Organs-on-chip and proteomics as tools to identify key molecular players responsible for human OA initiation

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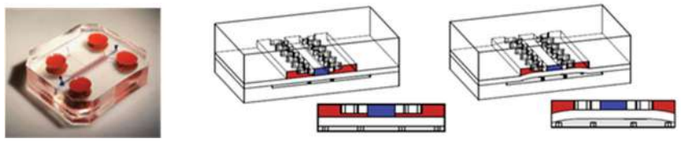
INTRODUCTION

Osteoarthritis (OA) is the most prevalent degenerative joint disorder and is a major cause of disability in the aging population[1,2]. However, despite its high prevalence, **no reversing therapies are currently available**[3]. This is mainly due to the gap of knowledge on initial OA mechanisms, linked to the disease multifactorial etiology and to the unavailability of reliable preclinical models. Here, we exploited a microscaled platform to induce a shift towards an **OA phenotype on a human cartilage-on-chip model**[4], and by means of **proteomics analysis** on cell culture supernatant we aimed at identifying possible **molecular players responsible for OA initiation**.

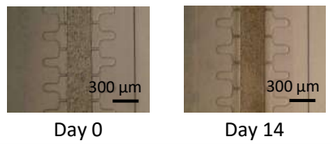
MATERIALS & METHODS

Generation of a human OA cartilage-on-chip model

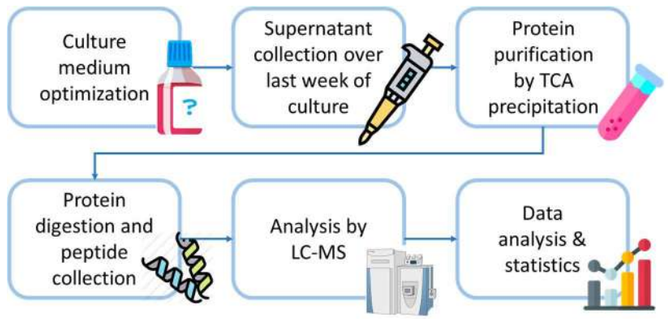
The microfluidic device described by Occhetta *et al.*[4] was used to recapitulate a **cartilage on-chip** model. After cartilage maturation, an **hyperphysiological cyclical compression (HPC culture)** was applied for 7 days to the constructs to induce an **OA phenotype**.



Occhetta et al., Nat. Biomed. Eng. 2019

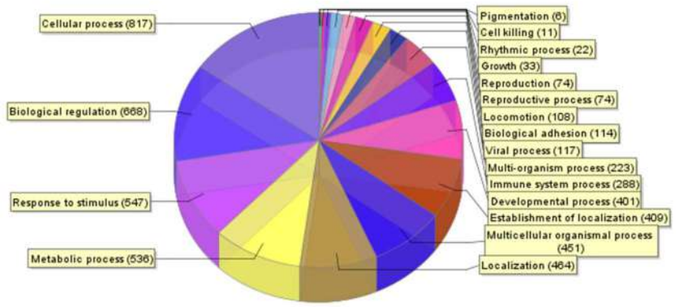


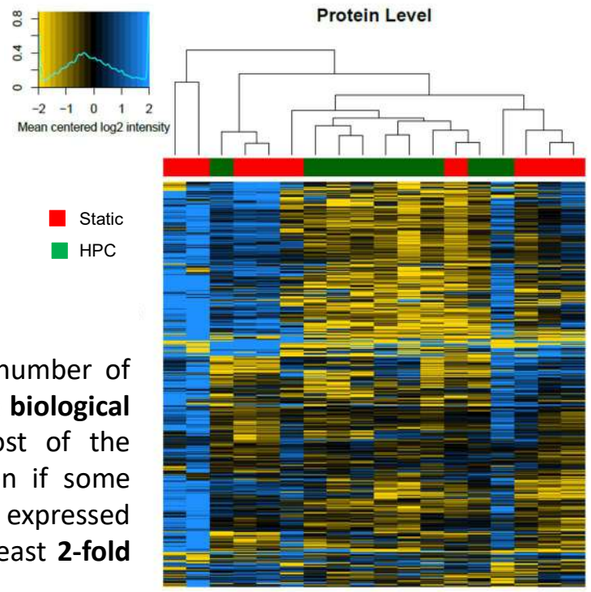
Proteomics analysis on cell culture supernatant

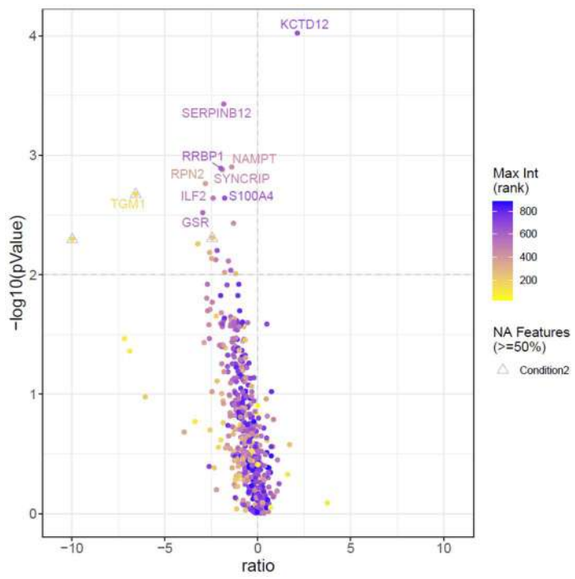


During the last week of HPC culture, culture medium was optimized to remove components that could interfere with the analysis (i.e. human serum albumin, TGFβ3 and dexamethasone). **Supernatant** was then collected at every medium change. After sample preparation, **LC-MS** was performed comparing static and mechanically stimulated samples to investigate **proteins** that were **significantly differentially expressed upon OA HPC induction**.

RESULTS







The proteomics analysis allowed us to identify a huge number of **proteins** synthesized by chondrocytes, related to **multiple biological processes**. The protein level heatmap shows that most of the proteins were **downregulated** in the HPC samples, even if some outliers are present. The most significantly differentially expressed proteins were then identified and only proteins with at least **2-fold change** were considered for further investigation.

OUTLOOK

Organs-on-chip are promising solutions as OA preclinical models, as they recapitulate complex environment, while allowing to reduce the use of animals in accordance with **3R principles**. However, analysing the outcomes of interest can be tricky due to the low amount of available material. Here, we performed **proteomics analysis on cell culture supernatant of human cartilage-on-chip models** and could detect significant differences between **HPC-induced OA samples** and controls. Differentially expressed proteins were identified and will be used to investigate **key molecular players** triggered by a mechanical damage, that in turn may trigger other events in the **OA cascade**.

AFFILIATIONS

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ACKNOWLEDGEMENTS

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