Reconstruction of the intra-articular environment of osteoarthritis with a 3D co-culture system of osteoarthritis patient-derived cells

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Disclosures: All authors have nothing to disclose.

INTRODUCTION: The inflammatory environment of osteoarthritis (OA) is a three-dimensional (3D) environment formed by the reaction of various cells of articular cartilage with each other. Cells administered for cartilage regeneration in OA interact with various inflammatory factors secreted from surrounding cells in this 3D environment and are affected by the mechanical factors of the joint. Therefore, the in vitro experimental method of administering inflammatory cytokines such as interleukin-1 beta (IL-1ß) to create an inflammatory environment in cells in two-dimensional (2D) monolayer culture is inevitably different from the inflammatory environment of OA in vivo. In addition, the effects of various factors affecting the survival/engraftment of cells on the administered cells can be evaluated in a 3D co-culture system composed of spheroids of intra-articular cells to better mimic the inflammatory environment of OA. In this study, a culture method using a 3D co-culture system consisting of chondrocytes, synovial cells, and adipocytes derived from OA patients was established to simulate the inflammatory environment in the joint cavity of OA patients. In addition, it was confirmed that the in vivo environment of OA could be better simulated as compared to the inflammatory environment in the 2D monolayer culture or spheroid culture of chondrocytes.

METHODS:
Cartilage, adipose, and synovial tissue samples from osteoarthritis patients were obtained during osteoarthritis surgery. After the cells were isolated from each tissue, a culture method was established. In this study, a 3D co-culture dish was designed in which each cell was not mixed, and each cell was cultured in this dish for one day to form spheroids. In a culture dish for a 3D co-culture system of osteoarthritis patient-derived synovial cells, chondrocytes, and adipocytes, 4x105 cells from patient tissues were inoculated with 400 μL of culture medium. At the same time, the 2D monolayer cell control group cultured chondrocytes in a total of 3 wells (to match the number of cells in the co-culture construct) so that 4x105 cells are cultured in each well of a 6-well plate culture dish. As another comparative group, spheroids using only chondrocytes were used. Cells were incubated for one day at 37 ℃ under an atmosphere of 5% CO2. After 24 hours of culture, IL-1β (10 ng/ml) was treated for 48 hours to induce an inflammatory response. After 48 hours of IL-1β treatment, each cell and culture medium were collected and cell viability (MTT assay), nitric oxide (NO) synthesis by inflammatory stimuli, and secretion of inflammatory cytokines (ELISA assay) was measured.

RESULTS SECTION: In this study, we confirmed that cells from each osteoarthritis patient's tissue can be cultured by forming spheroids in our newly designed 3D co-culture dish. Cell viability between spheroids of chondrocyte, spheroids of adipocyte and spheroids of synovial cell did not show a significant difference. However, the amount of NO synthesis was confirmed to be significantly higher in the case of spheroids of chondrocyte. Therefore, it was confirmed that the response in the inflammatory environment has a large difference depending on the tissue of origin. In addition, we confirmed that co-culture of cells isolated from the major constituent tissues of articular cartilage is required to properly simulate the inflammatory environment of OA. There is no significant difference in cell proliferation and cell viability according to the cell culture method (2D monolayer or spheroid culture) and cell type (spheroids of chondrocytes or co-culture of chondrocytes, adipocytes and synovial cell spheroids). However, it was confirmed that there was a difference between the amount of NO synthesis and the amount of secreted inflammatory cytokines (IL-6, PGE2 and MMP-13).

DISCUSSION: We reconstructed the intra-articular environment of OA with spheroid from OA patient-derived cells. Comparing osteoarthritis's inflammatory environmental response when using 2D monolayer culture, 3D spheroid culture, and 3D co-culture system confirmed that a better OA environment was formed when the 3D co-culture system was used. When using the 3D co-culture system developed in this study, it is expected that the limitations and problems of drug screening using 2D monolayer culture can be supplemented, and furthermore, the use of experimental animals can be replaced.

Acknowledgements: This research was supported by a grant of the National Research Foundation of Korea (NRF-2020R1A2C2008266 and 2023R1A2C1004164).

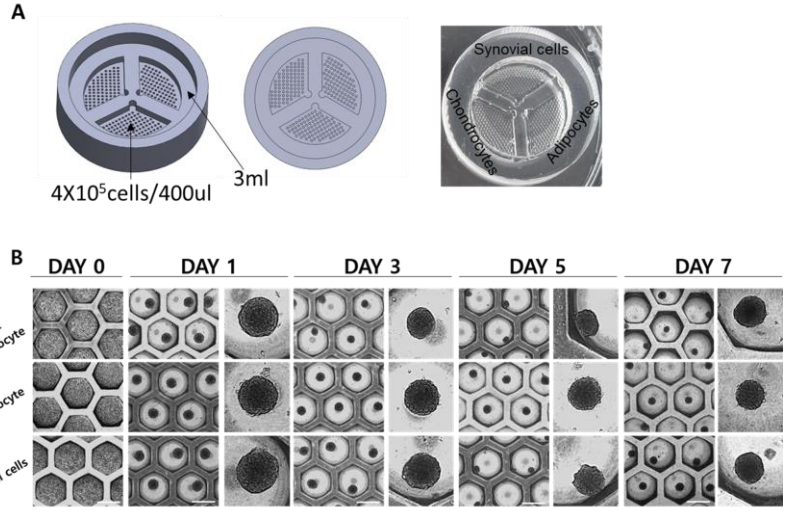

Figure. A culture method using a 3D co-culture system consisting of chondrocytes, synovial cells, and adipocytes derived from OA patients was established to simulate the inflammatory environment in the joint cavity of OA patients. In addition, it was confirmed that the in vivo environment of OA could be better simulated as compared to the inflammatory environment in the 2D monolayer culture or spheroid culture of chondrocytes.