Investigating the contribution of cartilage and synovium to osteoarthritis development through a compartmentalized human joint-on-chip model

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Osteoarthritis (OA) is **the most prevalent degenerative joint disorder**, causing pain and disability

No reversing therapies have been developed yet, due to due to the **disease complexity** and to the lack of understanding on **initial disease mechanisms**

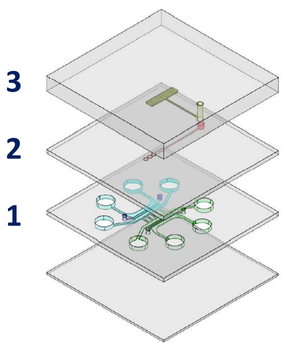
Organs-on-chip can be used to unravel joint tissues interactions during OA early stages , and can help to determine the **cause-and-effect relationships** between the various factors involved in the disease development.

AIM OF THE STUDY

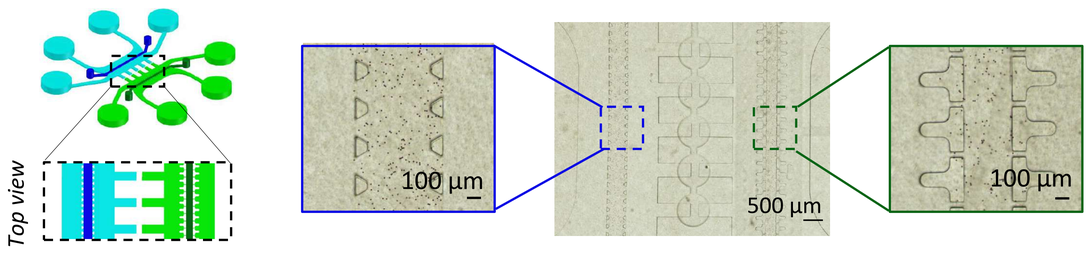
Purpose of this study was to develop a **compartmentalized joint-on-chip model** allowing for the **co-culture cartilage and synovium tissues** and for the **induction of OA traits**, aiming at investigating how the **communication between these tissues is disrupted and contributes OA pathogenesis**

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.

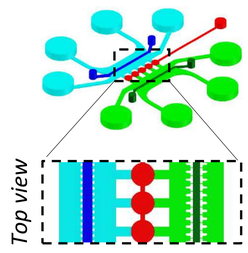


1. Chambers layer

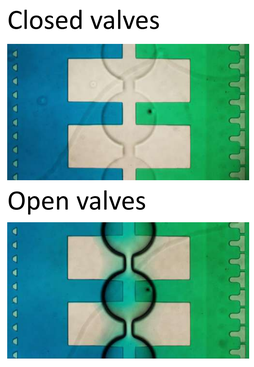


Synovial compartment — Cartilage compartment

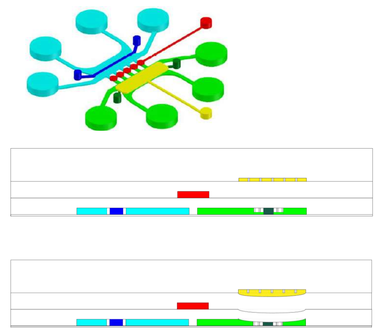
2. Valves layer



Communication valves



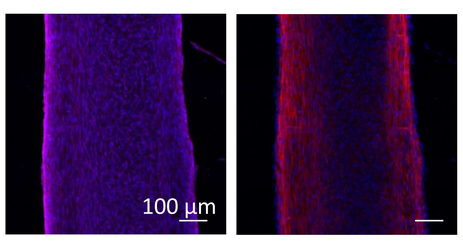
3. Actuation layer



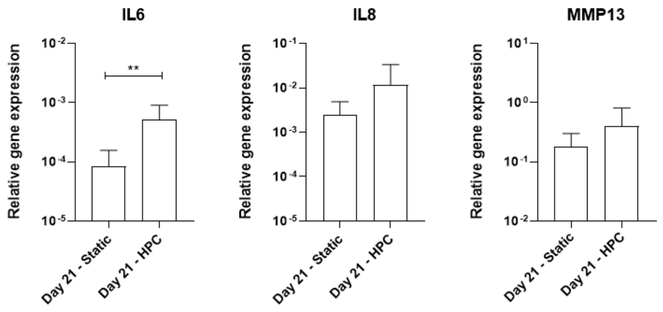
Cartilage OA model

Human articular chondrocytes (hACs) embedded in fibrin gel were cultured for **two weeks** and **cartilage maturation** was demonstrated by deposition of matrix rich in **collagen type-II** and **aggrecan**.

A **cyclical hyperphysiological compression (HPC)** was then applied for one week to induce a **shift towards an OA phenotype**, as assessed through upregulation of inflammatory markers (e.g. IL6, MMP13).

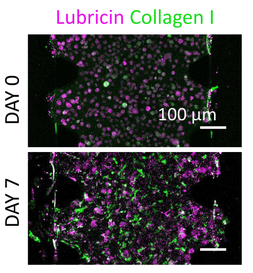


DAPI Collagen II DAPI Aggrecan

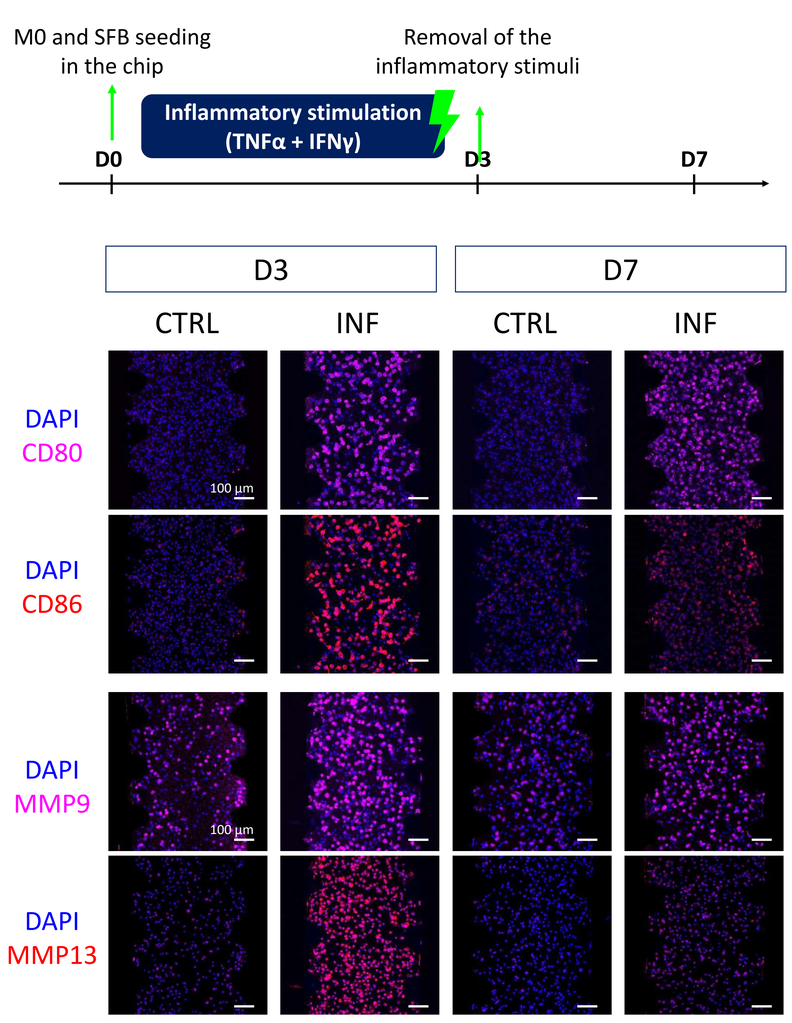


Synovium model and inflammation

Human synovial fibroblasts (SFBs) and monocytes-derived macrophages (M0s) embedded in a mix of fibrin gel and collagen type-I were cultured for up to **7 days**, and synthesis of **collagen type-I** and **lubricin** was shown.

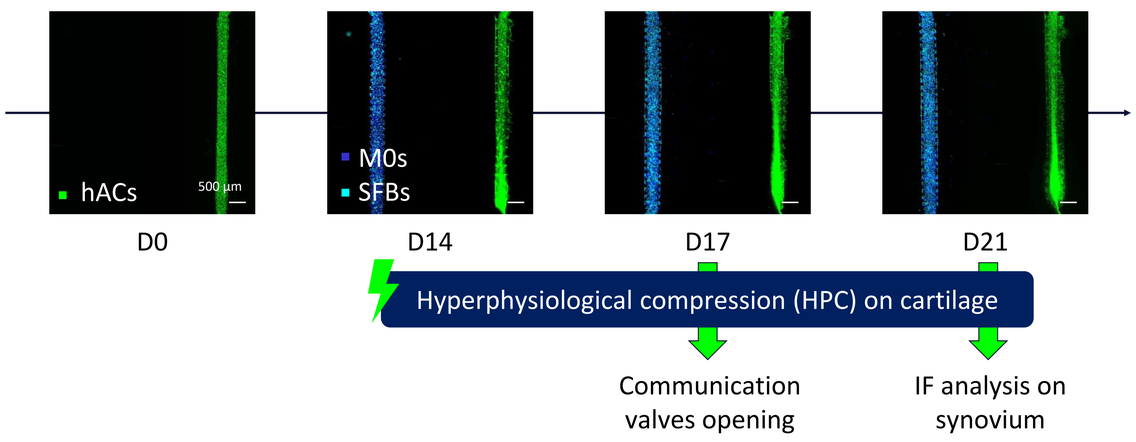


A protocol was then established to induce an **inflammatory state in the synovium**: an **inflammatory stimulus (TNF-α + IFN-γ) was administered for 3 days, followed by 4 days without stimuli**. The defined inflammation protocol could successfully **enhance the synthesis of MMPs** and could induce **macrophage 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 **inflammatory phenotype is stable**.

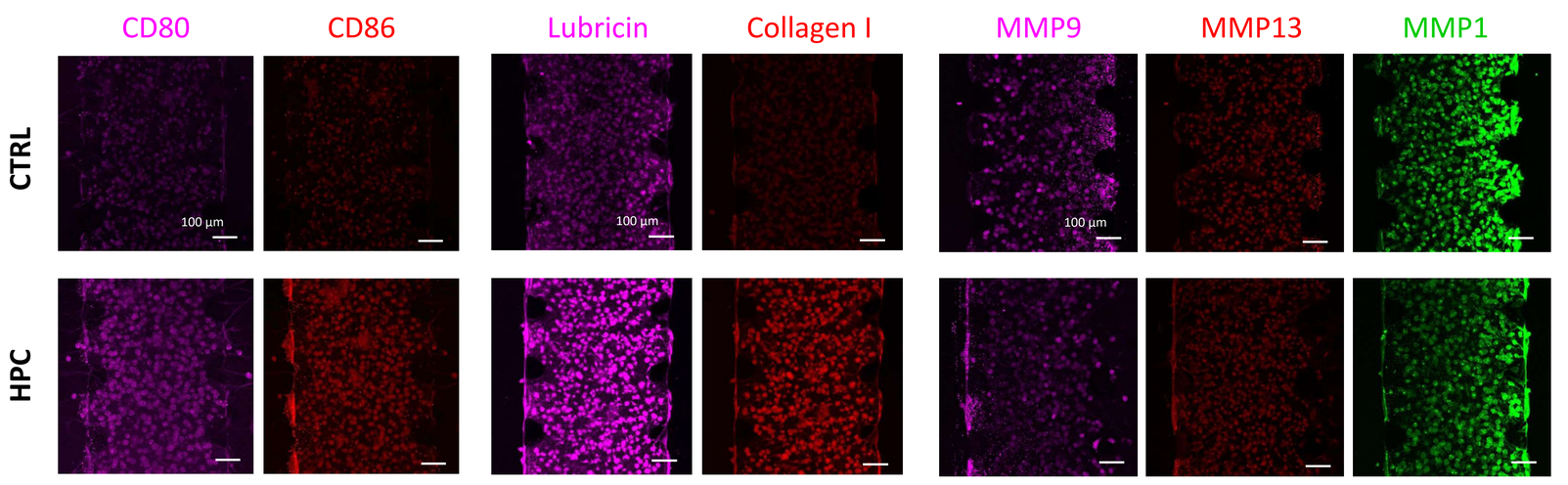


Effect of HPC cartilage on healthy synovium

An experimental set-up was optimized to **co-culture** cartilage and synovial microtissues in the platform, and to assess the effect of HPC OA cartilage on a healthy synovial tissue.



Immunofluorescence stainings performed on synovial tissue **revealed macrophage polarization towards pro-inflammatory state M1** and an **increase in collagen-type I and lubricin synthesis**, as found in literature during early OA stages. **No differences in MMPs** synthesis were detected with respect to controls, probably indicating that during the disease onset there is an increase in synovial matrix production and anabolic activity, substituted by catabolic activity in the later stages of the disease.



CONCLUSIONS

The compartmentalized microfluidic platform offers a solution **to independently mature 3D cartilage and synovial constructs**, as well as **to induce OA traits** in one compartment specifically, through the implementation of **temporal control over chambers communication**. The device was used to demonstrate that mechanically-induced damages to cartilage triggers inflammatory changes in the synovium. Further studies will help to determine **which of the two tissues plays the primary role in early OA stages**.

REFERENCES

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