SUPPORTING INFORMATION

Confirmation of motility of kinesin-driven microtubules on Si and Au substrates.

Although motility assays of kinesin-driven microtubules (MTs) have usually been performed on coverslips, it is difficult to fabricate grooves with steep sidewalls on glass substrates (Gl) using MEMS techniques. One of the reasons for the difficulty is that the coverslip contains not only silicon dioxide (SiO_2), but also impure substances such as aluminum oxide (Al_2O_3) and sodium oxide (Na_2O). Therefore, a Si substrate was selected in order to fabricate steep sidewall grooves on the substrate with precise control of the width. Although some studies have reported that the gliding velocities of MTs are dependent on the type of substrate [S1,S2], there have been no reports of motility assays of kinesin-driven MTs on Si substrates, although previous studies have employed Au-coated Si for motility assays [S3,S4]. However, motility assays of kinesin-driven MTs on Au substrates have not yet been evaluated. Therefore, motility was investigated for MTs on both Si and Au substrates to determine whether motility on each substrate was comparable to that on the Gl. For the Gl substrate, a coverslip (18×18 mm^2, No.1, Matsunami, Osaka, Japan) was used.

To evaluate the effect of the substrate type on the motility of MTs, the density and gliding velocity of MTs on the substrate surface were measured, because these factors would reflect the efficiency of a transport system. For the gliding velocity, forty randomly selected MTs were measured at 10 min after the flow cell was filled with the motility assay buffer, and their displacement was divided by the interval time. Differences among the data were analyzed using a Bonferroni test after a one-way analysis of variance (ANOVA) for equal variance, and a Steel-Dwass test after a Kruskal-Wallis test for unequal variance. The hypothesis tests were performed with a significance level of 0.05. Data from four independent experiments were expressed as a mean ±standard deviation (SD).

The densities of attaching MTs were $(9.6\pm2.2)\times10^{-4}$ counts/μm^2 (Si), $(18.7\pm3.4)\times10^{-4}$ counts/μm^2 (Au), and $(18.0\pm5.0)\times10^{-4}$ counts/μm^2 (Gl) (Fig. S1A). The densities of attached MTs on the Au and Gl surfaces was nearly double compared to that on Si ($p < 0.05$), and no significant difference was observed between the Au and Gl substrates. The gliding velocities on Au (0.59±0.06 μm/s) and Gl (0.56±0.10 μm/s) were similar and significantly higher than that on Si (0.48±0.15 μm/s) (Fig. S1B). These results confirm that motility on the Au substrate is comparable to that on the G1 coverslip.

REFERENCES

S1. C. Brunner, H. Hess, K-H. Ernst, and V. Vogel, *Nanotechnology*, 2004, **15**(10), S540.

S2. V. Verma, W. O. Hancock, and J. M. Catchmark, *IEEE Trans. Adv. Pack.*, 2005, **28**(4), 584.

S3. M. G. van den Heuvel, C. T. Butcher, S. G. Lemay, S. Diez, and C. Dekker, *Nano Lett.*, 2005, **5**(2), 235.

S4. M. G. van den Heuvel, C. T. Butcher, R. M. Smeets, S. Diez, and C. Dekker, *Nano Lett.*, 2005, **5**(6), 1117.

S5. S. Sugita, N. Sakamoto, T. Ohashi, and M. Sato, *J. Biomech. Sci. Eng.*, 2008, **3**, 510.

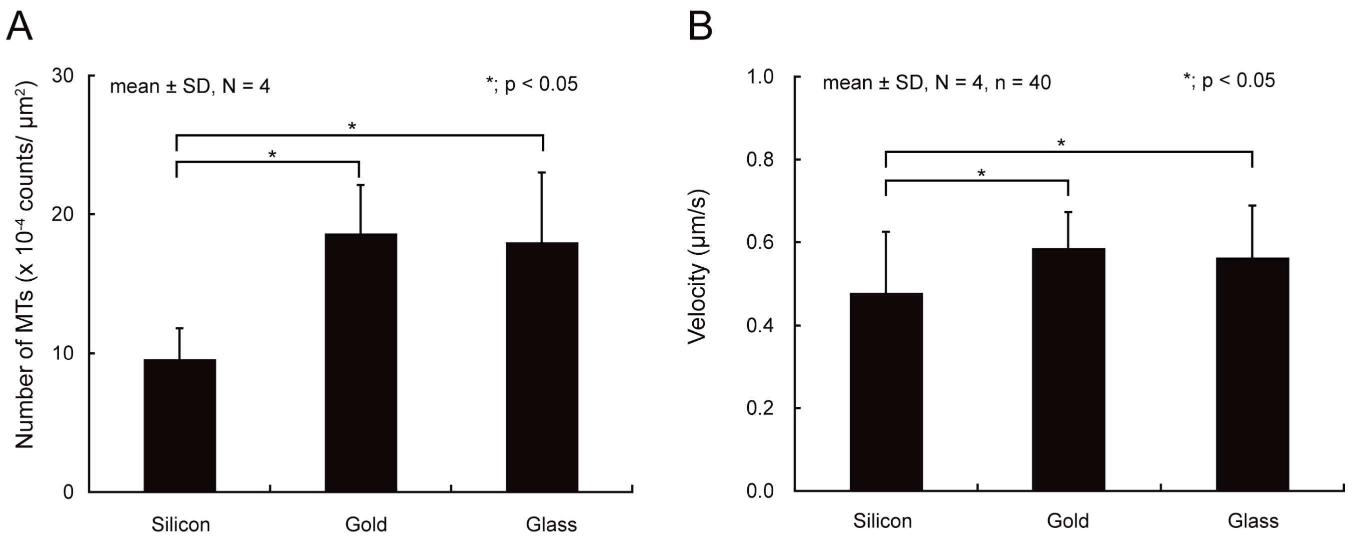

Figure S1. Effect of substrate type on (A) the number of MTs attached to the substrate surface and on the (B) gliding velocities of MTs.