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

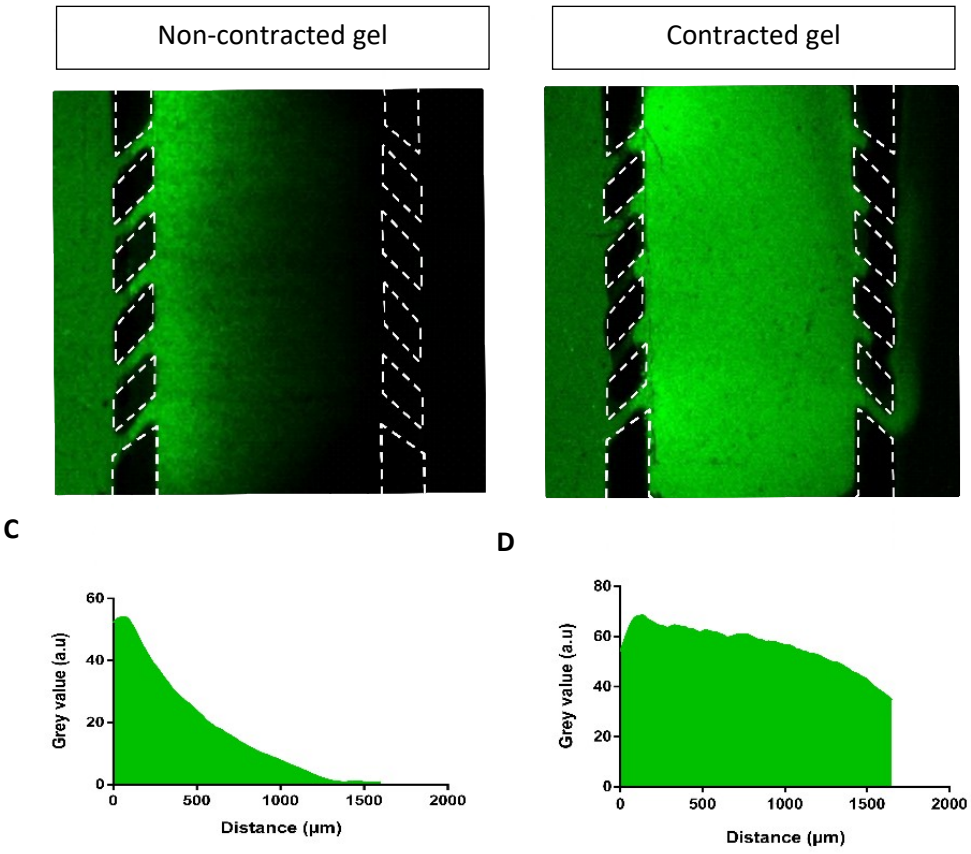
Supplementary experimental and figures

Experimental

Supplementary experimental information figure 5. The surface of rectangular COP channels (200 µm height, 1200 µm width) was modified with the different surface treatments studied and coated with collagen I at a final concentration of 0.1 mg/mL in cold PBS (Cytiva SH30256.01). Collagen was fluorescently labelled with carboxylate-modified microspheres (1:100 w/w Invitrogen™ F8807) by passive adsorption in order to allow spatial visualisation. After incubation overnight at 37°C, the channels were exposed to sequential shear stress forces (0.15, 25 and 150 dyn/cm^2). Confocal microscopy was performed in different fields of every channel and the area of fluorescent collagen was quantified and compared with the increase in flow rate.

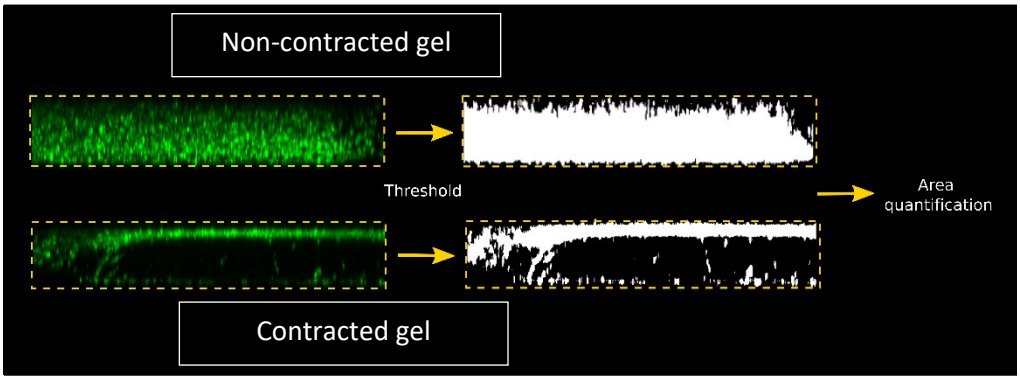
Supplementary figure 6. Fibronectin (Corning 354008) and laminin (Merck CC095) were diluted in cold PBS (Cytiva SH30256.01) at 0.1 mg/ml. Both proteins were fluorescently labelled with carboxylate-modified microspheres (1:100 w/w Invitrogen™ F8807) by passive adsorption in order to allow spatial visualization. Equally, laminin and fibronectin were injected in rectangular microchannels (200 µm height, 1200 µm width) with control and PAA-PG treated surfaces and incubated overnight at 37°C. Then, the channels were exposed to 150 dyn/cm^2 for 3 minutes. Confocal microscopy was performed before and after the flow. Fluorescence intensity was measured and compared in plot profiles using Fiji ® software.

Figures

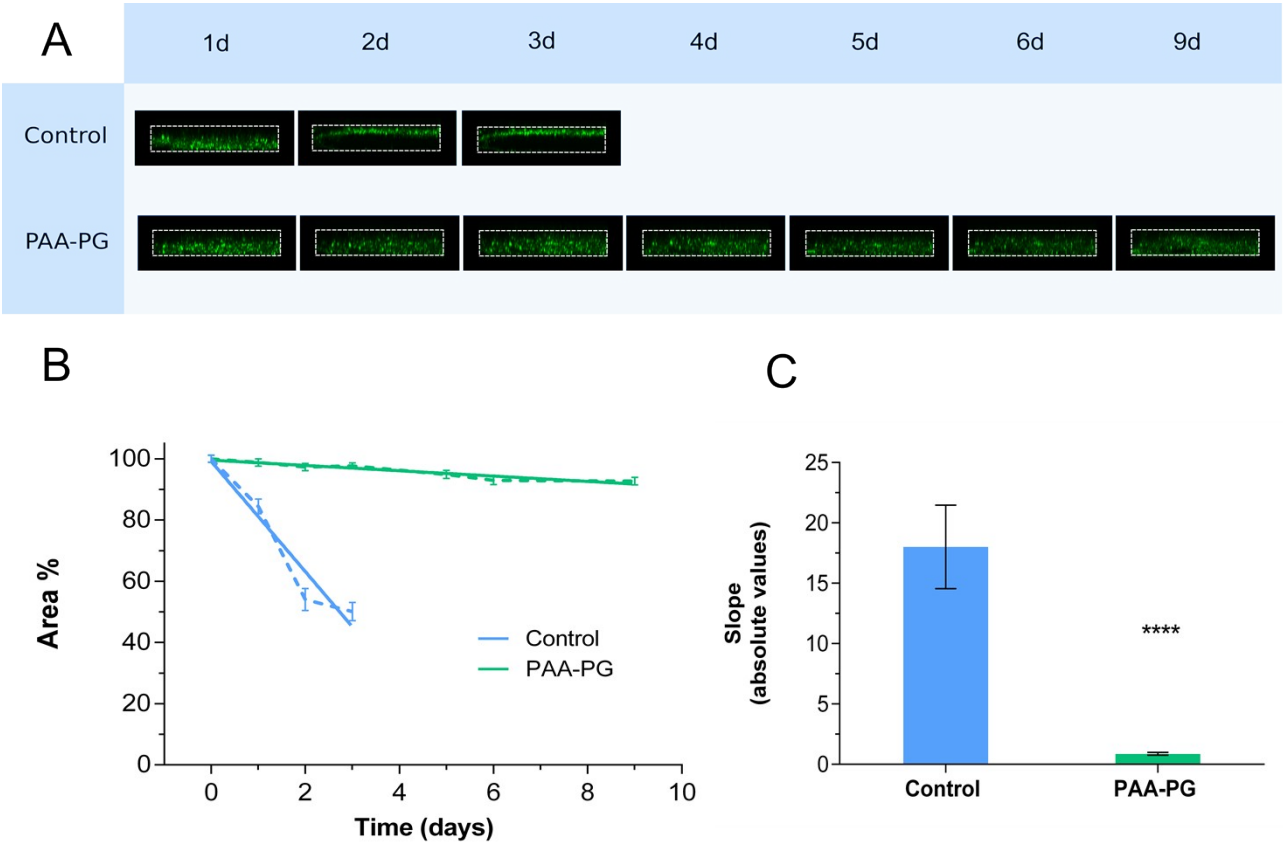


Supplementary figure 1. COP microfluidic device with collagen hydrogel (1.2 mg/mL) in the central chamber and culture medium at both lateral channels. Left medium channel contains fluorescent microspheres (FluoSpheres ™ 0.2 µm, F8811 Invitrogen) to visualize the culture medium trajectory through the collagen gel. Non-contracted gel creates a barrier that makes medium gradually pass through, generating a fluorescence gradient with a high intensity in the area near the left channel that decreases as it approaches to the right channel with non-fluorescent culture medium (A). Contracted gel does not completely confine to the walls of the device, resulting in gaps that allow the massive entry of culture medium

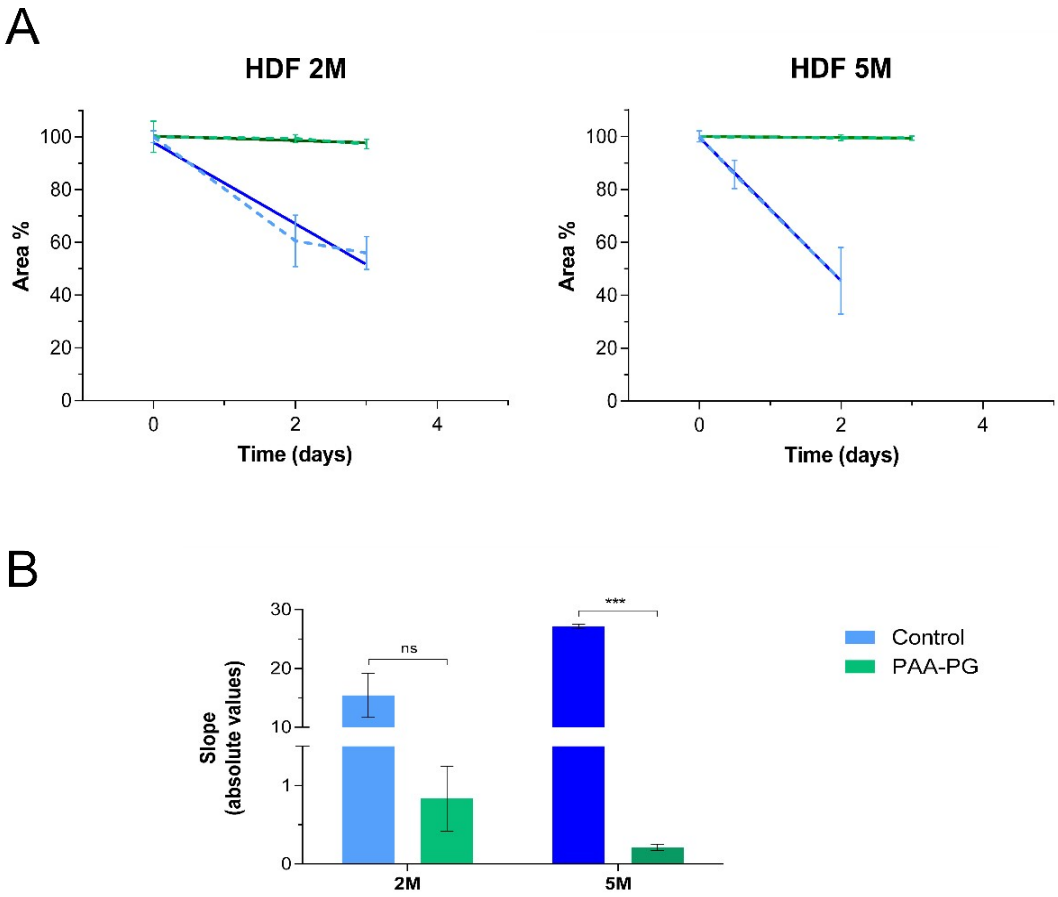
throughout the central chamber and therefore fluorescence (B). Fluorescence intensity profile of microspheres included in left medium channel along the central chamber device of non-contracted (C) and contracted (D) gels.



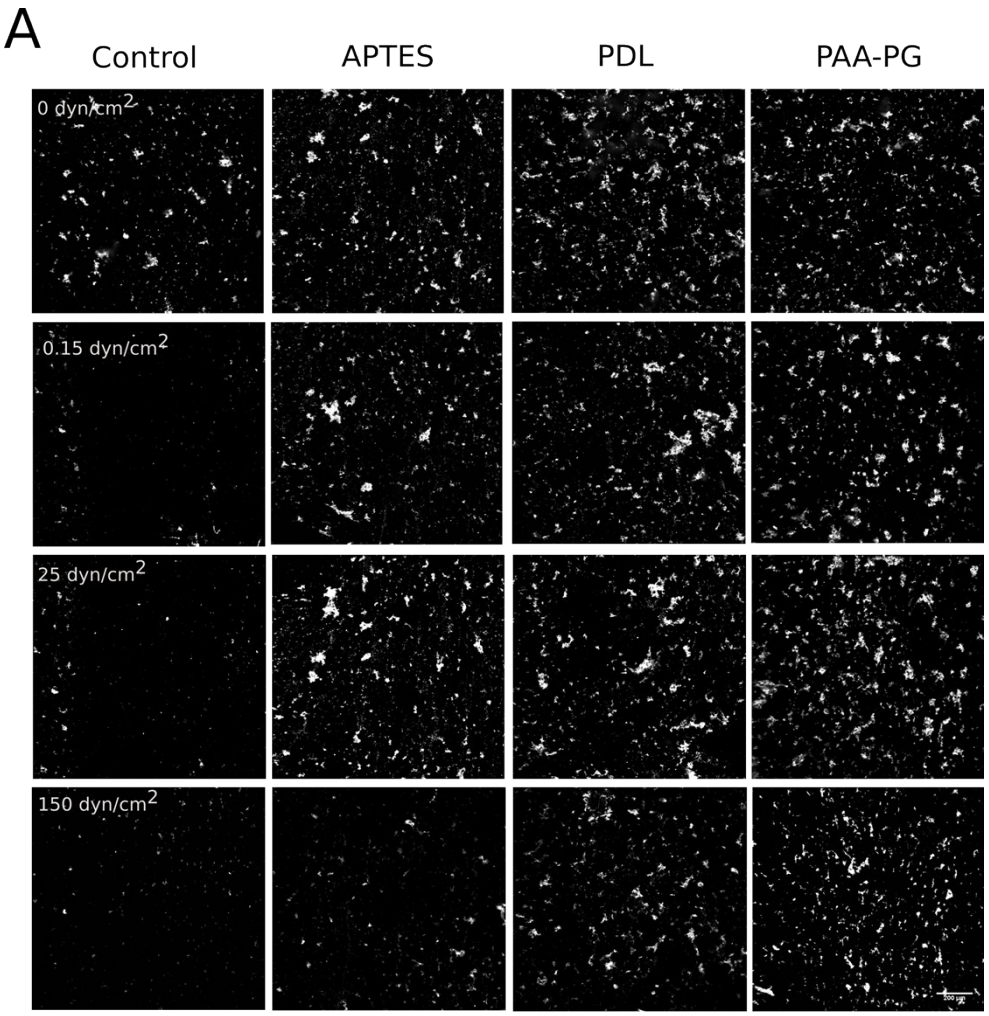
Supplementary figure 2. Workflow scheme of image analysis and area quantification with Fiji ® software.

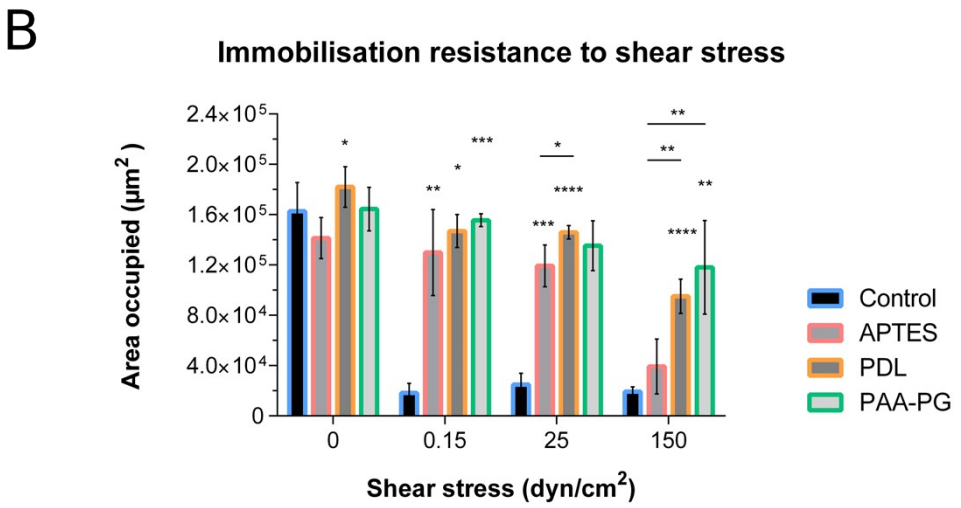


Supplementary figure 3. Culture of $4x10^7$ U87-MG embedded in 1.2 mg/mL collagen hydrogels in PAA-PG treated microdevices. (A) Transversal area evolution overtime of U87-MG labelled with green-fluorescent DiO Vybrant™ dye. (B) Area percentage of U87-MG embedded in collagen hydrogel within the microdevice chamber over nine days (dashed line) and trend lines (continuous line). (C) Statistical comparison among the slopes (absolute values) of trend lines from each condition. Note: ****p <0.0001, *** p <0.001, ** p< 0.01, * p < 0.05. Scale bar 100 µm.

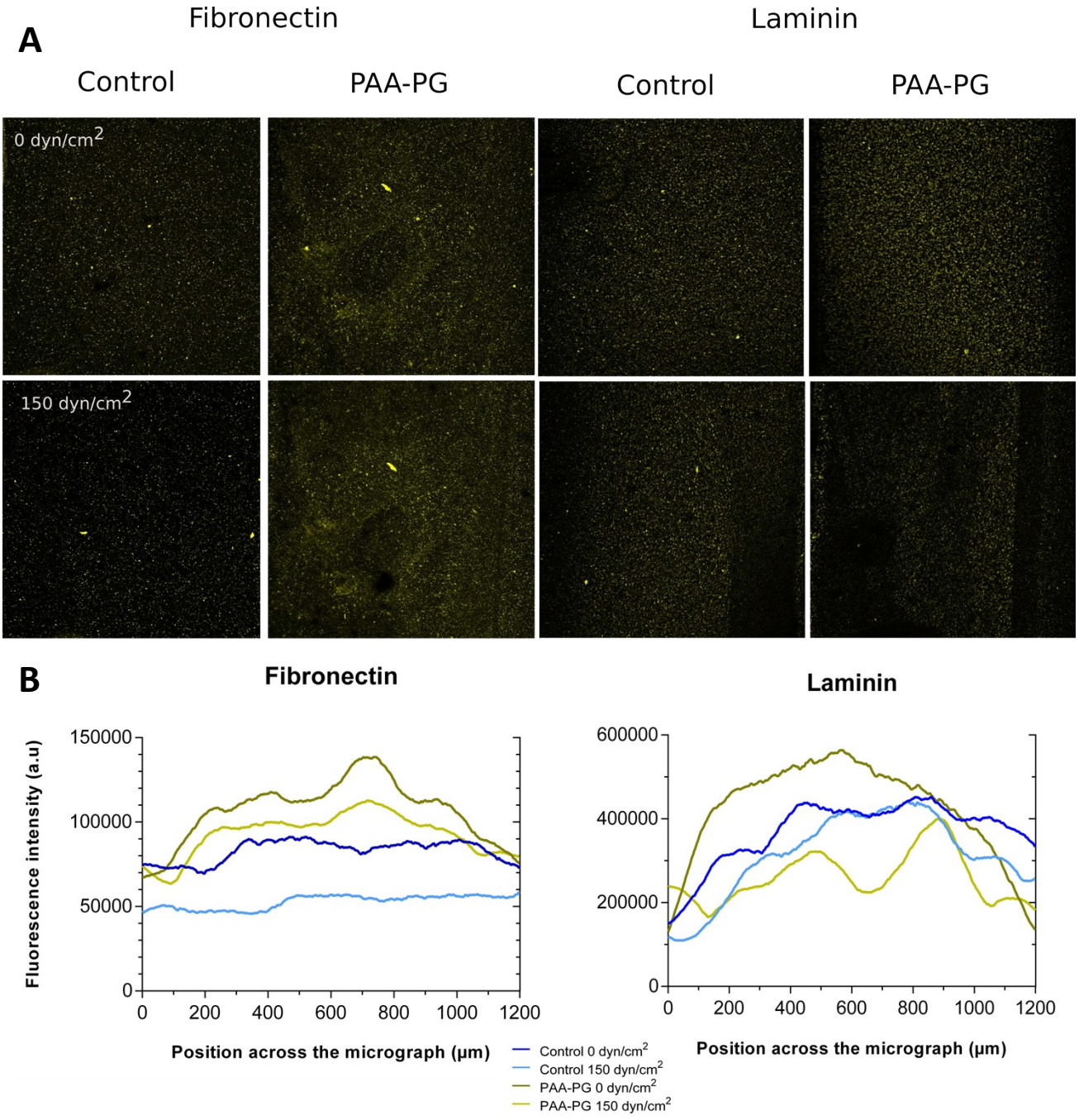


Supplementary figure 4. Culture of $2x10^6$ and $5x10^6$ HDF embedded in 1.2 mg/mL collagen hydrogels in PAA-PG treated microdevices. (A) Transversal area evolution overtime of U87-MG labelled with green-fluorescent DiO Vybrant™ dye. (B) Area percentage evolution of HDF embedded in collagen hydrogel within the microdevice chamber over three days (dashed line) and trend lines (continuous line). (C) Statistical comparison among the slopes (absolute values) of trend lines from each condition. Note: ****p <0.0001, *** p <0.001, ** p< 0.01, * p < 0.05. Scale bar 100 µm.

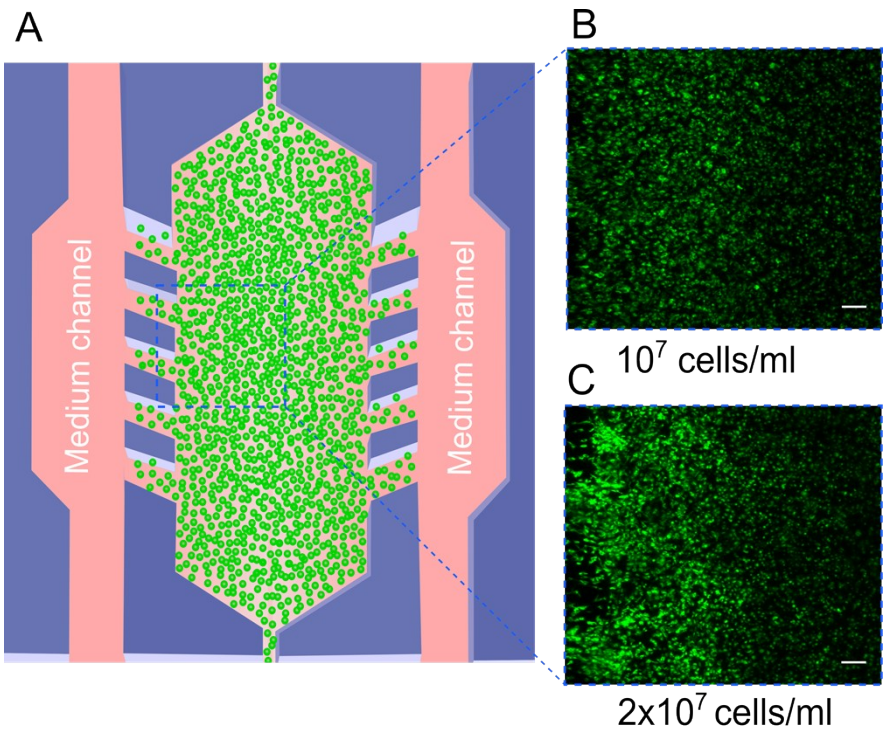


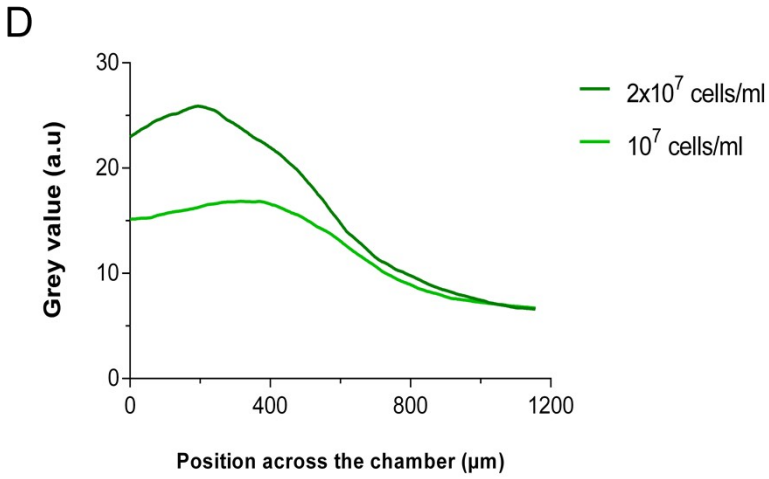


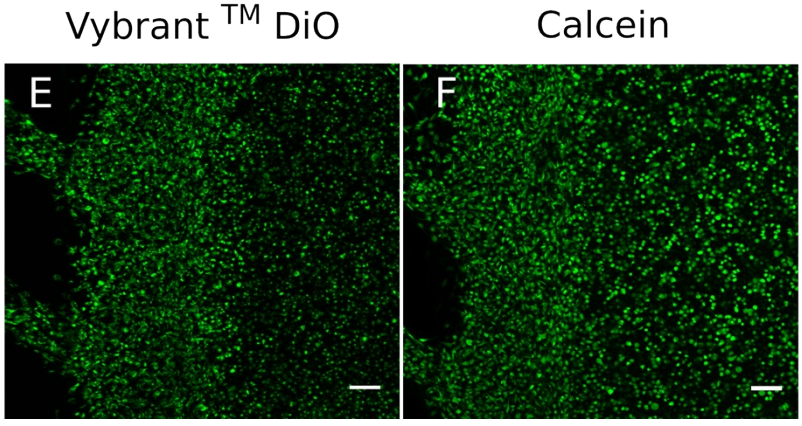
Supplementary figure 5. Collagen I coating on COP channels and shear stress resistance. (A) Confocal micrographs of fluorescently-labelled collagen I in surface modified COP-channels with no shear stress, 0.15, 25 and 150 dyn/cm^2. (B) Immobilization resistance to shear stress represented by collagen area occupied (mean and SD) after shear stress. Note: ****p <0.0001, *** p <0.001, ** p< 0.01, * p < 0.05. Scale bar 200 µm.



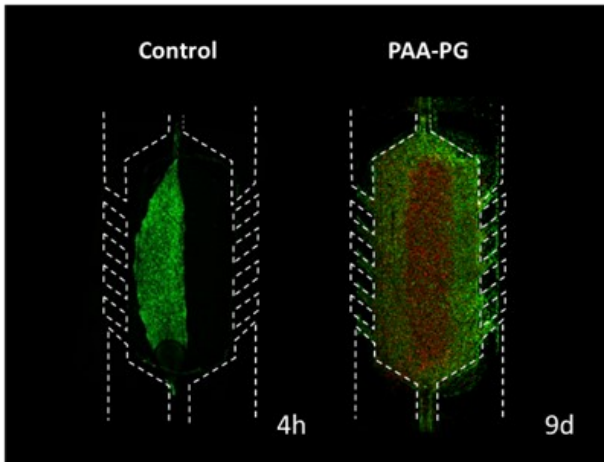
Supplementary figure 6. Fibronectin and laminin coating on COP channels and shear stress resistance. (A) Confocal micrographs of fluorescently-labelled fibronectin and laminin in control and PAA-PG channels with no shear stress and 150 dyn/cm^2. (B) Graphs showing fibronectin and laminin fluorescent probe intensity profile along the micrographs. Scale bar 200 µm.







Supplementary figure 7. Culture of HCF at 10^7 and $2x10^7$ cells/mL embedded in collagen (1.2 mg/mL) after PAA-PG treatment. (A) Schematic diagram of the microfluidic device with green circles representing cells in 3D culture within the central chamber and blue bordered regions square indicating top view region of analysis. Confocal top view micrographs of 10^7 (B) and $2x10^7$ (C) HCF/mL labelled with green-fluorescent DiO Vybrant™ dye after 24 hours. (D) Graph showing DiO Vybrant™ quantification of fluorescence intensity profile along the chamber position (where 0 µm is closest to the medium channel and 1200 µm is the centre of the culture chamber). $2x10^7$ HCF/mL after 48 hours culture labelled with (E) Vybrant TM DiO and (F) calcein (CAM) viability stain. Images E and F were taken with 1 hour of difference and presented the cell state similarly. Both exhibited two different areas, being the area on the left densely populated whereas that the right area displayed less cells, separated from each other and rounded. Note: Scale bar 100 µm.



Supplementary video 1. Real time micrograph acquisition of control and PAA-PG treated devices with $2x10^7$ cells/ml over 11 hours.

Supplementary figure 8. CAM-PI viability staining of U87-MG after 4 hours (control device) and 9 days (PAA-PG) preserving three-dimensional gel structure and allowing nutrients and gases gradients that generate a necrotic core in the central region of the culture chamber.