Cell vacuum: measuring micro-newton cell adhesion forces using micropipette suction

The force with which cells adhere to their substrate is of interest in the study of various diseases. For example, in the case of atherosclerosis, the disease that leads to heart attacks and strokes, the "leakiness" of cell-substrate adhesion may play an important role in the development of plaque in arteries. Cells adhere to their substrate through specific bonds that form multi-protein clusters called focal adhesions. We developed a technique to directly measure the force of detachment of a single adherent cell from its substrate (Fig. 1).

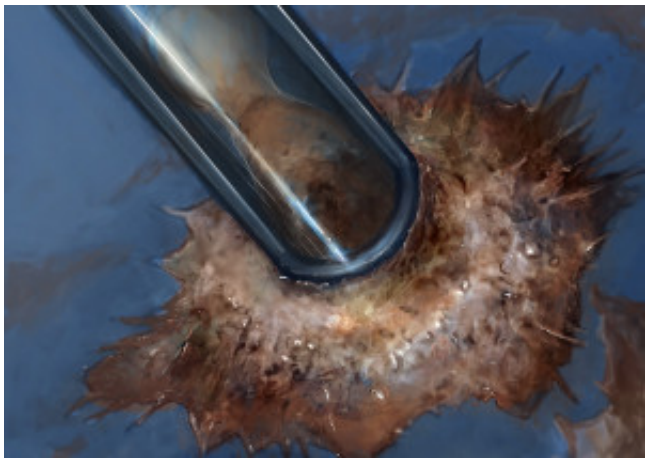

Fig. 1. Artistic representation of the suction of an endothelial cell with a micropipette.

The experimental setup consists of a micropipette: a thin and hollow glass tube that is only several microns in diameter at the tip. The back of the micropipette is connected to a syringe pump, and its tip is brought into contact with the surface of the adherent cell which is cultured on the bottom of a petri dish (Fig. 2). An aspiration pressure is applied inside the syringe pump and consequently inside the micropipette, and the aspiration pressure is increased at a constant rate. The pressure at which the cell is detached from the substrate is recorded. Using this pressure, we can calculate the force that was needed to detach the cell from its substrate.

Under brightfield illumination, we were able to quantify the projected area of the cell on its substrate. We observed that the detachment force scaled directly with cell area. That is, a larger cell required a larger force to detach it from the substrate. Using a microscopy technique known as Interference Reflection Microscopy, IRM, we were able to quantify cell adhesion area, which can be computed as the sum of the area of all focal adhesions. We found that for a given rate at which the aspiration pressure is increased, the detachment force scales with cell adhesion area. That is, the more focal adhesions, or the greater the cell-substrate contact area, the harder it is to detach the

cell. Interestingly, our results demonstrate that the critical stress, which represents the force needed to detach the cell divided by the cell adhesion area, is conserved among cells. Thus, if we were to know the adhesive area of any adherent cell, for example, through an image of the cell in IRM, we could predict the force that would be needed to detach it from its substrate.

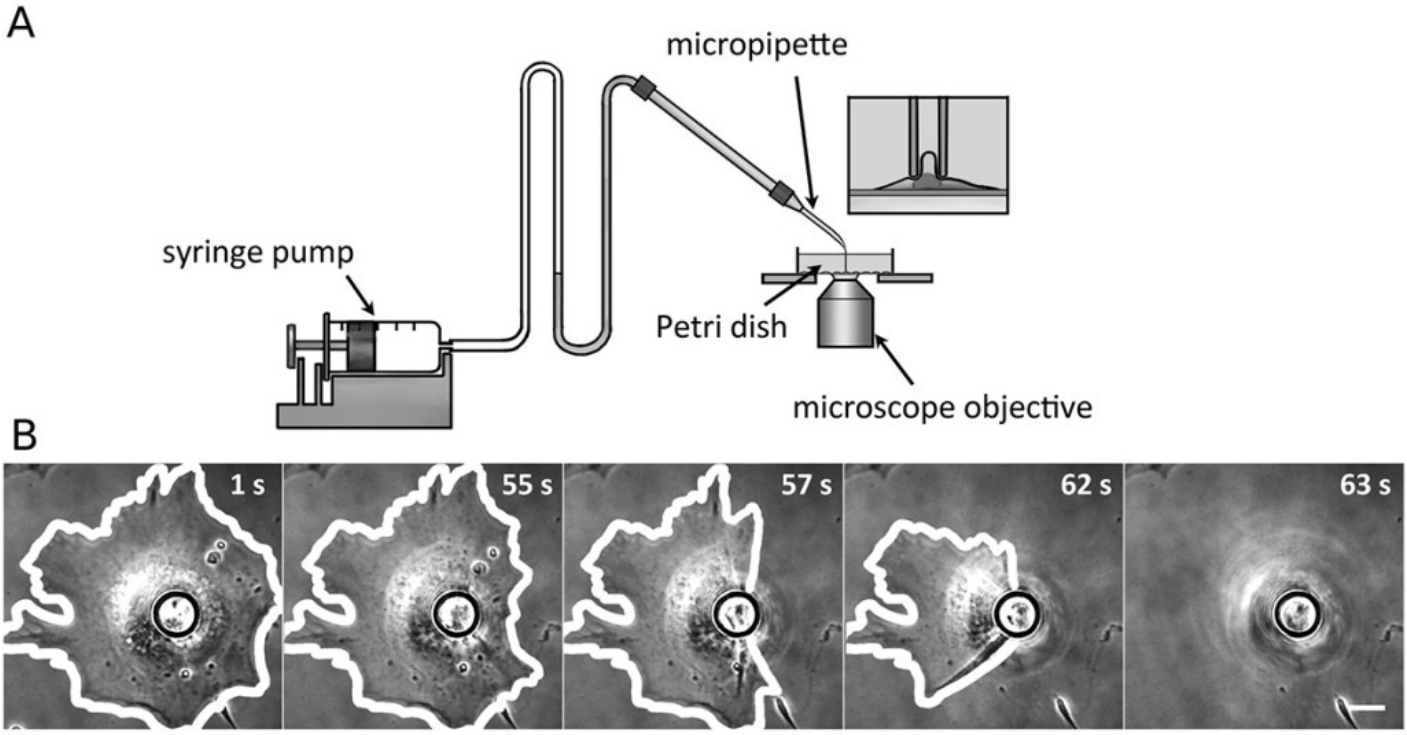

Fig. 2. (A) Experimental setup for aspiration experiments. A micropipette is positioned perpendicular to the surface of adherent endothelial cells. The syringe pump on the left creates an aspiration pressure. (B) Images of a cell being detached. The bar represents 10 microns. ©Biophys J. 2015 Jul 21

To rationalize our experimental results, we developed a simple analytical model that captures the essential physics of cell adhesion and detachment upon suction. The model results confirm a dependence of the critical stress on the geometrical properties of the micropipette and the cell, the rate at which the aspiration pressure is increased, as well as microscopic parameters characteristic of the adhesive bond present within the focal adhesions.

The single-cell manipulation technique reported here can be used to study changes in cell-substrate adhesion that occur during a variety of diseases or are induced by the presence of other types of cells or chemicals.

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