EncapsuLab Notebook

Original Bioencapsulation Protocol: Adapted from Aksan / Wackett Paper: J. Mater. Chem. A, 2013, 1, 11051

Cell Production

Plate validated bacteria culture on LB + appropriate antibiotic (Km for pBBR-BB::mer).

SOLUTIONS

Super Optimal Broth (SOB)
20.0 g tryptone
5.0 g yeast extract
0.584 g NaCl
0.186 g KCl
1.204 g $MgSO_4$ (anhydrous)
Fill to 1L with dH_2O
Adjust pH to 7.0 with 1M NaOH if necessary.
Autoclave.

PROTOCOL

1. Create starter culture with a single colony in 5 mL SOB.
2. Incubate at 37°C in 250 rpm shaker overnight
3. Grow intermediate culture by inoculation with 3% starter culture
4. Incubate at 37°C in 250 rpm shaker until OD600 of 0.5-0.75
5. Inoculate 100 mL with 3 mL intermediate culture
6. Incubate at 37°C in 250 rpm shaker for ~16 hrs (until saturation)
7. Centrifuge at 5000-6000xg for 10 min at 4C. Discard supernatant
8. Measure mass of pellet and resuspend in dH_2O to concentration of 0.1-0.2 g of cells/mL Note: PBS is better

MATERIALS NEEDED

Falcon tubes (10 mL)
LB Plates
Dry ingredients (SOB)
Pipette tips (100uL/1mL)
dH_2O

Bioencapsulation

Gel encapsulation of bacteria

SOLUTIONS

Phosphate-Buffered Saline (PBS)
8.0 g NaCl
0.2 g KCl
1.44 g Na_2HPO_4
0.24 g KH_2PO_4
Fill to 1L with dH_2O
Adjust pH to 7.4 with 1M HCl if necessary.
Autoclave if desired.

PROTOCOL

1. Dilute Ludox TM40 in ultrapure water at _:_ ratio
2. Add SPEG to Ludox mixture at 1:4 ratio
3. Separately, hydrolyze TMOS with water and 0.01M HCl by sonication with a typical ratio 1:1:0.1
4. Mix with SPEG solution at 1:2 ratio
5. Combine with cell mixture at 1:1 ratio
6. Transfer to molds and to cure (room temperature)
7. Wash with PBS (to detach non-anchored E. coli)

Note: will get ratio from Aksan lab
Note: temperature and duration not noted

MATERIALS NEEDED

Ludox TM40
silica-PEG
Tetramethyl orthosilicate (TMOS)
HCl
Molding apparatus
Sonicator

<u>Viability Assessment</u>

Gel encapsulation of bacteria

SOLUTIONS

Phosphate-Buffered Saline (PBS)
8.0 g NaCl
0.2 g KCl
1.44 g Na_2HPO_4
0.24 g KH_2PO_4
Fill to 1L with dH_2O
Adjust pH to 7.4 with 1M HCl if necessary.
Autoclave if desired.

PROTOCOL

1. Extract 0.1 g aliquot of gel
2. Pulverize by compressing between two glass slides
3. Resuspend in 3 mL PBS
4. Dilute samples and plate (in triplicate)
    a. Dilute 10uL in 990uL PBS
    b. Dilute 50uL in 950uL PBS
    c. Plate 20 uL of each sample

Resulting colony counts:
    Dilution 1: (100x dilution)*(3mL/20uL)=15,000 cells/colony
    Dilution 2: (2000x dilution)*(3mL/20uL)=300,000 cells/colony

MATERIALS NEEDED

Glass slides
LB Plates
Pipette tips (100uL/1mL)
Dry materials (PBS)

June

1/6/2014

Researching previous iGEM models. -Di
Encapsulation meetup, design formulation, research into device design. -Niko

10/6/2014

Discussing kind of models possible for our project. Brainstorms parameters for the system level model, brainstorm cellular model, layout plan to see where things go, tried different CFD software. -Di

16/6/2014

Downloaded student version of solid works, tried student version and found out limitations of the software, put together cellular model in simbiology, searched for parameters for the cellular model and put together model folder and presentation slides. -Di

17/6/2014

Met with Baris from Aksan lab to run through encapsulation; method does not encapsulate live cells, look for alternative methods.

Baris comments:

1) It is better to suspend your cells in PBS.
2) I can't give you a ratio for diluting TM40. I do not do it myself, and do not know if Eduardo did it in his paper. If so, just follow the protocol in his paper. If not, I do not know why you would want to do it.

Overall comment: I suggest you guys follow a protocol exactly as it is written in a paper. If it works-- great. If not, then try to fix it by changing things. It is easier to modify an established protocol then coming up with a new one.

22/6/2014

Simplified cellular model and tried to run sensitivity analysis, compared our model results to those of the other igem teams from previous competitions. -Di

Mercury Testing research. -Niko

Materials list, procedure design. - Niko

July

4/7/2014

Updated weekly powerpoint, put together parameter list that need to be measured by wet lab, brainstorm system level model. -Di

Looked for alternative encapsulation methods, testing methodology, device design. - Niko

6/7/2014

Developed framework encapsulation protocol, based of Wackett's atrazine remediation paper. Not yet published.

14/7/2014

Srijay, Patrick, Stephen, David, Niko

Revisions made by Kelly Aukema, adapted protocol

*Tried 2:2:1 TM40+PBS+PEG600:bacteria:TEOS crosslinker, gel solidified nicely. Limited mechanical strength.

*Attempted a mineral oil bath with 2:1 ratio oil:encapsulation, failed to entirely emulsify approximately sized particles, next time try 5:1 or 10:1. Must use larger stir bar that fits to the exact side of our container next time to avoid donut of encapsulate formation.

*Need to test mechanical strength with experiment. Set up flow through with a control gel (2:2:1, 0% bacteria, keep PBS ratio the same), and then 0.1g bacteria /mL encapsulate, 0.2g/mL, 0.3g/mL. Run over 24-48h, measure compactness vs. control. Need to maintain tube length, diameter, flow rate constant. Contact Jim Parejko to use his Ismatec peristaltic pump, also need to purchase some fritted glass buchner funnels.

http://www.ismatec.com/int_e/pumps/t_reglo/reglo_digital.htm

18/7/2014

Meet with Kelly Aukema, introduced us to all necessary methods/chemicals

+Lots of suggestions for viable cell encapsulation:

"I have been harvesting at ~5000-6000xg for 10 min only, so they could shorten their spin time if they want. And I would use PBS to resuspend the cells instead of water. For the gel recipe, something isn't quite right, but I'm not sure what is meant by step #4. This is how I wrote the protocol for the gel I used:"

Solution A: Tetramethoxysilane (TMOS)/Ultra Pure Water (UPW)/1 M HCl = 1/1/0.001 v/v/v
To prepare solution A: Hydrolyze TMOS mixture by sonicating in an ice bath for 15 minutes or stiring for 2 hrs at room temperature. This will look like a homogeneous mixture after it's done. Keep on ice or in the fridge.

Solution B: Ludox TM-40 silica nanoparticles/PBS/PEG 600 = 2/2/1 v/v/v
Solution C: Cells suspended at 0.2g/mL PBS
To make the final gel, mix 1/2/2 A/B/C v/v/v. It should solidify within 2 minutes. If a slightly longer gelation time is desired, keep all solutions on ice.

20/7/2014

Niko

*Bought ½” plastic tubing and hose clamps, as well as 2 3.5GPH Aqualifter pumps for testing, will drop off at lab.

22/7/2014
Dry lab meeting, discuss future aims for acquiring materials

24/7/2014
Picked up reagents for encapsulation from Aksan lab. PEG, TEOS, TM40.

25/7/2014
*Contact Chris for DEHS organic waste disposal ,
Jar labeled **A1 waste**; 80% water, 20% mineral oil

Experiment 1: 2:2:1 ratio, no bacteria. Mineral oil:gel in 3:1 ratio, spun too fast and ratio too low, subsequent gel was too small and not uniform. Looked at under microscope; gel fragmented and non-spherical. Fail.

Experiment A: 2:2:1 ratio, no bacteria. Mineral oil:gel in 10:1 ratio, spun at 300rpm. Subsequent gel was good. Removed supernatant oil, phase separated with water, and then vacuum filtered resulting gel. Resuspended in water, gel looks good. Now must try with bacteria-infused gel.
Stored in container labeled **A1**

7/28/2014
Established workflow of all upcoming encapsulation experiments (see PDF)

7/29/2014
Patrick, Srijay

Acquired 50 mL TEOS from Kelly (no stock of TMOS)
Attempted cross-linker hydrolysis with 1:1:0.001 TEOS/UPW/HCl (8 mL total volume); however, after 1 hr no phase separation was still very distinct without any signs of mixing so HCl was raised to 1:1:0.005 to accelerate reaction
*Raise stir speed to ensure full mix of TEOS/UPW, possibly useful
Attempted mock encapsulation without bacteria-
+Used mineral oil 2:1 ratio, TMOS as crosslinker; no beads formed- largely, globs of material

August

2/8/2014
Another attempt at producing silica beads
+Used petroleum oil 5:1, stir stick speed, flask size, and stick size, TEOS as crosslinker; some beads form, but large variations in size, formation of bubbles in mineral oil seem to cause varying viscosity in

6/8/2014
+Used mineral oil 5:1. Changed viscosity of oil prior to encapsulation and compared the resulting bead size; definitive improvement in bead formation and consistent size with this oil, final settings are 25 mL flask, >5:1 mineral oil to encapsulation ratio.

4/8/2014
Srijay, Patrick, Niko
Hydrolyzed TEOS for 2 hours, 1:1:0.01 ratio of TEOS:H2O:0.01HCl no phase separation = awesome. No cells added, filler amount if PBS added for the 0.2g/mL of cells to normally be added. Mixing into 5:1 ratio with mineral oil

+Checked for viability using finalized protocol; crushed beads better two glass slides, and scraped off mesh into 1 mL PBS, back calculated to find post-encapsulation cell survival

11/8/2014:
Results of previous viability tests
+Dilution plates under diluted, data inconclusive

13/8/2014
Repeat of encapsulation experiment

15/8/2014
Results of previous viability tests
+E. Coli (no plasmid) look like 5-10% cell viability (high enough to proceed with future experiments)

25/8/2014
Srijay, Patrick, Niko

Mineral Oil, funnels, and TMOS have arrived. Prepared 7.5mL of control beads (bacteria free) using TMOS. Labeled and set aside

New method for mineral oil removal: In a 500mL sep. funnel, phase separate between aqueous phase and organic, removing the aqueous with the encapsulated beads. Repeat 2x in order to get rid of bacteria present in supernatant.

26/8/2014
Repeat encapsulation with E.Coli + Mer operon

29/8/2014
Srijay, Niko
Results of viability tests
+ Approximately 15% viable cells immediately following encapsulation, 5% 42 hours following encapsulation

Shewanella pBBRBB GFP, Pseudomonas pBBRBB GFP, Pseudomonas pBBRBB Mer, k12 pBBRBB Mer prepared.

0.1g/mL bacteria encapsulated in TMOS hydrolization.

Set up Ismatec peristaltic pump, 3 funnels in fume hood in CSD268 lab. Retrieved 5 flasks for mercury testing from Nater lab, labeled appropriately.

0.08 g of Pseudo GFP, 0.05 g of Pseudo Mer, 0.07 g of K12 and 0.05 g of Shewanella suspended to 0.05 g/mL in PBS

September

1/9/2014

Labor day, classes start

12/9/2014

Encapsulation with E. Coli w&w/o Mer Operon

14/9/2014

PBS contaminated, dilution plates provided no useful data

17/9/2014

Repeat of previous encapsulation, also with Psedemonas with Mer operon, samples used for mercury remediation testing

18/9/2014

Results suggest pre-encapsulation cell viability is largely dependent on OD prior to centrifugation and resuspension; from now on, grow cells only to ODs below 1.0

+Mer positive samples reduce 1ppt methyl mercury to 0, controls do not.

25/9/2014

Encapsulation with Pseudomonas/E.Coli Mer Operon, non-Mer Operon, and blank beads for mercury remediation test

+Samples given to Aunica

October

7/10/2014

Results collected and analyzed for mercury remediation effectiveness.

For all further results between 1/9/2014 and 16/10/2014, refer to the wet-lab notebook. EncapsuLab's successes led to EncapsuLab members helping out wetlab with data and results.