CARTILAGE ON CHIP

INTRODUCTION

Articular cartilage covers the bone friction surfaces in joints (Figure 1). Among all the cartilage present in the human body the knee is the largest.

The principal function of the cartilage is to provide a smooth, lubricated surface for articulation and to facilitate the transmission of loads between the bones [1].
This function is ensured by the collagen distribution present in the cartilage. Cartilage tissues are neither vascularized nor innervated. They are mainly composed of dense extracellular matrix (ECM) with a sparse distribution of highly specialized cells called chondrocytes.

Nutrients diffusing from the blood vessels partially reach the chondrocytes forming gradients from the bone edge to the cartilage layer close to synovial fluid [2].

Due to the absence of blood vessels within the cartilage tissue, mechanical stimulation created upon motion promotes the delivery of nutrients to the chondrocytes. Moreover, depending on the specific mechanical stimulation, chondrocytes can regulate their own microenvironment through the production of specific proteins. One particular example is lubricin, which is found only in the superficial layers of the cartilage and which is produced by the cell due to the combination of compression and shear strain generated during movement. This protein has an essential function in the reduction of the friction during movement.

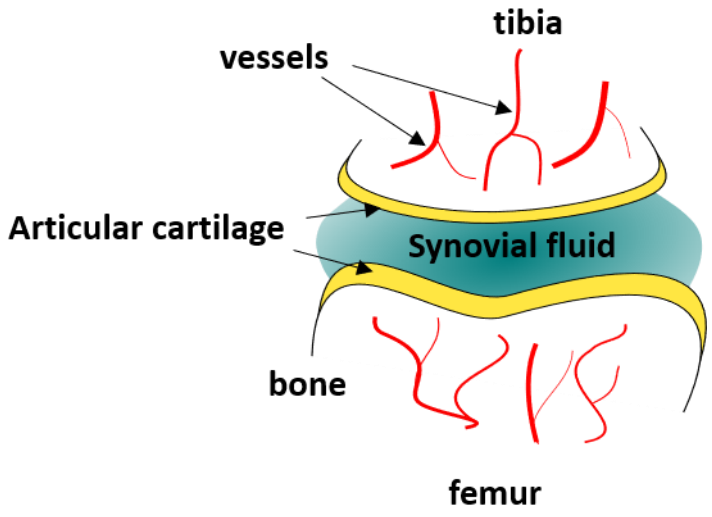

Figure 1: Schematic representation of a joint

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Elucidating how chondrocytes react to external stimuli (mechanical or chemical) is important to understand processes triggering cartilage diseases like osteoarthritis [3]. To this end, various approaches, as briefly summarized below, have been explored so far, while they all suffer from specific limitations:

Traditionally, cells are cultured in vitro in Petri dishes or culture flasks, in which the biological response to specific soluble stimuli such as drugs or inflammatory markers can be evaluated. 2D culture is widely used due to the high experiment reproducibility, and as such to the consistency of the obtained results.
However, in this approach, cells adhere from only one of their side to a hard plastic substrate, while in vivo, cells like chondrocytes are in a 3D homogeneous environment consisting of extracellular matrix. As such, the in vitro culture conditions induce a non-physiological phenotype, which has a significant impact on the results of the in vitro assays and in turn gives to misleading conclusions. Moreover, in this approach the culture is static, while in vivo a cell microenvironment is highly dynamic.

To overcome some of these limitations, three-dimensional (3D) culture approaches have been introduced. This 3D configuration better reproduces the in vivo cell environment. For cartilage specifically, collagen I and II, which are important components of the extracellular matrix in cartilage, can only be produced by chondrocytes cultured in 3D.
Although 3D models provide cells with an environment similar to the in vivo situation, they are again static and subsequently cannot incorporate the physiological stimulations occurring in cartilage tissues, such as continuous nutrient supply and, more importantly, specific mechanical stimulation.

To create more advanced culture platforms and address some of the aforementioned limitations, microfluidics has been introduced. It notably allows implementing mechanical stimulation on 3D cell culture and studying in situ the cell response to these stimulations, as well as creating dynamic culture conditions. Microfluidics is a powerful tool for in vitro experiments as it allows not only to control but also to couple and uncouple the biological, mechanical and chemical parameters of a cellular assay.

In this application note, we report on the use of Fluigent products to create complex mechanical stimulation patterns on 3D cell culture in a microfluidic platform, or so-called organ-on-a-chip device, with a specific focus on creating a cartilage-on-a-chip model.

MATERIALS AND METHODS

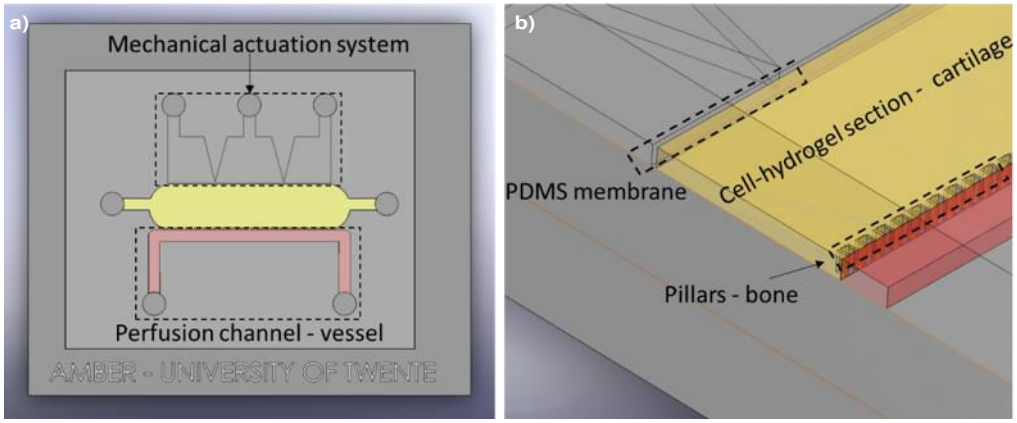
Microfluidic platform

The platform was fabricated using soft-lithography in PDMS, and assembled to a PDMS-coated glass slide. This platform allows replicating the following main cartilage parts and functions:

Culture chamber:
3D culture of chondrocytes in a hydrogel matrix

Nutrient supply:
Nutrients delivered to the 3D cell culture from the blood vessels present in bone.

Mechanical stress:
Application of various compression and shear stress modalities to the 3D cell culture using 3 chambers individually addressable, and through which either negative or positive pressure could be applied.



a) Top view of the device, showing the cell culture chamber in yellow, the perfusion channel in red and the mechanical actuation system;

b) Cross-section of the platform showing the structure of the cell culture chamber, located between a thin PDMS membrane and an series of pillars.

Figure 2: Cartilage-on-a-chip platform

The cartilage-on-a-chip platform (Figure 2) comprises several key-features:

A mechanical stimulation unit, composed of three actuation chambers connected to each other, yet independently addressable through individual inlets. Through each inlet, a positive or negative pressure of various amplitude can be applied to generate various mechanical stimulation patterns: compression only, or shear stress, to eventually mimic the mechanical stimulation experienced by cartilage tissue.

A thin PDMS membrane, whose deflection upon application of various pressure patterns, actually induce mechanical stimulation on the chondrocytes.

A 3D cell culture chamber, in which chondrocytes are grown in a 3D hydrogel matrix. This chamber is delimited by the PDMS membrane and an array of pillars.

A perfusion channel used to provide nutrients to the cells in the 3D hydrogel matrix, acting as the blood vessels in the bone.

Mechanical stimulus generation

A MFCS-EZ pressure controller (Fluigent) coupled to three 2-switch valves (Fluigent) has been used to generate the mechanical stimulation in the cartilage-on-a-chip platform (Compression and/or shear stress). Fluigent OxyGEN software (Fluigent) was used to automate the process and produce complex stimulation patterns, by changing the type of stimulus (positive or negative pressure) and its amplitude applied to the three actuation chambers (Figure 4).
A camera (ORCA-flash 4.0 LT, Hamamatsu Photonics) was used to monitor and analyse the PDMS membrane deformation upon application of various pressure patterns (negative or positive pressure, the latter ranging from 0 mbar to 1500 mbar).

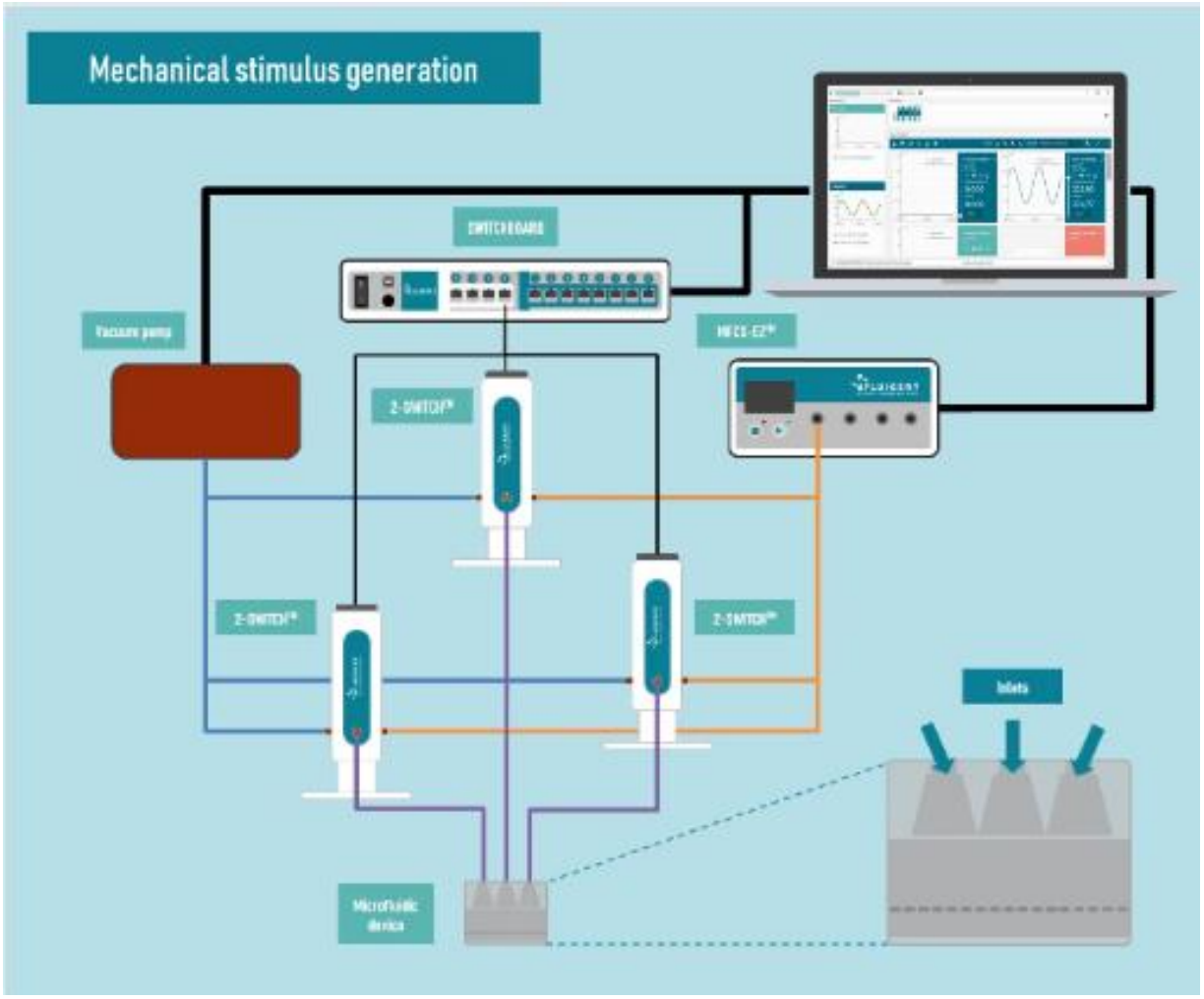


Figure 3: Schematic representation of the fluidic set up

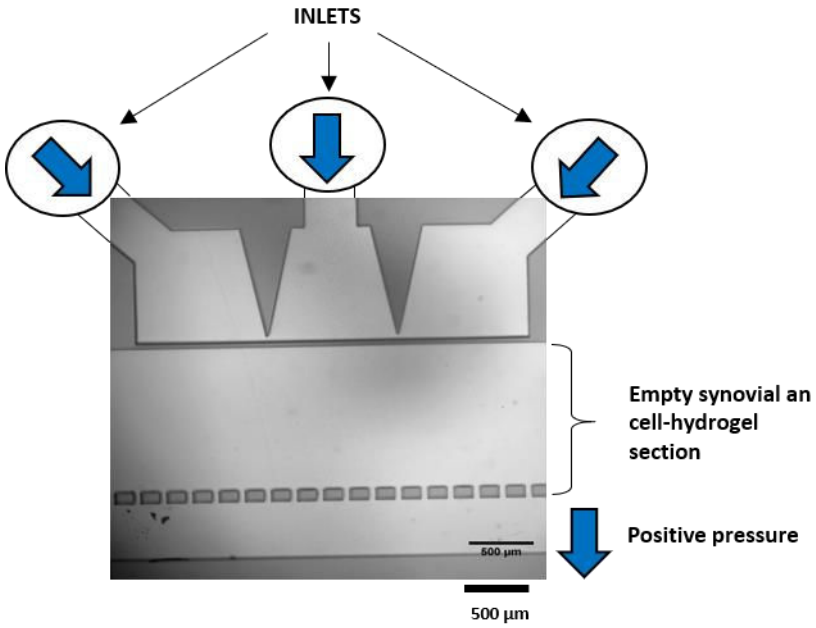


Figure 4: Picture of the culture chamber and the three actuation chambers

Cell culture and isolation

Human chondrocytes were obtained from a patient undergoing total knee replacement. Chondrocytes coming from a healthy-looking cartilage were expanded in culture flasks with chondrocyte proliferation medium for cell culture (DMEM supplemented with 10% FBS, 0.2 mM ascorbic acid 2-phosphate (AsAP), 0.1 mM non-essential amino acids, 100 U/ml penicillin and 100 µg/ml streptomycin, 4 mM proline). Medium was replaced every 3 days. Before any experiment, cells were counted to yield a 1 million cells/mL suspension.

Cell viability

Cells were cultured in the microfluidic device in an agarose 3D matrix for 3 or 6 days with or without mechanical stimulation. At the end of the experiment, cell viability was evaluated using a standard live/dead assay. Briefly, culture medium was removed from the microfluidic device, the devices were washed once with PBS medium, and medium containing two fluorescent dyes to detect both living (green) and dead cells (red) was perfused. After 30 min incubation, fluorescence images were acquired using a fluorescent microscope (EVOS FL, Thermo Fisher Scientific, United States).

RESULTS

Generating complex stimulation patterns

To test the deflection of the PDMS membrane and optimize the device parameters, different positive pressures (0-1500 mbar) were applied in the actuation chambers, in an all compression mode (same positive pressure applied to the three actuation chambers).

The positive pressure was applied using a MFCS-EZ pressure controller (Fluigent) ranging from 0 to 2000 mbar, and negative pressure using a vacuum pump.

These two pressure-generation systems were connected to three different 2-switch valves (Fluigent), which allowed switching in a well-controlled manner between positive and negative actuation, using a script written in Fluigent software.

Figure 5 shows an example of an experiment where the applied pressure was changed independently in the three actuation chambers, at different time points, to generate complex membrane deflection patterns and customized stimulations on the 3D cell-laden hydrogel. Here, the hydrogel (agarose) was supplemented with 15-micron beads, used as surrogates of cells, to characterize the cell displacement upon deflection of the membrane. This specific configuration (Figure 5) allows applying bulk shear stress on the cells (steps 2 to 3, and steps 3 to 4) at given locations in the cell culture chamber.

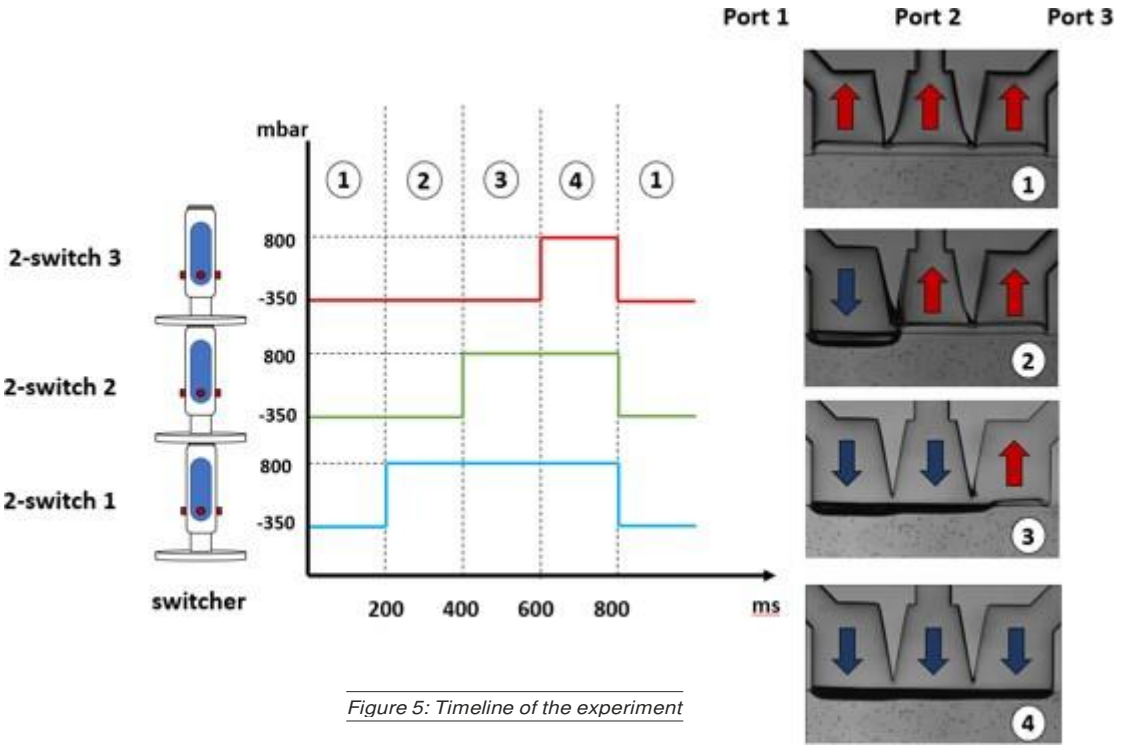


Figure 5: Timeline of the experiment

Legend:

E*xperiment showing the position of the 3 independent switches and the corresponding pressure applied in the three actuation chambers. Corresponding microscope pictures of the cartilage-on-a-chip platform, showing the actual membrane deformation and the mechanical stimulation applied on the 3D hydrogel matrix (supplemented with 15-micron beads, here).*

Cell culture experiments

Chondrocytes were cultured in the microfluidic platform for 6 days under static (Figure 6) or dynamic conditions, and stained afterwards to evaluate their viability. Dynamic conditions were applied in the form of 1.5 h of stimulation of the cell-laden hydrogel per day, using uniform compression (application of 800 mbar on all three actuation chambers with a 1-Hz frequency).

A live/dead assay revealed that the chondrocytes were not altered by the mechanical stimulation since the ratio of living cells to the total number of cells was similar in static and dynamic conditions (data not shown).

Furthermore, the cell deformation across the chamber was characterized (Figure 6); it amounted to 13% close to the membrane, and decreased across the cell culture chamber.
Future experiments will examine the impact of various stimulation patterns on cells, in terms of gene expression levels, extracellular matrix production and cell deformation.

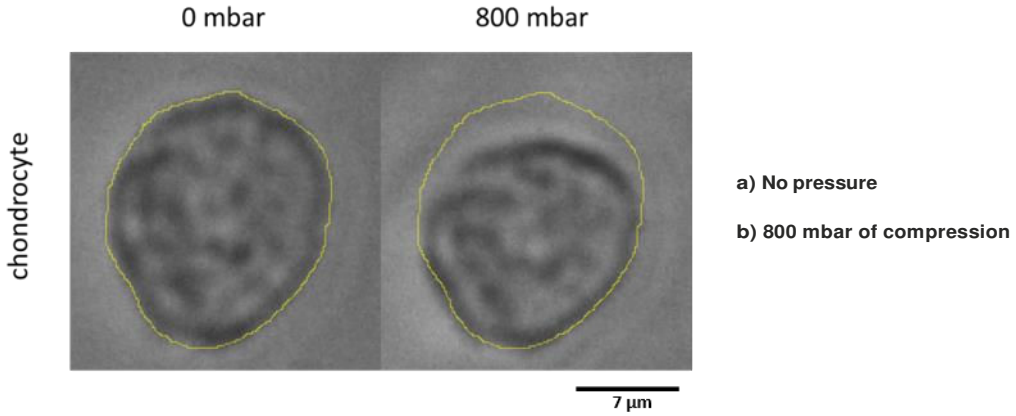


Figure 6: Chondrocyte deformation

Altogether, this new cartilage-on-a-chip platform is a good alternative to current experimentation approaches and cartilage in vitro models since it allows cultivating cells in a 3D extracellular matrix, while applying different mechanical stimulation patterns, providing a continuous flow of nutrient on cells and examining in situ the cell response to the applied stimuli. Fluigent pressure controlled, 2-switch valves and software are all instrumental to conduct such research, since it allows applying customized mechanical stimulation, at given frequencies and for specific time durations, and programming it in a user-friendly manner.

CONCLUSION

In the past years, transitioning from traditional 2D culture to 3D culture was a big improvement for in vitro systems. 3D culture is more physiologically relevant as they induce a phenotype, genetic expression, differentiation and proliferation rate that are similar to what occurs in vivo. However, these models are still simple compared to living organs. In vivo, most cells are constantly submitted to chemical and mechanical stimulations. Microfluidics and microfabrication are powerful methods to reproduce the complexity of living organs.

The example illustrated in this application note is the use of microfluidic to reproduce the mechanical forces exerted on the articular cartilage. Fluigent products were decisive to reproduce such stimulation. Their precision and the ability to fully automate different fluidic component at once were determinant to reproduce the amplitude and frequency of stimulation over long term experiment. The automated setup is able to exert compression and reproduce different cartilage physiological function at the same time without altering cell viability.
Its versatility allows to transpose this platform for many further studies such as gut, vessels or esophagus on chip.

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