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Characterization of Motility Properties of Kinesin-Driven Microtubules Towards Nano-Scale Transporter: Focusing on Length of Microtubules and Kinesin Density*

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Abstract

Kinesins, biomolecular motors moving along microtubules (MTs) in cells, can potentially be utilized as nano-scale transport systems with an inverted gliding assay, in which the MTs glide on a kinesin-coated surface. Although the key requirements include controls of gliding direction and velocity of MTs, the details of motility properties of MTs have not been well known. In this study, we quantitatively measured angular and gliding velocities, particularly focusing on the effects of MT length and kinesin density. The gliding assay of MTs of up to 20 μm in length was performed on a substrate coated with the kinesin density of 7.5, 38, and 75 μg/ml that resulted in the kinesin spacing of 7.8, 4.2, and 3.1 μm, respectively. The angular velocity for MTs shorter than kinesin spacings significantly decreased with increasing their length, and that for MTs longer than kinesin spacings was not affected by their length. Moreover, the angular velocity was substantially higher at lower kinesin density. These results suggest that both the number of associated kinesins with MTs and the kinesin spacings may contribute to the gliding direction. In contrast, the gliding velocity was independent of the MT length, ranging from 0.3 to 0.5 μm/s with decreasing the kinesin density. This may potentially imply the existence of an underlying mechanism with respect to the number of kinesins per the unit length of MTs. Towards development of high throughput nano-scale transport systems, long MTs and low kinesin densities would be effective for high directionality and high velocity, respectively.

Key words: Nanoscale Transport System, Motor Protein, Microtubule, Angular Velocity, Gliding Velocity

1. Introduction

Kinesin-1 (formerly conventional kinesin) is one of motor proteins that move unidirectionally along microtubules (MTs) in cells. Kinesins are constituted of the motor domains at the N-terminal, coiled-coil regions, and tail regions, with approximately 60 nm in contour length [1] and transport cargos such as vesicles and organelles using energy derived from ATP hydrolysis [2, 3]. Recently, many attempts utilizing kinesin motor proteins have been made for developing nano-scale transport systems *in vitro* [4, 5] because the biological nanomachines have several advantages compared to artificial actuators. Firstly,

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the nature-made motors can power the transport system at the nano-scale. Although recent nanofabrication techniques have enabled the production of devices with dimension less than 10 nm, such nano-scale motors are not still available. Secondly, kinesin motor has high-energy conversion efficiency to 50% [6]. Finally, one can easily observe MTs gliding on kinesins by constructing an *in vitro* system.

As the basic geometry of the nano-scale transport systems, the kinesin-MT systems have been employed in a so-called inverted gliding assay in which MTs are propelled by surface-bound kinesins and act as carriers that transport attached cargos [7]. MTs used in the gliding assay system are usually grown from tubulin *in vitro* and have a length ranging from less than one micrometer to over tens of micrometers. It has been reported that a short MT captured by a single kinesin can rotate due to thermal diffusion depending on its size [8]. For a long MT, it is speculated that the leading tip can still fluctuate due to thermal diffusion as illustrated in Fig. 1, leaving the remaining part of the MT fixed on the kinesins due to crossbridges formed between the MT and the kinesins. This may potentially still lead to a change in the gliding direction of the MT. Since, in fact, MTs glide over a kinesin-coated surface in random directions, many studies have been attempted to control the gliding direction of MTs with chemical patterning of motor proteins [9], fabricated topographical patterns [5, 10], or their combination [11, 12, 13]. However, little is known of how the gliding direction of MTs can change with respect to their length, kinesin density and so on.

It has been reported by Böhm *et al.* [14] that the gliding velocity increases with decreasing kinesin density bound to glass surfaces, indicating the dependence of the number of kinesins per the unit length of MTs on the gliding velocity. Interestingly, they suggested that a high number of kinesins bound to the same MT may interfere each other with the gliding motion due to binding of kinesins to numerous tubulin dimers, possibly resulted in an inefficient generation of motility. It is of great interest here to study the effect of the length of MTs on the gliding velocity because conventionally available MTs have a wide range of length as noted above and also because this, of course, may be related to a high efficient motility assay. It is thus hypothesized that if the gliding velocity solely depends on the number of kinesins per the unit length of MTs, the gliding velocity will not change with the length.

Although fundamental motility properties of MTs are prerequisite for the development of reliable and high efficient nano-scale transport systems, only a few studies have been reported on characterization. Here, we characterize the motility properties of MTs gliding on kinesins by using an *in vitro* motility assay system. Characteristic parameters such as angular velocity and gliding velocity were determined, particularly focusing on the length of MTs and kinesin density, i.e. the spacing between neighboring kinesins.

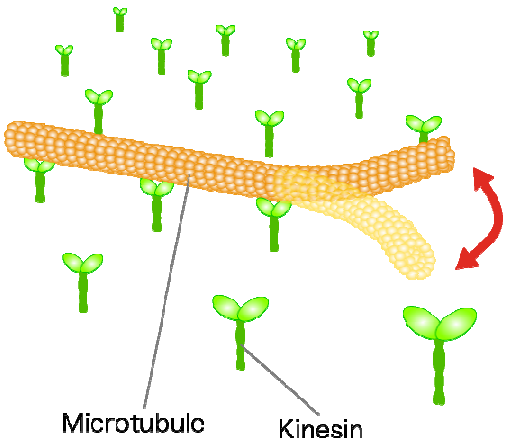

Fig. 1 Schematic illustration of an MT gliding on a kinesin-coated surface. The leading tip of the MT fluctuates in plane parallel to the surface, which is due to thermal diffusion.

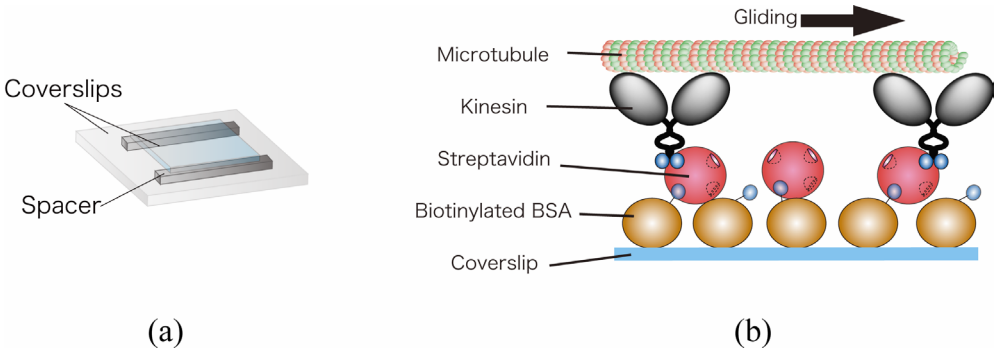

Fig. 2 Schematic illustrations of (a) experimental setup of an assembled flow cell to observe the gliding MTs and (b) an MT gliding on biotinylated kinesins immobilized on the surface via streptavidin and biotinylated BSA in the flow cell.

2. Materials and Methods

2.1 Protein preparation

MTs were prepared from bovine tubulin (TL238, Cytoskeleton, Denver, CO, USA) containing 20% rhodamine-labeled tubulin (TL331 M, Cytoskeleton, Denver, CO, USA). The tubulin was polymerized into MTs by incubating with 1 mM guanosine-5'-triphosphate (GTP) in BRB80 buffer (80 mM PIPES, 1 mM EGTA, 4 mM $MgSO_4$, pH 6.9) at 37°C for 1 h. Then the rhodamine-labeled MTs were diluted two-fold with BRB80 buffer containing 100 μM paclitaxel.

Kinesin was kindly provided by Prof. H. Higuchi (University of Tokyo, Japan). The kinesin gene (pGEX-DK411-BCCP) included the coding sequences for the 411 N-terminal residues of *Drosophila* kinesin heavy chain (DK-411) that has motor domain of kinesin required for movement [15], and biotin carboxyl carrier protein (BCCP) at the C-terminal [16].

2.2 Motility assay

Motility assay was performed as previously described [17] with some modifications. To observe the gliding MTs *in vitro*, a flow cell was fabricated as shown in Fig. 2(a). The flow cell was assembled by sandwiching 22 μm-thick spacers with two different sizes of glass coverslips (18 × 18 mm^2 and 26 × 36 mm^2, No. 1, Matsunami, Japan). BRB80 was used as the buffer for all experiments. Protein configuration including MTs and kinesins in association with streptavidin and biotinamidocaproyl-labeled bovine serum albumin (biotinylated BSA) in the flow cell is shown in Fig. 2(b). The flow cell was filled with 1 mg/ml biotinylated BSA for 2 min. After flushing with the BRB80 buffer at least twice, the flow cell was filled with 1 mg/ml streptavidin solution for 2 min. The flow cell was flushed twice again and was then filled with 38 μg/ml of kinesin solution for 2 min. Other two different concentrations of kinesin solution, 75 μg/ml (high density) and 7.5 μg/ml (low density) were also used in order to assess the effect of the spacing between kinesins on the MTs motility. The flow cell was finally filled with 1 mM adenosine-5'- [(β, γ)-imido] triphosphate, triethylammonium (AMP-PNP) solution containing MTs, followed by replacement with 1 mM adenosine triphosphate (ATP) solution containing oxygen scavenger additives (1.5% β-mercaptoethanol, 1.5 mg/ml bovine serum albumin, 15 μM paclitaxel, 30 mM glucose, 120 μg/ml glucose oxidase, 30 μg/ml catalase) and 0.1% methylcellulose. The experiments were performed at room temperature of 20°C controlled by air conditioning. The change in the temperature of the solution monitored with a thermometer during experiments was ± 0.4°C. Although it has been demonstrated that temperature affects the gliding velocity [18], this range of temperature difference can be assumed negligible. Time-lapse images of the gliding MTs in the flow cell were captured using an inverted fluorescent microscope (IX-71, Olympus, Tokyo, Japan) equipped with a CCD camera (Cascade 512B, Nippon Roper, Tokyo, Japan) and transferred to a PC for the

following image analysis.

2.3 Characteristic parameters

Image analysis was performed with image analysis software (Image J, National Institutes of Health, Bethesda, MD, USA). Characteristic parameters such as angular velocity and gliding velocity of MTs were determined with respect to the length of MTs (L) as follows. Firstly, the gliding velocity (V) was calculated from displacement of the leading tips of MTs every 5 s. The angular velocity (ω) was then determined as the rate of change in the orientation angle of the gliding velocity vector. Both the parameters were averaged over the period of observation and expressed as a mean. MTs shorter than 20 μm were analyzed because MTs longer than 20 μm in length was rarely constructed in this study. The spacing between neighboring kinesins was assessed roughly by following the trajectories of the MTs. Figure 3 shows the images of MTs to estimate the spacing between kinesins. We observed some MTs transiently showing partial winding track (Fig. 3(b)). By superimposing the time-lapse images of those MTs, the points that the MTs always passed through were assumed to reflect the kinesin positions, and the distance between the points was measured (arrows in Fig. 3(c)).

2.4 Statistical analysis

Because of an unequal variance in several variables, mean angular velocities and mean gliding velocities between kinesin densities were compared by the Steel-Dwass test. The association between MT length and mean angular velocity or mean gliding velocity was assessed by the Pearson's correlation coefficient. All hypothesis tests were performed using a significance level of 0.05. Data were shown as mean ± standard deviation (SD).

3. Results

3.1 Angular velocity

Typical time-lapse images of the gliding MTs under 38 μg/ml of kinesin solution are shown in Figs. 4(a) and (b) for a long MT with 8.0 μm long and a short MT with 2.9 μm long, respectively. The images clearly showed that the long MT glided over the surface along a rather straight path, whereas the short MT created a random path. Figures 4(c) to (g) show the trajectories of the gliding MTs in different ranges of the length. The trajectories are summarized by the length at 2 μm intervals up to 10 μm in order to prevent confusing their tendency and are plotted every 5 s up to 120 s. The trajectories of MTs shorter than 4 μm showed that the movement is random over the period of time (Figs. 4(c) and (d)). In contrast, the trajectories of MTs longer than 6 μm showed more straight and smooth movement (Figs. 4(f) and (g)).

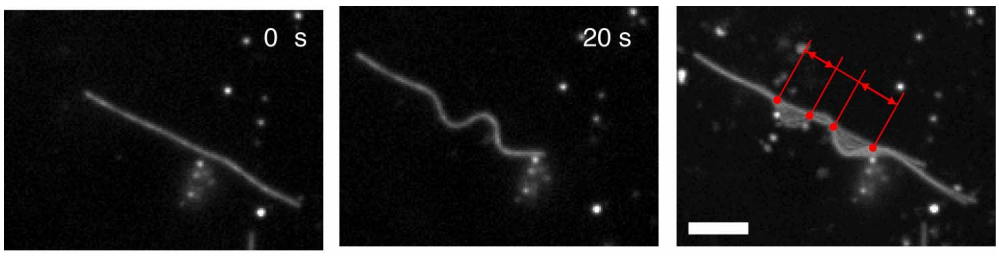

Fig. 3 Typical images of gliding MTs for estimation of the spacing between kinesins. (a and b) Sequential images of a gliding MT. The MT traveling in a straight track (a) suddenly showed winding shapes (b). (c) The points MTs always passed through on superimposed time-lapse images for 0–20 s (filled circles) gave the position of kinesin, and the spacings between kinesins were measured as the distance between points (arrows). Bar = 5 μm.

The measured kinesin spacings were 7.8 ± 2.7 (n = 29), 4.7 ± 1.3 (n = 43), and 3.1 ± 0.9 (n = 46) μm for 7.5, 38, and 75 μg/ml of kinesin densities, respectively. Figures 5(a) to (c) shows the length-dependency in the angular velocity at the three different kinesin densities. Under all the kinesin densities, the angular velocity substantially tended to decrease as the length of MTs increased, and finally achieved the plateau level. We separately analyzed the correlation between the angular velocity and the MT length by dividing the MTs into two groups: MTs shorter or longer than kinesin spacings. For the MTs shorter than the spacing between kinesins, the correlation coefficients of the linear regression lines were significant under all kinesin densities (r = -0.63, p < 0.001 (7.5 μg/ml); r = -0.50, p < 0.01 (38 μg/ml); r = -0.68, p < 0.05 (75 μg/ml)). On the other hand, the correlation coefficients of the regression lines were not significant for the MTs longer than kinesin spacings (r = -0.01, p = 0.958 (7.5 μg/ml); r = -0.01, p = 0.750 (38 μg/ml); r = 0.03, p = 0.854 (75 μg/ml)), indicating that the angular velocity of MTs longer than the kinesin spacing seemed to be independent of their length. The mean angular velocity of gliding MTs longer than the kinesin spacings was significantly higher at low kinesin density (3.1 ± 1.3, n = 23 (7.5 μg/ml)) than at the middle (1.9 ± 0.9, n = 38, p < 0.001 (38 μg/ml)) and high kinesin densities (1.8 ± 0.7, n = 44, p < 0.001 (7.5 μg/ml)) (Fig. 5(d)).

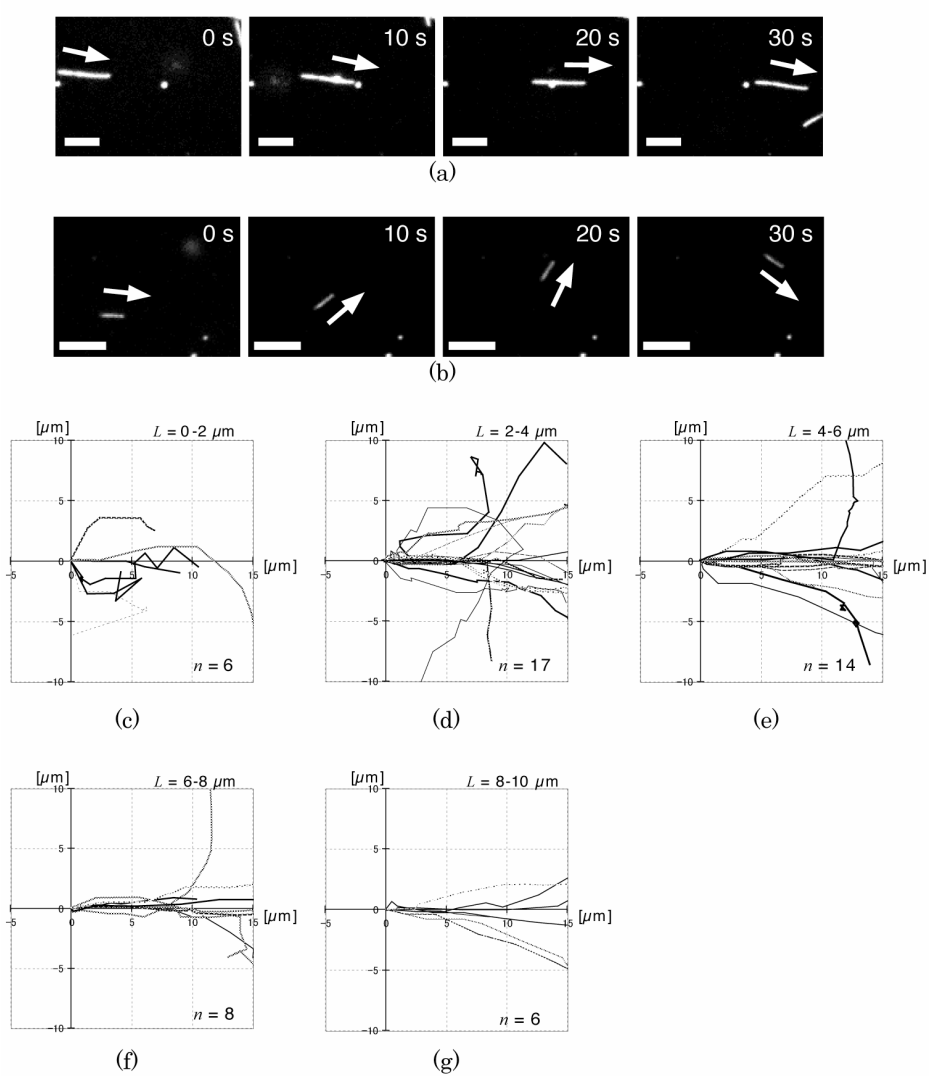

Fig. 4 Time-lapse observation of gliding MTs. (a, b) Typical fluorescent images of (a) a long and (b) a short gliding MT. Bar = 5 μm. Arrows indicate the gliding directions of MTs. (c–f) Trajectories of gliding MTs for (c) L = 0–2, (d) 2–4, (e) 4–6, (f) 6–8, and (g) 8–10 μm. The gliding direction at the initial time is expressed from left to right.

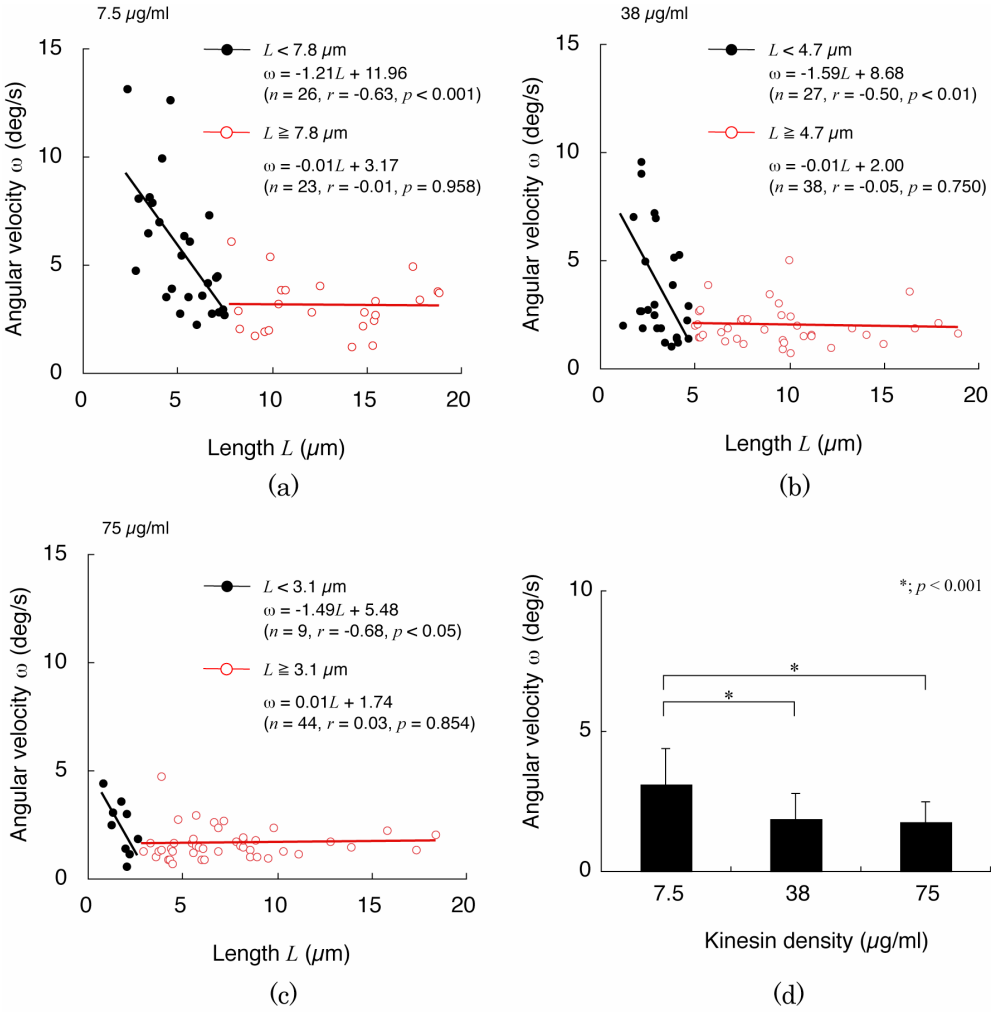

Fig. 5 The angular velocity of gliding MTs. (a–c) The angular velocity plotted against MT length for kinesin densities of (a) 7.5, (b) 38, and (c) 75 μg/ml. The filled and open circles show the data for MTs shorter and longer than the estimated kinesin spacing, respectively. The solid lines were obtained by least squares regression. (d) The averaged angular velocity for the MTs longer than the estimated kinesin spacings.

3.2 Gliding velocity

Figures 6(a) to (c) shows the length-dependency in the gliding velocity at the three different kinesin densities. For all the kinesin densities, the correlation coefficients between the gliding velocity and the MT length were not significant ($r = 0.28$, $p = 0.054$ (7.5 μg/ml); $r = 0.19$, $p = 0.13$ (38 μg/ml); $r = 0.24$, $p = 0.079$ (75 μg/ml)). The gliding velocities for all the length were 0.45 ± 0.15, 0.32 ± 0.10, and 0.35 ± 0.10 μm/s for 7.5, 38, and 75 μg/ml kinesin densities, respectively (Fig. 5(d)). The gliding velocity at the low kinesin density was significantly higher than those at the middle ($p < 0.001$) and high kinesin densities ($p < 0.001$). The independency of MT length on the gliding velocity was also observed for either case of the analysis for only MTs shorter or longer than kinesin spacings (data not shown).

4. Discussion

For developing high throughput nano-scale transport systems, fundamental motility properties of carriers such as directionality and velocity should be well controlled. As shown in Fig. 5, the angular velocity significantly decreased with increasing the length of MTs for the MTs shorter than the estimated kinesin spacings. This result can be explained by the larger thermal fluctuation for longer MTs captured by a single kinesin [8]. On the

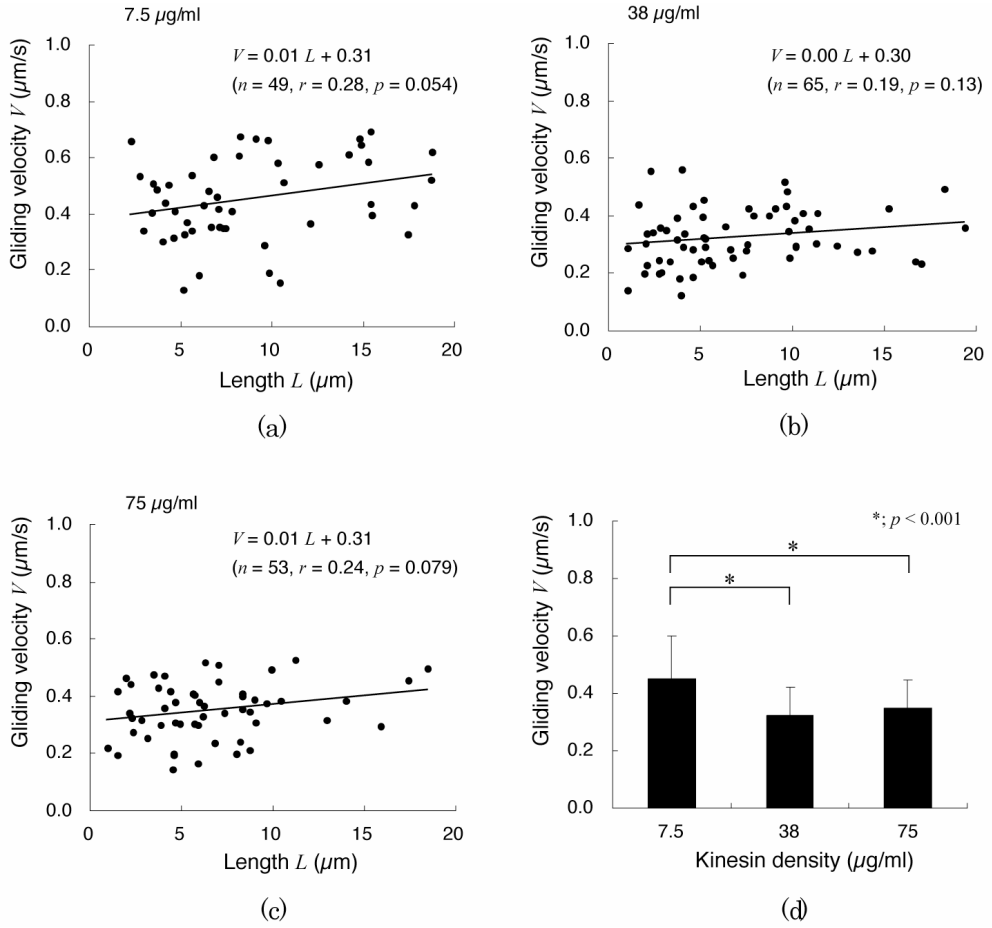

Fig. 6 The gliding velocity of MTs. (a–c) The gliding velocity plotted against their length for kinesin densities of (a) 7.5 μg/ml, (b) 38 μg/ml and (c) 75 μg/ml. The solid lines were obtained by least squares regression. (d) The averaged gliding velocity with regard to the kinesin densities.

other hand, the angular velocity for MTs longer than the spacing between kinesins was independent of the MT length. This is probably due to the fact that, within that range of the MT length, not a single kinesin but multiple kinesins could form crossbridges with the MTs and the crossbridges could work as fixed points against which reaction moments can be generated by the externally-driven thermal fluctuations. Although it is also possible that more crossbridges could decrease the deflection angle of the MT leading tip due to thermal fluctuations given by the strength of materials theory, the result obtained in this study shows that the effect was negligible small. In addition, as shown in Fig. 5(d), the angular velocity substantially increased as the kinesin density decreased, in other words, as the spacing between kinesins increased. These results strongly indicate that the number of associated kinesins with MTs would be a critical factor in the gliding direction of MTs. It is thus possible that longer MTs associated with multiple kinesins at higher kinesin density may have higher directionality of the gliding MTs.

It is important to note that detaching of MTs from the kinesin surfaces was frequently observed, with the rate depending on the length of MTs. The maximum lengths that the detaching was observed were 7.9, 4.1, and 2.3 μm for 7.5, 38, and 75 μg/ml kinesin densities, respectively. These values of the lengths were closely related to the estimated spacing between kinesins. Gliding MTs shorter than the kinesin spacing are likely to fail to catch next kinesin to glide ahead. It is also important to address the possible gliding distance. Since it is difficult to observe the gliding MTs until detaching due to the limited observation area, the gliding distance cannot be directly measured. In a separate experiment, it was confirmed that MTs were found to still glide even after 1 h. If the gliding velocity is,

for example, assumed to be 0.3 μm/s from Fig. 5, the gliding distance can be estimated more than 1 mm at 1 h. Stracke *et al.* [19] have also observed MTs gliding longer than 1 mm. Both preventing the detaching of MTs and the MTs gliding for a long distance would be also important for the transport system.

The gliding velocity of MTs obtained in this study had no significant correlation with the length of MTs for all the kinesin densities. Although Hunt *et al.* [20] have showed that the MT length did not affect their gliding velocity, the mechanism is still unclear. A single kinesin can produce the force of ~ 6 pN at maximum in a single step [21], presumably indicating that an increase in the number of kinesins interacting with MTs may result in an increase in the driving forces. Therefore, it is supposed that the driving forces by kinesins and drag forces acting on the gliding MTs due to the solution viscosity should be in equilibrium. However, the drag forces was, for example, estimated to be 0.05 and 0.1 pN for the MT length of 5 μm and 10 μm, respectively, which are much lower than the driving forces. Böhm *et al.* [14] have reported that the gliding velocity showed a logarithmic decrease with increasing kinesin density bound to glass surfaces, indicating the dependence of the number of kinesins per the unit length of MTs on the gliding velocity. They have also suggested that binding of a high number of kinesin molecules to the same MT may hinder movement of each kinesin, resulting in an inefficient generation of motility. Although this mechanism is still unknown, we also showed that the gliding velocity of MTs was not affected by the change of the MT length. These results indicate that the gliding velocity of MTs is potentially affected not by the MT length but by the number of kinesins per the unit length of MTs. Therefore, it is important to consider the effect of the MT length and kinesin density simultaneously.

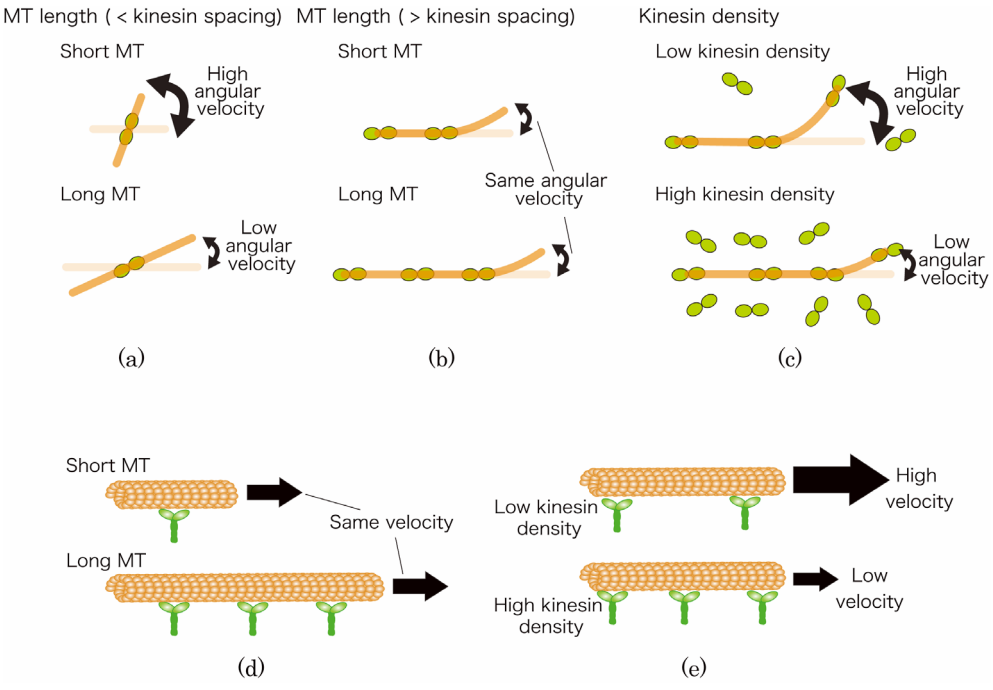

Fig. 7 A possible underlying mechanism to explain the motility properties of MTs with respect to the their length and kinesin density. (a) Dependence of the MT length for MTs shorter than kinesin spacings and (b) independence of the MT length for MTs longer than kinesin spacings. (c) Dependence of kinesin density on the angular velocity of MTs. (d) Independence of the MT length and (e) dependence of kinesin density on the gliding velocity of MTs.

Taken all the results together, we propose a concept to explain the possible mechanism for the angular velocity and the gliding velocity, as illustrated in Fig. 7. In Fig. 7(a), an MT shorter than the critical spacing between kinesins is supposed to be captured by a single kinesin and can rotate due to thermal diffusion depending on its length and shows high angular velocity. For an MT longer than the spacing captured by more than two kinesins, the angular velocity remains low and constant value (Fig. 7(b)). In Fig. 7(c), angular velocity is lower for higher kinesin density, probably because the increase of kinesin density results in an increase of the number of kinesins that MTs can be in contact with before showing large deflection. As shown in Fig. 7(d), the gliding velocity is independent on the MT length, whereas, Fig. 7(e) shows the gliding velocity depends on kinesin density; that is, increase in velocity with decreasing the kinesin density.

In summary, we characterized the motility properties of MTs gliding on kinesin-coated surface with respect to their length and kinesin density. The angular velocity of the gliding MTs significantly decreased with increasing their length for MTs shorter than kinesin spacings and achieved a plateau for MTs longer than kinesin spacings. Moreover, the angular velocity was substantially higher at lower kinesin density. These results suggest that both the number of associated kinesins with MTs and the kinesin spacing may contribute to the gliding direction. In contrast, the gliding velocity of the gliding MTs was not significantly affected by their length and was significantly increased at the low kinesin density compared to the other two densities, indicating the dependence of the gliding velocity on the number of kinesins per the unit length of MTs. These results reveal that long MTs associated with many kinesins would have high directionality, and that low kinesin densities would produce the MTs gliding of high velocity, towards development of high throughput nano-scale transport systems.

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