High-Throughput Microfluidic Platform for Modelling Inflammatory Responses of Human Articular Chondrocytes under Variable Fluid Shear Stress

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Supplementary Information

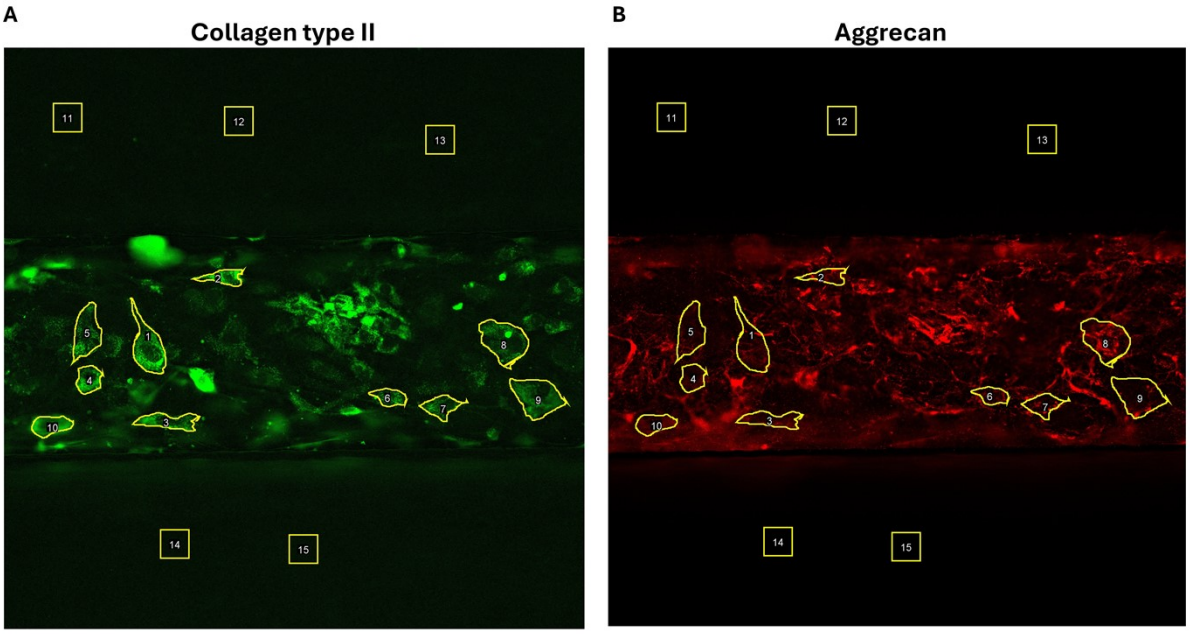

Figure S1: ROI selection for fluorescence quantification in Fiji ImageJ. Cells located on the z-slice corresponding to the base of the microchannel were selected by manually drawing regions of interest (ROIs) around individual cells in (A) the green channel (collagen II). The same ROIs were then transferred to (B) the red channel (aggrecan) for consistent quantification across markers. In this example, 10 cells were selected, and 5 additional ROIs positioned outside the microchannels were used to measure background signal. Cells appearing blurred or out of focus were excluded from analysis. Integrated density, background intensity, and cell area were measured in Fiji ImageJ for subsequent fluorescence quantification.

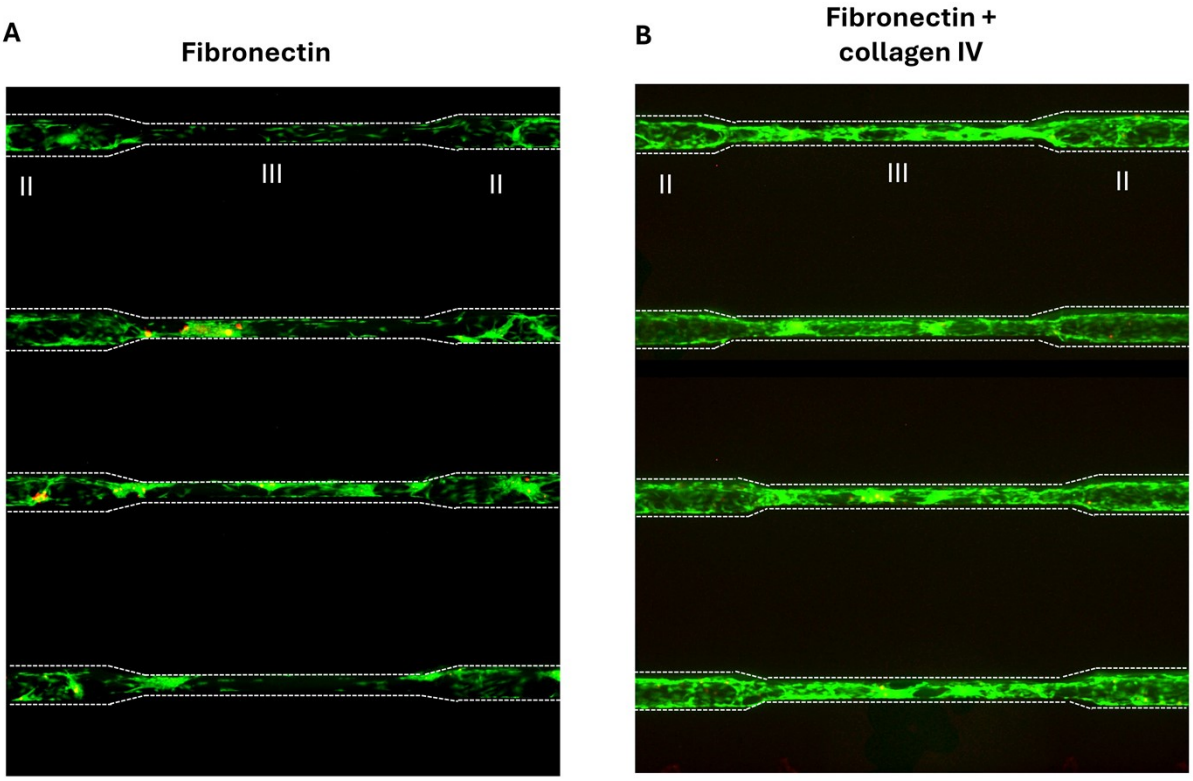

Figure S2: Low-magnification (2.5×) wide-field Live/Dead fluorescence images from the middle of a microfluidic unit showing four parallel microchannels (Regions II and III) for (A) fibronectin and (B) fibronectin–collagen IV coatings.

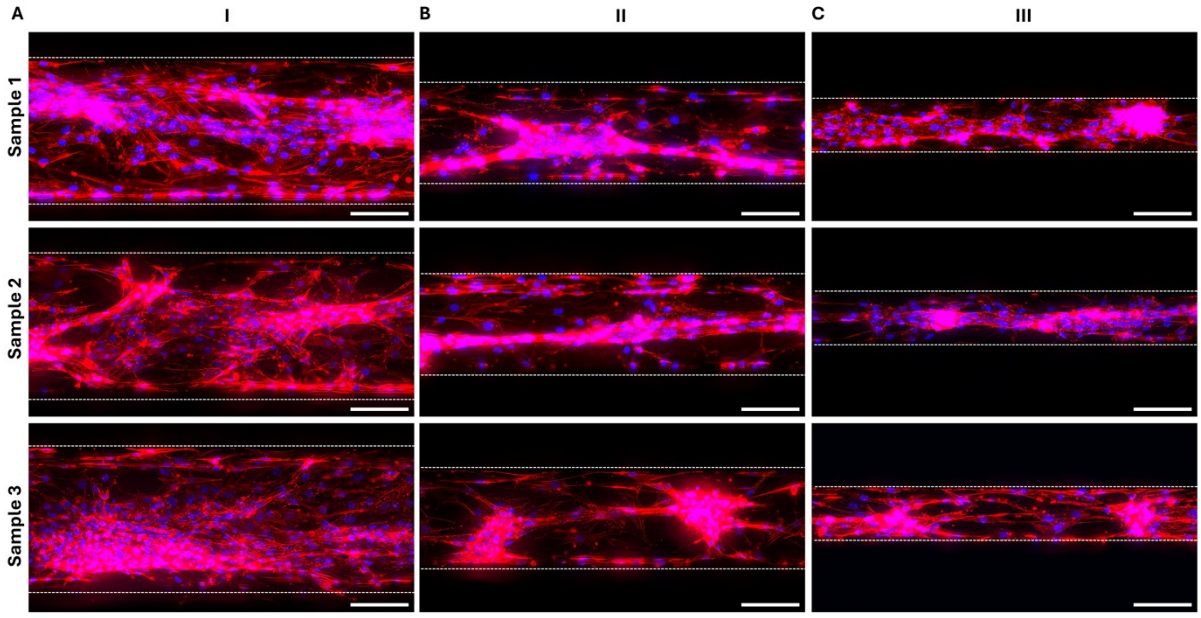

Figure S3: Representative fluorescence images acquired for human articular chondrocytes cultured for 21 days in the microfluidic chips and staining with TRITIC-phalloidin to visualize F-actin filaments (red) and DAPI to visualize cell nuclei (blue). The images correspond to regions (A) I (low FSS), (B) II (medium FSS), and (C) III (high FSS) for three different samples

without cytokine stimulation. Each image represents a z-projection of slices acquired between the bottom and the top of the microchannels. White dashed lines indicate microchannel boundaries. Scale bar= 100 μm.

Video S1: Z-stack slices captured from the bottom to the top of the microchannel in Region I (low FSS), corresponding to Sample 1 in Figure S1.

Video S2: Z-stack slices captured from the bottom to the top of the microchannel in Region II (medium FSS), corresponding to Sample 1 in Figure S1.

Video S3: Z-stack slices captured from the bottom to the top of the microchannel in Region III (high FSS), corresponding to Sample 1 in Figure S1.

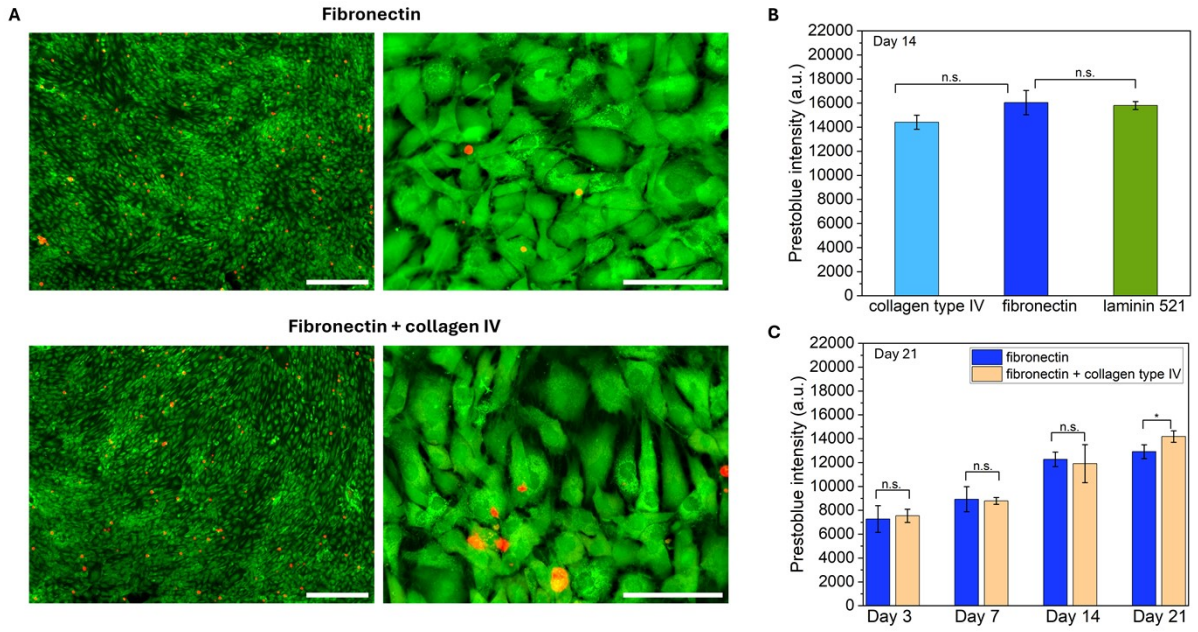

Figure S4: Cell viability and metabolic activity of healthy human articular chondrocytes cultured on 2D glass surfaces coated with PDMS and various proteins prior to cell seeding. (A) Wide-field fluorescence images of samples coated with fibronectin or a fibronectin/collagen type IV mixture. Left: scale bar = 500 μm; Right: scale bar = 100 μm. (B) Samples coated with collagen type IV, fibronectin, or laminin, evaluated on Day 14. (C) Samples coated with fibronectin alone or a combination of fibronectin and collagen type IV, assessed at different time points up to Day 21. N = 3 independent samples per group. Statistical analysis: Kruskal–Wallis test with Dunn's post hoc comparison; significance indicated as *$p < 0.05$; n.s. = not significant.

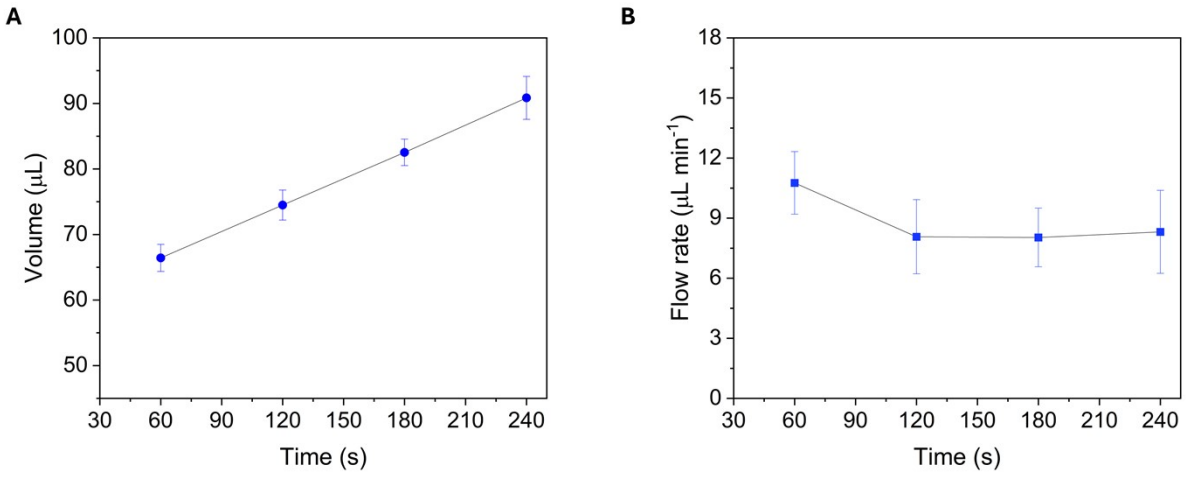

Figure S5: Gravity-driven flow dynamics at a 25° rocker tilt. (A) Cumulative volume of culture medium transferred from the inlet to the outlet reservoir over the 300 s dwell period at a 25° tilt angle, measured at four time points taken every 60 s. Measurements were performed on five independent microfluidic units. (B) Instantaneous flow rate calculated from the volume increments. The flow rate exhibited a rapid decline and stabilization at lower values.

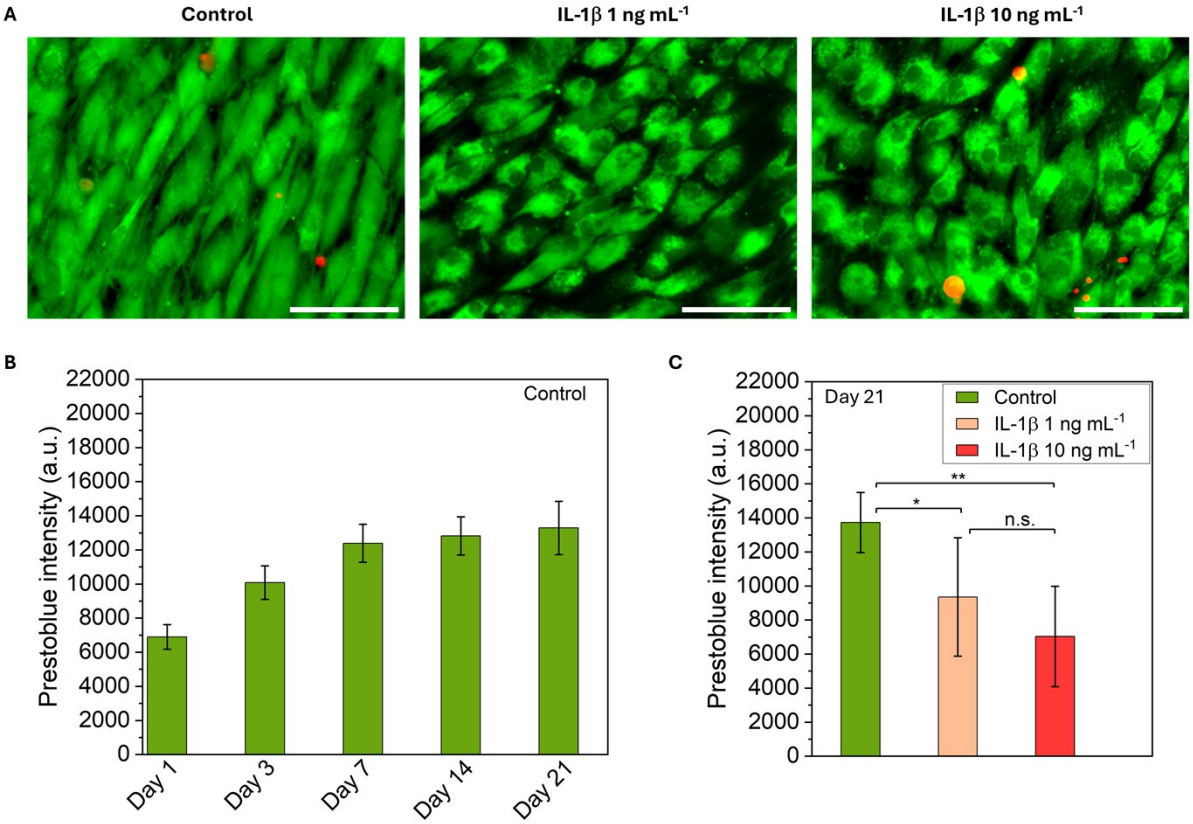

Figure S6: Cell viability and metabolic activity of human articular chondrocytes cultured on 2D surfaces (48-well plates coated with PDMS and fibronectin/collagen type IV mixture). (A)

Wide-field Live/Dead fluorescence images of control and IL-1β–stimulated samples acquired on Day 21. Scale bar = 100 µm (B) Metabolic activity of control samples measured at multiple time points. N = 6 per group. (C) Metabolic activity of control and IL-1β–stimulated samples measured on Day 21. N = 6 independent samples per group. Statistical analysis: Kruskal–Wallis test with Dunn's post hoc comparison; significance indicated as **p < 0.01, *p < 0.05; n.s. = not significant.

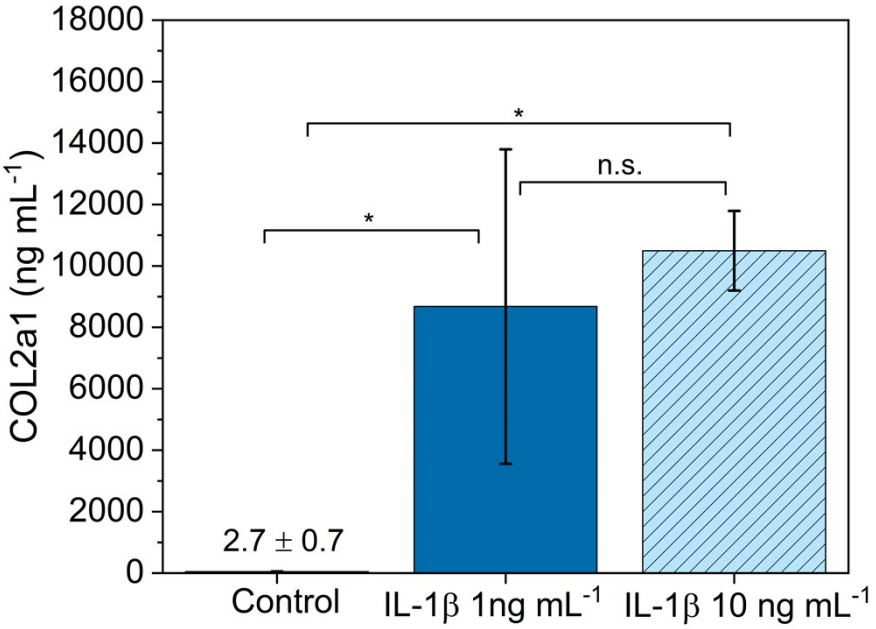

Figure S7: Concentration of collagen type II alpha 1 (COL2A1) secreted into the culture medium by human articular chondrocytes cultured in microfluidic chips for 21 days. Measurements were performed using ELISA for control samples and those stimulated with IL-1β at 1 ng mL^{-1} or 10 ng mL^{-1}. Cytokine stimulation occurred between Day 15 and Day 21. N = 3 independent samples per group. Statistical analysis: Kruskal–Wallis test with Dunn's post hoc comparison; significance indicated as *p < 0.05; n.s. = not significant.