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Polarized Alignment and Surface Immobilization of Microtubules for Kinesin-Powered Nanodevices

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ABSTRACT

Kinesin is a nanometer-scale, ATP-powered molecular motor protein that steps along the surface of microtubules. The microtubule tracks are cytoskeletal filaments, 24 nm in diameter, with an overall structural polarity that results from their asymmetric tubulin subunits. Kinesin recognizes the structural polarity and moves toward only one end of a microtubule. A major obstacle in the way of creating kinesin-powered devices is the development of methods for the controlled positioning and alignment of the microtubule tracks. Only by positioning microtubules with defined polarity can kinesin transport, or force generation, be directed efficiently within a device. Here, we report a method to surface immobilize microtubules with approximately 90% parallel alignment using a single-chain antibody that binds α-tubulin exposed at the microtubule minus end.

Natural kinesin is an elongated heterotetrameric protein, about 80 nm in length, consisting of two dimerized heavy chains and two light chains.[1] Force generation occurs in the amino-terminal motor domains, 4 × 7 nm pear-shaped regions,[2] with a mass of about 40 kd each. The motor domains contain adenosine triphosphate (ATP) and microtubule binding sites. Mechanistically, kinesin is a stepping motor. The side-by-side motor domains alternately bind and release the tubulin subunits of a microtubule in a walking or waddling type of motion.[3] The energy for the stepping motion is derived from the hydrolysis of ATP. For each ATP hydrolyzed by one of the motor domains, kinesin takes an 8 nm step along the microtubule surface,[4] a distance corresponding to the periodicity of the tubulin subunits in the microtubule lattice and the spacing between adjacent kinesin binding sites. From load-velocity experiments, it has been shown that single kinesin molecules can generate peak forces of 6−7 pN[5,6] during each step with about 50−70% efficiency. The nm dimensions suggest that kinesin can theoretically be surface immobilized with packing densities approaching 10^5 motors per μm^2, which makes cumulative forces on the order of 10s of nN per μm^2 and power densities on the order of 0.5 pW/μm^2 theoretically possible. Considering its size, efficiency, and our ability to genetically manipulate kinesin, we think that it will be possible to integrate kinesin into useful nanomechanisms or devices.[7] Other research groups have reported similar goals.[8−10]

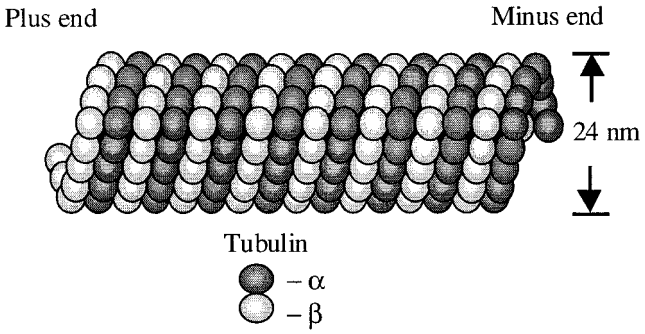

Figure 1. Microtubule structure. Microtubules are 24 nm hollow tubes formed by the self-assembly of tubulin heterodimers. The asymmetry of the tubulin subunits gives the microtubule polymer an inherent structural polarity. One end of the microtubule terminates with α tubulin subunits, whereas the opposite end terminates with β subunits. These termini are referred to as minus ends and plus ends, respectively.

Kinesin's microtubule tracks are rigid, hollow tubes formed by the self-assembly of tubulin protein subunits. Each tubulin subunit is an asymmetric heterodimer comprised of α and β tubulin. The asymmetry of the tubulin subunits and the head-to-tail assembly of the subunits into parallel protofilaments give microtubules an overall structural polarity (Figure 1). The kinetically more dynamic end of the microtubule (referred to as the plus end) is terminated with β tubulin, whereas the opposite end (the minus end) is terminated with α tubulin.[11] The microtubule structural polarity is recognized by motor proteins, which determines the direction of their motion along the microtubule surface.

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Kinesin steps only toward the plus end. So to efficiently direct kinesin motion, or force, as part of a mechanism, the polarity of the microtubule tracks must be controlled. Toward this end, we have explored several conditions for flow-aligning and immobilizing microtubules on the surface of microscope flow chambers.

Open ended flow chambers with dimensions of 24 mm × 5 mm × 100 μm and volumes of approximately 15 μL were constructed on microscope slides with coverslips and two strips of double-coated Scotch tape. Dilute suspensions of paclitaxel-stabilized microtubules (0.24 μM tubulin; ~9 × 10^6 microtubules/μL) were introduced into dry flow chambers by capillary action and observed by video microscopy. A rigid rod in dilute suspension will line up along the flow direction if the aspect ratio is large and if the hydrodynamic forces dominate Brownian forces.[12] Microtubules are rigid; the persistence length has been estimated to be between 2 and 10 mm.[13−15] The average length of microtubules polymerized in vitro is typically between 10 and 30 μm, resulting in an average aspect ratio on the order of 1000. The last condition occurs when the rotational Peclet number $P_e = \gamma/D_r \gg 1$, where γ is the shear rate and D_r is the rotational diffusion coefficient. The average shear rate when microtubule solutions were introduced into flow chambers at an average flow rate of 6.5 μL/s was on the order of 400 s^{-1}, and the rotational diffusion coefficient of a 10-μm microtubule is on the order of 0.02 s^{-1}, resulting in a P_e of 20 000 (see Supporting Information). Not surprisingly, therefore, microtubules immobilized on the surface of the flow chamber under these conditions were aligned in the direction of fluid flow.[7] An implication of the large aspect ratio, rigidity, and small rotational diffusion constant of microtubules is that they will align under the influence of much smaller shear forces than used in our experiments.

To determine microtubule orientation, polarity-marked microtubules were assembled by polymerizing dimly labeled tubulin onto bright flourescently labeled microtubule seeds.[16] The polarity of the resulting microtubules can be determined from the asymmetric position of the bright seed since tubulin adds to the plus end of the seed at about 3 times the rate as at the minus end. The labeled microtubules were observed by epifluorescence video microscopy in the presence of an enzyme-based system[16] to slow photobleaching of the fluorescence. In initial experiments, the effect of coverslip surface chemistry on microtubule polar orientation was examined. The expectation was that microtubules would have random orientations. As shown in Table 1, microtubules aligned and immobilized on the surface of flow chambers assembled with coverslips cleaned with KOH saturated ethanol approached a random distribution of orientations with equal numbers pointing in both directions. The deviation from a random distribution may be due to systematic experimental errors in scoring the orientation of the marked microtubules. For example, long microtubules that break on the plus side of the bright segment while flowing into the chamber could be scored incorrectly based on the apparent position of the mark. The distribution of microtubule orientations on amino-silane (trimethoxysilylpropyl-diethylenetriamine; DETA) treated surfaces was also determined. Electrostatic interactions of microtubules, which have a net negative charge at neutral pH, with positively charged amino-silanized surfaces is a common method used to immobilize microtubules.[17,18] Interestingly, on DETA surfaces the deviation from a random distribution of orientations was opposite from KOH/EtOH coverslips; the distribution is skewed with the minus ends pointing in the direction of flow. Apparently surface chemistry affects the orientation of flow-aligned microtubules, although the microtubules are still far from suitably oriented.

Table 1. Microtubule Alignment in Microscope Flow Chamber

	minus end		
coverslip surface	downstream (%)	upstream (%)	MTs counted
clean	45.3	54.6	150
DETA[a]	58.9	41.1	586
clean (VαNT1[b] fletching)	43.3	56.6	369
DETA (VαNT1[b] fletching)	52.5	47.5	505
DETA (K685H[c] fletching)	58.8	41.2	629
F108−NTA[d]−Ni^{2+} [e]−VαNT1	9.7	90.3	506[f]

[a] DETA − trimethoxysilylpropyl-diethylenetriamine. [b] VαNT1- histidine-tagged α-tubulin single-chain antibody. [c] K685H − truncated kinesin motor protein. [d] NTA − nitrilotriacetic acid. [e] F108−NTA−Ni^{2+} − metal-chelating Pluronic surfactant with chelated Ni^{2+}. [f] In the absence of VαNT1, zero microtubules bound to the F108−NTA-Ni^{2+} treated surfaces.

As an approach to orienting microtubules in the flow field, mass was added asymmetrically to the microtubules before flowing into the chamber. The rationale was that asymmetry in their hydrodynamic shape might cause the microtubules to orient during flow. Since kinesin has been shown to accumulate at the plus end of microtubules due to its motor activity,[19] it was possible that kinesin could asymmetrically alter the shape of microtubules and affect their flow. To test this possibility, a recombinant kinesin (K685H) was premixed with microtubules at a ratio of 1 kinesin to 10 tubulin subunits in the presence of ATP before flowing the microtubules into a DETA chamber. The presence of K685H on the microtubules had no effect on the distribution of microtubule orientations on DETA coverslips (Table 1). As an alternative method, mass was selectively added to the minus ends by premixing microtubules with a single-chain α-tubulin antibody (VαNT1) that binds specifically to the N-terminus of α-tubulin exposed only at the minus end of microtubules. The VαNT1 antibody was developed by phage display techniques.[11] The presence of the antibody on the minus end during flow alignment had no effect on the distribution of orientations on KOH/EtOH coverslips, but on DETA coverslips shifted the distribution closer to random (Table 1).

The specificity of the VαNT1 antibody for the tip of the microtubule minus end allowed us to bind one microtubule end to the surface before flow alignment as another approach to controlling microtubule polarization. The recombinant VαNT1 single-chain antibody has a C-terminal his tag, which was used to immobilize the antibody on flow chamber surfaces using a metal-chelating surfactant, Pluronic F108−NTA.[20] The chelating surfactant has the additional advantage

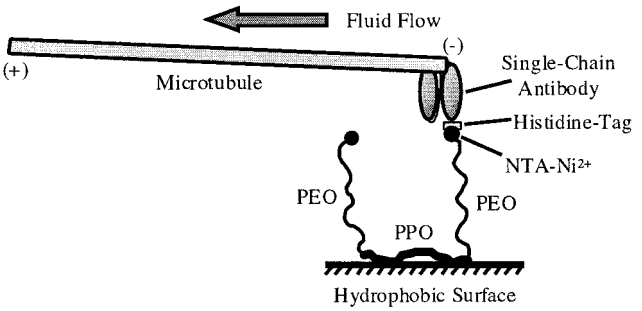

Figure 2. Immobilization and polar alignment of microtubules. A metal-chelating Pluronic surfactant is used to immobilize the minus end of microtubules on a hydrophobic microscope coverslip using a histidine-tagged α-tubulin single-chain antibody. The microtubules are polar aligned by fluid flow in a flow chamber.

of limiting nonspecific protein adsorption onto the flow chamber surfaces. Coverslips were first treated with dimethyldichlorosilane (DDS) to create a hydrophobic surface. The F108−NTA surfactant (1% w/v in water) was precharged with Ni^{2+} and loaded into the flow chamber to coat the hydrophobic coverslips. After washing, a solution of VαNT1 was added to the flow chamber and allowed to bind through affinity for nickel ions immobilized on the chelating surfactant. Excess VαNT1 was washed out, and a solution of polarity-marked microtubules was added. Microtubules bound to the surface antibodies were then aligned by flowing fluid through the flow chamber, as diagrammed in Figure 2. Because the microtubules were immobilized through only one end, a 0.2% (w/v) methyl cellulose solution was added to the chamber to restrict Brownian motion of the aligned microtubules. Low concentrations of methyl cellulose, through a volume exclusion effect, press microtubules onto the coverslip surface, which prevented reorientation and allowed clear images to be acquired by video microscopy. Methyl cellulose does not interfere with kinesin motility.[21,22] Flow alignment of microtubules held by one end by the minus-end-specific antibody were highly oriented (Table 1). Approximately 90% of the microtubules were oriented with their minus ends pointing upstream, as would be expected. The orientations might be even more polarized. As discussed above, breakage of microtubules during handling, which is more likely to occur on the longer plus-end side of the fluorescent mark, may have skewed the results away from 100%. In control experiments, microtubules did not bind to the F108−NTA−Ni^{2+} surface in the absence of the antibody.

In an earlier report,[7] we demonstrated that kinesin-driven silicon microchips interacting with multiple aligned microtubules can follow the path of parallel microtubules or can rotate at a stationary point if the microtubules are antiparallel. It is evident from these earlier results, that the ability to control microtubule orientation will now allow us to precisely direct kinesin forces and design efficient and sophisticated mechanisms. For example, on an array of aligned and polarized microtubules, large numbers of kinesins could be "ganged" by coupling through a rigid mechanical element−a machine part−to produce relatively large, directed, cumulative forces. To illustrate, the density of microtubules aligned and oriented with VαNT1 in our experiments was approximately 19 600 per mm^2. If only one kinesin motor, capable of generating 6−7 pN of peak force, interacted with each microtubule, directed, cumulative forces on the order of 100 nN could operate on mm^2 machine parts. If several thousands of kinesins were interacting with each microtubule, then cumulative, directed forces on the order of $\mu N/mm^2$ could be generated. Such forces could be used to power pumps or valves in fluidic devices over a wide range of length scales. With optimization, much higher densities of immobilized and oriented microtubules should be possible using the VαNT1 antibody, which would result in much higher directed forces.

In future experiments, we intend to develop methods of patterning the metal-chelating surfactant and VαNT1 antibody to create patterns of microtubules with opposite, orthogonal, or oblique orientations. Microtubules will be end bound to a pattern of VαNT1, aligned in a chosen direction by fluid flow, and tacked down using a secondary immobilization chemistry, e.g., photocrosslinking. The Pluronic F108 surface modification reagent can be adapted conveniently for multiple immobilization chemistries by mixing Pluronics with different end-group modifications.[23] Two strips of microtubules, oriented in opposite directions, could be used to rotate stationary gears or rotors equipped with surface-bound kinesin. The most practical application of such mechanisms might be in implantable medical devices where endogenous ATP could potentially fuel the device.

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Supporting Information Available: Fluid mechanical analysis of microtubule flow alignment, coverslip preparation, and VαNT1 expression and purification. This material is available free of charge via the Internet at http://pubs.acs.org.

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