Supplementary Data

Supplementary Data 1

Microfluidics based semi-quantitative analysis of ligand tethering on PDMS substrates

Protein tethering has been considered as one of the important factors that interplay with stiffness dependent cellular mechanotransduction. The common practice of determining the tethering strength of the ligand is AFM. Recently, Wen *et al.* [1] showed that PDMS do not support protein tethering whereas other substrate like polyacrylamide do. Here we have performed a microfluidics based wash-off study to confirm that the binding interaction of protein to PDMS substrate is independent of the matrix stiffness. In this experiment, the adsorbed proteins were subjected to microflow induced shear stress under 'partial slip' flow condition. It is already known that microfluidic shear force can alter the configuration of the matrix bound macromolecules (elongation or bending) [2]. We exploited the same to analyze the quantitative retention of ligands on PDMS matrices of different stiffness after exposing them to microfluidic shear force.
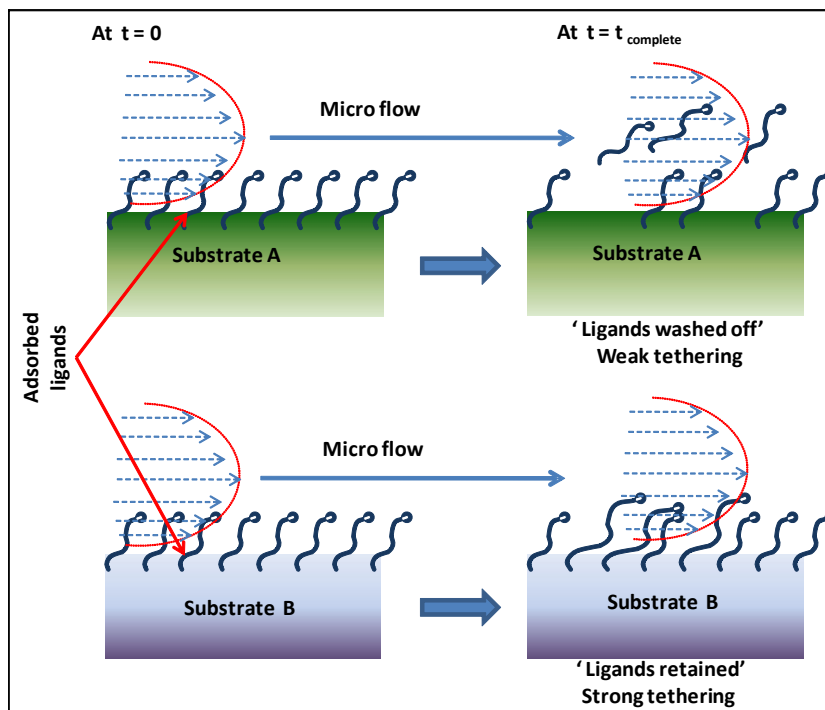

Fig. S1. A schematic representation of the philosophy of the experiment.

Experimental protocol: PDMS elastomeric substrates of varying stiffness were prepared by mixing PDMS and curing agent in various proportions (10:1 (P10), 20:1 (P20) and 30:1 (P30) w/w) as per the protocol given in section 2.2.1 and cast as thin layer on rectangular cover slip (24 x 60 mm). They were further treated degassed, beaked and treated with piranha solution for oxidation. A set of PDMS based microchannels (length (L): 25mm, width (W): 1.4 mm and height (H): 100 μm) were fabricated using the standard procedure of soft lithography. The channels were then fixed on the top of the PDMS substrates. Thereafter the channels were incubated with FITC-gelatin solution (0.1% w/v) for 30 min at 37 °C. After the incubation, the excess protein solution was removed and fluorescent intensity of the adsorbed protein was imaged using a confocal microscope (Fluoview 1000, Olympus). Then the microchannel (with the adsorbed protein at base PDMS layer) was exposed to pressure driven micro-flow of PBS. For this purpose, a syringe pump (PHD 2000, Harvard Apparatus) was employed. The details of the flow parameters are given below:

The fully developed axial velocity field (u_x) for a pressure driven flow through a rectangular channel with W>> H, can be described as,

$$u_x(y) = \frac{6Q}{WH^3} y(H-y) \; ; \; Q \text{ is the volumetric flow rate}$$

and the wall shear rate would be $6Q/WH^2$ which is proportional to the shear stress τ. The flow rate was used monitored in such a way that the wall shear becomes 6 dyne /cm^2. The shear stress was chosen on the basis of the recent work done by Kusunose et al. [3] . After the flow the intensity of the FITC-gelatin coated surfaces were again measured using the same protocol mentioned earlier. Thereafter, all the images were subjected for intensity analysis to profile the ligand distribution using NIH ImageJ software. For each substrate 15 random ROIs (region of interest) were selected and data were expressed as mean ± SD.

Results: The intensity analysis showed that the decrease in fluorescence intensity after flow exposure was almost same for all the substrate. These data clearly implied that protein tethering to the PDMS substrate is independent of substrate stiffness. This is in accordance to the Wen *et al.* [1] who suggest a lack of protein tethering mechanism in PDMS.
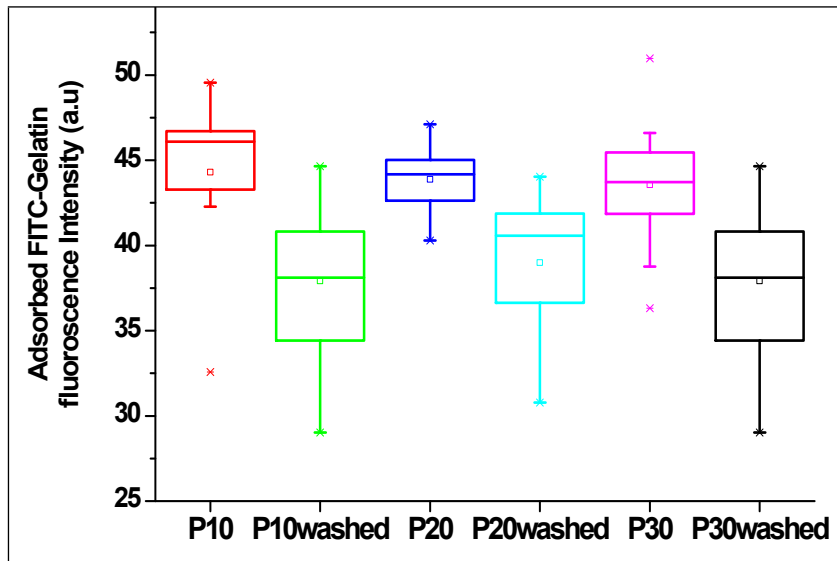
Fig. S2. Analysis of the fluorescent intensity of adsorbed FITC-gelatin molecules on PDMS surface before and after micro-flow exposure. For each substrate, 15 random ROI were selected and measured. Data was expressed as Mean ± SD. Datasets were found statistically insignificant ($p > 0.05$). The data showed a decrease in the mean intensity after micro-flow exposure, which is stiffness independent. This suggests a similar binding strength of the protein molecules in all the cases.

References:
1.   J. H. Wen, L. G. Vincent, A. Fuhrmann, Y. S. Choi, K. C. Hribar, H. Taylor-Weiner, S. Chen and A. J. Engler, Nature materials, 2014.
2.   B. Roy, T. Das, T. K. Maiti and S. Chakraborty, Analytica chimica acta, 2011, 701, 6-14.
3.   J. Kusunose, H. Zhang, M. K. J. Gagnon, T. Pan, S. I. Simon and K. W. Ferrara, Annals of biomedical engineering, 2013, 41, 89-99.

Supplementary Data 2
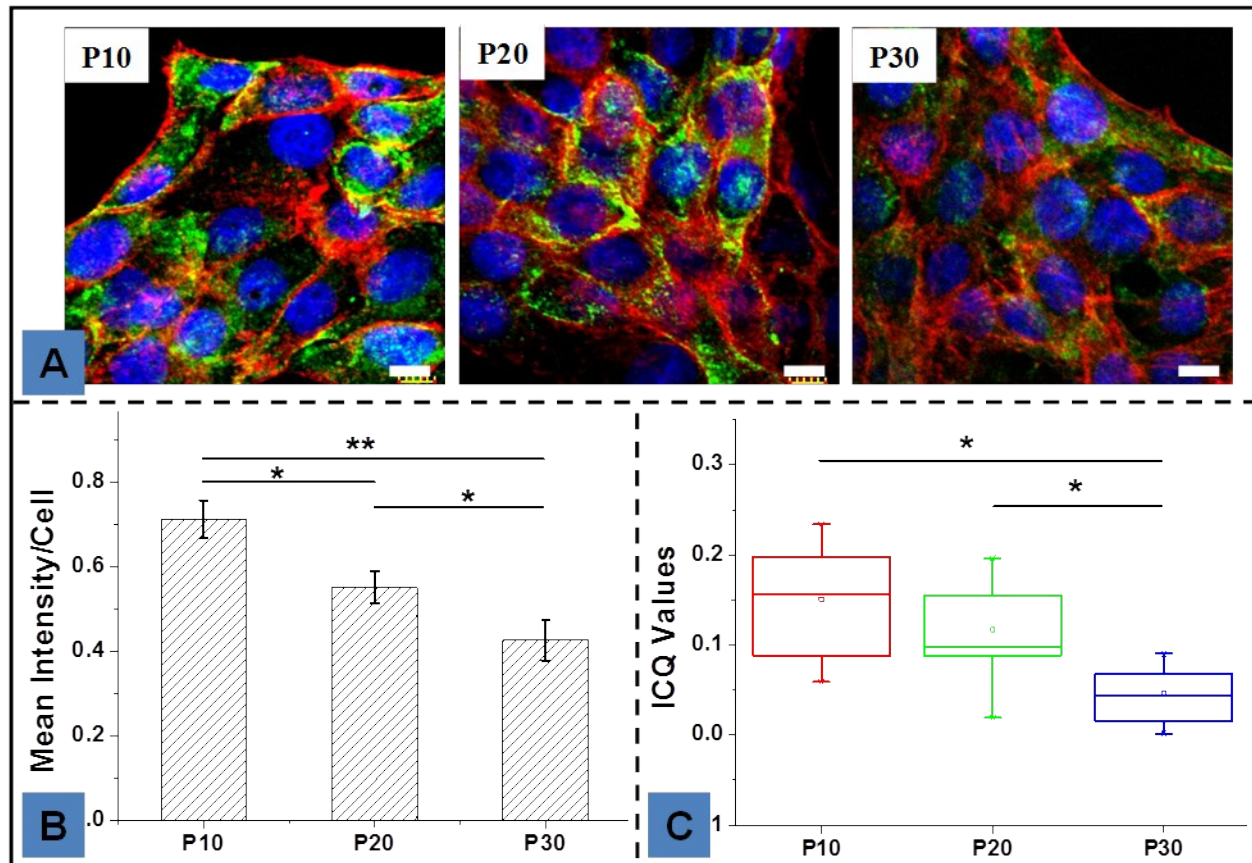
Fig. S3. Expression of E cadherin in HaCaT cells cultured on PDMS substrates of varying stiffness. (A) Confocal microscopic images of HaCaT cells stained with FITC tagged anti-E cadherin antibody (green), DAPI for nucleus (blue) and TRITC-phalloidin for actin cytoskeleton (Red). Quantification of E cadherin expression, (B) Mean Intensity and (C) ICQ value. (*p<0.05, **p<0.01, Scale bar 10µm). Result revealed that there was an overall increase in E cadherin expression at cell–cell junction with an increase in PDMS substrate stiffness.