Nanoscopic Control of Cell-Adhesion

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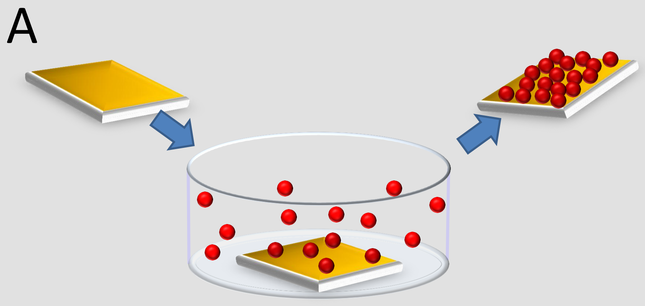
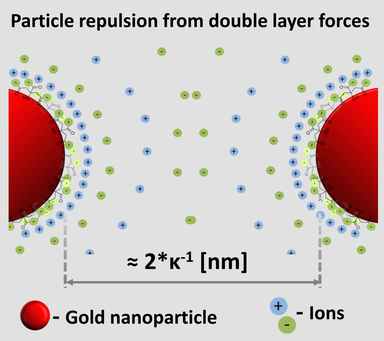
Introduction: Morphogenesis, wound healing and infection are all governed by cellular adhesion to supporting tissue. The aim of this research is to design nanopatterns that mimic natural tissue, organized at the protein level. Such patterns can be used to explore cellular adhesion mechanisms and to construct surface cues that control cell fate.

Self-assembly of nanopatterns

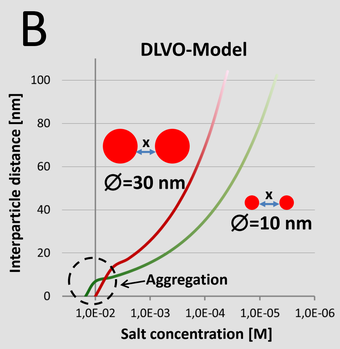
Nanopatterns were fabricated by simple self-assembly of protein-sized gold nanoparticles (5-50 nm) onto surfaces, e.g. gold substrates modified with dithiols that form a covalent bond with the nanoparticle (fig **A**).

A



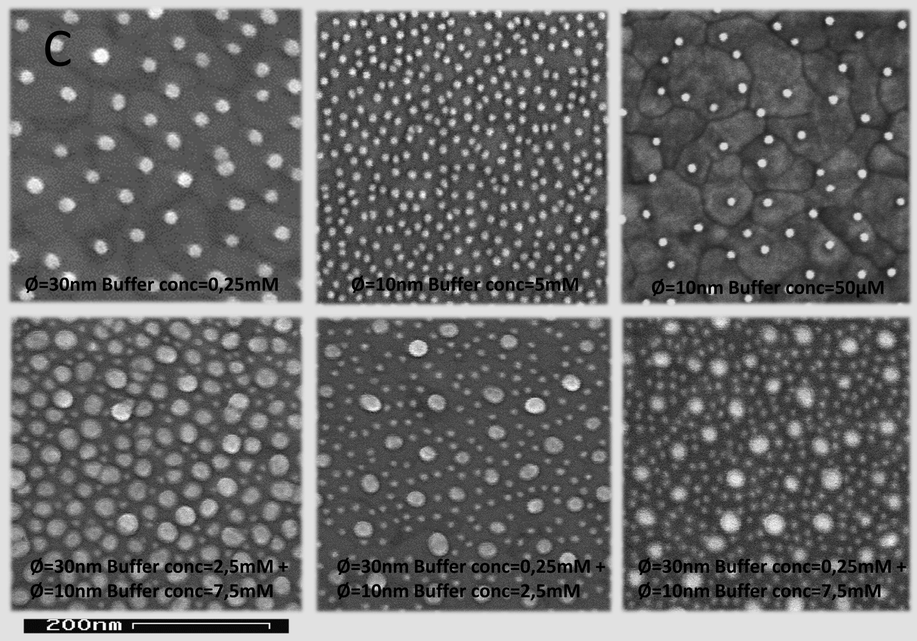


B



The particles have a negative surface charge, thus the distance between particles can be controlled by the type and amount of ions in the particles sol, in accordance with DLVO-theory (fig **B**). [1] Different patterns can be designed by variation of particle size, ionic strength and backfilling with smaller particles (fig **C**).

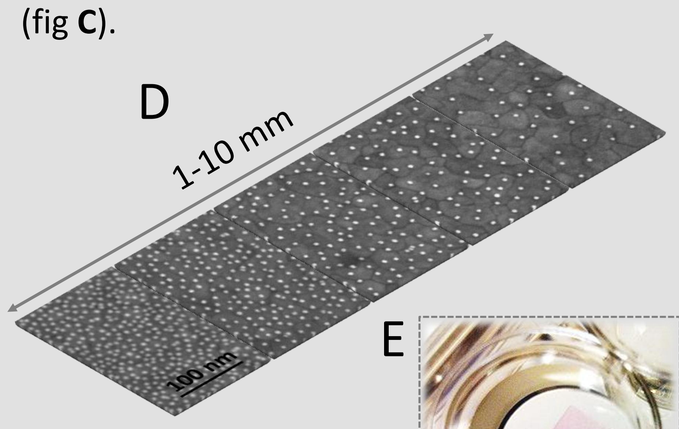
C



Nanoparticle gradients

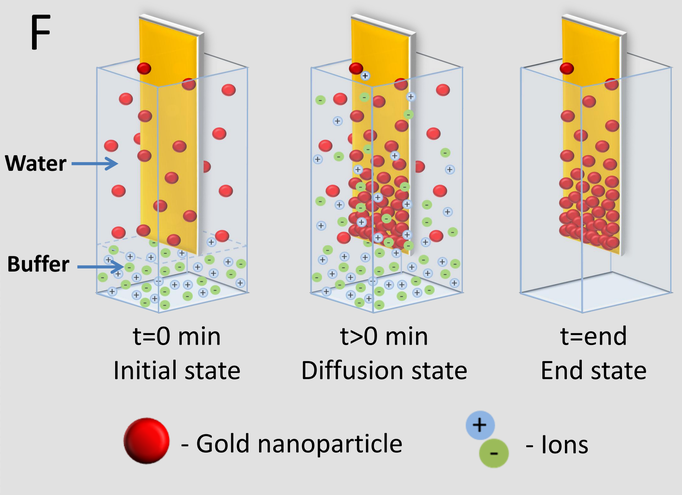
Surfaces were fabricated with a continuous gradient of nanoparticles [2] as shown by the montage of SEM micrographs (fig **D**). The photo (fig **E**) shows a particle gradient applied on a glass substrate. Gradient surfaces were made using a diffusion set-up (fig **F**). The particle gradient reflects the gradient of ions that appears in the chamber as buffer is diffused into the particle sol. Gradients with different steepness can be designed using Fick's law of diffusion (fig **G**).

D

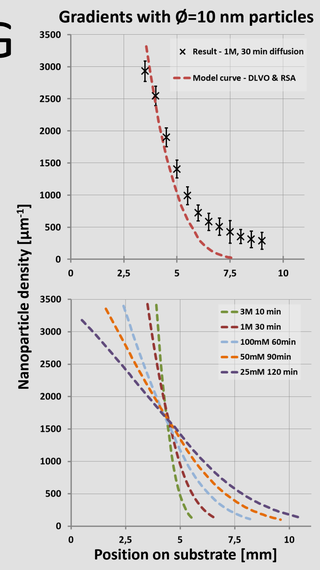


E

F



G

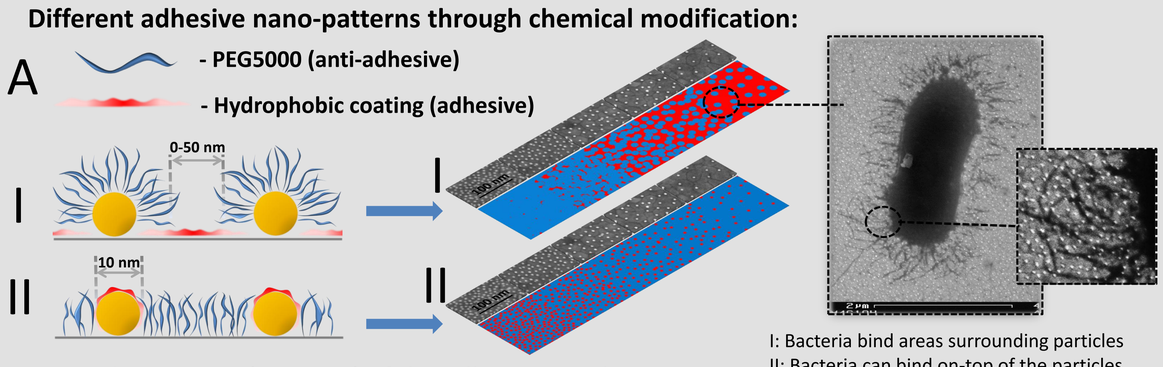


Fimbria-mediated adhesion of *E. coli*-bacteria

Fimbriae are very thin (5 nm), but relatively long (µm) proteinaceous extensions of many bacteria. For *E. coli*, fimbriae is a known virulence factor since they bind specifically to mannose at the surface of host cells, e.g. epithel. The involvement of fimbriae in colonization of material surfaces is however not well described.

Different adhesive nano-patterns through chemical modification:

A

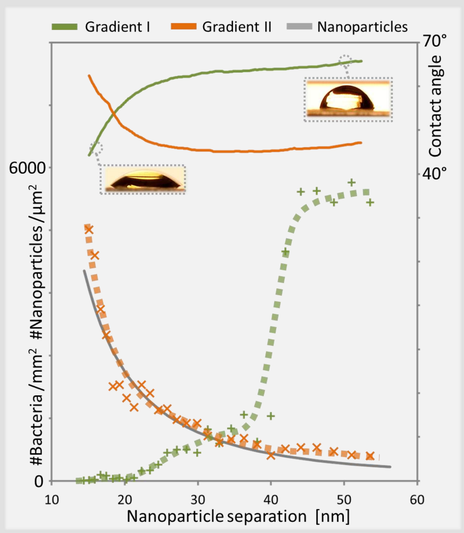


I: Bacteria bind areas surrounding particles
II: Bacteria can bind on-top of the particles
SEM-micrograph from gradient I.

I – Gradient in size (0-50 nm) of the adhesive patches
II – Gradient in number of adhesive 10 nm-patches

Two different adhesive nano patterns (fig **A**) were formed through chemical modification of nanoparticle gradients. [3] On gradient **I**, bacteria bound in three distinct levels (fig **B**): For large particle separation many but weakly bound bacteria were observed. For smaller particle separation, much lower binding was observed. The formed bonds were however stable towards flow pressure, suggesting that bacteria here mainly bind via fimbriae (fig **C**). [4] On gradient **II**, bacteria bound in direct relation to particle number, forming strong bonds. Together our results indicate a role for fimbriae in establishing efficient binding to surfaces, also when only small adhesive patches are available.

B



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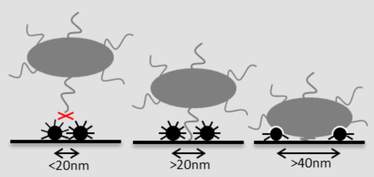
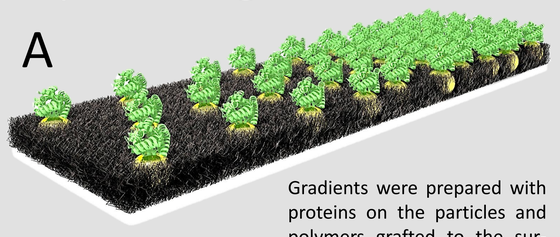


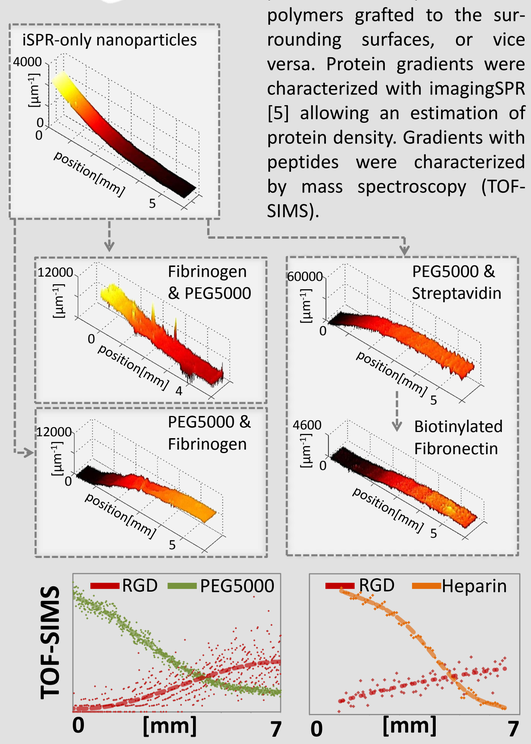
Extracellular matrix-inspired protein gradients

Surface patterns of ECM-proteins can support cell growth, differentiation and migration. Here, nanoparticle gradients were modified with matrix-proteins, cell-binding RGD-peptides and (bio)polymers (fig **A**). These surfaces allowed us to investigate adhesion, spreading and migration of epithelial cells in a dose-dependent manner. A gradient of Streptavidin was also prepared as a platform for further modifications.

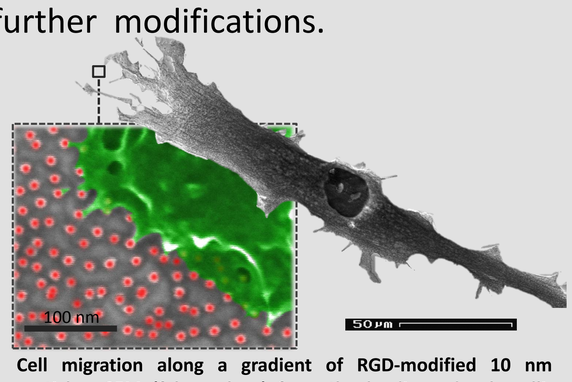
A



Gradients were prepared with proteins on the particles and polymers grafted to the surrounding surfaces, or vice versa. Protein gradients were characterized with imagingSPR [5] allowing an estimation of protein density. Gradients with peptides were characterized by mass spectroscopy (TOF-SIMS).

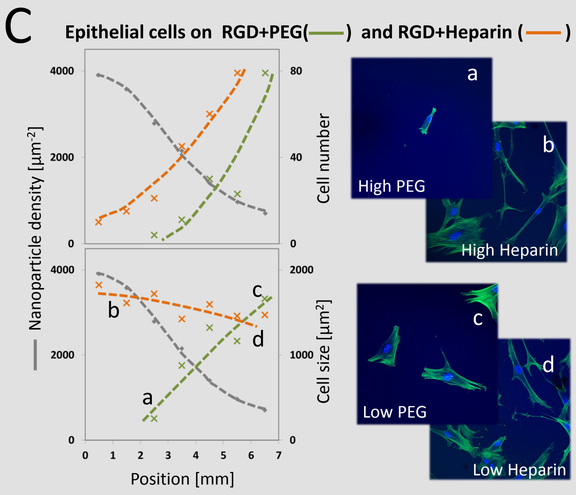


B



Cell migration along a gradient of RGD-modified 10 nm particles. SEM (false colour) from the leading edge lamellipodium, showing how the cell form adhesions to the particles.

C



Several patterns induced cell-migration (fig **B**) along the gradient, indicating that the cells responded to concentration differences as low as 100 binding points per µm^2. Co-grafting RGD-peptides with biologically active heparin, instead of inert PEG gave a general positive effect on cell spreading and cellular organization (fig **C**). This underlines the potential impact of multifunctional patterning for cell models.

[1] A. Lundgren et al (2008) *Nano Lett.* **8**:3989-92.
[2] A. Lundgren et al, *Swedish patent* SE1050866-7
[3] A. Lundgren et al (2011) *Angew. Chem. Int. Ed.* Ed.**50**: 3450-3453
[4] E. Sokurenko (2008) *Cell Host Microbe*. **4**: 314–323
[5] O. Andersson et al (2009) *Biomacromol.* **10**:142-48.

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Vetenskapsrådet