

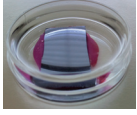
Determination of Cell Adhesion Forces and Cell Heights by Atomic Force Microscopy



Sebastian Geiser¹, Jochen Köser², and Bert Müller¹

¹Biomaterials Science Center, University of Basel, 4031 Basel, Switzerland
²University of Applied Sciences MuttENZ, Switzerland

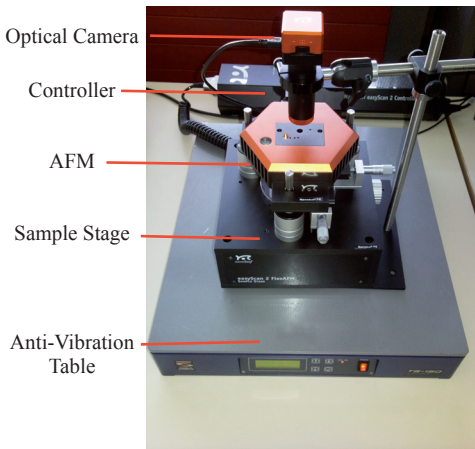
INTRODUCTION



For many dental implant materials cell adhesion is desired. It is the prerequisite for cell locomotion and other cell-relevant functions. Therefore, techniques to quantitatively assess cell adhesion forces have been developed. Rotating plates allow determining the mean attachment force over thousands of cells, while atomic force microscopy (AFM) measurements are suited for the local inspection of single cells. Nevertheless, dedicated data on cell adhesion forces on dental materials are rare. We aim to establish local and global methods for adhesion force measurements and to demonstrate the feasibility of these experimental approaches for cell-substrate combinations relevant for dentistry.

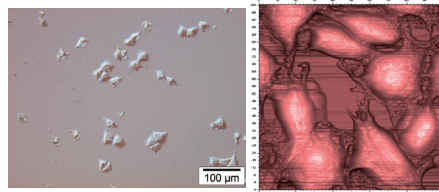
AFM SETUP

AFM measurements were performed with a Nanosurf Flex (Nanosurf, Switzerland).



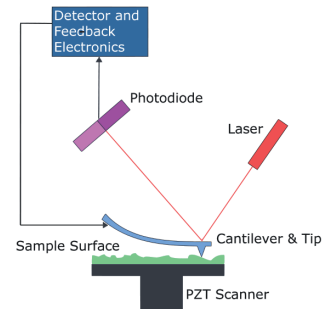
RAT2 CELLS

Rat2 cells were grown on monocrystalline silicon wafers in DMEM with 10% fetal bovine serum (FBS) as culture medium. To inspect the influence of cell density on the substrate, samples with different concentrations of rat2 cells were prepared. Previous to the measurements, DMEM was replaced with phosphate saline buffer (PBS). Measurements in DMEM were not feasible as the phenol red absorbs the red AFM laser light. Below, a light microscopy (left) and an AFM image of cells on silicon.



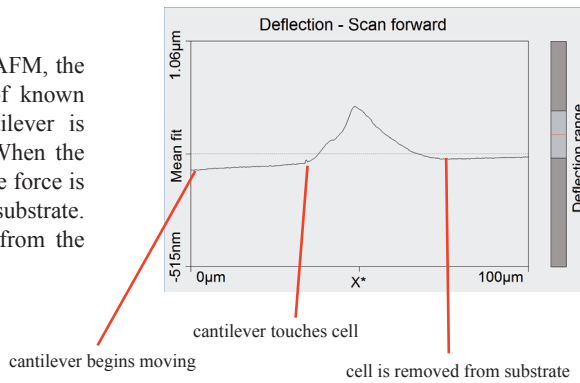
AFM

The AFM consists of a cantilever with a sharp tip (probe) at its end that is used to scan the specimen surface. The cantilever is typically silicon or silicon nitride with a tip radius of curvature on the order of nanometers. When the tip is brought into proximity of a sample surface, forces between the tip and the sample lead to a deflection of the cantilever. The deflection is measured using a laser spot reflected from the top surface of the cantilever.

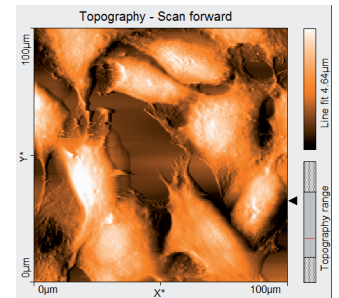


ADHESION FORCE MEASUREMENTS

In adhesion force experiments using AFM, the elastic properties of the cantilever of known spring constant are used. The cantilever is moved along the substrate surface. When the cantilever hits a cell, it bends until the force is sufficient to detach the cell from the substrate. The related force can be calculated from the degree of bending.



With this method, cell heights of 24 rat2 cells were measured. The average cell height amounts to $(3.38 \pm 0.45) \mu\text{m}$.



CONCLUSION AND ACKNOWLEDGEMENT

AFM is a suitable method to quantify cell adhesion force on individual cells on dental material substrates, and allows measuring cell heights with higher precision than conventional confocal microscopy.

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