Electronic Supplementary Information

Controlling the length of self-assembled microtubes through mechanical stress-induced scission

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Materials and Methods

Preparation of tubulin and kinesin

Tubulin was purified from porcine brain using high-concentration PIPES buffer (1 M PIPES, 20 mM EGTA, 10 mM $MgCl_2$; pH adjusted to 6.8 using KOH).[1] High-molarity PIPES buffer (HMPB) and BRB80 buffer were prepared using PIPES from Sigma, by adjusting the pH using KOH. The concentration of the purified tubulin was estimated from the absorbance at 280 nm and using the molar absorption coefficient=115,000 $M^{-1}cm^{-1}$. GFP-fused kinesin-1, consisting of the first 560 amino acid residue of human kinesin-1 (K560-GFP), was prepared as previously reported.[2] The concentration of the purified kinesin was estimated from the results of sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE). Bovine serum albumin (BSA) solutions of various concentrations were used as the standard. Using the image processing software 'ImageJ', a standard curve was prepared from the concentration of BSA solutions and intensity of corresponding BSA bands in the SDS-PAGE result. The concentration of kinesin was estimated from kinesin band intensity and the standard curve of BSA solutions.

Preparation of fluorescence dye-labeled tubulin and microtubules

Rhodamine-labeled tubulin was prepared using 5/6-carboxytetramethylrhodamine succinimidyl ester (TAMRA-SE; Invitrogen) following the standard protocol.[3] The labeling ratio was 1.0 as determined by measuring the absorbance of the protein at 280 nm and that of tetramethyl-rhodamine at 555 nm. Rhodamine-labeled microtubules (MTs) were prepared by polymerizing a mixture of rhodamine tubulin (RT) and non-labeled tubulin (WT) at 37 °C (RT/WT = 4:1; final tubulin concentration = 56 μM). The prepared MTs were kept in paclitaxel buffer (50 μM in BRB80 buffer) overnight at 25 °C. The microtubule solution was diluted

with motility buffer (80 mM PIPES, 1 mM EGTA, 2 mM $MgCl_2$, 0.5 mg mL^{-1} casein, 1 mM DTT, 10 μM paclitaxel, and ~1% DMSO; pH 6.8) before use.

Controlling the length of MTs by applying tensile and compressive stress

A piece of polydimethylsiloxane (PDMS, elastic modulus: ~1.33 MPa; Fuso Rubber Industry Co. Ltd.) with dimension 7.0×5.0×0.2 mm^3 (L×W×T) was fixed to the stretcher of the mechanical chamber.[4] A narrow channel at the top surface of the PDMS sheet was plasma treated for 3 min (10 Pa, 8 mA) by a plasma etcher (SEDE-GE; Meiwafosis Co. Ltd.). Anti-GFP antibody (Invitrogen) of the concentration of 0.1 mg mL^{-1} (10 μL) was applied to the plasma treated PDMS surface (flowcell). Then, 10 μL kinesin-1 solution (K560-GFP) of prescribed concentrations (~80 mM PIPES, ~40 mM NaCl, 1 mM EGTA, 1 mM $MgCl_2$, 1 mM DTT, 10 μM paclitaxel; pH 6.8) was introduced and incubated for 3 min to bind the kinesin to the antibody. The flowcell was washed with 10 μL of wash buffer (~80 mM PIPES, 1 mM EGTA, 1 mM $MgCl_2$, 0.5 mg mL^{-1} casein, 1 mM DTT, 10 μM paclitaxel, ~1% DMSO; pH 6.8). Next, 10 μL of 200 nM MT solution was introduced and incubated for 3 min, followed by washing with 20 μL of wash buffer. The mechanical chamber was closed, and humid nitrogen gas was kept passing through the chamber. After passing the nitrogen gas for 1 h, the chamber was mounted to the stage of the fluorescence microscope. Tensile stress was applied to the MTs by applying tensile strain to the PDMS, at a rate of 0.44%s^{-1}, using the computer-controlled stretcher as described in a previous report.[4] The compressive stress was applied to the MTs by releasing the applied tensile strain to PDMS at the same rate as previously reported.[5] All the aforementioned experiments were performed at room temperature (25 °C). In this work, the tensile and compressive strain were quantified by calculating the strain applied to the PDMS matrix. It is to note that, only the MTs aligned parallel to the direction of applied tensile and compressive strain were considered for analysis.

Image capture and analysis

MTs were illuminated with a 100 W mercury lamp and visualized by using an epifluorescence microscope (Eclipse Ti; Nikon) using an oil-coupled Plan Apo 60 × 1.40 objective (Nikon). Filter blocks with UV-cut specification (TRITC: EX540/25, DM565, BA606/55; GFP-HQ: EX455-485, DM495, BA500-545; Nikon) were used in the optical path of the microscope. Images were captured using a cooled CMOS camera (Neo sCMOS; Andor) connected to a PC. ND filters (ND4, 25% transmittance) were inserted into the illuminating light path of the fluorescence microscope to avoid photobleaching. Images of microtubules captured under a fluorescence microscope were analyzed using the image processing software "ImageJ". Length of MTs and MT fragments at different experimental conditions were measured manually using the image processing software.

Checking the activity of MTs after applying mechanical stress

In order to check the functionality of the length-regulated MTs, motility buffer (~80 mM PIPES, 1 mM EGTA, 1 mM $MgCl_2$, 0.5 mg mL^{-1} casein, 1 mM DTT, 10 μM paclitaxel, 1% DMSO, 4.5 mg mL^{-1} D-glucose, 50 U mL^{-1} glucose oxidase, 50 U mL^{-1} catalase, 5 mM ATP; pH 6.8) was applied to the flowcell using a capillary syringe after application of 28% tensile strain. This experiment was performed at 25 °C. The gliding MT fragments were monitored using a fluorescence microscope.

Definition of polydispersity index (PDI)

The number-average MT length (L_n) and weight-average MT length (L_w) are defined in analogy to the definitions of number-average and weight-average molecular weight in polymer science:

$$L_n = \frac{1}{n} \times \sum_{i=1}^{n} L_i$$

$$L_w = \frac{\sum_{i=1}^{n} L_i^2}{\sum_{i=1}^{n} L_i}$$

From the number-average MT length (L_n) and weight-average MT length (L_w) the PDI was calculated as shown below:

$$\mathrm{PDI} = \frac{L_w}{L_n}$$

Fitting of the length distribution of MTs

The histograms of MT length distribution were fitted according to the following equation of Gaussian distribution:

$$f(x) = \frac{1}{\sqrt{2\pi\sigma^2}} e^{-\frac{(x-\mu)^2}{2\sigma^2}}$$

where, μ is the mean and σ is the standard deviation.

Live subject statement

The porcine brain was purchased from Hokuren (Livestock Sales Division, Tomakomai Branch, 695 Toasa, Abira-cho, Yufutsu-gun, 059-1433, Hokkaido, Japan). All experiments were performed in compliance with the relevant laws and the guidelines of the institutional safety committee on genetic recombination experiments. This study involved no experimentation with human subjects.

Supplementary Figures

Amino acid sequence of α-tubulin

MRECISIHVGQAGVQIGNACWELYCLEHGIQPDGQMPSDKTIGGGDDSFNTFF
SETGAGKHVPRAVFVDLEPTVIDEVRTGTYRQLFHPEQLITGKEDAANNYARG
HYTIGKEIIDLVLDRIRKLADQCTGLQGFSVFHSFGGGTGSGFTSLLMERLSVD
YGKKSKLEFSIYPAPQVSTAVVEPYNSILTTHTTLEHSDCAFMVDNEAIYDICR
RNLDIERPTYTNLNRLIGQIVSSITASLRFDGALNVDLTEFQTNLVPYPRGHFPL
ATYAPVISAEKAYHEQLSVAEITNACFEPANQMVKCDPRHGKYMACCLLYRG
DVVPKDVNAAIATIKTKRTIQFVDWCPTGFKVGINYEPPTVVPGGDLAKVQRA
VCMLSNTTAIAEAWARLDHKFDLMYAKRAFVHWYVGEGMEEGEFSEARED
MAALEKDYEEVGVDSV

Amino acid sequence of β -tubulin

MREIVHIQAGQCGNQIGAKFWEVISDEHGIDPTGSYHGDSDLQLERINVYYNE
AAGNKYVPRAILVDLEPGTMDSVRSGPFGQIFRPDNFVFGQSGAGNNWAKGH
YTEGAELVDSVLDVVRKESESCDCLQGFQLTHSLGGGTGSGMGTLLISKIREE
YPDRIMNTFSVVPSPKVSDTVVEPYNATLSVHQLVENTDETYCIDNEALYDICF
RTLKLTTPTYGDLNHLVSATMSGVTTCLRFPGQLNADLRKLAVNMVPFPRLH
FFMPGFAPLTSRGSQQYRALTVPELTQQMFDAKNMMAACDPRHGRYLTVAA
VFRGRMSMKEVDEQMLNVQNKNSSYFVEWIPNNVKTAVCDIPPRGLKMSAT
FIGNSTAIQELFKRISEQFTAMFRRKAFLHWYTGEGMDEMEFTEAESNMNDLV
SEYQQYQD

Figure S1: Amino acid sequence of pig brain α-tubulin (top) and β-tubulin (bottom).[6,7]

The sequences are obtained from protein data bank (PDB code: 1TUB).

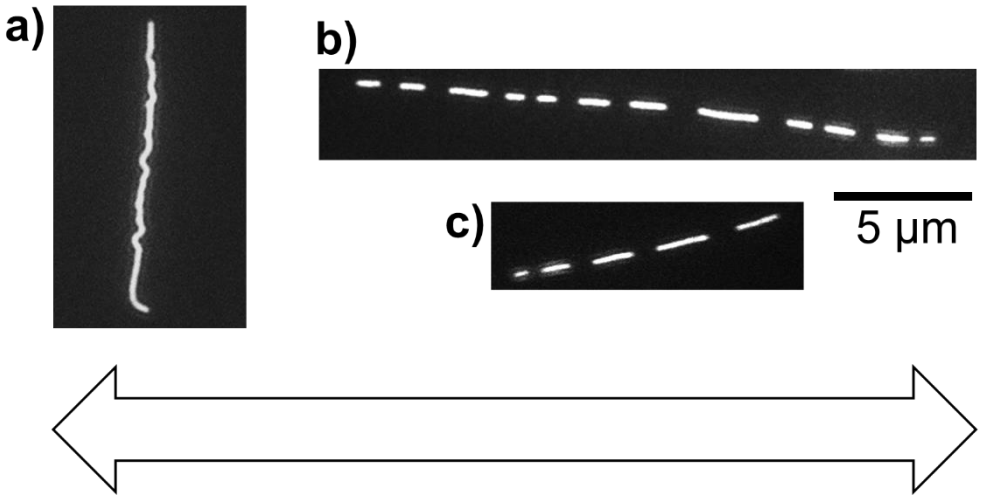

Figure S2: Fluorescence microscopy images show microtubules were deformed even when the microtubules were not aligned along the direction of the applied tensile strain (shown by the arrowhead). When the microtubules were aligned perpendicular to the direction of tensile strain (a), the microtubules underwent buckling upon stretching of the PDMS substrate. This is probably due to compression of the PDMS substrate along the longitudinal direction of the microtubules. Except for such perpendicularly aligned microtubules, fragmentation of microtubules was observed upon stretching of the PDMS, even when the microtubules were not aligned parallel to the direction of tensile strain (b, c). The images were captured after application of 100% tensile strain, where the kinesin concentration was 1300 nM.

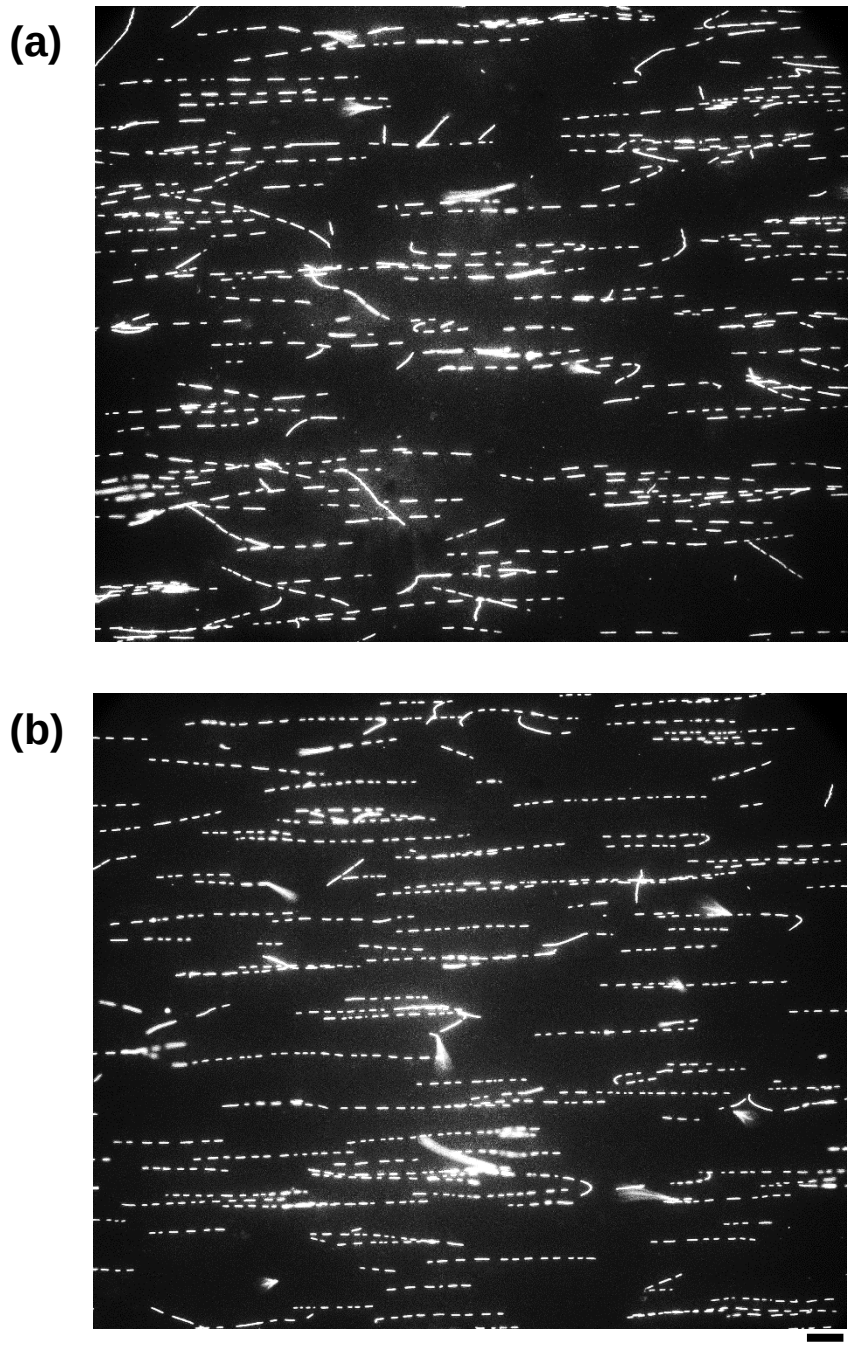

Figure S3: Fluorescence microscopy images of fragmented microtubules captured after application of 'only tensile stress' (a), and 'tensile and compressive stress' (b). The images were captures after applying 100% tensile strain. In (b), 14% compressive strain was applied after application of 14% tensile strain, which was then followed by increase in tensile strain to 100%. Scale bar: 5 μm.

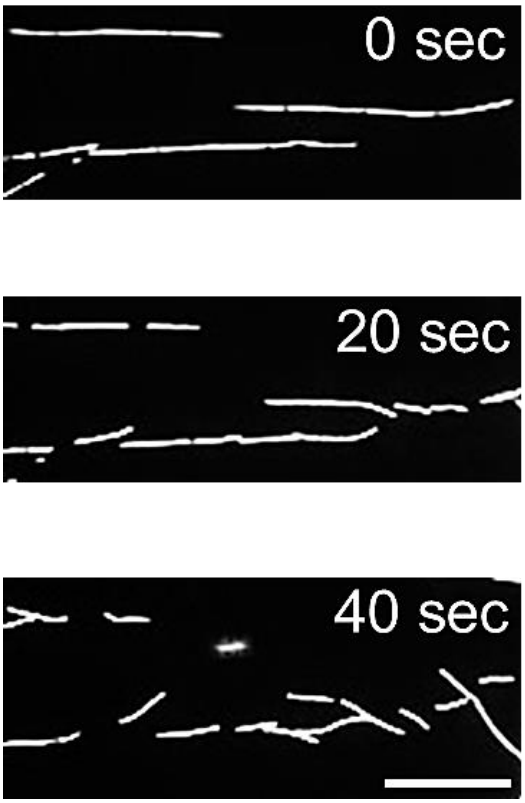

Figure S4: Time-lapse fluorescence microscopy images show motility of MT fragments at 25 °C when they were propelled by kinesins in the presence of ATP (5 mM). The images were captured after applying 14% tensile strain at 200 nM in-feed kinesin concentration. Scale bar: 20 μm.

Captions for Supplementary Movies

Movie S1: Three-dimensional structure of a pig brain tubulin dimer (PDB code: 1TUB).

Movie S2: The experimental procedure employed in this work to control the length of microtubules by applying tensile and compressive stress.

References

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