INTRODUCTION: Cartilage tissue engineering, which is also referred to as autologous chondrocyte implantation (ACI), is one of the most potent therapies for articular cartilage defects. However, recent clinical trials have shown no significant superiority of ACI over other techniques [1, 2]. One reason for this unexpected outcome is considered to be adverse effects resulting from its invasiveness, including chondrocyte harvesting, periostial coverage, wide arthroscopy, and multiple operations. To overcome these limitations, we developed an in situ forming gel based on alginate [3]. Our previous results led to a scenario in which, if host bone marrow stromal cells (BMSCs) were effectively recruited to the defects, acellular ultra-purified alginate gel (UPAL gel) implantation could improve reparative tissue of osteochondral defects [3]. Stromal cell-derived factor-1 (SDF-1) acts as a key chemokine of stem cell homing to bone marrow for tissue repair after injury [4]. A recent study suggested dose-dependent migration potential of human BMSCs in response to SDF-1 stimulation with UPAL gel [5]. Our hypothesis was that the combined administration of SDF-1 and UPAL gel could enhance the repair of osteochondral defects by recruiting host BMSCs to the defect site, even through acellular implantation. The objective of this study was to determine whether the local administration of SDF-1 using UPAL gel could improve reparative tissues of osteochondral defects compared with those without treatment.

METHODS:

Preparation of alginate gel: Ultra-purified alginate gel with a molecular weight of 1,700 kDa (endotoxin level of 5.76 EU/g; Sea Matrix®, Mochida Pharmaceutical Co., Ltd., Tokyo, Japan) was used in this study. In vivo study: Expression of SDF-1 protein at the site of the osteochondral defect. All procedures were performed under established ethical guidelines approved by the local animal care committee. An osteochondral defect 4.5 mm in diameter and 3 mm deep was created in the patella groove. The expression of SDF-1 at the site of the osteochondral defect was analyzed by both immunohistochemistry and Western blotting. Cell homing assessment. To evaluate cell homing into the defect site after local administration of SDF-1, the number of cells that migrated into the implanted site of UPAL gel was counted. The experimental vehicle, SDF-1, and AMD3100 groups are described below. Rabbit cartilage repair model. The defects were divided into 4 groups as follows: defect group, no treatment; vehicle group, UPAL gel containing bovine serum albumin; SDF-1 group, UPAL gel containing SDF-1; and AMD3100 group, UPAL gel containing AMD3100, an antagonist of CXCR4. Macroscopic, histological and immunohistochemical evaluations. At 16 weeks after operation, the center of each defect was stained with safranin-O. The macroscopic and histological findings were scored with 8-point and 28-point grading scales [6]. Immunohistochemical staining was performed with anti-type II and I collagen antibodies. Mechanical properties. The mechanical properties were measured using an indentation test.

In vitro study: Cell viability assay. BMSCs encapsulated in purified alginate beads were cultured in DMEM with 10% FBS. The number of viable cells was counted with Cell Counting Kit-8. Cell migration assay. Cell migration was measured using the CytoSelect™ cell migration assay module. Immunohistochemical differentiation of BMSCs in UPAL gel. Forty-μm thick sections of UPAL gel containing 1 x 10⁶ BMSCs were cultured in chondrogenic medium containing TGF-β3 for histological and immunohistochemical evaluations.

Statistical Analysis: All data are presented as mean ± standard error. Significant differences among 3 or more groups were assessed by one-way ANOVA followed by multiple-comparison post hoc tests. Comparisons between 2 groups were performed using unpaired t-tests.

RESULTS:

In vivo study: Expression of SDF-1 protein at the site of the osteochondral defect. Immunohistochemical analysis revealed cells expressing SDF-1 protein in the injury site at 1 week after creation of the osteochondral defect (Fig. 1A), whereas the expression was not found at 3 hours, 2 weeks, or 4 weeks after injury. After sham operation, no local expression of SDF-1 protein was observed at any time point. Western blot analysis showed enhanced expression of SDF-1 protein only in the tissue obtained at 1 week after injury (Fig. 1B). Cell homing assessment. Histological findings of the SDF-1 group showed more host cells in the rabbits with UPAL gel implanted into the osteochondral defect than in the other groups (n=5; p<0.01 vs. the vehicle group; p<0.001 vs. the AMD3100 group). Gross morphology. The defects were entirely replaced with cartilage-like tissue only in the SDF-1 group (Fig. 2). The scores in the SDF-1 group were significantly higher than in the other groups (Fig. 3). Histological findings. SDF-1 improved the reparative tissue compared to other groups (Fig. 3). This treatment group exhibited nearly normal cartilaginous structures and strong type II collagen staining, reconstruction of the normal subchondral bone structure, a smooth cartilage surface, and a tidemark. Type I collagen staining was not found in the cartilaginous layer. The neocartilaginous reparative tissue was integrated into the adjacent cartilage and bone. The total histological scores were significantly higher in the SDF-1 group than in the other groups. A limitation of this study was that we speculate that UPAL gel enhances chondrogenesis of BMSCs recruited by the chemotactic effect of SDF-1.

DISCUSSION: The current results showed that the SDF-1 treatment (UPAL gel containing SDF-1) histologically and biomechanically enhanced the reparative process of osteochondral defects. We can speculate that UPAL gel enhances chondrogenesis of BMSCs recruited by the chemotactic effect of SDF-1. A limitation of this study was that macroscopic and histological analysis was performed using rabbit samples at 16 weeks postoperatively. Long-term assessments using a large animal model need to be performed to adapt our approach for use in humans.

SIGNIFICANCE: The cell-free approach with local administration of SDF-1 may be an effective strategy for developing a minimally invasive technique for cartilage tissue regeneration.