

TISSUE REACTION TO POLY (LACTIC-CO-GLYCOLIC ACID) COPOLYMER MEMBRANE IN rhBMP USED RABBIT EXPERIMENTAL MANDIBULAR RECONSTRUCTION

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Abstract

A rabbit experimental mandibular defect was reconstructed with 1% atelocollagen gel including rhBMP-2 10µg and a covering a poly (lactic-co-glycolic acid) copolymer (PLGA) membrane. For this experiment, eight male rabbits were used and a histological study was conducted. Our study purpose was to examine the effects and fate of PLGA membrane during bone reconstruction. PLGA membrane was phagocytized by foreign body giant cells and macrophages in the healing course of reconstruction osteogenesis. These histological data suggest that the PLGA membrane was gradually absorbed and replaced by fibrous connective tissue or bone tissue. In the osteogenesis course, the outer periphery of the new bone was maintained by PLGA membrane without expansion.

Key words: tissue reaction; poly (lactic-co-glycolic acid) copolymer membrane; rhBMP-2; atelocollagen gel; bone reconstruction; rabbit

INTRODUCTION

We have previous conducted a series of research studies on rabbit mandibular with the application of 1% atelocollagen gel (ACG) including recombinant human bone morphologic protein-2 (rhBMP-2) and covering poly (lactic-co-glycolic acid) copolymer (PLGA) membrane. In these studies, bone processes were observed by µCT. Using µCT data, 3D images were obtained[1].

The data from that earlier study suggested that the atelocollagen gel is effective as a carrier of rhBMP-2. Furthermore, it indicated that PLGA membrane was very effective for maintaining bone morphology, since bone defects were completely covered by the membrane even though fluid gel was utilized in the defect areas.

The purpose of present examination is to examine the fate of the PLGA membrane during bone reconstruction in the bone defect through histopathological observation.

MATERIALS AND METHODS

The materials and methods were detailed in our previous paper. To summarize briefly, using eight Japanese male white rabbits (JW/CSK, Japan SLC Inc, Hamamatsu, Japan) under anesthetized condition with intravenous injection of sodium pentobarbital, a bone defect (4 x 6 mm) was made in the mandibular inferior border and was filled with 1% atelocollagen gel (ACG) including rhBMP-2 10µg (Astellas Pharma Inc, Tokyo, Japan). The region was covered with a PLGA membrane to fit with the contour of the mandible (Fig. 1).

For histological observations, the animals were killed at various times ranging from 5 to 28 days, and tissue reactions to the PLGA membrane were examined with histological method. Immediately after the animals were killed, the regions were removed and prepared as follows. The specimens were fixed in neutral buffered formalin solution. The specimens were demineralized with 10% EDTA after fixation and embedded in paraffin sections, and were then cut at 5µm and stained with hematoxylin-eosin.

RESULTS

Regarding the histological findings of the osteogenesis, mesenchymal cells, including osteogenic progenitor cells, proliferation and immature bone formation occurred from bone marrow in the mandibular bone defect at day 7 (Fig. 2). At day 14, the newly formed trabecular bone gradually increased in the defect area. Finally mature bone gradually increased and filled in at day 28.

Concerning tissue reaction to the PLGA membrane, numerous small round cells were observed around the PLGA membrane at day 7. The membrane was already invaded by granulation tissue, which mainly contained in macrophages and small number of foreign body giant cells, at day 14. Cell density of fibroblasts and macrophages in the PLGA membrane increased with the passage of time (Fig. 3). These infil-

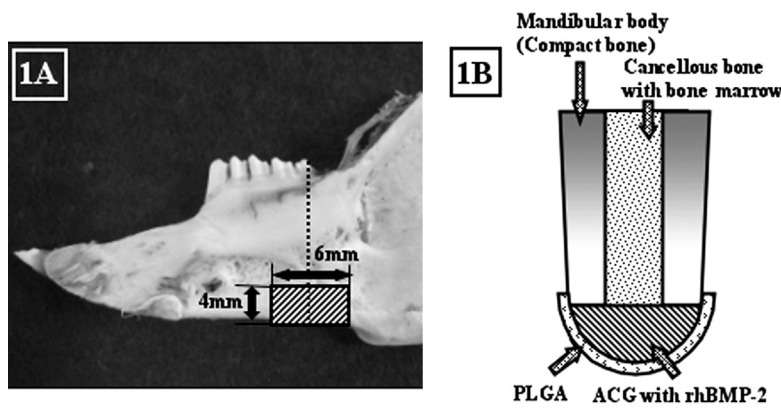


Fig. 1. Schematic illustration of correct experiment.

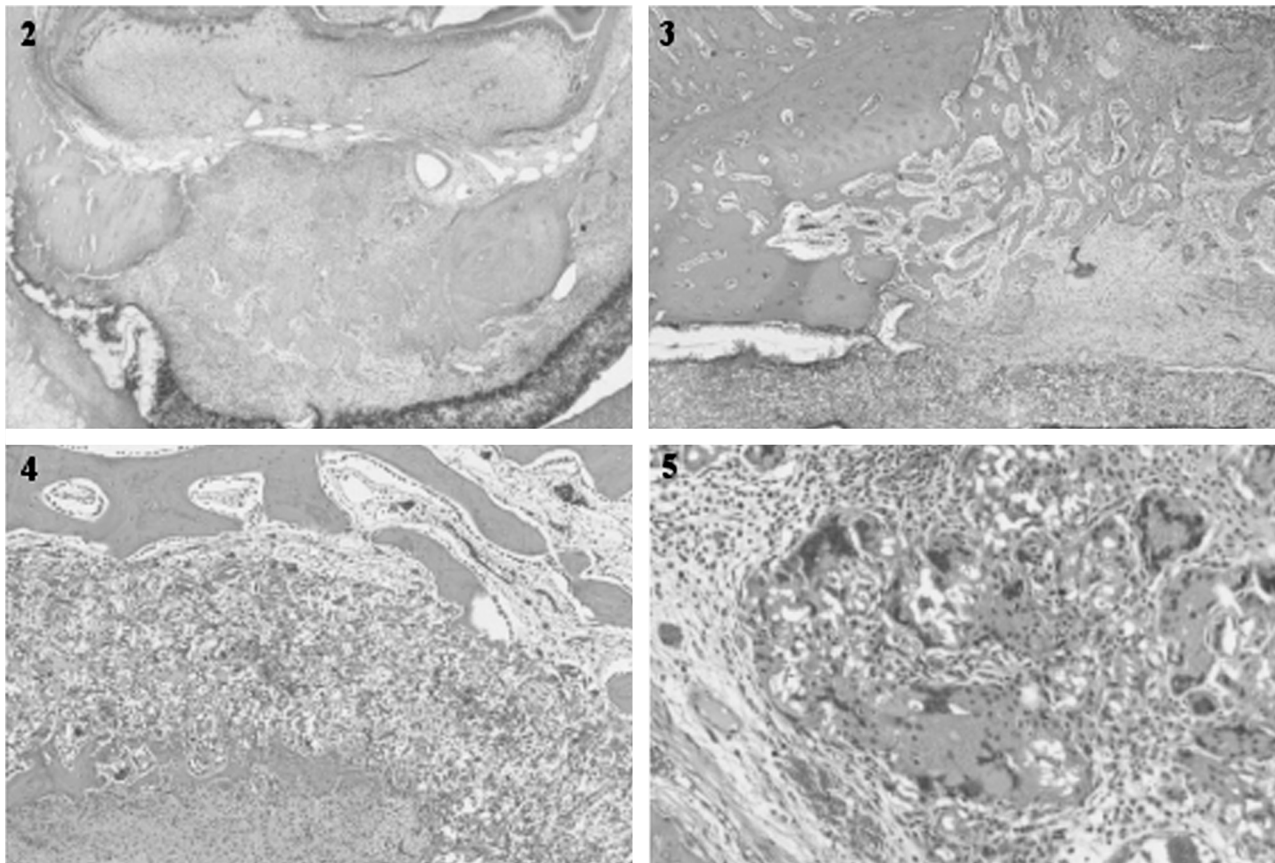


Fig. 2. PLGA membrane was already invaded by foreign body granulomas, which mainly contained in the macrophages. (7-days specimen, x 20).

Fig. 3. Cell density of fibroblasts and macrophages in the PLGA membrane increased over time. (14-days specimen, x20).

Fig. 4. PLGA materials were phagocytized by giant cells. (28-days specimen, x 40).

Fig. 5. The new bone was formed on the outer layer of the PLGA. (28-days specimen, x 80).

trating cells were only confined inside the PLGA membrane, and there was no influence on the connective tissue around the membrane. PLGA membrane was locally transformed to the granulation tissue, and PLGA material was phagocytized by foreign body giant cells and macrophages in the granulation tissue (Fig. 4). At day 28, the entire area of PLGA membrane was replaced by granulation tissues. At this stage, new

tenuis and feeble bone formation was recognized outside of the membrane (Fig. 5).

DISCUSSION

Numerous studies of bone reconstruction utilizing BMP, autogenic particulate cancellous bone and marrow and hydroxyapatite at the bone defect area and

which effectiveness have been reported [1-6]. Furthermore, PLGA [1], bioabsorbable poly (L-lactic acid) mesh tray [2] and titanium mesh plate [5] for bone transplantation, and the artificial materials in the bone defect area for the bone reconstruction, are effective in maintaining reconstruction of the bone morphology. In general, there have been many papers published about the effectiveness of the absorbable membrane, and fate of the absorbable membrane during bone formation in bone defects.

In the healing process of bone defects, periosteum supplies the osteoprogenitor cells which are essential for the bone formation, but also it has a certain role in preventing fibroblastic invasions from the outside to the defective part [7]. Guided bone regeneration (GBR) has been clinically applied in the field of periodontal surgery, and it provides an artificial membrane instead of actual periosteum [7, 8]. This artificial membrane prevents invasion of fibrous connective tissue origin cells into the bone defect from outer area by placing a cell interception membrane on the surface of the alveolar bone defect. Regarding the absorbable material, PLGA is widely available for clinical application. In our previous report [1], new bone formation did not expand outside the membrane because the PLGA membrane was replaced with new bone. Therefore, PLGA application for these bone recovery cases is effective for maintaining bone morphology, similar to the GBR method in periodontal surgery. However, there are few histological studies which have observed the fate of the absorbable membrane during bone formation in the bone defects.

PLGA, which we used in this examination, does not affect the tissue, and is classified as an absorbable material. Therefore, we examined the fate of PLGA membrane in the BMP used in bone recovery experiments. From the viewpoint of histopathology, we found no histopathological changes, degeneration or necrosis in the surrounding tissue at the material. Based on these results, we believe PLGA could be used safely as a bio-absorbable material. Tissue reaction to the material was as follows. The membrane was already invaded by granulation tissue 7 days after operation. This phenomenon shows that the PLGA material was phagocytosed and gradually disappeared by the passage of time. In general, materials from the external environment are taken up through a process generically called endocytosis; up-take of larger particulate or phagocytosis-difficult matters is treated by phagocytosis. Endocytosed vacuoles and their contents eventually fuse with a lysosome, resulting in degradation of the engulfed material, while heterophagy is comparatively conspicuous in the phagocytes, such as macrophages. In case of difficult material for phagocytosis, multinucleated giant cell appear. These histopathological findings suggest that the appearance of foreign body giant cells means the PLGA material is difficult for phagocytosis.

At the end of this experiment, 28 days after operation, there were not completely organized by fibrous tissue. The region was occupied by foreign body granulation tissue, including many giant cells, although PLGA was gradually phagocytosed and finally disappeared with the osteogenesis course. Our present examination results showed that the PLGA material stayed in comparatively long period; becomes organization of replaced granulation tissue still not completed.

REFERENCES

- Okafuji N, Shimizu T, Watanabe T, Kimura A, Kurihara S, Arai Y, Furusawa K, Hasegawa H, Kawakami T: (2006) Three-dimensional observation of reconstruction course of rabbit experimental mandibular defect with rhBMP-2 and atelocollagen gel. *Eur J Med Res* 11(8): 351-354
- Kinoshita Y, Kobayashi M, Hidaka T, Ikada Y: (1997) Reconstruction of mandibular continuity defects in dogs using poly (L-lactide) mesh and autogenic particulate cancellous bone and marrow: preliminary report. *J Oral Maxillofac Surg*. 55: 718-723; discussion 723-714
- Yasko AW, Lane JM, Fellingner EJ, Rosen V, Wozney JM, Wang EA: (1992) The healing of segmental bone defects, induced by recombinant human bone morphogenetic protein (rhBMP-2). A radiographic, histological, and biomechanical study in rats. *J Bone Joint Surg Am*. 74: 659-670
- King GN, King N, Hughes FJ: (1998) Effect of two delivery systems for recombinant human bone morphogenetic protein-2 on periodontal regeneration in vivo. *J Periodontol Res*. 33: 226-236
- Seto I, Asahina I, Oda M, Enomoto S: (2001) Reconstruction of the primate mandible with a combination graft of recombinant human bone morphogenetic protein-2 and bone marrow. *J Oral Maxillofac Surg*. 59: 53-61; discussion 62-53
- Kent JN, Finger IM, Quinn JH, Guerra LR: (1986) Hydroxylapatite alveolar ridge reconstruction: clinical experiences, complications, and technical modifications. *J Oral Maxillofac Surg*. 44: 37-49
- Dahlin C, Linde A, Gottlow J, Nyman S: (1988) Healing of bone defects by guided tissue regeneration. *Plast Reconstr Surg*. 81: 672-676
- Hammerle CH, Schmid J, Lang NP, Olah AJ: (1995) Temporal dynamics of healing in rabbit cranial defects using guided bone regeneration. *J Oral Maxillofac Surg*. 53: 167-174

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