UTILIZING PDMS STAMPING FOR MASS PRODUCTION OF MICROTUBULE FUNCTIONALIZED DETECTION DEVICES

Oya Koc, Mehmet C. Tarhan, Yslam Orazov, Hiroyuki Fujita and Beomjoon Kim
Institute of Industrial Science, University of Tokyo, Japan

ABSTRACT

Developments in micro technologies showed high potential in novel hybrid devices integrating biomaterials with electrical or chemical systems aiming at biomedical applications. However, biomaterial functionalization usually requires delicate microfluidic systems, complicating the overall structure of the devices. To use the upmost benefits of the micro technologies, hybrid devices should be designed to be mass-production compatible. Here, we propose to utilize PDMS stamping for functionalizing active regions of multiple hybrid devices in a short time and cost efficient way. Furthermore, we demonstrate capturing of some biomaterials, i.e. microtubules (MTs), on the PDMS stamp as the first step to achieve the proposed technique aiming at device functionalization at mass numbers.

KEYWORDS

Micro stamping, PDMS, biomaterials, hybrid devices, microtubule

INTRODUCTION

Micro technologies miniaturized the dimensions of 3-D structures allowing scaled-down-hybrid devices that can be used for novel biomedical applications. Smaller dimensions provided faster devices due to shorter diffusion distances and lower cost due to decreased amount of required materials. However, handling of biomaterials became a great challenge. Specific assembly and immobilization at the active regions of devices require additional components, such as channels, pumps or electrodes, complicating the devices. Microtubule-based systems are good examples to see how important the assembly and immobilization techniques are. To functionalize a micrometer sized active region of a device requires centimeter-sized channels [1] that are usually integrated with syringe pumps. For proper assembly of microtubules, complicated processes are necessary and thus, device-by-device assembly is not feasible. Therefore, a mass-production friendly technique is crucial to minimize the complexity of the devices while rapidly functionalizing the active regions of devices with biomaterial in a time and cost efficient way.

PDMS stamping is widely used for microcontact printing for patterning molecules such as self-assemble monolayers. Fast and easy patterning as well as simple protein stamping [2] using PDMS has already been demonstrated. However, PDMS stamping has much higher potential than just protein printing on a surface. We propose using PDMS stamps for relocating orientation-specific assembled microtubules to functionalize active regions for devices in mass numbers (Figure 1). As microtubules are essential components of intracellular transport, they are crucial for neuron functioning. Therefore, MT-based systems are promising tools for *in vitro* sorting and transport devices [3] as well as some neurodegenerative disease studies [1]. However, orientation-specific assembly of microtubules is cumbersome [4]. The proposed method might lead to mass-production of hybrid devices by functionalizing only the necessary regions in a time and cost efficient way. Here, we are demonstrating the first step of the proposed method: capturing microtubules from a glass surface using PDMS stamp.

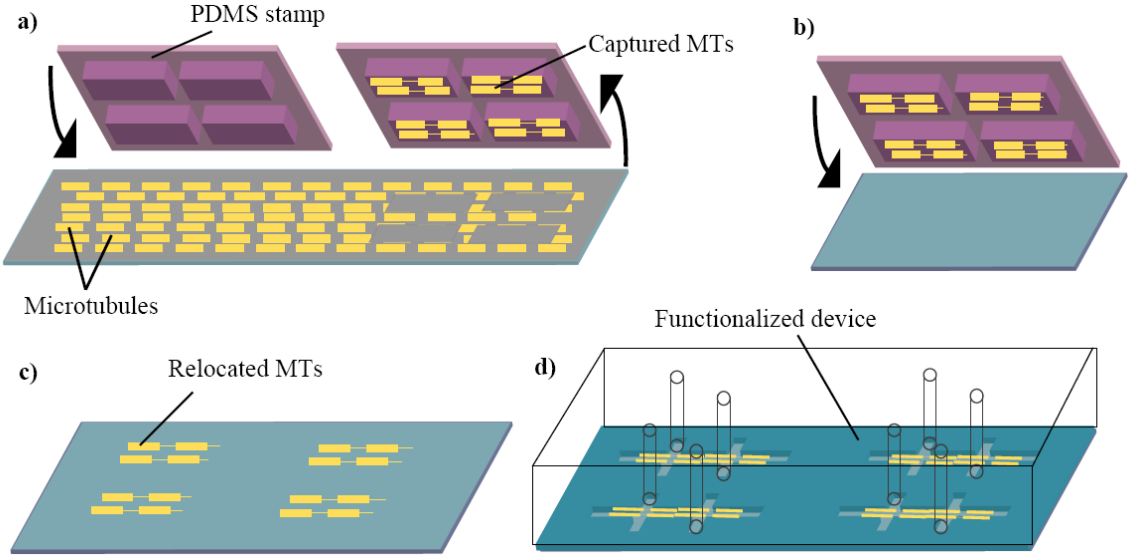

Figure 1.Schematic view of the method proposed. Microtubules are first oriented on a glass surface and then captured using a surface-treated PDMS stamp. The captured microtubules are relocated on target surface. Repeating the capturing and relocation process can functionalize several different devices to mass-produce functionalized detection devices.

978-0-9798064-5-2/μTAS 2012/$20©12CBMS-0001

EXPERIMENTAL

Materials

Microtubules: Tubulin was purified from four porcine brains and stored at a concentration of 4 mg/ml in liquid nitrogen. Part of the tubulin was labeled with tetramethyl rhodamine. Tubulin was mixed with labeled tubulin (with a ratio of 10:1, tubulin:labeled tubulin) and polymerized into microtubules in BRB80 buffer (80mM PIPES-NaOH pH=6.8, 1 mM MgCl2, 1 mM EGTA) containing MgSO4 (1 mM) and GTP (1 mM). After incubating at 37°C for 30 min, microtubules were stabilized with taxol (0.4 mM) and diluted in BRB80, containing taxol (20 μM).

Kinesin:. Inactive Kinesin was used in the experiments to immobilize microtubules on the glass surface. Kinesin molecules were diluted to 25 μg/ml in 0.7 mg/ml casein solution.

Experimental Setup and Procedure

Experiments were conducted on an inverted microscope (Olympus IX-71) stage. Fluorescence imaging was used to visualize labeled MTs. A photometrics camera (Cascade512 II) was used to monitor the experiments. Imaging software (Metamorph) was used to analyze the images after the experiment to measure the number and the length of the attached microtubules.

First, different surface conditions were examined to choose the best configuration for the capturing process (Figure 2). Three different attachment conditions were compared: (1) kinesin-coated glass, (2) poly-L-lysine (PLL) coated PDMS, and (3) PLL coated glass surfaces. Kinesin molecules bind on glass surface via their tail and attaching to microtubules with their heads for immobilization. Similarly, PLL-coating of glass was performed by incubating 0.01% PLL solution in a flow cell for 5 minutes. PLL-coated PDMS device, on the other hand, required a preliminary preparation. PDMS was first spincoated (1300 rpm for 30 seconds) on a glass surface followed by 30 minutes baking at 90°C and then exposed to oxygen plasma for surface treatment. The flow cell was assembled and 0.01% PLL was incubated in the flow cell for 5 minutes. After washing the unbound molecules for each condition, 100-fold diluted labeled MTs were injected in the flow channels. After incubating for 5 minutes, unbound MTs were washed away with BRB80 solution. The resulting flow cells were observed under the microscope. Microtubules shorter than 2 μm were discarded and the number and the total length of remaining microtubules were measured and compared (Figure 3).

Demonstration of MT capturing with a PDMS slab was performed in two steps. At first, a flow cell was assembled using two cover slips and 25 μg/ml kinesin coating of the glass surface. Then 20-fold diluted MTs were injected and incubated for 5 minutes in the flow cell. Finally, the top cover slip was removed (Figure 4a). In the second step, previously prepared and PLL-coated PDMS slab (around 3 mm thick with a surface area of half of the flow cell) was located on the flow cell (Figure 4b,c). The PDMS slab was gently pushed and kept undisturbed for 10 seconds. Then, PDMS slab was removed with the captured MTs (Figure 4d).

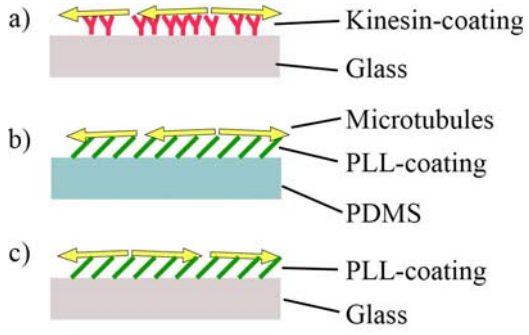

Figure 2. Schematic view of different surface coatings: a) kinesin coating of glass surface, b) PLL-coating of PDMS surface and c) PLL-coating of glass surface.

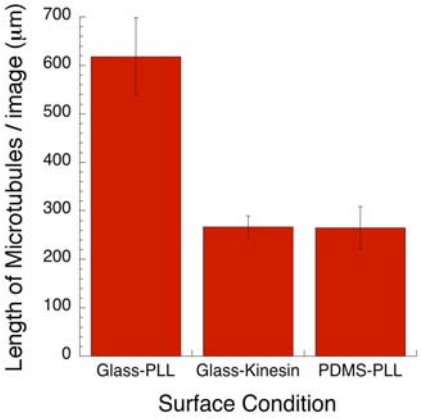

Figure 3. Graph showing total length of MTs averaged per image for three different surface conditions.

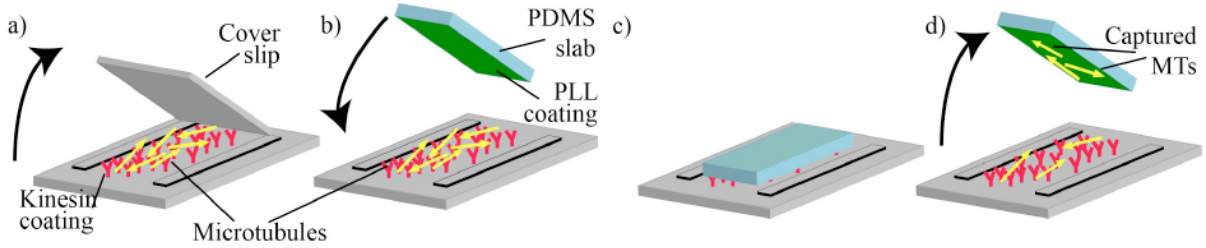

Figure 4. Schematic view of the demonstrated microtubule capturing process using a PDMS stamp. a) Microtubules were immobilized on kinesin-coated glass surface in a flow cell. b) After removing the upper cover slip, PLL-coated PDMS slab was approached and c) touched on the glass surface. d) Some of the microtubules were transferred on the PDMS slab.

RESULTS AND DISCUSSION

The effect of different surface conditions on microtubule attachment was compared using the total length of microtubules. Microtubule length was varying from few micrometers to tens of micrometers. Averaging the total length of microtubule per image (n>15) provided the attachment characteristics of different surface conditions (Figure 3). According to the results, PLL-coated glass surface provided the highest amount of microtubule attachment (658±40 μm/image). The remaining two cases, i.e. kinesin-coated glass and PLL-coated PDMS, showed similar characteristics (267±71 μm/image and 253±45 μm/image respectively).

Considering the overall system, PDMS surface should be considered as the surrogate surface capturing and relocating microtubules because PDMS can easily be shaped, transported and manipulated. According to the attachment rates, capturing microtubules from a PLL-coated glass surface would be more difficult when compared to the kinesin-coated glass surface. In fact, using kinesin-coated glass surface has a crucial benefit for the overall system. Kinesin coating on the glass surface can be used for microtubule orientation [5]. Therefore, succeeding microtubule capturing on the kinesin-coated glass surface can easily be extended to orientation specific capturing of microtubules.

Successful demonstration of microtubule capturing was performed (Figure 5). Microtubules on the kinesin-coated glass surface (Figure 5a) were captured by PLL-coated PDMS slab. Figure 5b shows the PDMS surface after capturing. Some of the microtubules were transferred from glass surface to the PDMS surface. Comparing the concentrations, we can conclude that only a portion of the microtubules was captured. Considering that microtubule attachment rates, thus the attachment strengths, were quite similar for the surfaces, partial capturing is quite understandable.

Despite successful demonstration, repeatability of the capturing process is still an issue. Applying too much pressure on the PDMS slab during the capturing process damages the microtubules while applying not enough pressure results in poor capturing. Therefore, further optimization is required to increase the success rate of the capturing process.

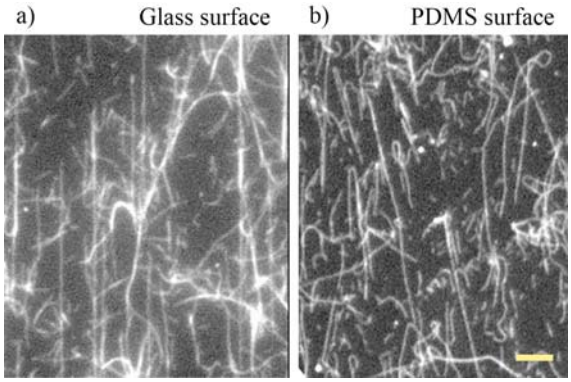

Figure 5. Fluorescence images of a) MTs on kinesin coated surface before capturing and b) PLL-coated PDMS surface after capturing. Some of the MTs were transferred to the PDMS stamp. Scale bar corresponds to 5 mm.

CONCLUSION

We proposed utilizing PDMS stamps as surrogate surfaces for capturing orientation-specific microtubules from a surface and transferring them to active areas of hybrid biomolecular devices. We have demonstrated the first step as microtubule capturing from a surface using a PDMS slab. The proposed system is a promising technique to simplify the targeted devices while providing the necessary functionalization in a short time and cost efficient way. This attempt to produce molecular chips in mass numbers may lead to develop the necessary technology to move from laboratory-based devices to point-of-care devices.

REFERENCES

[1] M.C.Tarhan, R.Yokokawa, H.Qiu, S.Karsten, and H.Fujita, *Motor protein motion along microtubules for molecular detection*, Proc. Transducers, p. 886, (2011).

[2] K. Shen, J.Qi, and L.C. Kam, *Microcontact Printing of Proteins for Cell Biology*, J Vis Exp., 22: 1065, (2008).

[3] J.L.Malcos, W.O.Hancock, *Engineering tubulin: microtubule functionalization approaches for nanoscale device applications*, Appl Microbiol Biotechnol.; 90(1): 1–10, (2011)

[4] M.C.Tarhan, R.Yokokawa, L.Jalabert, D.Collard, and H. Fujita, *Biomotor-based nanotransport system constructed by pick-and-place assembly of individual molecules*, Proc. IROS, pp.5628-5633, (2010).

[5] J.R.Dennis, J.Howard and V.Vogel, *Molecular shuttles: directed motion of microtubules along nanoscale kinesin tracks*, Nanotechnology 10:232 (1999)

CONTACT

Oya Koc, tel: +81-3-5452-6226; oyakoc@iis.u-tokyo.ac.jp