Single Cell Force Measurements and Cell Adhesion

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This short review describes the relevance of cell adhesion in cell biology. Starting with a short overview of the force range of adhesion related biological events and the current biophysical techniques for investigating these events, it will conclude with a description of the use of single cell force spectroscopy for quantifying mechanical properties such as stiffness, surface tension, and bond disruption forces.

Motivation

The past decade has seen the development of molecular cell biology and related biomedical/ pharmacological research. At the cell-level, the fundamental processes of genetics, metabolism, and cell communication are under investigation. Looking ahead, processes in the life sciences will be redefined in the new concept of system biology.

Adhesion is one of the crucial mechanisms of interaction between living cells and their environment. Cell adhesion is a complex process that involves non-specific and specific binding of glycocalyx and plasma membrane surface molecules (e.g. integrins, selectins or cadherins) to the extracellular matrix proteins (ECM, mainly fibronectin, collagen) or to other cells respectively, down stream signalling that adhesion has occurred and a possible cellular response to this binding (e.g. cell shape, migration, proliferation). It is a dynamic and complex process, which consists of physical interaction, biochemical response and physiological adaptation. Understanding this complexity and identifying the key triggers in specific biological responses of cell adhesion is of fundamental importance in a wide range of fields including cancer and stem cell research, developmental and infection biology, immunology and allergology, tissue engineering and implant research.

There are many well-established techniques for studying cell adhesion; from fluorescence microscopy to biochemistry and molecular biology (e.g. gene and protein expressing assays or aggregation and migration assays). Based on the finding that the elasticity of the extracellular matrix varies with the lineage of stem cells, new thoughts are discussed that not only (bio)chemical properties of the ECM/microenvironment but also their mechanical properties.

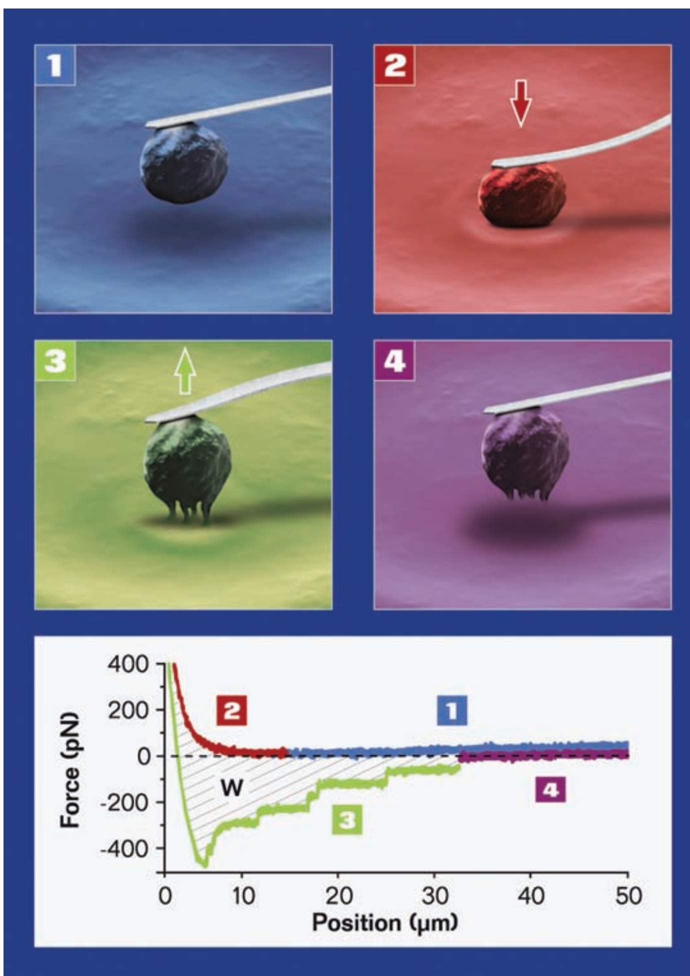

Figure 2: The basic principle for cytomechanical probing using SCFS technique with four steps: 1-approach, 2-contact, 3-retract and pulling 4-far away from the surface. Below we illustrate the corresponding part of the force distance curve.

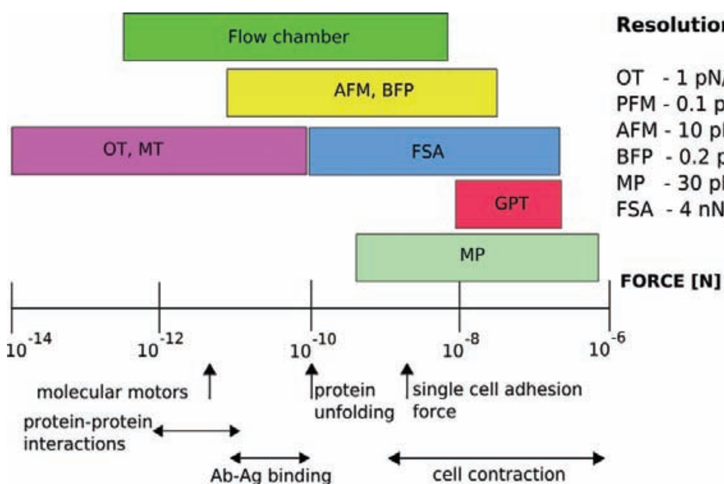

Figure 1: Overview of force range of adhesion relevant biological events and biophysical techniques used to investigate forces and mechanical properties of molecules and cells (at an approximate drawing speed of 500nm/s), for reviews see [3,4,5]. PFM—photonic force microscopy—is an OT variant

and forces can influence the connection to the plasma membrane and the cytoskeleton via adherence junction and focal adhesion as mechano-sensors [2,3].

Current Techniques to Measure Forces on Single Cells

The forces involved in single molecules and single cells range widely from less than 1pN up to 1µN (Fig.1). Single protein-protein bonds are in the range of 1-20 pN, whereas single molecule unfolding requires up to 100pN. To remove a single cell from a substrate demands at least 1nN of force and sometimes more.

Optical and magnetic tweezers are best suited to measuring weaker forces on single molecules or local viscoelastic properties of cells. Force sensor arrays (FSA) and soft gel embedded particle tracking (GPT), and micro-plates cell pulling (MP) are mainly used to investigate cell responses to topographic patterns, and to measure traction and migration forces respectively. With a micro-plates cell puller, viscoelastic properties of adhered cells can be determined.

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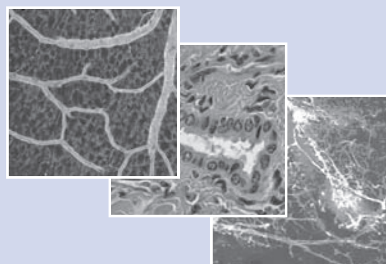

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Flow chamber, bioforce membrane probe technique (BFP) and atomic force microscopy (AFM) are characterized by a very wide force range from pN up to µN, and allow both measurements of unbinding forces of whole cells from ECM decorated substrates and of various pairs of receptor/ligand interactions.

All these methods have their limitations. The AFM based single cell force spectroscopy (SCFS) is a versatile tool that uniquely fulfils many needs. These include

(i) Large vertical movement for cell/cell interaction measurements of up to 100µm,
(ii) Providing reproducible quantitative results for single cells with precision down to the single molecule level,
(iii) Combining with fluorescence microscopy or even confocal techniques,
(iv) Supporting flat as well topographical surfaces, cell monolayers and aggregates, and
(v) Dedicated professional software.

Atomic Force Microscope-Based Force Measurements

1. AFM uses a laser to measure the deflection of a flexible cantilever (Fig. 2) during probing. To run a typical cell adhesion experiment, the basic steps are as follows:
2. An inverted optical microscope is used to facilitate the chemical binding of a cell to the cantilever.
3. The cell is brought into contact with the target surface using a defined force. This will cause deformation of the cell.
4. After a user-controlled contact time, the cell is withdrawn from the substrate by retracting the cantilever. The cell initially resists this process (the cantilever will bend in proportion to the unbinding force). Cell unbinding often requires effective pulling lengths of up to 100 µm [6] due to extrusion of membrane tethers during cell separation.

After complete separation, the cell can be used again to address a new target surface.

The main results extracted are:

(i) Maximum unbinding force,
(ii) Number and size of jumps,
(iii) Number and length of tethers,
(iv) Work (W) of separation until bonds start to break and
(v) Slope of indentation for calculating contact stiffness and the elasticity of the cell.

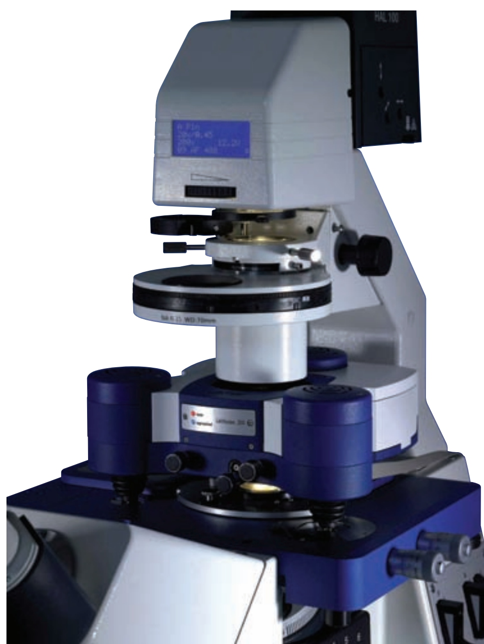

Figure 3: CellHesion® 200 setup in combination with an inverted fluorescence microscope.

Example for SCFS in Developmental Biology

How do zebra fish stem cells form the three germ layers during gastrulation? Very recently, biologists were able to explain this cell sorting and organization process using a CellHesion® system from JPK Instruments combined with a fluorescence microscope (Fig. 3). They quantified both adhesive forces and cell-cortex tension of individual endo-, meso-, and ectoderm cells [6] as basis for modelling simulations.

In the first part of this work, the authors have investigated the homotypic adhesion between all three cell types using the workflow illustrated in Figure 2. They found that mesoderm and endoderm progenitor cells are more cohesive than ectoderm cells (maximum adhesion force at 30 s contact time is 6 nN, and 3 nN, respectively).

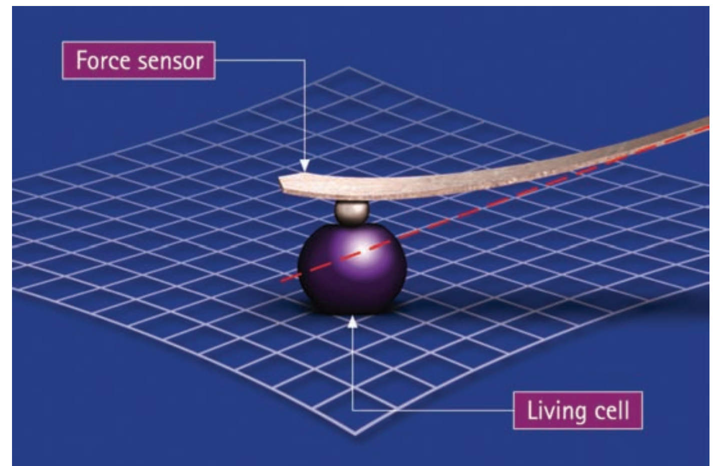

Figure 4: Schematic principle of cell elasticity and stiffness probing. An artificial sphere (e.g. silica, or polystyrene) with a diameter of approximately 1-10 µm is attached with glue to the cantilever. Precise positioning of the cantilever over the bead can be realized with the "cell capture" tool of JPK's software. The bead attached sensor is moved towards the target cell, whose surface is deformed by the indentor bead. Considering the degree of cantilever deflection and actual position of the piezo movement (height measured), the indentation depth can be calculated. The JPK Image processing software is an ideal tool for the data processing, from baseline correction to the contact point finding and slope analysis.

To measure the actomyosin related cell-cortex tension of all 3 different germ-layer progenitor cells, the authors used a setup schematically drawn in figure 4. They calculated the tension from the force-indentation curves using the liquid droplet model. The authors found a rising value for cell-cortex tension from endoderm cells to mesoderm and ectoderm progenitor cells (60µN/m to 45 and 35 µN/m respectively). Additionally, Blebbistatin reduced cell tension to the same level in all three cell types. Furthermore, they found strong evidences for modulation of tension by Nodal/TGGβ-related signalling based on single cell force spectroscopy data. ■

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For additional information, check the applications section of the web site. http://www.jpk.com/cellular-adhesion-cytomechanics.234.html_)

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