

## Part 1: Tissue Engineering Simulation Activities

### I. Introduction

Tissue Engineering can be used to replace damaged tissue and organs. A tissue is defined by its cells and extracellular matrix. To engineer tissue, scientists need three things: the right cells, a scaffold for 3D structure, and growth factors. With these components, scientists can construct a functioning tissue that can be transplanted into the body. Since the job of our immune system is to distinguish our cells from other types of cells, it is best to use cells from the person for which they are engineering the tissue. The scaffolding that creates the 3D space for the cells in the tissue can be made of biomaterials that are either synthetic or natural. The challenge then becomes to find the right scaffolding that will create the right spatial relationship for the cells and allow them to interact with each other in the correct way.

The process of tissue engineering consists of collecting the cells from a biopsy, letting them grow to a large number (expansion) and then to seed them onto the scaffolding. One improvement to this process has been with the use of bioprinting in which a computer controls a bioprinter to place the scaffolding and the cells in the correct space as determined by the designed program.

### II. Goal

The goal of this activity is for students to experience a simulation of tissue engineering. Since it is not feasible to grow cells or make biopolymers in a classroom, students will simulate the process by making various types of gels to use as scaffolding and hydrogels.

There are several different options for presenting the activities listed:

Option 1: The teacher can choose one or more of the materials below and demonstrate how tissue engineering would occur. (10 minutes)

Option 2: Students can be given one or more of the procedures below to perform and create their own engineered "tissue". (30 minutes)

Option 3: Students can be given all of the materials below and allowed to experiment until they find the best solutions to use to create a bioprint model. (60 minutes)

Which option you take will depend on the time you have, the number and level of students you have, and your comfort with the content and classroom discovery.

### III. Procedures

#### **Part A. Using Sodium Alginate to Simulate Tissue Engineering**

##### Materials

- 2% Sodium Alginate (Ward's \$15.19 for 25 g) or Gaviscon® Liquid (Target, 12 oz \$6.79)-2%
- CaCl<sub>2</sub> (Carolina Biological \$6.10, or use DampRid® from Walmart \$7.87)
- Syringes (CVS \$3.19 each)
- Transfer Pipets (Carolina Biological \$5-10/100)
- Petri dishes (Carolina Biological \$5.95/20)

## Procedure

1. Make up 200 ml of 4% CaCl<sub>2</sub>.
2. Pour CaCl<sub>2</sub> into the bottom of a petri dish.
3. Pull up 5 ml of Alginate (Gaviscon®) in the syringe or pipet. If you have a needle or micropipette tip to add onto the end of the syringe, it will prevent the syringe from getting clogged.
4. To make “scaffolding”, place the tip of the syringe or transfer pipet below the surface of the solution and release slowly while moving it to create a ribbon of gel. The liquid in the petri dish can then be removed and the gel ribbons can then be placed on a surface to represent the scaffolding.
5. If you hold the syringe under the surface of the solution without moving it, the gel will form a globular structure similar to a bag.
6. To make “hydrogels”, allow drops of the alginate solution to drop into the CaCl<sub>2</sub> solution to make drops. Alternatively, you can use one of the other methods below to create the “hydrogel”.



## **Part B. Using Gelatin to Simulate Tissue Engineering**

### Materials

- Unflavored Gelatin (any grocery store)
- Food Coloring (any grocery store)
- Petri dishes (Carolina Biological \$5.95/20)

### Procedure

1. Make a gelatin solution with warm water that is at least 2% w/v. Higher concentrations take less time to set. You can add food coloring to it at this point if you wish.
2. Pull up 5 ml of the gelatin solution into a 10 ml syringe.
3. To make “scaffolding” allow the gelatin mixture to set in the syringe and then eject it onto the surface as you move in the syringe to create the gelatinous ribbons. You can create a matrix pattern that represents the scaffolding.
4. To make “hydrogels”, use a lower concentration of gelatin that will not completely set but will remain rather viscous as you release it onto the “scaffolding”. Add a different color to this. Release this solution onto the scaffolding to represent seeding the scaffolding with the cells in hydrogel.
5. An alternative to the gelatin scaffold is to use play dough to create a mold for the “hydrogels”.



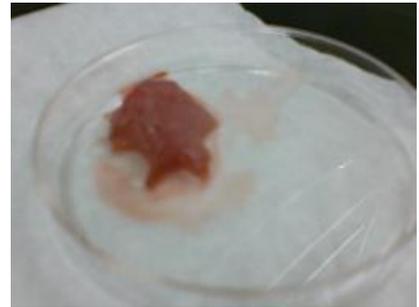
## **Part C. Using Agar and Yeast to Simulate Tissue Engineering with Live Cells**

### Materials

- Nutrient Agar Powder ( Carolina Biological for \$21)
- Yeast cells (active dry yeast, any store)
- Petri dishes (Carolina Biological \$5.95/20)
- Cast molds of different shapes – play dough molds will work.

### Procedure

1. You will need to make a liquid culture of cells to place on your scaffolding. Follow the directions on the active dry yeast packets to create the culture. Use warm water but not hot water to activate the cells. Add more or less water to adjust the viscosity of the mixture.
2. Make up an agar solution with a minimum concentration of 1.5% in hot water. Make sure the agar is completely dissolved; it can be microwaved if needed. The agar solution will solidify (gel) once it reaches room temperature.
3. You can create a 3D object by pouring the agar into a mold and allowing it to set. A simple mold can be made by taking a piece of aluminum foil and disinfecting one side with alcohol. Crumple the foil and use then use it as a mold. Alternatively, you can pour it into a flat surface, like the petri dishes, and then make a depression in it once it is solidified. Whatever method is used, the agar should fit into the petri dish to minimize its further exposure to the outside environment.
4. To seed your “scaffolding” with cells, pour the liquid yeast culture over the solidified agar and then place the petri dish aside to incubate overnight.



## Part D. Using Wax to Simulate Scaffold and Environmental Factors

### Materials

- Paraffin wax (any grocery store)
- Scented wax cubes (any grocery store)
- Paper cups coated with wax (any grocery store)
- Crushed ice

### Procedure

1. Begin with the plain paraffin wax with no additives. The wax will need to be heated in a double boiler to 175° - 185°F. It should be placed in the upper portion so that it does not receive direct heat from any side. Check the temperature of the wax occasionally to make certain it is not getting too hot. Do not leave the wax unattended.
2. Once the wax has melted completely, cut off the heat and prepare the mold.
3. Fill the paper cup with crushed ice. Carefully pour the hot wax over the ice until it reaches the top. Set it aside and allow the wax to cool and the ice to melt.
4. Return the remaining wax to the double boiler. Add two or more scented wax cubes and allow them to melt.
5. Repeat steps 3 and 4.
6. Tear off and discard the paper cups. The plain wax simulates a scaffold with support and sites for cells to inhabit. The scented wax simulates a scaffold embedded with environmental factors, such as growth factors or oxygen generators.
7. If desired, you can cut the wax “scaffold” into discs with a knife. Students can add “hydrogels” such as agar, alginate or gelatin or yeast cells to simulate tissue engineering.



## Part 2: Generating Oxygen for Tissue Engineering

### I. Introduction

One of the biggest challenges with engineering three dimensional tissues is vascularization. If the tissue is thicker than 0.1 – 0.2 mm, it needs a blood supply to bring in oxygen and nutrients. While there are growth factors present in tissue that help it build these blood vessels, it still takes time. To help maintain the tissue while it is growing blood vessels, scientists are working on Particulate Oxygen Generators (POGs) that can deliver oxygen to the growing tissues.

### II. Goal:

The goal of this activity is for students to simulate creating a POG using a couple of household chemicals. They will create the POG, and then test it in vitro. The POGs will be made by mixing Oxyclean™ granules, which contain sodium percarbonate, and a catalase solution. Sodium percarbonate, an adduct of sodium carbonate and hydrogen peroxide, will react with the catalase to produce water and oxygen. The oxygen release can be seen as bubbles in solution. To make the catalase solution, calf liver will be homogenized. Alternatively, potatoes can be homogenized as well.

### III. Procedures

#### Materials

- Oxyclean™ Product (any store) – has sodium percarbonate, an adduct of sodium carbonate and hydrogen peroxide.
- 2% Sodium Alginate (Ward's \$15.19 for 25 g) or Gaviscon® Liquid (Target, 12 oz \$6.79)
- CaCl<sub>2</sub> (Carolina Biological \$6.10, or use DampRid® from Walmart \$7.87)
- Calf or chicken liver (at any store) – contains catalase that will decompose peroxide into water and oxygen.

#### Procedure

1. Prepare 20 ml of 4% CaCl<sub>2</sub>. Add 5 ml of solution into two separate test tubes.
2. Mix 5 ml of Gaviscon® or alginate with ½ teaspoon of Oxyclean™.
3. Use a transfer pipette, that has been cut in half to increase bore size, to drop 5 drops of the mixture into one of the tubes of CaCl<sub>2</sub>. These drops represent the POGs.
4. Using a separate cut transfer pipette, drop 5 drops of unmix Gaviscon into the second tube of CaCl<sub>2</sub>. These drops will serve as controls
5. Make the liver homogenate by adding a small piece of calf liver to a cup of water and place into a blender. Allow the large chunks to settle out of solution or filter through cheese cloth if available.
6. Pour the CaCl<sub>2</sub> solution out of each of the two test tubes with the POGs and controls, and replace with 5 ml of the liver homogenate. Allow to sit and observe the two tubes. Do you see any evidence of oxygen production from the POGs?

## Part 3: Simulating Cell Encapsulation for Tissue Engineering

### I. Introduction

Another issue in tissue engineering is the host immune system. The immune system's job is to attach any cells that are not recognized as self. In this way, the body can fight off infections from bacteria and viruses. However, in some cases, the cells used in regenerative medicine and tissue engineering must come from another source (allograft) rather than from the host (autograft). Scientists have been working long and hard on how to prevent the host's immune system from attacking these foreign cells and thus rejecting them. One solution has been to encapsulate the cells in an alginate that protects them from the immune system but still allows the diffusion of molecules to and from the cells.

### II. Goal

The goal of this activity is for the students to simulate the encapsulation of "cells" with alginate and observing the diffusion of chemicals out of the "cells". The "cells" will be granules of borax, which is an alkaline compound. They will then place the encapsulated "cells" into phenolphthalein, a pH indicator, and observe a slow color change as the borax diffuses out of the alginate. This simulates how cells can produce a protein, such as insulin, that is released into the patient's body.

### III. Procedures

Materials:

- 2% Sodium Alginate (Ward's \$15.19 for 25 g) or Gaviscon® Liquid (Target, 12 oz \$6.79)
- $\text{CaCl}_2$  (Carolina Biological \$6.10, or use DampRid® from Walmart \$7.87)
- Borax Powder (at any store)
- Phenolphthalein Indicator

Procedure

1. Prepare 20 ml of 4%  $\text{CaCl}_2$ . Add 5 ml of solution into two separate test tubes.
2. Mix 5 ml of Gaviscon® or alginate with 1/4 teaspoon of borax.
3. Use a transfer pipette, that has been cut in half to increase bore size, to drop 5 drops of the mixture into one of the tubes of  $\text{CaCl}_2$ . These drops represent the encapsulated cells.
4. Using a separate cut transfer pipette, drop 5 drops of unmixed Gaviscon® into the second tube of  $\text{CaCl}_2$ . These drops will serve as controls.
5. Pour the  $\text{CaCl}_2$  out of the tubes and replace with 5ml of the phenolphthalein solution.
6. Observe the color changes.

### Acknowledgements

*These activities were developed by Terry Howerton of [Atkins High School](#) and Melanie Stancill of [Davie High School](#) in collaboration with faculty and staff at the Wake Forest Institute for Regenerative Medicine, with funding support from [North Carolina New Schools](#).*