Cell Sheet Technology for Cartilage Repair

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Introduction

The repair of the partial thickness defect of articular cartilage fills the one of the requirements for the future treatment of Osteoarthritis. We used the temperature-responsive culture dishes, which show both hydrophilic and hydrophobic property alterations in response to temperature change, because of coating with N-isopropyl-acrylamid polymers. Cultured cells reach to confluent at 37 and only reducing the temperature from 37 to 20 , and they are detached from the surface without any enzymatic treatment because the surface property becomes more hydrophilic (Fig.1). We already reported chondrocyte sheet, synovial cell sheet, and anterior cruciate ligament sheet were successfully collected. Chondrocyte sheets have good adhesion to the partial thickness defect of rabbi articular cartilage. The purpose of this study was to clarify the property of layered chondrocyte sheet compared to conventional monolayer culture, and to examine the potential of cell sheet engineering as a new treatment for the repair of partial thickness defect of articular cartilage, as a means to directly care for the osteoarthritis.

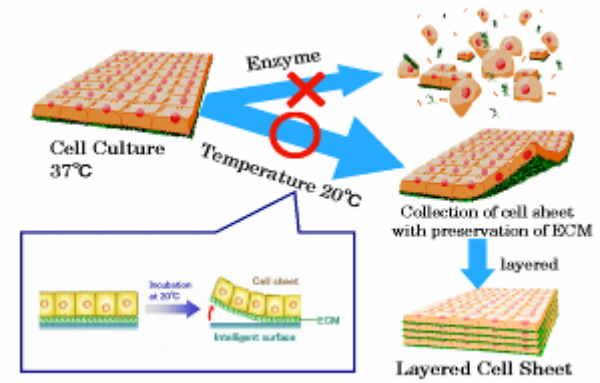

Fig. 1. Cell Sheet Technology

Materials and Methods

Consent was obtained from 10 patients who were to undergo reconstruction of the anterior cruciate ligament of the knee. During the surgery, synovial membrane and cartilage were obtained and enzymatically treated to isolate the cells. Temperature-responsive culture dishes were then seeded with a high cell concentration (more than 10000 cells/cm^2), and cell sheets were prepared (Fig.2). The cell sheets were then layered. To analyze the phenotypic expression of layered cell sheets and adhesive factors, single-layered cultured cells prepared according to the conventional methods and multi-layered cell sheets were examined using microarrays and real time PCR.

Results & Discussions

Compared with conventional single-layer culture methods, greater expression of proteins such as fibronectin1, collagen type II, aggrecan, and TIMP 1, which are related to adhesion and to synthesis of exteracellular matrix., was seen with layered cartilage cell sheets. In addition, activation was confirmed of genes such as SOX9 and HAS, which are important in maintaining articular cartilage characteristics.

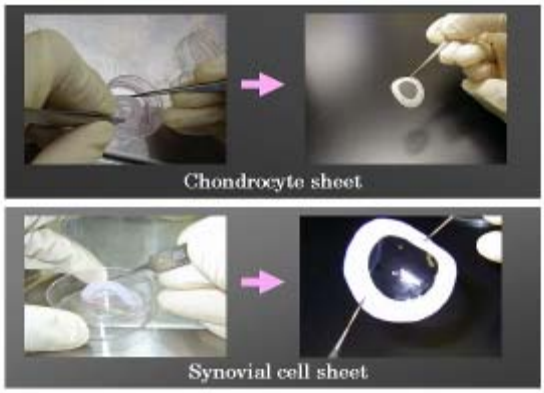

Fig. 2. Collection of chondrocytes and synovial cell sheets

Good adhesiveness was also seen *ex vivo* with layered cell sheets, which adhered and grafted to the damaged portion of the joint. From the above we considered that layered cell sheets function as a barrier that protects the surface of damaged cartilage from enzymes existing within the joint and prevents defluxion of proteoglycans from the damaged area, thereby contributing to repair and regeneration of partial thickness defect of articular cartilage (Fig.3). We are now studying to develop the focal gene delivery system using this adhesiveness of the cartilage cell sheet.

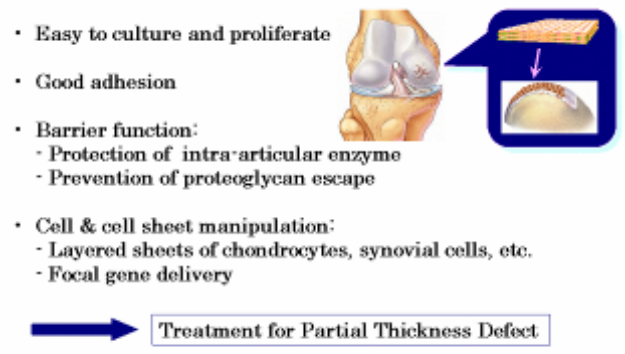

Fig. 3. Advantages of Cell Sheet

Conclusions

1. The cell sheet adheres well to the surface of articular cartilage.
2. The cell sheet technology and focal gene delivery might be a useful tool for the treatment of partial thickness defect of articular cartilage.