5E Learning Cycle Lesson Plan

1. **Title:** ONPRC Module 5 Alginate Laboratory

2. **Overview and Statement of Purpose**: The alginate lab is designed to teach students one of the cutting edge research procedures in oncofertility and to explore a bioengineering application that can be used to preserve the fertility in female cancer patients.

3. **Grade Level and Objective(s)**: High School lab activity on fertility preservation

4. **Student Background Knowledge:** Students should know that oncofertility is a new approach in medicine for working with cancer patients under 40 years old and encompasses comprehensive medical approaches to preserving fertility in these patients before their cancer treatment begins. One method of fertility preservation is to freeze the patient's oocytes (eggs) or sperm cells in liquid nitrogen prior to the cancer treatment. Sperm have been successfully frozen and thawed for many years and have resulted in live births. Oocytes are a different matter since the oocyte is much larger than a sperm cell and contains much more water. Oocytes can be easily frozen in liquid nitrogen but thawing them and having a live birth result has been problematic. The oocyte resides in a follicle until the follicle is mature and can ovulate or burst open, thus releasing the oocyte. One oocyte a month is released from an ovary during the monthly cycle in post-pubertal females. As the frozen oocytes within their follicles are thawed, they need a structure within which the follicles can continue to grow as they mature to ovulation. Follicles are 3-dimensional spheres and will collapse if not given a scaffolding.

Alginate is a natural choice of a biomaterial for this scaffolding. It is derived from large brown algae such as *Macrocystis* (such as giant bladder kelp), *Nereocystis* (such bullwhip kelp), and *Laminaria* (such as Devil's apron). Alginate is composed of polymers of two monomers, β-D-mannuronic acid and α-L-guluronic acid. The former polymer is called an M block and the latter is called a G block because of their distinctive ring structures. An alginate molecule is a polymer or long chain of M, G, and MG monomers bonded together. When there are a high proportion of G blocks in the molecule, calcium salts, like calcium chloride, can be added to form a strong gelatinous hydrogel of calcium alginate. This hydrogel is composed of one or more polymers suspended in water by crosslinking two or more of these molecules by covalent bonding. When the hydrogel is made, a follicle with an oocyte in it can be placed into the gelatinous sphere which will support it structurally while the follicle continues to mature and grow.

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5. **21st Century Skills**

A. Please list 21st Century Skills targeted by this lesson.

- Communication
- Collaboration
- Critical Thinking and Problem Solving

6. **Essential Knowledge (For teacher/instructor)**: The strength of the gel depends on several factors including the concentration of the alginate. (Other factors include the species of the alginate, its degree of polymerization, and the calcium concentration.) Using sodium alginate beads, 0.5%, 1.0%, and 2.0% solutions can be made in 50 ml screw-cap tubes by adding 0.25 grams of beads in 50 ml distilled water, 0.50 g in 50 ml distilled water, and 1.0 g in 50 ml distilled water, respectively. Label the tubes and add a different food coloring to each tube so the students can tell them apart when doing the lab. Seal each lid with Parafilm and lay each tube on its side on a rocker platform at room temperature overnight to fully dissolve the alginate. Shelf life is about 4 months.

The calcium chloride solution used to form the gel in this lab is made by dissolving 2.77 g of CaCl2 and 4.1 g of NaCl in 500 ml of distilled water. Stir with a magnetic spin bar until thoroughly dissolved. Label and store at room temperature. Shelf life is about 1 year.

7. **List of Materials:** Solutions of 0.5%, 1.0%, and 1.5% sodium alginate solutions made in 50 ml screw-cap tubes, calcium chloride solution, plastic droppers with different diameters, 1 ml syringe (without needle), small Petri dishes, forceps, and small plastic vials. As an extension of the lab, follicle encapsulation can be demonstrated by using small glass beads which imitate the follicles. The alginate gel spheres can be attached to small plastic pieces of mesh and the small glass beads can be inserted using very small diameter pipettes.

8. **Safety Considerations:** Since alginate, calcium chloride, and sodium chloride can be found in foods, no special safety considerations are needed. As always, students should be instructed not to eat and/or drink anything while in a lab setting. Students should wear goggles and gloves just to be on the safe side.

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9. **Detailed Lesson Plan**

A. **ENGAGE** - Engagement with transition question, challenge, or problem

i. **What will you do to engage the students?** Most students have eaten jello and might know how gelatin is prepared by dissolving the gelatin powder in hot water. Cold water is then added, stirred, and the liquid solution is put in the refrigerator to gel or (solidify). The teacher might demonstrate the preparation of jello in the classroom. Some already prepared jello might help show what happens to it as it sits at room temperature. What is happening to the molecules to cause the liquid to become a solid that doesn't readily melt at room temperature like solid ice would?

ii. **How will you connect to students' everyday lives?** We know that polysaccharides from algae (seaweed) – alginates, agars, carrageenans, and furcelleran- are found in the gel form in foods. Ask the students to look at home and see which of these substances they can find in their food, as listed on the label.

iii. **What questions will you ask? (Provide sample answers.)** What is the purpose of adding these algae to food? *(The algal additives provide gelling, thickening, and stabilization of the food. Alginates come from brown algae and agar, carrageenans, and furcelleran are derived from red algae.)*

What are other uses for algae (think science labs)? (Agar used to grow bacteria and yeast in a Petri dish.)

B. **EXPLORE** – Student-centered exploration of the topic/content

i. **How will you set up this exploration?** Students enjoy seeing and playing with calcium alginate formation. They soon learn that if they inject the sodium alginate solution into the calcium chloride solution too fast, they get long, worm-like structures when the goal is to make small, round spheres of calcium alginate gel. They become intrigued with experimenting with ways in which to make the smallest and roundest sphere. They might try slowly dropping it from a dropper at various heights and using different diameter droppers until they get the desired results.

ii. **What data will students gather or guided inquiry will student complete?** Students will be able to make a collection of the results of their trial and error. They should take notes about measurements taken as they attempt each trial. Each trial should be run 3 times and results noted. For instance, what is the diameter of the dropper, from what distance did they drop the sodium alginate solution into the calcium chloride solution, what is the shape and diameter of the gel formed (a drawing would be required here), and what percent solution was used.

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iii. **How will you help students generate their own questions?** Students will be asked to formulate questions regarding their data. Which solution formed the smallest and roundest spheres? How do the 3 solutions compare? Does the height from the solution matter? How? Does the diameter of the dropper matter? How? Does speed of delivery matter? How? What would be your recommendation to someone trying the same experiment in terms of % solution, distance, dropper diameter, and technique of delivery?
iv. **What questions will you ask as a means of formative assessment?** (Provide sample answers.)

SAMPLE QUESTION(s): Why do you take three measurements? *(In order to be as accurate as possible. By taking three different measurements, one can see that the results are repeatable.)*

Why do you measure from the same spot? *(Measuring from the same spot keeps the measurements accurate. If you measured from a different spot every time you would not be able to relate the data between trials).*

What are the controlled variables? What are the experimental variables? *(Controlled= height of the dropper, diameter of the dropper used, amount of calcium chloride solution used, the three different percent solutions used and the amount. Experimental=shape and size of the gel formed.)*

How could you make the data gathering process more accurate? *(Have the same person measure, have the same person deliver the drops of sodium alginate, start the measurement from same position)*

What type of people might be interested in this experiment? *(Food manufacturers, physicians, including fertility specialists, cancer survivors and their parents, and research scientists)*

How does this experiment connect to real life? *(Food additives, growing of frozen and then defrosted follicles taken from cancer patients prior to their chemotherapy and/or radiation treatments and taken from young women who want to postpone have children until later in life)*

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Which solution would you recommend to a research scientist who was using calcium alginate gel to grow follicles and why? *(The 0.5% solution forms the smallest and roundest spheres without being too flimsy or too rigid. The follicle could be placed into the sphere and would be able to expand the gel as it grew.)*

C. **EXPLAIN** – Students generate explanations for what they observe; conduct further research

i. **How will you get students to share their data?** Each student could display their data and results for others to see and compare with their own.
ii. **What guiding questions will you ask? (Provide sample answers.)**

SAMPLE QUESTION(s): What is occurring when the sodium alginate comes in contact with the calcium chloride solution? (*An alginate molecule is a polymer or long chain of M, G, and MG monomers bonded together. When there are a high proportion of G blocks in the molecule, calcium salts, like calcium chloride, can be added to form a strong gelatinous hydrogel of calcium alginate. This hydrogel is composed of one or more polymers suspended in water by crosslinking two or more of these molecules by covalent bonding.)*

What caused some of the gel to form in wormlike strands and what caused some to form nice round spheres? *(The crosslinking wormlike strands formed as the liquid alginate was dropped more quickly from a greater height while more spherical structures formed when the dropper was closer to the surface of the $CaCl_2$ solution and delivered more slowly.)*

How will the spherical gel benefit the oocyte in the follicle as it thaws? *(The spherical shape will support the follicle's 3-dimensional shape and keep it from flattening out in the Petri dish. In that shape, the follicle can continue to grow and mature until the oocyte is released.)*

iii. **What ideas will you try to develop?** Students have seen many slides of tissues under the microscope but the tissue is so thinly sliced with a microtome that it appears to be 2-dimensional. With the lab, the idea that cells are actually 3-dimensional and need a support scaffolding to further grow is clearly shown.

iv. **What terminology will you introduce and how will you relate this to the data?** Ideas relating to cancer and to cancer treatment and how fertility is affected would be added as well as the normal structure of the ovary, including primordial, primary, secondary, and antral follicles with the oocyte inside. The idea of the 3-dimensional nature of the follicle will further be shown using nuts – a mature mouse antral follicle is the size of a sunflower seed, a mature monkey antral follicle is the size of a pistachio, and a mature human antral follicle is the size of a pecan. Students would be asked to imagine how they might make an alginate gel sphere scaffolding to accommodate those sizes.

D. **ELABORATE –** Students apply the knowledge they have learned and engage in activities that extend their learning

i. **What will students do in the elaborate phase?** Have the students place a small glass bead representing the follicle into the calcium alginate sphere they made. Does it work? Is the sphere large enough to contain the glass bead?
ii. **What concepts will you have them apply?** What happens when the glass bead (or follicle) grows? How would you make a new gel sphere that is larger? What would need to be in the solution to allow an actual follicle to grow – water alone, nutrients alone, hormones like FSH and LH alone, or all of these? Are there other substances that could be added to enhance growth?
iii. **How is this different but related to what came before?** Earlier, the process was being applied to an alginate gel sphere that could contain a small follicle. Now we are trying to imagine how to construct a gel sphere that is larger so the growing follicle could be moved from the small sphere into a larger one as need be.
iv. **What guiding questions will you ask? (Provide sample answers.)**

SAMPLE QUESTION(s): Explain why forming larger calcium alginate spheres is helpful in the process of thawing oocytes from cancer survivors' frozen tissue. *(As the follicle which contains the oocyte grows, a larger scaffolding would be necessary to support the larger follicle.)*

With all the discussion in the news and on talk shows about young women being encouraged to freeze their eggs now so they can continue with their career and later defrost the eggs and have children, is this a good idea? *(Actually now, this is not a such good idea, except for cancer patients prior to their chemotherapy and radiation treatments. In 2012, the American Society of Reproductive Medicine lifted the experimental ban for freezing oocytes in cancer patients only. The follicles containing the oocytes can be harvested and frozen in liquid nitrogen; however, growing the defrosted follicles to maturity so they are capable of ovulation has not been perfected yet. The thawed follicles do begin to grow but don't reach full maturity. There have, however, been live births with using mature follicles in humans from frozen oocytes. Freezing embryos and sperm, if possible, continue to be the best options for live births in monkeys and humans.*)

E. **EVALUATE** - Monitor student understanding

i. What formative assessments will you use and when in the lesson will you use them? By asking the students questions before, during, and after the lab activity, the teacher would be able to assess understanding and misunderstanding of the ideas presented.

ii. What specifically will you look for in these? The teacher would be looking mainly for misunderstanding of ideas so they could be corrected.

iii. What summative assessment will you use? The teacher would have the students write a short essay on the usefulness of harvesting follicles from cancer patients for freezing and then growing them later in calcium alginate gel spheres until the follicle reaches maturity and releases the oocyte.

iv. What specifically will you look for in this? The teacher would look for clear understanding of definitions and applications.

v. Provide a sample response to the summative assessment. One method of fertility preservation for cancer patients is to freeze the patient's oocytes or sperm in liquid nitrogen prior to the cancer treatment. Sperm have been successfully frozen and thawed for many years and have resulted in live births. Oocytes are a different matter since the oocyte is much larger than a sperm cell and contains much more water. Oocytes can be easily frozen in liquid nitrogen but thawing them and having a live birth result has been problematic. The oocyte resides in a follicle until the follicle is mature and can ovulate or burst open, thus releasing the oocyte. One oocyte a month is released from an ovary during the monthly cycle in post-pubertal females. As the frozen oocytes within their follicles are thawed, they need a structure within which the follicles can continue to grow as they mature to ovulation. Follicles are 3-dimensional spheres and will collapse if not given a scaffolding. Alginate made from brown algae is a natural choice of a biomaterial for this scaffolding. When a sodium alginate solution is placed in a calcium chloride solution, a strong gelatinous hydrogel of calcium alginate forms. This hydrogel is composed of one or more polymers suspended in water by crosslinking two or more of these molecules by covalent bonding. When the hydrogel is made, a follicle with an oocyte in it can be placed into the sphere which will support it structurally while the follicle continues to mature.

10. **Modifications**

A. How would you modify this lesson to meet the needs of students with ADD/ADHD?
These students might need to be monitored so they don't work so hastily that they contaminate the stock solutions by putting their droppers into the wrong bottle.

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B. How would you modify this lesson to meet the needs of learning disabled students?
These students might need guidance in getting started but once underway, they would probably find this lab very interesting.

C. How would you modify this lesson to meet the needs of ELL students?
These students might need guidance in getting started but once underway, they would probably find this lab very interesting.

11. **Sources**

Sodium alginate and calcium chloride can be ordered from Sigma Chemical.

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