A compartmentalized joint-on-chip model as tool to investigate cartilage-synovium interactions in early stages of osteoarthritis

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The absence of reversing therapies for osteoarthritis (OA) is mainly due to the **disease complexity** and to the **gap of knowledge on initial disease mechanisms**, linked to the **unavailability of reliable human preclinical in vitro OA models.**

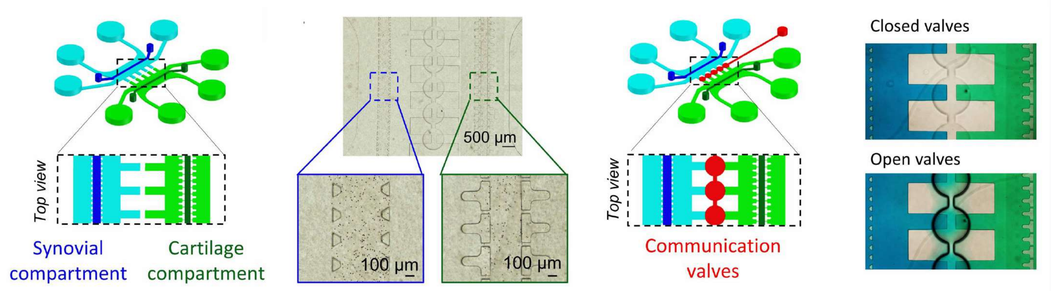
Organs-on-chip have the potential to provide in-depth insights into the **interactions between different joint tissues during early OA stages**, and can help to determine the **cause-and-effect relationships** between the various factors involved in the disease development.

AIM OF THE STUDY

Purpose of this study was to develop a **compartmentalized joint-on-chip model to co-culture cartilage and synovium tissues and induce OA traits**, aiming at **investigating how the communication between these tissues is disrupted and contributes to the development of OA.**

COMPARTMENTALIZED MICROFLUIDIC PLATFORM FOR CARTILAGE-SYNOVIUM CO-CULTURE

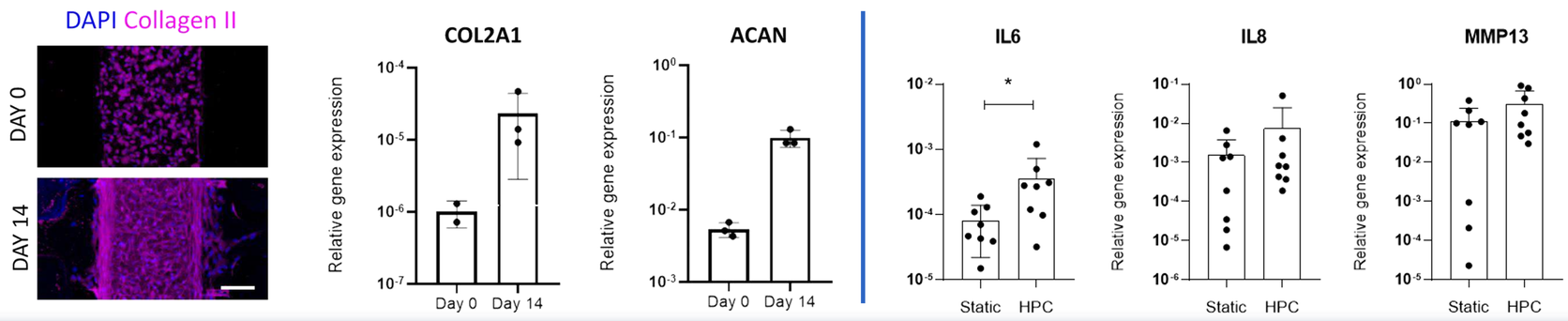
The proposed microfluidic device is composed of a culture chamber layer consisting of **two separate culture areas**, intended for **synovium and cartilage cultures**, each of them composed of a central channel to host **3D micro-constructs**, flanked by two lateral medium channels. The **paracrine communication** between the two compartments is controlled through **normally closed curtain valves** that can be opened through vacuum application in a dedicated valve layer. An additional **actuation layer** allows to apply a **mechanical compression to the cartilage compartment** upon pressurization[4].



3D CARTILAGE MODEL AND HYPERHPYSYIOLOGICAL COMPRESSION

Human articular chondrocytes (hACs) embedded in fibrin gel were cultured for **two weeks** statically in chondrogenic medium, recapitulating **mature cartilage micro-constructs**, as proven by a matrix rich in **collagen type-II** and by the **up-regulation of *COL2A1* and *ACAN*** expression at day 14.

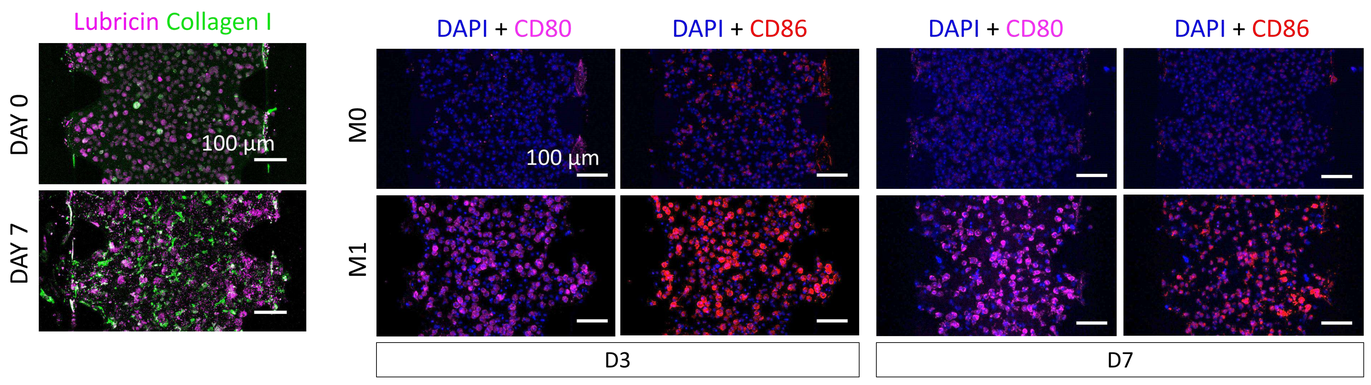
After maturation, a **cyclic hyperphysiological compression (HPC)** was applied for **one week** to induce a **shift towards an OA phenotype**, as indicated by a significant increase in *IL6* expression, and by an increasing trend of *IL8* and *MMP13* gene expression.



3D SYNOVIUM MODEL AND INFLAMMATION

Human synovial fibroblasts (SFBs) and monocytes-derived macrophages (M0s) were embedded in a mix of fibrin gel and collagen type-I, and cultured up to **7 days** in serum-free culture medium. Immunofluorescence stainings of synovial micro-tissues proved an **increased presence of lubricin and collagen type-I** seven days after seeding.

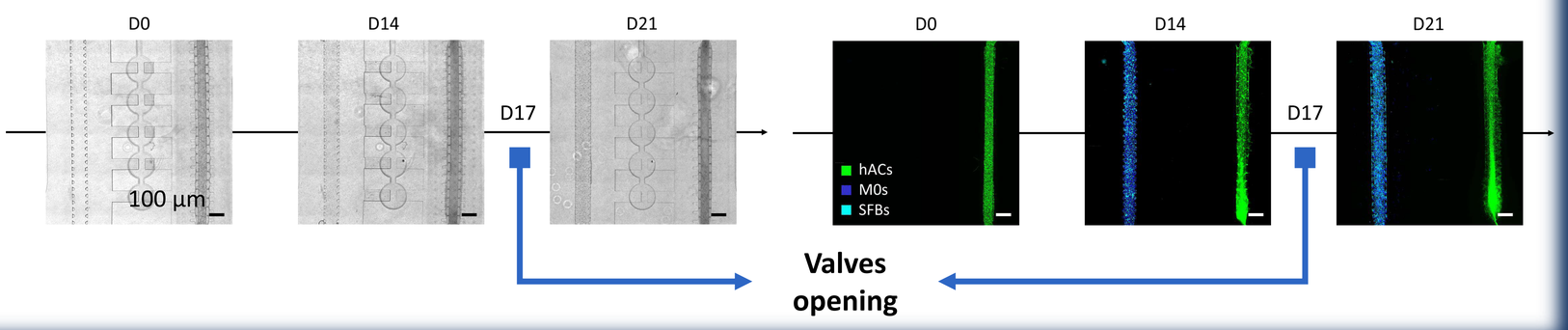
A protocol was then established to induce an **inflammatory state in the synovium**: an **inflammatory stimulus (TNF-α + IFN-γ) was administered for 3 days, followed by 4 days without stimuli**. The defined inflammation protocol could successfully enhance the synthesis of MMPs operated by SFBs and could induce **macrophage polarization towards pro-inflammatory state M1**, as shown by an increased presence of CD80 and CD86 markers at day 3. These results were maintained also at day 7, meaning that the **pro-inflammatory phenotype is stable**.



OPTIMIZATION OF CARTILAGE-SYNOVIUM CO-CULTURE

The experimental plan for the co-culture was optimized as it follows: hACs and synovial cells were seeded in the platform at day 0 and at day 14, respectively, and co-cultured up to day 21 with closed valves.

On day 17, i.e. either after three days of HPC on cartilage construct or after three days of inflammatory stimuli in the synovial tissue, valves can be lifted up and the effect in the other compartment assessed.



CONCLUSIONS

The proposed compartmentalized microfluidic platform allows the **generation of 3D human cartilage and synovial micro-constructs and the induction of OA traits in one of the two micro-tissues**, by controlling the communication between the compartments in both space and time. This joint-on-chip model offers a valuable solution to assess whether mechanically-damaged cartilage triggers inflammatory changes in the synovium, and vice versa whether an inflamed synovium triggers cartilage degradation, aiming at **determining which of the two tissues plays the primary role in early OA stages**.

REFERENCES

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ACKNOWLEDGMENTS

This work was partially financed by CARIPLO Foundation, Grants No. 2018-0551 and 2021-1564, has received funding from the European Union's Horizon 2020 research and innovation programme under the Marie Sklodowska-Curie grant agreement No 841975.

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