Reconstruction of Rabbit Experimental Mandibular Defect with rhBMP-2 and Atelocollagen Gel —μCT Observation in Vivo and Histological Examination —

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Abstract: A rabbit experimental mandibular defect was reconstructed with rhBMP-2 and atelocollagen gel, and examined using μCT (R_mCT, Rigaku Mechatronics, Tokyo) in vivo and histological techniques. Using eight rabbits, we made an experimental mandibular defect and filled it with 1% atelocollagen gel including rhBMP-2 10ìg (Astellas Pharma Inc.). In μCT observation, the density was slightly elevated at the bone marrow side at one week, and the phenomenon gradually expanded during the course of this experiment. Histologically, mesenchymal cell proliferation and immature bone formation occurred at one week , and mature bone gradually increased and filled in at four weeks.

Key words: rhBMP-2; atelocollagen gel; bone reconstruction; μ CT; rabbit

Introduction

Reconstruction of local bone defects in the jaw is one of the major problems in oral maxillofacial surgery. Bone morphogenetic protein (BMP) is assumed to be a protein which induces new bone in heterotopic sites and is extracted from cortical bone [1]. By molecular cloning, several types of BMP have been isolated, and recombinant human BMP (rhBMP-2) have been synthesized. When BMP is applied for the purpose of inducing new bone, it is necessary to develop a more suitable carrier for BMP that can be used clinically. Numerous papers have been published about experimental animal models with some carriers [2, 3]. However, there are few papers of rabbit model experiments.

In this examination, we used a rabbit experimental mandibular defect model. The defect was reconstructed with rhBMP-2 and atelocollagen gel composite, and conducted our examination using an experimental animal model μCT and a histological technique.

Materials and Methods

Using eight rabbits (Slc: JW/CSK, Japan SLC Co., Hamamatsu) under an anesthetized condition with an intravenous injection sodium pentobarbital, a bone defect (4x6 mm) was made in the mandibular inferior border, and was filled with 1% atelocollagen gel including rhBMP-2 10μg (Astellas Pharma Inc.). The region was covered with the poly (lactic-co-glycolic acid) copolymer membrane.

Under anesthesia, a X-ray μ CT (R μCT, Rigaku Mechatronics, Tokyo) was taken just after the operation and in the course of reconstruction period (0, 1, 2 and 4 weeks). Immediately after removal from rabbits, specimens were examined histologically.

Results

In μCT observation, the experimental bone defect was clearly observed just after the operation (Figure 1). After one week, there was a slight rise of X-ray absorption from the bone marrow side (Figure 2). At two weeks, the X-ray absorption level rose up and expanded (Figure 3). This was gradually expanded during the time course of this experiment.

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Histologically, there were various round- or spindle-shaped mesenchymal cell proliferations in the five day-specimens. At one week, trabecular immature bone was formed in the proliferating mesenchymal cells, especially at the bone marrow side of the defect region (Figure 4). The newly formed trabecular bone gradually matured according to the time course. At the end of the four-week experiment, the experimental bone defect was filled with matured trabecular bone. Regarding the poly (lactic-co-glycolic acid) copolymer membrane at one week, many phagocytotic cells were observed within the material. The material was replaced by granulation tissue, including numerous macrophages and foreign body giant cells, at the end of the experiment (4 weeks).

Discussion

BMP is an active factor identified in demineralized bone matrix [1]. It has been reported that the rhBMP-2 induced bone formation in large segmental bone defect and fracture of animal models [2, 3]. However, it is necessary to develop a more useful and/or suitable delivery system or carrier for BMPs that can be used clinically.

In this examination, atelocollagen gel was used as a carrier of rhBMP, and the poly (lactic-co-glycolic acid) copolymer membrane was applied as an interception from peripheral tissue and for bone shape recovery. Histological evaluation results suggested that the atelocollagen gel was effective as a carrier of rhBMP in these bone defect models because bone formation occurred quickly in the implanted site of bone defects. Furthermore, the poly (lactic-co-glycolic acid) copolymer membrane was also useful for the recovery of the bone defects because the replaced time was suitable for rhBMP-induced bone formation.

Regarding theμCT observation, rhBMP-induced bone formation in the defect site was clearly observed. The newly formed immature trabecular bone was observed as slightly elevated density mass at the bone marrow side one week after the operation, and the phenomenon gradually expanded during the course of this experiment in μCT observation. This was histologically confirmed as immature bone formation in one week-specimens, and mature bone had gradually increased and filled in at four weeks.

In summary, in μCT observation of this examination, the X-ray density was slightly elevated at the bone marrow side at one week,

and the phenomen on gradually expanded during the time course of this experiment, which lasted four weeks. This was histologically observed as followed: mesenchymal cell proliferation and immature bone formation occurred in one week-specimens, and mature bone gradually increased and filled in at four weeks. The histological data suggests that atelocollagen gel is effective as a carrier of rhBMP-2. Furthermore, μCT observation is extremely useful for follow-up in reconstruction of animal bone defect model.

Acknowledgments

I would like to thank Professor Toshiyuki Kawakami, Hard Tissue Pathology Unit, Matsumoto Dental University Graduate School of Oral Medicine, for his support and encouragement throughout this presentation. A part of this presentation was supported by a large number of earnest researchers.

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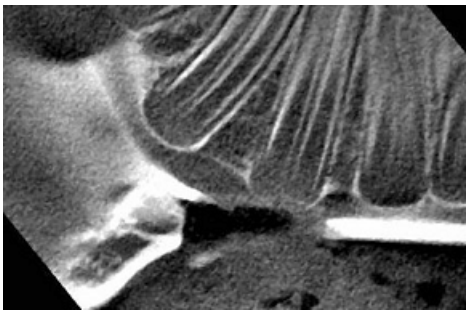

Figure 1: The experimental bone defect was clearly observed (iCT just after the operation).

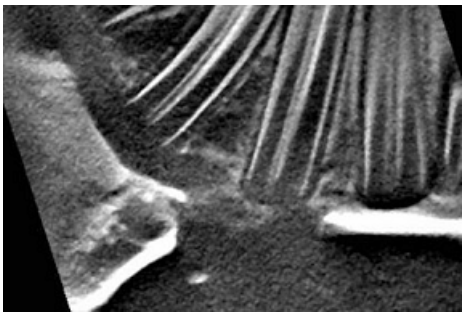

Figure 2: The slightly elevated X-ray density mass at the bone marrow side (iCT at one week).

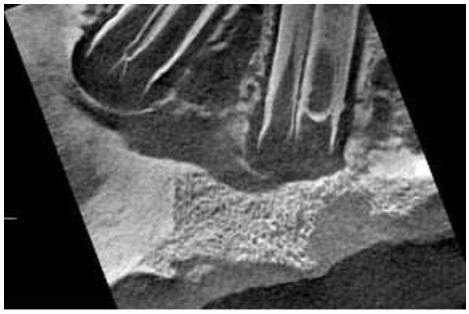

Figure 3: The X-ray absorption level was rise up and expanded (μCT at two week).

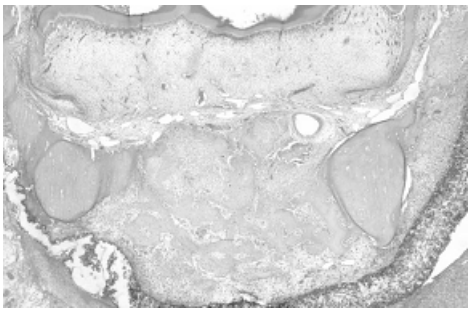

Figure 4: Trabecular immature bone was formed (HE, one week-specimen).