Centre for
Energy Research

Tamás Gerecsei

Novel biophysical techniques for single-cell adhesion research

PhD Thesis

Statistical Physics, Biological Physics and the Physics of Quantum Systems
program directed by professor Dr. Jenő Kürti PhD of the
Physics Doctoral School directed by professor Dr. Jenő Gubicza DSc.

Department of Biological Physics
Faculty of Science
Eötvös Lordánd University
and
Nanobiosensorics Laboratory
Centre for Energy Research

Supervision

Dr. Robert Hovath & Dr Balint Szabo

2021

Abstract

Cell adhesion is one of the most important processes in the life of an eukaryotic cell. Survival, growth and normal functioning are all intricately connected to the molecular machinery that serves to anchor the cell to its environment. More than a purely mechanical connection, adhesion is an active biological process in which a myriad of components play a role on both sides of the membrane. The most widely studied family of proteins connecting a cell to the extracellular matrix (ECM) is the family of integrins. The ligands binding and activating these proteins are short peptide sequences found on ECM proteins, such as Arg-Gly-Asp (RGD).

With the development of biophysical techniques, a large variety of approaches has been introduced with the aim of measuring cell adhesion. Each of these methods have their own unique set of advantages and disadvantages regarding throughput, versatility and single-cell capabilities. The work presented here focuses on the development of some of these techniques and on finding new fields of applications for them. First, the computer-controlled micropipette (CCMP) system is analyzed and compared to fluidic force microscopy (FluidFM). The former technique is based on a glass capillary that applies a negative pressure on surface attached cells or microbeads in a serial manner, providing subpopulation level information on adhesion. FluidFM is a novel variant of atomic force microscopy in which a microfluidic channel is incorporated into the cantilever, allowing a variety of live cell manipulations including detachment force measurements. Through measurements on funtionalized microbeads, I demonstrated the capability of CCMP to be used as a colloid force spectroscopy tool. I recorded adhesion spectra of the same microbead immobilization system using FluidFM and correlated the results. I characterized the avidin-biotin-based surface functionalization using optical waveguide lightmode spectroscopy to estimate the detachment force of the noncovalent bond, which resulted in a value of $F = 188 - 375\,\mathrm{pN}$.

Turning towards cellular biology, I developed a cell-based assay to measure the two dimensional dissociation constant of integrin-RGD binding as $K_d^{2D} = (4503 \pm$

1673)$\frac{1}{\mu m^2}$. This series of experiments is the first instance of measuring a molecular-level parameter of cellular adhesion with a computer-controlled micropipette. Results were confirmed by optical biosensor measurements. Furthermore, I devised a subpopulation analysis making use of the full capabilities of CCMP to provide single-cell level data. Results showed that a significant ratio of the cellular population is in a weakly adhered state on all surface coatings.

While one of the benchmark methods of high-throughput, label-free cell adhesion measurements is the application of resonant waveguide grating (RWG) biosensors, the interpretation of the provided signal had been dubious. Here, by the development of an experimental workflow using FluidFM and single-cell level RWG imaging, the optical signal could be calibrated into detachment force and detachment energy values. This way, not only the intricate structure of the adhesion spectrum could be resolved, but to this day the only truly parallelized detachment force measurement technique was introduced. This powerful approach is also able to determine the time evolution of mechanical adhesion parameters.

The results in this work represent a contribution to the development and application of single-cell level cell adhesion measurement techniques.

Motivation and Objectives

The field of cell-substrate interaction measurements is rapidly developing nowadays. A general tendency, also observable in many other biology related fields, is the shift towards single-cell level measurements. The greatest challenge of this technological revolution is achieving a high throughput, which is essential to gather a statistically relevant number of data points. Furthermore, with the development of the techniques an increasing number of quantitative measures are proposed to describe cell adhesion, such as adhesion force, detachment vacuum, resonant wavelength and angle shift signals etc. The principal aim of this work is to further develop and explore three single-cell level cell adhesion measurement techniques: the computer-controlled micropipette, fluidic force microscopy and the resonant waveguide grating biosensor. In this process I aimed at building on our previous results and experiences with these systems, especially regarding CCMP-based cell manipulations. In concrete terms the objectives are the following:

- The computer-controlled micropipette had been used as a cell adhesion assay previously, however the in-depth analysis of some of the measurement parameters could further improve applicability. The objective of this work was to introduce the measurement of functionalized microbeads as a new application of CCMP and to compare it to an already established colloidal force spectroscopy technique, fluidic force microscopy. The role of different measurement parameters was uncovered, such as the valve opening time and the detachment vacuum in terms of their relationship to traditional CFS parameters.

- Another objective was to present an improved single-cell adhesion assay which is able to quantitatively characterize cell adhesion by measuring the two-dimensional dissociation constant (K_d^{2D}) of the integrin-RGD binding. The created setup is a live-cell assay that can be used to determine the binding characteristics of transmembrane proteins in their native environment (in the membrane of a live cell) to a surface immobilized compound. The demonstration of such a workflow was a principal goal of this work. It was also important

to fully exploit the ability of CCMP regarding single-cell level information. Therefore, a novel methodology was elaborated to provide a population level quantitative assessment (in the form of the K_d^{2D}) as well as a detailed analysis of sub-populations as allowed for by the resolution of the technique. As a comparison, the measurements were correlated with results from a plate-based RWG biosensor. To further apply CCMP-based cell adhesion measurements, the interactions of CR3 and CR4 integrins to fibrinogen was studied in inflammatory conditions.

- The final goal of the work presented here was to push the limits of single-cell adhesion measurements using RWG biosensors. This optical method has several significant advantages, most importantly parallelization which allows for the measurement of hundreds of cells at the same time. Two problems had to be solved to fully exploit this system, both related to the interpretation of the spatially resolved signal. First, a protocol had to be devised for the identification and definition of a single-cell on the images provided by the biosensor. Secondly, the goal was to test the long standing assumption that an integrated version of the RWG signal is in direct correlation with the adhesion force of a cell. To this end a combined experimental protocol was developed which allowed for the sequential measurement of cell adhesion first by the RWG sensor and then with fluidic force microscopy. By processing the data sourced by the two techniques, the correlation coefficients between the biosensor signal and the adhesion force and energy were determined. Based on these results, a new biosensor-based workflow is proposed which is the highest throughput method to measure single-cell adhesion to this day.

Thesis points

1. I established a protocol for the measurement of microbead adhesion using a computer-controlled micropipette. I devised an immobilization system based on avidin-biotin bonding and characterized the molecular buildup of the layers. I executed measurements to determine the adhesion spectra of microbeads as a function of increasing negative pressure and of varying the opening time of the corresponding fluidic valve. The latter measurement mode, providing a method to measure bond lifetime, had not been previously demonstrated using a computer-controlled micropipette. I executed detachment experiments on surfaces with various biotin surface densities to examine the ability of CCMP to differentiate between surface treatments. A strong correlation ($C = 0.927$) between the strength of adhesion and the biotin content of the surface confidently demonstrated this possible application. **[T1]**

2. I measured the force distribution of the same system using fluidic force microscopy in colloid force spectroscopy mode. I compared the results acquired by the two techniques and I found that the measured distributions can be well correlated. Based on this principle, I executed an elementary calibration of the detachment vacuum to detachment force. The unbinding force of an individual avidin-biotin bond could be estimated as $188 - 375\,\mathrm{pN}$ which corresponds well with previously measured values in the literature. Through these results I established the CCMP method as an alternative to cantilever-based colloid force spectroscopy to measure microbead adhesion. The principle advantage of this method is the increased throughput in applications requiring bead exchange, as it is capable of measuring roughly 150 beads in 1 h compared to $12 - 15$ beads per hour achieved by FluidFM. **[T1]**

3. The effect of targeting offset on the detachment vacuum was also determined. Experiments with manual and automated targeting revealed that offsets larger than $5\,\mu\mathrm{m}$ cause a decrease in the negative pressure needed to detach beads. **[T2]**

4. I devised a protocol to measure the dissociation constant of integrin-RGD binding in live cells using a computer-controlled micropipette. Cell adhesion measurements on surfaces with various RGD densities were executed and adhesion histograms were generated. I found that the weighted average of histograms was an applicable measure of adhesion on the population level and it could be fit with the kinetic mass action law to acquire the value of the two-dimensional dissociation constant: $K_d^{2D} = (4503 \pm 1673)\frac{1}{\mu m^2}$. RWG biosensor measurements yielded a value in reasonable agreement, confirming the ability of CCMP to be used as a live-cell assay in dissociation constant measurements. **[T3]**

5. I analyzed the dependence of cell adhesion on ligand density at a single-cell level. I could show that most cells remain in the weak adhesion region and that increasing RGD density results in the appearance of a strongly attached subpopulation rather than the increased attachment of all cells. **[T3]**

6. I used CCMP-based cell adhesion measurements to determine the differing role of CD11c and CD11b integrins under inflammatory conditions in monocyte derived primary cells. I found that adhesion is reduced in inflammatory conditions in MDDCs and that CD11c plays a dominant role in the adhesion of dendritic cells to fibrinogen. **[T4]**

7. I executed and designed experiments to calibrate the signal of a resonant waveguide grating biosensor. Fluidic force microscopy was used to detach cells directly from the surface of the biosensor after measurement of the kinetic biosensor curves. The definition of an appropriate integrated version of single-cell imaging data made it possible to correlate the optical signal to the principal mechanical adhesion characteristics: adhesion force and adhesion energy. The strong correlation between the optical and mechanical parameters allowed for the determination of the calibration coefficients. Based on these results, the adhesion force and energy of 300 cells were measured in a time-resolved, paralellized way revealing a lognormal distribution of adhe-

sion strength whose parameters could be followed in time by the biosensor. This technique constitutes the adhesion force measurement with the highest throughput in literature so far. **[T5]**

Publications related to the thesis points

T1 Gerecsei, Tamás, István Erdődi, Beatrix Peter, Csaba Hős, Sándor Kurunczi, Imre Derényi, Bálint Szabó, and Robert Horvath. Adhesion force measurements on functionalized microbeads: An in-depth comparison of computer-controlled micropipette and fluidic force microscopy. Journal of colloid and interface science 555:245-253 2019.

T2 Ungai-Salánki, Rita, Benjamin Csippa, Tamás Gerecsei, Robert Horvath and Bálint Szabó. Nanonewton scale force measurements on biotinylated microbeads with a robotic micropipette. Journal of colloid and interface science (under review)

T3 Gerecsei, Tamás, Péter Chrenkó, Nicolett Kanyo, Beatrix Péter, Attila Bonyár, Inna Székács, Balint Szabo, and Robert Horvath. Dissociation Constant of Integrin-RGD Binding in Live Cells from Automated Micropipette and Label-Free Optical Data. Biosensors 11, no. 2:32 2021. (February issue cover)

T4 Lukácsi, Szilvia, Tamás Gerecsei, Katalin Balázs, Barbara Francz, Bálint Szabó, Anna Erdei, and Zsuzsa Bajtay. The differential role of CR3 (CD11b/CD18) and CR4 (CD11c/CD18) in the adherence, migration and podosome formation of human macrophages and dendritic cells under inflammatory conditions. Plos one 15, no. 5, 2020.

T5 Sztilkovics, Milan*, Tamás Gerecsei*, Beatrix Peter, Andras Saftics, Sandor Kurunczi, Inna Szekacs, Balint Szabo, and Robert Horvath. Single-cell adhesion force kinetics of cell populations from combined label-free optical biosensor and robotic fluidic force microscopy. Scientific reports 10, no. 1:1-13 2020.

*: equal contribution.

Further publications

- Gerecsei, Tamás, Beatrix Péter, Rita Ungai-Salánki, Sándor Kurunczi, Inna Székács, Bálint Szabó and Róbert Horváth. Prospects of Fluidic Force Microscopy and related biosensors for medical applications. Nanobioanalytical Approaches to Medical Diagnostics, Elsevier 2021. (in press)

- Gerecsei, Tamás, Rita Ungai-Salanki, Andras Saftics, Imre Derényi, Robert Horvath and Balint Szabo. Characterization of the dissolution of water microdroplets in oil. Journal of colloid and interface science (under review)

- Ungai-Salánki, Rita, Tamás Gerecsei, Péter Fürjes, Norbert Orgovan, Noémi Sándor, Eszter Holczer, Robert Horvath, and Bálint Szabó. Automated single cell isolation from suspension with computer vision. Scientific reports 6, no. 1:1-9, 2016.

- Salánki, Rita, Tamás Gerecsei, Norbert Orgovan, Noémi Sándor, Beatrix Péter, Zsuzsa Bajtay, Anna Erdei, Robert Horvath, and Bálint Szabó. Automated single cell sorting and deposition in submicroliter drops. Applied Physics Letters 105, no. 8:083703, 2014.

- Saftics, Andras, Barbara Türk, Attila Sulyok, Norbert Nagy,Tamás Gerecsei, Inna Szekacs, Sándor Kurunczi, and Robert Horvath. Biomimetic dextran-based hydrogel layers for cell micropatterning over large areas using the FluidFM BOT technology. Langmuir 35, no. 6: 2412-2421, 2019.

- Ungai-Salánki, Rita, Beatrix Peter, Tamás Gerecsei, Norbert Orgovan, Robert Horvath, and Bálint Szabó. A practical review on the measurement tools for cellular adhesion force. Advances in Colloid and Interface Science 269:309-333, 2019.

- Jankovics, Hajnalka, Boglarka Kovacs, Andras Saftics, Tamas Gerecsei, Éva Tóth, Inna Szekacs, Ferenc Vonderviszt, and Robert Horvath. Grating-coupled

interferometry reveals binding kinetics and affinities of Ni ions to genetically engineered protein layers. Scientific reports 10, no. 1:1-11 2020.