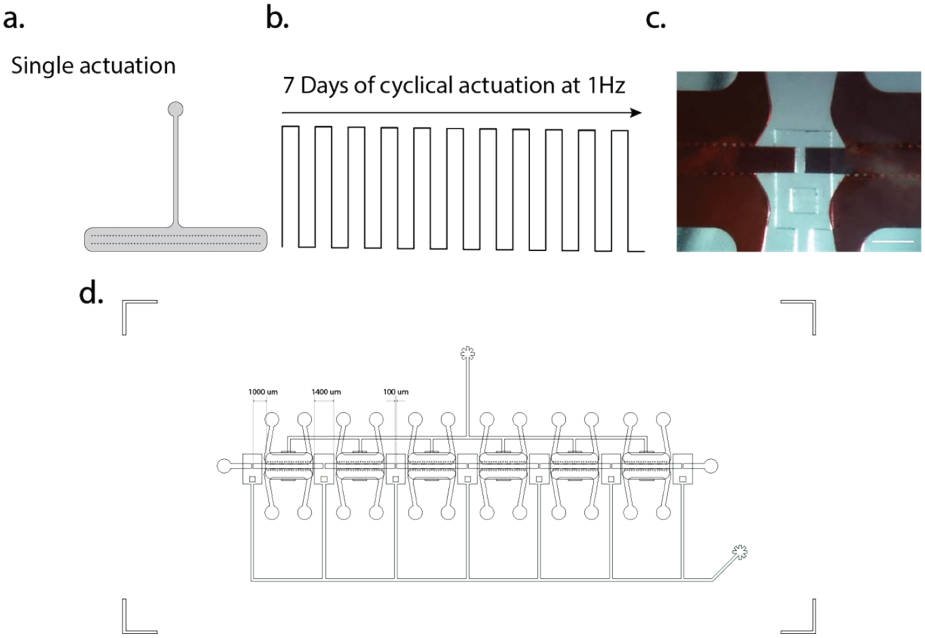
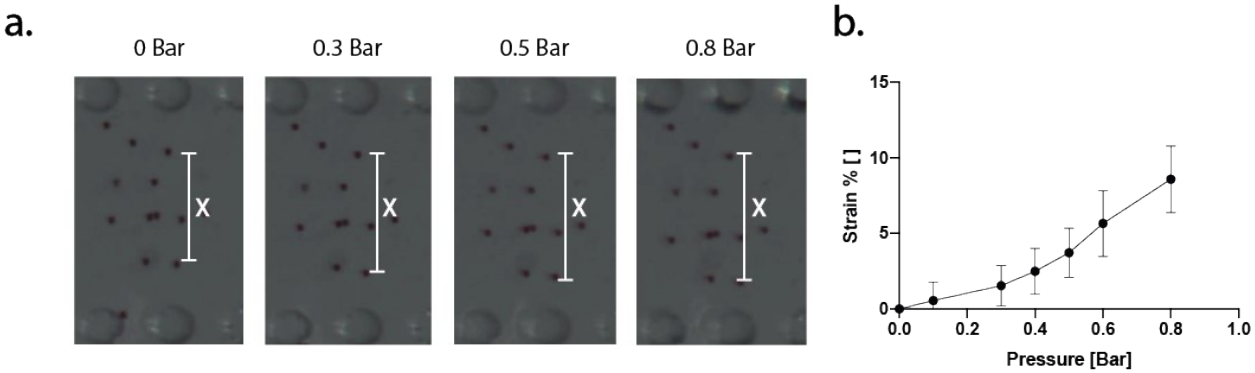
Electronic Supplementary Material (ESI) for Lab on a Chip.

Supplementary Images

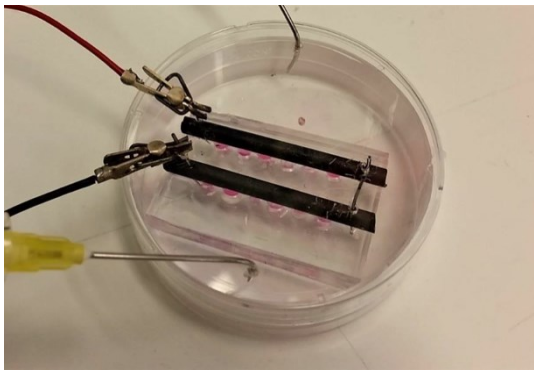


SI1: Alternative layout of the actuation chamber. a. Alternative actuation chamber layout; The actuation is long enough to cover the first three culture chambers. b. Stimulation pattern adopted in the experiment to verify culture chambers independence upon mechanical stimulation. A frequency of 1 Hz was adopted for 7 consecutive days. C. Picture of two adjacent chambers after 7 days of stimulation with the united actuation chamber. The united actuation causes the valve in between the chambers to partially open thus allowing diffusion of compounds from one chamber to the other. Hence the mixing of blue and red dye resulting in the brownish color visible in the picture. Scale bar 500 μm. d. Alternative layout of the device. Incremented dimensions of the space between adjacent chambers allowed for pneumatic chambers to be positioned under each of the culture chambers, thus increasing

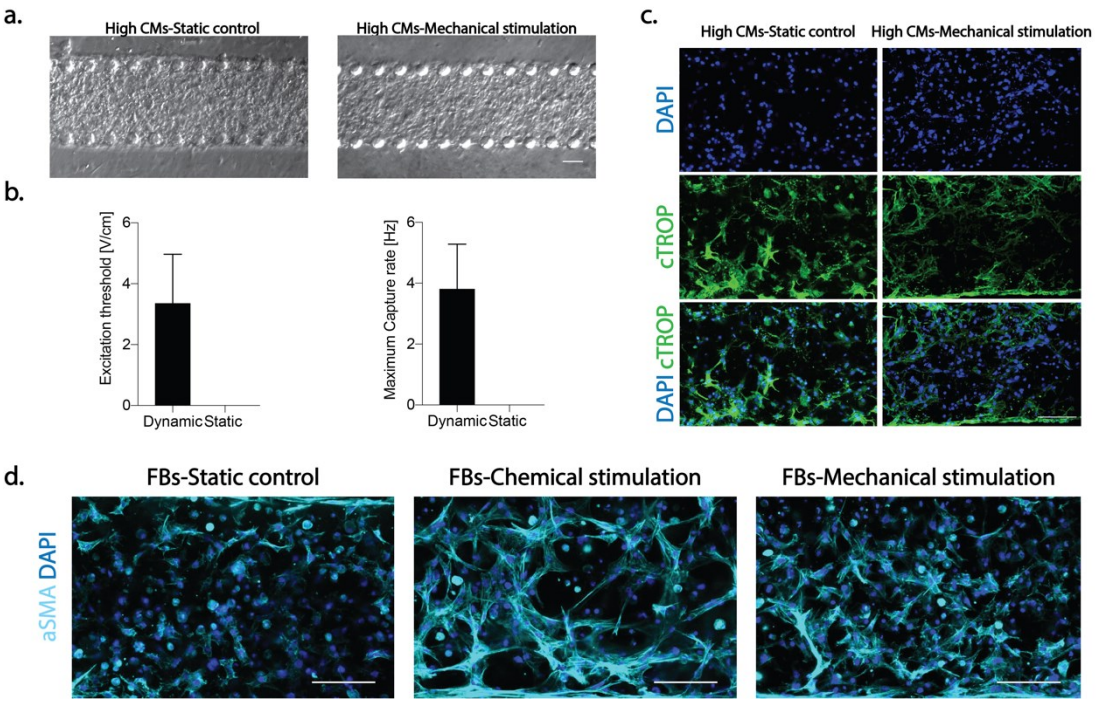


the throughput of the mechanically active replicates present in the same device.

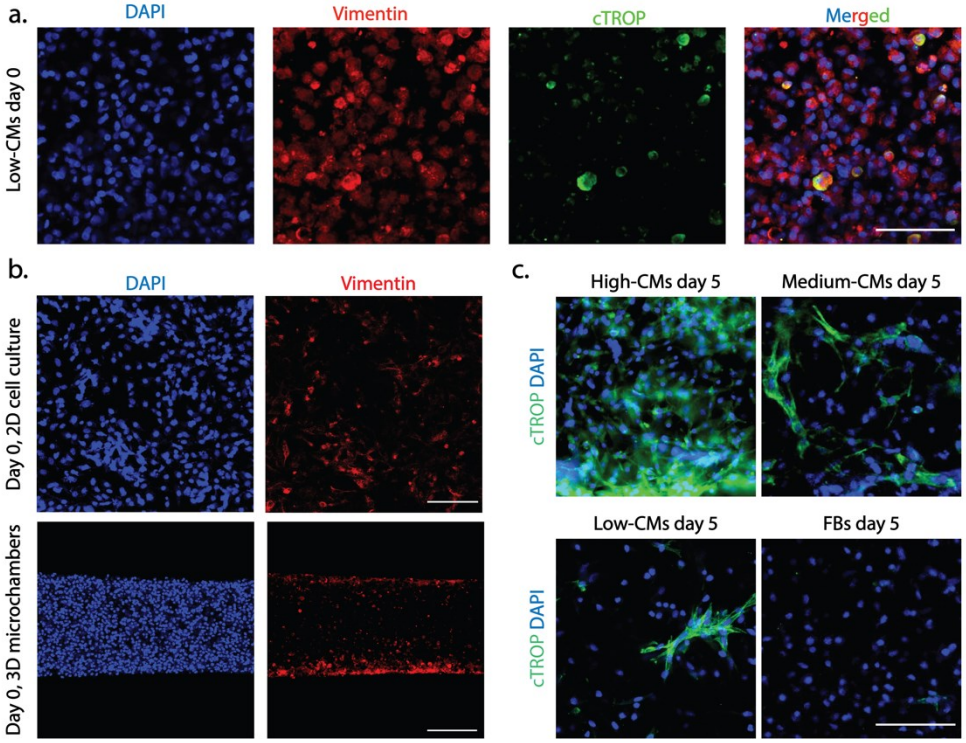
SI2: Evaluation of the strain field within constructs. a. Polystyrene beads (diameter 10 μm) incorporated in fibrin gel and injected into the central channel of the device. Different pressure levels are depicted, from 0 to 0.8 Bar. 5 different couples of beads were considered per chamber; 6 different chambers belonging to 2 devices were measured. The distance along the X direction of the chosen couples of beads at different pressures was measured. The result was used as an indication of the stretch level experienced by cells in the same conditions. b. Graph of the deformation level when different pressures were applied.



SI3: Electrical stimulation setup. Picture of the metallic electrodes used for construct electrical stimulation. Rods, visible in black, are in direct contact with the culture medium filling the appropriate reservoirs but not directly immerged in the reservoirs.

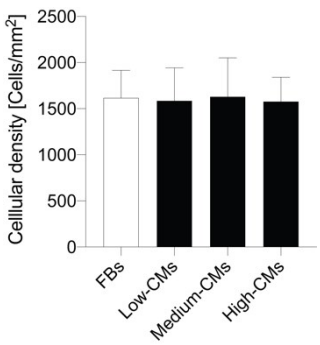

a.
High CMs-Static control
High CMs-Mechanical stimulation
b.
Excitation threshold [V/cm]
Maximum Capture rate [Hz]
Dynamic
Static
c.
High CMs-Static control
High CMs-Mechanical stimulation
DAPI
cTROP
DAPI cTROP
d.
FBs-Static control
FBs-Chemical stimulation
FBs-Mechanical stimulation
aSMA DAPI

SI4: Device biological validation. Device usability for biological experiments was assessed replicating previously obtained results. a. Brightfield images of a population constituted by 80% CMs and 20% FBs (High-CMs) seeded in the device and cultured for 5 days under static conditions or cyclic mechanical stimulation. At the end of the culture periods construct still completely fill the



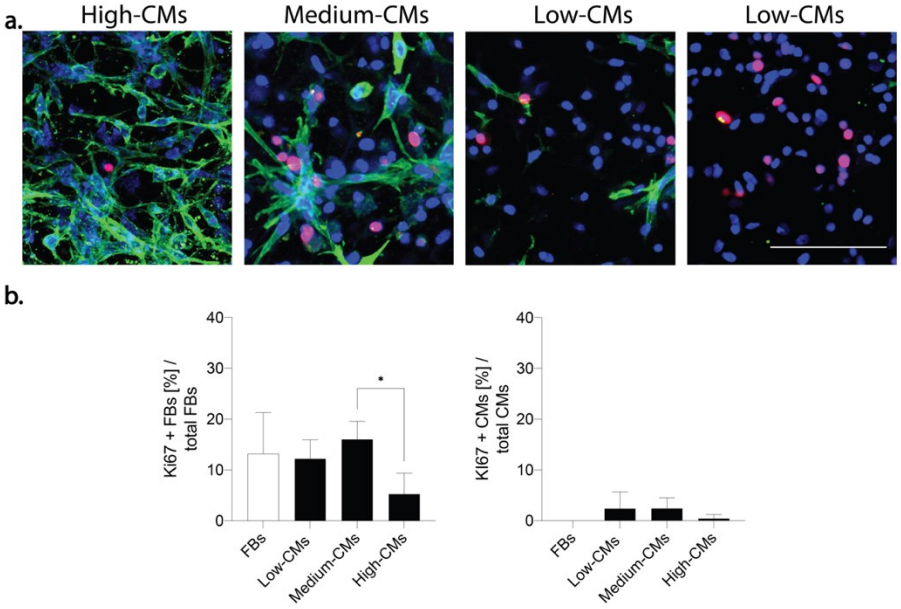
central culture channel. Scalebar 100 μm. b. Confirmation of the effect of mechanical stretching on maturation was obtained. After 5 days of culture within the same device, only mechanically stimulated micro-constructs exhibited synchronous beating, while no homogeneous beating activity was detected for the static condition. Upon electrical pacing, mechanically stimulated construct exhibited an excitation threshold of 3.35V±1.62 and a maximum capture rate of 3.8Hz ±1.48, while no values could be detected for static constructs. At least N=4 biologically independent samples were considered in ETH and MCR measurements. c. Immunofluorescence images of High-CMs population cultured under static conditions or mechanical stimulation for 5 days. DAPI is represented in blue, cTROP in green. Scalebar 100 μm. Images were analyzed exclusively in a qualitative fashion in this phase. No major differences in cell morphology were detectable in the two conditions. d. A 100%FBs population was used to assess the possibility of obtaining a hypertrophic phenotype induction, either through administration of TGF-β1 (5 ng mL-1) or through the application of cyclical stretching (8.5%) for 5 days. Both the pro-fibrotic factor TGF-β1 and mechanical stimulation were sufficient in increasing the number of fibroblasts expressing hypertrophic alpha-smooth muscle actin (αSMA), thus assuming a hypertrophic phenotype, as compared to the static condition. Images were analyzed exclusively in a qualitative fashion in this phase. DAPI is represented in blue, αSMA in cyan. Scalebar 100 μm. Static culture without TGF-β1 was used for controls.

SI5: Populations' quantifications. Populations were quantified both at day 0, (4 hours after seeding) and at day 5 at the end of the culture period. a. Representative images of the low-CMs population at day 0. DAPI is represented in blue, Vimentin in red, cTROP in green. Close to no cells were negative for both markers. Scalebar 100 μm b. Populations' quantifications at day 0, high-CMs population only. Staining for Vimentin was performed both in 2D culture and in 3D within microfluidic devices. No differences in the assessment of the population in between the two quantification methods was visible. DAPI is represented in blue, Vimentin in red. Scalebar 100 μm. c. Populations' quantifications at day 5. Representative images of the four coculture ratios are represented.



At the end of the culture, CMs were defines as cTROP positive cells and FBs as cTROP negative. DAPI is represented in blue, cTROP in green. Scalebar 100 μm.

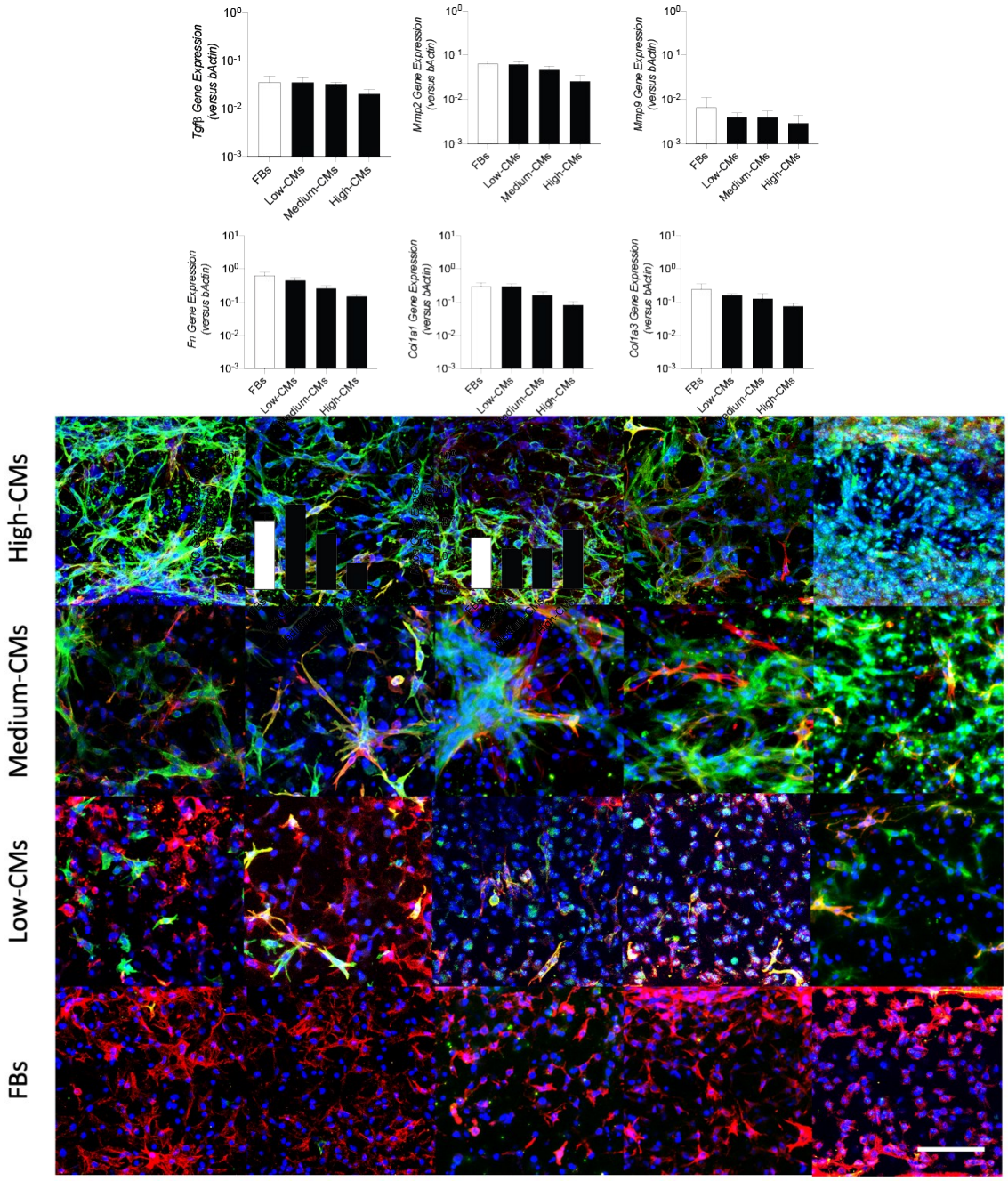
SI6: Assessment of the cellular density after 5 days of culture. In light of the results of a previous FBs-based fibrotic model [21] all co-culture populations were seeded with an initial cellular density of 100 x 10^6 cells/ml while the control group (i.e. the 100% FBs population) was prepared with a cell density of 14.5 x10^6 cells/ml as previously established. The different seeding densities were



*chosen to account for the different proliferation rates. After 5 days of culture in 3D microchambers, no statistical difference could be detected in cellular density (i.e. the number of cells per mm^2). At least n=5 images belonging to N=3 biologically independent samples for each condition were used for quantifications. Statistics by one-way ANOVA with Tukey's post hoc test for Gaussian populations and Kruskal-Wallis test with Dunn's post hoc test for non-Gaussian populations. **p< 0.01, ***p<0.001 and ****p<0.0001.*

*SI7: Determination of the Ki67 positive populations. a. Representative images of the different populations. DAPI is represented in blue, cTROP in green, and Ki67 in red. Scalebar 100 μm. b. Left: percentage of Ki67 positive FBs (cTROP negative cells) over the total amount of FBs; Right: percentage of Ki67 positive CMs (cTROP positive cells) over the total amount of CMs. At least N=4 biological independent images were considered in quantifications. Statistics by one-way ANOVA with Tukey's post hoc test for Gaussian populations and Kruskal-Wallis test with Dunn's post hoc test for non-Gaussian populations. *p< 0.05.*

SI8: Images used for quantifications in Figure 4. α-SMA (red) and cardiac Troponin I (green) of the four considered conditions after 5 days of culture in dynamic condition. Nuclei were stained with DAPI (blue). Scalebars 100 μm. At least N=5 images belonging to n=3 different experiments were adopted in quantifications. Acquisition areas were chosen randomly within the constructs. Cellular distribution and density varied in between experiments, also among samples belonging to the same condition.



SI9: Gene expression without normalization. Gene expression was registered through RT-qPCR. bActin was adopted as housekeeping gene. At least N=4 biological independent samples were considered in each analysis.

*SI10: Analysis at the mRNA level of fibrosis-related markers (namely, Mmp2 and Mmp9, TGF-β1, TnC, and Nppa) of the different CM and FB ratios (Low-, Medium- and High- CMs) at day 0 (i.e. of the populations before injection in microfluidic devices) quantified by RT-qPCR. bActin was used as housekeeping gene. Results were normalised to the expression of the FBs population expression. *p< 0.05, Kruskal-Wallis test with Dunn's post hoc test for non-Gaussian populations. N=3 samples per kind of cellular ratio were analysed.*