A MULTISCALE APPROACH TO STUDY CHONDROCYTE MECHANOBIOLOGY USING A CARTILAGE-ON-CHIP SETUP

Satanik Mukherjee[1], Laurens Spoelstra[3], Thanh Le[3], Carlo Alberto Paggi[3], Raphaelle Lesage[1], Jorge Barrasa Fano[1], Hans van Oosterwyck[1], Marcel Karperien[3], Liesbet Geris[1,2]

[1]KU Leuven, Belgium;[2]University of Liège, Belgium ;[3]University of Twente, the Netherlands

Introduction

Mechanical loading is an essential factor that regulate the health of chondrocytes. Understanding the mechanobiology of chondrocytes not only helps in unravelling factors that contribute to cartilage degeneration in Osteoarthritis (OA), but also in devising mechanical cues to stimulate chondrocytes to regenerate cartilage in a tissue engineering scenario. Given that chondrocyte mechanobiology is a multiscale and multifactorial process, studying it *in vivo* is a daunting task. To circumvent this, we propose a multiscale *in silico - in vitro* approach using a combination of numerical modeling and a cartilage-on-chip microfluidic device [1] that mimics the mechanical environment of the chondrocyte in the knee joint. Using this approach, we investigate how mechanical loading might affect the synthesis of relevant matrix proteins by the chondrocyte that results in a change in the chondrocyte microenvironment.

Methods

Primary human chondrocytes (1.5 million cells/mL) were seeded in 2% w/v agarose hydrogel, together with fluorescent beads (3.17 microns, 5 millions/mL) and injected in the cartilage-on-chip device. The device was actuated for dynamic compression with a pressure of 300 mBar and a frequency of 1Hz. An in-house algorithm [2] was used to track the beads to obtain mechanical strains around the chondrocyte due to the mechanical loading imposed. Immunofluorescence staining was performed for Collagen 2 and 6, followed by confocal microscopy to obtain cell specific matrix deposition. Mechanical characterization of the agarose was executed separately using unconfined stress relaxation experiments. Subsequently, a multiscale *in-silico* model of the setup was developed (Figure 1). The multiscale model consisted of 3 different length scales: i) **Gel-level finite element (FE) model**, containing the cell and bead laden hydrogel in the setup; ii) **Cell-level FE model**, containing individually segmented cells in hydrogel from the setup, and iii) **Intracellular gene/protein regulatory network**, which is an additive, semi-quantitative gene and protein regulatory network for chondrocyte mechanotransduction and inflammation developed using a combination of knowledge-based and inference-based approach [3].

Results

Mechanical characterization of the agarose hydrogel revealed that the stiffness of the hydrogel was reduced by 12% on addition of the chondrocytes. There was a further reduction of the stiffness by 10 % on addition of the beads. However, increasing the density of the beads from 1.5 million/mL to 10 million/mL did not cause significant change. By tracking the beads, the distribution of strain across the hydrogel in the cartilage-on-chip was obtained. Furthermore, on zooming in to individual cells, and obtaining brightfield and fluorescent microscopy images, we were able to calculate both cellular deformations as well as deformations of the cellular microenvironment (Figure 1). The gel level and cell level deformations obtained experimentally corresponded closely to the numerical predictions from the model. After a week of static culture of the cells in the setup, immunofluorescent staining revealed deposition of Coll2 and Coll6 by the cells in their near vicinity, thereby indicating the formation of a pericellular matrix by the cells (Figure 2).

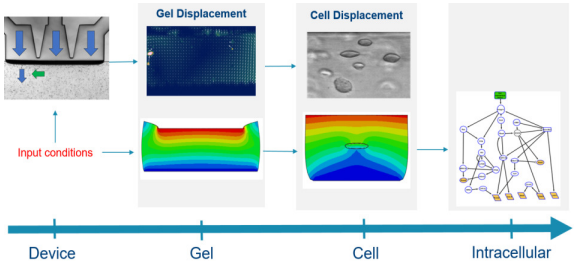

Figure 1: Multiscale workflow for the cartilage-on- chip

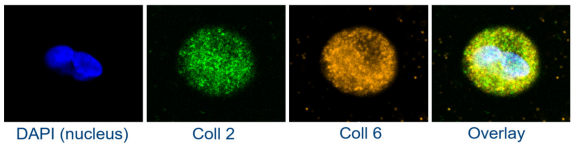

Figure 2: Matrix protein synthesis by a single cell

Discussion

Using the cartilage-on-chip device together with *in silico* modeling, we were able to study chondrocyte mechanobiology in a multiscale manner from an external mechanical stimulus to a cellular response. The established workflow not only allowed measuring cell-specific deformations, but also measuring local deposition of matrix constituents by the cells that represent the pericellular matrix. The developed approach has huge potential to facilitate cartilage tissue engineering by unravelling suitable conditions to ensure gradual development of healthy cartilage.

References

1. Paggi et.al. *Lab Chip*, 2022,22, 1815-1828
2. Barrasa-Fano et al. *SoftwareX*, 2021, 15: 100723.
3. Lesage et. al. *Bmc Biology*, 2022, 20.1: 253.

Acknowledgements

We acknowledge funding from the EC (MSCA 721432 & ERC CoG 772418), FWO and ESB Mobility Award.