Supplementary Material

Electric field-mediated adhesive dynamics of cells inside bio-functionalised microchannels offers important cues for active control of cell-substrate adhesion

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Image processing and cell tracking

Cell tracking was performed using an open source software "Tracker". The cell tracking videos recorded using high speed camera were converted into image frames and uploaded in the software. Thereafter, cellular motions were tracked using Auto-Tracker functionality to obtain the displacement and velocity data of the individual cells within a particular time window. The displacement and velocity data were further imported to MATLAB for post-processing.

Surface Characterisation using Atomic Force Microscopy

The surface morphological changes between collagen-coated and bare PDMS substrates were observed on flat substrates by mapping 20 × 20 μm^2 area with the help of atomic force microscopy (AFM). Since, it would be difficult to characterize curved surfaces of microchannels using AFM, flat PDMS substrates were prepared and modified accordingly for the AFM characterization. PDMS flat substrates were prepared by spin-coating PDMS and crosslinker mixture (in 10:1 ratio) on clean glass slides, followed by curing on a hot plate. Thereafter, 1 cm^2 square chips were prepared by peeling off the PDMS substrate and cutting it using scalpel. The pieces were subjected to oxygen plasma treatment in the plasma cleaner (FEMTO ver., Diener Electronics, Germany) for 30 secs at a power input of 40 W. Next, the samples were immersed into 10% APTES solution at 55 °C for 2 hours. After washing the excess APTES with DI water, the samples were immersed in 2.5% Glutaraldehyde (GA) solution for 1 hour. The excess GA solution was washed thoroughly with DI water and the samples were thereafter immersed in 0.1 mg/mL of Collagen type I solution. Finally, the samples were stored at 4 °C overnight and were used for characterization experiments the following day after removing the extra protein solution with DI water. The tentative order of

magnitude of the collagen layer was determined by calculating the average height of the deposited protein elements relative to the average height of roughness elements on bare PDMS substrate, as obtained from the respective surface topography scans in Fig. S1(a)

and S1 (b). Fig. S1(c-e) show the 2D AFM data with different positions of the line elements along which AFM scans have been performed.

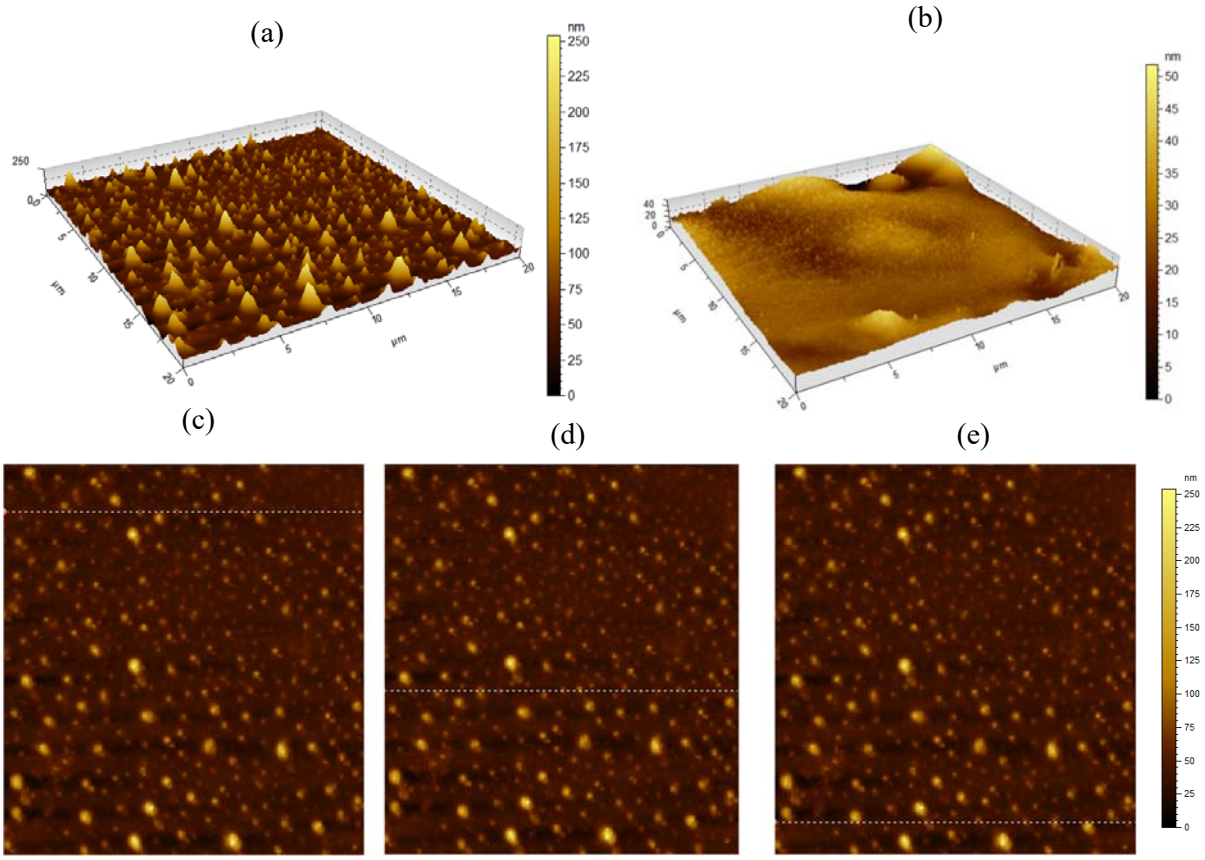

Fig S1: AFM characterisation of the collagen-coated PDMS substrate: (a) shows the AFM scan of the collagen-coated PDMS substrate and (b) shows the AFM scan of the bare PDMS substrate. (c-e) show the 2D AFM data with different positions of the line elements along which AFM scans have been performed.

Determination of adhesion strength parameter

We have quantified an adhesion strength parameter Ø, by performing shear flow experiments using our microfluidic channels, as per previously reported methodologies[1]. In brief, we coated our microfluidic devices with collagen-I protein and allowed the HeLa cells to adhere to the channel wall by filing up the channels with cell suspensions and incubating it at 37^{o}C and 5% CO_2 atmosphere for around 45 minutes. Post incubation, different levels of shear stress was applied on the cells by gradually increasing the flow rate (5 µl/min, 50 µl/min, 250 µl/min, 500 µl/min, 1000 µl/min) controlled using a programmable syringe pump. The cells were exposed

to a particular shear stress for 4 minutes before ramping up the shear level. The number of adhered cells before and after a particular shear level was determined using microscopy and image analysis, so that the fraction of adhered cells could be found at the end of each shear stress level. Finally, an arithmetic mean of these adhered cell fractions was obtained which has been considered as an adhesive strength parameter Ø, highlighting the relative tendency of cells stay adhered to the substrate under a range of applied shear stress. To estimate the adhesion strength parameter in presence of electric field, axial electric fields of strength 1 V/cm, 2 V/cm and 3 V/cm (all in FFD configuration) were applied through the connector electrodes of the channel, while this shear flow experiment was taking place.

Table S1: Physical properties of cells

Cell properties	HeLa	MDA-MB-231
Cell diameter (µm)	19.86 ± 2.05	11.14 ± 1.58
Young's modulus (MPa)	69.61 ± 5.12	56.19 ± 8.00

Table S2: Categorizing different types of cellular motions based on the velocity data

Electric field strength (V/cm)	V_{ch} (µm/s)	v_c/V_{ch}	Motion type
0	338.2	0.064	Adhesive Rolling
1	384	0.820	Adhesive Rolling
2	798.6	0.426	Adhesive Rolling

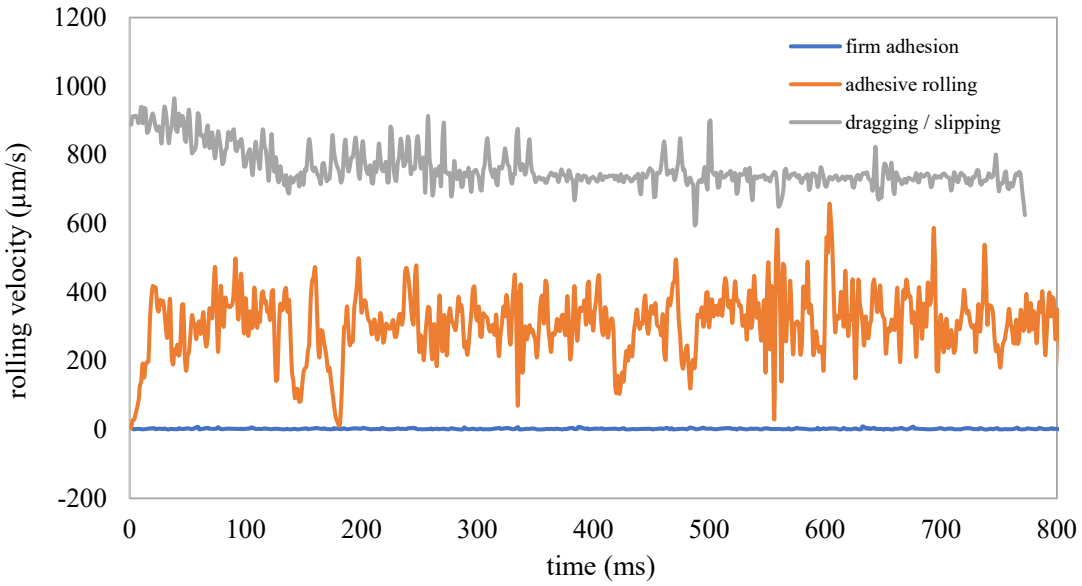

Fig S2: Representative example of three different types of cellular motions observed with HeLa cells

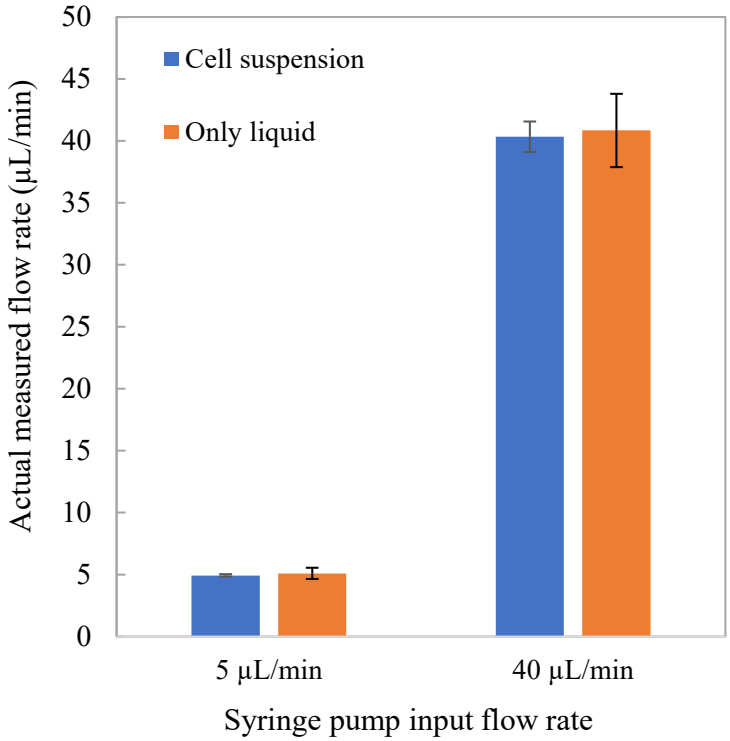

Fig S3: Comparison between input flow rate set in syringe pump and actual measured flow rate for cellular and acellular suspensions.

It is true that although the syringe pump is set to a specific flow rate, there might be slight alterations in the actual flow rate because of the performance of the mechanical parts of the pump. In order to test this issue, we set the syringe pump to particular flow rates and measured the rate of volumetric output in an eppendorf tube. For all our experiments, the tygon tubing that was used as fluidic connectors had a uniform diameter of ~ 508 µm, as specified by the manufacturer. The cell concentration was maintained at 10^5 cells/ml in all our experiments, by careful counting of the cells using hemo-cytometer.

The flow rate measurement test was carried out for 2 different flow rates (5 µL/min and 40 µL/min) both with cellular and acellular suspensions. Fig.S3 highlights the comparison between the input syringe pump flow rate and actual measured flow rate obtained for both cellular and acellular suspensions. It is observed that the actual flow rate for cellular suspensions is almost identical to that obtained for the acellular one, for both the flow rate levels, thereby highlighting the fact that the actual flow rate in this case, is not significantly sensitive to the presence of cells and their respective volume fractions. Moreover, there is only ~ 1% deviation between the actual measured flow rate and the input set flow rate in case of the cellular suspensions for both the flow rate levels. Hence, we could safely rely on the input flow rate set in the syringe pump for further experimental analysis.

Theoretical prediction of adhesive tendency of cells of different sizes

From experiments, we found that the smaller cells (MDA-MB-231) exhibit lesser adhesion frequency compared to the bigger cells (HeLa) for the same background flow rate and electric field strength. In order to complement our experimental results, we analysed the forces acting on the cells of both types. The net force acting on the HeLa cells due to fluid shear is ~ 3 times (both for E = 0 V/cm and E= 1 V/cm) than that acting on the smaller breast cancer cells [calculated based on Eq.7a-Eq.14 of Section 3.2 of the main manuscript]. However, the smaller the cells, the smaller will be the contact area with the substrate and lesser will be the number of bonds present in the contact area. Since initially in our model, we considered the number of bonds in the contact area of HeLa cells to be ~ 400 [2], for the smaller breast cancer cells, the number of bonds get proportionally decreased to the order of ~ 100. Hence, the net force acting per bond due to shear on the smaller cells comes out to be ~26.4% greater than that acting on the larger cells [as per Eq. 14. of Section 3.2 of the main manuscript]. Similarly, in case of application of electric field strength of 1 V/cm, the net force per bond acting on the cells is also ~26.1% greater for smaller cells compared to HeLa cells. Moreover, the ratio of reverse rate constant (for bond breakage) in presence and absence of electric field comes out to be ~ 7.12 for the smaller cells while it is ~ 4.73 for the larger cells [refer to Eq. 15 of Section 3.2 of the main manuscript]. These calculations suggest the fact that the smaller cells, owing to an enhanced degree of external forcing, should have reduced adhesion tendency compared to larger cells for the same background flow and electrical stimulus, which is exactly obtained in our experiments too.

Reference

1 E. W. K. Young, A. R. Wheeler and C. A. Simmons, *Lab Chip*, 2007, **7**, 1759.

2 A. B. Yasunaga, Y. Murad, V. Kapras, F. Menard and I. T. S. Li, *Biophys. J.*, 2021, **120**, 2511–2520.