Microcontact printing of PLL-g-PEG and its derivatives

semester thesis

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Semester Thesis

Microcontact printing of PLL-g-PEG and its derivatives

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7. Literature
1. Introduction

Microcontact printing is a process that has been used for years. The technique works very well with some specific systems such as alkane thiolates on gold and siloxanes on hydroxyl terminated surface. Some less known systems are alkane thiolates on Cu, GaAs, and InP; alkane sulfinites and alkyl phosphines on Au; carboxylic, phosphoric and hydroxamic acids on metal oxides.

This work deals with microcontact printing of Poly-L-lysine-g-poly(ethylene glycol) (PLL-g-PEG) and its derivatives. Surfaces with adsorbed PLL-g-PEG are known to be highly protein resistant.

The aim of the work is to determine minimal features size that can be stamped, the stability of the molecules on the surface and the homogeneity of the produced patterns.

One possible use of this technique is the production of microarrays for use in the biomedical area. If it is possible for microcontact printing to create patterns where one can transfer well defined amounts of a certain substance on geometrically well defined places, it will be quite easy to do hundreds of tests automatically with one single testing plate. This technique is versatile and flexible in pattern size. The pattern size can easily be adapted to the desired conditions.

Within this semester thesis, different characterisation methods such as X-ray photoelectron spectroscopy, fluorescence microscopy and atomic force microscopy were used to investigate the stamped patterns.
2. Theory

2.1. Monolayers

Monolayers are thin films of molecules. The molecules can be either ordered or non-ordered.

One example of an ordered monolayer is the thiol/gold system in which the thiol-containing alkyl chain molecule is bound through the thiol group onto the gold surface, and Van-der-Waals interactions between the chains provide a structural order similar to that of a crystal unit cell. The molecules normally have two different end groups and a long hydrocarbon chain between as a spacer in the form of \( Y(CH_2)_nX \). The head group physisorbes or chemisorbes to the surface.

Silanes, phosphates and carboxyl acids on metal surfaces or alkanthiols on silver or copper are just a few know systems. [1, 2]

The PLL-g-PEG/TiO\(_2\) system is non-ordered. PLL-g-PEG is a grafted brush copolymer and the spaces between the PEG side chains are too large for an ordered system. The TiO\(_2\) surface is negatively charged at pH 7.4 (isoelectric point at around pH 4.5). The NH\(_3^+\) groups of the poly-l-lysine adsorb to the surface by electrostatic forces. The adsorption of PLL-g-PEG depends on the pH of the solution. [3]

2.2. Microcontact printing

Microcontact printing is a simple technique for producing well defined self assembled monolayers or patterns on different surfaces. There are different combinations of substrate and ink.

To produce a stamp, a liquid polymer is poured over a master made of a siliconwafer. After hardening, the stamp can be peeled of the wafer. In another step the stamp is incubated with solution. This step is used to transfer the molecules onto the stamp. It takes about 15 minutes until the system is in the equilibrium.

The following stamping is quite quick, taking only a few seconds. The stamping time depends on the “Ink-Surface-system”. For thiol/gold it takes just a few seconds and for PLL-g-PEG/TiO\(_2\) the stamp can be removed within seconds to minutes. This longer contact time could be an effect of the much longer polymer chains in PLL-g-PEG.

For several experiments it is important to cover the unstamped area of the substrate with another molecule. This is normally achieved by dipping the sample in solution for a defined time. During this backfill process the second molecules adsorb to the non-stamped areas.

The maximal resolution in soft lithography for micro contact printing is at ~30 nm and the minimum feature size is below 100 nm. [1, 2]

Figure 1: Microcontact printing
2.3. Fluorescence microscopy

Fluorescence is a term used to signify a compound's ability to absorb light at one wavelength and emit light at a higher wavelength. Fluorescent microscopy is used by scientists or clinicians to detect and localise minute amounts of substances, where the fluorescence is observed as luminosity against a non-fluorescent background.

The basics of fluorescent microscopy are quite simple. First, a fluorochrome has to be attached to the molecules or samples. A fluorochrome is a molecule that has the ability to absorb light of one wavelength and emitting or releasing the absorbed energy in a longer wavelength. [4]

In most fluorescent microscopes a dichoric mirror is being used to separate the excitation and emission light paths.

![Schematic FM microscope](image)

An innovation in the fluorescent microscopy field is the confocal microscope. Confocal microscopy works such that the final image has the same focus as the object or the focus corresponds to the point of focus in the object. The object and its image are confocal. The microscope is able to filter out the out-of-focus light from above and below the point of focus in the object. The confocal microscope eliminates this out-of-focus information by a confocal pinhole located in the front of the image plane, which acts as a spatial filter and allows only the in-focus light to be imaged.

This particular microscope can be used for three-dimensional reconstruction of samples, e.g. stained cells.
2.4. X-ray Photoelectron Spectroscopy (XPS)

X-rays cause emission of core electrons (photo emission) from the surface. The kinetic energy of these electrons depends on their electronic state in the atomic or molecular orbital. Since the state is unique for each element, XPS provides information about the chemical composition of the sample surface. It is not only quantitative but also provides information about the state of the electron (direct neighbour atom, e.g. C-C, C=O, etc.). Even information of binding relations can be observed. This information is in the shape and the energy shift of the peak.

We can describe the kinetic energy as:

\[ E_{\text{kin}} = h \nu - E_B - \Phi \]

- \( E_{\text{kin}} \): kinetic energy of emitted electron
- \( h \nu \): energy of the stimulating X-ray photon
- \( E_B \): binding energy of the electrons before photoionisation
- \( \Phi \): work function of the spectrometer

The information-content of an XPS-spectrum is very surface-sensitive, meaning about 5-10 nanometers deep. This method is particularly used to examine the chemical composition of thin films on substrates.
2.5. Atomic force microscopy (AFM)

The atomic force microscope allows taking direct topographical images of surfaces. The technique is based on the interaction forces between surface and a small tip. There are no specific demands on the sample such as electroconductivity or a special sample environment such as ultrahigh vacuum. The resolution exceeds SEM or optical interferometer. The sample is fixed on a piezo scanner, which moves the sample in the x-y-plane with a high lateral resolution (subangstroms). The z-component is measured with the cantilever. The deformation of the cantilever is proportional to the force between the tip and the surface and is registered by a reflected laser beam.

There are different working modes:

- Constant force: the cantilever is moved to have every time the same forces between cantilever and sample.
- Constant height: the cantilever is fixed in the z-axis.
- Non-contact-mode (Tapping mode): This method is based on frequency measurements.
- Friction mode: the sample is moved in x-direction.
2.6. Proteins

Proteins are biopolymers built up of 20 different amino acids with L-configuration. If there are more than 100 amino acids, it can be considered a protein. Below 100 amino acids, biopolymers are called peptides. In comparison to the other biopolymers (polysaccharides and nucleic acids) proteins show a large variety of biological functions.

The side chains of the proteins can be hydrophobic or hydrophilic, positively, negatively or neutrally charged. The side chains possess important properties, as they are able to bind ligands and to catalyse biochemical reactions. Therefore, the side chains play a major role in the adsorption of protein to metallic and organic surfaces.

In general, when a material is in contact with blood or connective tissue, proteins immediately adsorb to form a thin layer of 2–5 nm. This primary adsorption is unspecific and based on Van-der-Waals and electrostatic forces. [6] The adsorption of these biomolecules is the first step in the interaction of an implant in the human body. Many factors determine protein adsorption on a surface, such as chemistry, topography, texture, wettability and electrostatic charges. [3]

In a second state cells can produce ligand bonds with the surface proteins such as fibronectin and vitronectin (wound healing), which are connected with integrin to actin filaments in the inside of the cell membrane. This process is necessary for a cell to adhere to a surface, meaning if a surface is protein resistant, it is in most cases also cell resistant. [7,8]
3. Materials and Standard Procedures

3.1. Stamps

All stamps were made of polydimethylsiloxane (PDMS, Sylgard 184, Dow Corning, Wiesbaden). Firstly the two components silicone and hardener were mixed 10 : 1 (volume/volume) for 10 minutes. To remove the bubbles from the still liquid PMDS-mixture it was kept in the vacuum for about a half an hour. After this step the solution was poured over the master made of silicon. Again the mixture was put in the vacuum to remove the bubbles and then heated up to 70°C for at least 72 hours. The last step was to remove the PDMS from the master and cut it into sized pieces.

3.2. Substrates

- Silicon wafers 20x20 mm and 10x10 mm coated with 12 nm TiO₂ (PSI, Villigen)
  These substrates were used for XPS experiments and AFM measurements

- Commercial float glass ca. 15x15x1.5 mm
  These substrates were used for most of the experiments with fluorescent measurements.

- Microscopic cover glass 10x10x0.1 mm
  Used for special stamping experiments with stamps from the Prof. Dr. M. Bastmeier, Konstanz

- Tissue culture polystyrene (TCPS), gamma sterilised, tissue culture-treated
  (TPP, Switzerland)
  Used for cell experiments.

3.3. PLL-PEG

Poly-(L-lysine)-g-poly(ethylene glycol) (PLL-g-PEG) consists of a poly-(L-lysine) (PLL) (20kDa) backbone, which is at a physiological pH (7.4) highly cationic and interacts with the negatively charged surface of any metaloxide by electrostatic forces due to the amino groups (NH₃⁺).

The polyethyleneglycol (PEG) (2kDa) side chain is grafted with a ratio of 3.5. [3]

The PEG side chain is believed to drastically reduce protein adsorption by steric repulsion and excluded volume effects. [9]

The radius of gyration \( R_g \) of the PEG side chains is estimated...
using an empirical equation based on static light scattering measurements. This provides radii of gyration of about 1.65 and 2.82 nm for PEG of mol wt 2000 and 5000. [10]

3.4. PLL-g-PEG derivatives

PLL-g-PEG-fluorescein fluorescein terminated 5 kDa PLL-g-PEG. This molecule was used for all the fluorescence measurements.

PLL-g-PEG-RGD RGD terminated 3.4 kDa PEG side chains. RGD is a amino acid sequence that is necessary for vitronectin to adsorb on a surface. This modification was used for cell experiments.

3.5. Solutions

Buffer solution (HEPES Z1): 10 mM HEPES (4-(2-hydroxyethyl-piperazine-1-ethane-sulfonic acid, Fluka) in ultrapure water adjusted to pH 7.4 with NaOH

PLL-g-PEG solution: 1 mg/ml PLL-g-PEG in HEPES Z1

3.6. Cleaning protocols

Stamps:
- soak in distilled hexane for 1 hour
- ultrasonicate for 15 minutes (3 times)
- dry at air

Surfaces:
- rinse with ethanol
- dry with nitrogen
- clean in O2-plasma (plasma cleaner, sterilizer PDC-32G, Harrich) for 2 minutes

HEPES Z1:
- filtered with a 0.2 microns filter

3.7. Stamping

The cleaned stamps were incubated for at least 15 minutes with the referring solution. Just before stamping the solution was removed and the surface dried with nitrogen until there was no moisture visible anymore. The PDMS was put to the surface with one edge and then dropped onto the sample. If there was no wetting visible, it was necessary to press very softly with the tweezers on the PDMS until the stamp clearly was in contact with the sample. 15 seconds after the wetting the stamp was removed and the same immediately rinsed with ultra pure water for several seconds. The final step was drying with a nitrogen stream.
3.8. Soaking with PLL-g-PEG

To obtain a full layer of PLL-g-PEG, the sample was immersed for 15 minutes in PLL-g-PEG solution. After removal, it was rinsed for several seconds with ultrapure water and finally dried in a nitrogen stream.

3.9. Backfill

The already stamped sample was kept for 15 minutes in PLL-g-PEG (or its derivatives) solution and after removal rinsed with ultrapure water for several seconds, then finally dried under a stream of nitrogen.

3.10. Storage

The solutions were kept in the fridge at 4°C. Fluorescein containing samples and solutions were wrapped up in aluminium foil to protect of light, which is necessary to reduce the bleaching of the fluorescein groups.

3.11. Cells

For the cell test, Human Foreskin Fibroblasts (HFF), 7500 per well were used in 10% serum-containing medium.
4. Results and Discussion

4.1. XPS-Experiments

All experiments were done with the SAGE 100 (Specs, Berlin, Germany) with nonmonochromatic Mg-K\(_\alpha\)-Rays at 240 W. The take-off-angle was 90° between the sample plane and the X-ray gun.

The overview spectra were scanned from 0 to 1100 eV at a detector energy of 50 eV.

The detailed spectra were scanned at a detector energy of 14 eV and a resolution of 0.1 eV.

All XPS-results are given in atom %.

Clean reference sample

The cleaned sample is used as a reference. The amount of carbon is form aduaiticious hydrocarbons on the surface. This is nearly impossible to prevent, since in air there are always some hydrocarbons that immediately upon cleaning stick to a high energy surface such as TiO₂.

<table>
<thead>
<tr>
<th>Chem. element</th>
<th>sample 1</th>
<th>sample 2</th>
<th>average</th>
</tr>
</thead>
<tbody>
<tr>
<td>C</td>
<td>7.7</td>
<td>11.1</td>
<td>9.4</td>
</tr>
<tr>
<td>O</td>
<td>69.1</td>
<td>65.9</td>
<td>67.5</td>
</tr>
<tr>
<td>Ti</td>
<td>23.2</td>
<td>23.0</td>
<td>23.1</td>
</tr>
</tbody>
</table>

Soaked PLL-g-PEG sample

The samples soaked with PLL-g-PEG show good reproducibility. Only the value of the nitrogen content differs about 20 %, but this may be an artefact of the small content.

<table>
<thead>
<tr>
<th>Chem. element</th>
<th>sample 1</th>
<th>sample 2</th>
<th>average</th>
</tr>
</thead>
<tbody>
<tr>
<td>C</td>
<td>27.3</td>
<td>27.2</td>
<td>27.3</td>
</tr>
<tr>
<td>N</td>
<td>3.6</td>
<td>2.9</td>
<td>3.3</td>
</tr>
<tr>
<td>O</td>
<td>54.4</td>
<td>54.4</td>
<td>54.4</td>
</tr>
<tr>
<td>Ti</td>
<td>14.7</td>
<td>15.5</td>
<td>15.1</td>
</tr>
</tbody>
</table>

Stamped PLL-g-PEG sample

Stamped PLL-g-PEG with non-structured stamps shows a large difference in the silicone contamination. This is probably caused by different hardening times of the PDMS and different times between cleaning with hexane and stamping. The carbon content is about 80% of the soaked value. Therefore we
conclude that a submonolayer of PLL-g-PEG has been transferred with microcontact printing.

Table 3: Si-Wafer, TiO$_2$ 12nm; stamped PLL-g-PEG

<table>
<thead>
<tr>
<th>Chem. element</th>
<th>sample 1</th>
<th>sample 2</th>
<th>sample 3</th>
<th>sample 4</th>
<th>sample 5</th>
<th>average</th>
</tr>
</thead>
<tbody>
<tr>
<td>C</td>
<td>23.1</td>
<td>22.8</td>
<td>21.3</td>
<td>23.7</td>
<td>23.1</td>
<td>22.8</td>
</tr>
<tr>
<td>N</td>
<td>1.1</td>
<td>0.3</td>
<td>0.3</td>
<td>1.3</td>
<td>1.1</td>
<td>0.8</td>
</tr>
<tr>
<td>O</td>
<td>56.5</td>
<td>55.0</td>
<td>56.2</td>
<td>57.1</td>
<td>57.8</td>
<td>56.5</td>
</tr>
<tr>
<td>Si</td>
<td>4.1</td>
<td>7.9</td>
<td>7.0</td>
<td>0.9</td>
<td>2.0</td>
<td>4.4</td>
</tr>
<tr>
<td>Ti</td>
<td>15.2</td>
<td>14.0</td>
<td>15.3</td>
<td>16.9</td>
<td>16.0</td>
<td>15.5</td>
</tr>
</tbody>
</table>

Soaked PLL-g-PEG fluorescein sample

The samples soaked in PLL-g-PEG fluorescein solutions indicate a much higher content of carbon related to the PLL-g-PEG soaked samples. This must be an effect of the much longer side chains of the fluorescein terminated PEG. The values are more scattered than in the PLL-g-PEG soaked samples. An explanation may be that the fluorescein terminated PLL-g-PEG does not produce a single monolayer, even if there is sufficient time and concentration. This may be caused by the longer PEG side chains, repelling adsorption and hinder the PLL-g-PEG to transfer to the metal oxide or glass substrate.

Table 4: Si-Wafer, TiO$_2$ 12nm; soaked in PLL-g-PEG fluorescein

<table>
<thead>
<tr>
<th>Chem. element</th>
<th>sample 1</th>
<th>sample 2</th>
<th>average</th>
</tr>
</thead>
<tbody>
<tr>
<td>C</td>
<td>46.8</td>
<td>52.0</td>
<td>49.4</td>
</tr>
<tr>
<td>N</td>
<td>1.8</td>
<td>2.4</td>
<td>2.1</td>
</tr>
<tr>
<td>O</td>
<td>44.8</td>
<td>40.3</td>
<td>42.6</td>
</tr>
<tr>
<td>Ti</td>
<td>6.7</td>
<td>5.3</td>
<td>6</td>
</tr>
</tbody>
</table>

Stamped PLL-g-PEG fluorescein sample

Stamped PLL-g-PEG fluorescein samples with non-structured stamps show only 30 % of the carbon content than the soaked samples. The larger molecules of PLL-g-PEG fluorescein indicate a lower transfer ratio in comparison to the PLL-g-PEG molecules. This may be proof: that the longer side-chains do not only sterically hinder the proteins to adsorb, but also the PLL backbone itself from adsorbing on the surface.

Table 5: Si-Wafer, TiO$_2$ 12nm; stamped PLL-g-PEG fluorescein

<table>
<thead>
<tr>
<th>Chem. element</th>
<th>sample 1</th>
<th>sample 2</th>
<th>average</th>
</tr>
</thead>
<tbody>
<tr>
<td>C</td>
<td>16.6</td>
<td>13.9</td>
<td>15.3</td>
</tr>
<tr>
<td>N</td>
<td>0.7</td>
<td>1.2</td>
<td>1.0</td>
</tr>
<tr>
<td>O</td>
<td>61.8</td>
<td>62.8</td>
<td>62.3</td>
</tr>
<tr>
<td>Si</td>
<td>1.7</td>
<td>2.4</td>
<td>2.1</td>
</tr>
<tr>
<td>Ti</td>
<td>19.2</td>
<td>19.6</td>
<td>19.4</td>
</tr>
</tbody>
</table>
4.2. Fluorescence Experiments

The fluorescence measurements were done with the LSM 510 Zeiss scanning confocal microscope. All samples were stamped on glass.

Experiments without backfill

The first experiments were carried out without backfill. The aim was to determine the minimal pattern size. These experiments demonstrate the reproducibility of this technique very well. Firstly the fluorescent intensities of the patterns showed only 5\% deviation. And secondly the patterns were homogeneous over a wide range, which means over several mm in each direction.

The intensity ratio between the signal and the background is 35 : 1 or better. This is independent on the pattern size and stamping area size.

The smallest pattern size was achieved with a stamp from the group of Prof. Martin Bastmeier, University Konstanz. This stamp contains patterns from 3\(\mu\)m squares down to 300 nm squares with different lateral dimensions. There were various problems with this stamp: the small stamping area of 4x5mm and the small feature height of several hundred nanometers was one of them. Another problem was the relatively bad wetting behaviour of the stamp, such that a high pressure was necessary to achieve stamped patterns.

The smallest patterns we could obtain were 800 nm squares (see figure 7). Even those squares were not very well sized or could not be determined accordingly.

The smallest line stamps that were available were about 2-3 \(\mu\)m wide and 6 mm long. These lines show the homogeneity of stamping with this technique. Also the edges of the patterns are well defined and the non-stamped areas
showed nearly no fluorescence, which meaning that contamination with PLL-g-PEG-fl* of these regions is minimal.
The thinnest lines observed were about 2 μm in width (see figure 8).

![Figure 7: PLL-g-PEG – fl* without backfill (Bastmeiser stamp); pattern size 800 nm](image)

![Figure 8: PLL-g-PEG-fl* stamped without backfill; lines 2-3 μm wide](image)

**Stability of monolayer**

To procure some information about the stability of the monolayer, a sample was stamped with PLL-g-PEG-fl* and the intensity measured with a confocal
fluorescence microscope. For the 12 following days the sample was conserved in HEPES. After the removal and drying under a stream of nitrogen the fluorescence intensity was measured again with the fluorescence microscope. The patterns were still well defined and there was no difference visible in the clearness and resolution of the patterns. The intensity of the smallest patterns (5x5µm squares) showed a loss of intensity of 34% and the bigger lines (10x100µm) showed a loss of 17%. There are probably several effects. The main effect will by the bleaching of the fluorescein during the handling by experiment and the fluorescence measurement. Another reason may be due to some loss of polymer molecules by rinsing and handling the sample after the 12 days again with ultrapure water. Another effect will be some desorption of PLL-g-PEG-fl* during the 12 days.

**Experiments with backfill**

In order to examine the stability and compactness of the stamped pattern, experiments were carried out with a backfill of different PLL-g-PEGs. By stamping PLL-g-PEG and backfilling of PLL-g-PEG-fl* the ratio between the fluorescence intensities of the stamped area and backfilling is 0.8. This may be an effect of exchange of the stamped and backfill polymers. Another aspect may be that a stamped layer is not a full layer. This was also shown in the XPS experiments. There are still areas left, where PLL-g-PEG-fl* polymer molecules can adsorb.

The reverse experiment was done by stamping PLL-g-PEG-fl* with a PLL-g-PEG backfill. The intensity ratio between PLL-g-PEG-fl* and PLL-g-PEG backfill is much higher, by arbitrary value about 25. This may be because the additional adsorbed PLL-g-PEG on the patterns is not visible in the fluorescence microscope. There is also an exchange of the two molecules, which is seen in the loss of intensity of about 40% in relation to the samples without a backfill. Therefore we cannot obtain a surface that is structured with two different molecules in a manner such that there is only one specific molecule on a specific area.

![Figure 9:](image)

*Figure 9:* left: PLL-g-PEG stamped and PLL-g-PEG-fl* backfill (with bleached area)  
right: PLL-g-PEG-fl* stamped and PLL-g-PEG backfill
4.3. Cell Experiments

The PLL-g-PEG is known to drastically reduce protein adsorption [9]. Some experiments were carried out to test whether this works well with stamped PLL-g-PEG patterns.

Samples for cell studies were prepared according to the stamping protocol mentioned above using PLL-g-PEG-RGD (the side chains are RGD terminated) with an RGD content of 1, 20 and 100 %. A PLL-g-PEG backfill step using standard dip coating and a subsequent HEPES Z1 wash were applied to the sample before use for cell studies. Stamping was performed on glass slides as well as directly in the wells of tissue culture polystyrene 24-well plates. Cell experiments were performed using human foreskin fibroblasts (HFF) kept under standard cell culture conditions during the incubation, performed according to standard protocols, phase-contrast microscopy was used to monitor cell behaviour.

The first experiments were done with PLL-g-PEG-RGD 100% and a backfill of PLL-g-PEG. The PLL-g-PEG-RGD was stamped a non-patterned square of about 4x4mm on TCPS and normal glass slides. On the glass slides samples, no defined areas where cells did adhere. The HFF did not adhere very well to the surface. On the TCPS samples, the stamped areas were clearly defined (see figure 10, left). Even after 21 days the cells still did not adhere on the PLL-g-PEG. These experiments were also done with different concentrations of the RGD terminate groups. The result with the lowest concentration of 1% showed the same result as the highest conc. about 100% RGD.

The experiments with patterned samples did not work as well. Some structures could be seen, but not as well defined as the patterns itself were.

Figure 9:  left:  PLL-g-PEG-RGD 100% stamped, PLL-g-PEG backfill, cell adsorption experiment, imaged after 20 days, one corner of the stamped area is visible.

right:  PLL-g-PEG-RGD 100% stamped, PLL-g-PEG backfill, cell adsorption experiment, imaged after 21 days

On the TCPS samples, the stamped areas were clearly defined (see figure 10, left). Even after 21 days the cells still did not adhere on the PLL-g-PEG. These experiments were also done with different concentrations of the RGD terminate groups. The result with the lowest concentration of 1% showed the same result as the highest conc. about 100% RGD.

The experiments with patterned samples did not work as well. Some structures could be seen, but not as well defined as the patterns itself were.
The cell experiments were not entirely reproducible. The reason may be in the PLL-g-PEG, because there were two different synthesised PLL-g-PEG used and the experiments worked only with one. To prove that the cell adsorption is caused by the RGD group, we conducted another experiment. First the stamp was incubated once with ultrapure water and once with HEPES Z1 and normally stamped with a PLL-g-PEG backfill to see whether the PDMS contamination is sufficient to adhere cells. This experiment showed that the contamination of the PDMS is not sufficient for cell adhesion. Another experiment with stamped PLL-g-PEG and PLL-g-PEG backfill also proved that the adsorption was caused by the RGD groups and not by some other effects.

Figure 10: left: PLL-g-PEG-RGD 20% PLL-g-PEG-fl* pattern stamped and PLL-g-PEG backfill, cell adsorption experiment, photographed after 3 days
right: PLL-g-PEG-fl* stamped, same pattern as in left picture, fluorescence microscope picture
4.4. AFM-Experiments

In comparison of the cell studies, the measurements with the AFM were direct proof of the patterned surfaces. The samples used were TiO$_2$ coated Si-Wafers with stamped PLL-g-PEG. The patterns are stamped with a stamp from A. Goessel. [11]

The patterns were as expected from the fluorescence measurements well defined and homogeneous. The edges were sharp, meaning less than 0.5 µm in width.

The AFM-images showed that the stamped area was made of polymer clusters with a size of about 125 nm, which means about 3-5 polymer molecules. This is different to a full layer of PLL-g-PEG adsorbed out of a solution. That showed that the adsorption mechanism is different. By the adsorption in the stamping process the polymer molecules adsorbed probably during the inking time to the PDMS and while the stamp is on the substrate the molecules adsorb at the substrate.

Figure 11: AFM images, PLL-g-PEG stamped on TiO$_2$; range: a) 50 nm, b)25 nm, c)75 nm, d)25 nm
5. Conclusions and Outlook

PDMS contamination

On every stamped surface a certain degree of PDMS contamination was always obvious. It was not possible to prevent this, since there was always some unreacted PDMS monomers or short fragments in the stamp. During the stamping process, these PDMS molecules were transferred to the substrate. One possibility for improvement may be to change the type of the polymer. Another point will be shorter contact times while stamping. Both measures may help but it is likely that there will always be some contamination transferred.

Resolution

The limiting factor of stamping PLL-g-PEG with PDMS is the clustering of the PLL-g-PEG molecules. These clusters are about 125 nm in width (about 3-5 molecules) and they are statistically spread on the substrate. Therefore the minimal pattern size that can realistically be achieved with this system and optimally designed stamp is around 0.5 μm. A further aspect is the precision of the mechanical movement by hand. In this respect and for this dimensional range a mechanical stamp handling system would be helpful.

Homogeneous pattern

As the AFM and the fluorescence measurements showed, the stamped patterns are quite homogenous. This and the high reproducibility are basic features making the technique interesting for industrial applications. The quality of the pattern is independent of the pattern size in a wide range, i.e. from micrometers to millimetres.

Cell tests

Based on the few simple cell experiments, performed within this thesis, we can prove that the stamping of a polymer molecule such as PLL-g-PEG-RGD allows one to produce cell-adhesive areas, followed by backfilling with unmodified PLL-g-PEG thus making the background non-adhesive. Contamination of PDMS did not disturb the cell experiments. These experiments also proved that living cells react very sensitively to small differences, such as minor chemical changes in the PLL-g-PEG quality.
### Outlook

- **Mechanical aided stamping**

  It would be very helpful to have mechanical support in the stamping process. This could be achieved by using an aligner or an automated system with pressure control.

- **Other polymers**

  A different polymer without continuous monomer diffusion would probably help to reduce the contamination on the stamped surfaces. D. Trimbach, block copolymers, called Kraton, currently evaluates. [12]

- **Smaller patterns**

  For smaller patterns down to 500 nm it will be necessary to have well defined and designed (relations between height and width of the structures) stamps for the stamping of PLL-g-PEG.

- **Cell tests**

  It will be necessary to continue experiments to find out why and how the cells adhere on the stamped patterns.

- **Different chemical functions of the stamped patterns**

  For biomedical use it will be interesting to have a different chemical function on every single spot of the well-defined pattern for the use in microarray-type of sensing experiments.

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6. Literature


[12] Personal communication with D. Trimbach