

Regeneration of Assembled, Molecular-Motor-Based Bionanodevices

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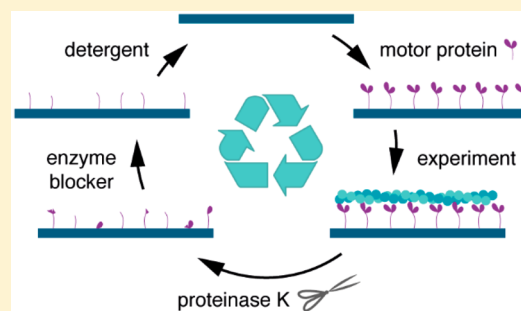
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S Supporting Information

ABSTRACT: The guided gliding of cytoskeletal filaments, driven by biomolecular motors on nano/microstructured chips, enables novel applications in biosensing and biocomputation. However, expensive and time-consuming chip production hampers the developments. It is therefore important to establish protocols to regenerate the chips, preferably without the need to dismantle the assembled microfluidic devices which contain the structured chips. We here describe a novel method toward this end. Specifically, we use the small, nonselective proteolytic enzyme, proteinase K to cleave all surface-adsorbed proteins, including myosin and kinesin motors. Subsequently, we apply a detergent (5% SDS or 0.05% Triton X100) to remove the protein remnants. After this procedure, fresh motor proteins and filaments can be added for new experiments. Both, silanized glass surfaces for actin–myosin motility and pure glass surfaces for microtubule–kinesin motility were repeatedly regenerated using this approach. Moreover, we demonstrate the applicability of the method for the regeneration of nano/microstructured silicon-based chips with selectively functionalized areas for supporting or suppressing gliding motility for both motor systems. The results substantiate the versatility and a promising broad use of the method for regenerating a wide range of protein-based nano/microdevices.

KEYWORDS: Nano/microdevice, regeneration, protein desorption, molecular motor, proteinase K, detergent



Protein adsorption to surfaces is a ubiquitous and extremely important phenomenon.^{1–4} Whereas it may be unwanted in several instances, it has also been widely exploited in biotechnology as well as in fundamental biophysical and biochemical investigations.⁵ For instance, antibodies, or other protein-based recognition molecules, are adsorbed to detector surfaces in biosensing^{6–10} and proteomics^{11,12} applications. In addition, fundamental functional studies often rely on surface adsorption of receptors and protein nanomachines.^{13–15} One widely used assay of the latter type is the *in vitro* gliding motility assay. In this assay,¹⁶ biomolecular motors isolated from cells are adsorbed onto a surface, enabling the propulsion of cytoskeletal filaments that can be observed by microscopy. Two commonly studied systems are actin filaments propelled by myosin motors^{16–19} and microtubules propelled by kinesin²⁰ or dynein motors.²¹ These motor systems are responsible for cell motility, muscle contraction, and intracellular cargo transport. Modified versions of the assay may also be applied to noncytoskeletal motors, such as processive DNA enzymes.²² *In vitro* gliding motility assays have also enabled a variety of nanotechnological applications, for

example, in nanoseparation and biosensing^{6,23–30} and more recently, parallel biocomputation.³¹ Many of these applications require nano/microstructured surfaces for physically and chemically confined filament transport along predefined paths, involving widely differing surface materials and chemistries. It is also expected that such devices will become increasingly complex in the future, for example, by integrating (opto)- electronic components for automated read-out of filament positions and velocities.³¹

The nano/microstructures used for molecular-motor-based devices are both expensive and time-consuming to produce. Furthermore, processes to fabricate them (such as electron beam lithography) consume a fair amount of energy.³² It is therefore highly desirable to develop methods that enable the effective regeneration and reuse of these nano/microstructures. In such processes, all proteins that have deteriorated over time are ideally replaced by fresh, functional proteins without the

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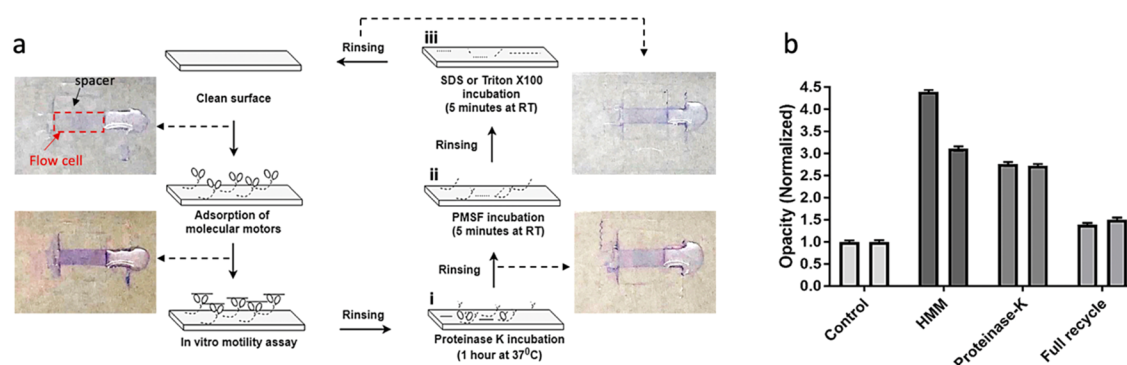


Figure 1. Optimized method for regeneration of molecular-motor-based nano/microdevices. (a). After performing an in vitro motility assay (left panel), the main steps of the regeneration process (right panel) are (i) incubation with proteinase K (200 $\mu\text{g}/\text{mL}$; preactivated by Ca) for 1 h at 37 $^{\circ}\text{C}$ to digest the surface-adhered proteins, followed by (ii) incubation with PMSF (5 mM) for 5 min at room temperature (RT) to inactivate any remaining proteinase K, and (iii) incubation with SDS (5%) or Triton X100 (0.05%) for 5 min at room temperature to remove protein fragments from the surface. Afterward, fresh proteins can be added for new motility assays. Inset photos: Coomassie brilliant blue stain (0.1%) of the flow cell before addition of HMM or any other component (control), immediately after incubation with HMM at surface-saturating concentration (120 $\mu\text{g}/\text{mL}$) and after steps (i) and (iii) of the regeneration process. (b) Flow-cell opacity (average intensity measured from region of interest (ROI) in flow cell area subtracted by the average intensity in similar ROI on the glass outside the flow cell). Data from two experiments using Triton X100 (0.05%) as detergent (given $\pm$ pixel standard error of the mean) normalized to control value. See SI for details.

need to disassemble the chips from their microfluidic environments. At the same time, it is important that the surface regeneration is achieved without destroying the nano/microstructures. Ideally, the process also preserves the motility-contrast between motility-supporting and motility-suppressing areas, which are often sustained by the use of different surface chemistries.^{33–35}

In previous efforts to regenerate protein-coated surfaces, various approaches have been tested. These range from gentle methods, such as incubation with high-ionic-strength solutions³⁶ and detergents^{37–39} or a combination of detergents and acids^{39,40} to harsher methods including highly concentrated acids, oxygen plasma,⁴¹ or laser treatment.⁴² Whereas the gentle methods often fail in effectively removing all aged proteins, harsher methods often alter the surface chemistry as well as the physical properties of the nano/microstructures and electronic components. Moreover, some of the procedures, such as oxygen plasma treatment, require disassembly of the devices.

We present here a novel method for regenerating molecular-motor-based nano/microdevices that keeps both the surface chemistry and surface topography intact and that does not require disassembly of the devices. We applied the described regeneration procedure to in vitro motility assays with both the actin–myosin and the microtubule–kinesin systems. Furthermore, we varied the substrates as well as the chemically and topographically structured surfaces. In the case of actin–myosin assays, we use the soluble myosin motor fragment heavy meromyosin (HMM), chymotryptically cleaved off from myosin. This is the most commonly used motor fragment in nanotechnological applications of actin–myosin as well as in most fundamental studies using the in vitro motility assay. Different surface chemistries were tested because the most optimal HMM-driven actin-motility is obtained on a distinct surface chemistry compared to kinesin-driven microtubule motility, investigated here by using kinesin-1. Likewise, the required surface chemistry for motility-suppression varies for the two motor systems. It was also essential to verify that the latter chemical properties are not altered by the regeneration procedure because motility-suppressing areas are central in functional devices. Importantly, using our approach, we find

that repeated regeneration is possible for both motor systems, with maintained selectivity of motor function between different areas on a chip. The extension of the regeneration method to a range of nonmotor applications is discussed in view of the importance of protein adsorption in diverse fields.

To investigate and optimize different protocols for surface regeneration, we compared the performance of motor-driven filament gliding before and after surface treatment. Specifically, we adhered motor proteins to the surfaces, added fluorescently labeled filaments in the presence of the chemical energy molecule adenosine triphosphate (ATP) and recorded the gliding motility on a fluorescence microscope. Subsequently, for surface regeneration we aimed to clean the surface without device disassembly, by application of proteinase K and/or PMSF and/or detergents to the devices, including intermediate washing steps. After reapplication of fresh motors and filaments to the same device we recorded the resulting gliding motility.

In initial studies, using the actin–myosin system, we found that treatment with proteinase K or with different detergents alone only led to partial regeneration (Figures S1–S3, Table S1). Proteinase K treatment prevented surface binding of newly added actin filaments if no fresh HMM motors had been added after the treatment. In partial analogy, newly added microtubules exhibited only rare binding (with no motility), if no fresh kinesin motors had been added after the treatment. These findings suggest that functional motors are effectively digested by proteinase K. If fresh HMM motors were added after proteinase K treatment, however, there was motor-driven transport of subsequently added actin filaments albeit with lower velocity and increased filament lengths compared to our controls. The latter characteristics suggest a low density of functional motors,^{43,44} likely due to protein remnants on the surface with inhibition of high-density binding of fresh HMM. This is consistent with results from Coomassie brilliant blue staining to assess the total protein, peptide, and amino acid surface binding. Such staining (Figure 1) reveals only limited removal of motor protein remnants following proteinase K treatment. Because we expected the remnants to be removed by detergents,² we cleaned the surfaces with detergent after proteinase K treatment. A subsequent Coomassie stain

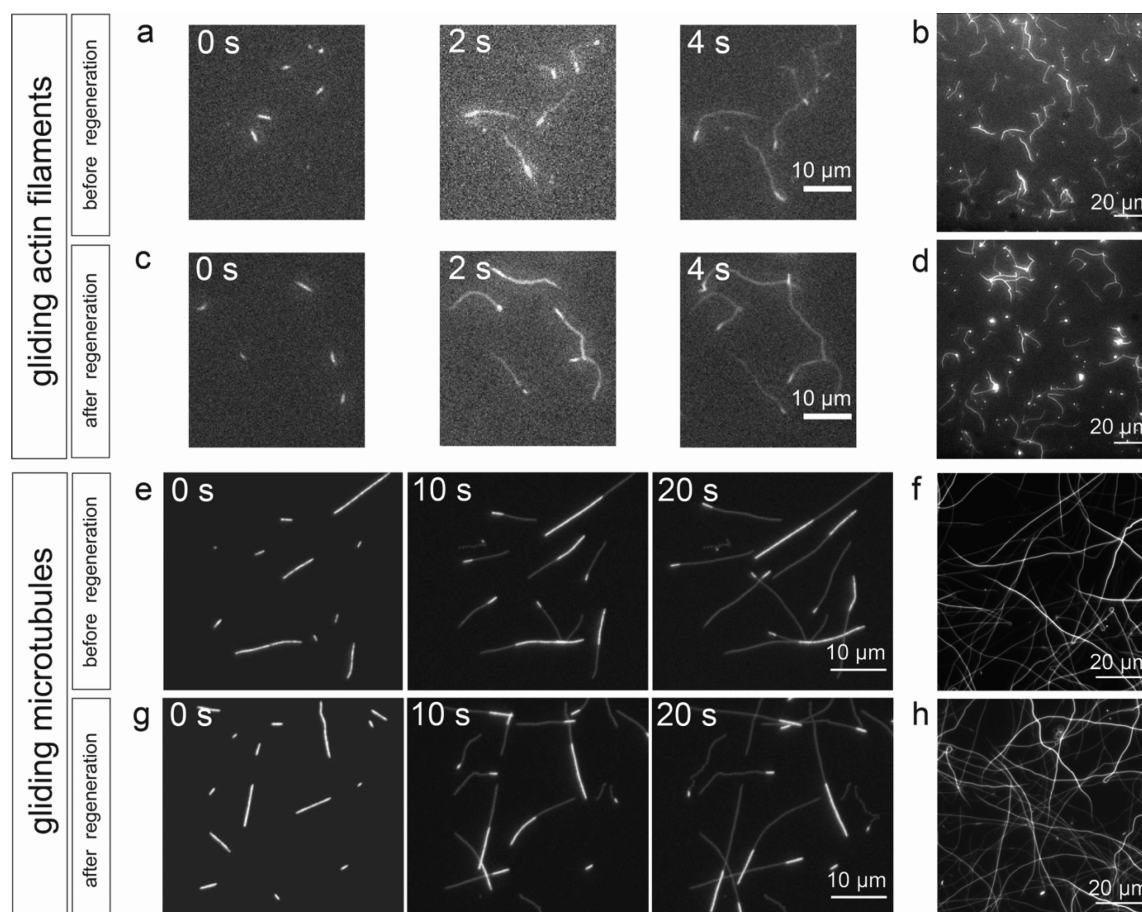


Figure 2. Gliding motility assays before and after regeneration. (a,c) Fluorescence micrographs zooming in on five gliding actin filaments on an HMM-coated, TMCS-derivatized SiO_2 surface before (a) and after (c) regeneration. The images show maximum projections (derived using ImageJ⁴⁶) of time-lapse movies from time 0 s to the time points indicated. Actin filament positions at the latter time point are highlighted. (b,d) Maximum projections (from 4 s time-lapse movies at lower magnification) of gliding actin filaments before (b) and after (d) regeneration. (e,g) Fluorescence micrographs zooming in on few microtubules gliding on kinesin-coated glass substrates before (e) and after (g) regeneration. Again, the images show maximum projections of time-lapse movies from time 0 s to the time points indicated. Microtubule positions at the latter time point are highlighted. (f,h) Maximum projections (from 121 s time-lapse movies at lower magnification) of gliding microtubules before (f) and after (h) regeneration. Note that the time between frames for HMM-propelled actin filaments is appreciably shorter than for kinesin-propelled microtubules due to higher velocity. Also, the gliding paths are appreciably more curved for the actin filaments due to >10-fold lower persistence length than for microtubules. See [Movies S1, S2, S3, and S4](#) for videos corresponding to data in this figure.

corroborated the idea that almost all protein remnants had now been removed ([Figure 1](#)). Also, the contact angle of water droplets after full regeneration of a trimethylchlorosilane derivatized surface was largely unchanged when measured on one control surface in three different locations ($71.3 \pm 1.5^\circ$; mean $\pm$ SD) compared to the preregeneration value ($72.8 \pm 2.0^\circ$). This supports the idea that the regeneration procedure preserves the key surface properties. In accordance with this idea, the motor propelled actin filament velocity, the fraction of motile filaments, and the average filament length all recovered to the initial control values after regeneration. Intermediate PMSF treatment (to terminate proteolysis; [Figure 1](#)) was of value by yielding higher relative gliding velocities after regeneration and was therefore included as a standard step in our optimized protocol.

More generally, using standard surface substrates, we found that the motile function (measured by gliding velocity, fraction of motile filaments, and filament lengths) after regeneration was fully restored compared to the control, both for actin–myosin on trimethylchlorosilane [TMCS] derivatized glass and SiO_2 and for microtubule–kinesin on cleaned glass surfaces

([Figure 2](#); [Movies S1, S2, S3, and S4](#)). With regard to their innate material properties, we here consider SiO_2 and glass as equivalent substrates.⁴⁵

We first consider the actin–myosin system in more detail. Regeneration restored smooth gliding with a high fraction of motile filaments and a gliding velocity that was similar as before the treatment ([Figure 2a–d](#), [Movies S1 and S2](#), and [Figure 3a](#)). Evaluation of filament lengths ([Figure 3b](#)) and plots of velocity versus filament lengths ([Figure S4](#)) provides more insight. Because of the short on-time of a myosin motor domain on the actin filament in each cyclic interaction, low actin gliding velocities are expected if only few motors interact with the actin filaments, such as in the case of short actin filaments and/or low motor densities.⁴⁴ Therefore, velocity versus filament-length plots sensitively reflect the HMM surface density. Moreover, if there are fewer motors on the surface, the average length of the gliding filaments is expected to be higher because of reduced filament fragmentation due to myosin imposed forces.⁴⁴

Our results, showing that both the velocity versus filament-length relationship ([Figure S4](#)) and the average filament length

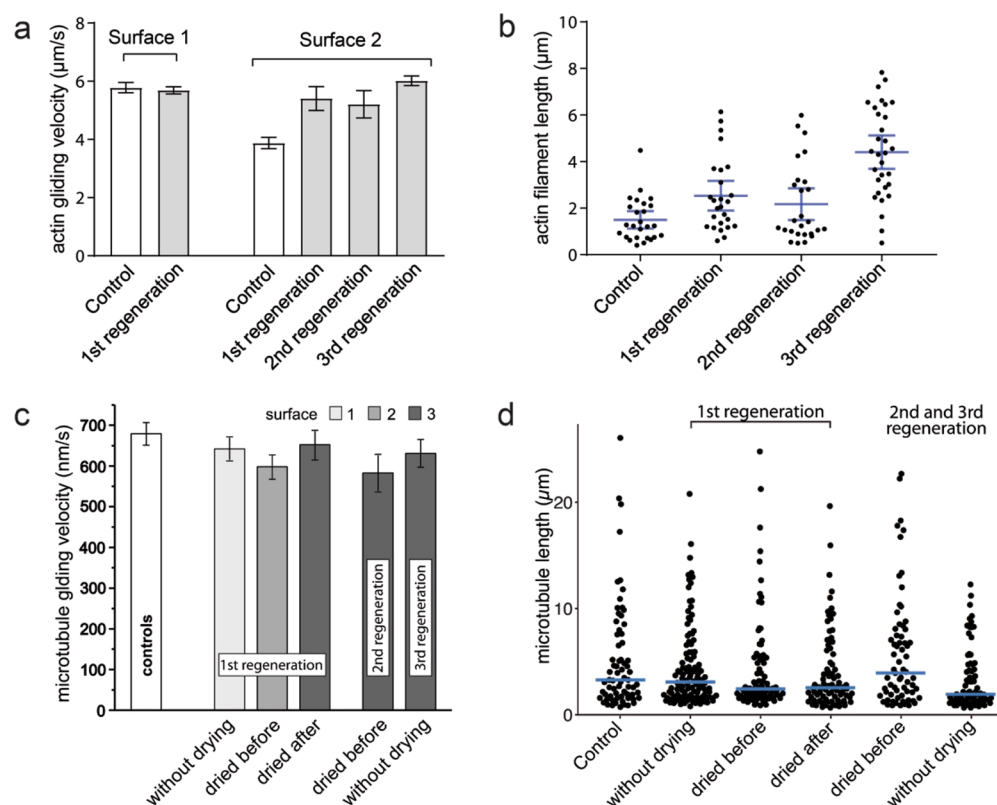


Figure 3. Analysis of motility performance after regeneration. (a) Actin gliding velocity at 25.1–26 °C produced by HMM adsorbed to TMCS-derivatized SiO₂ surfaces; “Surface 1” and “Surface 2” represent two different surfaces tested in two different experiments and were regenerated either once (no further regeneration was attempted intentionally) or three times. In each individual experiment, 10–24 actin filaments were analyzed. (b) Actin filament length distribution for filaments propelled by myosin adsorbed to one TMCS-derivatized SiO₂ surface before and after each of three regeneration cycles for “Surface 2” in (a). (c) Average microtubule gliding velocity at 25 °C in a kinesin motility assay on glass surfaces. The control value shown is the mean gliding velocity of surfaces 1–3 before regeneration. Surface 1 was regenerated directly after the control gliding assay and was tested without letting the channel dry in between. Surface 2 was processed similarly but the channel was dried before the regeneration procedure. Finally, surface 3 was regenerated and dried afterward before performing a new gliding assay. Subsequently, surface 3 was regenerated a second time (drying before regeneration) and a third time (without drying). In each individual experiment, the velocity of 277–731 microtubules was analyzed. (d) Microtubule length distribution and median length of the assays depicted in (c). The control bar represents an assay before regeneration. Velocity data are given as mean $\pm$ 95% confidence interval.

(Figure 3b) are similar before and after regeneration, therefore support efficient regeneration. This finding is consistent with contact angle measurements and analysis by Coomassie stain discussed above (Figure 1). Moreover, we could demonstrate a proof-of-principle repeatability of the regeneration procedure without decline in HMM-driven actin filament velocity (Figure 3a) by studying three subsequent regeneration cycles. Because each cycle is, nevertheless, likely associated with some deterioration either due to minimal accumulations of protein remnants on the surface or accidental failure of some step in a given regeneration cycle it is not surprising that we find a slightly increased actin filament length after the third cycle in Figure 3b. However, importantly, the lack of any trend toward a decline in velocity with repeated cycles suggests (Figure 3a) that any accumulating negative effects are of limited significance.

The results with the microtubule–kinesin system (Figures 2e–h, 3c,d) were analogous to those with the actin–myosin system. After regenerating the flow cell using the optimized protocol (Figure 1) and adsorbing new kinesin molecules, microtubules moved smoothly and the fraction of nonmotile filaments did not increase (Figure 2e–h, Movies S3 and S4, and Table S2). The microtubule gliding velocities, which are independent of the kinesin density on the surface,²⁰ remained

nearly constant upon regeneration (Figure 3c). Although the length distribution of gliding microtubules is generally dependent on the actual population, the length of the shortest gliding filaments allows some conclusion about the actual active kinesin density.⁴⁷ Thus, because the length distributions before (control) and in all conditions after the regeneration are comparable with respect to the shortest filaments (Figure 3d), we can assume that a similar motor density is achieved after regenerating the surface. When testing whether it was possible to repeatedly regenerate surfaces for kinesin–microtubule motility, we observe, in analogy to our results earlier for the actin–myosin system, no decrease in microtubule gliding velocities even after a third regeneration (Figure 3c). This suggests that there is no significant accumulation of negative effects in each regeneration cycle and that substantially more regeneration cycles will be possible than the three cycles demonstrated here. The small differences in velocity before and after regeneration (Figure 3c) are within the range of variability resulting from small temperature differences (1 K) in our setup.⁴⁸ The fact that repeatable regeneration was verified for both motor systems suggests no critical dependence of the regeneration procedure on the motor system or on the underlying surface substrate.

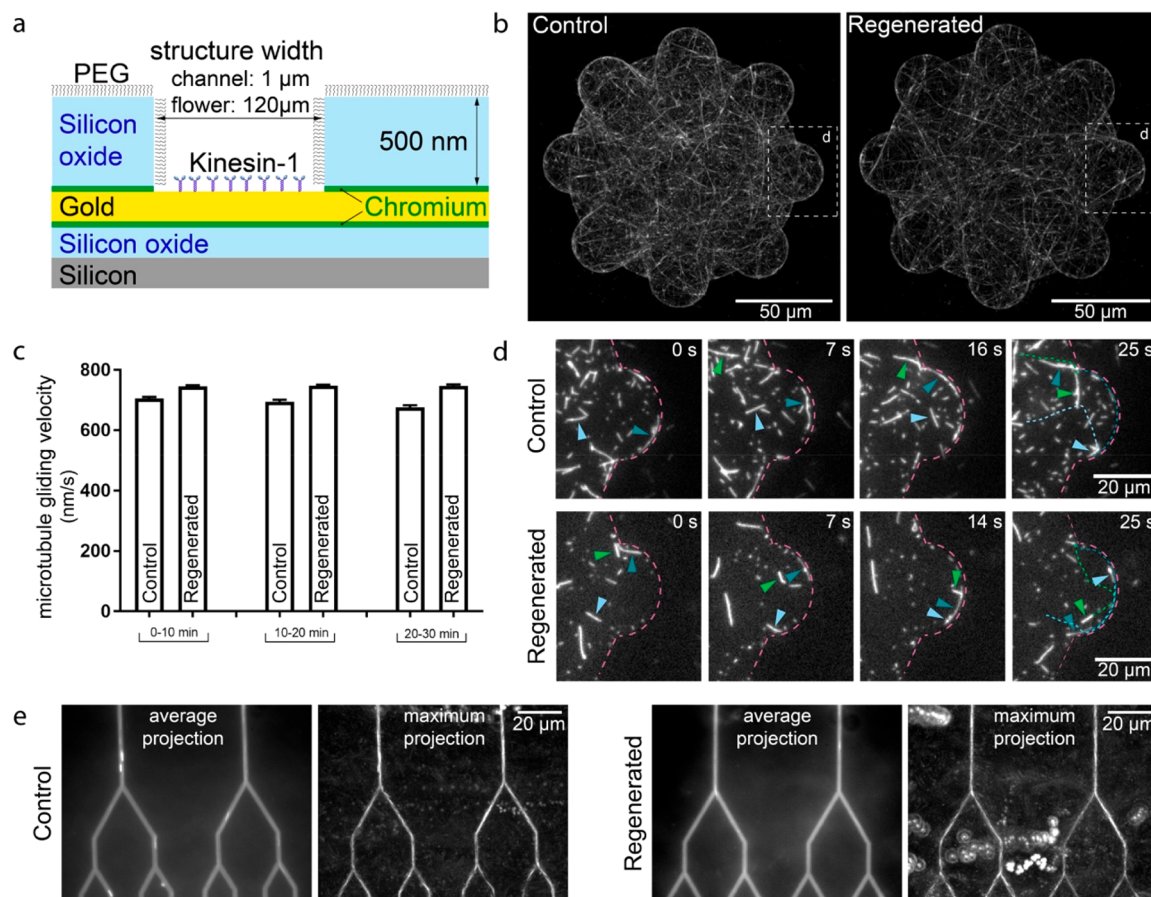


Figure 4. Regeneration of an assembled nano/microdevice with embedded microstructures for the microtubule–kinesin system. (a) Schematic cross-section of a microstructured SiO₂ surface used for regeneration. On these surfaces, motility was restricted to the gold layers on the patterned, gold-coated floor. The rest of the surface was rendered protein-repellent by a PEG coating. (b) Maximum projections (from 10 min time-lapse movies) of fluorescently labeled microtubules gliding on kinesin-coated gold floors before (“Control”) and after regeneration. (c) Microtubule gliding velocity at different time points before and after regeneration; $N = 3$ surfaces. At each individual time point, 637–1216 microtubules were analyzed. Data are given as mean $\pm 95\%$ confidence interval. (d) Time series of fluorescence micrographs showing microtubule gliding within one “petal” of the flower structure as depicted in (b) before and after regeneration. The positions of three microtubules are marked along with their trajectories. (e) Average projections as well as maximum projections of fluorescently labeled microtubules gliding within 1 μm wide channels with kinesin-coated gold floors before (“Control”) and after regeneration. The projections were derived from 30 min time-lapse movies. See also [Movies S5](#) and [S6](#).

Because the motility-supporting surfaces may dry out between repeated cycles of usage, we examined the possibility of drying the surfaces before or after regeneration. The procedure was tested for the microtubule–kinesin system on glass surfaces and was found to yield almost as successful a regeneration as without drying (Figure 3c,d; Table S2). However, a slight decrease of microtubule gliding velocity was observed, especially in the case of drying the surface before regeneration. The decrease of the mean velocity was probably due to some transiently nonmotile, short filaments as the fraction of permanently nonmotile filaments was only slightly increased. Therefore, regenerating the device immediately after usage and subsequently drying it for storage might be more reliable.

After having demonstrated the successful regeneration of planar surfaces, we tested our optimized protocol on topographically structured surfaces that were also chemically patterned to support motility only in predefined areas. For the microtubule–kinesin system, this was realized using structured SiO₂ on top of a gold-coated silicon wafer (Figure 4a). The SiO₂ walls were chemically modified with PEG to prevent

protein binding, that is, to restrict motility to the gold floors of the structures. Similar structures have been used earlier for kinesin-based nano/microdevices.^{31,49} The control motility assay showed smooth gliding of filaments and motility was restricted to the gold-coated floors of the “flower” structures (Figure 4b). The microtubule gliding velocities were around 700 nm/s and remained approximately constant over time (Figure 4c). After regenerating the surface and adsorbing new proteins, motility was still observed only on the floors, clearly demonstrating that the chemical selectivity due to the PEG-coating of the SiO₂ walls was not compromised by the regeneration procedure. The microtubule gliding velocities after regeneration slightly increased above the control value to about 740 nm/s but remained constant over time. The small velocity increase may again be explained by a slight increase of the ambient temperature in the course of the experiment. Moreover, microtubules were reliably guided at the silicon oxide walls (Figure 4d) and did not get stuck more frequently: the fraction of stuck microtubules after regeneration $f_{\text{regeneration}} = 0.09$ was comparable to the control value $f_{\text{control}} = 0.07$. Besides the flower structures, with rather large motility-

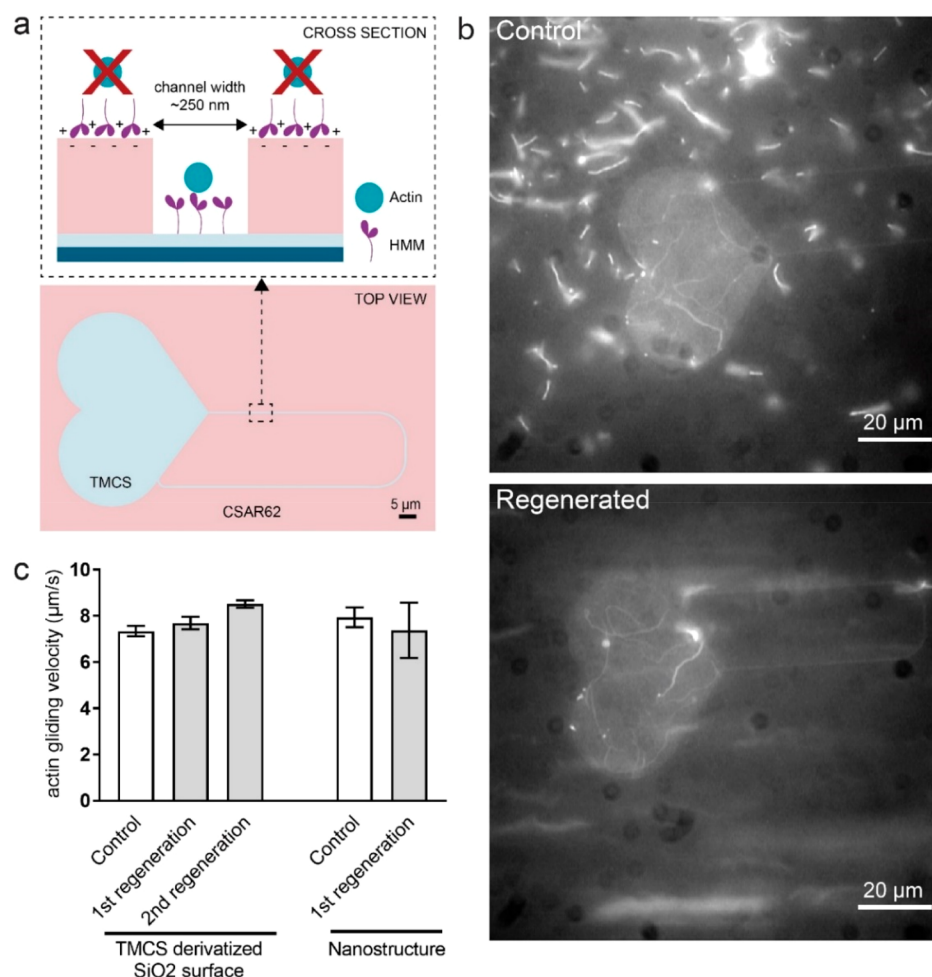


Figure 5. Regeneration of an assembled motility device with embedded nano/microstructures for the actin–myosin system applying the detergent Triton X100 (instead of SDS). (a) Schematic design of the structured SiO₂ surface used for regeneration with topographically and chemically defined areas. The structure was produced by electron beam lithography to produce CSAR62 polymer walls and surroundings (pink) of trimethylchlorosilane-derivatized-SiO₂ channels (blue). HMM motors bind to the TMCS-derivatized floors in a motility-promoting manner, whereas binding to the surrounding CSAR 62 polymer walls is inhibited.⁵¹ The large heart shaped structure acts as a loading zone for the actin filaments with the aim to guide them into the 500 nm wide feedback channel. (b) Maximum projections (from 4 s time-lapse movies) showing actin filament gliding driven by HMM motors adsorbed to TMCS-derivatized SiO₂ in the heart-shaped structure before (“Control”) and after regeneration. Feeding filaments into the channels were observed in this particular structure neither before nor after regeneration. Note, filaments floating in solution are seen as quite bright objects (due to relatively stationary position) above areas outside the loading zone in the Control. See also [Movies S7](#) and [S8](#). (c) HMM-propelled actin gliding velocity on TMCS-derivatized SiO₂ surfaces; $N = 2$ surfaces, where the first surface was a flat chip and regenerated twice and the second surface had an embedded nanostructure. On the flat SiO₂ chip, 18–25 actin filaments were analyzed and on the heart-shaped structure, 3–12 filaments were analyzed. Temperature 26.0–26.6 °C. Data are given as mean $\pm$ 95% confidence interval.

supporting areas, structures with narrow channels ($<1 \mu\text{m}$ in width) are useful for nano/microdevices, for example, in biocomputation and biosensing. When regenerating such structures we likewise obtained fully restored motility ([Figure 4e](#)) and the number of nonmotile filaments did not increase.

We also tested the method by regenerating a chemically and topographically patterned SiO₂ surface adapted for the actin–myosin system and produced using selective spin-cleaning with PDMS ([Figure S5](#)). In this case, half of the surface was covered with the motility-inhibiting CSAR62 polymer resist⁵⁰ that may be used for nanofabrication of structures on a SiO₂ chip.⁵¹ We found that the motility inhibition in the area covered with CSAR62 polymer resist was not altered by the regeneration procedure whereas the TMCS derivatized SiO₂ surface area without CSAR62 resist was fully regenerated. When regenerating a topographically and chemically patterned SiO₂ surface produced using electron beam lithography (EBL; [Figure 5](#)), we

found that a similar treatment as described above generally does not lead to successful regeneration of nano/microstructures for actomyosin motility with TMCS-derivatized SiO₂ surrounded by motility suppressing CSAR62 polymer resist. We considered the possibility that this is due to a lack of compatibility of CSAR62 with extended aqueous treatment. To test this idea, we submerged nanostructures under water for 8 h but did not observe resist lift off or other changes. We then hypothesized that the use of SDS may cause the problem as the long hydrocarbon chains of SDS may interact with the ester groups of the CSAR62 polymer resist and change the polymer properties. Moreover, SDS is anionic with appreciable net negative charge and may transfer negative charges to the CSAR62 polymer resist. The idea that the use of SDS is the basis for the problems with regeneration of nanostructured surfaces is supported by results of experiments where SDS was substituted with the nonionic detergent Triton X100 (0.05%).

In contrast to SDS, Triton X100 has neither the long hydrocarbon chains nor negative charges. Interestingly, we found that Triton X100 is equally effective as SDS for regeneration of both trimethylchlorosilane derivatized glass and SiO₂ surfaces (Figure 5) that otherwise use the standard protocol (Figure 1). Both the actin gliding velocities and the mean filament lengths were maintained at the control value after regeneration suggesting that the HMM surface density is unchanged compared to motility assays before regeneration (Figure S4). Furthermore, motility was maintained on the TMCS-derivatized loading zone of the nanostructured chips (produced by EBL) with similar quality as before regeneration (Figure 5b,c; Movies S7 and S8). Most importantly, as desired there was no motility on the surrounding resist areas. It will be of value to test and, if necessary, optimize the regeneration protocol also for narrow, submicron-wide TMCS-derivatized channels applied for the actin–myosin system.

Taken together, our results indicate that the described regeneration procedure is equally suitable for topographically structured surfaces as for flat surfaces and that it preserves chemical selectivity. Although the use of SDS as detergent is suitable in most cases it may need to be substituted by other detergents (e.g., Triton X100; Figure 5). Regeneration of 1 μm wide channels, applicable in biosensing and biocomputation devices based on the microtubule–kinesin system, could be successfully demonstrated (Figure 4e) whereas the regeneration of nanoscale channels suitable for actin–myosin based devices remain to be evaluated.

In summary, we developed and tested a method to regenerate biomolecular-motor-based nano/microdevices, assembled together with a fluidic system. The method is useful for both the actin–myosin and microtubule–kinesin systems and it allows repeated use of the devices without damaging or disassembling the fluidic system. Our findings are important because the regenerated structures were surface-functionalized as in recent nanostructured devices^{31,51} for motor-driven biocomputation or biosensing applications. In the future, possibly after further optimizations, the method may become even more important for more sophisticated bionanodevices, for example, devices with integrated nanowires and/or nanoelectronic structures for automated electrical or optical signal read-out. Furthermore, we expect that the method will find general use for the regeneration of bionanodevices as well as of nonpatterned surfaces, for example, in biosensors, also when nonmotor proteins are the key biological elements.

■ ASSOCIATED CONTENT

● Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.nanolett.9b02738.

Methodological details for all parts of the manuscript, supplementary results considering Figures S1–S5 in greater detail. Supplementary figures and tables contain information as follows: Regeneration of trimethylchlorosilane derivatized glass surfaces only with SDS (Figure S1), efficiency of regenerating trimethylchlorosilane derivatized glass surfaces with only Proteinase K. (Figure S2), efficiency of SDS (0–5%) inclusion after Proteinase K (1h) treatment for regenerating trimethylchlorosilane derivatized glass surfaces (Figure S3), efficiency of the optimized regeneration protocol on flat as well as on

micro/nanostructured surfaces with Triton X100 as detergent (Figure S4), regeneration of TMCS-derivatized and ARP (CSAR62) micropatterned surface for actomyosin motility (Figure S5), summary of findings with different approaches to test recycling of trimethylchlorosilane derivatized glass surfaces (Table S1), and fraction of stuck microtubules in a motility assay before (control) and after surface regeneration (Table S2) (PDF)

In vitro motility assay, using actin–myosin system, on HMM-coated TMCS-derivatized SiO₂ surface before surface regeneration (AVI)

In vitro motility assay, using actin–myosin system, on HMM-coated TMCS-derivatized SiO₂ surface after surface regeneration (AVI)

In vitro motility assay, using microtubule–kinesin system, on a glass-surface before surface regeneration (AVI)

In vitro motility assay, using microtubule–kinesin system, on glass-surface after surface regeneration (AVI)

In vitro motility assay, using microtubule–kinesin system, within channels (width = 1 μm) on a structured SiO₂-surface before surface regeneration (AVI)

Representative in vitro motility assay, using microtubule–kinesin system, within channels (width = 1 μm) on a structured SiO₂-surface after surface regeneration (AVI)

In vitro motility assay, using actin–myosin system, on HMM-coated TMCS-derivatized nanostructured surface before surface regeneration (AVI)

In vitro motility assay, using actin–myosin system, on HMM-coated TMCS-derivatized nanostructured surface after surface regeneration (AVI)

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Notes

The authors declare no competing financial interest.

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