14th Swiss Conference on Biomaterials, May 8th, 2008, Basel Switzerland

Detection of the Forces and Modulation of Cell-Substrate Interactions

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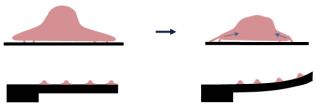
INTRODUCTION

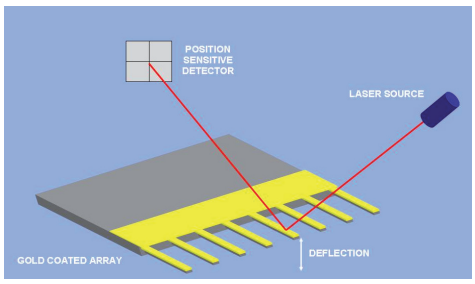


The cell's organization and the maintenance of tissues depend on the complex interplay between cells and the surrounding extracellular matrix and substrate. It is established by diffusible signalling molecules and direct mechanical interactions linking the intracellular cytoskeleton with the extracellular matrix or substrate hence creating a supra-cellular architectural framework. To detect and monitor changes in this framework, several methods have been developed ranging from the labelling of specific cytoskeletal components to the measurement of forces generated by individual cells. In this paper, we present the concept using nanomechanical cantilever sensors to quantify forces generated from the interplay of cells and cell layers with the supporting micrometer-thick substrate.

CANTILEVER SENSORS

The development of methods to determine cell forces date back to the early 80s. The idea of using deformable, elastic substrates to study cell forces was conceived by Harris et al. (A.K. Harris, P. Wild, D. Stopak (1980) Science 208:177-9). Substrate was a 1 µm-thin silicone rubber film. The cells caused the film to buckle. The consequent wrinkle field provided a visible pattern. The wrinkles in the substrate, however, were larger than the cells causing them.

Nanomechanical cantilever sensors are defined as tiny plate-like structures, which are fixed at the one end to the 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 x 100 x 1 μm^3, 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.



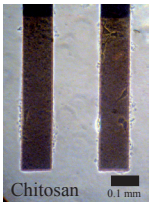
Cantilever sensors have been successfully applied for the determination of forces created by conformational changes of proteins and nucleic acids as well as the forces created by the expansion of lipid membranes upon the insertion of biomolecules (see e.g. S. K. Vashist, Journal of Nanotechnology, June 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 potentially can be used as another parameter to describe the cytoskeletal organization in attached cells/cell layers.

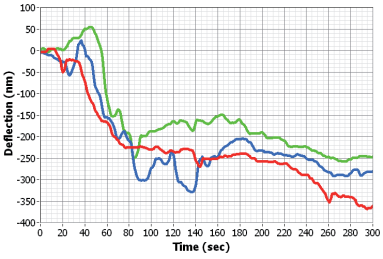
Arranged in an array cantilevers can be used as references and several experiments can be performed simultaneously.

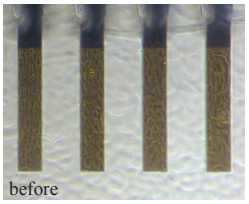
CELL FORCE MEASUREMENTS

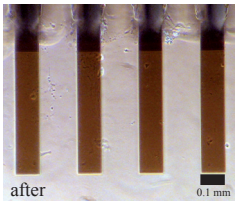
Cell force measurements applying microcantilever sensors allow for investigations on the influence of chemical and architectural variations of the cell substratum on the force generation of adherent cells. Today cantilever sensors are made from silicon and are covered with a metal layer, mostly titanium and gold. This offers the possibility to immobilize the cells on two different basic surfaces. In a first test experiment two different chemical surface modifications were tested for their suitability to support adhesion and growth of rat fibroblasts (Rat2, ATCC 1764): The gold surface of the sensor was coated with either an amino-terminated molecule (mercaptoethylamine MEA) or an amino-reactive crosslinker followed by the immobilization of chitosan. Afterwards the sensors were incubated with serum containing culture medium followed by addition of trypsin-released fibroblasts. As can be seen from the pictures below (taken 20 h after cell seeding) Rat2 fibroblasts preferentially attached to the MEA-coated sensor surface and show typial healthy morphology.



The Cantisens Research system (Concentris GmbH, Basel, Switzerland) is a flexible cantilever sensor readout system with intergrated temperature control, which allows real time monitoring under constant liquid flow. Its suitability for cell force measurments was investigated using cells growing on MEA-functinalized cantilevers. It has turned out that cells are sensitive to drying out during transfer of the cantilever array from the cell culture dish into the sensor readout system. Dense cell layers seemed to be slightly more robust during the transfer. The forces exerted onto the cantilevers during the treatment of such cell layers with trypsin/EDTA can be seen in the graph below.





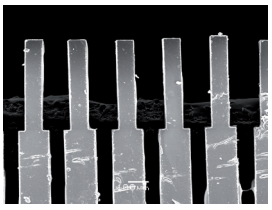


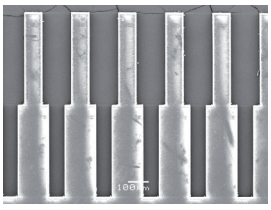
Trypsin/EDTA was injected at times 40 s untill times 260 s and a fast release of cellular forces on three different cantilevers was observed. Before and after the experiment the cantilever array was observed under the microscope to correlate the changes in surface force with the number and density of cells.

POLYMERIC CANTILEVERS

The acceptance of cantilever sensors as a standard tool in research crucially depends on their robustness, their ease of use, their reproducibility and finally their price.

Today microcantilevers are produced from silicon in a rather complex process. We are going to develop polymeric microcantilever arrays which can be produced at reduced cost by e.g. injection moulding. First results of such polymeric cantilever arrays realized with different methods are shown on the right. The cantilevers have typical dimensions of 100 µm x 500 µm and a thickness of 20 to 50 µm. In addition microstructures can be much easier realized on polymeric surfaces as compared to silicon. Microstructures of defined size and spacing are known to effect the colonization of substrates by cells and cantilever sensors offer the possibility to investigate the influence of such microstructures on cell attachment and organization.





CONCLUSION AND ACKNOWLEDGEMENT

This communication presents an approach for the quantitative measurement of contractile cell forces on dedicated substrates with tailored morphology and function. 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 applications.

The presented research activities belong to the project 'DICANS', a collaborative initiative between the BMC, PSI, FHNW and Concentris GmbH funded by the Swiss Nanoscience Institute located at the University of Basel.

We thank S. Nedunkanal and S. Granacher for their contribution towards the development of polymeric cantilever structures.

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