Bone Regeneration with Local Controlled Application of BMP-2 from a biodegradable sponge composed of gelatin and βTCP in a bone defect of Rabbit Ulna

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Introduction
To overcome the problems to treat bone defects, bone tissue engineering has been attracted much attention as a new therapeutic technology which induces bone regeneration by making use of osteoinductive growth factors and scaffolds or their combination. Yamamoto et al have reported a successful drug delivery system using unique biodegradable sponge incorporating bone morphogenic protein-2 (BMP-2) which could control the release of BMP-2 for a prolonged time in an in vivo environment, leading to an enhanced ectopic bone formation1 and bone regeneration2. In addition, biodegradable sponge composed of gelatin and β-tricalcium phosphate (βTCP), gelatin-βTCP sponge, was also fabricated to investigate more ideal scaffold to enhance bone regeneration and reported3. The purpose of this study was to investigate the effectiveness of this gelatin-βTCP sponge for the promotion of bone regeneration at a bone defect site in vivo environment.

Materials and methods
Gelatin sponges (Nitta Gelatin Co., Osaka Japan) incorporating βTCP (Taihei Chemical Industries, Nara, Japan) were prepared by chemical crosslinking of gelatin with glutaraldehyde in the presence of βTCP at 50 weight percentage according to the method described previously1,3. To prepare the gelatin hydrogel incorporating BMP-2, 100µL of phosphate-buffered saline solution (PBS, pH7.5) containing 17µg of BMP-2 was dropped onto freeze-dried gelatin hydrogel and left overnight at 4°C. Similarly, 100µL of BMP-2-free PBS was dropped onto a freeze-dried hydrogel to obtain the BMP-2-free empty hydrogel. To investigate the effectiveness of βTCP and BMP-2 with this material for the promotion of bone regeneration at the fracture site in vivo environment, we used a segmental ulna bone defect model of skeletally mature Japanese white rabbit (Kitayama Labs, Nagano, Japan), according to the surgical procedure previously reported1 and divided them into four groups: Group1; treated with gelatin sponge only, Group2; treated with gelatin sponge incorporating BMP-2, Group3; treated with gelatin sponge incorporating BMP-2, Group4; treated with gelatin-βTCP sponge incorporating BMP-2. We evaluated the healing of fracture radiographically (performed by an X-ray), histologically (stained with hematoxylin, eosin and alcan blue) and biomechanically (performed by a standardized three-point bending test).

Results
Radiographical findings
Radiographs at 4 and 8 weeks after surgery are shown in Figure 1. It is apparent that bone regeneration at the bone defect of ulna was detected radiographically in the group of 3 and 4. On the other hand, no bone formation was radiographically observed at the bone defects in the group of 1 and 2.

Histological findings
Figure 2 shows histological sections of ulna defects at 4 and 8 weeks after surgery. The degree of fracture healing was evaluated using a five point scale proposed by Allen et al2. The bone defect was histologically occupied by newly regenerated bone tissue in group 3 and 4. The score of fracture healing was better in group 3 than in group 4 at 4 weeks after surgery. To the contrary, no bone regeneration was detected at the defect in group1 and 2.

Biomechanical findings
Figure 3 shows the data of three-point bending test at 4 and 8 weeks after surgery. The group 4 showed the greater tendency of the fracture load, deflection at fracture, the stiffness and the toughness than group 3 at 8weeks after surgery, however there was no significant difference between them.

Discussion
The present study demonstrated the in vivo bone regeneration at the critical-sized bone defect of rabbit ulna induced by gelatin sponge and gelatin-βTCP sponge incorporating BMP-2. No apparent bone regeneration was induced by the gelatin and gelatin-βTCP without BMP-2 incorporation, indicating that BMP-2 was necessary to heal the critical-sized bone defect. To investigate the quality of the regenerated bone, we also evaluated it biomechanically with three-point bending test. We found no significant difference between groups of gelatin sponge and gelatin-βTCP sponge incorporating BMP-2. Incorporation of biodegradable βTCP granules has been reported to give an enhanced resistance to compression to the gelatin sponge3 and the gelatin-βTCP sponge was expected to enhance biomechanical strength during the initial bone regeneration. Our data showed that the gelatin-βTCP sponge did not impair the effectiveness for the promotion of bone regeneration by BMP-2 in the bone defect. However it did not significantly improve the quality of regenerated bone from the view point of biomechanical assessment.

Reference:
2. Yamamoto M et al. Biomaterials 2005
3. Takahashi Y et al. Biomaterials 2005