A Novel Double-network Hydrogel Induces Spontaneous Articular Cartilage Regeneration

In Vivo In A Large Osteochondral Defect

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Introduction: Articular cartilage defect is a significant and increasing health care concern. It has been a commonly established concept that the articular cartilage tissue cannot spontaneously regenerate in vivo. Therefore, it has been the prevalent strategy to fill an osteochondral defect with a tissue-engineered cartilage-like tissue or a cell-seeded scaffold material by implantation surgery (1). However, it has been pointed out that this strategy has many problems for current clinical application (2,3,4). In such a scientific situation, studies on spontaneous in vivo regeneration of the cartilage in an osteochondral defect have not yet been attempted in the previous literature. Is articular cartilage tissue really unable to spontaneously regenerate in the case of a defect in vivo? We have considered that the mesenchymal stem cells (MSCs) contained in the blood clot from the bone marrow may differentiate into chondrocytes if a certain material having idealized quality is implanted just beneath the blood clot. Then, we have paid attention to an originally developed double-network (DN) hydrogel (5) composed of the two independently cross-linked networks of poly-(2-acrylamido-2-ethylpropanesulfonic acid) (PAMPS) and poly-(N,N'-dimethylacrylamide) (PDMAAm), which is a bioactive hydrogel (6,7) having a unique mechanical properties (8). Thus, we have developed a novel method to induce spontaneous hyaline cartilage regeneration in vivo for a large osteochondral defect by implanting a PAMPS/PDMAAm DN gel plug to the bottom of the cavity, leaving the defect itself vacant. The purpose of this study is to quantitatively evaluate the cartilage regenerated with this innovative method in comparison with the untreated control.

Materials and Methods: A total of 23 mature female rabbits, weighing 3.5 ± 0.3 kg, were used in this study. All experiments were performed under the Rules and Regulation of the Animal Care and Use Committee.

Method to induce cartilage regeneration: An osteochondral defect having a 4.5-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 1.5-mm depth remained after surgery (Fig 1). In the left knee, an osteochondral defect having 1.5-mm depth was created and remained without any treatment. All animals were allowed unrestrained activity in their cages, postoperatively. Five rabbits were sacrificed at 1, 2, 3, and 4 weeks after implantation, respectively. Their knee joints were used for histological evaluations. The remaining 3 rabbits were sacrificed at 4 weeks and served for real time PCR analysis.

Results: Histologically, the treated defect was filled with a blood clot at 1 week (Fig 2A). A tissue rich in proteoglycan appeared in a localized zone close to the bony wall and the implanted gel at 2 weeks (Fig 2B), increasing at 3 weeks. At 4 weeks, the defect filled by a sufficient volume of the proteoglycan-rich tissue with a regenerated bone tissue resembling the normal subchondral bone (Fig 2C). The most superficial part is devoid of cells, resembling the lamina splendens in the normal articular cartilage (Fig 2E). Thus, the regenerated proteoglycan-rich tissue showed a 4-layer structure similar to the normal articular cartilage structure (Fig 2C). At 1 week, we could find elliptic and spindle-shaped cells resembling MSCs in the triangular zones (Fig 2a). These zones subsequently became proteoglycan-rich zones at 2 weeks (Fig 2b). Type 2 collagen was abundantly expressed in the proteoglycan-rich tissue. On the other hand, the untreated (control) defect was filled with the fibrous and bone tissues even at 4 weeks (Fig 2F). Wayne’s scores (Fig 2E) were significantly higher in the gel-treated specimens than in the untreated control (p<0.0001, p=0.0062). In the real time PCR analysis, type 2 collagen, Aggrecan, and Sox9 mRNAs were obviously expressed and comparable to that in the normal chondrocytes, while it was seldom seen in the tissues regenerated in the untreated controls (Fig 3).

Discussion: A series of these evaluations demonstrated that the implantation of the DN gel plug on the bottom of an osteochondral defect could induce spontaneous hyaline cartilage regeneration in vivo. This fact has given a significant modification to the commonly established concept that the articular cartilage tissue cannot spontaneously regenerate in vivo. In the present study, we could not clarify the mechanism of spontaneous cartilage regeneration. However, we speculate that the in vivo biochemical and biomechanical environment created by existence of the DN gel may differentiate some undifferentiated cells including MSCs into chondrocytes. As to clinical relevance, this fact has prompted an innovative strategy to repair an osteochondral defect. That is induction of the spontaneous cartilage regeneration with the artificially synthesized gel material. This novel strategy should be studied in greater detail in the near future as a realistic research focus.