HUMAN MENISCUS CELL: CHARACTERIZATION OF THE PRIMARY CELL CULTURE AND ATTACHMENT TO POROUS COLLAGEN SHEET

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Introduction: Meniscus is one of the cartilaginous tissues in the knee joint which play important roles in joint motion, load transmission or lubrication. It is well known that intrinsic healing of injured meniscus is limited even if repaired surgically, and that degenerative joint disease develops following loss of its function. Therefore, it is a reasonable way to employ cell-based tissue engineering technique for treatment of meniscus injuries. Although many studies have been performed on meniscus cells from animals including rabbit, rat, pig or dog, little is known about human meniscus cells. In this study, we investigated human meniscus cells in the primary culture to characterize their potential of proliferation and expression of cartilaginous matrix proteins. Furthermore, we studied the attachment of the cultured cells to a porous collagen sheet in attempt to develop cell-seeded meniscus implant.

Materials and Methods: Cell preparation: We investigated forty-seven human menisci which were excised during arthroscopic knee surgeries from the patients who gave the informed consent and needed excision of meniscus due to irreparable tears such as multiple and/or degenerative tear. Weight of the excised meniscus ranged from 0.03 to 6.6 gram with a mean of 2.01 g. They were cut into small pieces and digested by 0.4% collagenase (Wortington, USA) in Hunk's F-12 medium (F-12) for 2-3 hours. Liberated cells were counted by at day 1, 3, 7, and 14 of the culture. Expression of cartilaginous matrix proteins and cytokines: mRNA was extracted from the primary cultured cells in F-12/DMEM and F-12, while the cultured meniscus cells were harvested at day 7 of the primary culture, and seeded onto the porous collagen sheet at the cell density of 1 x 10^6 per 35mm diameter culture dish in attempt to develop cell-seeded meniscus implant. Quantitative RT-PCR analysis revealed that mRNA expression of type II collagen and aggrecan. Cell attachment to porous collagen sheet: A sterilized porous collagen sheet which was made of type I collagen extracted from bovine skin (Integran®, Koken, Tokyo Japan) was cut to fit the size of 48 well and pre-incubated with F-12/DMEM at 37°C . The cultured meniscus cells were harvested at day 7 of the primary culture, and seeded onto the porous collagen sheet at the cell density of 1 x 10^6 cells per well, and incubated in the 5% CO_2 at 37°C . After one day and five days of incubation, the cell-seeded porous collagen sheets were fixed and processed for histological analysis after staining with hematoxylin-eosin. Results: Morphology of the human meniscus cells in the primary culture: Meniscus cells were successfully released from human meniscus by collagenase digestion. The average cell number obtained from human meniscus was 6.9 x 10^6 / wet weight gram. One day after the primary culture, there were cells plated on a plastic culture dish and suspended in the medium. The mean plating efficiency at one day after culture was 38%, and this value was not statistically different among the three kinds of culture media. The morphological analysis of the plated cells revealed that there were consistently three distinguishable cell types; elongated fibroblast-like cells, polygonal cells and small round chondrocyte-like cells (Fig.1). The elongated fibroblast-like cells proliferated well in all three culture conditions, whereas small round cells unchanged or decreased in number during the culture. Cellular proliferation rate was the highest in DMEM, and the lowest in F-12 (table 1). Three cell types were maintained in F-12/DMEM and F-12, while the elongated fibroblast-like cells became predominant in DMEM with the time course of the primary culture: mRNA expression of cartilaginous extracellular matrices and cytokines and chondroitin sulfate production: RT-PCR analysis revealed that the primary cultured meniscus cells exhibited the mRNA expression of type II collagen and aggrecan. Quantitative RT-PCR analysis revealed that mRNA expression of type II collagen tended to decrease during the culture and that the same samples showed increase of mRNA expression of aggrecan. Furthermore, mRNA expression for IGF and bFGF were also detectable by RT-PCR analysis at day 1, 4, 7. Concentration of chondroitin sulfate in the media was 10.2 ± 1.8mmol/ml, which was much more higher that that of tendon fibroblast culture medium. Discussion: Although it is already known that meniscus contained fibrochondrocytes with fibrous collagen bundles and ground substances consisting of type I and type II collagens as well as proteoglycans, cellular heterogeneity is little understood. To our knowledge, this is the first report that investigated human meniscus cell in the primary culture using different culture conditions and identified three distinguishable subpopulations of the human meniscus cells. The fact that these cells proliferated in vitro may indicates the potential of intrinsic healing of meniscus. These cells proliferated in the primary culture and maintained their heterogeneous subpopulation, suggesting that this culture condition might be useful for cell-based tissue engineering for re-implantation of human meniscus cells. We also demonstrated the mRNA expression of cartilage matrix proteins and bFGF, IGF mRNA expression. It is noteworthy to point out that mRNA expression of aggrecan gene increased during the monolayer culture, while that of the type II collagen gene decreased. This characteristic of the human meniscus cells which differs from that of chondrocytes could be attributed to their unique biomechanical environment where they are subjected to both compression load and hoop stress at the same time. The expression of bFGF and IGF appears to indicate the possibility that cell proliferation or matrix production is stimulated by these cytokines. We finally demonstrated the possibility of seeding cells onto porous collagen sheet. Since there was inconsistency in histological appearance of cell seeded-collagen sheet, further investigation is needed to optimize the method of cell seeding and the structure of biological scaffold. However, the fact that the cultured human meniscus cells in some extent attached to the porous collagen sheet by seeding suggests that this culture method potentially makes it possible to develop autogenous cell-seeded collagen meniscus implant.