Electronic Supplementary Material (ESI) for Lab on a Chip.

Emulating the chondrocyte microenvironment using multi-directional mechanical stimulation in a cartilage-on-chip

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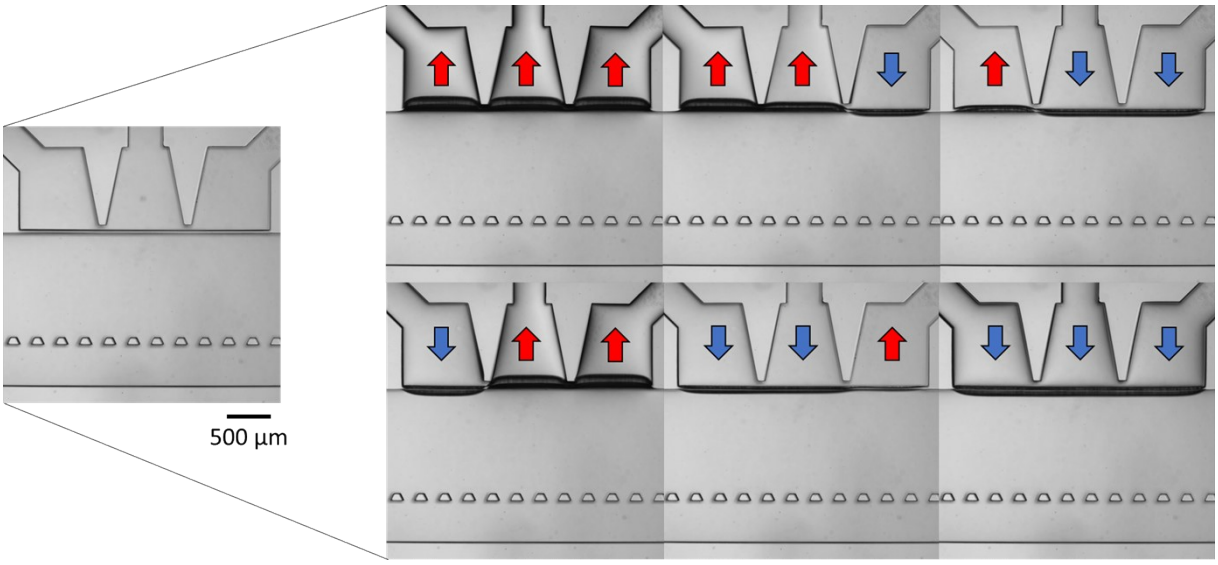
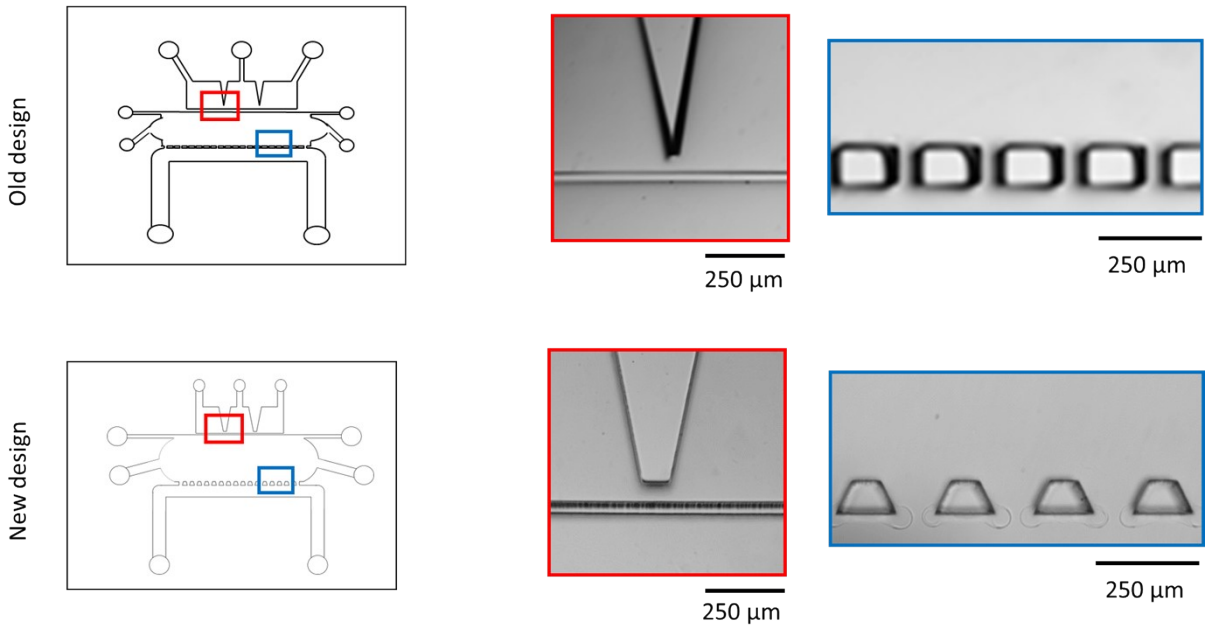
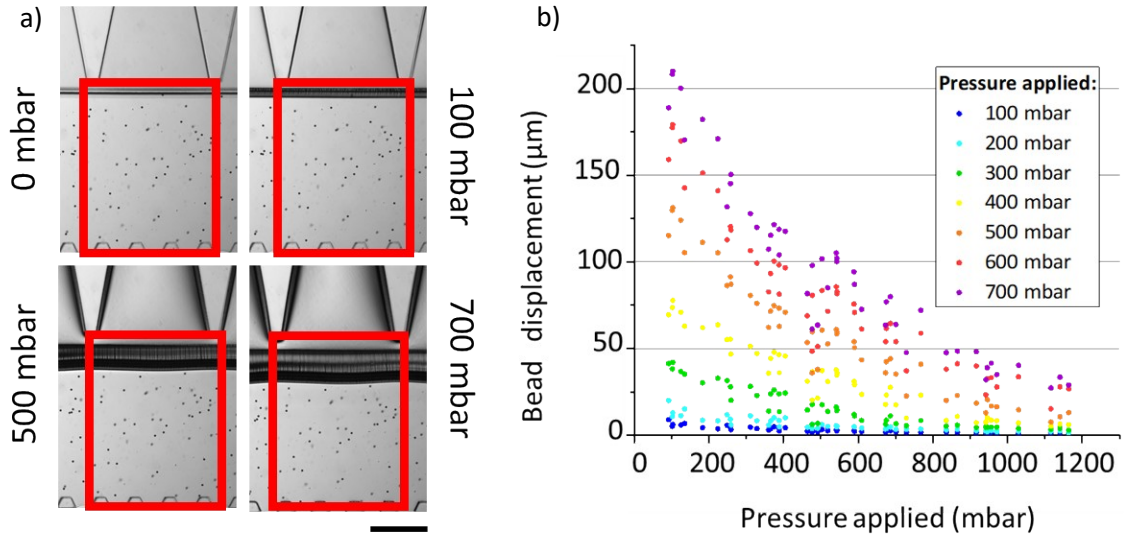


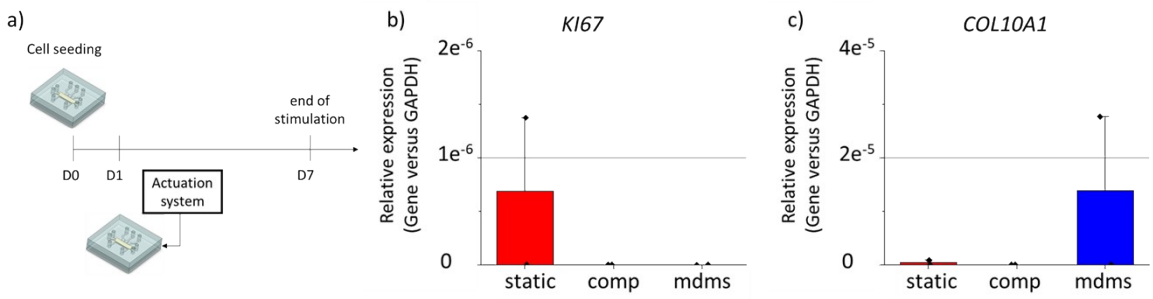
Supplementary Figure 1: Top view of the cartilage-on-chip system without any hydrogel matrix in the cell culture chamber. Deformation of the 50 µm thick PDMS membrane is achieved by applying positive (blue arrow) and/or negative (red arrow) pressure in the three individual actuation chambers generating a multi-directional mechanical stimulation pattern.



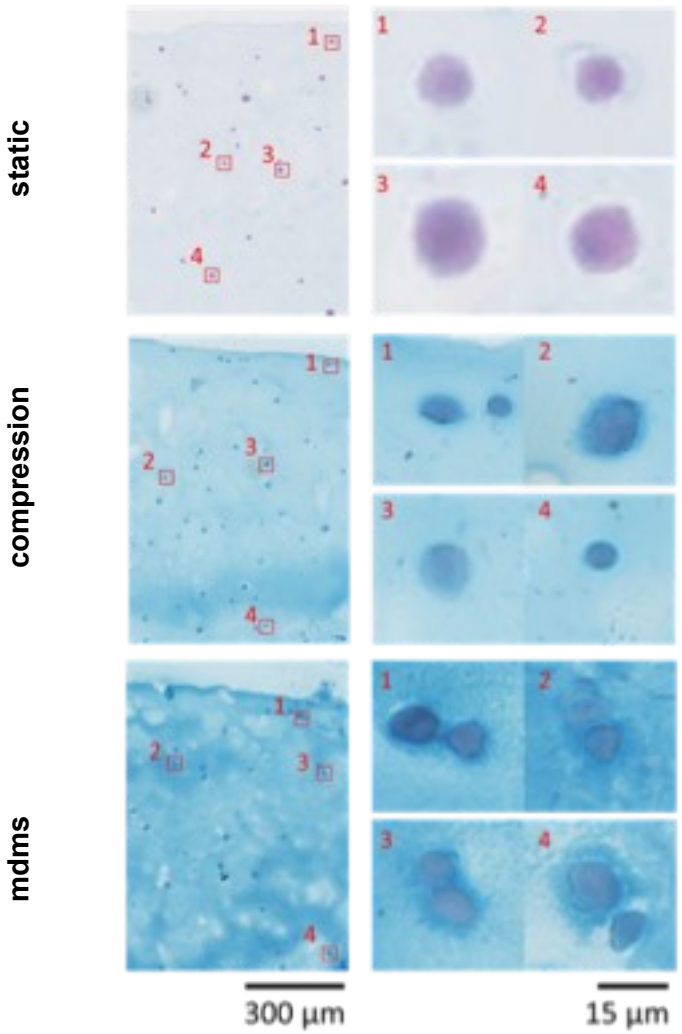
Supplementary Figure 2: *From left to right*: schematic representation of the top view of the old and new cartilage-on-chip devices, highlighting the applied modifications to the mechanical actuation (red square) and the pillars that separate the cell-hydrogel chamber from the perfusion (blue square). The aspect ratio of the membrane is increased as well, which is not depicted on this top view of the system.



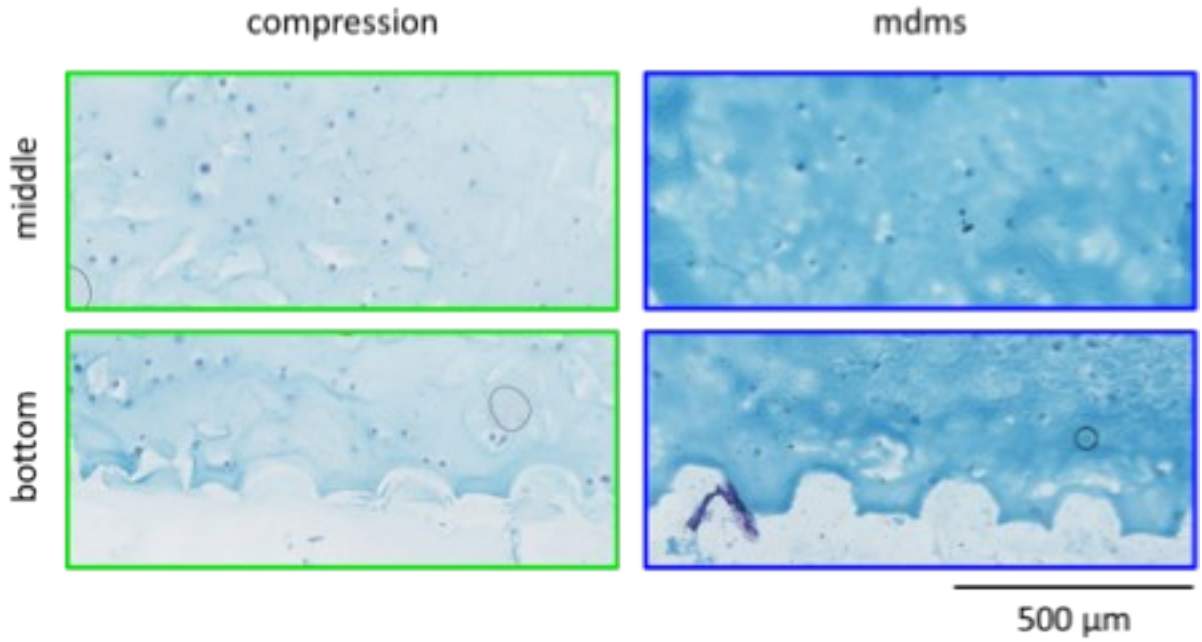
Supplementary Figure 3: Determination of the hydrogel deformation under application of compressive forces only, by evaluating the displacement of microbeads (15-µm in size) in the agarose matrix. a) Top view of the middle actuation chamber with microbeads embedded in 2% low melting temperature agarose in PBS (w/v) at different pressures. Scale bar: 500 µm. b) Graph depicting the displacement of 47 microbeads at different distances from the actuation chamber and for different applied pressures; all microbeads were selected in front of the middle actuation chamber as highlighted in the pictures in a.



Supplementary Figure 4: a) Experimental design with three culture conditions (static, compression only and multi-directional mechanical stimulation - mdms). Expression level of *KI67* (b) and *COL10A1* (c) normalized to that of the house-keeping gene *GAPDH*.



Supplementary Figure 5: Histology slides stained with Alcian blue of 2% low melting temperature agarose in which human chondrocytes are embedded after 15 days of static culture, exposure to compressive forces or multi-directional mechanical stimulation (stimulation being applied from day 1 for 14 days).



Supplementary Figure 6: Histology analysis of the cell-laden hydrogel: comparison of the GAG production on the middle of the chamber and next to the pillars. Agarose (2% low melting temperature agarose) stained with Alcian blue, in which human chondrocytes were embedded and exposed to compressive forces (*left*) or multi-directional mechanical stimulation (mdms) (*right*) for 14 days, while being in culture for 15 days in the device.

Supplementary table 1: Statistical analysis of the different pericellular matrix of chondrocytes in the 5 sections (from 0 to 1000 µm) in the compression (c) and multi-directional mechanical (mdms) stimulation.

Comparison of hydrogel sections	P_{value}
0-200 mdms 0-200 c	n.s
200-400 c 0-200 c	n.s
200-400 c 0-200 mdms	n.s
200-400 mdms 0-200 c	n.s
200-400 mdms 0-200 mdms	n.s
200-400 mdms 200-400 c	n.s
400-600 c 0-200 c	**
400-600 c 0-200 mdms	**
400-600 c 200-400 c	n.s
400-600 c 200-400 mdms	n.s
400-600 mdms 0-200 c	***
400-600 mdms 0-200 w	**
400-600 mdms 200-400 c	n.s
400-600 mdms 200-400 mdms	n.s
400-600 mdms 400-600 c	n.s
600-800 c 0-200 c	n.s
600-800 c 0-200 mdms	n.s
600-800 c 200-400 c	n.s
600-800 c 200-400 mdms	n.s
600-800 c 400-600 c	n.s
600-800 c 400-600 mdms	
600-800 mdms 0-200 c	n.s
600-800 mdms 0-200 w	n.s
600-800 mdms 200-400 c	n.s
600-800 mdms 200-400 mdms	n.s
600-800 mdms 400-600 c	n.s
600-800 mdms 400-600 mdms	n.s
600-800 mdms 600-800 c	n.s
800-1000 c 0-200 c	n.s
800-1000 c 0-200 mdms	n.s
800-1000 c 200-400 c	**
800-1000 c 200-400 mdms	*
800-1000 c 400-600 c	***
800-1000 c 400-600 mdms	***
800-1000 c 600-800 c	n.s
800-1000 c 600-800 mdms	**
800-1000 mdms 0-200 c	***
800-1000 mdms 0-200 mdms	***
800-1000 mdms 200-400 c	n.s
800-1000 mdms 200-400 w	**
800-1000 mdms 400-600 c	n.s

800-1000 mdms 400-600 mdms	n.s
800-1000 mdms 600-800 c	**
800-1000 mdms 600-800 mdms	*
800-1000 mdms 800-1000 c	***

Supplementary information S1: Estimation of the forces experienced by single chondrocytes in the culture chamber as a function of their position and the applied pressure (compressive forces).

By looking at the observed deformation as a function of the pressure applied to the system it is possible to determine the force exerted on the cell. To simplify the calculation, we assume that the forces applied on the membrane are like the ones exerted on the hydrogel. Hence, we can use the following equations:

$F = \frac{\Delta L}{L0} E A$ and $\Delta L = L0 - L$

Where: *F* is the force applied; *L0* is the initial length of the cell (*e.g.*, its diameter in first approximation); *L* is the final length of the cell upon mechanical stimulation; *E* is the Young's modulus of the chondrocyte (considered equal to 4 kPa, here[1]); A is the projected surface area of the cell, which is, assuming this projection is circular in a first approximation: $A=\pi(L0/2)^2$.

Using this and data extracted from Figure 2 in the manuscript, we can estimate the forces exerted on chondrocytes located at different positions in the chamber, for different applied pressures. For these calculations, we only consider cells with similar size of 13 μm (L0) not to introduce any confounding factor arising from the exact size of the chondrocytes in their deformation.

For an applied pressure of **300 mbar**, and considering a cell (L0 = 13 μm in diameter) close to the membrane which slightly deforms (L=12 μm), the force it experiences amounts to:

$$F = \frac{13x10^{-6} - 12x10^{-6}}{13x10^{-6}} 4x10^{3} x\, \pi\, (6.5x10^{-6})^2 = 40.82 \text{ nN}$$

For an applied pressure of 300 mbar, and considering a cell (L0 = 13 μm in diameter) in the middle of the chamber which slightly deforms (L=12.1 μm), the force it experiences amounts to:

$$F = \frac{13x10^{-6} - 12.1x10^{-6}}{13x10^{-6}} 4x10^{3} x\, \pi\, (6.5x10^{-6})^2 = 36.74 \text{ nN}$$

For an applied pressure of 300 mbar, and considering a cell (L0 = 13 μm in diameter) close to the pillars which slightly deforms (L=12.3 μm), the force it experiences amounts to:

$$F = \frac{13x10^{-6} - 12.3x10^{-6}}{13x10^{-6}} 4x10^{3} x\, \pi\, (6.5x10^{-6})^2 = 28.57 \text{ nN}$$

For an applied pressure of **700 mbar**, and considering a cell (L0 = 13 μm in diameter) close to the membrane with deforms more significantly (L = 9.8 μm), the force it experiences amounts to:

$$F = \frac{13x10^{-6} - 9.8x10^{-6}}{13x10^{-6}} 4x10^{3} x\, \pi\, (6.5x10^{-6})^2 = 130.62 \text{ nN}$$

For an applied pressure of 700 mbar, and considering a cell (L0 = 13 μm in diameter) in the middle of the chamber which moderately deforms (L=11.3 μm), the force it experiences amounts to:

$$F = \frac{13x10^{-6} - 11.3x10^{-6}}{13x10^{-6}} 4x10^{3} x\, \pi\, (6.5x10^{-6})^{2}$$

= 69.39 nN

For an applied pressure of 700 mbar, and considering a cell (L0 = 13 μm in diameter) close to the pillars with deforms slightly (L = 11.7 μm), the force it experiences amounts to:

$$F = \frac{13x10^{-6} - 11.7x10^{-6}}{13x10^{-6}} 4x10^{3} x\, \pi\, (6.5x10^{-6})^{2}$$

= 53.07 nN

References

[1] N. D. Leipzig and K. A. Athanasiou, Biophys J, 2008, 94, 2412-2422.