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Microtubule Ring Formation in a PDMS Microfluidic Device

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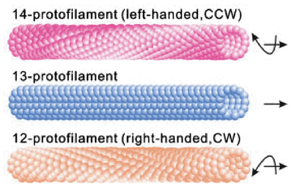
Abstract

We developed a polydimethylsiloxane (PDMS) microfluidic device that decreases photodamage to biomolecules via deoxygenation to enhance the lifetime of kinesin motor proteins in an *in vitro* inverted motility assay with microtubules. Biotin labeled microtubules powered by kinesin motors spontaneously form ring structures in the presence of streptavidin. The improved photoprotection in the microfluidic device enabled us to study the initial stages of ring formation that were formerly unobservable due to photodamage. Valves on the devices were used to control the addition of microtubules and streptavidin. There have been two primary mechanisms proposed to explain the formation of rings: pinning and bundle twisting. The pinning mechanism entails a single microtubule pinned at the head looping around to contact the tail forming a single ring, followed by bundling as other microtubules encounter the ring and become attached. The bundling mechanism is hinged on the fact that non-13mer microtubules rotate as they are translated by kinesin, which would twist a bundle of microtubules and introduce strain that would coerce the bundle to move in a curved path and then form a ring. We show that both proposed mechanisms occur and characterize the conditions under which each mechanism predominates.

Microtubules

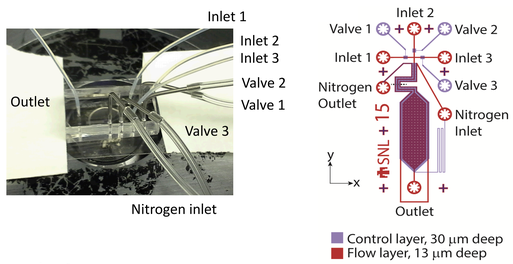
Microtubules (MTs) are cytoskeletal polymeric filaments that serve two primary roles: (1) structural support for the cell, and (2) intracellular network for motor protein transport. The fundamental building blocks of MTs are α-tubulin and β-tubulin, which bind together tightly to form a heterodimer approximately 8 nm in length. Assembly of these heterodimers in the presence of guanosine triphosphate (GTP) forms protofilaments with an intrinsic polarity where one end is terminated by an α-tubulin (i.e., minus-end) and the other end is terminated by a β-tubulin (i.e., plus-end). Lateral association of protofilaments with similar polarity leads to the formation of extended sheets and eventually the mature MT filament.



Molecular motors can be harnesses for nanobiotechnology applications. The self-assembly of MTs can be used to create non-equilibrium, nanoscale structures, such as long filaments and spools, in which the gliding MTs adopt tightly bent or buckled configurations with radii of curvature that far exceed the natural curvature of freely fluctuating MTs.

Microfluidic Deoxygenation and Buffer Exchange

An elastomeric PDMS microfluidic device for buffer exchange and deoxygenation. The device was designed with normally closed valves to switch controllably between 3 different buffers.



Flow velocity

The flow velocity in the observation chamber is U=1 µL/min during switching of buffer.

Shear rate

The shear rate experiences by microtubules is : $\tau_w = \frac{6\mu\bar{v}}{h}$

At U=1 µL/min, t_w = 0.22 Pa. In agreement with previous reports, we did not find this shear rate to detach microtubule from the surface.

Buffer switching time

The time to switch from one solution to another can be calculated using Taylor-Aris dispersion theory. The concentration as a function of distance and time is given by:

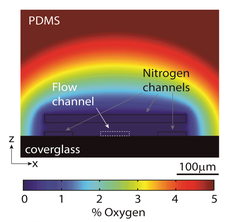
$$c(x,t) = \frac{c_0}{2}\operatorname{erfc}\left(\frac{x-t}{2\sqrt{t}}\right)$$

Valves Open  Valves Closed 

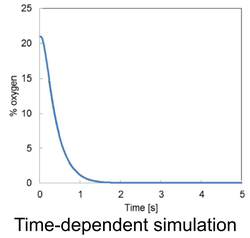
Switching time for solution to go to 98% new solution is less than 5 s.

Dexoygenation without enzymatic oxygen scavenging :

Device exploits permeability of PDMS to gases to replace oxygen in solution with nitrogen by diffusion. Channels for the flow of nitrogen were located to the sides and above the observation channel (separated by a 35 mm membrane).



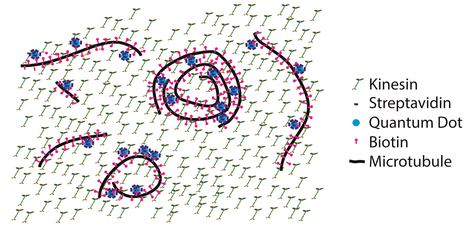
2D finite element steady-state simulation of the oxygen concentration in the device.



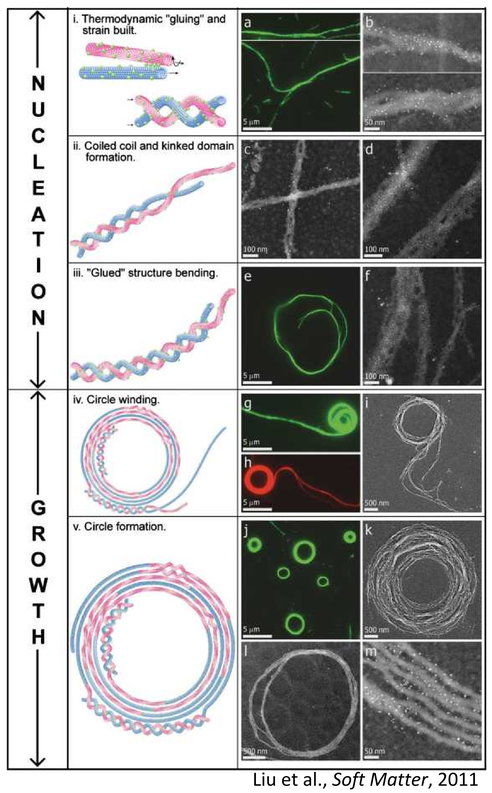
Time-dependent simulation showing time to reach steady state oxygen concentration

Spool formation

Set up of in vitro inverted motility assay on glass coverslip:



SEM and fluorescence images of ring nucleation and growth. Microtubules with 12 or 14 protofilaments rotate during translation, causing coiling of microtubules in bundles. The handedness of the microtubules influences the direction rotation direction of the rings.

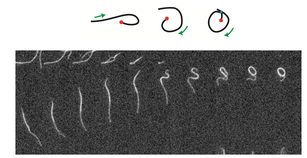


Liu et al., *Soft Matter*, 2011

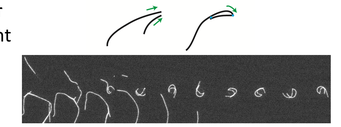
Spool Initiation Mechanisms

We use the microfluidic device to directly monitor spool initiation. The following initiation mechanisms were observed:

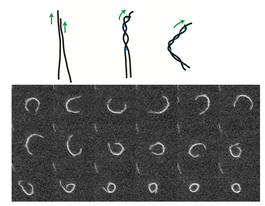
Pinning
- The tip of the MT becomes pinned, causing the tail to loop around
- Increased frequency under high excitation intensity



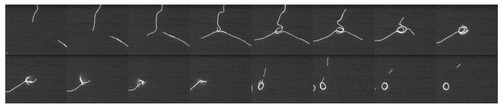
Tip binding
- MTs get attached together at two points with different arc lengths in between, causing the bound tip to describe a circle
- Relatively uncommon



Coil twisting
- The winding of two MTs around each other creates strain that causes the bundle to curve
- Requires MTs with pitch (not 13 protofilaments)
- Phenomenon is even more pronounced with actin



Interactions
- "Sticky" MTs change tip direction when interacting
- Previously unobserved mechanism
- Only occurs at lower Streptavidin concentrations



References

Vandelinder and Bachand, *Anal. Chem.*, 2013; Liu et al., *Advanced Materials*, 2008; Liu et al., *Soft Matter*, 2011; Luria et al., *Soft Matter*, 2011; Schaller et al., *PNAS*, 2011.

Acknowledgements

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