Synovial Stem Cell-Derived Extracellular Vesicles Enhance ECM Production in Cell-Laden Nanofiber Scaffolds

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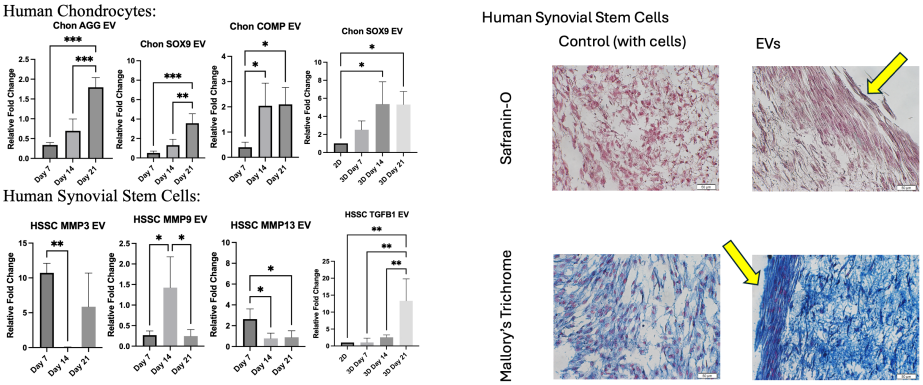
Introduction: Scaffolds have been a popular medium for influencing cells to assist with cartilage regeneration and proliferation [1]. Preliminary studies using nanofiber scaffolds, made of polyglycolic acid and poly (L-lactide-co-ε-caprolactone), have shown greater tissue regeneration and cartilage phenotype compared to other commonly used scaffolds [2]. In addition to scaffolds, extracellular vesicles have been showing regenerative capabilities for cartilage repair [3]. To investigate the abilities of novel nanofiber scaffolds co-cultured with extracellular vesicles, this study aimed to compare the chondrogenic potential of cells in a 2D monolayer culture vs 3D nanofiber scaffold culture in the presence of extracellular vesicles.

Methods: Human chondrocytes were cultured in DMEM F12 + 10% FBS and human synovial stem cells were cultured in DMEM + 15% FBS. Human synovial stem cells were starved for 3 days after which the conditioned media was collected for isolation of extracellular vesicles (EVs). EVs were collected using ultracentrifugation and particle size and count were determined using ZetaView NTA. EVs were stored in -80°C until experimentation. For the 2D portion of this experiment, human chondrocytes and human synovial stem cells were seeded at a density of 50,000 cells into each T25 flask, half of which were control group while the other half were treated with EVs. Cells were harvested at 7, 14, and 21 days with n=1 flask for each timepoint in each group. For the 3D portion of this experiment, human chondrocytes and human synovial stem cells were seeded at a density of 1,000,000 cells onto nanofiber scaffolds. Each scaffold was 200 µm thick and 6mm in diameter. One group served as the control while the other was treated with EVs. Every 1,000,000 cells were treated with 2.5 x 10^9 particles of EVs in each treatment cycle. Scaffolds were harvested at 7, 14, and 21 days. The control 3D group had n=6 scaffolds for each timepoint and the 3D EV group had n=4 scaffolds for each timepoint. N=2 scaffolds for the control and EV group were collected at day 22 for histology. Media changes and re-treatments for treated groups was performed every 2-3 days. At each timepoint cells were harvested for total RNA purification. RNA purification and cDNA synthesis were performed, and real-time PCR was used to measure gene expression. Histology samples were processed after collection on day 22.

Results: For human chondrocytes, gene expressions of aggrecan, SOX9, and COMP were compared between 3D control and 3D EVs, and 2D EVs with 3D EVs. For 3D control versus 3D EVs, aggrecan expression was significantly higher on day 21 compared to day 7 ($p<0.001$) and day 14 ($p<0.001$). SOX9 also showed a similar trend of upregulation overtime with significantly high expression on day 21 compared to day 7 ($p<0.001$) and day 14 ($p=0.003$). COMP expression was significantly higher on day 14 ($p=0.02$) and day 21 ($p=0.01$) compared to day 7. For human synovial stem cells, gene expressions of MMP3, MMP9, and MMP13 were compared between 3D control and 3D EVs, and 2D EVs with 3D EVs with the addition of TGFβ1. For 3D control versus 3D EVs, MMP3 expression was highest on day 7, but by day 14 it had significantly decreased ($p=0.004$). However, after day 14, expression increased at day 21 again but was not significant. Similarly, MMP9 expression was lowest on days 7 ($p=0.01$) and 21($p=0.01$) but peaked on day 14. MMP13 expression was highest on day 7 but on days 14 ($p=0.01$) and 21 ($p=0.02$) was significantly lower compared to day 7. When looking at 2D versus 3D in human chondrocytes, SOX9 expression was significantly higher on days 14 ($p=0.02$) and 21 ($p=0.02$) compared to the 2D EV group. In human synovial stem cells for 2D versus 3D, TGFβ1 expression was significantly higher on day 21 compared to the 2D EV group ($p=0.003$), day 7 EV scaffolds ($p=0.002$), and day 14 EV scaffolds ($p=0.005$). Gross visual assessment was performed on scaffolds stained with Safranin-O and Mallory's Trichrome.

Discussion: In human chondrocytes at day 21, aggrecan and SOX9 were upregulated. SOX9 stimulates expression of aggrecan and this combination is known to reduce inflammation in osteoarthritic environments [4]. COMP, a key player in cartilage regeneration, was also significantly high on day 14 and day 21 [5]. In the 2D versus 3D model, SOX9 was significantly upregulated on days 14 and 21 in the nanofiber scaffolds compared to the 2D group. This trend may suggest that EVs have therapeutic and regenerative effects in human chondrocytes. In human synovial stem cells, MMP3, MMP9, and MMP13 are known to degrade cartilage ECM in osteoarthritis [6]. Initially, MMP3 expression was high on day 7 but significantly decreased by day 14 in the presence of EVs. MMP9 expression was significantly high on day 14, but significantly lower on days 7 and 21. The therapeutic effects of EVs are evident through MMP13 profile. Expression was significantly high on day 7 but by day 14 and 21, it was significantly lower. TGFβ1, known to aid chondrogenic differentiation, was significantly upregulated on day 21 in the scaffolds compared to the other timepoints and the 2D group treated with EVs [7]. The results show that groups treated with EVs upregulate cartilage repair markers and lower markers of cartilage degradation. Gross visual assessment of Safranin-O and Mallory's Trichrome with and without EVs revealed an increased ECM production on scaffolds treated with EVs. In scaffolds treated with EVs, cells formed a more linear and organized structure compared to the sparser structure of the untreated scaffolds.

Conclusion: EVs promote chondrogenic phenotypes in a 3D model overtime and compared to a 2D monolayer culture of chondrocytes. EVs demonstrated anti-inflammatory effects on human synovial stem cells. Histological findings show the synovial cells also demonstrated differentiation potential into chondrocytes visualized through the increased ECM production. These results support the chondroprotective and regenerative capabilities of extracellular vesicles.



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