September 14, 2012: 3D Microtubule Structure – Testing Construction

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Goal: The goal was to observe the interaction between the mesh and microspheres.

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

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 MT100. 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. Ensure that the cell does not dry out by maintaining the BRB80 on the flow-in side of the cell. Remove from microscope stage.
11. Repeat steps 6-10 but image just below the top surface. (Record how many focus knob notches this corresponds to.)

Structure Stability

12. Prepare a 1-2mm wide strip of coffee filter paper. Place the first slide back on to the microscope stage. Place the filter paper on the exit side of the flow cell and the black petri dish on top with room to pipette in on the flow-in side.
13. Pipette 20μL of BRB80 **slowly** through the cell by dropping the solution in front of the flow-in side and begin imaging at the same time. Record for 10 minutes.
14. See how many flushes until the structure breaks

Microsphere Solutions

Microsphere solution

Volume	Reagent
99 ul	BRB80T
1 ul	Carboxylated 0.5 um microspheres

Microsphere solution 2

Volume	Reagent
10 ul	1/100 microsphere solution
90 ul	BRB80

Flush 20 ul BRB80PL into flow cell.

1. Wait 15 mins
2. Flush with BRB80
3. Flow in 20 ul microsphere solution
4. Image
5. Repeat steps 1-5 with microsphere solution 2

Results:
The 3D mesh structure was very stable. After 40 flushes of BRB80, it still maintained its structural integrity.

Upon imaging, some of the beads remained in the mesh structure. They were primarily in solution as opposed to on the surface. Some of the beads did appear to adhere to the 3D structure. In the future, we will evaluate the interaction between the structure and microspheres of different sizes and charges.