A compartmentalized joint-on-chip to unravel cartilage-synovium crosstalk in osteoarthritis

Palma C.[1], Salehi S.[2], Moretti M.[2], Occhetta P.[1], Lopa S.[2], Rasponi M.[1]

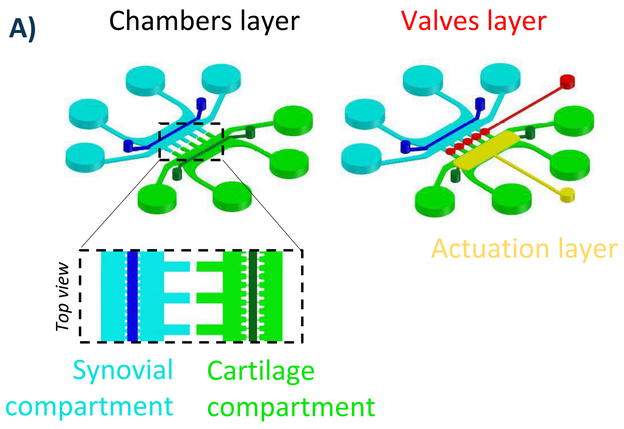
[1]Department of Electronics, Information and Bioengineering, Politecnico di Milano, Italy; [2]Cell and Tissue Engineering Laboratory, IRCCS Istituto Ortopedico Galeazzi, Milan, Italy

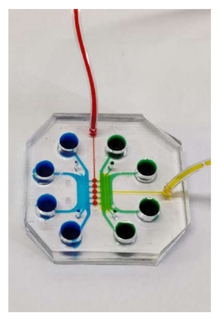
INTRODUCTION

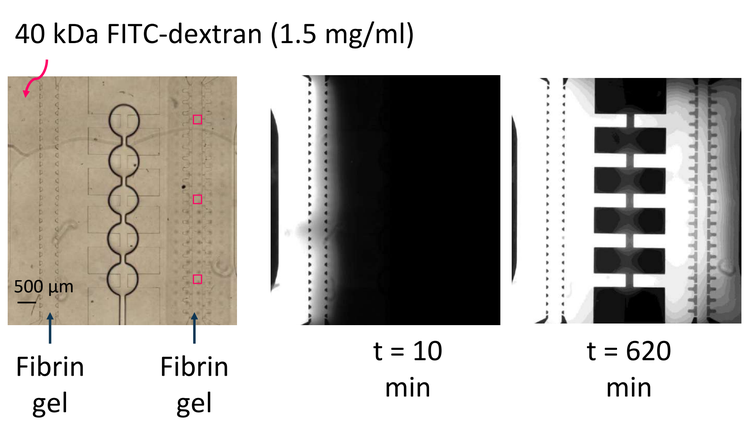
Osteoarthritis (OA) is **the most prevalent degenerative joint disorder**, but **no reversing therapies** have been developed yet[1], due to the **disease complexity** and to the **disease multifactorial aetiology**[2]. A gap of knowledge still exists on initial disease mechanisms, linked to the unavailability of reliable human preclinical in vitro OA models[3]. In this scenario, **organs-on-chip** are promising candidates to elucidate cause-effect relationship among **players in early OA stages**. To this end, we developed a compartmentalized **joint-on-chip model for the co-culture of cartilage and synovium**, aiming at evaluating the disruption of the physiological cross-talk between these tissues that contributes to the pathogenesis of OA[4].

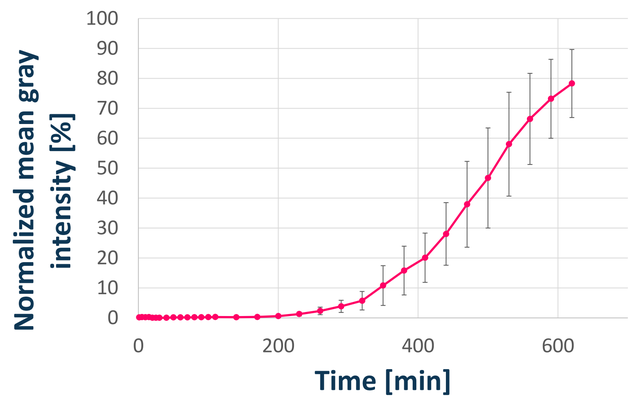
COMPARTMENTALIZED MICROFLUIDIC PLATFORM FOR CARTILAGE-SYNOVIUM CO-CULTURE

The microfluidic device (A) comprises a culture chamber layer consisting of **two separate culture areas**, intended for synovium and cartilage cultures, respectively, whose paracrine communication is controlled through **normally closed curtain valves**. An **actuation layer** allows to apply a mechanical compression to the cartilage compartment upon pressurization[5]. The platforms were fabricated in PDMS through soft lithography (B) and technically characterized to estimate **cytokine diffusion** from one comportment towards the other one, upon central valves opening (C).





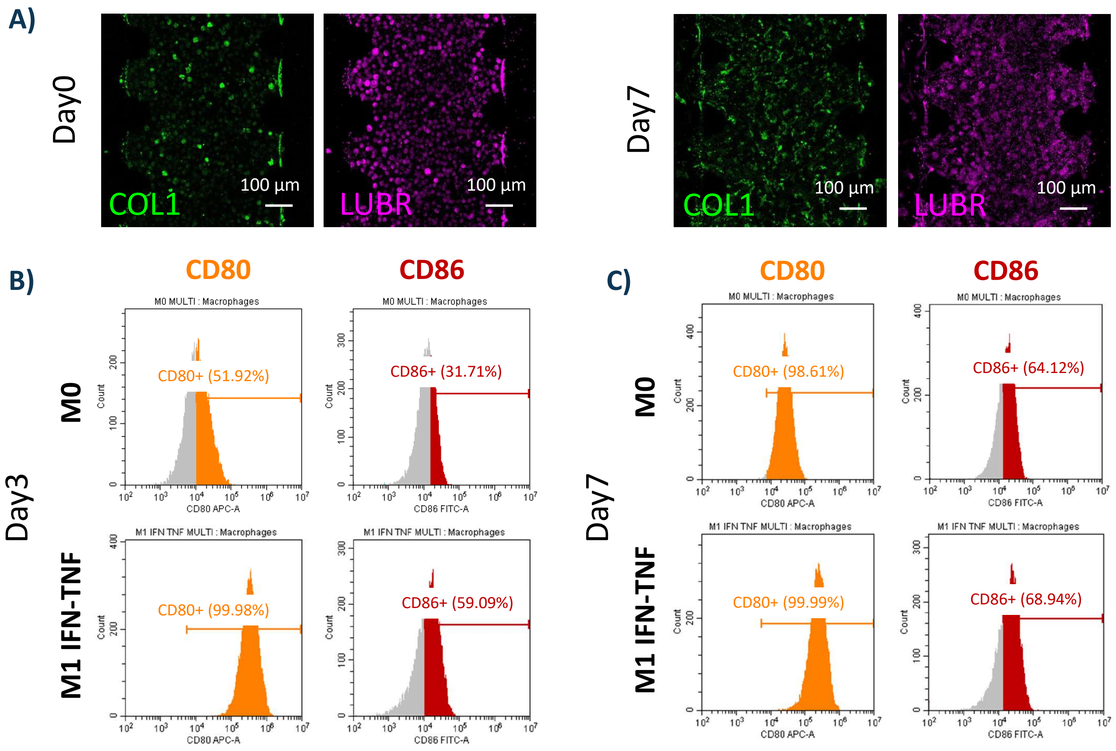




SYNOVIUM MODEL

Human synovial fibroblasts (SFB) and **macrophages** (M0) were embedded in a mix of fibrin gel and collagen I (50/50 ratio v/v), with a total cell density of 25 M/ml (SFB/M0 ratio 50/50) and cultured for up to 7 days. Immunofluorescence stainings proved proper synthesis of **collagen I** and **lubricin** (A).

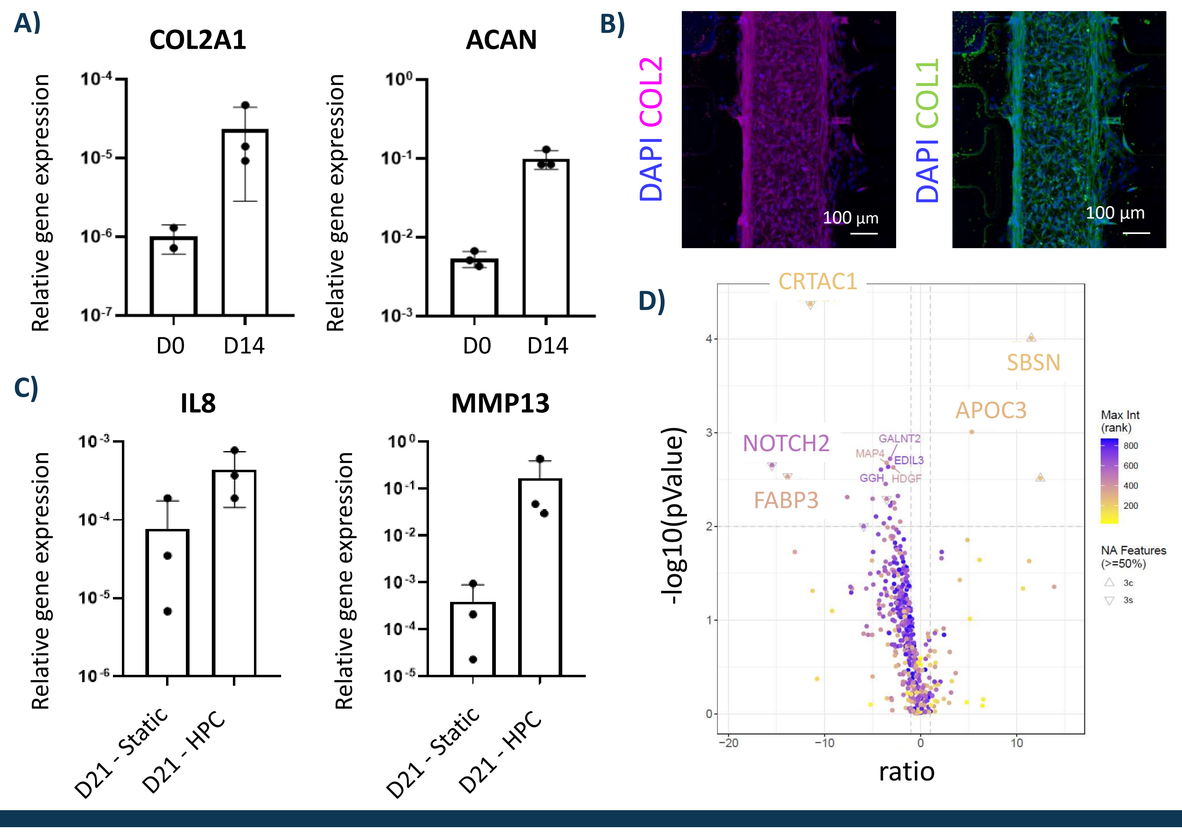
A protocol was established to induce **M0 polarization towards pro-inflammatory state** (M1): an **inflammatory stimulus** (TNF-α+IFN-γ) was administered for 3 days to M0 cultured in 2D, followed by 4 days without stimuli. As proven by flow cytometry (FACS), an increase in the expression of inflammatory markers (CD80, CD86) was detected at day 3 (B), and maintained until day 7 (C). The same protocol was applied to the 3D synovial model, confirming induction of an **inflammatory phenotype** on resident macrophages.



CARTILAGE MODEL

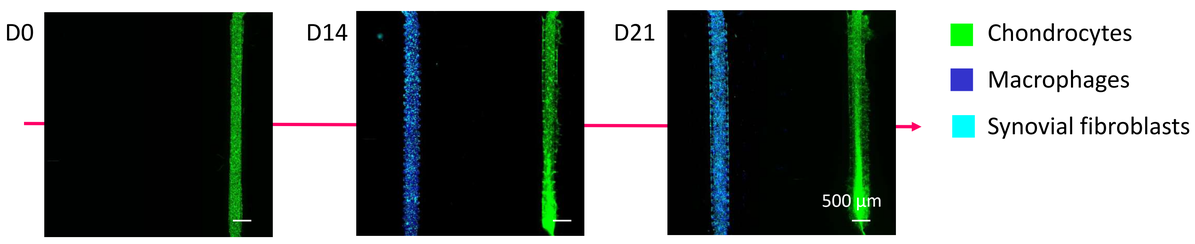
Human articular chondrocytes embedded in fibrin gel (50 M/ml) were cultured for two weeks in chondrogenic medium, and **cartilage maturation** was demonstrated by upregulation of *COL2A1* and *ACAN* (A), and deposition of matrix rich in collagen (B).

A **cyclical hyperphysiological compression** (HPC) was applied for one week to induce a shift towards an **OA phenotype**[5], as assessed through upregulation of cytokine *IL8* and degrading enzyme *MMP13* (C). Liquid chromatography-mass spectrometry (LC-MS) was performed on cell supernatant: **differentially expressed proteins** were identified, suggesting pathways possibly correlating with OA onset (D).



CO-CULTURE OF 3D SYNOVIAL AND CARTILAGE MODELS

An experimental set-up was optimized to **co-culture** cartilage and synovial microtissues in the platform, exploiting compartmentalization to comply with specific maturation timings.



DISCUSSION and CONCLUSIONS

The proposed device offers a solution to mature **cartilage and synovial constructs** in separated compartments, by enabling a **temporal control over chambers communications**. The platform is being currently used to assess whether mechanically-damaged cartilage triggers inflammatory changes in the synovium, and whether synovitis has a role on cartilage degradation, eventually allowing to understand which of the two tissues has a **predominant role in early OA stages**.

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cecilia.palma@polimi.it