Effects of Low-Intensity Pulsed Ultrasound on Activation of Three-Dimensional Cultured Chondrocytes

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[Introduction]
Articular cartilage is an avascular musculoskeletal tissue with a relatively low capacity for self-regeneration or repair. Various treatments for osteochondral lesions have failed to achieve success. There were some reports that low-intensity pulsed ultrasound (LIPUS) stimulation had an effect on the healing of osteochondral defects in animal models. In some in vitro studies, LIPUS resulted in an increase in aggrecan and proteoglycan synthesis although chondrocytes proliferation and Type II procollagen mRNA were not affected.

In the current study, we show for the first time that it is possible to enhance the proliferation of chondrocytes cultured using a three-dimensional support system through LIPUS stimulation. Moreover, one of the intracellular signaling pathways involved in the proliferation of chondrocytes was identified.

[Materials and Methods]
Articular cartilage tissue was obtained from the metatarsophalangeal joints of freshly slaughtered six-month-old porcines. Twenty-four-well plates containing Type I honeycomb collagen sponges as three-dimensional support matrix for the cell culture were used. Isolated chondrocytes in the collagen gel and the culture medium composites were added to each sponge and incubated at 37 °C for one hour. The final cell density was adjusted to 2×10^5 cells/well/mL.

Low-intensity pulsed ultrasound (LIPUS) at a frequency of 1.5 MHz and a repetition rate of 1 KHz was applied to chondrocytes cultures from day 2 for 20 minutes every day. Experiments were performed under two different culture conditions: a control (US-) group and (US+) group. At days 3, 7, 10 and 14, number of cells and levels of chondroitin sulfate isomers such as chondroitin 6-sulfate and chondroitin 4-sulfate synthesized by the cultured chondrocytes were measured by high-performance liquid chromatography (HPLC) and fluorometry. The specimens were stained with Safranin O and Alcian blue at post-culture weeks 1 and 2 for a histological evaluation. Anti-type II collagen antibodies were used to examine the chondrocytes phenotype and to detect type II collagen production at post-culture weeks 1 and 2. For immuno-staining and Western blot analysis, the following primary antibodies were used: AKT, Mib 1 (Ki67), ss-DNA, Cyclin B1, MAPK, anti-paxillin, FAK, anti-beta catenin, anti-beta-1 integrin and alpha-1 integrin.

Statistical comparisons of the mean values were performed using multivariate ANOVA. A p-value of <0.05 was considered statistically significant.

[Results]
Acid mucopolysaccharide was detected around the cells in both groups. A layer structure of the chondrocytes and matrix was evident on the surface of the cells. In particular, a thicker layer and wider area of the surface of the cells. In particular, a thicker layer and wider area of the surface of the cells. In particular, a thicker layer and wider area of

[Discussion]
In general, it has been considered that signal transduction Integrin→MAPK to nucleus is the most important for the proliferation of chondrocytes under mechanical stress 2). However, in the current study, it was shown that in addition to that general pathway, the signal transduction Integrin→Akt to nucleus was also activated under LIPUS stimulation. Moreover, Akt controls the metabolism of beta catenin by controlling GSK3, which carries out the phosphorylation of beta catenin, and also raises intracellular beta catenin concentration and makes it move into the nucleus. Moreover, it is thought that Akt also plays a role similar to the Wnt signal, which controls the metabolism of beta catenin by controlling GSK3. From the result of ss-DNA antibody staining it was demonstrated that apoptosis was not involved in the difference in the number of cells between the two groups. The results of immunostaining with Cyclin B1 and Mib1 antibodies showed that LIPUS enhanced the proliferation of chondrocytes in our three-dimensional culture system. One reason for this phenomenon was the differences in the procedure of chondrocyte culture. A suitable environment in which nutrition and oxygen were appropriately supplied to chondrocytes by cultivating them in layers on collagen sponge was constructed.

[Conclusion]
LIPUS promoted the proliferation of cultured chondrocytes in a three-dimensional system using collagen sponge to create an environment similar to cartilaginous tissue.

References: