Team 2 - Lab Notebook (A'21)

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Lab 1 - Fibrin Microthreads - (09/02/2021)

Procedure (AM Sep 7, 2021),(CB, Sep 8, 2021)

1. Prepare 600 mL of 1X (10 mM) HEPES buffer solution (60 mL of stock solution and *540 mL diH_2O) and store in a labeled bottle.
2. Place blunt end needle (25 gauge, BD) into 0.86 mm I.D. polyethylene tubing.

CAN REUSE THESE MATERIALS IF PREVIOUS USER WASHED PROPERLY

3. Luer lock the blunt end needle/tubing assembly onto the front end of the blending connector.
4. Place a metal non-stick pan next to the syringe pump.
5. Label syringes – Thrombin and Fibrinogen
6. Check that the syringe pump is out and set to the correct values for syringe diameter (8.2 mm) and speed (0.225 mm/min).

In Lab

1. Fill pan with 600 mL HEPES buffer solution.
2. Do a “dry” run of the entire procedure, before loading the syringes with fibrinogen and thrombin, to make sure all materials and equipment are set up and working properly (as shown schematically in Figure 1 above). Make sure you know how to use the syringe pump and that when it is running the plungers are being depressed.

Co-Extrusion Procedure

1. Add 150 µL of thrombin aliquot to 850 µL of calcium chloride solution in a new 1mL microcentrifuge tube and mix well.
2. “Prime” both 1 mL syringes by moving the plunger.
3. Collect all of the thrombin OR fibrinogen solution into the appropriate 1 mL syringes.

COLLECT THE FIBRINOGEN SOLUTION SLOWLY AND CAREFULLY, FAILURE TO DO SO MAY RESULT IN INSOLUBLE FIBRINOGEN FORMATION!! (it’s ~~vicious~~ viscous!!!). Do not vortex or shake.

4. Invert syringe, remove all bubbles, and ensure that both syringes have equal volumes.
5. Place each 1 mL syringe of fibrinogen and thrombin solutions into the back end of the blending applicator.

ALWAYS PUT FIBRINOGEN SOLUTION IN THE BLENDING APPLICATOR OPENING WITH THE CIRCLE ON IT.

6. Secure syringe/blending applicator construct into syringe pump.
7. Press RUN on the syringe pump and wait for fibrin solution to flow out of the tip of the tubing.
8. Draw threads into the buffer solution, taking 6-10 seconds to draw each thread.
9. If the pump does not automatically stop when the syringes empty, press STOP.
10. Immediately wash tubing/blending applicator with cold water and a 20 mL syringe, plugging the other opening with your thumb (at least 5 water rinses per blending applicator opening).
11. Flush water out of blending applicator/tubing repeating step 9 using an empty 20 mL syringe.
12. Fibers can be removed from the bath after 10-15 minutes (not longer than 15 minutes!!!).

13. Label a cardboard box with your team number and team member names.
14. Stretch threads and secure along the cardboard box (~7.5 inch threads).
15. Leave stretched fibrin threads to dry overnight.

After Lab

1. Place a dry microthread into a clean petri dish. Place on microscope and adjust magnification. Take a picture using the microscope software. Make sure your thread images are all taken at the same magnification and are in focus.
2. Use a hemacytometer or other measurement reference to create a calibration image for the objective/magnification you use to acquire images of fibrin microthreads. This will allow you to convert your measurements in ImageJ from pixels to a unit of length.
3. Capture images of a dry microthread and use ImageJ to take several measurements (>3 per thread) of microthread diameter. Repeat this procedure for at least two microthread samples. (4 were completed) There is an ImageJ tutorial in the Week 2 folder for you to use.
4. Re-hydrate microthreads by immersing them in PBS for at least 30 minutes.
5. Repeat the image capturing and measurement steps for the rehydrated microthreads, taking at least 3 measurements per microthread from at least two individual microthread samples.(4 were completed)

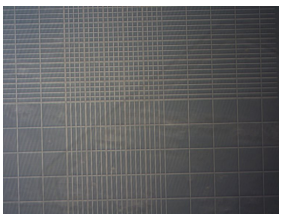
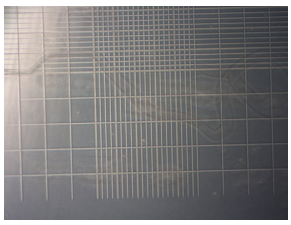
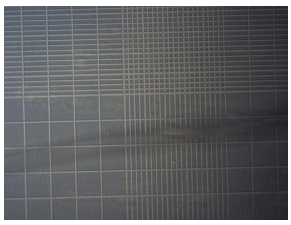
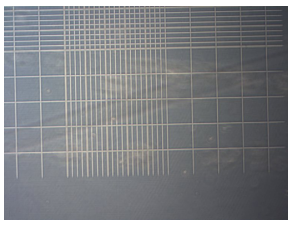
Observations

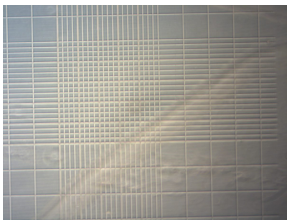
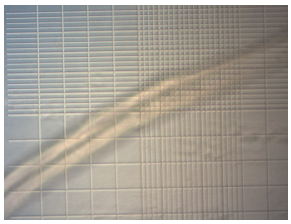
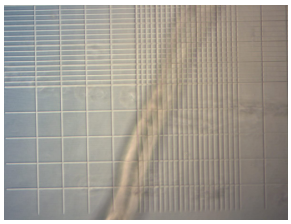
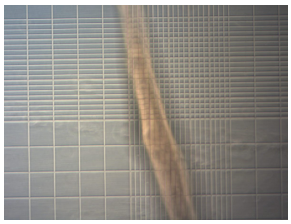
- While the Fibrinogen used in the lab exercise normally takes around 10 minutes to thaw, the fibrinogen ~~in~~ took about an hour to thaw (AM, Sep 7, 2021)
- The first half of threads that were extruded were inconsistent in size across the thread and had many bubbles on the ends and in the middle as a result of ~~going~~ extruding too slow (AM, Sep 7, 2021)
- While we waited the full, recommended 10 minutes between extruding the threads and removing them from the sheet, the threads became visible and solidified after about 8 minutes (AM, Sep 7, 2021)
- The longer threads that were extruded towards the end of the “extrusion period” were much easier to extract/remove from the sheet/HEPES solution (AM, Sep 7, 2021)
- The shorter threads were easier to extract, but broke a lot easier when transferred to the cardboard box for storage (AM, Sep 7, 2021)
- Post-extrusion the threads had inconsistent content uniformity, where parts of the threads looked disconnected ~~through~~ in the HEPES (CB, Sep 8, 2021)
- ~~One of the threads~~ The 3rd thread didn’t seem to rehydrate as much as the other threads and was hard to see through the PBS after soaking for 30 min as recommended (CB, Sep 8, 2021)

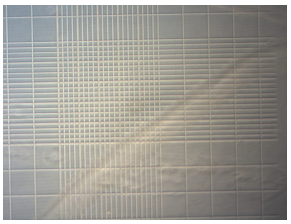
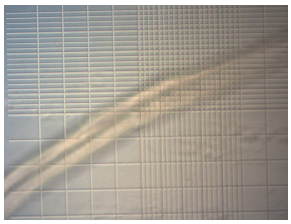
Raw Data

Images (AV, Sep 7, 2021)

Dry thread 1	Dry thread 2	Dry thread 3	Dry thread 4

Rehydrated thread 1	Rehydrated thread 2 (1)	Rehydrated thread 2 (2)	Rehydrated thread 3
			

Rehydrated thread 4 (1)	Rehydrated thread 4 (2)
	

Analysis (w/ explanation of process) (CB, Sep 8, 2021)

A table with the measured diameters for the dry and hydrated microthreads was made using three measurements from each thread to produce an average. Each thread standard deviation and coefficient of variance was then found using these diameters for both the dry and hydrated microthreads. Then, an overall standard deviation and coefficient of variance were found using the averages from each thread.

Thread #	Diameter (mm)	
	Dry	Hydrated
1	0.193	0.273
	0.189	0.280
	0.172	0.256
Avg:	**0.185**	**0.270**
SD:	**0.011**	**0.012**
COV:	**0.060**	**0.046**
2	0.141	0.472
	0.132	0.434
	0.141	0.348
Avg:	**0.138**	**0.418**
SD:	**0.005**	**0.064**
COV:	**0.038**	**0.152**
3	0.241	0.403
	0.222	0.579
	0.270	0.485
Avg:	**0.244**	**0.489**
SD:	**0.024**	**0.088**
COV:	**0.099**	**0.180**
4	0.202	0.321
	0.202	0.296
	0.202	0.328
Avg:	**0.202**	**0.315**
SD:	**0.000**	**0.017**
COV:	**0.000**	**0.053**
Standard Deviation:	**0.0446610693**	**0.1003780631**
Coefficeint Of Variance:	**0.9673877827**	**1.48892059**
Avg of Avg	0.192	0.373
Avg of COV	0.049	0.108

Conclusions (AS, Sep 8, 2021)

This specific technique for fabrication of fibrin microthreads resulted in larger diameters than recorded in the previous lab entry reported by Cornwell et al. and a discrepancy between different threads. This technique produced dry threads around 160 μm and rehydrated threads about 311 μm larger in diameter than the data displayed in the paper by Cornwell et al. The differences between experiments could lead to multiple different factors. The speed in which the coextrusion occurred between the thrombin and the fibrinogen was around 19 times slower in this fabrication procedure than in the Cornwell et al. procedure. The slower the extrusion may have led to build up of larger threads and therefore greater diameters. The discrepancy of diameters between separate thread samples in this fabrication process could be explained by the specifics of the technique itself. Fibrin microthreads were extruded into multiple lines, and between each line, ~~a group member~~ the group member extruding lifted the tubing out of the HEPES buffer. This break in between samples may have affected the dimensions of the strands.

Lab 2 - Biodegradable Microtubules - (9/10/21)

Procedure (AS, Sep 12, 2021) (CB, Sep 12, 2021)

In Lab

Fabricate DCPC Lipid Microtubules

1. Set up your stir plate, stir bar, beaker, and thermometer.
2. Label the outside of your tube (not the lid) with your group number, the date, and the contents.
3. Wrap your 15 mL conical tube in aluminum foil to prevent light from damaging the microtubules.
4. Make a plan for steps 5-10. These all need to be done rapidly to prevent the DCPC solution from cooling too quickly.
5. ~~Go over to the oven and, using a oven mitt, pour ~500 mL of pre-heated 55 ºC water into your beaker.~~ Fill up the beaker with 500 mL of DI water and heat up to 55 ºC using a hot plate and the stir bar.
6. While minimizing the amount of time the pre-heated EtOH is out of the oven, pipette 2.8 mL 100% EtOH into the glass vial with 5 mg of DCPC.
7. Transfer the EtOH and DCPC into the 15 mL conical tube.
8. Slowly add 1.2 mL diH2O (pre-heated to 55 ºC) from your beaker to the EtOH/lipid solution.
9. Microtubules should be protected from light from this point forward.
10. Place conical tube in 55°C water bath (your beaker) and allow to cool to 25°C at an approximate rate of 1°C/min. Record the time and bath temperature every 5 minutes in your lab notebook. Use these values to calculate the rate of cooling. This rate can be adjusted (if necessary) by adding ice to the bath. Make a note of the time points
11. Remove 40 µL of the microtubule solution and mix with 360 µL of diH_20 in a microcentrifuge tube. Repeat so your team will have two samples. This will be used in the “Measure Microtubules” section below.
12. To the remaining microtubules, add 75 mg of trehalose, a cryoprotectant which stabilizes the microtubules, to bring it to a final concentration of 50 mM.
13. Bring the remaining ~4 mL of microtubule solution to the TA. The microtubules you made in lab this week will be lyophilized (freeze-dried) and returned to you next week for protein (BSA) loading.

After Lab

1. Load a hemocytometer with the microtubule sample you diluted in class and collect images (enough to measure 25 microtubules per sample).
2. Using the known distances of the squares on a hemacytometer (look this information up online), measure the length of the microtubules using ImageJ. Measure the length of at least 25 microtubules and obtain at least 2 images from your sample of microtubules. The second sample is just a back-up and is not required for this step unless you were unable to locate microtubules from the first sample.

Observations

- While the DI water was meant to cool at a rate of about 1℃ per minute, our cooling process was taking significantly longer. The process as a whole took around 50 minutes to complete. (AS, Sep 12, 2021), (AM, Sep 12, 2021)

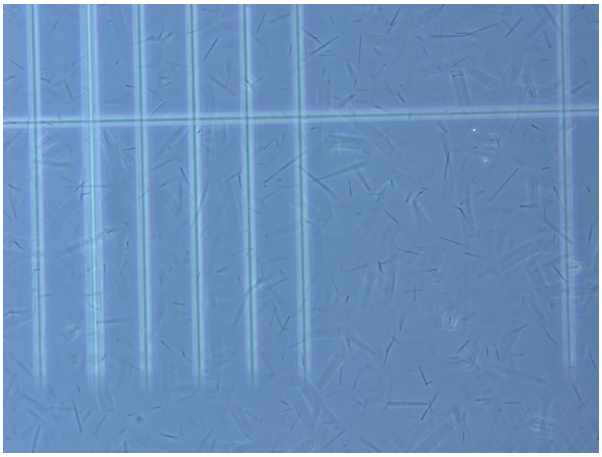
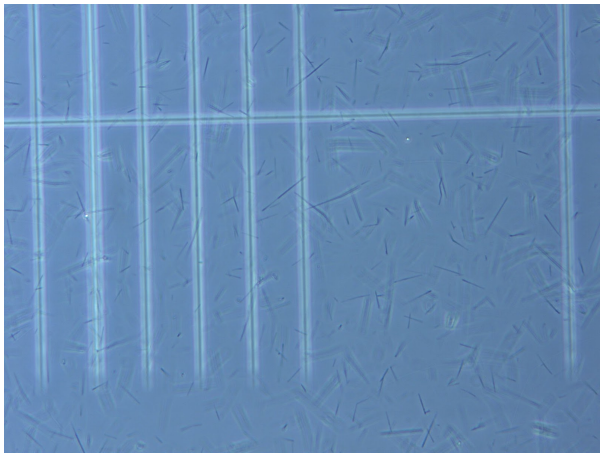
- Room temperature water was added to move along the cooling process. Water was also removed from the beaker with a pipette so the water wouldn't overflow. (CB, Sep 12, 2021)
- Difficulties with locating microtubules under the microscope with correct brightness,magnification, and focus -> this was likely due to incorrect loading of the microtubule solution into the hemocytometer initially (AV, Sep 16, 2021)

Raw Data

(AM, Sep 12, 2021) (AM, Sep 16, 2021)

Time	**Minutes Since Cooling (min)**	**Temperature (℃)**
02:45 PM	0	52
02:46 PM	1	51.2
02:47 PM	2	50.3
02:48 PM	3	50.0
02:49 PM	4	49.6
02:50 PM	5	49.1
02:51 PM	6	48.7
02:52 PM	7	47.8
02:53 PM	8	47.3
02:54 PM	9	46.9
02:55 PM	10	46.2
02:56 PM	11	45.8
02:57 PM	12	45.4
02:58 PM	13	44.9
03:00 PM **(*H_2O added*)**	15	43.9
03:01 PM	16	42.9
03:04 PM **(*H_2O added*)**	19	42.1
03:05 PM	20	41.6
03:07 PM	22	41.4
03:08 PM	23	39.8
03:09 PM **(*H_2O added*)**	24	39.6

03:10 PM	25	39.2
03:11 PM	26	38.8
03:12 PM **(*H_2O added*)**	27	38.6
03:13 PM	28	37.2
03:14 PM	29	37.0
03:15 PM **(*H_2O added*)**	30	36.5
03:16 PM	31	35.9
03:17 PM **(*H_2O added*)**	32	35.7
03:18 PM	33	35.1
03:19 PM	34	34.9
03:20 PM	35	34.7
03:21 PM	36	34.6
03:22 PM	37	33.8
03:23 PM	38	33.7
03:24 PM	39	33.5
03:25 PM	40	32.3
03:26 PM **(*H_2O added*)**	41	31.9
03:27 PM	42	31.6
03:28 PM	43	30.5
03:29 PM **(*H_2O added*)**	44	30.4
03:30 PM	45	29.3
03:31 PM **(*H_2O added*)**	46	28.5
03:32 PM	47	27.7
03:33 PM **(*H_2O added*)**	48	26.9
03:34 PM **(*H_2O added*)**	49	26.9
03:35 PM	50	25.6

Microscopic Picture #1 (9/10)	Microscopic Picture #2 (9/10)
	

Analysis

It was found that the cooling process was noticeably slow throughout the cooling process as can be seen from the table of data above. The lab recommends a cooling rate of 1 degree per min, but the actual was about 1 degree for 3-5min depending how long the water had been cooling for. To speed up this process we added small amounts of tap water into the beaker to speed up the cooling process. We were careful not to add too much of the room temperature tap water because cooling the microtubules too quickly could denture the formation. (CB, Sep 16, 2021)

Leftover microtubule solution in the 15mL conical tube was preserved via the addition of trehalose which acts as a cryoprotectant and freeze-dries the lipid structures within the microtubules that are still in the tube for future use/analysis. (AV, Sep 16, 2021)

When ~~measuring~~ capturing images of the microtubules under the microscope we found that they were best observed under the second phase of the microscope. Once the microtubules were located in the hemocytometer, we were able to capture two images that had at least 25 microtubules to measure with ~~image J.~~ ImageJ. (CB, Sep 16, 2021)

Bellamkonda et al. observed average microtubule lengths of 46 +/- 21 µm whereas our data had average lengths of (inserts length value here) (AV, Sep 16, 2021)

Signatures: Caitlin Bonavita, Andy Voronin, Andrew Sifferlen, Ana Mina
Date:9/16/2021

Lab 3 - BCA Assay Calibration Curve - (9/16/21)

Procedure (AS, Sep 18, 2021) (CB, Sep 22, 2021)

In Lab

Protein Standard Curve Preparation:

1. Label a microcentrifuge with the date, your team number and the solution: “1 mg/mL BSA”.
2. Create the 1 mg/mL BSA solution by diluting the 100 mg/mL stock solution (prepared in Week 2) 1:100 in PBS in this microcentrifuge tube (final volume = 1 mL).
3. 2) Label 7 microcentrifuge tubes with the date, initials and each of the following BSA concentrations: a. 0, 31.25, 62.5, 125, 250, or 500 µg/mL
4. 3) Generate samples at the concentrations listed above by serial dilution of the **1 mg/mL BSA** working solution (we will go through this in lab).
5. 4) In the tube labeled 500 µg/mL pipet 500 µl of the 1mg/mL solution you just made and mix it with 500 µl PBS, to dilute the solution in half. *Note: at this point you will have 500 µl of the 1mg/mL solution. This is more than enough to do the whole experiment.
6. For each subsequent dilution, take 500 µl of the previous solution and dilute it 1:1 with PBS for a final volume of 1 mL; For the 0 µg/mL sample, use PBS without protein.

Protein Measurement:

1. Obtain ~~4 mL~~ 3mL of BCA working reagent in a 15 mL conical tube from the TA.
2. Label a 96 well plate. You will pipet known (standard concentrations) into separate wells in this plate.
3. Add 25 µL of each of your BSA samples IN DUPLICATE to the 96 well plate.
4. Store in the fridge, labeled with your group number and the date

~~***TA will do steps 5-9 on Thursday at 2pm**~~

5. Add 200 µL of the BCA working reagent to each well/protein sample.
6. Gently swirl the plate to mix. Cover the plate; label the time on the lid and place in the oven (37ºC) for 30 minutes.
7. The BCA reagent will change color from green to purple in the presence of protein.
8. Turn in your labeled plate to the TA to read the absorbance on a spectrophotometer (plate reader).
9. Turn in any leftover BCA reagent to the TA to collect for disposal as hazardous waste
10. Steps 5-9 recompleted on 9/21/2021 due to a malfunction with the measuring tool.

Loading Microtubules with BSA Protein

1. Pick your microtubules and a centrifuge tube of an unknown microtubule sample from the front of the room.
2. Add 400 uL of 100 mg/mL BSA solution to lyophilized microtubule samples in the 15 mL conical tube
3. Leave the samples in the BSA solution overnight at room temperature.

4. Add 5 mL PBS to the samples and centrifuge at 400g (~~2,000 RPM on the large centrifuge in the lab~~ 3250 RPM in the small centrifuge in the lab) for 5 minutes. Remove supernatant to remove unloaded, extra-tubular protein. Repeat 2X.
5. Make sure to change out pipette tips as you switch from unknown to known.
6. Add 1000 uL PBS to resuspend the microtubules in each centrifuge tube, and aliquot the resulting solution into two separate microcentrifuge tubes. This should result in a total of 4 microcentrifuge tubes (2 of your microtubes, and 2 of the instructor provided tubes), each containing 500 uL of solution.
7. Place vials on the rotator in the oven and incubate at 37°C

Post Lab

Compiling a Release Profile

1. At three different time points after initiating incubation (e.g., 1, 3, 6 days), remove the microtubule samples from the incubator and collect a supernatant sample by centrifuging the particles at 400g (3250 RPM in the small centrifuge in the lab) for 5 minutes. Remove the tube from the centrifuge. The solution should be clear, and the microtubules should be concentrated in a pellet at the side of the tube as shown in Figure 1.
2. Remove 250 µL of supernatant (PBS with released protein). Transfer each supernatant sample to a labeled microcentrifuge tube and store it in your team's tube box in the refrigerator.
3. Add 250 µL of fresh PBS back to each microtubule sample to replace the supernatant volume.
4. Resuspend microtubule pellet by pipetting back and forth until there are no solids left on bottom of the centrifuge tubes. (AV, Sep 23, 2021)
5. Place the microtubule samples back on the rotator in the incubator.
6. Repeat steps 1-4 for each time point for each sample.

Observations:

- The spectrophotometer (plate reader) was not functioning correctly when attempting to develop a calibration curve for the BCA Assay of BSA in PBS. The standard curve should produce higher absorbance with higher concentration of BSA protein. Instead the absorbance was around 0.4 and had no correlation among the concentrations. (AS, Sep 18, 2021)
- There was some BSA assay left over in the tube after pipetting into the wells (CB, Sep 22, 2021)
- The micropipette would sometimes click over 5 microliters when pipetting into the wells and during serial dilution. (CB, Sep 22, 2021)
- The pipette tips were changed when switching from the known to unknown samples to ensure no cross contaminations. (CB, Sep 22, 2021)

Raw Data: (AS, Sep 22,2021)

BSA Concentration (µg/mL)	Mean Absorbance	Standard Deviation	Coefficient of Variance
1000	1.055	0.056568542	0.053619472
500	0.661	0.025455844	0.038511111
250	0.468	0.021213203	0.045327358
125	0.375	0.009899495	0.026398653
62.5	0.319	0.002828427	0.008866543
31.25	0.2725	0.006363961	0.023353985
0	0.243	0.011313708	0.046558471

The table above represents the statistical calculations from our data in the lab. Data we collected in the lab displays the absorbances of differing BSA protein concentration in PBS.

Analysis: The BSA assay data shown in table above shows a sustained release profile of protein filled lipid microtubules. As BSA protein concentration decreases the absorbance also decreases. This regression is ideal for an optimal drug delivery profile. (AV, Sep 23, 2021)

Preparing Buffers and Solutions - (08/26/21)

Procedure (AS, Sep 23, 2021)

In Lab

Phosphate-buffered saline (PBS; 155 mM NaCl, 1 mM KH_2PO_4, 3 mM Na_2HPO_4, pH 7.4; 250 mL total volume)

1. Calculate the mass of each salt needed for the PBS
2. Use an appropriate balance, weigh boat and spatula to weigh each salt. Use a different spatula for each salt – do not cross-contaminate salts!!!
3. Add salts to ~~a~~ your 500 mL bottle.
4. Add ~ 200 mL deionized water to the bottle and place on a magnetic stir plate to dissolve.
5. After salts have dissolved, measure the pH using the pH meter. Add small droplets of either HCl or NaOH solution to adjust pH up or down as necessary. Re-measure the pH and repeat measuring and adjusting the pH to 7.4.
6. Pour the solution into a graduated cylinder and fill to 250 mL with deionized water.
7. Pour the PBS back into the glass bottle. Label the bottle with lab tape and a marker. List the name of the reagent, the pH, the date, and your team's initials.
8. Store the PBS at room temperature

Bovine serum albumin (100 mg/ml BSA dissolved in PBS; 1 mL total volume)

1. Label a microcentrifuge tube with the date and your team's initials, and label it "100 mg/mL BSA"
2. Weigh BSA and add it to a 1.5 mL microcentrifuge tube.
3. Gently add 1 mL of the PBS you prepared to the same tube.
4. Gently invert to mix. DO NOT SHAKE the solution, as this will cause foaming (which denatures proteins!). Invert gently several times until mixed.
5. Store at 4 °C in a personal fridge box for your team. You can pick this up in the front of the room

HEPES buffer 10X stock solution (100 mg/mL BSA dissolved in PBS; 1 mL total volume)

1. Add HEPES (MW 238.3 g/mol) to diH_2O
2. Adjust pH to 7.4 using NaOH/HCl (
3. Bring final volume to 100 mL using a graduated cylinder.
4. Transfer to a clean glass bottle.
5. Transfer to a clean glass bottle.

Calcium Chloride (10 mM solution; prepare 40 mL total volume)

1. Add $CaCl_2$ (MW 110.98 g/mol) to 40 mL of diH_2O
2. Mix in a clean glass bottle.

3. Store at 4 °C on the shelf in the fridge labeled with your class.

Observations

- While gently inverting the BSA and PBS, the two did not mix easily. A pipette tip was used to mix easier. In some attempts, too much BSA clung to the pipette tip, but as long as the mixture did not foam, the proteins did not denature. (AS, Sep 23, 2021)

Raw Data (AS, Sep 23, 2021)

Salt/Solute	**Concentration (M, or mol/L)**	**Molecular Weight (g/mol)**	**Final Volume (L)**	**Mass (g)**
PBS				
$NaCl$	0.155	58.44	0.250	2.26
KH_2PO_4	0.001	136.086	0.250	0.034
Na_2HPO_4	0.003	141.96	0.250	0.1
HEPES	0.100	238.3	0.100	2.38
$CaCl_2$	0.040	110.98	0.040	0.18

Analysis (AM, Sep 23, 2021)

Four buffer solutions were made during the lab period in preparation for future biomaterial laboratory endeavors. It was important to initially only add water to about 70-80% of the final amount of desired solution. This is because ~~there needs to be~~ pH'ing the solution will require an addition of a large amount of liquid in the form of either an acid or a base (depending on the buffer being made). A stir bar was used to mix the solution as the acid or base was added to the solution to ensure gradual and even integration/mixing.

Conclusions (AM, Sep 23, 2021)

Buffers are used to prevent the pH of a solution from fluctuating. In this lab period, 4 buffer solutions were made: Phosphate-buffered saline (PBS), Bovine serum albumin (BSA), HEPES, and Calcium chloride. The PBS and BSA will be helpful when making microtubules and tracking their protein release rate. The HEPES buffer will be useful in slowing down the cross-linking reaction between fibrinogen and thrombin when making fibrin microthreads.

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Date:9/23/2021

Lab 4 - Measuring Protein Concentrations in Standards and Supernatant Samples - (09/23/2021)

Procedure (AS, Sep 25, 2021)

In Lab

1. Obtain 6 mL of BCA protein detection reagent in a 15 mL conical tube from the TA.
2. Label a 96 well plate. You will pipet your known (standard concentrations prepared in Week 4) and unknown (supernatant samples collected from both sets of DCPC microtubules) into separate wells in this plate.
3. Add 25 µL of each of your BSA standard curve samples IN DUPLICATE to the 96 well plate.
4. Add 25 µL of each of your BSA standard curve samples IN DUPLICATE to the 96 well plate.
5. ~~Store in the fridge, labeled with your group number and the date~~.
    a. ~~***TA will do steps 6-9 on Tuesday and Thursday at 2pm**~~
6. Add 200 µL of the BCA working reagent to each well/protein sample.
7. Gently swirl the plate to mix. Cover the plate; label the time on the lid of the place and place in the oven (37ºC) for 30 minutes.
8. Turn in your labeled plate to the TA to read the absorbance on a spectrophotometer (plate reader).
9. Turn in any leftover BCA reagent to the TA to collect for disposal as hazardous waste.

Observations:

- The samples from 9/21/2021 were not clear solutions, but somewhat clouded. The absorbance measured from these samples was greater than all of the other samples. Clearly there was some error in collecting samples as the absorbance should be decreasing with every day. (AS, Sep 25, 2021)
- The samples were taken on day 2, 4, 6 rather than 1,3,5 as the lab suggests. This was run by the professor and deemed fine. (CB, Sep 30, 2021)

Raw Data: (AS, Sep 25, 2021)

Standard Curve		
BSA Concentration (µg/mL)	Absorbance 1	Absorbance 2
1000	1.343	1.303
500	0.848	0.825

250	0.568	0.552
125	0.418	0.416
62.5	0.329	0.328
31.25	0.27	0.268
0	0.233	0.236

The table above represents the absorbance measured by the spectrophotometer for our standard curve data. The standard curve data includes the known concentration of BSA in PBS in μg/mL.

Date	Sample ID	Absorbance
Sep 19, 2021	Known 1	0.524
		0.521
	Known 2	0.489
		0.48
	Unkown 1	1.203
		1.17
	Unkown 2	1.041
		1.004
Sep 21, 2021	Known 1	0.895
		0.868
	Known 2	0.879
		0.858
	Unkown 1	1.78
		1.429
	Unkown 2	1.521
		1.329
Sep 23, 2021	Known 1	0.277
		0.276
	Known 2	0.298
		0.299
	Unkown 1	0.526
		0.532
	Unkown 2	0.491
		0.495

The table above represents the absorbance measured by the spectrophotometer for our supernatant samples. Two known and unknown samples were taken each day. Using the standard curve from the data in the previous table, the concentration of BSA in PBS for each sample can be calculated.

Analysis: The absorbance values of each for each of the samples in the well plates were measured by the spectrophotometer and reported in the table shown. A standard curve was created using the serial dilution samples and was used to find the unknown concentrations of the BSA protein in the PBS in each of the samples.

Conclusions: The values found are based off of the light let through the samples, and therefore corresponds to how dark the samples turned with the presence of the BSA assay. The samples with the highest absorbance will be purple while the lowest will look more green. After the concentrations are calculated they can then be used to determine how much protein was released on each day. For our lab something went wrong with the day 2 sample that would throw off the final calculations, therefore RP4 (released protein on day 4) and RP6 are used to represent the amount of protein released on each of those days. (CB, Sep 30, 2021)

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Date:9/30/2021

Lab 5 - PDMS and Hydrophobicity - (9/23/2021)

Procedure:

In Lab

1) Weigh 10 parts Sylgard silicone elastomer base and 1 part Sylgard silicone elastomer curing agent. 5g of base and 0.5g of curing agent (10:1 mass/mass ratio)

*Note: DO NOT MIX THE STOCK SOLUTIONS!!! Use separate weighing materials for each reagent.

2) Pour reagents together and thoroughly mix the elastomer base and curing agent.
3) Pour the well mixed solution into your mold (in our case, we will just use the weigh boat you mixed it in).

*One important thing to keep in mind is that the uncured reagents are very tacky and can make a big mess of anything they contact (the degassing chamber, the scale used to weigh reagents). Students should wear gloves when handling PDMS, be careful not to spill, and make sure they clean up the space and equipment they use when preparing PDMS. We keep “Goo Gone” in the lab for this reason…

4) Degass the PDMS by putting it into a vacuum chamber for at least 30 minutes (larger/thicker volumes of PDMS may require more time).
5) After degassing, visually inspect the PDMS to ensure that there are no more bubbles. If there are, repeat steps 4 and 5.
6) Cure the PDMS by placing the weighboat into an oven set for 60 °C for at least 30 minutes (larger/thicker volumes of PDMS may require more time).
7) While you are waiting for PDMS to cure, complete the contact angle measurements on glass coverslips and the material of your choice.
8) Measure the contact angle of the glass by placing a glass coverslip on a clean, flat surface. **Only handle samples with gloves or forceps to avoid contaminating the surface!**
9) Place a droplet of deionized water (~50μL) on the surface.
10) Take a digital image of the droplet/surface interface with a digital camera (bring your own, or we will have some that you can borrow).
11) Repeat for a total of four drops and corresponding measurements, taken at different locations on the surface.
12) Repeat steps 8-11 to measure the contact angle of a material of your choosing and your PDMS once cooled. All teammates must select a material and make at least one contact angle measurement on that material. However, for the lab assignment, only one additional material needs to be completely analyzed.

Open each image in ImageJ, and use the angle measurement tool to measure the contact angle between the water droplet and the surface.

Observations:

- The drop of DI water on the Ti-84 cover was difficult to take a picture of since it was white and the surface had such high wettability that the contact angle was small. (AS, Sep 26, 2021)
- The PDMS was very thin when taken out of the tray, so the drops were taken from the thickest middle section. (CB, Sep 30, 2021)
- The PDMS was left in the oven with the heat too high so the plastic on the boat started to melt, but the professor said it was okay, as PDMS is a highly resilient material. (CB, Sep 30, 2021) (AM, Sep 30, 2021)

Raw Data: (AS, Sep 26, 2021)

Sample	Angle 1	Angle 2	Angle 3	Angle 4
PDMS	109.702	109.997	125.065	117.05
Glass	36.666	32.337	30.853	28.862
Polypropylene (PP)	102.441	92.23	106.978	90.07

The table above represents the measured contact angle of DI water on the surfaces of our samples. Each measurement was made through ImageJ.

Analysis: After the drops were pipetted onto the different surfaces, a picture was taken of each and imported to imageJ. Here the contact angle of each of the drops was found using the angle tool. This was done for 4 on each of the surfaces. The different contact angles on each of the surfaces relates to the wettability of each. The PDMS contact angle averaged at about 121 degrees, the Glass averaged at about 32 degrees, and the polypropylene averaged at about 98 degrees. (CB, Sep 30, 2021)

Conclusion: The contact angle for the PDMS was much greater than that of the Glass and the Polypropylene in between at 90 degrees. These contact angles all correspond to the wettability of each of the surfaces. With the highest contact angle measurements, the PDMS would be the least wettable because the contact angle is much more and therefore the water clings to the surface much less. The polypropylene would be in the middle and the glass would be the most wettable. (CB, Sep 30, 2021)

Signatures: Caitlin Bonavita, Andy Voronin, Andrew Sifferlen, Ana Mina
Date:9/30/2021