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Comment

# Commentary on "Adhesion and membrane tension of single vesicles and living cells using a micropipette-based technique" by M.-J. Colbert et al.

P. Nassoy[a]

Laboratoire Physico-Chimie Curie, Institut Curie, CNRS, 75005 Paris, France

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Cell adhesion is a ubiquitous phenomenon in biology. It is involved in most cellular physiological responses such as survival, growth, differentiation, proliferation, migration, as well as in many pathological situations such as metastasis formation, tissue inflammation and in host-biomaterial interactions. Cell-cell and cell-extracellular matrix adhesion are determined and finely regulated by both the nature and the density of the adhesion molecules involved in adhesive contacts. For instance, cell migration has been shown to strongly depend on cell-substratum adhesiveness: cells cannot move on weakly adhesive (too slippery) substrates nor on strongly adhesive (too sticky) surfaces. A maximal migration speed is observed for intermediate adhesiveness [1]. For practical biomedical purposes, such as implant design, it is also crucial to assess cell-substrate adhesiveness.

Consequently, there has been a great deal of research over the last three decades to probe the strength of cell adhesion to surfaces. Numerous assays have been developed towards this goal. The oldest and simplest attachment assay is based on rinsing the surface to remove weakly attached cells from the substrate and counting the remaining cells [2]. Cells adhering with a force less than the rinsing force are removed, but this force is difficult to control and therefore ill-defined. More quantitative methods have been proposed. While their working principle is similar and consists of measuring the ability of adherent cells to remain attached when exposed to a detachment force, they differ by the geometry of the experimental arrangement, and thus by the nature of the distractive force. The most prevalent techniques may be classified into three main categories: centrifugation [3], hydrodynamic shear [4], and micromanipulation (using AFM [5], magnetic tweezers [6], or micropipettes [7]). Characterization of cell-substrate adhesiveness is here provided by the measure of separation forces or peeling shear stresses, which result from either ensemble measurements or single-cell experiments.

Although separation forces or stresses are measured precisely, closer inspection of the results obtained with different techniques in similar conditions (same cell line, same surface) surprisingly reveals strong discrepancies. Whereas centrifugal forces required to displace cells attached to surfaces usually fall in the 1–10 nN range, separation forces measured by single-cell micromanipulation are typically one or two orders of magnitude higher, and hydrodynamic peeling forces are at least 10-fold lower. Therefore, even though each of these adhesion assays was proved to be useful for comparative studies and practical purposes, the fact that the measured separation forces or stresses are assay-dependent suggests that additional care must be taken to reach a rigorous and intrinsic quantification of cell adhesion.

As explained by P.-G. de Gennes and F. Brochard-Wyart in 2003, separation forces or stresses are not the proper parameters characterizing cell adhesivity. They should be replaced with the adhesion energy, which is the intrinsic parameter in unbinding assays. This proposal was guided by previous works in polymer adhesion, which demonstrated that the critical stress required to separate two glued blocks depends on the thickness and the elastic modulus of the glue layer [8]. Similarly, cell-cell or cell-substrate adhesion should also be defined by the adhesion energy. De Gennes and Brochard-Wyart have also proposed a mechanical arrangement, inspired from the classical Johnson-Kendall-Roberts (JKR) assay in polymer adhesion, and derived the complete theoretical analysis to extract the adhesion energy from force measurements [9]. They came up with an astonishingly simple finding: in the so-called "cellular JKR test", the density of adhesion energy (in $\mathrm{J\,m^{-2}}$) is given by the ratio between the unbinding force and the radius of the stretched cell when detachment

[a] e-mail: pierre.nassoy@curie.fr

occurs: $W = f/\pi R$. Moreover, the variation of the contact radius as a function of the applied pulling force was computed in detail. The validity of the model was experimentally confirmed by Brochard-Wyart and coworkers in the peculiar case of red blood cells [10]. The mechanics of human erythrocytes was extensively characterized and red blood cells have been commonly used as force transducers in force techniques (*e.g.* Biomembrane Force Probe [11]). Therefore, Pierrat *et al.* [10] used red blood cells both as a force transducer and as an adhesive object of interest. Yet, this experimental set-up did not permit any extension to other cell types.

In this issue, Dalnoki-Veress and coworkers [12] report a novel experimental approach, which uniquely allows them to measure the adhesion energy between cell and substrate. The versatility of the technique originates from the use of an L-shaped pipette, which serves both to manipulate individual cells and to measure the applied force via the measurement of its deflection. The technique is first validated on lipid vesicles adhering to gold. Then, their Micropipette Deflection (MD) method was applied to living cells. Quite interestingly, by simple application of the Young-Dupré law, the membrane tension of adhering cells can be derived from the visualization of the adhesive contact and from measurement of $W$. The paper by Colbert *et al.* [12] thus provides for the first time a quantitative and non-invasive assay to estimate the tension of eukaryotic cells. This exploratory study is expected to be pursued and should help in investigating the influence of putative molecular players on the homeostatic tension of cells. Finally, it is worth mentioning that the technique developed by the authors can be seen as a multipurpose mechanical device. Not only does the MD method provide a measure of $W$ through cell unloading, but it also allows them to monitor the force relaxation upon cell compression. It was found that compressed cells undergo a logarithmic force relaxation. This effect, which has never been reported to date, could be due to the fact that experiments were carried out at room temperature instead of 37 °C, or, more interestingly, reveal material properties of cells; as suggested by the authors, logarithmic relaxation is strongly reminiscent of spin-glasses or granular materials.

In my opinion, the MD technique developed by Colbert *et al.* [12] should shortly become a standard adhesion assay, since it combines the ideal design of the cellular JKR imagined by P.-G. de Gennes and F. Brochard-Wyart [9] and an unprecedented dynamical range, which enables to monitor fast detachment events as well as ultra-slow glassy relaxation.

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