Contractile Cell Forces on Rigid Substrates

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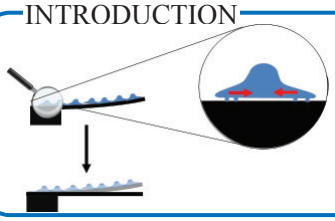
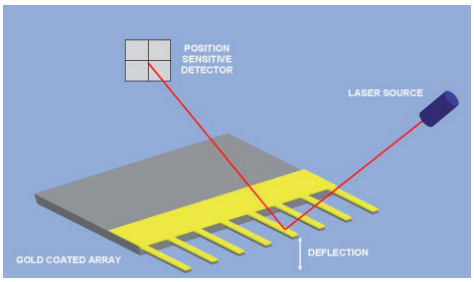
INTRODUCTION



The cell's organization and the maintenance of tissues depend on the complex interplay between cells and the surrounding extracellular matrix and substrate. In recent years biomechanical parameters have been evolved as crucial factors for the determination and characterization of cell fate, e.g. for the differentiation of stem cells or the malignant transformation in cancerogeneous cells. Here we present results from a novel approach to determine cell created forces on stiff substrates using nanomechanical cantilever sensors. The technology can quantify cell forces generated on different substrates and can thus be applied to characterize cell-materials interactions in biomedical applications, aiding e.g. the development of novel medical implant materials and discover and investigate basic cell characteristics.

CANTILEVER SENSORS

Nanomechanical cantilever sensors are defined as tiny plate-like structures, which are fixed at one end to a solid support. Machined from thin, and therefore flexible materials as silicon or polymeric materials they bend in reaction to contractile cell forces acting along their longitudinal axis. With dimensions of 500 µm x 100 µm x 1 µm, forces as small as 10 µN/m have been detected via the deflection of a laser beam focused at the apex of the cantilever structure.



Cantilever sensors have been successfully applied for the determination of forces created by conformational changes of proteins and nucleic acids as well as the forces created by the expansion of lipid membranes upon the insertion of biomolecules (see e.g. S. K. Vashist, Journal of Nanotechnology, June 2007; H.P. Lang, M. Hegner, Ch. Gerber, Materials Today 8, 30-36, 2005). Now we extend their application to the detection of forces created by whole cells.

Furthermore, the resonance frequency of loaded cantilevers which reacts to mass and stiffness changes at the sensor surface can be monitored. This potential can be used as another parameter to describe the cytoskeletal organization in attached cells/cell layers.

Arranged in an array cantilevers can be used as references and several experiments can be performed simultaneously.

CELL FORCE MEASUREMENTS

Cell force measurements applying microcantilever sensors allow for investigations on the influence of chemical and architectural variations of the cell substratum on the force generation of adherent cells. In our first experiments we investigated the forces of fibroblast cell (Rat2, ATCC 1764) growing on bare silicon cantilever surfaces.

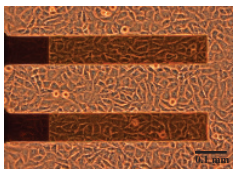
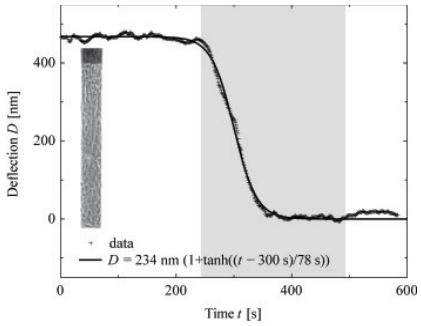


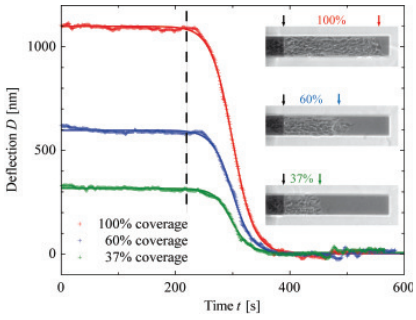
Figure: Phase contrast image of Rat2 fibroblasts growing on silicon cantilevers

Fibroblasts were seeded on silicon cantilevers in a tissue culture dish. The image, taken 20 h after cell seeding, shows Rat2 fibroblasts which attach to both the silicon cantilevers and the cell culture dish with comparable morphology.

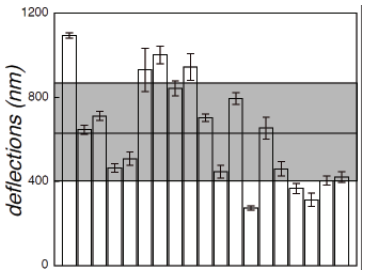
Cell force measurements were established using the Cantisens Research system (Concentris GmbH, Basel), a flexible cantilever sensor readout system with intergrated temperature control, which allows real time monitoring under constant liquid flow. In a typical experiment Rat2 fibroblasts were settled on silicon cantilevers followed by overnight incubation to allow cell attachment and contractile cell force generation, resulting in cantilever bending. Subsequently the cells are released from the cantilever and the concommitant cantilever relaxation is recorded. The graph below shows the force curve during such a cell release experiment.



The observed cell forces correlate well with the number of cells growing on the cantilever sensor (see figure below). Cantilever signals in the range of less than 10 nm can be detected which allows the assumption that, if required, forces generated by single cells can be detected with such sensors.



When we compared the cell force induced bending signals of several fully cell covered cantilevers we observed a considerable variation both within the cantilevers of an array and between sensor arrays (see below). This relates very much to reported cell force variations in single cell measurements and raises questions about the supracellular coordination of cytoskeletal networks as well as about the necessary number of cells to determine truly averaged contractile cell forces.



ABOUT CELL BIOMECHANICS

Cell biomechanics is about the mechanical parameters of cells and their interactions with their physiological environment. This involves a.o. the measurement and manipulation of cellular contractile forces, cell-cell and cell-substrate adhesion strengths or cell and susbstrate stiffness. The link between cell biomechanics and cell fate is highlighted by the following examples:

- The differentiation of stem cells into different cell types can be triggered by growth on matrices of different elasticity (Engler et al., Cell 126, 677-689, 2006)
- The elasticity of cancerogenous cells differs considerably from non-tranformed cells (Cross et al., Nature Nanotechnology, December 2007)
- Cell stretching induces the differentiation of muscle cells (Sadoshima and Izumo, EMBO 12(4): 1681–1692, 1993)
- Cell contraction, cell forces acting on the underlying substrate material depend on substrate stiffness; very limited data for cell forces on rigid substrates ->DICANS project

CONCLUSION AND ACKNOWLEDGEMENT

This communication presents an approach for the quantitative measurement of contractile cell forces on dedicated substrates with tailored functionalities. It can be used to realize biological or chemical sensors or to improve the surface biocompatibility of medical implants. Consequently it is of fundamental interest and important for different kinds of biomedical applications.

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