



# Cell Adhesion and Cell Detachment Forces on Micro-Nanopatterned Substrates



MAX-PLANCK-GESELLSCHAFT

J. Deeg<sup>1,2</sup>, I. Louban<sup>1,2</sup>, D. Aydin<sup>1,2</sup> and J.P. Spatz<sup>1,2</sup>

<sup>1</sup>Max-Planck-Institute for Metals Research, Dept. of New Materials & Biosystems Heisenbergstr. 3, D-70569 Stuttgart

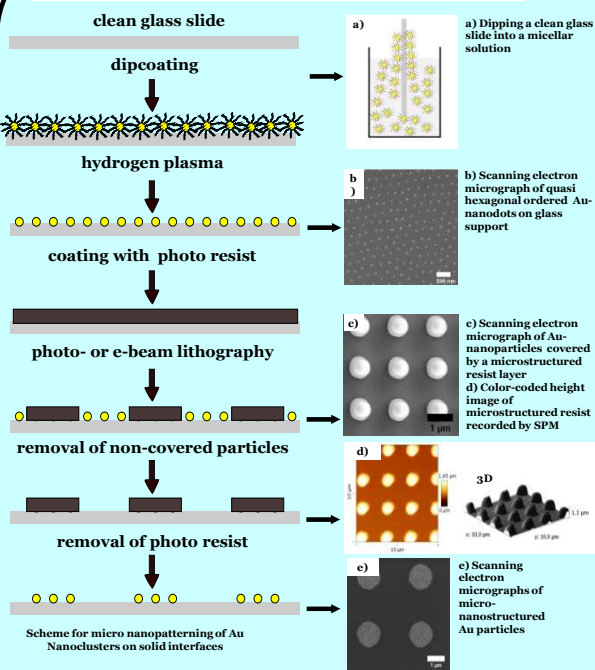
<sup>2</sup>University of Heidelberg, Dept. of Biophysical Chemistry

## Introduction

Many aspects of the intra- and extracellular organization of an organism are related to the adhesion of cells to their surrounding, the extracellular matrix. Cell adhesion is a complex, highly regulated process which plays an essential role in most fundamental cellular functions such as motility, differentiation, proliferation and apoptosis.

To investigate questions concerning cell adhesion surfaces with defined adhesion properties in terms of bioactivity are required. This work uses gold nanoparticles arranged in a quasi hexagonal pattern where each gold dot serves as a single anchor point for the cell's integrins, the receptors mediating the connection between cell and environment. On such surfaces a quantification of available binding sites is possible. Furthermore, a micro structuring of the gold particles allows for varying the global density of these integrin binding sites without changing the spacing between those. Such patterns can be used to investigate whether an effect is distance or density dependent. Former experiments have shown that adhesive interparticle distances of more than 73 nm reduce cell spreading as well as cell attachment and almost prevent the formation of focal adhesion and actin stress fibers<sup>1</sup>. To check if this finding is due to insufficient available binding sites or only to an existing critical binding site distance which may prevent clustering of adhesive sites, the quality of cell adhesion was investigated on different patterns by optical microscopy and Single Cell Force Spectroscopy (SCFS).

## Fabrication of micro-nanopatterns

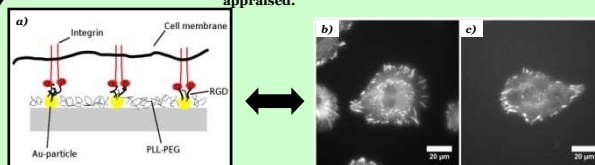


### Fabrication Procedure

The extended nanostructures of gold nanoparticles are produced by dipcoating based on the self assembly of diblock copolymer micelles<sup>2</sup>. Covering the surface with a polymer resist followed by photo or electron lithography leaves a microstructured pattern of covered and non-covered Au-particles. Removing the uncovered ones and a subsequent removal of the resist layer creates the desired micro-nanostructured pattern<sup>3</sup>.

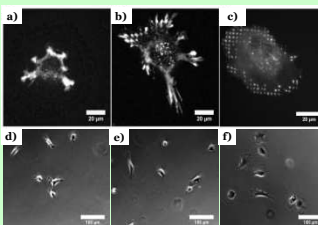
## Cell spreading

By means of spreading area (projected cell area) and circularity the quality of adhesion can be appraised.



a) Cell attachment to functionalized surfaces: The cell's integrins can only bind to the gold particles functionalized with RGD peptide whose strong affinity to integrin has been shown. The interspace is passivated with a highly protein resistant layer.

Fluorescence images of REF YFP pax 6h after seeding on a completely adhesive surface (b) and on a biofunctionalized nanostructured surface (c). The focal adhesions appear bright and are well developed on both surfaces. There is almost no difference between both surfaces.

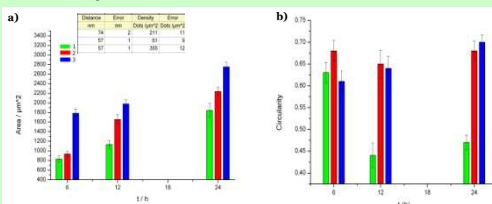


(a)-(c) Fluorescence microscopy images and (d)-(f) Phase contrast micrographs of REF YFP pax on micro-nano-structured surfaces after 6h, 12h and 24h. Such images were used for acquisition of binding sites is strongly reduced. On surface (1) the spacing of the binding sites is above a critical limit, while on surface (2) and (3) the spacing is below this limit.

### Cell spreading experiments:

Rat embryonic fibroblasts (REF 52 YFP pax) are plated and cultured on biofunctionalized nano- and micro-nanostructured substrates for 6, 12 and 24 hours, respectively. Cell spreading, their circularity and cell attachment is analyzed via phase contrast microscopy. Fluorescence microscopy visualizes the highly ordered focal adhesions (c) as a consequence of the provided micro-nanostructured binding sites. The figures below show spreading area and circularity of cells on three different structured surfaces (see table). Sample (1) and (3) are only nanostructured while (2) is micro-nanostructured and hence the number of binding sites is strongly reduced. On surface (1) the spacing of the binding sites is above a critical limit, while on surface (2) and (3) the spacing is below this limit.

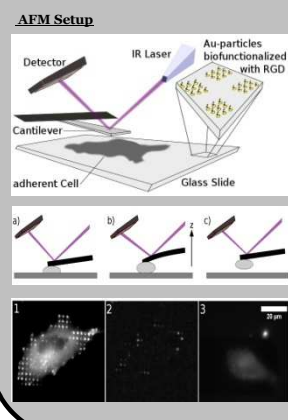
## Results (for interpretation see conclusion)



Spreading area (a) and circularity (b) of the cells after 6, 12 and 24 hours on three different structured surfaces

## Detachment forces determined by SCFS

Adhesion forces can be appraised by the detachment forces

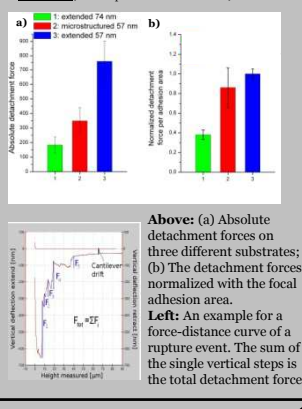


### Experimental Procedure

Cells are seeded on the functionalized surfaces 12 hrs prior to experiment. The cell is immobilized on the cantilever by a gentle approach (a). After a certain adhesion time which ensures a strong cell-cantilever connection (b), cell-surface adhesion forces are quantified by retracting the cantilever and rupturing the cell from the surface (c).

Simultaneous fluorescence microscopy ensures that the cell is detached from the surface

## Results (for interpretation see conclusion)



Above: (a) Absolute detachment forces on three different substrates; (b) The detachment forces normalized with the focal adhesion area.

Left: An example for a force-distance curve of a rupture event. The sum of the single vertical steps is the total detachment force.

## Conclusion and Interpretation

Specially arranged adhesive protein arrays were used to investigate cellular adhesion. The micro-nanostructured surfaces allowed for controlled variation of distance and density of specific proteins serving as binding sites. Two different characteristics to judge the quality of adhesion were analyzed, cell spreading and the strength of adhesion.

**Spreading Area:** The analysis reveals that the spreading area on surface (2) (red bar) is higher for all time points than on surface (1) (green bar), although the available binding sites are strongly reduced compared to surface 1 due to the microstructuring. It is lower than on surface (3) (blue bar) which has the same spacing but also remarkably more anchor points per area. **Cell spreading is reduced on surfaces with distances above a critical and still higher on surfaces with less binding sites but spaced below the critical distance.**

**Circularity:** The circularity is almost as high on surface (2) as on surface (3) for all time points. That means that only on surface (1) strong migration and hence filopodial structures are distinct, which is noticeable in a ragged and not pancake-like shape. Migration takes place when the underlying substrate does not fulfill the requirements for a successful attachment. **Circularity is strongly reduced when binding sites are arranged above the critical distance and is not when the number of binding sites is reduced but the distance kept below the critical.**

**Detachment Force:** The total detachment force on surface (2) is higher than on surface (1). This means that the distance seems to be more important for adhesion strength. But on the other hand the detachment force of surface (2) is notably smaller than on surface (3) which has the same spacing but is not microstructured meaning that the number of binding sites is significantly higher. Eventually, normalizing the force with the adhesive area shows that the value of surface (2) is now in the same range like the one on surface (3) and the value of surface (1) is significantly smaller. **The lack of binding sites results in less developed adhesion sites and therefore smaller absolute detachment forces. But the values of the forces related to the adhesion area evidently demonstrate that for the formation of stable adhesion clusters firstly the spacing is crucial.**

## References

- 1) Arnold M. et al. ChemPhysChem 2004
- 2) Glass R. et al. New J. Phys. 2004
- 3) Aydin D. et al. Small 2009

## Acknowledgement

University of Heidelberg & MPI for Metals Research, Dept. of New Materials and Biosystems, Julia Ranzinger, Jasmin Zahn, Dr. Babak Hosseini, Dr. Marco Schwider, Dr. Vera Hirschfeld Warneken

Horst Kessler, TU München, Benny Geiger, Wetzman Institute, Ulrich Schwarz & Thorsten Erdmann, BIOMS Heidelberg.