Cell-cell adhesion studied with Flex-FPM

Cells are the building blocks of life. Some thrive best individually, suspended, but most of them are integrated in a larger 3D matrix, like tissue. These cells interact with neighboring cells in the same tissue or those at the interface to adjacent, different, tissue. This can be a natural interface as between tendons and bones, or in the case of implants or biofilms, an artificial one. The forces governing cell-substrate and cell-cell interactions are important for their structure and function, and their quantification is of interest to better understand the mechanical strength of tissue and interfaces and related failures therein.

Flex-FPM - the standard tool for cell adhesion

AFM has been used extensively to study cell-substrate or cell-cell interactions at the single cell level [Helenius et al. (2008), Moreno-Encerrado et al. (2017)]. For this purpose cells are generally immobilized chemically to a cantilever. However, the chemical immobilization limits the maximum obtainable adhesion forces to a few hundred nanonewtons and it requires many cell experiments to obtain conclusive results. The limitation in force range is particularly troublesome when studying cells after prolonged incubation times (hours to days), where forces may exceed the micronewton range. This is particularly true when studying confluent layers of cells. Here cell-cell interaction contributes in addition to the substrate adhesion that is generally studied.

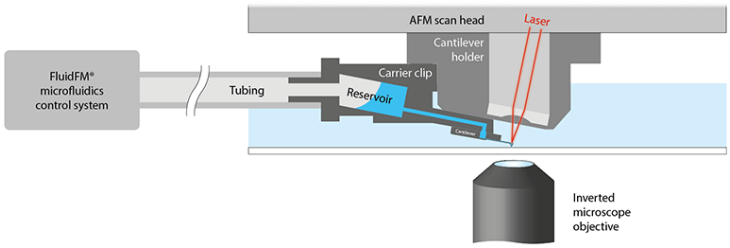

Fig. 1: Flex-FPM extending the FlexAFM functionality with FluidFM technology: Local sample manipulation using hollow cantilevers

Flex-FPM (Fig. 1) is a flexible tool overcoming these two main limitations. It was pioneered at the ETH Zurich in the groups of Prof. Julia Vorholt and Dr. Tomaso Zambelli. Using FluidFM™ technology the cell is attached to the cantilever via negative pressure through a channel inside the cantilever. Compared to chemical binding, much higher forces can be achieved within a few seconds, reaching into the low micronewton range [Potthoff et al. (2012); Potthoff et al. (2014)].

The binding via aspiration is not only strong and fast, but also reversible. Consequently, the same FluidFM™ probe can be used for multiple cells in a row. A magnificent number of over 200 different yeast cells were studied with a single cantilever in one day under different environmental conditions [Potthoff et al. (2012)]. This number cannot be reached for mammalian cells, but throughput is still higher than with chemical binding. Protocols have been established to clean the cantilever enzymatically with trypsin [Potthoff et al. (2014)] or chemically in a sodium hypochlorite solution [Jaatinen (2016)]. After cleaning, new cells can be aspired without need for a new coating step.

Cell-cell adhesion

Recently, FluidFM™ cell adhesion experiments were extended to study cell-cell interaction. This can be the force between a cell (on the cantilever) and a cell below on a substrate (fig. 2 A), but also between a cell and its surrounding cells in a confluent layer (fig. 2 B).

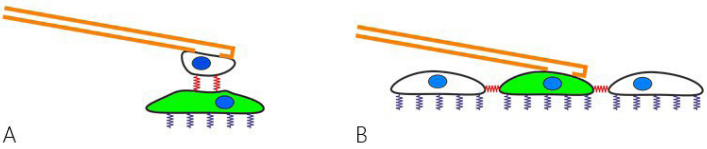

Fig. 2: Cell-cell interactions (red springs) studied by aspiration of single cells to a hollow FluidFM™ probe (orange). A) Probing the force between a cell immobilized on the cantilever and a cell on the substrate, B) picking a single cell from a confluent layer, probing cell-substrate (purple) and cell-cell (red) interactions.

Dr. Noa Cohen of Prof. Tanya Konry's group at Northeastern university in Boston studied cell-cell adhesion with a Flex-FPM system to gain more insight into tumor progression and metastasis [Cohen et al. (2017)].

Figure 3 shows an optical image of the method depicted in fig. 2 A that was used by Cohen for this study.

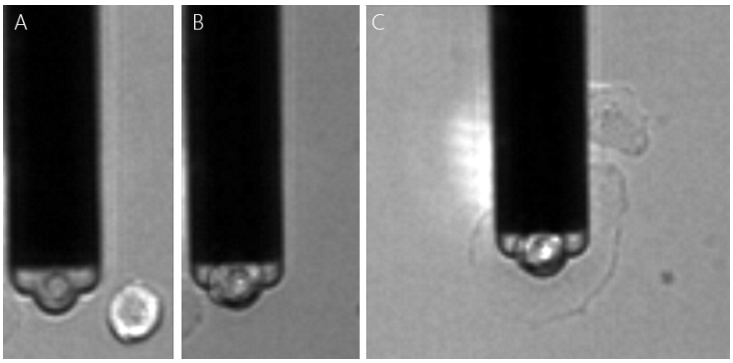

Fig. 3: Optical images showing A) a single cell to be picked up by a FluidFM probe B) the cell aspired to the cantilever and C) the FluidFM probe with aspired cell during a cell-cell adhesion measurement. Data courtesy of Tanya Konry group, Northeastern University, Boston, USA.

Interactions between single MCF7 breast cancer cells on the cantilever with different types of cells on the substrate were found to develop differently with incubation time. In these experiments, the reversible binding of cells allowed the different cell pairs to be studied with the same probe (fig. 4).

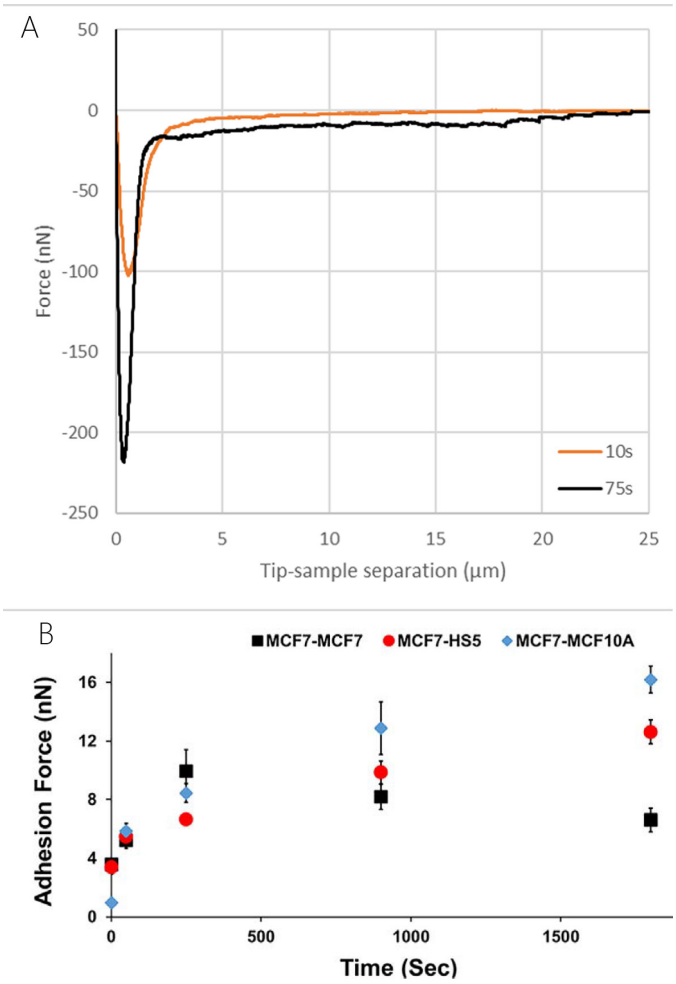

Fig. 4 A) Typical force spectra between a MCF7 cell aspired to the cantilever and a non-cancerous, fibroblast (HS5) on the substrate at different contact times. B) Development of the force with contact time between the cells. Data courtesy of Tanya Konry group, Northeastern University, Boston, USA.

Dr. Ana Sancho from the group Prof. Jürgen Groll's group at the University of Würzburg extensively studied the interaction between a cell and its neighbors in a confluent layer of cells (fig. 2 B) [Sancho et al. (2017)]. Fig. 5 shows the cantilever picking up a cell from a confluent layer (A) and the empty space from where the cell was removed (B).

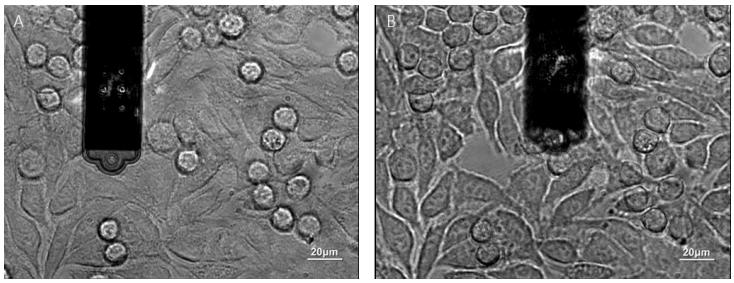

Fig. 5: Confluent layer of cells, where one is pulled out by FluidFM, adapted from: Sancho et al. (2017), Scientific Reports volume 7, 46152.

Human endothelial cells from the umbilical artery were found to exert strong intercellular force (figures 6 A & B) that could be decreased significantly by overexpression of Muscle Segment Homeobox 1, to induce endothelial-to-mesenchymal transition. This transition is a process involved in cardiovascular development and disease. Complementary to these adhesion experiments, the Flex-FPM system was also used to perform nano-indentation experiments using colloidal beads aspired to the cantilever.

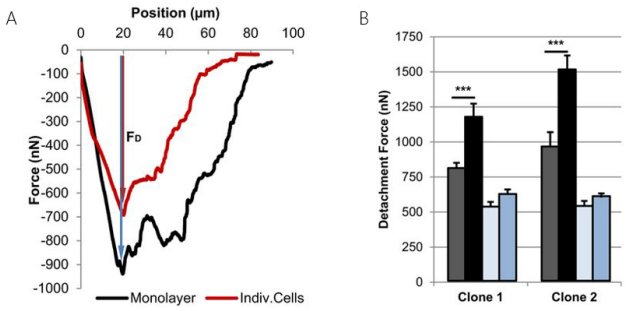

Fig. 6: A) Typical single cell force spectra of individual cells or cells in a confluent layer, depicting the increase in force by cell-cell interactions. B) Effect of MSX1 on the observed cell adhesion for individual cells and cells in a monolayer. Grey and black bars: control measurements on individual cells and monolayers, resp., pale and light blue MSX1 treated individual cells and cells in a monolayer, resp. Adapted from: Sancho et al. (2017), Scientific Reports volume 7, 46152.

Both examples strongly benefitted from FluidFM™ technology provided by the Flex-FPM solution. In case of the confluent layer the large forces of up to over 1.5µN eliminate chemical binding to study cell-cell adhesion. In both cases the reversible binding provided the experiments with the necessary speed-up to obtain sufficient statistics.

References

Jonne Helenius, Carl-Philipp Heisenberg, Hermann E. Gaub, Daniel J. Muller **Single-cell force spectroscopy** Journal of Cell Science 2008 121: 1785-1791; doi:10.1242/jcs.030999

Alberto Moreno-Cencerrado, Jagoba Iturri, Ilaria Pecorari, Maria D.M. Vivanco, Orfeo Sbaizero, José L. Toca, Herrera **Investigating cell-substrate and cell-cell interactions by means of single-cell-probe force spectroscopy** Microscopy Research & Technique 2017 80: 124-130; doi:10.1002/jemt.22706

Eva Potthoff, Orane Guillaume-Gentil, Dario Ossola, Jérôme Polesel-Maris, Salomé LeibundGut-Landmann, Tomaso Zambelli, Julia A. Vorholt **Rapid and Serial Quantification of Adhesion Forces of Yeast and Mammalian Cells** PLoS ONE 7(12): e52712; doi:10.1371/journal.pone.0052712

Eva Potthoff, Davide Franco, Valentina D'Alessandro, Christoph Starck, Volkmar Falk, Tomaso Zambelli, Julia A. Vorholt, Dimos Poulikakos, and Aldo Ferrari **Toward a Rational Design of Surface Textures Promoting Endothelialization** Nano Lett. 14, 2, 1069-1079; doi:10.1021/nl4047398

Leena Jaatinen **Quantifying the effect of electric current on cell adhesion studied by single-cell force spectroscopy** Biointerphases 11, 011004 (2016); doi:10.1116/1.4940214

Noa Cohen, Saheli Sarkar, Evangelia Hondroulis, Pooja Sabhachandan, Tania Konry **Quantification of intercellular adhesion forces measured by fluid force microscopy** Talanta Volume 174, 1 November 2017, Pages 409-413; doi:10.1016/j.talanta.2017.06.038

Ana Sancho, Ine Vandersmissen, Sander Craps, Aernout Luttun & Jürgen Groll **A new strategy to measure intercellular adhesion forces in mature cell-cell contacts** Scientific Reports volume 7, Article number: 46152 (2017); doi:10.1038/srep46152