Fabrication of Alginate beads

References:

- AB Yeatts, CN Gordon, and JP Fisher. Formation of an Aggregated Alginate Construct in a Tubular Perfusion System. Tissue Engineering Part C, Methods. 17: 1171-1178 (2011). (PubMed)
- BNB Nguyen, H Ko, RA Moriarty, JM Etheridge, and JP Fisher. Dynamic Bioreactor Culture of High Volume Engineered Bone Tissue. Tissue Engineering Part A. 22: 263-271 (2016). (Pubmed)

Reagents/Solutions

Maintain pH for solutions at 7.4

- Alginic acid sodium salt → Sigma Aldrich
- HEPES
  NaCl
- $CaCl_2$
- EDTA (dissolve in PBS)
- PBS
- Hyaluronic acid sodium salt from F. Strepto-coccus Equi Species (-20 degC) → Sigma Aldrich

Table 1: Calculated weight of reagents (g) for desired concentrations and volumes.

Reagent	MW [g/mol]	Desired Conc [M]	Calculated weight (g) for desired total volume (mL)					
			100	200	300	400	500	1000
HEPES	260.29	0.025	0.65	1.30	1.95	2.60	3.25	6.51
NaCl	58.44	0.15	0.88	1.75	2.63	3.51	4.38	8.77
$CaCl_2$	110.99	0.1	1.11	2.22	3.33	4.44	5.55	11.10
		0.3	3.33	6.66	9.99	13.32	16.65	33.30
EDTA	372.24	0.05	1.86	3.72	5.58	7.44	9.31	18.61
		0.1	3.72	7.44	11.17	14.89	18.61	37.22
		0.15	5.58	11.17	16.75	22.33	27.92	55.84
		0.2	7.44	14.89	22.33	29.78	37.22	74.45

** NOTE: Always pH solutions to 7.4. Filter if necessary.

Preparing x-amt % w/v alginate solution (can vary % w/v):

1. Pour desired volume of buffer solution with 0.025M HEPES and 0.15M NaCl into beaker.
2. Weigh **x**-amt g of alginic acid and add into beaker.
3. Heat (3) and stir (6) solution for 30 minutes. Break up clumps if necessary.
4. Cool for 5-10 minutes and pour alginate solution into a 50 mL Falcon tube.
5. Sterile filter solution using a Steriflip/syringe filter.
6. Store in 4degC for max 3-5 days.

Table 2: Alginate weight amounts (g) for desired percentage.

% Alginate	Calculated weight (g) for desired total volume (mL)			
	50	100	150	200
0.8	0.4	0.8	1.2	1.6
1.2	0.6	1.2	1.8	2.4
2.0	1.0	2.0	3.0	4.0

Making alginate beads:

1. Fill a beaker with ~10 mL of 0.1M $CaCl_2$ for each 1mL of alginate solution. Place beaker on stir plate.
2. Stir solution with a stir bar. Make sure that a funnel is not formed.
3. Suck up alginate into a syringe with a needle (22 gauge) slowly to avoid air bubbles.
    a. If bubbles are present, tap the syringe gently
    b. You can change the size of the bead by changing the needle gauge
4. Inject alginate into $CaCl_2$ solution. Beads will form automatically
5. Wait ~15 minutes for beads to stabilize.
6. Remove stir bar with tweezers.
7. Remove beads from solution with measuring spatula (spray down with EtOH well, before use).
8. Place beads into desired location (well plates).
9. Rinse beads with DMEM (1x).
10. Repeat 1-7 to make more beads. Remember to use new $CaCl_2$ solution.

**** If you are encapsulating cells within the alginate bead always use aseptic techniques (i.e., biohood, autoclave instruments):**

1. Determine # of cells (X) that are required to obtain desired cell density.
   EQN: X cells/mL x 1 mL/30 beads = cell density (cells/bead)
2. Pellet cells from cell culture.
3. Resuspend cell pellet with desired alginate concentration using an electric pipette.
4. Now follow the 'making alginate beads' procedure.
5. Incubate cell-beads with ~2mL of media (+ 10% FBS) in 12 well plate.
6. Change media every 2 days.

NOTE: While waiting for the beads to stabilize set up next alginate-cell solution.

**** To remove cells from alginate bead:**

1. Aspirate media from well plates.
2. Pipet 2 mL of 100mM EDTA (pH 7.4) to each well for ~25-30 minutes in the incubator.
3. Put total solution volume in a 15mL centrifuge tube.
4. Rinse well plates with 1 mL of PBS.
5. Add in additional PBS (~4mL) to make solution less viscous.
6. Centrifuge cells and solution at 1000*xg* for 8 minutes at a level of 2 increase and 9 decrease.
7. Isolate cells by remove supernatant and blot tubes dry.
8. Resuspend cell pellet with 10uL of PBS.
9. Aliquot into microcentrifuge tubes.
10. Start assays.