September 21, 2012: Magical Microtubule Forest

Goal: To investigate the properties of the 3D mesh structure when Poly-L-Lysine concentrations are changed as well as to observe the structure in the presence of kinesin.

Materials:

Name	**[Stock]**	**Abbreviation**
BRB80		
MgCl2	100mM	Mg
GTP	25mM	GTP
DMSO	5%	DMSO
Rhodamine Tubulin	20μg	
Taxol	1mM	TX
Casein	20mg/mL	CS
D-Glucose	2M	DG
Glucose Oxidase	2mg/mL	GO
ATP	100mM	ATP
Catalase	0.8mg/mL	Cat
Dithiotheritol	1M	DTT
Poly-L-lysine		PL
Kinesin		

Procedure:

1. Prepare **microtubule growth buffer** solution in a microcentrifuge tube:

Volume	**Reagent**	**[Stock]**	**[Final]**
21.8μL	BRB80		
1μL	Mg	100mM	4mM
1μL	GTP	4mg/mL	0.16mg/mL
1.2μL	DMSO	5%	0.0024%
25μL	**Final Volume**		

2. Add 6.25μL of growth solution to the aliquot of tubulin (prepare microtubules in the original aliquot of tubulin). **Wrap in foil, expose this to light as little as possible.** This results in a final tubulin concentration of **[*Tubulin*] = 3.2*mg/mL*.** Incubate this on ice for 5 minutes and then incubate at 37ºC for 30 minutes. (If incubating in water bath, wrap in parafilm.)

3. Clean a 35x50 coverslip with the sonicator, ethanol, then the ozone cleaner.

4. Prepare flow chamber – Use two strips of double sided tape on a 35x50 coverslip (on the long edges), and stick a 22x22 coverslip on top. (Results in ~100μm height, volume

of ~15μL.)

5. Prepare standard solution:

BRB80PL

Volume	Reagent	[Stock]	[Final]
90μL	BRB80		
10μL	Poly-L-lysine	10mg/mL	1mg/mL
100μL	**Final volume**		

Structure Formation

6. Flow in 20μL of BRB80PL, wait 15 minutes.
7. Flow in 20μL of BRB80, wait 5 minutes.
8. Prepare to image the flow cell. Flow in 20μL of doubly stabilized MT10. Place 5μL of BRB80 on the flow-in side of the cell to prevent drying. Immediately place on microscope focusing on the top surface of the flow cell. Image the flow cell for 10 minutes in one area.
9. Image any 3D structure that formed in the same area.

10. Repeat steps 6-9, but wait 1 hour with PLL in the flow cell for step 6.

Stability with 2 flushes of BRB80PL

1. Flow in 20 ul BRB80 PL
2. Wait 15 min, flush with BRB80
3. Flow in 20 ul BRB80 PL
4. Wait 15 min, flush with BRB80
5. Flow in 20 ul MT100
6. Image

Stability with a higher concentration of BRB80PL

Higher concentrationBRB80PL (2:9)

Volume	Reagent	[Stock]	[Final]
45μL	BRB80		
10μL	Poly-L-lysine	10mg/mL	1mg/mL
55μL	**Final volume**		

Repeat 6-11 of structure formation

Higher Concentration of BRB80 PL (1:3)

Volume	Reagent	[Stock]	[Final]
10μL	BRB80		
10μL	Poly-L-lysine	10mg/mL	1mg/mL

20μL	**Final volume**		

repeat 6-11 of structure formation

Kinesin solution: (Wait 2 minutes after adding BRB80CS, before putting kinesin in)

Volume	**Reagent**	**[Stock]**	**[Final]**
94µL	BRB80CS		
5µL	Kinesin		
1µL	ATP	100mM	1mM
100µL	**Final volume**		

1. Flow in 10 ul BRB80PL, wait 15 min
2. Flow in BRB80
3. Flow in MT100, wait 5 min
4. Flow in kinesin motor solution
6. Wait 10 min
7. Image

Results:

The 3D mesh structure with the PLL was slower to form with doubly stabilized microtubules than with GTP stabilized microtubules. The microtubules were stiffer and the structure appeared less curved and rounded than the normally stabilized microtubules. The next day, the structure was more elaborately formed.

With the higher concentration of poly l lysine, the webs took longer to form. The structures appeared to form more in the middle of the flow cell than they did in experiments with the .04 M PLL concentrations.

After flowing through kinesin, the microtubule structure sheared.