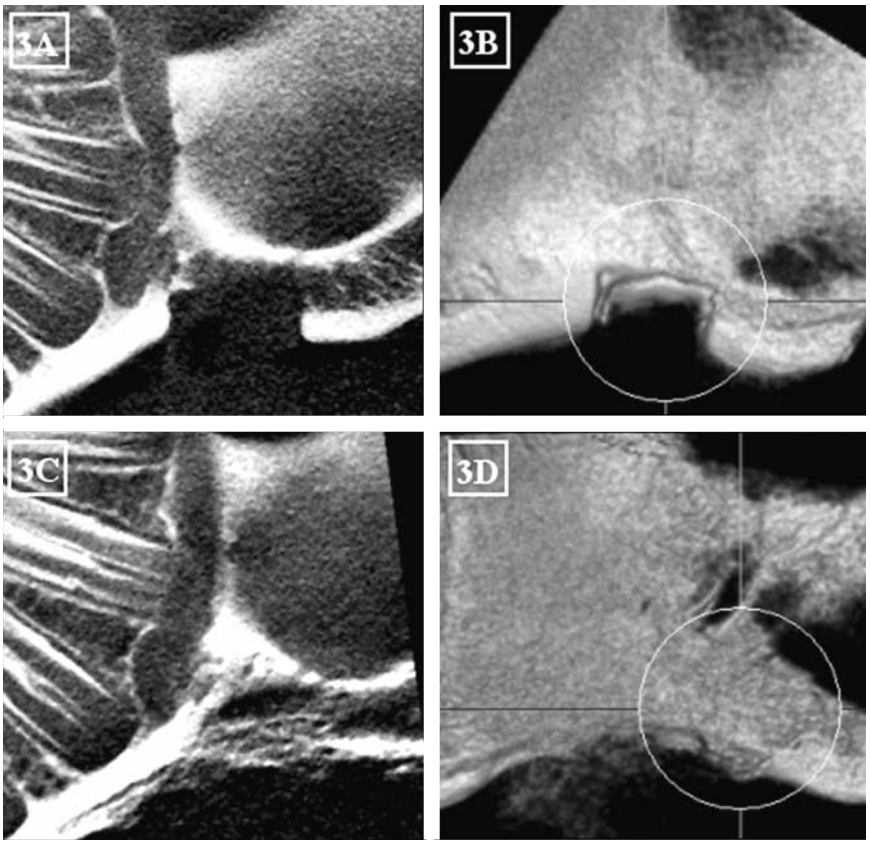

Fig. 3. Experimental group. μCT (A) and 3D (B) images of X-plane right after the operation, and at day 28 (C, D) the same subject shown in Fig. 3A, B.

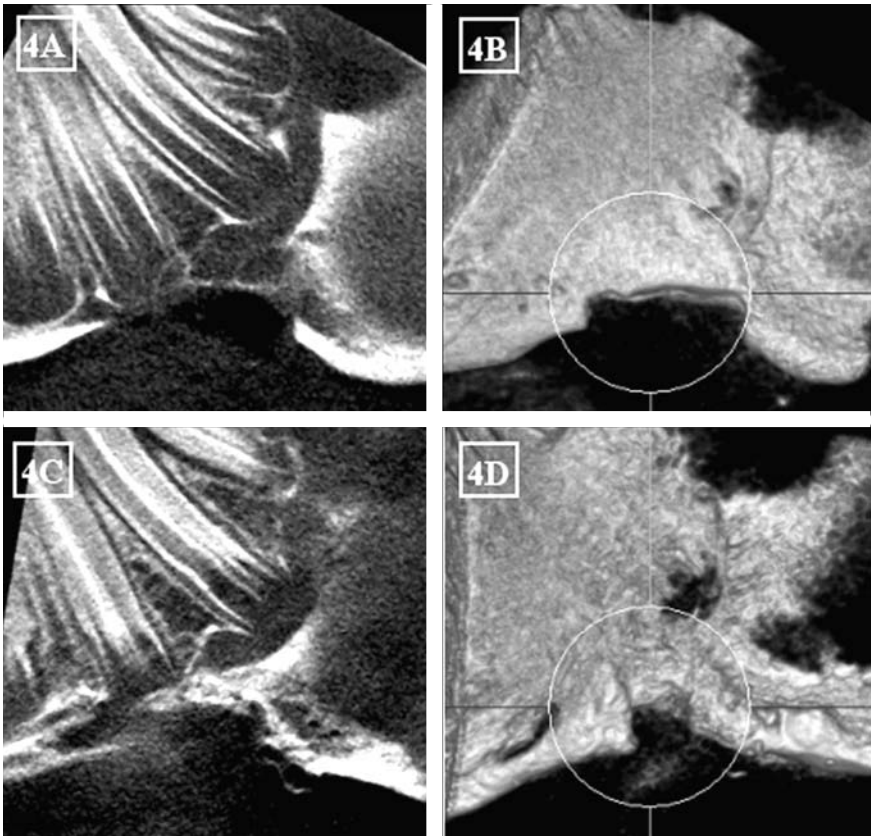

Fig. 4. Control group. μCT (A) and 3D (B) images of X-plane right after the operation, and μCT (C) and 3D (D) images of day 28, same subject shown in Fig.4A.

After μCT, i-view software (J. Morita, Kyoto, Japan) was utilized for image reconstruction. The voxel of this μCT was 512 x 512 x 384 and each exposure time was 17 seconds. By achieving cross-sectional images from three directions, the para-sagittal plane (X), frontal plane (Y) and horizontal plane (Z) directions, we were able to observe all three different images simultaneously. Throughout μCT observations, 100μm x 100μm voxel size and 51mm x 51mm x 38mm imaging volume were arranged (Fig. 2B).

The 3D images of the defect area were constructed by composing the μCT data. A comparison between 3D and μCT images was conducted.

Examination Results

Experimental Group

On the μCT image, which was taken from the X-plane right after the operation, a distinctive contrast between the quadrate bone defects in the mandible inferior border to the bone marrow was observed (Fig. 3A). At the same time, the bone defect was also visible from buccolingual direction on Y-plane image, and clear borders between defective area and others were confirmed on the Z-plane image. Using 3D images, the bone defect was clearly visible (Fig. 3B). At day 7, a slight rise of X-ray absorption from the bone marrow side was confirmed. At day 14, although the borders were still confirmed, the X-ray absorption level rose up and expanded in the bone defect area. At day 28, the range of X-ray absorption level had gradually increased, and the border between the bone defect and the surrounding structure had become indistinctive (Fig. 3C). This phenomenon was observed clearly in 3D images (Fig. 3D).

Control Group

Regarding two different types of control, both μCT images, which were taken from X-plane right after operation, were basically equal to those of the experimental group (Fig. 4A). The 3D images were also same (Fig. 4B).

In chronological μCT observations of the control group, the border between the bone defect and other region was still clear at day 14. In the group with ACG, only a slight rise of the X-ray absorption had been confirmed throughout μCT observations until the day 28 (Fig. 4C, D).

Discussion

There are many reports which support the use of rhBMP for the bone defect, fracture and reconstruction experiments of animal models. However, method and animal for each experiment vary. One observation report, consisting of X-ray and histological findings on rats, reveals that rhBMP application for the femur fracture and the alveolar bone defect models promoted ossification. In addition, there are other reports which applied rhBMP on larger animals' bone defect models: such as rabbit temporomandibular joint excision and dog alveolar part of mandible bone defect. Furthermore, monkeys were used for some final phase experiments before the clinical application since they

are close to human beings. In these studies, the bone defect in mandible inferior border was filled with rhBMP added hydroxyapatite granule and block[6], and for the other experiment model, a titanium plate was fixed on the bone transection in mandible which was created by 30mm osteotomy and filled with rhBMP to promote ossification[7]. After this bone transection experiment, a dental implant was applied on the reconstructed bone, which it lasted for about one year. Therefore there is much expectation for the clinical application of rhBMP on actual patients. However, a much higher concentration level of rhBMP was required to achieve this mandibular reconstruction: the rhBMP concentration levels were 5-10 times more than that of dog and 20-50 times more than that of rat mandibular reconstruction experiments. That is, the larger the animal, the greater the quantity of rhBMP required. In general, in these experiments a gelatin sponge covered by a PLGA was utilized for rhBMP. However, it is still uncertain which carrier is more appropriate to achieve effective application of rhBMP for the bone reconstruction. The rabbit mandibular defect models were filled with ACG, rhBMP and covered with PLGA, and the effectiveness of application has been studied thoroughly.

The most significant characteristics of our study are that we have employed a μCT for smaller experimental animals, developed by Arai[7], in order to verify the course of experiments. By this chronological μCT somatoscopy, we were able to observe a rise of the X-ray density only at day 7 and the expansion of the X-ray absorption range which generated from the bone marrow side chronologically. When we used this system, we were able to examine changes in reconstruction of the bone defect with rhBMP. In addition, the X-ray absorption range was in accord with the area where bone proliferation occurs. This rather active bone reconstruction appears to be due to the application of ACG including rhBMP-2.

It is very effective to apply CT which utilizes 3D constructions on the cranio-maxillofacial region clinically. The μCT images which have been obtained from this experiment enabled us to attain 3D images, and that allowed us to visualize bone formation processes. Employing these μCT and 3D images to observe the whole process should allow for further technical improvement for the operation. Finally, additional histological examination of the reconstruction course is in progress, and the results will be reported in the near future.

Achnowledgement

This μCT was developed with a joint study by, Matsumoto Dental University Institute for Oral Science (Shiojiri, Japan), Rigaku Mechatronics Co., Ltd (Tokyo, Japan) and NUBIC (Nihon University Incubation Center, Tokyo, Japan).

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