Selective Adhesion of Functional Microtubules to Patterned Silane Surfaces

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ABSTRACT We show that microtubule polymers can be immobilized selectively on lithographically patterned silane surfaces while retaining their native properties. Silane films were chemisorbed on polished silicon wafers or glass coverslips and patterned using a deep UV lithographic process developed at the Naval Research Laboratory. Hydrocarbon and fluorocarbon alkyl silanes, as well as amino and thiol terminal alkyl silanes, were investigated as substrates for microtubule adhesion with retention of biological activity. Microtubules were found to adhere strongly to amine terminal silanes while retaining the ability to act as substrates for the molecular motor protein kinesin. Aminosilane patterns with linewidths varying from 1 to 50 μm were produced lithographically and used to produce patterns of selectively adhered microtubules. Microtubules were partially aligned on the patterned lines by performing the immobilization in a fluid flow field. Patterns were imaged with atomic force microscopy and differential interference contrast microscopy. Motility assays were carried out using kinesin-coated beads and observed with differential interference contrast microscopy. Kinesin bead movement on the patterned microtubules was comparable to movement on microtubule control surfaces.

INTRODUCTION

The demonstration of selective adhesion of biomaterials (cells, proteins, carbohydrates, lipids, etc.) to solid substrates requires the selection of a surface and process that allow the biomaterial to bind without causing denaturation or compromising the biological activity of interest. In addition to identifying the adhesive surface, a surface that resists nonspecific adhesion of the biomaterial in question must also be discovered to create a selective pattern of the biomaterial on a solid substrate. Generally speaking, the identification of a nonadhesive surface is often more difficult to find because most proteins will irreversibly bind to surfaces for a number of reasons (Bohnert and Horbett, 1986; Norde, 1986; Elwing et al., 1987; Vandenberg et al., 1991). In specific cases, however, the goal of spatially controlled selective adhesion ("patterning") of biomaterials has been accomplished many different ways with varying levels of success. For example, live cells have been patterned by adsorption to patterned silanes on silica (Kleinfeld et al., 1988; Dulcey et al., 1991; Stenger et al., 1992; Britland et al., 1992; Spargo et al., 1994) and to patterned organothiols on gold (Lopez et al., 1993; Singhvi et al., 1994), and by using ultrafine topographical cues to guide attachment of the cell (Clark et al., 1991). Proteins have been patterned by selective adhesion or covalent attachment to patterned surfaces (Prime and Whitesides, 1991; Rozsnyai et al., 1992; Bhatia et al., 1992, 1993) and by lithographic photoresist "lift-off" approaches (Nakamoto et al., 1988; Lom et al., 1993). In this paper we describe the interaction of microtubules with a variety of silane-modified surfaces and show that microtubules can be selectively adhered to patterned aminosilane surfaces with a minimum linewidth of approximately 2 μm.

Microtubules play a central role in both the structural and biological function of cells. Structurally, they are part of the scaffolding that defines the shape of most cellular appendages and they are also known to maintain the cytoplasmic distribution of the endoplasmic reticulum and Golgi apparatus (Lee and Chen, 1988; Kreis, 1990). Perhaps of more importance, however, is the fundamental role microtubules play in the active distribution of material and organelles throughout the cytoplasm. Molecular motor proteins, such as kinesin and dynein, use energy derived from the hydrolysis of ATP to travel along a microtubule carrying cargo such as endosomes, lysosomes, granules, vesicles, mitochondria, actin, spectrin, and more (Vale, 1987; Schroer et al., 1988, 1989; Schnapp and Reese, 1989). Kinesin and analogues move in the anteretrograde direction along the microtubule (toward the distal end), and motors in the dynein family move in the retrograde direction (toward the basal end) (Vale et al., 1985; Vale, 1987; Gibbons, 1988). In this way material can be transported independently throughout the cell along the same microtubule network. Recently, a variety of new techniques have become available for the study of microtubules and molecular motors in vitro. Among these techniques, video-enhanced differential interference contrast (DIC) microscopy (Schnapp, 1986) and optical laser trapping (Kuo and Sheetz, 1993; Svoboda et al., 1993) have had the greatest impact on the understanding of the dynamic properties at the individual molecular level. A necessary component of the success of these two

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techniques is the ability to anchor biologically active microtubules and molecular motors to glass coverslips so that measurements can be made under the microscope. With this new technology many important characteristics of the microtubule-kinesin interaction have been elucidated, including the isometric force applied by a single kinesin molecule (Kuo and Sheetz, 1993; Svoboda et al., 1993; Hunt et al., 1994) and its minimum step size (Svoboda et al., 1993).

Polymer surfaces to which both microtubules and motors adhere include positively charged materials such as polylysine and polyornithine. In this paper we describe the binding of microtubules to surfaces modified with organosilane films. Organosilane films are useful as substrates because they can be obtained commercially with a wide variety of organic functional groups and can be used to modify almost any solid having surface hydroxyl groups. Their most important property in relation to this work, however, is their ability to be chemically altered by exposure to deep-UV light (Calvert et al., 1991). Thus, exposure of silane films to patterned UV light can be used to create solid surfaces with patterns of different chemical moieties. Four different alkyl silane films were chosen for this study: a long-chain hydrocarbon silane (*n*-octadecyldimethylmethoxysilane, ODMS), a fluorinated hydrocarbon silane ((tridecafluoro-1,1,2,2-tetrahydrooctyl)-1-dimethylchlorosilane, 13F), a thiol-terminal silane (3-mercapto-propyltrimethoxysilane, MTS), and an amino-terminal silane (trimethoxysilylpropyldiethylenetriamine, DETA). Tubulin protein was found to adhere to all of these surfaces to at least some extent. The amino-silane DETA, however, promoted the strongest adhesion of microtubules without destroying their ability to act as a substrate for kinesin motility. Microtubules are known to have a low binding affinity for clean, wettable silica and glass cover slides (Sale and Fox, 1988). Thus, a surface consisting of patterned aminosilane interspersed with regions of clean silica and glass was used to produce selectively adhered microtubules. Microtubules were partially aligned on these patterned surfaces by depositing them in a fluid flow field. Microtubules that were selectively adhered to the patterned aminosilane surfaces were shown to serve as normal motility substrates for the movement of the molecular motor kinesin. This ability to pattern microtubules while retaining many of their native properties has possible implications for studying the biological mechanisms of motor activity and microtubule structure, as well as the potential for cargo transport and computational network applications in active micro-devices.

MATERIALS AND METHODS

Tubulin protein was extracted from chicken brain and purified following the procedure of Murphy and Wallis (1986). Kinesin was isolated from bovine brain by using a modification of the procedure described by Palfrey and co-workers (Matthies et al., 1993). GTP, ATP, and casein were obtained from Sigma Chemical (St. Louis, MO) and taxol from Calbiochem (La Jolla, CA). DETA, MTS, ODMS, and (tridecafluoro-1,1,2,2-tetrahydrooctyl)-1-dimethylchlorosilane (13F) were obtained from United Chemical Technologies (Piscataway, NJ). Diethylaminoethyl dextran (DEAE-dextran; 500,000 MW) was purchased from Pharmacia (Piscataway, NJ). All solvents were of reagent grade or better, and water was deionized with a resistivity greater than 15 MΩ.

Positively charged polymeric control surfaces were produced by coating coverslips with DEAE-dextran. Coverslips were cleaned with sulfuric acid, rinsed with water, and then immersed in 10 mg/ml DEAE-dextran for 10 min. After removal from the polymer solution the coverslips are rinsed with water and air-dried.

Silanes were coupled to n-type, ⟨100⟩ polished native oxide silicon wafers (WaferNet, San Jose, CA) and number 0 coverslips (Corning Glass Works, Corning, NY) using the following procedure. Wafers or slips were immersed in 1:1 concentrated HCl:methanol for 30 min, rinsed three times with water, immersed in concentrated H_2SO_4 for 30 min, rinsed three times with water, heated to 100°C for 5 min in water, and then dried with a stream of filtered nitrogen gas. In addition, the coverslips were exposed to an oxygen plasma for 2 min before acid cleaning. The DETA silane reaction mixture was prepared in a dry box and contained 1 mM acetic acid in dry methanol:water:silane (94:5:1, v/v). Clean substrates were immersed in this mixture for a reaction time of 15 min and then rinsed four times with methanol. MTS, ODMS, and 13F silane reactions were carried out in a dry box by immersing clean substrates in a 1% mixture of silane in dry toluene (v/v) for 1 h and rinsing four times with toluene. After silanization, all substrates were dried using a stream of filtered nitrogen and cured at 120°C on a hot plate for 5 min. Before use with the microtubules, the silane thickness and advancing water contact angle were measured with a Gaertner Model 115C ellipsometer (Chicago, IL) and a Zisman-type contact angle apparatus (Zisman, 1964), respectively. Silane film thickness was determined by first measuring the optical constants for the silicon wafer and assuming that the refractive index of the film is given by the manufacturer's value for the (bulk) index of refraction (Wasserman et al., 1989).

DETA silane was patterned using the procedure described in Calvert et al. (1991). Briefly, the silane was cleared from the surface of the polished silicon wafers by exposure to pulsed 193 nm deep UV ArF excimer laser light at a total dose of ~28 J/cm^2, leaving a bare silicon oxide surface behind. Similarly, the coverslips were patterned with a dose of ~13 J/cm^2. Surfaces were either flood-exposed to clear silane from the whole surface or exposed through a chromium line mask to produce a patterned array of silane and deep ultrviolet (DUV) cleared areas. DETA line patterns were made with linewidths varying between 1 and 50 μm. After the patterning process, wafers were dipped in 0.1 M KOH for 2 s (see Results for a description), rinsed three times with water, and dried with a stream of nitrogen for immediate use with the microtubules.

Microtubules were assembled ("polymerized") from approximately 50 μM tubulin in PMG buffer (100 mM piperazine-N,N'-bis(2-ethanesulfonic acid) buffer at pH 6.95 containing 1 mM $MgCl_2$ and 1 mM GTP) by incubating for 10 min at 37°C. Taxol was added to a concentration of 100 μM, and the incubation was continued for another 10 min to stabilize the microtubules so that experiments could be carried out at room temperature. After polymerization, the microtubule suspension was centrifuged through a 40% sucrose cushion for 15 min at 120,000 × *g* in a Beckman L8–80 ultracentrifuge equipped with an SW-60 rotor. The resulting pellet was resuspended and used for the atomic force microscopy (AFM) experiments. The pelleting procedure removes excess tubulin monomer, which may foul the surface by competing with the microtubules for available binding sites. When necessary, the centrifugation was repeated until a satisfactory microtubule suspension was obtained.

AFM images were acquired by using a Nanoscope III AFM (Digital Instruments, Santa Barbara, CA) operated in air. Contact images were acquired with silicon nitride cantilevers (Digital Instruments) with a bending modulus of approximately 0.06 N/m. Tapping mode images were acquired with microfabricated silicon cantilevers with a bending modulus of approximately 30–50 N/m. Minimum contact force ($\leq$15 nN) was maintained for all imaging modes. Microtubule samples for AFM were prepared using the following protocol. One to three microliters of 5 mg/ml microtubule suspension was deposited on the wafer surface. After 1 min the wafer surface was rinsed four times with water to remove microtubules that did not adhere well to the surface. The wafer was then dipped in methanol or 1% uranyl acetate to fix the microtubules and dried with a stream of nitrogen. Fixing was found to be necessary because unfixed microtubules were destroyed during the nitrogen drying step.

Patterned samples for kinesin motility studies were prepared using a protocol similar to that for the AFM samples. Ten microliters of 5 mg/ml microtubule suspension was washed over a patterned coverslip surface followed by two 10-μl rinses with PMG buffer and one 10-μl rinse with 5 mg/ml casein in motility buffer (15 mM imidazole, 1 mM EDTA, 2 mM $MgCl_2$, 1 mM ATP at pH 7). Kinesin-coated beads at 0.05 mg/ml kinesin were then introduced in motility buffer. Bead motility was observed using video-enhanced DIC microscopy on a Zeiss Axiovert inverted microscope, and images were stored for subsequent analysis on super VHS videotape.

For both wafer and coverslip substrates, the microtubules could be aligned on the surface if the microtubule suspension was made to flow across the DETA substrate during the initial deposition. For consistency, this flow pattern was maintained through subsequent rinse steps. It should be noted, however, that alignment could not be achieved by putting the microtubule-coated surface into a fluid flow field after a random initial deposition of microtubules. By that time the microtubules were bound so tightly to the DETA surface that they could neither be moved or removed by any flow field we could attain.

RESULTS

The adhesion of microtubules to four silane surfaces (DETA, MTS, 13F, ODMS) was studied, as was the degree of kinesin motility on microtubules bound to the silane surfaces. Table 1 shows average advancing water contact angles and film thickness of the four silane films. DETA and MTS have similar wettability (contact angle ~40°), whereas 13F and ODMS are both quite hydrophobic (contact angle >90°). The contact angle alone, however, was not a useful parameter for distinguishing the interaction of microtubules with these surfaces, as can be seen from the composite of AFM images shown in Fig. 1. Microtubules bound cleanly to DETA, apparently depolymerized when binding to MTS, and weakly bound to both 13F and ODMS surfaces. Kinesin motility assays were carried out on microtubules bound to coverslips modified with each of the silane-treated surfaces. Sporadic kinesin motility was noted on the 13F and ODMS surfaces, indicating that the 13F and ODMS surfaces may partially depolymerize and denature the microtubules. No microtubules were ever observed to be bound to the MTS surfaces; only random aggregates of protein were observed. Only microtubules bound to the DETA-modified coverslips supported normal motility of kinesin. Bovine brain kinesin-coated beads moved with a typical speed of ~0.5 μm/s on the DETA surface, comparable to motility rates on the DEAE-dextran control surface-bound microtubules.

DETA wafers that were flood exposed with DUV to clear (remove) the silane completely from the surface were found to promote a moderate amount of nonspecific microtubule adhesion. Rinsing the wafers with organic solvents such as chloroform or methanol did not substantially reduce the amount of adhesion. DUV cleared wafers treated with 0.1 M KOH for 2 s, however, demonstrated a dramatic decrease in microtubule adhesion. Physically, it was observed that the KOH treatment improved the wettability of the DUV cleared region. Compilation of 30 measurements taken from six separate wafers showed a slight reduction of the magnitude and statistical variation of the advancing contact angle from 4.3 ± 2.2° to 2.8 ± 1.1° in the cleared region. We have not fully investigated the cause of this improved wettability, but it may be due to removal of residual organic debris and increased hydrolysis of the silicon oxide surface. Fortunately, the KOH treatment had only a mild effect on the DETA silane surface, resulting in a change in contact angle from 40 ± 3° to 38.5 ± 5° and a change in film thickness from 6.5 ± 2 Å to 6.0 ± 2 Å. No change in the affinity of microtubules for the KOH-treated DETA surface was observed.

High-contrast selective adhesion of microtubules on DUV patterned DETA surfaces was observed most often on wafers (or cover glass) that had been treated with KOH as described above. A ratio of the total length of microtubules bound (per unit area) on the DETA portion of the surface versus the total length bound on the DUV cleared portion (per unit area) was defined as the microtubule adhesion contrast between those regions of the surface. Wafers that were not treated with KOH had a typical microtubule adhesion contrast of 10:1 or less, whereas those treated with KOH were conservatively estimated to have an adhesion contrast of 100:1 or more.

Figs. 2 and 3 show several AFM images of microtubules selectively bound to patterned DETA surfaces. In Fig. 2, *A* and *B*, microtubules were selectively adhered to 35-μm lines of DETA silane and imaged by Tapping mode AFM. No attempt was made to produce alignment by fluid flow. The enlarged area in Fig. 2 *B* shows that the actual line edge resolution (edge roughness) is approximately 1 μm or less. Fig. 2 *C* shows another 35-μm-wide line pattern made with the same sample of microtubules used in Fig. 2 *A*. In this case the microtubules have been partially aligned (orthogonal to the line direction) by applying the microtubule suspension anisotropically such that a fluid flow field is created during the initial immobilization. Fig. 2 *D* shows the two-dimensional Fourier transform of the region of microtubules in the middle of the patterned line. The elliptical shaped scatter near the center of the transform reveals the anisotropic alignment of the microtubules normal to the line direction. The "downstream" edge of the line of microtubules shows a higher edge roughness (2–3 μm) than the "upstream" edge (~1 μm), consistent with microtubules bound at one end while hanging off at the

TABLE 1 Film thickness as measured by ellipsometry and advancing water contact angles for all silane films (on polished silicon wafers)

Silane film	Contact angle	Film thickness (Å)
DETA	40 ± 3°	6.5 ± 2.0
DUV-exposed DETA*	2.8 ± 1.1°	~0
MTS	43 ± 3°	7.3 ± 2.0
ODMS	95 ± 2°	5.0 ± 2.0
13F	94 ± 2°	5.0 ± 2.0

*After KOH treatment.

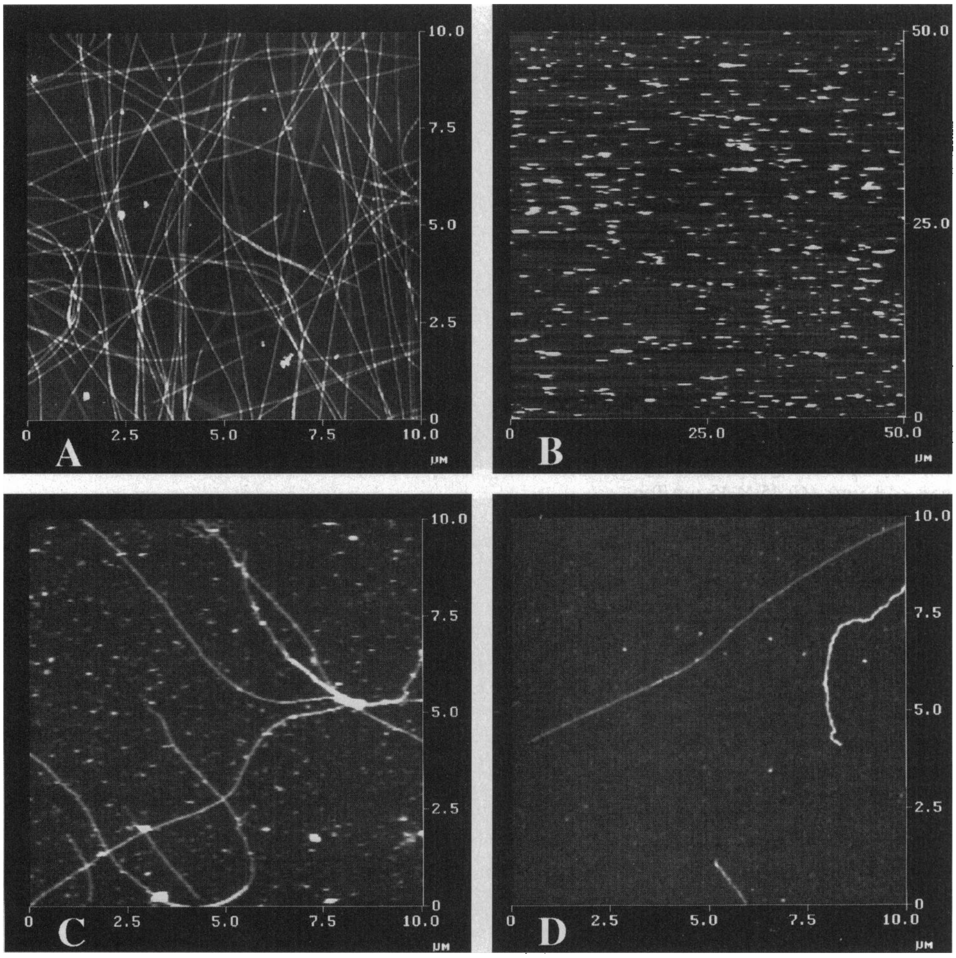

FIGURE 1 AFM images of microtubules on silane-modified silicon wafer surfaces. (*A*) 10 × 10 μm contact mode image on DETA; (*B*) 50 × 50 μm contact mode image on MTS; (*C*) 10 × 10 μm contact mode image on 13F; (*D*) 10 × 10 μm contact mode image on ODMS. The height range of the greyscale is 0 to 30 nm for all figures.

other. It is worth noting the increased fraction of nontubular aggregates seen in Fig. 2 *C* as compared to 2 *A*. This is due to the degradation and depolymerization of the microtubules during the several-hour period from the time when the samples were prepared until the images were acquired. Currently we are investigating methods for stabilizing the microtubules against this rapid degradation. Finally, Fig. 3 shows an AFM Tapping mode error signal image (see Putnam et al., 1992) of a field of microtubules patterned on 8-μm-wide lines. These short microtubules have been preferentially aligned along the length of the line.

Fig. 4 shows a series of captured DIC microscope images of kinesin-coated beads moving on an 8-μm-wide patterned line of microtubules. The microtubules were immobilized in a fluid flow field and thus have been partially aligned. The six images are a time sequence over the course of approximately 10 s. Arrows indicate beads whose movements are relatively easy to follow over this sequence of images. No preference for left or right movement was observed, indicating that although the microtubules are partially aligned by the fluid flow they are not oriented by the flow treatment, i.e., the distal ends of the microtubules are not all pointing in the same direction.

DISCUSSION

The interaction of microtubules with surfaces can be varied greatly by modifying the chemistry of the surface. Microtubules could be bound to hydrophobic surfaces (13F and ODMS), although the binding affinity was weak compared to DETA and positively charged DEAE-dextran control surfaces. Microtubules bound to hydrophobic surfaces were poor motility substrates for kinesin, exhibiting only sporadic bead movement in the presence of ATP. It is not surprising that the microtubules adhered to these surfaces, because proteins are often found to denature and bind irreversibly when exposed to hydrophobic surfaces (Bohnert and Horbett, 1986; Norde, 1986; Elwing et al., 1987; Vandenberg et al., 1991). In light of this potential for denaturation and subsequent partial depolymerization of the microtubule, kinesin would be expected to move only in the regions of the microtubule where the periodic array of kinesin-binding sites remained undamaged.

The stark contrast of the interaction of microtubules with the DETA and MTS surfaces was quite surprising because MTS has been used as a primary surface treatment for covalent coupling of proteins (Bhatia et al.,

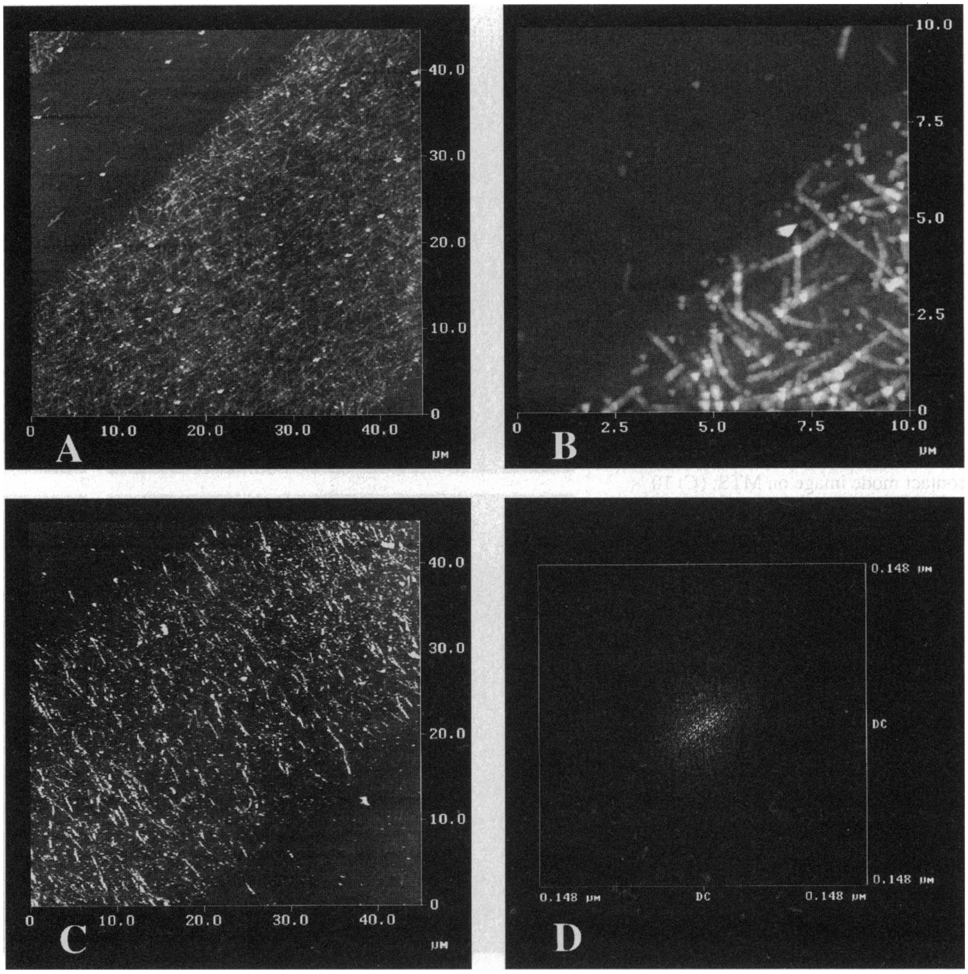

FIGURE 2 AFM images of microtubule patterns on patterned DETA surfaces. (*A*) 45 × 45 μm Tapping mode image of microtubules bound to 35-μm-wide DETA lines, height greyscale = 0 to 30 nm; (*B*) 10 × 10 μm enlarged area of the edge of the pattern in *A* showing the edge roughness (<1 μm); (*C*) 45 × 45 μm Tapping mode image of partially aligned microtubules on 35-μm-wide DETA lines, height greyscale = 0 to 30 nm; (*D*) Two-dimensional Fourier transform of the microtubule region in the center of the line in *C* showing the alignment of the microtubules orthogonal to the line direction.

1989, 1992, 1993). The MTS surface caused complete depolymerization of the microtubules. In fact, not a single intact microtubule was observed on the MTS surface when imaging with either AFM or DIC microscopy. In contrast, the DETA surface performed as well as control surfaces for immobilizing intact microtubules while preserving kinesin motility. Despite their similarity in wettability, the chemistries of the DETA and MTS surfaces are dramatically different. At neutral pH the amines of the DETA surface will be protonated, yielding a uniform surface with a net positive charge. In contrast, the MTS surface may have as many as three different chemical moieties present: electrically neutral sulfhydryls and disulfides, and negatively charged sulfonates (Bhatia et al., 1993). Because our surfaces were not directly exposed to a strong oxidizer or large doses of UV light we expect that the predominant moieties present are the sulhydryls and the disulfides. Because the surface of tubulin is highly acidic with a net negative charge at normal pH (Fields and Lee, 1984) one expects microtubules to bind strongly to the positively charged DETA. On the contrary, the strong interaction of the microtubules with the MTS surface was unexpected. The depolymerization of the microtubules on the MTS surface may be due to some structural rearrangement of the tubulin monomer itself. For example, the thiols present on the MTS surface could destabilize tubulin by chemically reducing the disulfide bridges within the folded tubulin structure. Evaluation of this scenario will require further study.

Treatment of patterned DETA surfaces with KOH was found to be essential for producing high-contrast selective adhesion of microtubules to the regions of DETA. Without this treatment, a substantial amount of nonspecific adhesion of the microtubules to the DUV cleared areas of the surface was observed. Perhaps the most significant physical change in the cleared areas after the KOH treatment is the reduction in the statistical variation of the advancing contact angle, going from ±2.2° to ±1.1°. This indicates the presence of a chemically more uniform surface, which may be due to at least two possible factors: 1) more uniform distribution of surface hydroxyl groups and 2) removal of residual organic debris left after the laser exposure. The latter is likely to be of greatest importance because the laser treatment is not expected to fully remove all organic residue from the surface. Unfortunately, we have been unable to quantify which of these two possibilities is most important because the amount of debris present on the surface is

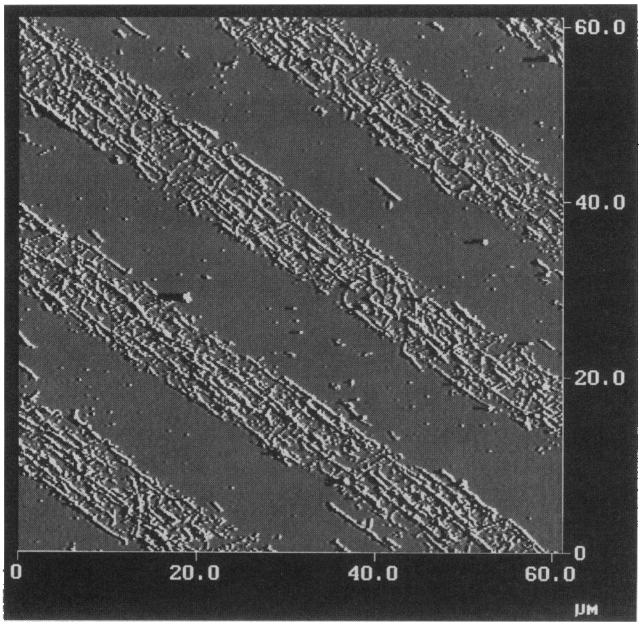

FIGURE 3 60 × 60 nm AFM Tapping mode error signal image of partially aligned microtubules on 8-µm-wide DETA lines. Height greyscale = 0 to 10 nm.

below the detection limit for ellipsometry and cannot be clearly distinguished with contact or Tapping mode AFM. In the future, however, it may be possible to observe residual debris by using lateral force microscopy.

Selective adhesion of microtubules on DETA patterns was observed for linewidths greater than 2 µm. The best examples of selectivity were observed on linewidths greater than 8 µm because the length of the microtubules often caused overlap between the patterned lines. Microtubules that were purposely shortened by vortexing or gentle sonication did show the best selective adhesion on the narrower lines; however, these microtubule suspensions were also the least stable and degraded rapidly at room temperature. No selective adhesion was observed on 1-µm lines because even the shortest microtubules bridged these lines. Long microtubules could be partially aligned by fluid flow on the patterned substrates (Figs. 2 *B* and 3), but the degree of alignment was not sufficient to overcome the problem of microtubule overlap on the narrow lines. This is evident even on the 8-µm lines in Fig. 3.

Microtubules bound to both patterned and uniform DETA surfaces showed normal kinesin motility. Bead velocities were approximately 0.5 µm/s, which is within the error of the kinesin bead velocity observed on microtubules bound to the control surfaces. Bead movement on flow-aligned microtubules was also normal, and there was no evidence that the alignment procedure produced a predominant orientation (polarity) of the microtubules. Movement along both directions of the aligned microtubules was equally probable. It may be possible to break this symmetry by modifying one end of the microtubule with a bulky group, which would act as a "sail" or an "anchor" in the flow field. Perhaps this could be accomplished by incubating kinesin-coated beads with a dilute suspension of microtubules until all microtubules ended up with a bead bound at their distal end.

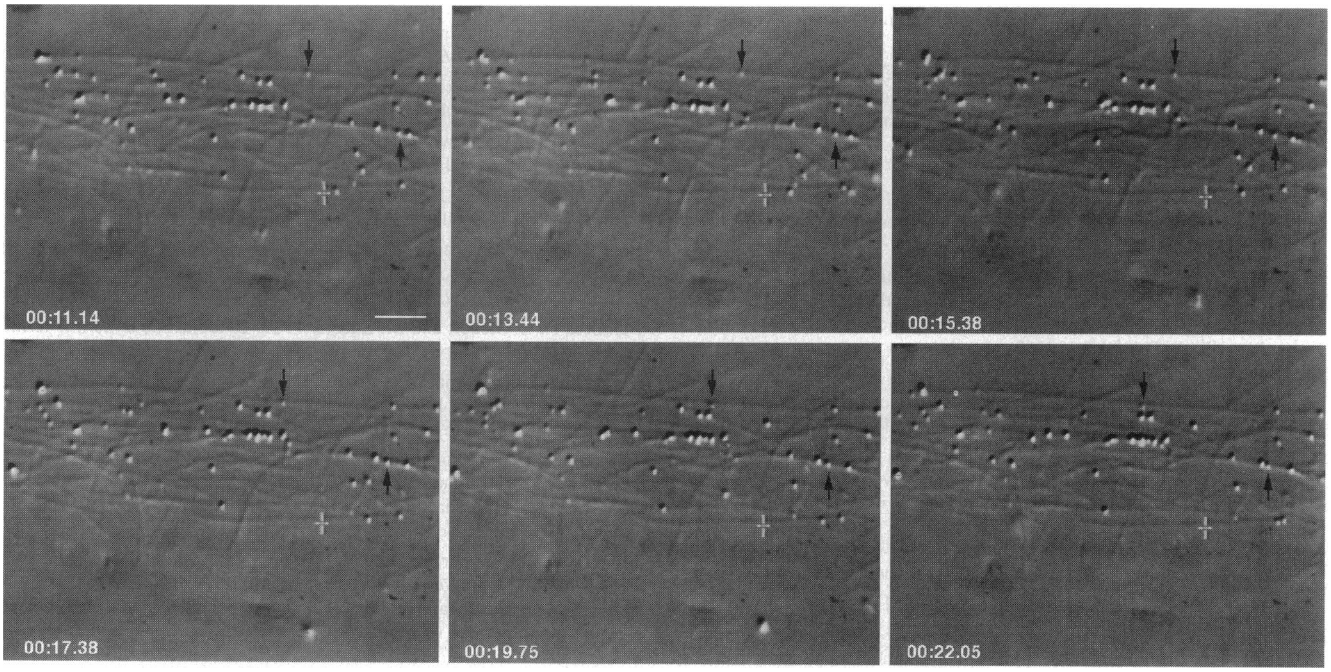

FIGURE 4 DIC microscope images of kinesin-coated beads moving on an 8-µm-wide patterned line of microtubules. The microtubules have been partially aligned. The six images are a time sequence over the course of approximately 10 s, as shown by the timer in the lower left corner of the images.

The ability to form patterned arrays of microtubules on surfaces may have an impact on several areas of research and technology, including novel micromechanical motors, control of live cell growth on solid substrates, and development of novel computation and cargo transport networks (Hameroff et al., 1992). To address these issues, several additional problems need to be overcome besides the selective adhesion of microtubules on patterned surfaces. Control of the orientation (polarity) and stability of the patterned microtubules are two of the most critical issues. The ability to create meandering paths of immobilized microtubules with well-defined polarity and long-term stability is essential for any potential technological application. We are currently studying how kinesin may be used to create microtubule patterns with defined polarity by directing microtubule "traffic" during the immobilization process. In addition, we are continuing to study methods for chemically stabilizing microtubules without destroying their ability to act as a substrate for kinesin (Turner et al., 1994).

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REFERENCES

Bhatia, S. K., J. J. Hickman, and F. S. Ligler. 1992. New approaches to producing patterned biomolecular assemblies. *J. Am. Chem. Soc.* 114: 4432–4433.

Bhatia, S. K., L. C. Shriver-Lake, K. J. Prior, J. H. Georger, J. M. Calvert, R. Bredehorst, and F. S. Ligler. 1989. Use of thiol-terminal silanes and heterobifunctional crosslinkers for immobilization of antibodies on silica surfaces. *Anal. Biochem.* 178:408–413.

Bhatia, S. K., J. L. Teixeira, M. Anderson, L. C. Shriver-Lake, J. M. Calvert, J. H. Georger, J. J. Hickman, C. S. Dulcey, P. E. Schoen, and F. S. Ligler. 1993. Fabrication of sufaces resistant to protein adsorption and application to two-dimensional patterning. *Anal. Biochem.* 208: 197–205.

Bohnert, J. L., and T. L. Horbett. 1986. Changes in fibrinogen and albumin interactions with polymers indicated by decreases in detergent elutability. *J. Colloid Interface Sci.* 111:363–377.

Britland, S., E. Perez-Arnaud, P. Clark, B. L. McGinn, P. Connolly, and G. Moores. 1992. Micropatterning proteins and synthetic peptides on solid supports: a novel application for microelectronics fabrication technology. *Biotechnol. Prog.* 8:155–160.

Calvert, J. M., M.-S. Chen, C. S. Dulcey, J. H. Georger, M. Peckarar, J. M. Schnur, and P. E. Schoen. 1991. Deep ultraviolet patterning of monolayer films for high resolution lithography. *J. Vac. Sci. Technol.* B9: 3447–3450.

Clark, P., P. Connolly, A. S. G. Curtis, J. A. T. Dow, and C. D. Wilkinson. 1991. Cell guidance by ultrafine topography in vitro. *J. Cell. Sci.* 99:73–77.

Dulcey, C., J. Georger, V. Krauthamer, D. Stenger, T. Fare, and J. Calvert. 1991. Deep UV photochemistry of chemisorbed monolayers: patterned coplanar molecular assemblies. *Science.* 252:551–554.

Elwing, H., S. Welin, A. Askendal, U. Nilsson, and I. Lundström. 1987. A wettability gradient method for studies of macromolecular interactions at the liquid/solid interface. *J. Colloid Interface Sci.* 119:203–210.

Fields, D., and J. Lee. 1984. Heterogeneity of vertebrate brain tubulins. *Proc. Natl. Acad. Sci. USA.* 81:4041–4045.

Gibbons, I. 1988. Dynein ATPases as microtubule motors. *J. Biol. Chem.* 263:15837–15840.

Hameroff, S., J. Dayhoff, R. Lahoz-Beltra, A. Samsonovich, and S. Rasmussen. 1992. Models for molecular computation: cellular automata in the cytoskeleton. *Computer.* 25:30–39.

Hunt, A. J., F. Gittes, and J. Howard. 1994. The force exerted by a single kinesin molecule against a viscous load. *Biophys. J.* 67:766–781.

Kleinfeld, D., K. Kahler, and P. Hockberger. 1988. Controlled outgrowth of dissociated neurons on patterned substrates. *J. Neurosci.* 8:4096–4120.

Kreis, T. 1990. Role of microtubules in the organization of the Golgi apparatus. *Cell Motil. Cytoskel.* 15:67–70.

Kuo, S., and M. Sheetz. 1993. Force of a single kinesin molecule measured with optical tweezers. *Science.* 260:232–234.

Lee, C., and L. Chen. 1988. Dynamic behavior of endoplasmic reticulum in living cells. *Cell.* 54:37–46.

Lom, B., K. E. Healy, and P. E. Hockberger. 1993. A versatile technique for patterning biomolecules onto glass coverslips. *J. Neurosci. Methods.* 50:385–397.

Lopez, G., M. Alber, S. Schrieber, R. Carroll, E. Peralta, and G. Whitesides. 1993. Convenient methods for patterning the adhesion of mammalian cells to surfaces using self-assembled monolayers of alkanethiolates on gold. *J. Am. Chem. Soc.* 115:5877–5878.

Matthies, H. J. G., R. J. Miller, and H. C. Palfrey. 1993. Calmodulin binding to and cAMP-dependent phosphorylation of kinesin light chains modulate kinesin ATPase activity. *J. Biol. Chem.* 268:11176–11187.

Murphy, D., and K. Wallis. 1986. Erythrocyte microtubule assembly in vitro. *J. Biol. Chem.* 261:2319–2324.

Nakamoto, S., N. Ito, T. Kuriyama, and J. Kimura. 1988. A lift-off method for patterning enzyme-immobilized membranes in multi-biosensors. *Sensors Actuators.* 13:165–172.

Norde, W. 1986. Adsorption of proteins from solution at the solid-liquid interface. *Adv. Colloid Interface Sci.* 25:267–340.

Prime, K. L., and G. M. Whitesides. 1991. Self-assembled organic monolayers: model systems for studying the adsoption of proteins at surfaces. *Science.* 252:1164–1167.

Putnam, C. A. J., K. O. van der Werf, B. G. de Grooth, N. F. van Hulst, J. Greve, and P. J. Hansma. 1992. A new imaging mode in atomic force microscopy based on the error signal. *SPIE Scanning Probe Microscopies.* 1639:198–204.

Rozsnyai, L. F., D. R. Benson, S. P. A. Fodor, and P. G. Schultz. 1992. Photolithographic immobilization of biopolymers on solid supports. *Angew. Chem. Int. Ed. Engl.* 31:759–761.

Sale, W., and L. Fox. 1988. Isolated b-heavy chain subunit of dynein translocates microtubules in vitro. *J. Cell Biol.* 107:1793–1797.

Schnapp, B. 1986. Viewing single microtubules by video light microscopy. *Methods Enzymol.* 134:561–573.

Schnapp, B., and T. Reese. 1989. Dynein is the motor for retrograde transport of organelles. *Proc. Natl. Acad. Sci. USA.* 86:1548–1552.

Schroer, T., B. Schnapp, T. Reese, and M. Sheetz. 1988. The role of kinesin and other soluble factors in organelle movement along microtubules. *J. Cell Biol.* 107:1785–1792.

Schroer, T., E. Steuer, and M. Sheetz. 1989. Cytoplasmic dynein is terminus end-directed motor for membranous organelles. *Cell.* 56:937–946.

Singhvi, R., R. Kumar, G. Lopez, G. Stephanopoulos, D. Wang, G. Whitesides, and D. Ingber. 1994. Engineering cell shape and function. *Science.* 264:696–698.

Spargo, B., M. Testoff, T. Nielson, D. Stenger, J. Hickman, and A. Rudolph. 1994. Spatially controlled adhesion, spreading, and differentiation of endothelial cells on self-assembled molecular monolayers. *Proc. Natl. Acad. Sci. USA.* 91:11070–11074.

Stenger, D., J. Georger, C. Dulcey, J. Hickman, A. Rudolph, T. Nielsen, S. McCort, and J. Calvert. 1992. Coplanar molecular assemblies of amino- and perfluorinated alkylsilanes: characterization and geometric definition of mammalian cell adhesion and growth. *J. Am. Chem. Soc.* 114: 8435–8442.

Svoboda, K., C. Schmidt, B. Schnapp, and S. Block. 1993. Direct observation of kinesin stepping by optical trapping interferometry. *Nature.* 365:721–727.

Turner, D. C., C. Chang, S. L. Brandow, and D. B. Murphy. 1994. Observation of kinesin motility on chemically cross-linked microtubules. *In* American Society for Cell Biology 34th Annual Meeting, San Francisco, CA. (Abstr.)

Vale, R. 1987. Intracellular transport using microtubule-based motors. *Annu. Rev. Cell Biol.* 3:347–378.

Vale, R., T. Reese, and M. Sheetz. 1985. Identification of a novel force-generating protein, kinesin, involved in microtubule-based motility. *Cell.* 42:39–50.

Vandenberg, E., H. Elwing, A. Askendal, and I. Lundström. 1991. Protein immobilization to 3-aminopropyl triethoxy silane/gluteraldehyde surfaces: characterization by detergent washing. *J. Colloid Interface Sci.* 143:327–335.

Wasserman, S., G. Whitesides, I. Tidswell, B. Ocko, P. Pershan, and J. Axe. 1989. The structure of self-assembled monolayers of alkylsiloxanes on silicon: a comparison of results from ellipsometry and low-angle x-ray diffraction. *J. Am. Chem. Soc.* 111: 5852–5861.

Zisman, W. 1996. Relation of equilibrium contact angle to liquid and solid constitution. *In* Contact Angles, Wettability and Adhesion, Vol. 43 of Advances in Chemistry. F. Fowkes, editor. American Chemical Society, Washington, DC. 1–51.