THE REPAIR OF FULL-THICKNESS ARTICULAR CARTILAGE DEFECTS WITH CULTURED CHONDROCYTES EMBEDDED IN PROTEOGLYCAN-CONTAINING ATELLOCOLLAGEN GEL IN THE RABBIT MODEL

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Introduction
In the articular cartilage, chondrocytes are surrounded by various extracellular matrices such as collagens, proteoglycan (PG), hyaluronan (HA) and noncollagenous proteins. These extracellular matrices maintain homeostasis of cartilage tissue through not only protecting the chondrocytes from mechanical damage by load to joint, but also by regulating modulators such as cytokine and growth factor to maintain cell function. Recent studies have demonstrated that type II collagen and HA were useful as a cell scaffold material for cartilage repair, but no studies have investigated whether or not PG is a useful material. Our previous in vitro study has demonstrated that PG treatment increased mRNA expression of aggrecan in rabbit articular chondrocytes embedded in atelocollagen gel and PG-treated chondrocytes showed a prominently increased alcinian blue positive matrix [1]. The purpose of this in vivo study is to investigate whether or not PG-containing atelocollagen gel embedding chondrocytes enhance the repair of full-thickness articular cartilage defect in rabbit knee.

Materials and Methods
All animal experiments in this paper followed the Guidelines for Animal Experimentation of Hirosaki University.

Chemical characterization of PG
PG was prepared from salmon nasal cartilage. The approximate protein content of the PG was 7%, and was consistent with that of PG from bovine nasal cartilage and Swann rat chondrosarcoma. Glycosaminoglycan from salmon nasal cartilage PG was composed of 58% 6-sulfated unsaturated disaccharide units, 26% 4-sulfated unsaturated disaccharide units, 8.6% nonsulfated unsaturated disaccharide units and 7% disulfated unsaturated disaccharide units.

Isolation and culture of chondrocytes
Chondrocytes isolated from the articular cartilages of Japanese white rabbits were maintained in monolayer culture for 5 days. Then, chondrocytes were released by trypsin-EDTA treatment and embedded in atelocollagen gel (Koken, Japan) mixed with PG (0, 100, 1000 µg/ml PG) at a concentration of 2×10⁵ cells/ml. A 50 µl sample of this mixture was placed on 35-mm diameter culture dishes and then gelled by incubation at 37°C for 30 min. The mixtures were cultured for 3 weeks before transplantation.

Chondrocyte transplantation
Under general anesthesia, full-thickness articular cartilage defects (5 mm in diameter and 3 mm in depth) were created in the trochlea groove of the femur in the rabbits. Chondrocytes embedded in PG-containing atelocollagen gel were transplanted into the defects. A periosteal flap was harvested from the ipsilateral anteromedial aspect of the tibia and the defects were then covered with the periosteal flap, the cambium layer of which was facing patella. After the operation, all animals were allowed to walk freely in their cages without splints.

Macroscopic and histological evaluation
The rabbits were killed at 12 weeks after the operation. The seven defects of each group were examined macroscopically. Then, the distal femurs were dissected and were fixed. After decalcification, they were embedded in paraffin. Four µm-thick sagittal sections were obtained from the center of the each defect and were stained with safranin O-fast green. Histological findings were scored according to a modification of the histological grading scale described by Wakitani [2]. The data were analyzed by one-way ANOVA followed by Scheffe test. Differences were considered significant for P<0.05.

Results
Macroscopic observations
Macroscopically, all defects were filled with the reparative tissue, which had a white, smooth, and glossy surface at 12 weeks. The new tissue showed almost the same hardness surrounding host cartilage. Although they appeared to integrate with the surrounding cartilage in most cases, one case of 1000 µg/ml PG group was poorly integrated with the surrounding cartilage. There were no signs of synovitis, adhesions, or adverse reactions.

Histological examinations
The defects were filled with small and round cells and rich extracellular matrices stained with safranin O at 12 weeks (Fig. 1). Although some cases of 1000 µg/ml PG group were stained better than 0 and 100 µg/ml PG group, surface regularity and integration with host cartilage of them was worse. Although histological scores for each group were not statistically different, the total score of 100 µg/ml PG group was the lower than that of 0 and 1000 µg/ml group (Table 1).

Fig 1. The representative histological findings at 12 weeks after the operation. Sections were stained with safranin O-fast green (A; 0 µg/ml PG, B; 100 µg/ml PG, C; 1000 µg/ml PG). Original magnification: (A-C) ×100.

Table 1 Mean value of histological grade of the reparative tissue

<table>
<thead>
<tr>
<th>Category</th>
<th>0 µg/ml PG (n=7)</th>
<th>100 µg/ml PG (n=7)</th>
<th>1000 µg/ml PG (n=7)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cell morphology</td>
<td>0.86 ± 0.38</td>
<td>0.71 ± 0.49</td>
<td>1 ± 0.59</td>
</tr>
<tr>
<td>Matrix staining</td>
<td>1.14 ± 0.69</td>
<td>0.86 ± 0.38</td>
<td>0.86 ± 0.90</td>
</tr>
<tr>
<td>Surface regularity</td>
<td>1 ± 0.82</td>
<td>0.86 ± 0.69</td>
<td>1.57 ± 0.98</td>
</tr>
<tr>
<td>Thickness of cartilage</td>
<td>0.86 ± 0.90</td>
<td>0.14 ± 0.38</td>
<td>0.43 ± 0.53</td>
</tr>
<tr>
<td>Integration of donor with host adjacent cartilage</td>
<td>0.71 ± 0.49</td>
<td>0.86 ± 0.69</td>
<td>1.14 ± 0.69</td>
</tr>
<tr>
<td>Average total score</td>
<td>4.57 ± 1.90</td>
<td>3.43 ± 1.40</td>
<td>5 ± 1.53</td>
</tr>
</tbody>
</table>

Discussion
In this study, we demonstrated that exogenous cartilage PG lowered histological score of reparative tissue. These results suggest that PG enhance the repair of full-thickness articular cartilage defect in rabbit knee. However, from our results that high concentration PG heightened histological score of reparative tissue, we suspect that high concentration PG inhibit the repair of articular cartilage defect. Our previous study has demonstrated that high concentration PG enhanced chondrocyte proliferation in both two-dimensional and three-dimensional culture [1]. Enhancement of chondrocyte proliferation by PG may induce chondrocyte dedifferentiation. We expect that exogenous cartilage PG may be useful for application to cartilage regeneration using chondrocytes as a cell scaffold material that enhances the repair of articular cartilage defect. Therefore, we need to fully investigate the effect of PG on chondrocyte metabolism as well as chondrocyte differentiation by the glycoconjugate approach.

References

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