Alginate Lab ONPRC Module 5 – Teacher Notes

Equipment Needs:

For each group of 2 -3 students

Small test tube rack containing:
- 1 mL needleless syringe
- 1 mL syringe with needle (optional)
- 1 mL plastic small diameter dropper with attached bulb
- 1 mL plastic graduated dropper with attached bulb
- 3 mL plastic graduated dropper with attached bulb
- 10 mL pipette (for $CaCl_2$)
- Pipette pump
- Pair of forceps/student
- Goggles and nitrile gloves/student
- Microcentrifuge tube and cap/student
- Small (1" diameter) plastic Petri dish/student
- 9 oz. plastic cup with water for rinsing/group

Larger test tube rack containing:
- 15 mL capped plastic vial of $CaCl_2$
- 15 mL capped plastic vial of 1% alginate solution
- 15 mL capped plastic vial of 2% alginate solution

Alginate Solutions

You can make different concentrations of alginate; 1.0%, 1.5%, and 2.0% are easy for students to pick up and move; 0.5% is more difficult; 0.25% is very difficult.

Using sodium alginate beads, 0.5%, 1.0%, and 2.0% solutions can be made by adding 0.25 grams of alginate in 50 ml distilled water, 0.50 g in 50 ml distilled water, and 1.0 g in 50 ml distilled water, respectively, made in 50 ml screw-cap tubes. [Do not add the alcian blue and acetic acid (see below)]. Label the tubes and add a few drops of different food coloring to each tube before putting on a rocker platform. Shelf life is about 4 months.

(An easy way to remember how to make the solutions is to add X grams of sodium alginate to 100 ml of water, where X is the percent you want to make. So for a 1% solution, add 1 gram sodium alginate to 100 ml of water. Then if you decrease the water, decrease the number of grams by the same amount.)

Calcium Solution (to gel alginate beads)

50 mM $CaCl_2$ (2.77 g)
140 mM NaCl (4.1 g)
500 ml distilled H_2O

- Measure 500 ml of distilled H_2O with a graduated cylinder
- Add $CaCl_2$ and NaCl to the water in a 500 ml beaker
- Stir with spin bar until thoroughly dissolved
  Sterile filter with a Millipore Stericup filter, in the hood
- Label with your name, the date, "Calcium Chloride Solution," and store at Room Temperature. Shelf life is 1 year.

For the alginate lab, go to www.nubio.northwestern.edu , then to the Labs tab at the top, then to the lab called Just Bead It.

Blue alginate bead solution:

0.375 gram sodium alginate (1.5% alginate; wt/vol)
Sigma Chemical A2158-100G 100 g = $28.10
0.125 g alcian blue (0.5% alcian blue; wt/vol)
Sigma Chemical 05500-5G = $85.00
0.0625 ml acetic acid (0.25% HAc, vol/vol)
25 ml distilled water or phosphate-buffered saline (Deionized water is best, if available)

- Measure 25ml distilled water with a graduated cylinder
- Pour water into a 50 ml screw-cap centrifuge tube
- Add acetic acid
- Add alginate and alcian blue to the tube
- Place tube on rocker platform overnight at room temperature to fully dissolve alginate.

Questions to Think About:

Page 3

- "A biomaterial is now defined as a substance that has been engineered to take a form which, alone or as part of a complex system, is used to direct, by control of interactions with components of living systems, the course of any therapeutic or diagnostic procedure" and "covers the wide range of physical, biological and chemical sciences that underpin the design of biomaterials and the clinical disciplines in which they are used. These sciences include **polymer synthesis** and characterization, **drug** and **gene vector** design, the **biology** of the host response, **immunology** and **toxicology** and self-assembly at the nanoscale. Clinical applications include the therapies of **medical technology** and **regenerative medicine** in all clinical disciplines, and diagnostic systems that reply on innovative contrast and sensing agents."
http://www.journals.elsevier.com/biomaterials/

Examples of biomaterials include alginate, medical implants, contact lenses, and scaffolding for tissue regeneration.

-Alginate comes from cell walls of brown algae, such as kelp and luminaria.

-You could find alginate in your home in some olives (the red filling rather than a pimiento), gum drops, and hair products.

-Alginate is a good biomaterial for ovarian follicle culture since it forms 3-dimensional spheres in which the follicles can grow. Without this alginate framework, the follicle wouldn't be able to maintain its 3-D shape.

Page 4

-If the follicle is cultured in just a Petri dish, it would try to grow as a collapsed, flattened structure.

-The alginate provides a 3-dimensional scaffolding in which the follicle can grow in its natural shape.