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


# Supporting Information

# *In situ* viscoelastic properties of cellular monolayers via graphene strain sensing of elastohydrodynamic phenomena

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**Finite element analysis (FEA).**

As a viscoelastic material, understanding the thickness dependence of relaxation time constant for the cell monolayer is helpful to interpret data which were collected from the mechanical property test using the presented microfluidic platform. Since it is not feasible to change MDCK cell

monolayer thickness during a mechanical property test, FEA was used to model the behavior of the composite structure (PDMS + cell monolayer) under hydrodynamic force inside the channel. The FEA is critical to the analysis of thickness dependence of relaxation for the cell monolayer because it controls the cell layer thickness while other parameters are kept consistent.

FEA was performed with the structural mechanics module of a standard simulation software (Comsol Multiphysics 6.1) for linear elastic analysis. The two domains of the finite element mesh, each with different material properties, were the PDMS channel and the MDCK cell monolayer. The PDMS was modeled as a linear elastic material with Young's modulus of 300 kPa and the Poisson's ratio was taken to be 0.49. The MDCK cell monolayer was also modeled as a linear elastic material with Young's modulus of 30kPa[2,3] and the Poisson's ratio was taken to be 0.49. The hydrodynamic force of the moving droplet acting on the channel wall was modeled as a rectangle wave function which changed its value between peak and baseline over time. The external load acted on the bottom surface of the cell layer (**Figure S1a**). A probe was embedded inside the PDMS domain 1 μm away from the PDMS-cell interface which was modeled as the Pd-graphene strain sensor (**Figure S1b**). The displacement over time at the position of the probe was extracted at each thickness that had been applied for the cell layer (**Figure S1d**). The same fitting procedure described in the main text was applied to extract the calculated relaxation time constant from the probe displacement-time curve. The characteristic decay of the stress relaxation showed two distinctive phases from the curve. The calculated relaxation time constant result of the first phase was thickness dependent while the result from the second phase was kept constant which is close to that of the pure PDMS.

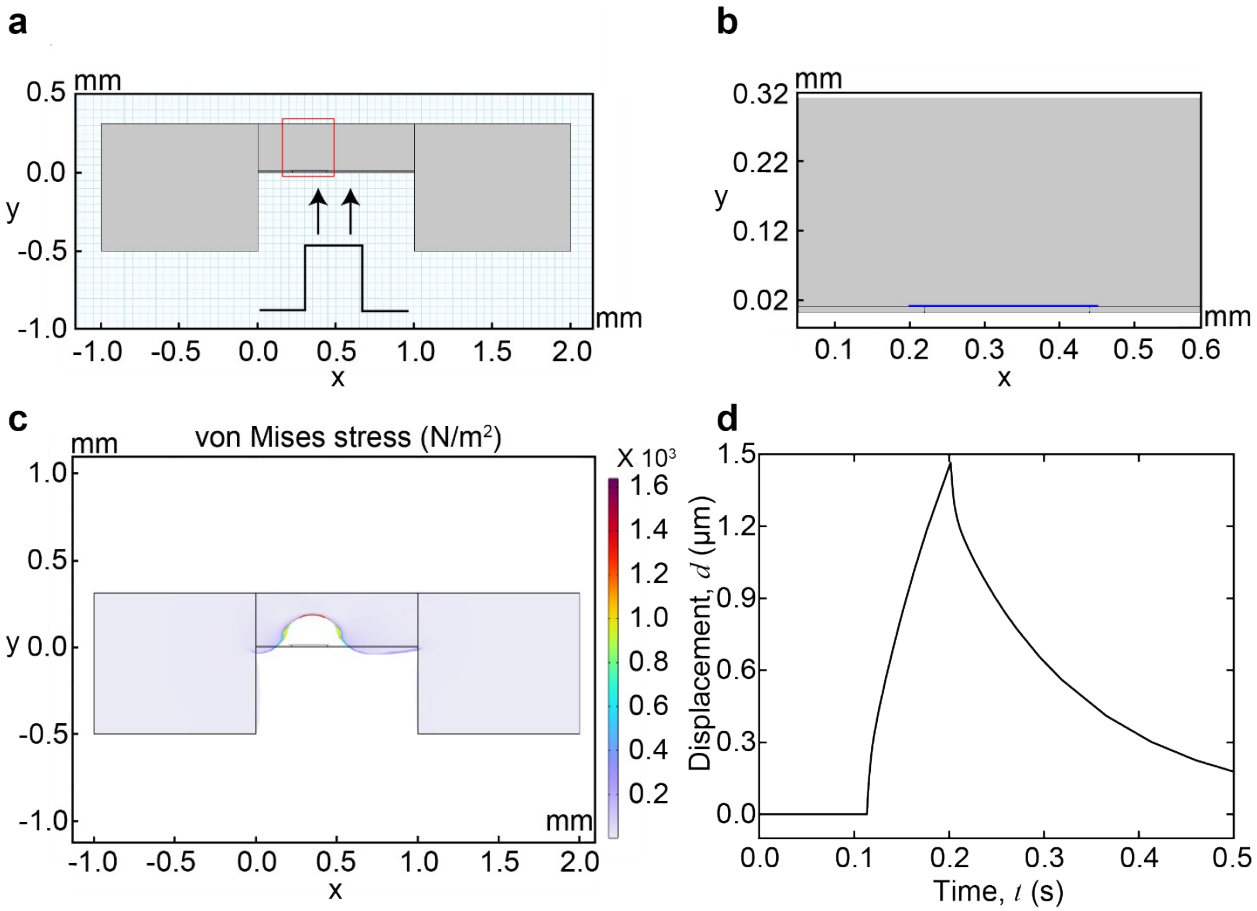


**Figure S1.** FEA simulation set-up. (a) Geometry of the channel. A line probe (red box) was placed in close proximity to the channel and acting as the strain sensor in real device. A rectangular function was used to model the applied stress of a droplet. (b) Line probe area in (c). There is a thin PDMS layer between the line probe and the channel. (c) Channel deformation under the stress (Cell layer thickness: 10 μm) (d) An example of displacement output from the line probe (Cell layer thickness: 10 μm). Simulation time is 0.5 second. The maximum deformation is ~15% of the cell layer thickness.

Cell layer thickness range was set from 10 μm to 20 μm to mimic the physiological state of a real cell monolayer and ensure the physical reliability of the simulation. From the fitting result of the first phase of the displacement curve, the calculated relaxation time constant kept increasing with the layer thickness from 15.8 $s^{-1}$ to 18.9 $s^{-1}$. The value was higher than that of the pure PDMS

layer (9.9 ± 1.3 $s^{-1}$) because of the stiffening effect of the cell layer. The simulation result was also higher than that of the experiment (14.5 ± 0.4 $s^{-1}$) when a MDCK cell monolayer was attached on the PDMS because the real thickness of the MDCK cell monolayer (8 μm) is smaller than the lower bound (10 μm) of the simulation. The difference between the lowest and the highest value of the calculated relaxation time constant from the simulation was larger than the standard deviation of the experiment data. These observations suggested that, as a viscoelastic material, the relaxation time constant was thickness dependent in a certain thickness range. As a result, an equation which was to describe the relaxation time constant relationship for a two-layer structure was obtained, $\lambda_{total} = \lambda_{PDMS} + \lambda_{cell} \times exp(1 - \frac{a}{h})$, where $a$ was a constant and $h$ was the layer thickness. In this situation, $a$ was chosen to be 9.4 μm and $\lambda_{cell}$ is 9.0 $s^{-1}$. It was shown by FEA that the relaxation behavior of the cell layer was thickness dependent and a thickness correction method was required along with the experiment data to acquire the true mechanical properties of the cell layer.

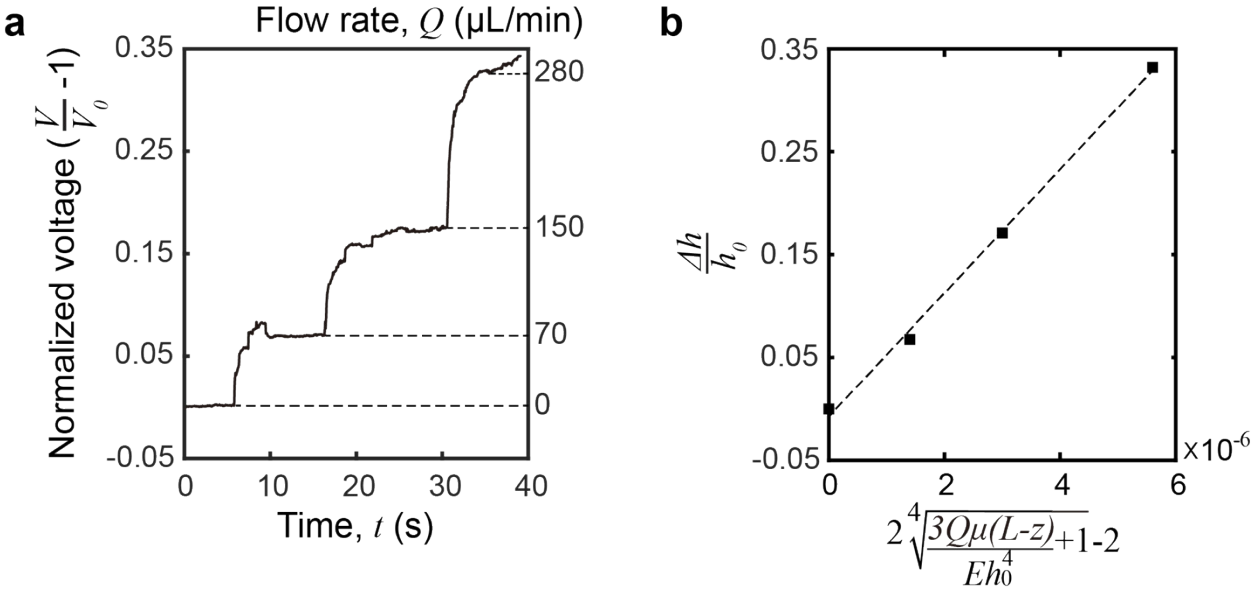

**Figure S2.** (a) Demonstrating sensing of elastohydrodynamic phenomenon by monitoring the voltage change of the device at different flow rates in an additional device. (b) Experimental data fitted against theoretical prediction of deformation. The relationship between flow rate and elastohydrodynamic deformation is approximately linear at low pressures. The linearity is shown by $R^2 = 0.99$.

**Additional replicates from cell trypsinization experiment.** Data obtained from additional individual devices during cell trypsinization experiments are shown in **Figure S3**.

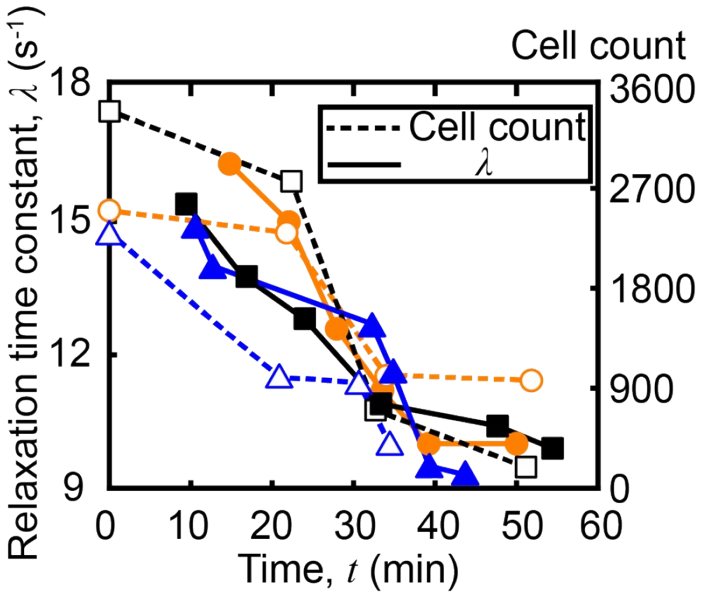


**Figure S3.** Trypsin-EDTA measurement. Relaxation time constant ($\lambda$) and cell number change with trypsinization treatment time (all three trials). Dash line shows cell number and solid line shows $\lambda$.

**Metabolic activity measurement of EMT.** Cell metabolism was assessed using the PrestoBlue Cell Viability Reagent (A13262, Thermo Fisher Scientific) on days 1 and 3 (**Figure S4**).

PrestoBlue was diluted in cell culture media at 10% (v/v). After aspirating media, each MDCK cell sample was incubated with 220 μL of PrestoBlue solution at 37 °C for 2 h. Then 80 μL of the solution from each sample was transferred to a 96-well plate, and fluorescence was measured using a SpectraMax i3x Multi-Mode Microplate Reader at 585 nm. Data were normalized with respect to cell metabolic activity on day 1.

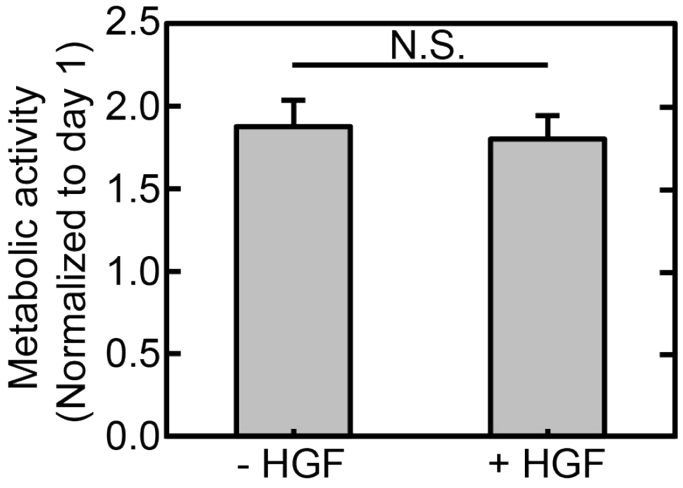


**Figure S4.** Metabolic activity measurement of EMT. Metabolic activity measurement of MDCK cell with/without HGF treatment. The result shows no significant difference in metabolic activity of MDCK cell with/without HGF treatment on day 3.

**Confocal z-scan of MDCK cell monolayer**

*Z*-scan of MDCK cell monolayers are shown in **Figure S5** labeled with CellTracker Red**.** Image taken through microfluidic device. Layer thickness is approximately 8 μm.

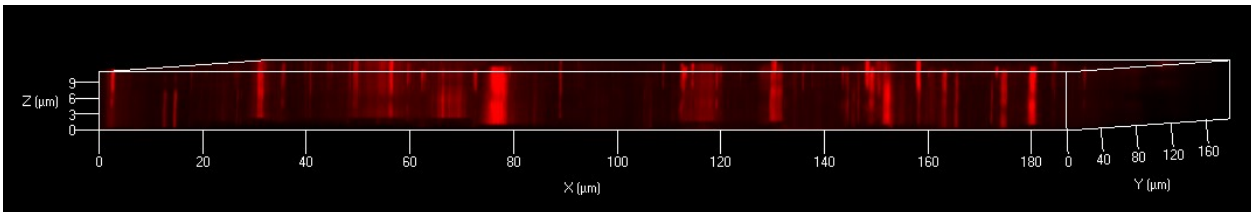


**Figure S5.** Z-scan confocal image of the MDCK cell monolayer.

## REFERENCES

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(2) Sorba, F.; Poulin, A.; Ischer, R.; Shea, H.; Martin-Olmos, C. Integrated Elastomer-Based Device for Measuring the Mechanics of Adherent Cell Monolayers. *Lab Chip* **2019**, *19* (12), 2138–2146.

(3) Schulze, K. D.; Zehnder, S. M.; Urueña, J. M.; Bhattacharjee, T.; Sawyer, W. G.; Angelini, T. E. Elastic Modulus and Hydraulic Permeability of MDCK Monolayers. *J. Biomech.* **2017**, *53*, 210–213.