Repair of Articular Cartilage Defects with a Novel Injectable In-situ Forming Material in a Canine Model

INTRODUCTION: Autologous chondrocyte implantation (ACI) is an ideal procedure for articular cartilage defects. However, recent comparative clinical trials have demonstrated no significant superiority of this procedure over other procedures. A potential reason for this unexpected outcome is the adverse effects resulting from invasive procedures such as periosteal coverage and wide arthrotomy, and from donor site morbidity. To overcome these limitations, we developed a cellular implantation system of bone marrow stromal cells (BMSCs) using a novel in-situ forming material based on alginate gel. Additionally, for its clinical application, the endotoxicity of this material was successfully reduced by the ultra-purification of alginate. Our previous study demonstrated that autologous BMSCs implantation using this system enhanced cartilage repair in osteochondral defects in rabbits [1]. For our novel system to become a clinical reality, we need to evaluate this technique using a larger animal model that more closely relates to the human clinical setting than does the rabbit model. The aims of this study were to evaluate the potential of treatment with BMSCs; implants using the current cellular vehicle system to enhance cartilage repair in a canine model and to determine whether this system was able to be arthroscopically performed in cadaveric knees.

METHODS: Material Preparation: A novel in situ forming material based on purified sodium alginate with quite a low endotoxin level of 5.76 EU/g (Sea Matrix, Mochida Pharma. Co. Ltd., Tokyo, Japan) was used in this study.

Cartilage Repair Model: According to ethical guidelines approved by the local animal care committee, autologous BMSCs were isolated by monolayer culture of bone marrow aspirated from 3-month-old beagle dogs. Two osteochondral defects (55 mm) were created in the patella groove of each beagle dog. The defects were filled with the developed material containing 2.5 x 10^7 autologous BMSCs/ml. Without any additional fixation to the implantation sites, the wound was closed in layers. The defects were divided into the following three groups (n=16 in each): defect group; material group, material implantation without BMSCs; material with BMSCs group, material embedded with BMSCs implantation.

Evaluation of Reparative Tissue: Dogs were euthanized at 16 weeks postoperatively. The sections of the center of each defect were stained with safranin-O and H-E. The reparative tissue were evaluated by microscopic and histological scoring [2]. The compressive modulus (MPa) of each sample was measured by an indentation test.

Histological Assessment: The histological scores were significantly higher in the material treatment groups than the defect group (p<0.05, Table 1). The defect group showed fibrous tissues and degenerative changes in the adjacent cartilage. The defect sites in the material group were mainly repaired with fibrocartilage (Fig. 1A). The material with BMSCs group exhibited normal cartilaginous tissue with a rich GAG matrix (Fig. 1C). Regarding the statistical comparison of each category, the material with BMSCs group significantly enhanced the reconstruction of subchondral bone (p<0.01, vs the defect group; p<0.05 vs the material group). No apparent infiltration of inflammatory cells at defect sites was found in the material treatment groups.

Mechanical Property: The compressive modulus in the material with BMSCs group was significantly higher, compared to that in other groups (p<0.05, Table 2), and approximately reached 75% of normal cartilage. BMSCs implanted using the current cellular vehicle system to enhance cartilage repair in osteochondral defects by standard arthroscopic technique. At 24 hours after implantation, the implanted material remained at the defect site and maintained its initial shape and hardness (Fig. 3).

RESULTS: Overall Scores: The mean overall scores, including histological and macroscopic scores, in the material treatment (material and material with BMSCs) groups significantly increased, compared to those in the defect group (p<0.01, vs material with BMSCs; p<0.05, vs material group, Table 1). No significant difference in the scores was found between the material treatment groups.

Macroscopic Assessment: The mean macroscopic scores of material treatment groups were significantly superior to the defect group (p<0.01, vs material with BMSCs; p<0.05, vs material group). In the defect group, the surface was rough and depressed. In contrast, the surface in the material treatment groups was smooth and continuous with the adjacent tissue. The material with BMSCs group showed firm and glossy white cartilage-like tissue at the implantation site.

REFERENCES:

DISCUSSION: The current study showed that the implantation of BMSCs using a novel ultra-purified in situ forming material induced matured hyaline-like cartilage repair of osteochondral defects in a canine model. Quite a low endotoxicity of this material was able to prevent the infiltration of inflammatory cells, which evoke an immunological reaction against the implanted material, at defect sites. Furthermore, we successfully established an arthroscopic injectable implantation technique with this in situ forming material using human cadaveric knees. The results obtained here indicate that our cellular implantation system can reduce the adverse effects of current invasive ACI techniques and provide significant advantages for patients, including a shorter postoperative recovery period and an earlier return to daily activities.