Supplementary Information (SI) for Lab on a Chip.
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**Supporting Information**

# A novel 3D-printed tool for *in vitro* cell interaction studies under flow conditions

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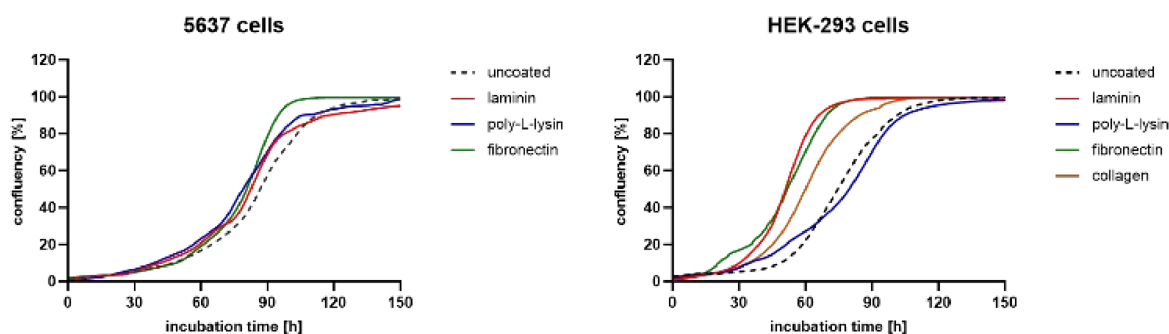
### Methods - Coating of Glass Coverslips

For the binding studies, cells were cultured on glass coverslips. To enhance cell adhesion during experiments, various coating materials were tested, and optimal coating procedures were determined in preliminary experiments for each cell line. For 5637 cells, coverslips were disinfected with ethanol 70%, rinsed with water, and incubated with a 0.01% (w/v) aqueous poly-L-lysine solution for 15 minutes on a shaker at room temperature. After this coating, the coverslips were washed three times with water. For HEK-293 cells, laminin-coated coverslips were prepared by first treating the coverslips with 0.01% (w/v) poly-L-lysine solution as described above, followed by incubation for 30 minutes at 37°C with laminin solution (40 µg/ml) in isotonic HEPES buffer (pH 7.4, 20mM). The coverslips were then rinsed with water. Cell growth on treated and untreated coverslips was monitored using the PHIO Cellwatcher M system (PHIO scientific GmbH). Additionally, cell adherence to the coverslips under flow conditions was confirmed by comparing the integrity of the cell layer before and after 20 hours of exposure to stirring motion in the FlowCube.

### Results - Coating of Glass Coverslips

Growth curves for 5637 cells on coated and uncoated coverslips showed similar trends, with a slight beneficial effect of poly-L-lysine. Across all conditions, final plateau levels were comparable (Figure 4A). Cell adherence on poly-L-lysine-coated coverslips under shear stress was sufficiently high. (Figure 4B) Based on these findings, poly-L-lysine was selected for subsequent binding experiments with 5637 cells in the FlowCube due to its beneficial effect on growth and the cost-effectiveness compared to other coatings. HEK-293 cells exhibited stronger coating-dependent differences compared to 5637 cells. On uncoated coverslips, growth progressed gradually, while laminin and fibronectin coatings enhanced proliferation, leading to an earlier onset of exponential growth and faster progression to the plateau phase. Collagen also promoted increased proliferation relative to uncoated glass, though less effectively than laminin or fibronectin. In contrast, poly-L-lysine had no significant effect on HEK-293 cell growth (Figure **S1 top**). As with 5637 cells, all conditions converged to similar confluence levels at the plateau. Based on these results and adhesion assays under shear (Figure **S1 bottom**), both laminin and fibronectin supported comparable HEK293 attachment and shear stability. Laminin was selected for subsequent FlowCube binding experiments due to its equivalent performance and greater cost-effectiveness.



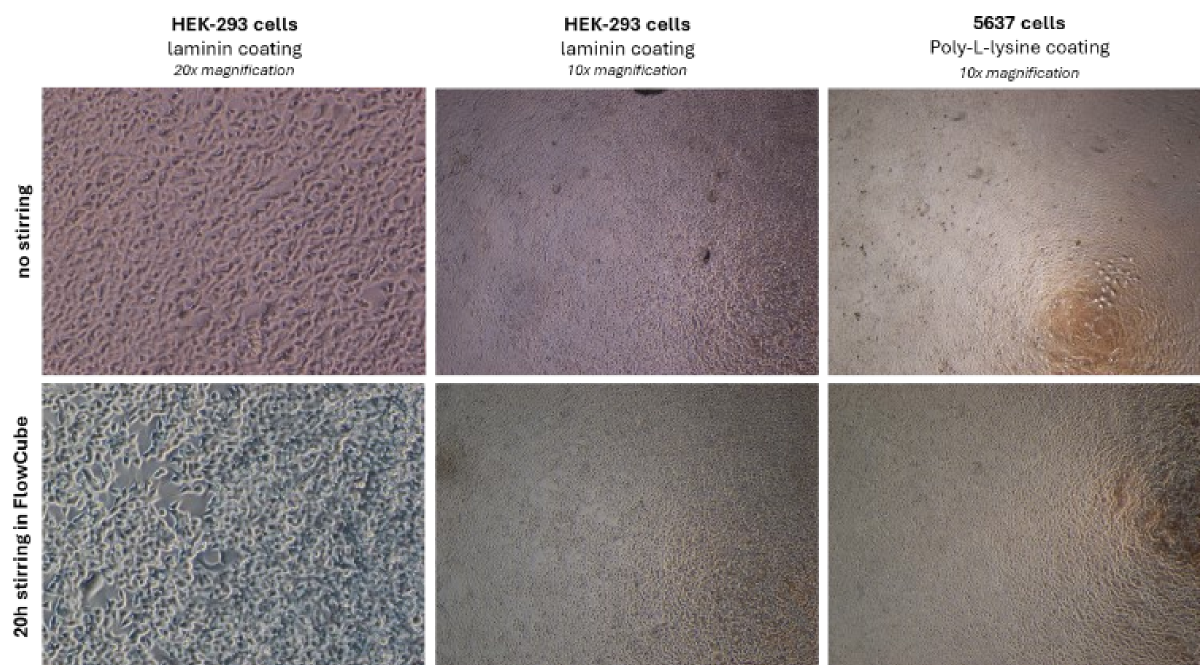



**Figure S1:** Cell growth on glass coverslips coated with different materials (top) and cell adherence on laminin-coated coverslips (HEK-293 cells) and poly-L-lysine-coated coverslips (5637 cells) before and after 20 hours of incubation under flow conditions in the FlowCube (bottom). The results demonstrate stable cell adherence to coverslips with the respective coating materials, even after exposure to shear stress.

**Cell Experiments in the FlowCube and Multiwell-plate**

Fluorescence intensities were normalized to the multiwell plate signal at each time point, and monensin-treated results were normalized to pre-treatment values to emphasize the fluorescence increase due to dequenching. Nanoparticle formulations showed similar cell interaction under static condetions, irrespective of cell layer orientation suggesting no relevant impact of sedimentation on cellular dose. Shear stress on the other hand resulted in slightly reduced cell association of the nanoparticle formulation (Figure S2A). For microparticles, a significantly higher cell interaction was observed in the multiwell plate setup, attributed to particle sedimentation (Figure S2B). The increasing difference over time between the FlowCube and multiwell plate setups is primarily due to enhanced particle binding in the multiwell plate caused by sedimentation.

For floating microcapsules (Figure S2C), normalization to monolayer data highlights significantly higher cell interaction in the FlowCube setup, with reproducible fluorescence intensity ratios between experiments. The fWGA ligand exhibited no significant differences between setups at most time points, though a trend toward higher cell interaction in the FlowCube was observed. Upon monensin treatment, dequenching of the fluorescein signal, this trend became more evident and increased cell interaction in the FlowCube with stirring was observed (Figure S2D).

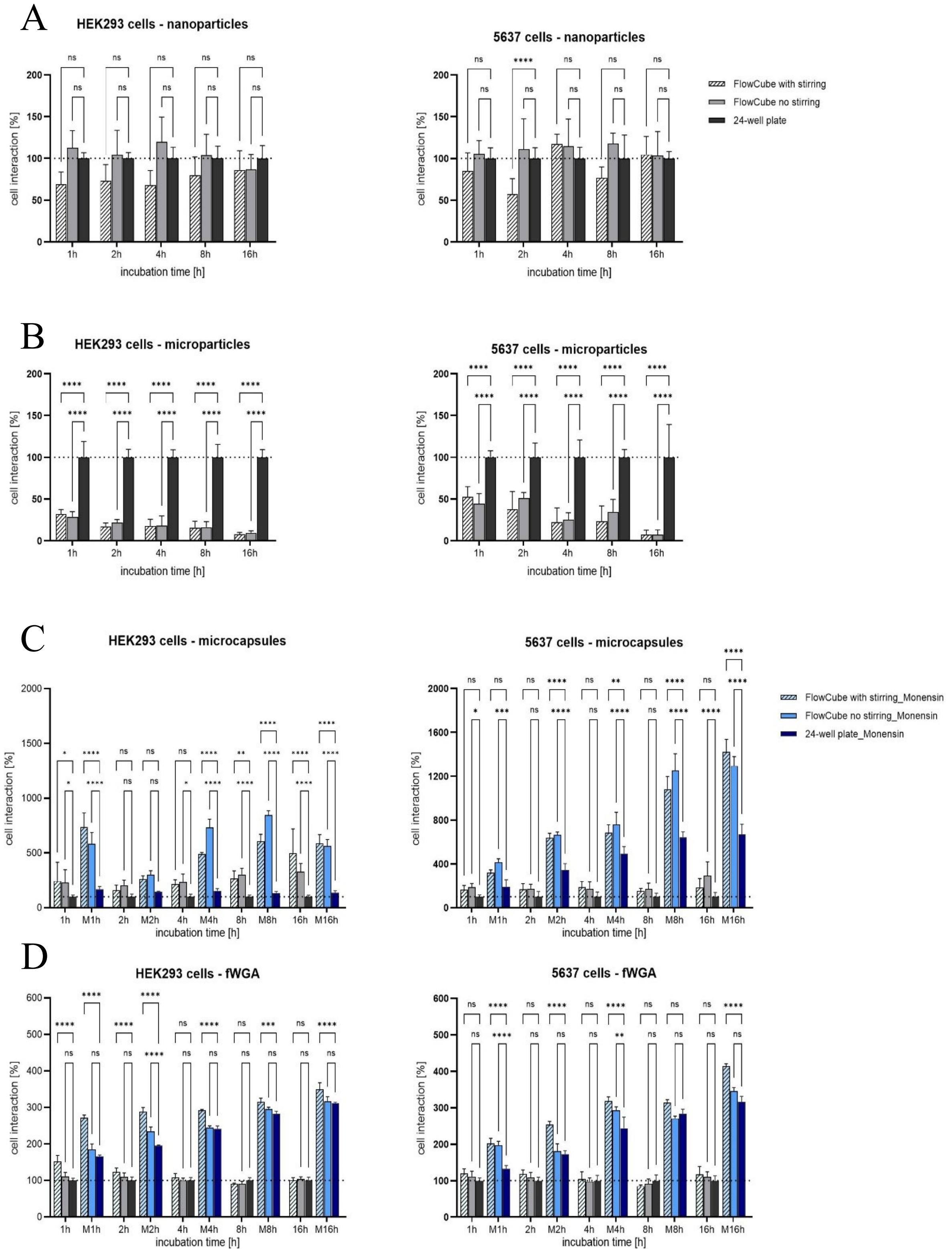



**Figure S2:** Comparative analysis of cell-associated fluorescence intensities between the FlowCube and multiwell plate experimental setups. All data were normalized to the fluorescence intensity of the multiwell plate experiments at each time point. (NP n=3, MP n=2, MC n=3, fWGA n=3; monensin experiments n=2) (* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$; Data shown as mean ± SD)

**Method – Cultivation and Staining of primary HUVEC cells**

Primary human umbilical vein endothelial cells (HUVEC; PromoCell, C-12200) were cultured in gelatine-coated 25 $cm^2$ flasks at 37°C in a humidified 5% $CO_2$ atmosphere. Culture flasks and coverslips, were treated with autoclaved gelatine solution (1%, 5 ml per flask, 0.5 ml per coverslip) for 1 h at room temperature to promote cell adherence. The gelatine solution was then removed, and surfaces were rinsed with PBS. Cells were seeded at a density of 200,000 cells/$cm^2$ and subcultured approximately every three days. Endothelial cell basal medium (PromoCell, C-22210) was supplemented with the endothelial cell growth medium supplement mix (PromoCell, C-39210) according to the manufacturer's instructions. For dynamic (FlowCube) vs static (well plate) experiments, HUVECs were grown on coated coverslips to confluence, then the cell-coated coverslips were transferred into the FlowCube and subjected to stirring for 16 h. Subsequently cells were fixed and stained with phalloidin–red for F-actin and Hoechst33342 for cell nuclei as described in the manuscript.

**Results – Microscopic Analysis of F-actin under Dynamic and Static Conditions**

Under static conditions, F-actin exhibited a predominantly cortical distribution with diffuse cytoplasmic staining and strong enrichment at the cell periphery. Under dynamic conditions, actin reorganized into thicker stress fibre bundles spanning the cell body, with modest alignment in the flow direction and evidence of reinforced junctional actin (Figure S3), consistent with shear-induced cytoskeletal remodelling.

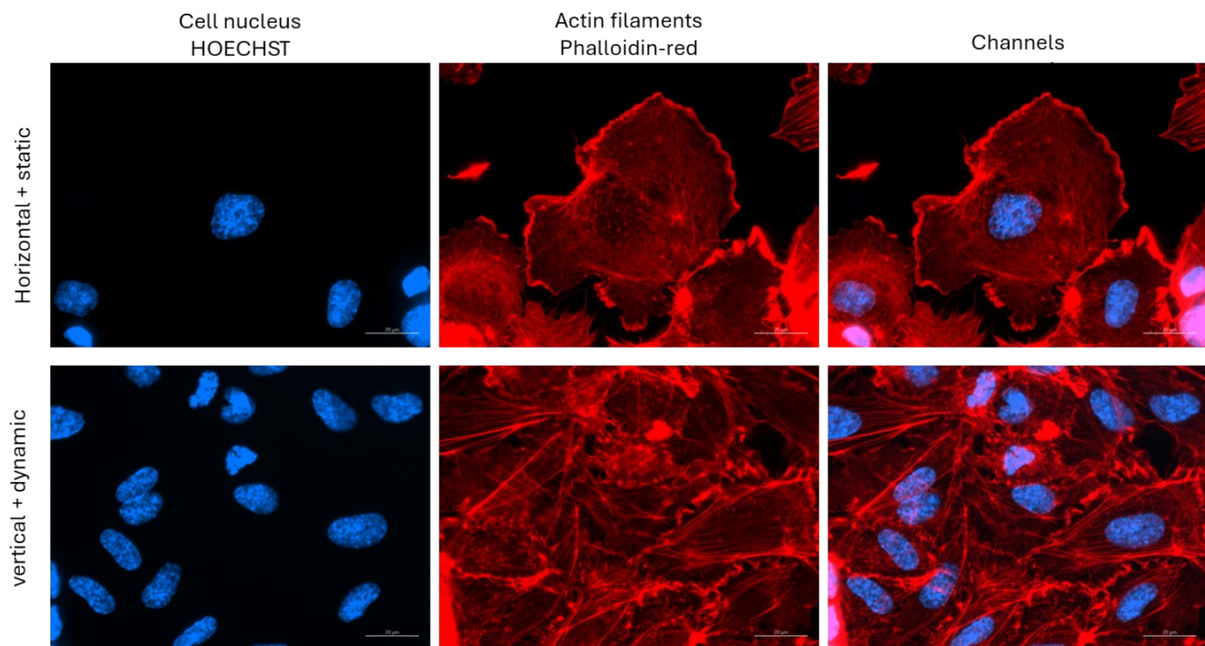



**Figure S3:** Microscopic analysis of F-actin under static and dynamic conditions. Under static conditions, F-actin shows a predominantly cortical distribution with diffuse cytoplasmic staining and strong enrichment at the cell periphery. Under dynamic conditions (FlowCube, 16h), F-actin reorganizes into thicker stress-fibre bundles spanning the cell body, with modest alignment in the flow direction (upper right to lower left) and reinforced junctional actin, consistent with shear-induced cytoskeletal remodelling. Phalloidin (red) labels F-actin; Hoechst33342 (blue) labels nuclei. Scale bars indicate 20 µm.