Induction of Spontaneous Hyaline Cartilage Regeneration Using a Double-network Gel: The Efficacy of a Novel Therapeutic Strategy for an Articular Cartilage Defect

INTRODUCTION: The most prevalent strategy to repair the articular cartilage defect is to fill an osteochondral defect with a tissue-engineered cartilage-like tissue or a cell-seeded scaffold material [1]. However, it has been pointed out that this strategy has many problems in clinical practice [2-4]. To solve such problems, we have developed an innovative method to induce spontaneous hyaline cartilage regeneration in vivo in a large osteochondral defect by implanting an originally developed PAMPS/PDMAAm double-network (DN) gel at the bottom of an osteochondral defect [5]. However, because the power of the statistical analysis in our previous study was insufficient, the efficacy of the above-described potential treatment to induce the spontaneous cartilage regeneration using the DN gel has not been established as of yet. In addition, as the previous study showed only the 4-week results, the long-term results should be clarified. Therefore, we have investigated the efficacy of this novel method using a sufficient number of animals at 4 and 12 weeks after surgery.

METHODS: A total of 76 mature female Japanese white rabbits, weighing 3.6 ± 0.5 kg, were used in this study. An osteochondral defect having a 4.3-mm diameter was created in the femoral groove of the right patellofemoral joint. A cylindrical DN gel plug was implanted into the defect so that a defect having 2-mm depth remained after surgery. The depth of 2 mm was chosen because this depth was the most effective to induce the spontaneous cartilage regeneration in our previous preliminary study [5]. In the left knee, an osteochondral defect with the same diameter was created and remained without any treatment. Thirty-seven animals were sacrificed at 4 and 12 weeks after implantation, respectively. Thirty of the 37 animals were used for histological and immunohistochemical evaluations, and another 4 of the 37 rabbits were used for surface assessment of the regenerated tissues using confocal laser scanning microscopy (CLSM). The remaining 3 were used for evaluation of gene expression of cartilage markers using real-time PCR analysis. Four intact knees from the 2 rabbits were used as normal control for surface observations with CLSM. [Macroscopic, histological and immunohistochemical examinations] The tissue regenerated in the defect was quantitatively evaluated with 2 scoring systems; the Wayne’s grading scale [6] and the O’Driscoll categorical grading scale [7]. Knee specimens were sliced from the center of each defect and stained with HE and Safranin-O. For immunohistochemical evaluations, monoclonal antibody (anti-hcCLIII), purified IgG, (Fuji Chemical Industries Ltd, Japan) was used as primary antibodies. [CLSM] The surface was observed with CLSM (OLS1200, Shimazu Corp, Kyoto, Japan). For each specimen, images of a scan size of 120 x 90 μm were obtained at three locations of the center of the defect surface. Scanned surface was observed to quantitatively analyze concerning the surface roughness. [Real time PCR] Total RNA was extracted from the tissues regenerated in the defect. The real time PCR for GAPDH, type-2 collagen and aggrecan was performed in Thermal Cycler Dice TP800 (TakaraBio, Japan) by using SYBR Premix Ex Taq (TakaraBio, Japan). The expression level of the gene was normalized to GAPDH. [Statistical analysis] For statistical comparisons, the ANOVA and the paired t test were used. The significance limit was set at p=0.05.

RESULTS: [Macroscopic, histological and immunohistochemical examinations] At 4 weeks, the defect was filled with a sufficient volume of the proteoglycan-rich tissue with a regenerated bone tissue resembling the normal subchondral bone in the DN gel-implanted knee. On the other hand, all defects in the untreated control were insufficiently filled with fibrous and bone tissues and were not stained with Safranin-O (Fig 1). At 12 weeks, the DN gel-implanted defect was filled with a hyaline-like cartilage tissue. In contrast, no staining with Safranin-O was found in the untreated defect (Fig 1). Immunohistochemical staining for type-2 collagen were consistent with Safranin-O staining. Histological evaluation using the grading scales revealed a significant higher score in the DN gel-implanted knees compared to the untreated defects (control) (p<0.0001) (Fig 2). [Real time PCR] The mean relative values of type-2 collagen mRNAs in the regenerated tissue were obviously higher in the DN gel-implanted defect than in the untreated defect at each period (Fig 3). [CLSM] The mean value tended higher in the DN gel-implanted knee than the untreated control, but there was no statistically difference between the DN gel-implanted knee and the untreated control. The mean surface roughness of the untreated control was significantly higher than the normal cartilage at 12 weeks (p<0.0106) (Fig 4, 5).

DISCUSSION: The present study demonstrated that the cartilage regeneration was induced by the DN gel implantation. Most specimens maintained a good cartilage matrix with the nearly normal structural integrity at 12 weeks, although the efficacy of the DN gel implantation seemed to reduce for a longer follow-up. This study also showed that the surface of the regenerated cartilage was morphologically comparable to the normal cartilage surface. These findings were encouraging as the defect usually deteriorates severely with time if untreated. Further studies are needed to establish the clinical safety of this hydrogel in the near future.