THU0058 ENHANCEMENT OF CARTILAGE REGENERATION EFFICIENCY WITH HUMAN ADIPOSE STEM CELL THREE-DIMENSIONAL SPHEROID

J. Y. Ko[1], E. Lee[1], J. Kim[1], G. I. Im[1]. [1]*Research Institute for Integrative Regenerative Biomedical Engineering, Dongguk University, Goyang, Korea, Rep. of (South Korea)*

Background: 3D (three-dimensional) cell culture technology has been researched steadily because of its high potential of biocompatibility compared to single cells since 1990s, and is being developed to 3D spheroids recently. Spheroids are considered to reflect the natural organization of cells better than 2D cell cultures, and stem cells spheroids have been studied extensively in therapeutic transplantation. Stem cells were considered as a method of replacing autologous chondrocyte in regenerative treatment of articular cartilage. Compared to conventional single cells, 3D cell culture is artificially created an environment similar to a living body in vitro so that all cells collectively, a cell culture model that allows growth or interaction with the environment. Therefore, the findings of this study indicate that enhancement of treatment efficiency of stem cells caused by potential of survival and proliferation of hASC spheroid in Osteoarthritis. In conclusion, spheroid positive subpopulation of hASCs has high cell proliferation and survival but not apoptosis and cell death potential, which may contribute to successful cartilage regeneration and the development of stem cell therapies in the future.

Objectives: Studied for 3D spheroids to investigate the mechanism of enhancement of survival and proliferationof hASC (human adipose stem cells) spheroid, which may contribute to successful improvement of therapeutic efficacy of stem cells.

Methods: Cell isolation and culture / 3D cell culture dish preparation / hASCs culture on 3D cell culture dish / Real-time PCR analysis / Western blotting / Alcian blue staining / ACLT + MM (Anterior cruciate ligament transection with Medial meniscectomy) model / In vivo fluorescence for cell tracking / In vivo effects of spheroids in OA joint / Histological analysis / Enzyme-linked immunosorbent assay (ELISA) results for inflamma -tory cytokines in rat synovial fluid / Statistical Analysis

Results: In order to see how the spheroid showed more residual than single, and how effective it was in actual cartilage regeneration, the result of paraffin tissues were confirmed by safranin O staining for each condition. The tendency of cartilage regeneration efficiency was good for spheroid. Although the differences between the single and spheroid groups were small, they reaffirmed that they could somewhat protect cartilage and help regeneration treatment. However, immunohistochemistry of HN(Human nucleic antigen) staining showed that cells of single and spheroid were not observed in the wound but disappeared by the paracrine effect.

Conclusion: Spheroids do not exhibit differentiation characteristics, but they could be seen as a result of expression of related genes such as Bax, Bcl-XL and Alcian blue staining. Spheroids tend to have low potential of cell death rather than proliferation and reduction in the proliferation. So, we conclude the fact that instead of hASCs going directly to the surgical site to regenerate cartilage, they can help catrilage regeneration.

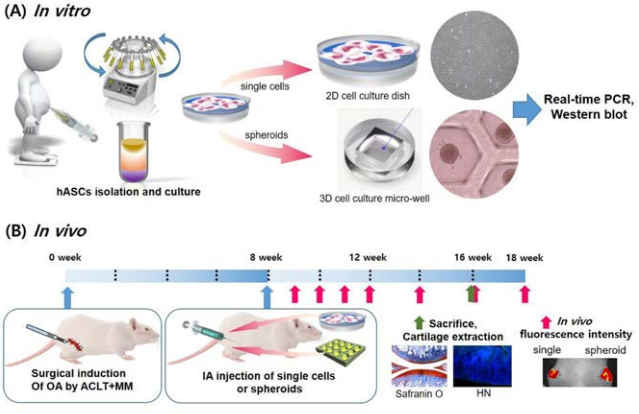

Figure 1. General scheme of *in vitro* and *in vivo* experiments using spheroid. (A) *in vitro* experimental procedures to identify single and spheroid. (B) *in vivo* experimental procedures in surgically-induced OA model to identify the cartilage regeneration efficiency of spheroid.

Acknowledgments: This research was supported by the National Research Foundation of Korea (NRF-2019R1H1A2039685 and 2019R1I1A1A01043778).
Disclosure of Interests: None declared
DOI: 10.1136/annrheumdis-2020-eular.4022

THU0059 INCREASING SURFACE LUBRICATION WITH POLY(2-METHYL-2-OXAZOLINE) HALTS DEGENERATIVE CHANGES IN THE CARTILAGE IN A COLLAGENASE INDUCED OSTEOARTHRITIS (CIOA) RAT MODEL

S. Kauppinen[1], D. Fercher[2], G. Barreto[3], G. Morgese[2], E. Benetti[2], S. Saarakkala[1], H. Nieminen[4], M. Zenobi-Wong[2], M. A. Finnilä[1]. [1]*University of Oulu, Oulu, Finland*; [2]*ETH Zürich, Zürich, Switzerland*; [3]*University of Helsinki, Helsinki, Finland*; [4]*Aalto University, Espoo, Finland*

Background: Degenerative lesions of articular cartilage (AC) surface are related to disruption of the well-organized collagen network and allow proteoglycans to escape from the tissue. Ultimately, this leads to the development of osteoarthritis (OA). Targeted therapy for early AC lesions could provide an effective way to halt the OA development process.

Objectives: This study aims to evaluate the effectiveness of an engineered surface lubricant; poly(2-methyl-2-oxazoline) (PMOXA)[1] to prevent the destruction of the AC surface. Our recently developed contrast-enhanced μCT (CEμCT) method was used to quantify AC surface erosion[2].

Methods: OA was induced in 12-18 week-old male Wistar rats (N=17) with an injection of 250 U Collagenase within 25 μL solution into the left hind limb. Both hind legs were treated with a second injection three days after the collagenase injection (CI). Three groups were formed by using either PMOXA (N=5), hyaluronic acid (HA; N=6), or saline (N=6) during the second injection. The animals were sacrificed after 45 days, and harvested knees were fixed in phosphate-buffered formalin for a week. Knees were stored in 70% ethanol, and tibia and femur were carefully dissected free of other tissue, stained with 1% phosphotungstic acid[3], and scanned with a desktop μCT with 2.8μm pixel size. The medial and lateral AC surfaces were manually segmented from 3D projections using an in-house developed program (Matlab sofware). These surfaces were analyzed by iteratively fitting a reference surface (RS) to a median-filtered smoothed surface representing a perfectly smooth surface, capturing the realistic shape AC. An offset of 5 pixels (14 μm) was added between the RS and the original surface (OS). Two quantitative parameters were calculated from the data: Average of Maximum Void Depth (MVD) (depth of lesion) and Degeneration-% (area exceeding 20 μm MVD / whole area) *100). Estimates of mean differences from all groups against the CI+Saline -group were determined using a linear mixed model.

Results: Boxplots from tested groups are shown in Fig. 1A and MVD results are visualized in Fig. 1B. Collagenase caused structural defects only on the medial and lateral tibial AC surfaces, which was seen as increased MVD and Degeneration-%. CI changes were not seen in PMOXA or HA treated groups. Furthermore, MVD and Degeneration% were lower in CI knees that were treated with PMOXA.

Conclusion: Our CEμCT analysis method was able to detect subtle changes of the AC surface in the medial and lateral tibial cartilage, caused by the CI. In contrast, the CI did not cause detectable changes in the AC of the femur, which indicates that in the CIOA model, the tibia is more susceptible to structural degradation. Our results show that early intervention with HA or PMOXA can halt the degenerative AC changes caused by CI. However, HA did not suppress the effects of CI in the medial tibia, which indicates that PMOXA could be more effective to prevent the development of OA.

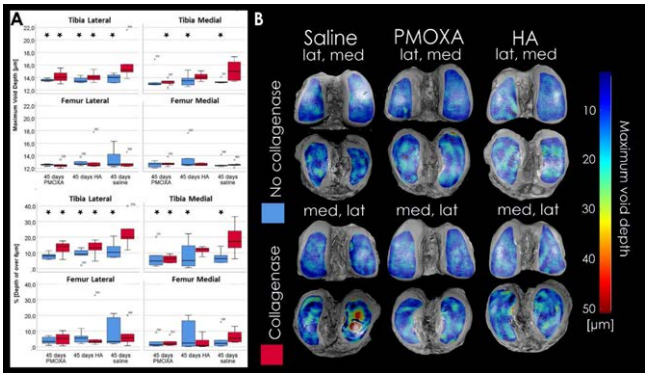

Figure 1. A) Boxplots of Maximum Void Depth (MVD) and Degeneration-%. Lateral and medial side are analyzed separately for both tibias and femurs. Stars indicate if a group was statistically different from control group (CI+Saline).CI= red, no CI= blue. B) Representative visualizations for maximum void depth overlayed on top of the 3D AC surface.

References:
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