was performed. Correlated imaging and high resolution mapping allowed unprecedented insights into dynamic membrane structure and behaviour.

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Biophysics of the Hyaluronan-Rich Pericellular Coat

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The articular cartilage consists of a complex extracellular matrix (ECM), which is subject to a high mechanical loading. To counteract the ongoing abrasion, a specialized cell type is embedded within the ECM. These so called chondrocytes constantly renew the ECM. To live and even divide in such a mechanically challenging environment, chondrocytes are protected by a several micron thick pericellular coat (PCC). This coat is of vital biological importance for example in cell proliferation and migration, but also in diseases like osteoarthritis or with age.

The PCC consists mainly of water. Thus it remains invisible in all light microscopy techniques. Where the individual components and even their molecular interactions are well understood, much less is know about the mesoscopic structure of the PCC, which in turn is vital to understand force transductions.

In order to analyze the molecular architecture of the PCC, we established a new set of tools to measure and manipulate the PCC on living cells. The micromechanical profile was measured with position-sensitive passive microrheology. In contrast to other mechanical techniques applied to the PCC, it is independent of the adjacent cell body and enables the measurement in the z direction normal to the cell surface. The observed profiles show a decreasing viscoelasticity within the PCC correlating to a decreasing concentration of the polymer backbone (hyaluronan).

Further, we demonstrate that the PCC expression depends on the cell's interactions with the ECM. In order to precisely control adhesion, we employed nanostructured surfaces. Chondrocytes not only change their adhesion areas in respect to the ligand density, but also the size of their PCC. The modified PCC size could also be related to changes in its mechanical properties analyzed in ongoing experiments.

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Functional Mapping of Single Molecules and Gels using Atomic Force Microscopy

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We have used Atomic Force Microscopy (AFM) to map the spatial distribution of epitopes in the peptide core of mucin glycoconjugates, and also their glycosylation. Using AFM in a physiological environment, and tips functionalised with antibodies against MUC-gene specific epitopes in the peptide core, and also with lectins which recognise terminal sugars, we mapped both single molecules and complex macromolecular structures.

From the simultaneous topographic and force-spectroscopy data, the force-volume maps, we directly quantified the localization, number, and rupture force of recognition bonds on different epitopes of mucin molecules (Figure 1). The specificity of the interactions was confirmed using antibody-blocking peptides, and blocking sugars, and as expected, we observed a loading rate dependence of the unbinding strength.

We have extended these single molecule measurements to more complex physiological gel systems, such as the soft gel layer of the tear film. Binding experiments confirmed the relative abundance of two sugars, previously established by chemical analysis of purified mucins. This comprehensive approach to single molecules and their macromolecular assembly provides an insight into extracellular configuration and packaging of mucins in a gel, and points towards new applications of AFM force mapping.

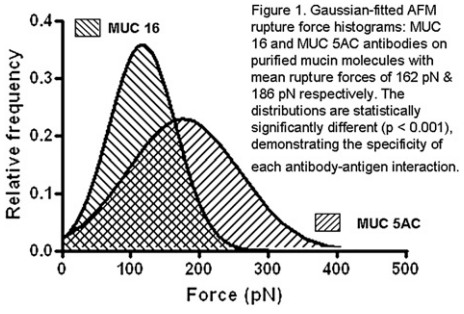

Figure 1. Gaussian-fitted AFM rupture force histograms: MUC 16 and MUC 5AC antibodies on purified mucin molecules with mean rupture forces of 162 pN & 186 pN respectively. The distributions are statistically significantly different ($p < 0.001$), demonstrating the specificity of each antibody-antigen interaction.

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Electroporetic Identification Of Cancer Cells By Afm And Fluorescence Techniques

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We carried out a comparative study of cancer cells and normal cells by AFM and found that there exists a sufficient electroporetic difference between them. We measured the population and size of the ''bulges'' on cell surface, possibly indicating a step directly preceding electroporation, and of the pores upon repeatedly applying an electric field (0 ~ 2 kV). We found that the bulge population of the cancer cell was much higher than that of the normal cell at low electric potentials (0 ~ 500 V), while the pore size of the cancer cell was much bigger by several times. This phenomenon is believed to be due to the elastic difference of the cell membrane, as is actually confirmed by our force measurement that the cancer cell was softer than the normal cell. We also carried out supplemental studies by fluorescence microscopy and electron microscopy, which also supported our AFM results.

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Optical trapping of coated microspheres

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In an optical trap, micron-sized dielectric particles are held by a tightly focused laser beam. The optical force on the particle is composed of an attractive gradient force and a destabilizing scattering force. We hypothesized that using anti-reflection-coated microspheres would reduce scattering and lead to stronger trapping. We found that homogeneous silica and polystyrene microspheres had a sharp maximum trap stiffness at a diameter of around 800 nm - the trapping laser wavelength in water - and that a silica coating on a polystyrene microsphere was a substantial improvement for larger diameters. In addition, we noticed that homogeneous spheres of a correct size demonstrated anti-reflective properties. Our results quantitatively agreed with Mie scattering calculations and serve as a proof of principle. We used a DNA stretching experiment to confirm the large linear range in detection and force of the coated microspheres and performed a high-force motor protein assay. These measurements show that the surfaces of the coated microspheres are compatible with biophysical assays.

Micro & Nanotechnology: Nanopores

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Urea-Induced Conformational Changes in dsDNA Probed by Solid-State Nanopores

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Urea is an uncharged, polar molecule used ubiquitously as a structural denaturant for proteins, a hydrogen bond destabilizer in nucleic acids, and to increase the resolution of similar DNA fragments in electrophoretic processes. Despite its widespread use, the precise effects of varying urea concentrations on dsDNA structure are unknown. Solid-state nanopores in the range 2.7 - 4.5 nm have recently been shown to accurately gauge the effective diameter of dsDNA in aqueous solutions[1]. This DNA sizing method involves measurement of the reduction of a high ionic-strength electrolyte current as DNA transverses the pore. In this study, we systematically vary urea concentrations in the electrolyte solution (0 - 8.6M) and measure its effects on the effective DNA cross-section. Using an 800 bp dsDNA fragment, our studies reveal that the mean DNA effective diameter increases by as much as 0.6 nm. Further, the effective DNA diameter as a function of urea concentration follows a sigmoidal trend, indicative of cooperative binding. This interpretation is also supported by the translocation dynamics of the DNA as a function of urea concentration. We will present our experimental results along with a simple model to explain these observations.

[1]Wanunu, M., Sutin, J., McNally, B., Chow, A. & Meller, A. (2008) DNA Translocation Governed by Interactions with Solid State Nanopores. Biophys J 95, in press.

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Atomic Scale Description of Ionic Behavior in Polymer Nanopores

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Polyethylene terephthalate (PET) is a carbon-based polymer commonly used in plastic containers. Due to its high melting point, mechanical strength, and