

# The biological role of the meniscus and the influence of synovium co-culture on meniscus healing.

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**INTRODUCTION:** The meniscus is critical to stabilizing the joint and transmitting loads. However, little is known about the communication between meniscus and synovium and the influence of this communication/crosstalk on meniscus healing. This study aims to evaluate the biological role of the meniscus and study the influence of synovium co-culture on meniscus healing. We hypothesize that there is crosstalk between the meniscus and synovium. Furthermore, we expect a co-culture with osteoarthritic synovium to have a negative impact on meniscus healing.

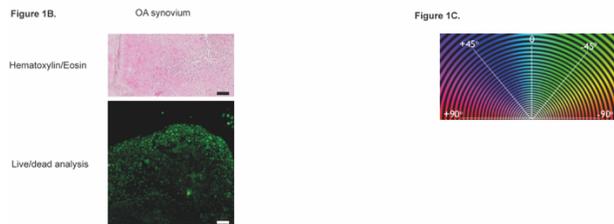
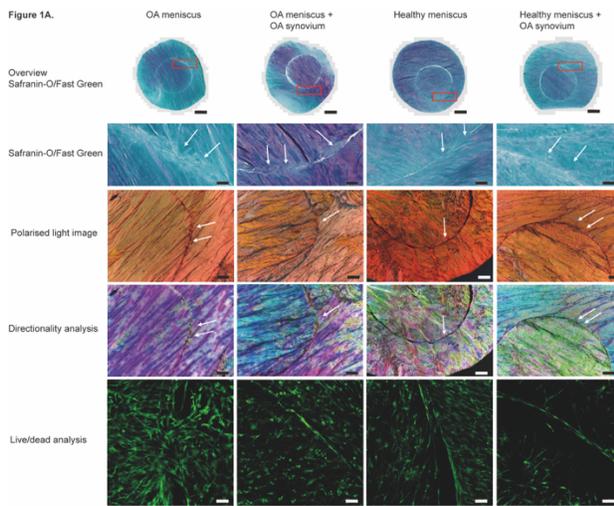
**METHODS:** To understand the impact of meniscus and synovium crosstalk, we used a meniscus donut model (MDM) to evaluate the following groups: osteoarthritic (OA) meniscus, OA meniscus with OA synovium, OA synovium, healthy meniscus, and healthy meniscus with OA synovium. Healthy menisci were obtained from JRF Ortho (3 male, 1 female) and OA samples were obtained from patients undergoing total knee arthroplasties (2 male, 3 female). We assessed viability and histological staining to evaluate tissue healing and cytokine and matrix metalloproteinase (MMP) secretion to evaluate the biological role of the meniscus. Picrosirius red-stained tissues were acquired under two polarization directions to visualize fiber orientation. Secretion of interleukins (IL) and MMPs in the cell-culture supernatant was evaluated using a Luminex® xMAP® technology through Eve Technologies Corporation. In this abstract we evaluate the secretion of IL-8, IL-10, TNF- $\alpha$  and MMP-2 between groups.

**RESULTS:** Synovium and meniscus tissue remained viable throughout the 21-day culture period. After 21 days, tissue repair occurred at the biopsy intersection in all groups with no pronounced differences between groups. Live/dead images showed live cells and cell accumulation along the border of the inner and outer region of the model. (Figure 1) Co-culture groups with synovium have higher IL-8 and MMP-2 secretion compared to meniscus monoculture. Co-culture groups of OA meniscus with synovium have a lower secretion of TNF- $\alpha$  than OA synovial tissue alone. IL-10 is elevated in all groups. (Figure 2)

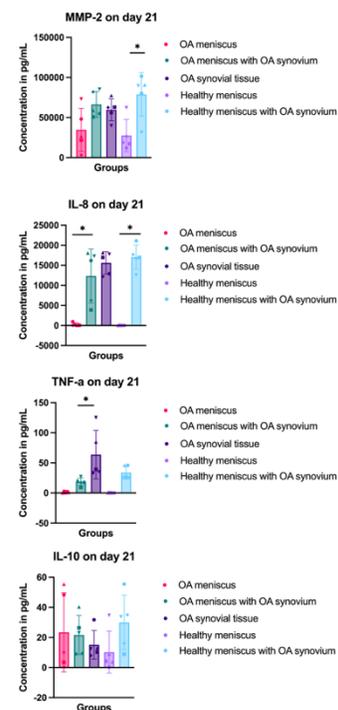
**DISCUSSION:** The co-culture groups of OA meniscus with synovium have a lower secretion of TNF- $\alpha$  than OA synovium alone, this might suggest that the OA meniscus modulates TNF- $\alpha$  secretion by the synovium. The elevated IL-10 secretion in the meniscus groups compared to the OA synovium group, suggests an anti-inflammatory effect of the meniscus on the pro-inflammatory cytokine profile of the OA synovium.

**SIGNIFICANCE:** Meniscus tissue and synovium show significant and measurable crosstalk. The interplay between IL-10 and TNF- $\alpha$  might suggest a modulatory role of the meniscus in inflammation. These results support the paradigm shift to a biological role of the meniscus in joint homeostasis compared to only a mechanical role.

## IMAGES and TABLES:



**Figure 1A.** Healthy and osteoarthritic meniscus mono- and cocultured with osteoarthritic synovium at 21 days. Safranin-O/Fast-Green images taken at 20x with the scale bar indicating 1000µm on the overview picture and 100µm on the zoomed in picture. Polarised light images of picrosirius red images taken at 4x with the scale bar indicating 1000µm. Live/dead images taken at 10x with the scale bar indicating 50µm. The red square indicates the zoomed in part of the overview meniscus. White arrows indicate unaligned collagen fibers on the Safranin-O/Fast Green, Picrosirius red and polarised picrosirius red images. Live cells are green, dead cells are red. Colors on the directionality analysis image correspond with different degrees as seen on Figure 1C.  
**Figure 1B.** Osteoarthritic synovium monocultured at 21 days.  
**Figure 1C.** Osteoarthritic synovium monocultured at 21 days.  
All pictures taken at 20x. Scale bar of the Hematoxylin and Eosin staining is 100µm. Live cells are green, red cells are dead.



**Figure 2.** Healthy and osteoarthritic meniscus groups mono- and co-cultured with OA synovium and OA synovium monoculture. The concentration of MMP-2, IL-8, IL-10 and TNF- $\alpha$  in the cell-culture supernatant on day 21 are shown for the following groups: healthy and osteoarthritic meniscus groups mono- and co-cultured with OA synovium and OA synovium monoculture. Each bar represents the mean cytokine concentration in cell-culture supernatant in pg/mL with each point representing a donor. A two-way ANOVA with a Tukey's multiple comparison test between groups was performed and its significant results are shown.