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Hyaluronic Acid Enhances In Vitro Induction Effects of Synthetic PAMPSAnd PDMAAm Hydrogels on Chondrogenic Differentiation of ATDC5 Cells

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Summary:
In vitro chondrogenesis of ATDC5 cells cultured on the PAMPS and PDMAAm gels was influenced by supplementation of hyaluronic acid, in which a low concentration (0.01 mg/mL) of hyaluronic acid significantly enhanced the chondrogenic differentiation of ATDC5 cells cultured on the PAMPS and PDMAAm gels.

Abstract:
Introduction:
Recently, we developed an innovative method to induce spontaneous hyaline cartilage regeneration in vivo by implanting a PAMPS/PDMAAm double-network (DN) hydrogel, which is composed of poly(2-acrylamido-2-methylpropanesulfonic acid) (PAMPS) and poly(N,N'-dimethyl acrylamide) (PDMAAm) [1, 2]. To clarify a part of the mechanisms of this phenomenon induced by the DN gel, we have studied in vitro behavior of the chondrogenic ATDC5 cells on single-network PAMPS and PDMAAm gels, which were components of the DN gel, and reported that these gels induce chondrogenic differentiation of ATDC5 cells in vitro in maintenance medium without insulin [3]. However, the medium used in the in vitro study did not contain all important molecules that existed in the joint fluid and might play significant roles in vivo. It is important to clarify in vitro effects of such molecules on the in vitro induction effect of these gels on chondrogenic differentiation of ATDC5 cells. We have noticed that the joint fluid contains hyaluronic acid (HA) because recent studies have reported that HA improves chondrocyte proliferation and increases matrix synthesis [4, 5, 6]. We have hypothesized that HA may significantly affect the in vitro induction effects of the PAMPS and PDMAAm gels on chondrogenic differentiation of ATDC5 cells, depending on the concentration. The purpose of this study was to test this hypothesis.

Methods:
This study consisted of 3 sub-studies: The first sub-study was performed to assess fundamental effects (without HA) of the PAMPS gel, PDMAAm gel, and polystyrene (PS: control) dish surfaces on chondrogenic differentiation of ATDC5 cells cultured in the maintenance medium and the differentiation medium (containing insulin). The second and third sub-studies were performed to analyze effects of supplementation of HA (3 concentrations) into the 2 media on chondrogenic differentiation of ATDC5 cells cultured on the PAMPS gel and PDMAAm gel, respectively. At 7 days of culture, gene expression of cartilage markers was examined using real-time PCR analysis and collagen-2 expression by immunocytochemistry. [Hydrogel preparation] PAMPS gel and PDMAAm gel disks were synthesized using the previously reported polymerization method. Then, the gel disks were placed in 24-well PS dishes for culture. [Cell culture] The ATDC5 cell line was obtained from the RIKEN cell bank (Tsukuba, Japan). The maintenance medium consisted of a 1:1 mixture of DMEM and Ham's F-12 medium supplemented with 5% fetal bovine serum, human transferrin and sodium selenite. To analyze the effects of HA on the chondrogenic differentiation of the ATDC5 cells, HA (ARTZ, Seikagaku Co., Japan) was supplemented into each medium so that the HA concentration

became 0.01, 0.10, and 1.00 mg/mL. [Real time PCR] The real time PCR for type-2 collagen (Col-2) and aggrecan was performed in Thermal Cycler Dice TP800 (TakaraBio, Japan). The expression level of each gene was normalized with that of GAPDH. [Immunocytochemistry] The primary immunoreaction was carried out with a mouse monoclonal antibody against type-2 collagen (Abcam). The secondary immunoreaction was carried out with Alexa 488-conjugated goat anti-mouse IgG (Invitrogen). [Statistical Analysis] The ANOVA was used with the Fisher's PLSD test for multiple comparisons. The significance limits were set at $p=0.05$.

Results:
[Sub-study 1] Expression of collagen-2 and aggrecan genes was significantly greater on the PAMPS and PDMAAm gels than on the PS surface in the cells cultured in the maintenance medium ($p<0.05$). In the cells cultured in the differentiation medium, expression of Col-2 and aggrecan genes was significantly greater on the PDMAAm gel than on the PS surface ($p<0.0001$), while there were no significant differences in expression of these genes between the PAMPS gel and the PS surface. [Sub-study 2] On the PAMPS gel with the maintenance medium, HA at the concentration of 0.01 and 0.10 mg/mL significantly increased expression of Col-2 gene ($p=0.0008$ and $p=0.0413$, respectively) and aggrecan gene ($p=0.0070$ and $p=0.0198$, respectively) in comparison to those without HA, while HA at the concentration of 1.00 mg/mL did not significantly affect expression of these genes. In the cells cultured in the differentiation medium, HA at the concentration of 0.01 mg/mL significantly increased expression of Col-2 and aggrecan genes ($p=0.0111$ and $p=0.0269$) in comparison to those without HA, while HA at the concentration of 0.10 or 1.00 mg/mL did not significantly affect expression of these genes. [Sub-study 3] On the PDMAAm gel with the maintenance medium, HA at the concentration of 0.01 or 0.10 mg/mL did not significantly affect expression of Col-2 and aggrecan genes, while HA at the concentration of 1.00 mg/mL significantly reduced expression of these genes ($p=0.0426$ and $p=0.0218$). In the cells cultured in the differentiation medium, no significant difference was detected in each comparison.

Discussion:
First, the PAMPS and PDMAAm gels significantly induced chondrogenic differentiation of the ATDC5 cell in the maintenance medium even without insulin, and the PDMAAm gel significantly enhanced chondrogenic differentiation of the ATDC5 cell induced by insulin. Secondly, supplementation of a low concentration (0.01 mg/mL) of HA significantly enhances chondrogenic differentiation of the ATDC5 cells cultured on the PAMPS gel, independent of existence of insulin, while this enhancement effect of HA supplementation decreased when the concentration increased. Thirdly, supplementation of a high concentration (1.00 mg/mL) of HA significantly reduced chondrogenic differentiation of the ATDC5 cells cultured on the PDMAAm gel, independent of existence of insulin, although this reduction effect was not significant in supplementation of a low concentration (0.01 mg/mL) of HA. It is well known that HA affects cells through signal transducing receptors on the cell surface, and that the effect of HA varies depending on the concentration [7, 8]. The present study suggested that mechanical signals from each hydrogel surface significantly affect chondrogenic differentiation of the ATDC5 cells, and that HA affects the mechanical signals from each hydrogel surface, depending on the concentration. In conclusion, a low concentration (0.01 mg/mL) of HA significantly enhances the chondrogenic differentiation of ATDC 5 cells cultured on the PAMPS and PDMAAm gels.

References:
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