Anti-Inflammatory Effects of Synovial Fluid-Derived Versus Gingival Cell-Derived Extracellular Vesicles on Human Chondrocytes

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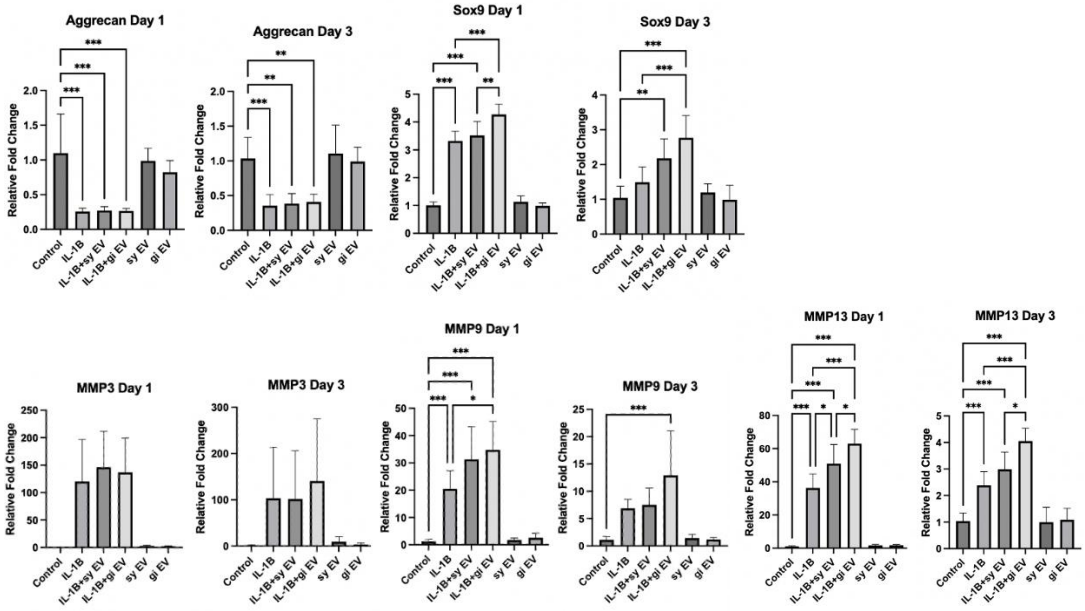
Introduction: Since recent years, extracellular vesicles (EVs) are emerging as new potential treatment to decrease the inflammation and cartilage degeneration caused by osteoarthritis (OA) [1]. Studies show that the therapeutic potential of EVs is determined by their parent cells. Synovial fluid derived exosomes have shown therapeutic effects on OA.[2] Gingival mesenchymal stem cells are easily accessible and have shown regenerative potential in bone, skin, and the trachea. [3] However, the therapeutic effects of gingival exosomes have not been explored. Therefore, the aim of this *in vitro* experiment was to compare the anti-inflammatory effects of synovial fluid and gingival fibroblast derived exosomes on chondrocytes.

Method: Human gingival fibroblasts and human synovial fluid cells were cultured in DMEM/F12 +10% FBS. At passage 10, gingival fibroblasts were starved for two days and then EVs were collected using ultracentrifugation. Similarly, EVs were also collected from Synovial fluid derived cells. The EV particle size and count were determined using ZetaView NTA. Human Chondrocytes were seeded onto 12 well plates with 100,000 cells per well and maintained in culture for 3 days prior to treatment. The chondrocytes were treated with IL-1β (15ng/ml), Synovial (Sy) EV ($5.7x10^8$ particles/ml), and Gingival (Gi) EV ($5.5x10^8$ particles/ml) in groups(n=6) as follows: control (media), IL-1β, IL-1β+sy EVs, IL-1β+gi EVs, sy EVs, and gi EVs. Cells were harvested at days 1, 3 and 8 for total RNA purification. Day 8 groups received a retreatment on day 3. RNA purification and cDNA synthesis were performed, and real-time PCR was used to measure gene expression.

Results: Gene expressions of aggrecan, SOX9, ADAMTS4, MMP3, MMP9, and MMP13 were compared among groups. On days 1 and 3, groups treated with IL-1β and IL-1β+EVs had statistically significant decreases in aggrecan expression and statistically significant increases in SOX9 expression. By day 3, IL-1β and EV groups still had statistically significant increased SOX9 expression. By day 8, there were no differences in SOX9 expression, but aggrecan expression was significantly lower in groups treated with IL-1β+EVs. MMP9 and MMP13 had significant upregulation in groups treated with IL-1β and IL-1β+EVs on day 1. After day 1, while MMP3 expression decreased to nonsignificant levels, MMP13 expression in these groups and MMP9 in the IL-1β+gi EV group remained significantly high on day 3. By day 8, there were no significant trends in MMP9 and MMP13 expression. However, MMP3 expression showed a similar trend to day 1 of MMP9 and MMP13 where groups treated with IL-1β and IL-1β+exosomes were significantly higher compared to the control group. Lastly, there were no significant trends shown in ADAMTS4 on day 1, 3, or 8. However, the relative fold change of ADAMTS4 did decrease from day 1 to day 3.

Discussion: SOX9 is one of the transcription factors that, when activated, will make more aggrecan [4]. SOX9 has also shown the ability to inhibit IL-1β induced inflammation in chondrocytes. Matrix metalloproteinases on the other hand are responsible for the degradation of cartilage and other extracellular matrix components of joints. MMP13 mainly degrades aggrecan [5]. SOX9 was significantly upregulated in IL-1β+EV groups. Specifically, IL-1β+gi EV group were significantly higher than IL-1β and IL-1β+sy EV on day 1 and remained significantly higher than IL-1β alone group on day 3. Meanwhile, the IL-1β+sy EV group did not have any significant differences compared to the IL-1β group. Upregulation of MMP13 in response to inflammatory cytokine could have contributed to degradation of aggrecan, however, SOX9 expression was simultaneously upregulated in inflammatory groups with EVs, specifically Gi EVs. It is likely that treatment with Gi EVs allowed the chondrocytes to resist damage by promoting aggrecan synthesis. This finding suggests that gingival EVs may have stronger anti-inflammatory tendency compared to synovial exosomes. The results also showed that in the groups treated with EVs alone, they do not contribute to any inflammation.

Significance/clinical relevance: It is possible that through the upregulation of SOX9, EVs do have potential therapeutic effects in inflammatory environments. Specifically, the differences between SOX9 expression in gingival and synovial EV groups may show that gingival EVs are effective in combating ECM degradation that occurs in inflammatory environment. It appears that the therapeutic effect of the EVs can be chondroprotective, not by inhibiting catabolic markers, but through promoting anabolic factors. Gingival cells, being relatively easy to procure, may have the potential to be a reliable source for EVs for applications in OA. However, different dose concentrations and treatment times should be tested to further explore the potential of gingival EVs.



1. Fan, W.J., et al., *Exosomes in osteoarthritis: Updated insights on pathogenesis, diagnosis, and treatment.* Front Cell Dev Biol, 2022. **10**: p. 949690.
2. Tao, S.C., et al., *Exosomes derived from miR-140-5p-overexpressing human synovial mesenchymal stem cells enhance cartilage tissue regeneration and prevent osteoarthritis of the knee in a rat model.* Theranostics, 2017. **7**(1): p. 180-195.
3. Jiang, S. and L. Xu, *Exosomes from gingival mesenchymal stem cells enhance migration and osteogenic differentiation of pre-osteoblasts.* Pharmazie, 2020. **75**(11): p. 576-580.
4. Song, H. and K.-H. Park, *Regulation and function of SOX9 during cartilage development and regeneration.* Seminars in Cancer Biology, 2020. **67**: p. 12-23.
5. Mehana, E.-S.E., A.F. Khafaga, and S.S. El-Blehi, *The role of matrix metalloproteinases in osteoarthritis pathogenesis: An updated review.* Life Sciences, 2019. **234**: p. 116786.