Nanometer Size Protein Patterning using nCP for the Investigation of Protein-Protein Interactions in Live Cells

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Microcontact printing (e.g. [1]) (µCP) is a well-known method for depositing proteins or antibodies in a well-defined pattern. These patterns can be used e.g. to investigate protein-protein interactions in live cells[2]. Usually µCP is performed using polydimethylsiloxane (PDMS), a relatively soft stamp material. The disadvantage of PDMS is that there are some restrictions on the structure design and achievable resolution due to the deformation (sagging and pairing) of the small features[3]. Using µCP protein patterns in the µm range can be achieved. Further reduction of the patterns size would be highly interesting to specifically probe cellular subregions, or to study smaller cells. In this work we used nanocontact printing (nCP) to create protein patterns in the nanometer range. During nCP hard stamps (made from e.g. Ormocomp from microresist technology GmbH) were used instead of PDMS in order to create features with much smaller sizes[4]. We used this method to print and immobilize bovine serum albumin (BSA) on epoxy modified glass slides. For our experiments we have chosen two different Si master one with recessed pyramids with a quadratic cross section and a distance of 6 µm between the individual pyramids and another one with 300 nm line width and a distance of 4 µm between the lines. These masters were replicated using the UV-curable Ormocomp material. The stamps were replicated on a PVC foil and supported by a compliant layer made of PDMS to achieve a conformal contact[5]. In order to get a good wettability of the stamps they were treated with O_2 plasma. Afterwards a BSA solution in phosphate buffered saline (PBS) was spin coated and the nCP process was performed using EVG®620. The nCP experiments were carried out with BSA solutions in PBS with concentrations between 10 mg/ml and 0.066 mg/ml. Also the contact time was varied from 1200 s to 10 s. By printing 10 mg/ml BSA using a contact time of 1200 s dots with diameters between 1.5 µm and 3 µm were created (figure 1). Since experiments have shown that the contact time seems not to have a big influence on the feature sizes we decided to use a contact time of 10 s for further experiments. We were able to get a reduction of the feature sizes from 1 µm dots for a concentration of 1 mg/ml BSA in PBS down to 180 nm by reducing the concentration of the BSA to 0.066 mg/ml (figure 2 and 3). We were also able to imprint BSA lines with a line width of about 300 nm (figure 4). Since the usual feature sizes that can be achieved with µCP using PDMS stamps are in the µm range, nCP is a promising method to fabricate protein arrays with features down to 180 nm or even below. In the upcoming months we will try to print nanometer size streptavidin patterns in order to immobilize biotinylated proteins or antibodies for the investigation of protein-protein interactions in live cells.

We acknowledge funding from the Austian Nanoinovative in the NILmaterials project.

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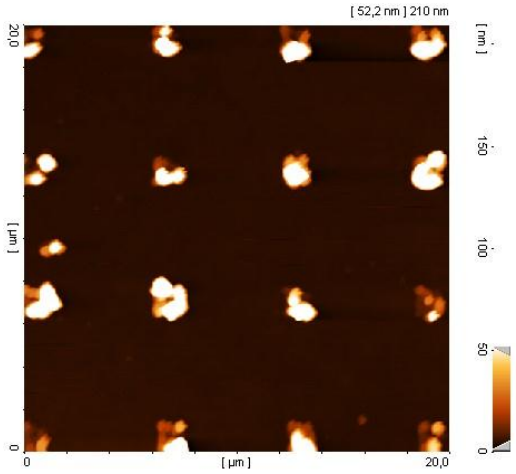

Figure 1. AFM image of BSA (10mg/ml) dots fabricated with a pyramids stamp and a contact time of 20 min with diameter between 1.5µm and 3 µm

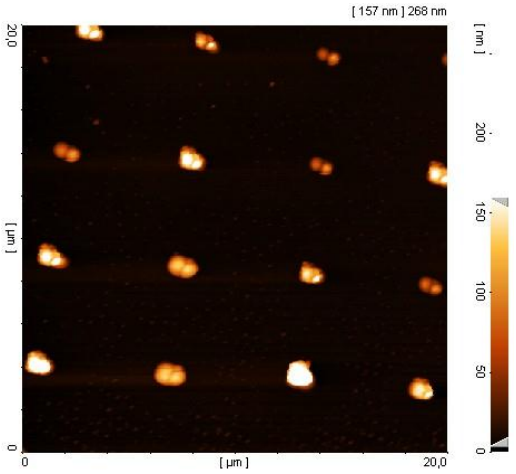

Figure 2. AFM image of BSA (1mg/ml) dots fabricated with a pyramids stamp and a contact time of 10s with diameter around 1 µm

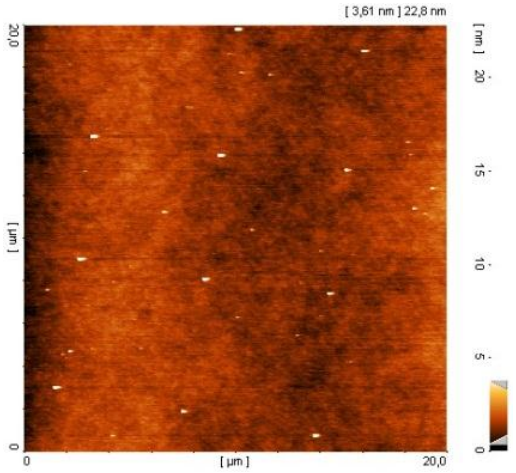

Figure 3. AFM image of BSA (0.066mg/ml) dots fabricated with a pyramids stamp and a contact time of 10s with diameter of about 180nm

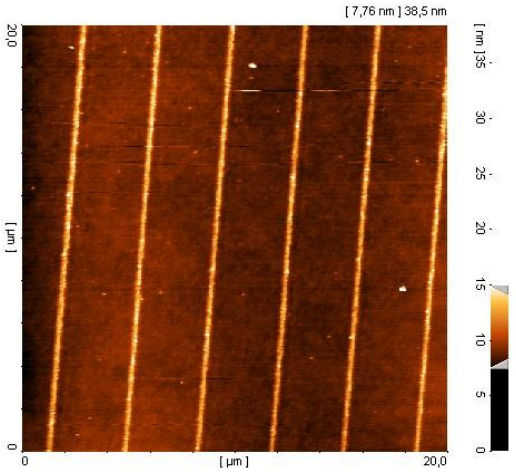

Figure 4. AFM image of lines of BSA (2,5mg/ml) fabricated with a lines stamp and a contact time of 60 s with a width of 300 nm