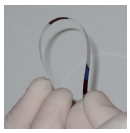
Contractile Forces of Myoblasts on Functionalized Micro-Plates

Raoul Enning[1,2], Alfredo Franco-Obregon[2], Janos Vörös[2], and Bert Müller[1,3]

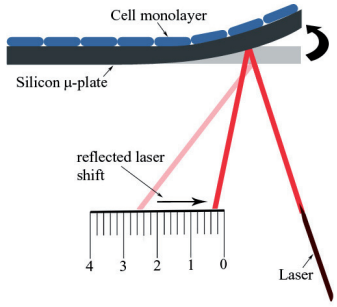
[1]*Computer Vision Laboratory, ETH Zürich, Switzerland*, [2]*Institute of Biomedical Engineering, ETH Zurich, Switzerland*,
[3]*Biomaterials Science Center, University of Basel, Switzerland*

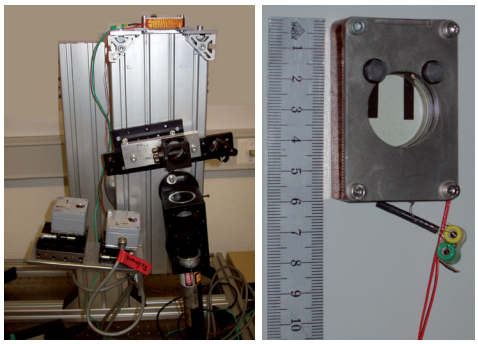
INTRODUCTION



Cells are capable of exerting mechanical forces on their surroundings. This important feature permits cell adhesion, motility and contraction; all are vital in the process of wound healing, tissue generation as well as muscle contraction. Adhering cells can strongly influence the shape on the surfaces they attach to, if the substrate is flexible enough. It has been seen experimentally that adhering cells can pull on elastic substrates through their focal adhesion sites. Methods have been developed for calculating these cellular forces. The crystalline silicon plate, the substrate of choice in this experiment, acquires the aforementioned elastic properties when it is manufactured quite thinly. Cantilever-like µ-plates (4 mm x 14 mm) are cut from a Si(001)-wafer only 30 µm thick.

µ-PLATE BENDING



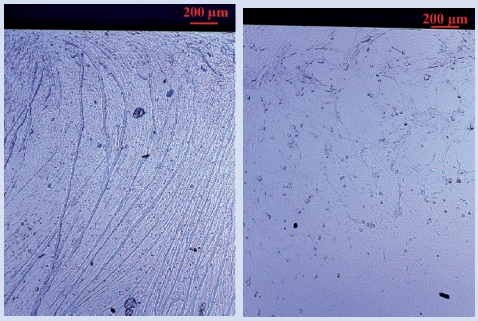


An approach that has much similarity with atomic force microscopy is the measurement principle for quantifying cellular forces. Laser light is reflected at the µ-plate and detected on the 2D position sensitive detector (PSD). As changes in cellular contraction or adhesion forces modify the curvature of the µ-plate, the reflection angle and thus the final resting position of the laser beam will change accordingly. This change in position is detectable and recordable allowing a quantification of the force using the Stoney Formula, a formula that governs the deforming effects of thin membranes attached to much thicker substrates of a different material.

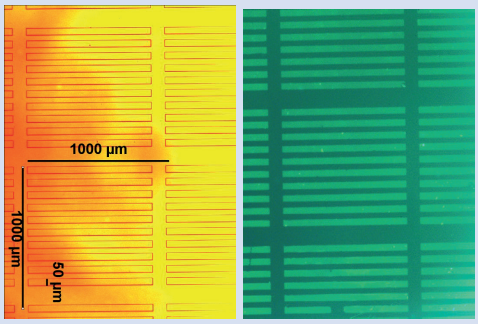
$$\sigma_f = \frac{M_s h_s^2}{6 \rho h_f}\left[\frac{(1+hm(4+6h+4h^2)+h^4 m^2)}{(1+h)}\right]$$

This formula gives the force exerted by the entire film (all cells). Thus, by counting and approximating the total amount of cells, an average force per cell can be found.

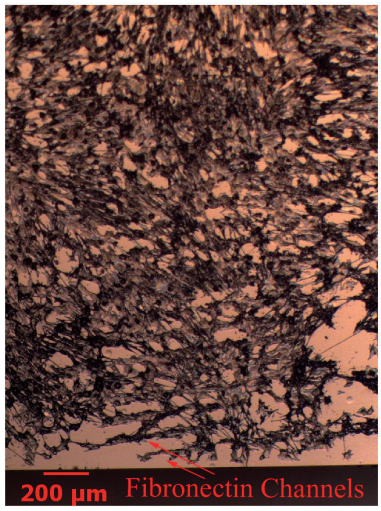
CELL ADHESION, CELL CONFLUENCE µ-CONTACT PRINTING



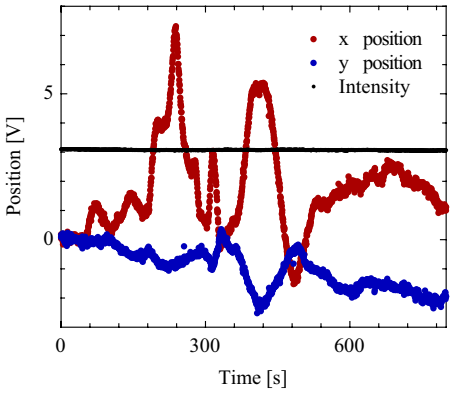
Cellular confluence over the length of one side of the µ-plates, a requirement that must be satisfied in order to apply the Stoney formula to this system, is difficult to fullfill. It was found that coating the µ-plates with the fibronectin glycoprotein solved this problem resulting in a full surface confluence at high cellular density. Simultaneously, a highly protein resistant polymer (PEG-*g*-PLL) was applied to the non-cellular side in order to reduce and repel cell adhesion. Each applied method worked extremely well. However, as seen in the picture above, the cells were growing laterally along the length of the µ-plates, and not in a longitudinal fashion that is desirable for measuring contraction forces. In an attempt to reduce cell proliferation in the lateral direction, the adhesion protein was "functionalized" or patterned as small groves along the length of the plate through a method known as µ-contact printing. The negative space of the print was covered by PEG-*g*-PLL.



CELL SHAPE AND CONTRACTION



The resulting zebra stripe of protein and polymer did not have the desired effect of controlling the direction of cell proliferation due to the channel width of the stripes being much too low; moreover, the cells simply bridged the PEG stripes, growing from one protein strip to another.



Nonetheless, the effects the cells exerted on the substrate after stimulation with caffeine were very apparent, and the forces per cell of (15 ± 5) µN and (12 ± 4) µN were observed on non-functionalized and functionalized plates, respectively.

CONCLUSION

The use of the cell adhesion protein fibronectin is a proper method to realize a uniform layer of cellular confluence. Similarly, the results also showed that the poly ethylene glycol polymer coated surface had little or no cell growth at all. The combination of the two allows functionalizing the µ-plates into long, narrow protein channels to work properly, as the silhouette of the print can be seen through the positioning of the cells in the picture. However, the channel width of the µ-contact print was rather too low. Many myotubes behaved in the exact opposite manner as intended, growing in a vector orthogonal to the long axis of the µ-plate. Repeating the experiment with an increased channel width of the µ-contact print should provide more appropriate results. The contractile forces of the myocytes are estimated to correspond to average values between 10 and 20 µN.