Microfabricatd cantilevers for parallelized cell-cell adhesion measurements.

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Abstract

Single cell adhesion measured with atomic forc microscopy (AFM) offers unpaired time and force resolution and allowed the investigation of many important phenomena with unmatched precision.
However this technique suffers of serious practical limitations that hinder its effective application to a broader set of situations. In the most common protocols, cells are cultured on a weakly adhering substrate and are then *fished* on a properly functionalized cantilever (the most common functionalizations are fibronectin, laminin or concanavalin). The cell is left stabilize on the cantilever for a few minutes and then brought in contact with the target substrate, or the target single cell, pressed with controlled force and time and then retracted, while adhesion forces are recorded. This process faces two main problems: i) the number of cells and cantilevers that can be tested during a normal working day rarely exceeds 2 unities; ii) the cell properties may take a long time before stabilizing on the cantilever surface, so limiting the kind of cells suitable for this approach.
Here we propose a different strategy based on the fabrication of large cantilevers and on the culture of the cells directly on the cantilevers. The experiment is schematically illustrated in Figure 1.
Cantilevers area fabricated by standard micromachining, with an active area of 500x500um. In order to create a flat surface parallel to the substrate a polymeric wedge is created starting from an UV curable polymer. The cantilever with a droplet of polymer is brought in contact with a flat surface and then exposes to UV light. The cantilever fabrication is schematically illustrated in Figure 2.
Human Embryonic Kidney cells, HEK 293A, have been grown on the cantilever and on plastic Petri dishes for three days prior to measurements. 30 minutes prior to measurement the cells where live-stained with NucBlue, to allow the direct counting of the interacting cells.
As shown in Figure 3, we measured the adhesion energy as the integral of the force distance curve and demonstrated that it is directly proportional to the number of cells involved in the contact, so obtaining a straightforward measurement of the average single cell adhesion energy.

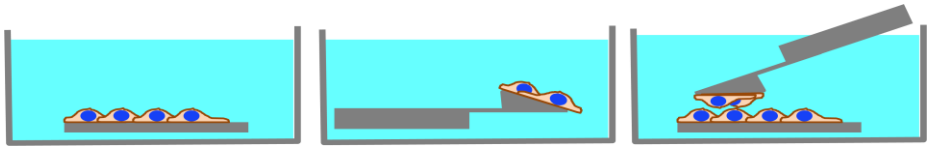

Figure 1: Scheme of the measurement strategy

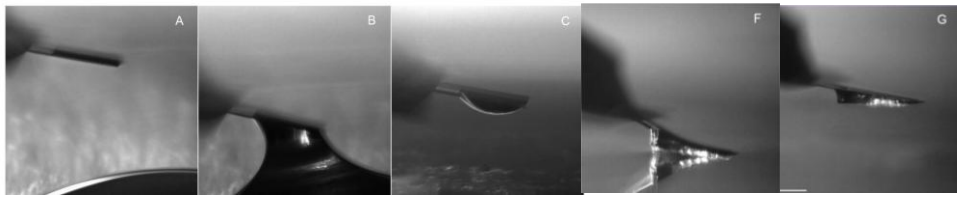

Figure 2: selected snapshot takne during the polymeric wedge formation. A) A fabricated Si3N4 cantilever is approached to a UV-curable resin droplet. B) the cantilever is wet by the resing and C) withdrawn. F) the cantilever is pressed against a non –interacting flat surface, and cured in situ. G) the formed canrilever with a polymeric wedge is ready.

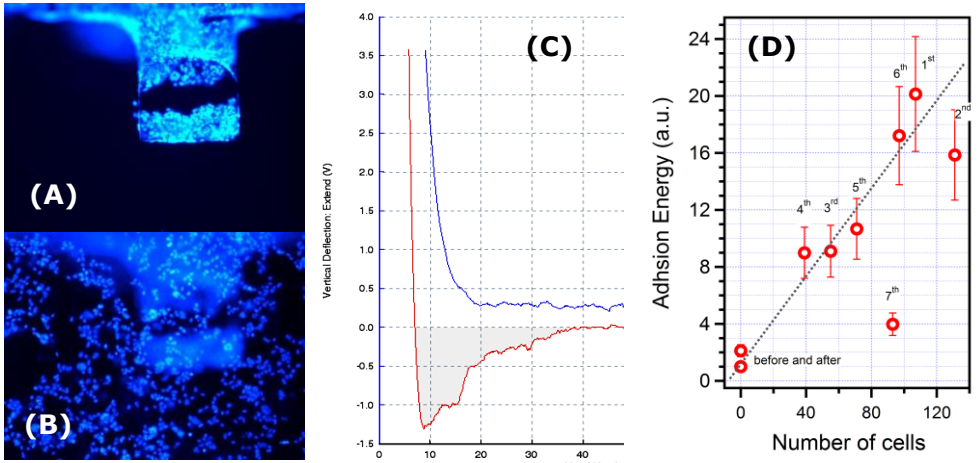

Figure 3: A) and B) imgase of the fluorescent stained nuclei of the cells on the A) contilever and B) the substrate. The overlap of the well defined nuclei on the substrates with the blurred out-of-focus signal from the cantilever nables the evalutauion of the number of cells involved. C) example of the force-vs-distance curve. D) Adhesion energy vs number of cells for an experiment with 240" and 3nN of contact time and force respectively