Study of chondrocyte mechanobiology using a cartilage-on-chip device: a multiscale approach

Satanik Mukherjee[1], Raphaelle Lesage[1], Laurens Spoelstra[3], Thanh Le[3], Carolina Serrano Larrea[3], Carlo Alberto Paggi[3], 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: Chondrocytes, the primary cells in articular cartilage require mechanical loading to maintain homeostasis. 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 cartilage regeneration is a multiscale and multifactorial process, studying it *in vivo* is a daunting task. To support this, we have developed 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 investigated how mechanical loading might affect the synthesis of relevant matrix proteins by chondrocytes embedded in agarose hydrogel. Furthermore, the gradual change in the chondrocyte microenvironment during cartilage regeneration can also be studied, highlighting the change in mechanics that chondrocytes experience during the regeneration process.

METHODS: Primary human chondrocytes (1.5 million cells /mL) were seeded in 2% w/v agarose hydrogel, together with fluorescent beads (3.17 microns diameter, 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 0.5 Hz. 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 to obtain individual cells' matrix deposition. Mechanical characterization of the cell-seeded 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 approaches [3].

RESULTS: Mechanical characterization of the agarose hydrogel revealed that the stiffness of the hydrogel was reduced by 12% upon addition of the chondrocytes. There was a further reduction of the stiffness by 10 % upon 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. In the cartilage-on-chip device, a 36 µm membrane deformation was observed upon application of 300 mBar actuating pressure. By tracking the beads, the distribution of strain across the hydrogel was obtained. A gradient in hydrogel deformation was observed, with the deformation decreasing on moving further away from the actuating membrane (Fig 1b). 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 (Fig 1d). 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, which was further increased following a week on dynamic mechanical stimulation with a frequency of 1Hz and amplitude of 300mBar. This thereby indicates the influence of external mechanical stimulation in aiding the formation of a pericellular matrix by the cells (Fig 1c).

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 measured 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 workflow can be used in the future to investigate specific mechanisms of chondrocyte mechanotransduction, as well as test the efficacy of pharmaceutical drugs in arresting mechanically induced catabolic processes in chondrocytes.

SIGNIFICANCE/CLINICAL RELEVANCE: The developed multiscale approach has huge potential in not only unraveling the role of mechanical loading in progression of OA but also to delineate the role of mechanical loading in cartilage tissue engineering by quantifying the properties of osteochondral implants that can provide appropriate mechanical stimulation to the embedded progenitor cells.

REFERENCES: [1] Paggi et.al. *Lab Chip*, 2022; [2] Barrasa-Fano et al. *SoftwareX,* 2021; [3] Lesage et. al. *Bmc Biology,* 2022.
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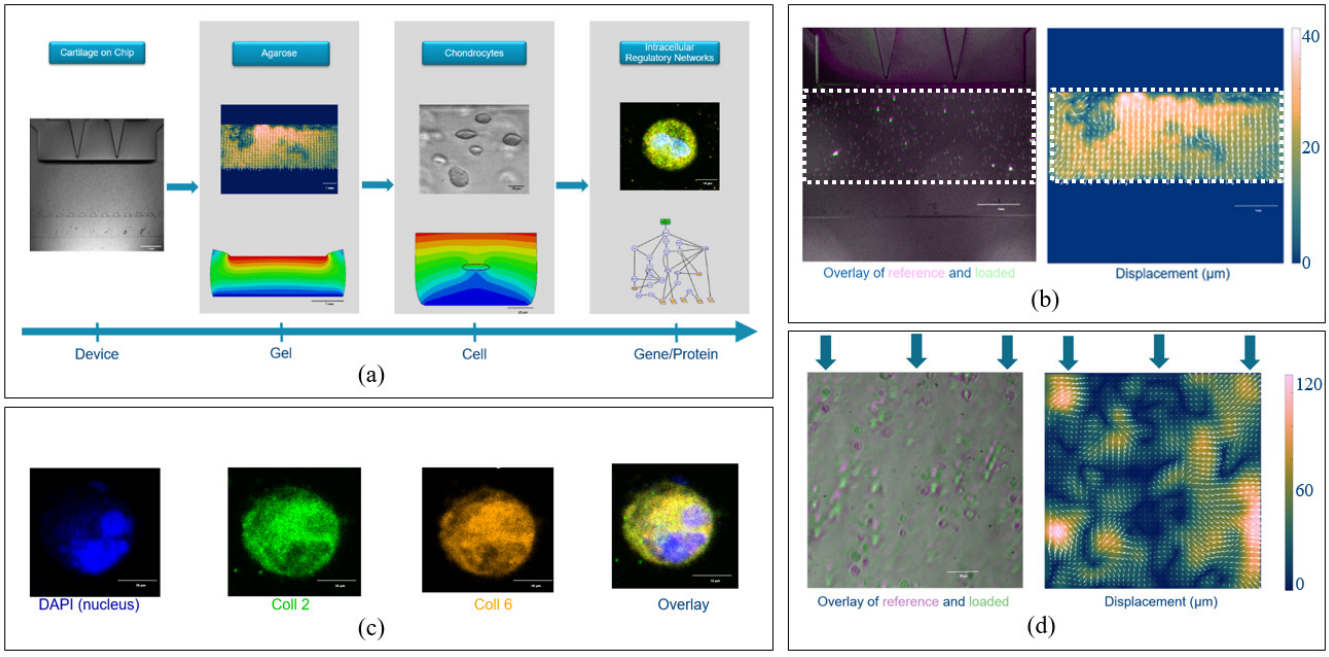

Figure 1: (a) Overview of the multiscale workflow for the cartilage on chip setup, (b) Deformation of the hydrogel measured by particle tracking, (c) Matrix protein synthesis by a single cell after 2 weeks of culture (1 week static +1 week dynamic loading). (d) Deformation of the cellular micro-environment