Design and development of nanoimprint-enabled structures for molecular motor devices

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Abstract

Devices based on molecular motor-driven cytoskeletal filaments, e.g., actin filaments, have been developed both for biosensing and biocomputational applications. Commonly, these devices require nanoscaled tracks for guidance of the actin filaments which has limited the patterning technique to electron beam lithography. Thus, large scale systems become intractable to fabricate at a high throughput within a reasonable time-frame. We have studied the possibility to fabricate molecular motor-based devices using the high throughput, high resolution technique of nanoimprint lithography. Molecular motor-based devices require wide open regions (loading zones) to allow filaments to land for later propulsion into the nanoscale tracks. Such open zones are challenging to fabricate using nanoimprint lithography due to the large amount of material displaced in the process. We found that this challenge can be overcome by introducing nanoscaled pillars inside the loading zones, into which material can be displaced during imprint. By optimising the resist thickness, we were able to decrease the amount of material displaced without suffering from insufficient filling of the stamp. Furthermore, simulations suggest that the shape and positioning of the pillars can be used to tailor the overall cytoskeletal filament transportation direction and behaviour. This is a potentially promising design feature for future applications that however, requires further optimisations before experimental realisation.

1. Introduction

Molecular motors are a class of proteins that govern the directed and active transport in eukaryotic cells. These motor-systems can be isolated from the cell and used in so called in vitro motility assays [1–4], where motors, adsorbed onto a surface, propel the corresponding cytoskeletal filaments on a chip, in a highly energy-efficient manner (~25 kT/10 nm) [5–7] compared to e.g., nanofluidics. Such molecular motor systems on a chip in nanotechnological applications require directed movement along chemically [8, 9] and/or physically [10–15] defined tracks, fabricated by lithography. The unidirectional movement is strongly dependent on the persistence length of the cytoskeletal filaments [8, 11, 15, 16], e.g., the actin-myosin II motor system of muscle [17–19] requires very narrow tracks <300 nm [8, 11] to prevent U-turns because of the rather high flexibility of actin filaments compared to e.g., microtubules. On the other hand, the velocity of myosin II propelled actin filaments is faster than of kinesin propelled microtubules.

Nanotechnological device applications of molecular motors includes e.g., biocomputation [7] where mathematical problems are encoded into graphical networks of channels. As the cytoskeletal filaments move along the tracks, they explore all possible pathways, each corresponding to different solutions depending on the problem encoded. Molecular motors are also used within biosensing [20] for e.g., nanoseparation of analyte
molecules to concentration regions to increase the rate of collection analyte on the detector and to increase the sensitivity in detection [21]. Both these types of devices require large open surface areas patterned for adsorption of the motors in active form. These areas, here denoted loading zones, are important for initial landing of a large number of cytoskeletal filaments from solution, for later transportation into the more complex channel networks, see figure 1.

Currently, the loading zones and network structures are patterned by electron beam lithography (EBL) for the actin-myosin system [7, 11, 20]. However, as patterns grow larger and more complex, the costs and fabrication time scale become unmanageable. Nanoimprint lithography (NIL) is a promising high-resolution [22–24] technique as it offers high throughput at lower costs relative other lithography techniques. The principle of NIL is based on displacement of resist under pressure to replicate stamp features, so this method is not limited by diffraction during sample fabrication. NIL is very suitable for single-step lithography applications, and with high-resolution stamps it is possible to obtain features as small as 20 nm or below [25]. Furthermore, the effects of electrostatic charging and beam scattering [26, 27] need not be taken into account as compared to EBL. However, NIL becomes challenging if the difference between the height and width dimensions of the desired material displacement is large [28], which is the case in some parts of molecular motor based devices, e.g., the above mentioned loading zones.

It has been previously shown that NIL can be used to pattern narrow tracks (100–400 nm wide, 150 nm deep) in poly(methyl methacrylate) for actin-myosin devices [29]. However, to the best of our knowledge, no studies have been done to develop a method to imprint the larger and thus, more challenging loading zones, combined with narrow tracks.

In this paper we show how we redesigned the loading zones from previous EBL fabrication efforts [8, 20] and optimised the resist thickness to be able to imprint structures with a large width to height ratio without suffering from insufficient filling of the stamp. By using a thin resist layer and by adding nanoscaled pillars we were able to provide regions into which the laterally displaced imprint resist could flow. Subsequent to residual resist removal, we were able to observe successful actin-myosin motility. Furthermore, we present simulations showing that the structural design and placement of these pillars can be used to tailor the emptying rate of the loading zone as well as to guide the filaments towards different directions. The implications of these theoretical findings for improvements in pattern design are discussed.

2. Experimental

2.1. Stamp design and fabrication

Previous research [20] has shown that a funnel shaped loading zone is preferred over a circular one as the filaments are guided towards the funnel tip, providing an improved loading, i.e., emptying rate of filaments exiting the loading zone and entering the nanoscale channels as desired. Therefore, this funnel shape was preserved also for the loading zones used here. All EBL designs were made using the Raith150 software (Raith GmbH). To imprint shallow, wide loading zones, different types of nanopillar shapes were introduced into the pattern. Initial testing without these pillars had a variety of issues including incomplete resist displacement, inhomogeneous residual layers and large areas of resist peeling off during stamp removal.
A hard NIL master stamp with negative features was fabricated using a 4" Si (100) wafer in thermally oxidized (500 nm) SiO2 (Siegert Wafer GmbH, Aachen, Germany). The wafer was patterned by electron beam lithography (EBL) and reactive ion etching (RIE). In the first processing step, a double layer hard-etch-mask consisting of 60 nm Cr and 30 nm SiO2 was deposited on top of the SiO2 layer by thermal evaporation (AVAC HVC600, Ltd) and atomic layer deposition (ALD) (Fiji F200, Veeco), respectively. The top SiO2 layer served as the etch mask for patterning Cr, which in turn, was used for relatively deep etching of thermal oxide SiO2. A two-step RIE was required due to insufficient stability of the EBL resist to pattern the Cr layer directly.

In the second processing step, a layer of EBL resist CSAR 62 (Allresist GmbH, Strasbourg, Germany) was spin-coated (Primus SB15, ATMgroup) onto the etch-mask at 3000 rpm for 30 s and baked on a hotplate for 2 min at 160 °C. The stamp pattern was defined by EBL (Raith150, GmbH) at 20 kV acceleration voltage, beam current ≈ 0.15 nA and dose 60 μC/cm². The pattern was developed in o-xylene (VWR, Radnor, PA, USA) for 5 min at room temperature and rinsed in 2-propanol (VWR, Radnor, PA, USA). Resist residues were removed by ashing in oxygen plasma (Plasma Preen, Plasmatic Systems Inc.) for 15 s at 5 mbar. In the third processing step, the top 30 nm SiO2 was patterned by inductively coupled plasma reactive ion etching (ICP-RIE) (Plasmalab System100, Oxford Instruments) using a mixture of C4F8 and O2 gases (chamber pressure 1.0 · 10⁻⁶ Torr, gas flow 78.7/3.2 sccm (C4F8/O2), RF power 268 W, ICP power 1194 W, DC bias 266 V). The CSAR 62 resist was used as a mask in this etch process. The underlying Cr was etched using a mixture of CI4 and O2 gases, creating a stable mask for deep etching the thermal SiO2 (chamber pressure 1.0 · 10⁻⁶ Torr, gas flow 20/5 sccm (Cl2/O2), RF power 97 W, DC bias 129 V). In the final etching step, the structures were etched down ~380 nm into the 500 nm thick thermal SiO2 by of C4F8/O2 ICP-RIE (chamber pressure 1.0 · 10⁻⁶ Torr, gas flow 78.8/0.9 sccm (C4F8/O2), RF power 248 W, ICP power 1986 W, DC bias 344 V). After the final etch, all fluorocarbon residues were removed by ashing the wafer in pure oxygen plasma for 10 min at 5 mbar. The remaining Cr mask was removed by wet etching in Chrome Etch 18 (Micro resist technology GmbH, Berlin, Germany) for 5 min. To provide an anti-sticking surface preventing resist adhesion during NIL, the structured SiO2 was coated with a monolayer of fluorodecyltrichlorosilane (FDTS) by ALD (Fiji F200, Veeco).

The master stamp was used to fabricate transparent intermediate polymer stamps (IPS*) through thermal imprinting. The IPS was replicated from the master stamp by heating it to 160 °C and imprinting at 50 bar for 2 min and then cooled down to 115 °C before lowering the pressure to atmospheric pressure.

### 2.2. Sample fabrication

All samples were fabricated on 2" SiO2/Si(100) wafers by imprinting the patterns in TU7-220 resist (Obducat, Lund, Sweden). A modified version of a standard IPS*-simultaneous thermal and UV-imprint (IPS*-STU) process developed by Obducat, was used for the nanoimprinting. The wafers were patterned by the STU*, and a separate IPS* was used for each wafer. Prior to the deposition of TU7, the wafers were treated in oxygen plasma at 5 mbar for 60 s to improve resist adhesion. To obtain an optimum resist thickness, a series of wafers were spin-coated for 30 s at five different spin-speeds: 1000, 1200, 1500, 2000 and 3000 rpm, and baked on a hotplate for 60 s at 95 °C.

During imprint, the TU7 layer was heated to 75 °C and subjected to a pressure of 20 bar with subsequent exposure to 20 s of UV-light, with a wavelength of 370 nm and intensity of 34 mW cm⁻², to cross-link the TU7 polymer. The pressure and temperature were maintained for 2 min and then decreased to ambient conditions. After imprinting, the wafers were examined in a scanning electron microscope (SEM) (Hitachi SU8010, Hitachi, Japan) to determine the minimum resist thickness which ensures sufficient filling of the stamp, see figure 2.

A new series of wafers with the spin-speed with the best results (1000 rpm) were fabricated and imprinted. These wafers were used to optimise the removal of the residual resist layer by reactive ion etching (Sirius T2, Trion Technology) in oxygen plasma. To examine the residual layer, a small scratch was made through the structured area and scanned in an atomic force microscope (AFM) (NaioAFM, NanoSurf) in non-contact mode with an ACLA tip, before and after etching for 10 s, 30 s and 110 s, see figure 3. The obtained data was analysed in the Gwyddion software [30].

The wafers were diced (Disco DAD 3320, Co) into to 10 × 10 mm samples and treated with trimethylchlorosilane (Sigma-Aldrich, Saint Louis, MO, USA) as previously described [31] for 64 min at 200 mbar.

### 2.3. In vitro motility assay

We performed in vitro motility assays [4] as previously described [31] at 26 °C, by infusing 120 μg/ml heavy meromyosin (5 min) and 200 nM Rhodamine-phalloidin labelled actin filaments (2 min) followed by an assay solution (pH 7.4) containing 1 mM MgATP and with anionic strength of 60 mM. The motility assay was observed in a Zeiss Axio observer inverted microscope with Hg-lamp illumination, Zeiss Plan-APOCHROMAT.
63 × 1.4 NA oil immersion objective, TRITC filter set (excitation 532–554 nm, emission 570–613 nm, dichroic cut-on 562 nm) and a Hamamatsu EMCCD camera. The recorded data was analysed in ImageJ \[32, 33\] to study the filament behaviour.

2.4. Monte Carlo simulations

To estimate the effect of different shaped pillars in the loading zones, we performed Monte Carlo simulations of the movement of filament tips on open surfaces and of their guiding at wall edges. The simulations take into account the filament rigidity, thermal motion and the guiding effect of the channel walls as described in previous work \[15\]. Briefly, the effect of the thermal fluctuations of filament tips moving at velocity (v) was taken into account by updating the direction of motion at time intervals (Δt) with an angular change, which was obtained from a Gaussian distribution with zero mean value and standard deviation (σ) according to equation (1):

\[
\sigma = \left( v \Delta t / L \right)^{0.5}
\]

where \( L \) is the persistence length of the filament (in this case 10 μm). The MATLAB (Mathworks, Natick, MA) randn function with a Mersenne-Twister algorithm was used as random number generator for normal distributions. For each simulated device, the random number generator was initialised with the ‘rng(‘shuffle’)’ command. Thereafter, individual random seeds for each filament were generated with the ‘randi’ function, ensuring that the random number generator for each filament was initialised with a unique random seed. After a
wall collision was detected, the direction of the filaments was changed such that the change in angle was minimal and the angle after collision followed the angle of the smooth wall at the collision point. Similar algorithms have been successfully used to optimise nanofabricated structures for the guiding of cytoskeletal filaments [7, 34].

3. Results and discussion

In standard NIL procedures, the resist is spun thicker than the stamp protrusions to prevent deformation and damaging of the stamp during imprinting. However, by using a two-step imprinting procedure with a soft IPS® this risk is completely eliminated. Due to the large difference between height and width being imprinted in the structures presented here (up to \( \sim 1:200 \)), we deliberately spun a thinner resist layer than the intended depth of the structures to try and obtain more capillary flow upwards to displace as much resist as possible vertically, into the stamp cavities. The thickness of the resist was varied by changing the spin-speed from 1000 rpm up to 3000 rpm. We found that the thinnest resist possible to use is just above 350 nm, while the thinner resists cause insufficient filling, see figure 2.

The residual layer of TU7 was removed by reactive ion etching in oxygen plasma. Initially \( \sim 80 \) nm TU7 remained after imprinting. After a 30 s full wafer etch, only 40 nm remained. To ensure full removal of all TU7 in order to promote later myosin driven actin motility, a longer 110 s full wafer etch was performed, completely removing all remaining TU7 on the channels floors, see figure 3. A slightly shorter etch time can also be used to maintain higher pillars.

The pattern designs and resulting imprints that were fabricated with use of the optimisations described above, are depicted in figure 4.

The imprinted structures supported motility inside both the loading zone and the connecting nanochannel, see figure 5, although the majority of filaments remained inside the loading zone without entering the channel. We observed filaments tracing the pillar-structures when approaching at a grazing incidence angle, but detachment was observed upon head-on collision, similar to what previous findings have suggested [8, 35]. The overall number of filaments observed in these structures is low, which is likely due to the increased number of obstacles as compared to open loading zones, causing an increased number of exiting events due to head-on collisions. Moreover, the decreased number of filaments is also attributed to a decreased surface area available for motor adsorption. The surface area is decreased to different extents from 1200 \( \mu \text{m}^2 \) without any pillars, depending on the different designs (figure 4). Nevertheless, as the filaments come in contact with the pillars, their trajectory path is also redirected, providing a good spread of filaments across the entire loading zones (figure 5). Such properties are desirable in applications where filament behaviour in a confined area is studied, e.g., in response to different chemical environments. Therefore, we hypothesized that a hexagonal and square pattern repetition would generate different filament spreading due to constant redirection upon impact, as filaments otherwise tend to trace the walls in confined spaces [11, 15]. However, no obvious difference was observed between the hexagonal and square pattern positions, both geometries provided good motility, this can likely be improved by further optimisation to increase the number of filaments studied.

In addition, Monte Carlo simulations suggest that the pillars introduced could also be able to tailor both the overall direction of filament movement as well as the emptying rate of filaments exiting the loading zone. For these simulations, the same number of filaments (240) where simulated for a time equivalent of 1500 s for each design. In an experimental set-up, the total number of filaments will also depend on the free SiO\textsubscript{2} surface area available for molecular motor binding. Most design have the same SiO\textsubscript{2} area in both the square and hexagonal pattern repetitions (figure 4). However, depending on the number of pillars included and the pillar shape, the imprint areas vary slightly. Thus, the total number of filaments would not be consistent in each experiment. However, as is clear from figure 4, the difference would be small.

The overall simulated behaviour of the filaments can be seen in the heat maps presented in figure 6. The designs with three sharp corners (designs A, B, F, G in figure 4) present a larger difference in emptying rate (figure 7) between the square and hexagonal pattern repetition as compared to the pillar shapes with just one sharp corner (designs D and E in figure 4). The emptying rate with the completely symmetrical pillar shapes (see designs H and I) are close to identical, despite the difference in pillar diameter. Moreover, the symmetrical pillar shapes provide more evenly distributed filaments inside the loading zone (figure 6).

Another noticeable difference is that the emptying rate (figure 7) for square pattern repetitions is lower than for hexagonal pattern repetition if a sharp corner is pointing towards the exit (compare A and B, D with E, F and G). However, the overall emptying rate is still higher for patterns with a sharp corner pointing towards the exit (compare A, D with B, E). Interestingly, the pillar shape in designs F and G provide a slightly different filament movement. The heat map of design F (figure 6) clearly shows a larger density of filaments closer to the loading zone exit, while the opposite is true for design G. This is likely caused by the concave arc in the pattern that changes the angle of any filament hitting the concave wall towards (design F) or away from (design G) the exit,
Figure 4. Designs of imprinting patterns with square and hexagonal placement of the pillars, and SEM images of the resulting structures after processing optimisation and motility tests. The imprinting area corresponds to the amount open SiO₂ available for molecular motor binding. Sample E was imaged at a different occasion to the others, the contrast difference compared to the other images does not reflect a material difference.

Figure 5. (A) Fluorescence microscope image of actin filaments moving across a loading zone and (B) a time series showing an actin filament moving away from the loading zone through the connecting channel (see recordings in supporting information is available online at stacks.iop.org/MRX/6/025057/mmedia). Note that the images in (B) have been rotated 90° counter clockwise as compared to (A). The scale bar is 5 μm in all images.
which will affect the proceeding pathway [9]. The effect becomes more evident after comparison of emptying rates (figure 7) for filaments pushed towards an end of the loading zone, where designs F and G provide the two most extreme emptying rates.

The filament behaviour will of course vary for different molecular motor systems as a key factor affecting the trajectory path is the persistence length. Here we have used parameters that match the behaviour of the actin-myosin system in an in vitro motility assay, namely a persistence length of 10 μm and a velocity of 10 μm/s. Similar simulations have been successfully used to predict the behaviour of both actin-myosin and kinesin-microtubule motor systems in nanostructures [7, 34].

4. Conclusion

We have shown that it is possible to fabricate structures with an aspect ratio up to ~1:200 (height-to-width) for molecular motor devices by nanoimprint lithography. Key parameters are resist thickness and introduction of pillar shapes into large open areas such as loading zones for cytoskeletal filaments. The pillars provide sinks in the stamp into which polymer can be displaced. Furthermore, Monte Carlo simulations indicate that the introduced pillars could be used to tailor the behaviour of filament movement by varying the shape and configuration of the pillars. In contrast to previous attempts that created simple line patterns for the guiding of cytoskeletal filaments [29], our approach allows fabrication of complex molecular motor-powered nanodevices such as network-based biocomputers [7] or lab-on-a-chip diagnostic devices [6]. We envision that this technology will enable rapid mass-production of such devices at greatly reduced cost, bringing them closer to market maturity. Furthermore, the possibility to imprint structures with large differences in dimensions by

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**Figure 6.** Heat maps of simulations showing the filament distribution across the different loading zone designs. The colour bar on the right indicates the number of filaments that visited each pixel over a simulated duration of 1500 s. The colour map is the same for all images.

**Figure 7.** Emptying rate, number of filaments exiting the loading zone as a function of time for different pattern designs. The shape of the pillars and the pattern repetition affects the emptying rate, e.g., the hexagonal pattern repetition provides a higher emptying rate for patterns with sharp pillar corners pointing towards the loading zone exit ((A), (D) and (G)).
resist thickness optimisation may also find future applications within several other fields including nanopillar patterning for e.g., superhydrophobic surfaces [36] or cellular behaviour studies [37].

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