Electronic Supplementary Material (ESI) for Soft Matter.

Supplementary Information

Probing local lateral forces of focal adhesions and cell-cell junctions of living cells by torsional force spectroscopy

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Cell viability assessment after torsional force spectroscopy

After completing the mapping using torsional force spectroscopy, we conducted a live/dead viability/cytotoxicity assay on the investigated cell sample to determine its viability. Subsequently, fluorescence microscopy imaging was performed at the same region. Figure S1a displays the camera view of the atomic force microscopy (AFM) setup, illustrating the position of the cantilever relative to the cell sample. Figure S1b presents the corresponding bright field image obtained after torsional force spectroscopy mapping, where the cells were stained with the polyanionic dye calcein and Ethidium homodimer-1 (EthD-1). Calcein is retained within live cells, while EthD-1 enters cells with damaged membranes. The fluorescence emission wavelengths of calcein and EthD-1, detected using the calcein and rhodamine optical filters, respectively, are 517 nm and 617 nm. The overlay of the camera view and the bright field image can be observed in Fig. S1c, confirming the alignment of the sample areas. The scan area is highlighted by the red frame, and the topographical map of the initial overview scan, displayed in Fig. S1d, reflects the peripheral region of the cell indicated in Fig. S1b. Fluorescence images of live cells (captured using the calcein optical filter) and dead cells (captured using the rhodamine optical filter) are presented in Figs. S1e and S1f, respectively. Based on these images, we can conclude that both the mechanically investigated cell and the neighboring cells were not significantly damaged by the AFM tip, and their cell membranes remained intact after performing more the 65.000 force distance curves with a setpoint force of approx. 2 nN that caused tip indentations or sample deformation of up to 2 µm.

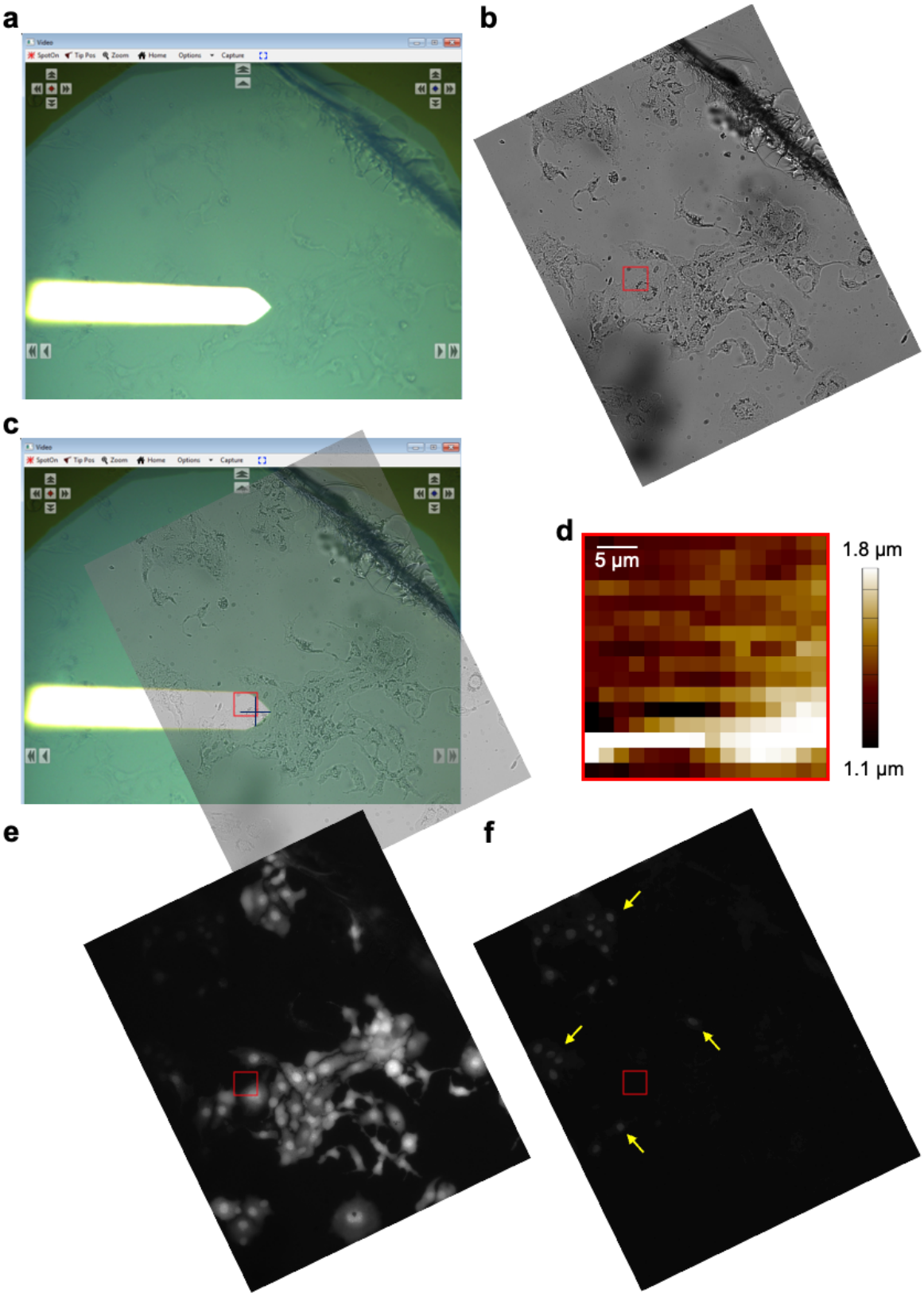

Fig. S1 Live/dead viability/cytotoxicity assessment of the cell after the torsional force spectroscopy experiment. **a** Optical view of the cell sample and relative alignment of the cantilever. **b** Brightfield image of the same area in the subsequent fluorescent microscopy assessment. The red square marks the area that was investigated by force spectroscopy. **c** Overlay of the AFM optical view and the brightfield images taken by the fluorescence microscope to match the positions. **d** Topographical map of the first overview scan by torsional force spectroscopy. **e** Fluorescence microscopy image exhibiting cells with intact cell membrane. f Fluorescence microscopy image of dead cells. The read square indicates the measurement area of the torsional force map, yellow arrows point out some dead cells.

Proposed tip-sample interaction mechanism

During the approach of the tip towards the cell sample (indicated by the blue line in Fig. S2a), it initially makes contact with the cell membrane (stage 1, Fig. S2a). As the approach continues, the lipid bilayer membrane undergoes vertical stretching, causing the molecules to slide against each other (stages 1-2, Fig. S2b). At a specific position (stage 2, Fig. S2a), the tip penetrates through the membrane, creating a small pore between the lipid bilayer molecules. With further force application, this pore enlarges. Evidence for this phenomenon is observed in the force curve as a small force plateau (encircled by dashed line in Fig. S2a). Similar characteristics have been observed in a study involving cell membranes and nano needles [1]. Hence, the tip has the ability to sense subsurface features and their mechanical properties beneath the cell membrane, such as cytoskeleton components, desmosomes, or focal adhesions. Based on the viability assessment, which confirmed the integrity of the cell membrane after the completion of force spectroscopy maps, we propose that when the tip is retracted (indicated by the red line in Fig. S2a) the lipid layer reorganizes and closes the pore.

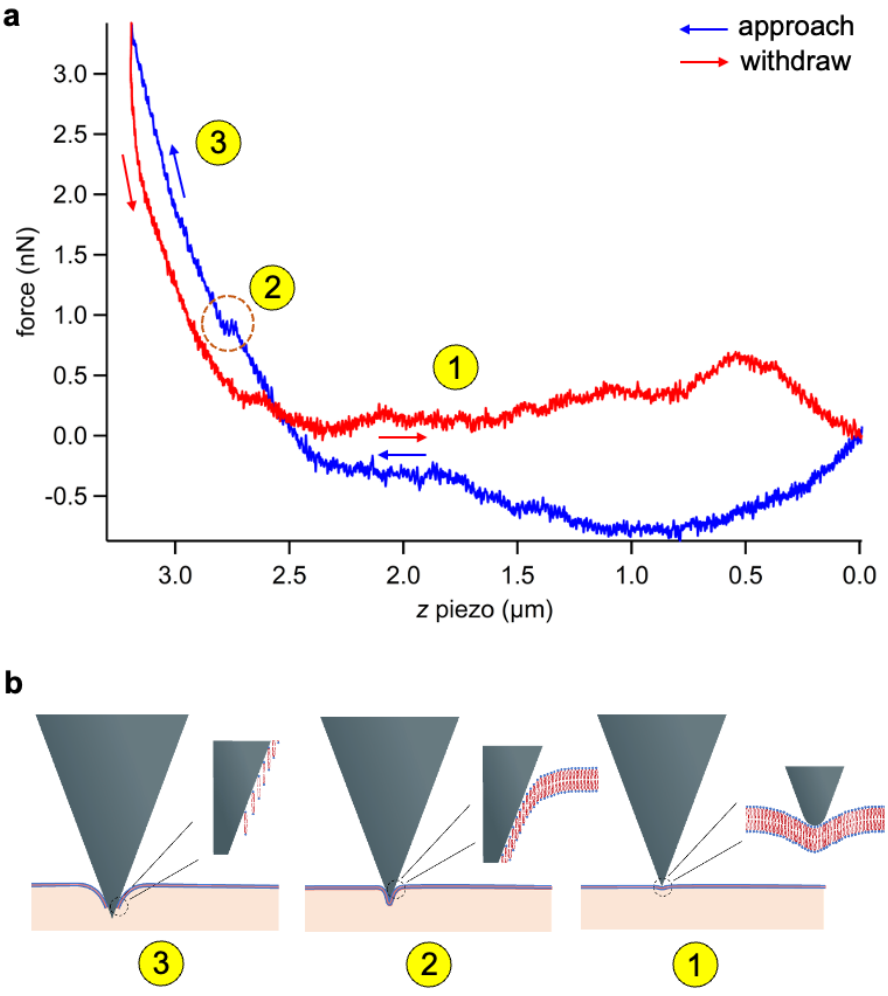

Fig. S2 (a) Measured force vs. *z*-piezo position curve. (b) Proposed tip-sample interaction mechanism at different stages (1 – 3).