

Critical evaluation of the interpretation of AFM stiffness measurements on living cells

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Critical evaluation of the interpretation of AFM stiffness measurements on living cells

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Introduction

In blood vessels, shear stress is an important factor for the behavior of endothelial cells (ECs) covering the internal vessel wall. When ECs are subjected to varying shear stress, their mechanical properties change due to cytoskeletal actin fiber remodeling which influences the signaling function towards smooth muscle cells.

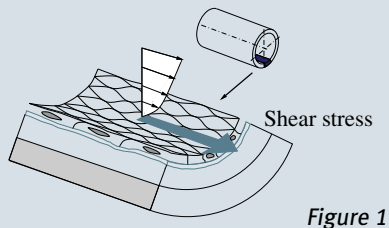


Figure 1

Objective

To investigate changes in mechanical properties of ECs subjected to a varying fluid shear stress and to correlate those changes to adaptation of the actin cytoskeleton.

Methods

As a first step, we investigated a model cell, i.e. a cardiac myoblast (H9c2), which has an abundant actin cytoskeleton comparable to that in ECs subjected to shear stress. Local mechanical properties of H9c2 cells were investigated with an Atomic Force Microscope (AFM) and correlated to the actin cytoskeleton, as visualized with confocal scanning microscope (CLSM).

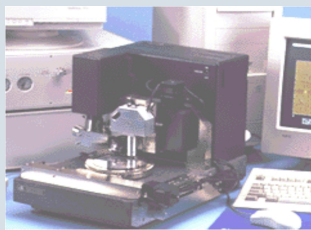
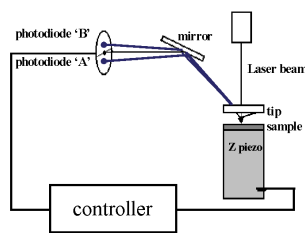


Figure 2



Hertz model

The Young's modulus E can be calculated using the linear Hertz model that gives the relationship between the indentation δ and the loading force:

$$F = \frac{3}{4} \tan(\alpha) \delta \frac{E}{(1 - \nu^2)} \delta$$

The first part of the equation accounts for the contact stiffness, the second part for the sample stiffness. When interested in cell stiffness, the contact stiffness should be kept constant, meaning that the indentation should be constant.

Results

The left figure below shows a combined AFM (top) and CLSM actin (bottom) image of a fixed cardiac myoblast. The right figure shows an elasticity map of an area on top of a living myoblast. Differences in elasticity may be caused by underlying cytoskeletal structures.

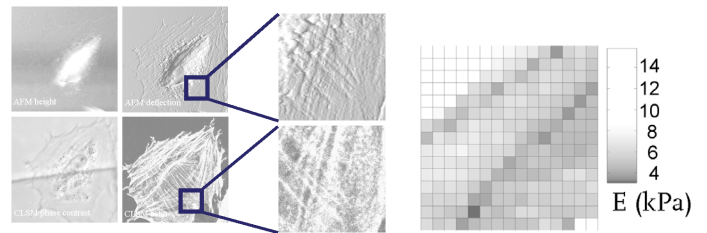


Figure 3

Fitting the force curves at different values of the force — which is often done in literature — or of the indentation depth results in different values of the Young's modulus.

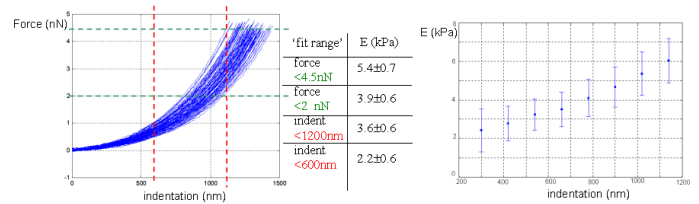
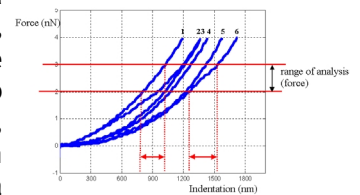


Figure 4

Results of 256 indentation measurements on an area of $5 \times 5 \mu\text{m}$ on top of a living myoblast $\uparrow \downarrow$

Besides stiffness also characteristic mechanical behavior can be measured with AFM. For example, force curve 1 and 2 (see opposite) are equal up to an indentation of 600nm, at higher indentation depths, curve 2 shows a more compliant behavior.

Figure 5



Conclusions

F-actin filaments of cardiac myoblasts can be visualized with CLSM and AFM. AFM indentation experiments yield information about stiffness and mechanical behavior. However, the interpretation of the data needs great care because of the non-linearity of cells and of the cell-AFM tip contact.

Future

To perform combined shearing and AFM indentation experiments on living ECs.