Repair of Osteochondral Defects Using Porous Hydroxyapatite Collagen composite Impregnated with Bone Morphogenetic Protein-2

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Introduction: Articular cartilage is an avascular tissue with low mitotic activity. Thus, damaged lesions in the articular cartilage are difficult to repair spontaneously. We previously reported good osteochondral defect repair using FGF-2 and porous hydroxyapatite collagen (HAp/Col), which we originally developed as a new bone void filler. Recently, several authors reported that bone morphogenetic proteins (BMPs) promote cartilage regeneration. Additionally, porous HAp/Col has a high affinity for BMP and can absorb a large amount of BMP, suggesting that porous HAp/Col is a suitable carrier for BMPs. These characteristics of HAp/Col may be effective for providing the sustained release of BMPs. Therefore, we hypothesized that porous HAp/Col impregnated with even a low dose of BMP would promote regeneration within an osteochondral defect. In the present study, we investigated the effect of porous HAp/Col impregnated with recombinant human BMP-2 (rhBMP-2) on the healing of a full-thickness osteochondral defect.

Methods: In this study, we used a cylindrical porous HAp/Col (5 mm in diameter and 3 mm in height, volume: 58.875 mm³, porosity: 95%). All animal experiments were performed after obtaining approval from the Animal Experiment Committee of Tokyo Medical and Dental University and were conducted in accordance with the guidelines on the protection and use of laboratory animals. [Measurement of BMP-2 adsorbed to HAp/Col] The quantity of rhBMP-2 that can be adsorbed to porous HAp/Col was evaluated. Briefly, a porous HAp/Col was impregnated with a BMP-2 solution including 25 μg of rhBMP-2 and then incubated at 37°C for 30 minutes. HAp/Col impregnated with BMP-2 was crushed in fetal bovine serum (FBS) by repeated compression, and the concentration of BMP-2 released from the HAp/Col into the serum was measured using ELISA. The total amount of BMP-2 adsorbed on the HAp/Col was calculated. [Transplantation of porous HAp/Col impregnated with BMP-2 into osteochondral defects in rabbits] A full-thickness osteochondral defect, 5 mm in diameter and 5 mm deep, was created at the trochlear groove of male Japanese white rabbits using a trephine drill and a steel bur. Animals were assigned to one of four treatment groups: the defect was left empty (defect group,n=6), filled with HAp/Col (B-0 group,n=6), filled with HAp/Col impregnated with 5 μg of rhBMP-2 (B-5 group,n=6), or filled with HAp/Col impregnated with 25 μg of rhBMP-2 (B-25 group,n=6). Animals were sacrificed at 3, 6, 12, and 24 weeks after the operation, and the repaired tissue was evaluated carefully. The evaluation included macroscopic observation and histological observation using hematoxylin and eosin, toluidine blue, and immunohistochemical staining for Type 1 & 2 collagens. For a semiquantitative analysis of the repaired tissue, histological sections were scored blindly by two expert observers according to a modified version of the histological grading scale, as described by Wakitani et al. The subchondral bone repair was evaluated using micro-CT images. The statistical analysis involved two-factor ANOVA followed by a multiple comparison test using the Tukey HSD test. A p value of less than 0.05 indicated a significant difference.

Results: [Quantity of BMP-2 adsorbed to HAp/Col] The ELISA assay showed that the porous HAp/Col could absorb 361.8 μg (14.8%) of rhBMP-2 per 1 cm³ even after the compression procedure in FBS. [Transplantation of porous HAp/Col impregnated with BMP-2] The macroscopic observation revealed that the defect in the defect group and the B-0 group was not fully covered by any tissue even at 24 weeks, whereas no cleft was observed in the defects treated with rhBMP-2 (fig1). In the micro-CT analysis, subchondral bone regeneration was very poor in the defect group throughout the experimental period, and abundant formation of regenerated subchondral bone was observed in the HAp/Col transplanted groups at each time point. However, significantly more subchondral bone was regenerated in the BMP-2 groups compared with the B-0 group, and there were no differences between the BMP-treated groups (fig2). No ectopic ossification was observed around the transplanted site in all samples. The histological evaluation showed that, at 3 weeks after surgery, fibrous tissue-like repair was observed in both the untreated defects and the defects of the B-0 group. However, the defects treated with rhBMP-2 were replaced with toluidine blue-stained tissue, which was also positive for type II collagen, as determined by immunostaining (fig3). At 24 weeks, no toluidine blue-stained tissue was observed in the defect and B-0 groups. In contrast, metachromasia was observed in the defects...
treated with rhBMP-2 (fig5). The histological scores of the BMP-2-treated groups were higher than in the untreated defect and the B-0 groups throughout the study. At 24 weeks, the score of the B-5 group was the highest among the groups. However, the score for the group treated with 25 μg of rhBMP-2 were lower at 24 weeks than at 6 weeks (fig6).

**Discussion:** The present study showed that porous HAp/Col adsorbed a large amount of rhBMP-2, indicating that porous HAp/Col would maintain a high BMP-2 concentration around the implant and that porous HAp/Col is a suitable carrier for rhBMP-2. BMP-2 was identified as a bone-inducing factor, and the clinical application of BMP for orthopedic surgery has begun in the USA. However, BMP is not widely used yet because of several side effects, which may be caused by the use of excessive amounts of BMPs. Hydroxyapatite has a strong affinity for BMPs, and in this study, porous HAp/Col adsorbed a large amount of BMP-2, even in the presence of serum. This fact suggests that porous HAp/Col may be a suitable carrier for BMPs, which would decrease the complications caused by BMPs. The histological evaluation revealed that the defect treated with 5 μg of rhBMP-2 showed the highest histological score. However, the osteochondral defects were not repaired with perfect hyaline-like chondral tissue in the groups treated with rhBMP-2. A previous report mentioned that the histological scores of defects treated with high doses (20 and 40 μg) of rhBMP-2 were lower at later time points than at early time points. The long-term effects of BMP-2 may cause excessive differentiation of the mesenchymal stem cells or progenitor cells that repair the cartilage tissue defects at early time points. However, the present study indicates that rhBMP-2 is effective for rigid subchondral bone repair, which is important for the repair of the smooth articular surface. To achieve better cartilage repair, supplying the appropriate quantity of BMPs at specific time points and including the supportive effects of additional growth factors might be necessary.

**Significance:** Articular cartilage injury is quite common and affects the patient’s QOL. In this study, we showed that transplanting porous HAp/Col impregnated with an appropriate amount of BMP-2 contributes to complete osteochondral defect repair.

**Acknowledgments:**

**References:**