Bone Marrow Stimulation Technique Augmented By Ultrapurified Alginate Gel Enhances Osteochondral Repair In A Rabbit Osteochondral Defect Model

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Introduction: The osteochondral repair procedures such as microfracture, osteochondral transplantation and ACI (Autologous Chondrocyte Implantation), are indicated for osteochondral injury. Although ACI is desirable, especially for relatively larger defects, this technique requires a two-step operation including donor cell harvesting. Bone marrow stimulation technique is characterized by technical simplicity and is less-invasiveness. However, it has been shown to result in fibrocartilage with inferior long-term results in comparison to original hyaline-like cartilage. In order to overcome the disadvantage of this bone marrow stimulation technique, an advanced technique is required. We previously reported a novel cellular implantation system using an injectable ultrapurified alginate gel (UPAL gel) [1, 2]. UPAL gel enhanced the cellular proliferation and chondrogenic differentiation of bone marrow derived mesenchymal stem cells (BM-MSCs), and the implantation of UPAL gel improved the reparative tissues in rabbit osteochondral defects [1]. In addition, UPAL gel containing Stromal cell-derived factor-1 (SDF-1) enhanced the recruitment of BM-MSCs and successfully achieved hyaline-like cartilage repair without BM-MSCs transplantation [2]. Based on these studies, we focused on using UPAL gel as an adjuvant scaffold in combination with a bone marrow stimulation technique. We hypothesized that bone marrow stimulation technique augmented by UPAL gel will enhance cellular differentiation resulting in better osteochondral repair. The objective of this study was to assess the efficacy of bone marrow stimulation technique augmented by UPAL gel in a rabbit osteochondral defect model.

Methods: An in situ forming material based on UPAL gel was used in this study. A full-thickness osteochondral defect 4.0 mm in diameter and 2.0 mm in depth was created in the patella groove in 30 male Japanese white rabbits. For bone marrow stimulation technique, seven holes were drilled into defect using a 0.5 mm-diameter drill. We divided the osteochondral defects into three groups as follows (n = 20 knees of 10 rabbits in each group): the defects without intervention (Group D, Fig.1A), the defects with bone marrow stimulation technique (Group MS, Fig.1B), and the defects with bone marrow stimulation technique and UPAL gel (Group MSG, Fig.1C). Fifteen rabbits were evaluated at the 4 weeks and the other 15 rabbits were evaluated at the 16 weeks. At each period of time, 10 knees of 5 rabbits in each group were used for macroscopic evaluation and 5 knees of them were used for histological evaluation. Macroscopic score and histological score for joint surface repair were evaluated as previously reported [3]. Mean scores were statistically compared by non-parametric Kruskal-Wallis tests between groups. Significance was accepted with a p value < 0.05.

Results: There were no perioperative complications and macroscopic signs indicating infection, including excessive joint fluid or purulent synovitis at each period of time. After 4 weeks of operation, the defects in Group MS and Group MSG had better covering with reparative tissues than in the Group D (Fig. 2A-C).
The macroscopic scores of Group MS (mean ± SD; 1.70 ± 0.95 [P = 0.0134]) and Group MSG (2.30 ± 1.82 [P = 0.0058]) were significantly higher than that of Group D (0.40 ± 0.70) (Fig. 2G). In the Group MSG, the defects were partially filled with hyaline-like cartilage. The histological scores of Group MSG (7.20 ± 1.92) were better than those of Group D (5.40 ± 2.88) and Group MS (5.60 ± 1.52) without statistical significances (Fig. 2I). After 16 weeks of operation, the reparative tissues of the Group D and Group MS were insufficient, and covered with cracked tissues (Fig. 2D,E). On the other hand, the defects of the Group MSG were covered with abundant transparent tissues (Fig. 2F). The macroscopic scores of Group MSG (5.60 ± 1.17) were significantly higher than those of Group D (2.20 ± 1.03 [P = 0.0005]) and Group MS (3.50 ± 1.18 [P = 0.0057]) (Fig. 2H). The histological scores of Group MSG (13.60 ± 2.88) were significantly higher than those of Group D (5.20 ± 2.05 [P = 0.0234]) and Group MS (7.00 ± 3.08 [P = 0.0422]) (Fig. 2J).

**Discussion:** Bone marrow stimulation technique augmented by UPAL gel elicited hyaline-like cartilage repair compared to bone marrow stimulation technique alone. Although bone marrow stimulation technique augmented by scaffolds is currently developed as one-step methods, most need to fix the materials to the defect with pins, sutures or glue. Our technique successfully filled the drilled defect with UPAL gel without any fixing material. Previously, we demonstrated that host cells from bone marrow were able to penetrate into the defect filled with UPAL gel, and local administration of SDF-1 enhanced host cells migration[2]. Based on these results, we speculate that the recruitment of host BM-MSCs caused by a bone marrow stimulation technique and the chondrogenic differentiation by UPAL gel resulted in better osteochondral repair in this study. In conclusion, the bone marrow stimulation technique augmented by UPAL gel had a positive effect on the reparative tissues in the rabbit osteochondral defects.

**Significance:** The augmentation by UPAL gel in bone marrow stimulation technique enhanced osteochondral repair rather than bone marrow stimulation technique alone in rabbit osteochondral defect models. This method has the potential of improving the clinical outcome after bone marrow stimulation technique in human articular cartilage injury.
Figure 1. The procedures for bone marrow stimulation technique and the injectable implantation system using UPAL gel. Cylindrical defect was created on the patellar groove (A), seven holes of subchondral penetration were made in the defect (B), gelation of the superficial layer of the implanted alginate by putting CaCl₂ on (C).

Figure 2. Macroscopic morphology of osteochondral defect at 4 weeks (A-C) and 16 weeks (D-F) postoperatively. Macroscopic scores at 4 weeks (G) and 16 weeks (H) postoperatively (n = 10 at each time point). Histological scores at 4 weeks (I) and 16 weeks (J) postoperatively (n = 5 at each time point). Niederauer's macroscopic scoring scale range: 0-8. Niederauer's histological scoring scale range: 0-18. Values are mean and SD. *p < 0.05, **p < 0.01