Tissue Engineering and Ionic Bonding

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Subject: Chemistry (Bonding, Tissue Engineering)
Level: High School

Standards: Teaching Standards
- Standard A - Teachers of science plan an inquiry-based science program for their students
- Standard B - Encourage and model the skills of scientific inquiry, as well as the curiosity, openness to new ideas and data, and skepticism that characterize science
- Standard C – Teachers of science engage in ongoing assessment of their teaching and of student learning
- Standard D – Teachers of science design and manage learning environments that provide students with the time, space, and resources needed for learning science.
- Standard E – Teachers of science develop communities of science learners that reflect the intellectual rigor of scientific inquiry and the attitudes and social values conducive to science learning

Content Standards
- Standard A – Abilities necessary to do and understand scientific inquiry
- Standard B – As a result of their activities in grades 9-12, all students should develop an understanding of structure and properties of matter and chemical reactions (specifically bonding)
- Standard C - As a result of their activities in grades 9-12, all students should develop an understanding of the cell.
- Standard E - As a result of their activities in grades 9-12, all students should develop an understanding of abilities of technological design and understandings about science and technology
- Standard G - As a result of their activities in grades 9-12, all students should develop an understanding of science as a human endeavor
Objectives:

Curriculum will teach students about ionic bonding by relating it to key tissue engineering principles and allowing them to experience ionic bonding through an inquiry based laboratory using alginate gel, a common tissue-engineering scaffold.

Students will:

- Be able to state the three main components used in Tissue Engineering
- Be able to state the advantages of tissue engineering over traditional treatments
- Be able to state why Tissue engineering is needed
- Be able to carry on an everyday conversation about tissue engineering
- Be able to define ionic bonding
- Be able to state that ionic bond formation causes gelation of alginate
- Be able to describe how model based inquiry is carried out in laboratories

Vocabulary:

<table>
<thead>
<tr>
<th>Ionic Bond</th>
<th>Alginate</th>
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<tbody>
<tr>
<td>Tissue Engineering</td>
<td>Gel</td>
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<td>Cell</td>
<td>Cation</td>
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<td>Scaffold</td>
<td>Monovalent</td>
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<td>Cell Signaling</td>
<td>Divalent</td>
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<td>Biomedical</td>
<td>Extracellular</td>
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<tr>
<td>Engineering</td>
<td>matrix (ECM)</td>
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Materials:

For Each Group:

- **Dry Activity**
  - 12 toothpicks
  - 8 water soluble packing peanuts
  - Bucket filled with water
  - Styrofoam ball
  - Modeling clay

- **Inquiry Activity**
  - 30 ml 2% CaCl₂
  - 30 ml 2% NaCl
  - 30 ml 2% alginate
  - Red food coloring
  - Yellow food coloring
  - Blue food coloring
  - 8 30 ml free standing conical tubes
  - 3 transfer pipettes

Safety:

Safety concerns are minimal within this lab exercise; however, students should be required to wear eye protection to promote proper lab safety protocol.
Science Content for the Teacher:

Tissue Engineering Content:

Tissue engineering is the production of novel living tissue using three main essential components: cells, scaffolding, and cell signaling. By producing these engineered tissues, one can implant and replace diseased or injured tissues. The act of creating novel engineered tissues is advantageous for three main reasons. First, traditional transplantation surgeries (e.g. kidney and heart transplants) can often have dramatic effects on saving and improving the quality of life of those receiving the transplants. Unfortunately, there simply are not enough transplants to go around and waiting lists for life saving organs are incredibly long. In fact, every 30 seconds someone dies from a condition that could have been treated by tissue engineering. By creating and growing new tissues, the supply problem can be solved.

Furthermore, often those receiving transplantations have to go on immune suppressing drugs that have some very undesirable side effects. Tissue engineering provides an avenue in which the tissues can be grown with the patient’s own cells, and thus, will not trigger an immune response. This eliminates the need for the patient to go on immunosuppressant drugs. Finally, tissue engineering provides an advantage over traditional polymer/metal implants (e.g. hip and knee replacements) by producing living and biocompatible tissues. Metal and polymer implants suffer from poor biocompatibility and mechanical failure. Tissue engineered tissues are made up of materials (cells, proteins, lipids, and carbohydrates) the body is familiar with and thus are quite biocompatible. The cellular component provides the advantage of having a self-repair mechanism that is not present in polymer/metal implants. Thus, tissue engineered tissues will last longer than traditional implant materials. Overall, it is for these main reasons that tissue engineering has become an active field of investigation in the scientific and engineering worlds.

The students will learn the three main tools/concepts of tissue engineering and what their role is in tissue engineering. The three main components of tissue engineering are the cells, the scaffold, and the cell signals. Tissue engineering is the combination of those three components in order to create tissue to replace damaged tissue caused by trauma or disease. Each of these components has a specific role in the artificial construct.

The central dogma behind tissue engineering is that cells are placed on a biodegradable “scaffold”. This scaffold is usually in the shape of the tissue to be created. Over time the cell will produce proteins, lipids, and carbohydrates that will make up the “extracellular matrix”. This is simply the material that is surrounding the cells. As the cells produce the extracellular matrix, the biodegradable scaffold will be degraded and the cell produced extracellular matrix will take its place resulting in a completely cell produced tissue with no synthetic aspects.

The cells are what provide the tissue with the “living” component. This allows the tissue to respond to trauma experienced during normal wear and tear that tissues undergo. Current synthetic implants made of plastics and metals are subject to wear and fatigue leading to failure of the implants. When these implants are damaged they cannot repair
themselves. So by creating a living implant, these tissues can respond to these traumas and self-repair, giving it the possibility to last longer than synthetic implants. Cells also provide the tissue with their function. For example, in cartilage the proteins and proteoglycans the cells produce provide the tissue with their mechanical properties. In other tissue such as the pancreas, the cells produce the protein insulin providing the pancreas with its function. As can be seen, the cell plays a vital role in the development of tissue-engineered tissues with the desired properties.

The scaffold provides the support and shape of the tissue. The scaffold provides a place for the cells to attach and develop on. One analogy that can be used: the scaffold is the framework of a house. You put up the framework for the shape of the house you want and then build upon that framework. This is essentially how the cells utilize this framework. A very important aspect of most scaffolds used in tissue engineering is that they are biodegradable. They will be broken down inside the body. This is important because the main goal of tissue engineering is to create a tissue with non-synthetic materials but with a composition of proteins, lipids and carbohydrates mimicking the natural tissue. So this original scaffold must be broken down for the extracellular matrix to take its place.

The signals provide the instructions to the cells. Once the cells are seeded in a scaffold they must produce the proteins that are appropriate to form the tissue desired. As a result, tissue engineering attempts to use signals that the cell sees in its natural environment. These signals can include hormones, growth factors, and mechanical signals. Tissue engineers attempt to harness these signals to control the growth and development of the new tissue.

Chemistry Content:

We will use one of the main tools of tissue engineering, scaffolds, to both teach the student about tissue engineering while also teaching them about ionic bonding. Alginate is a common scaffold used in cartilage tissue engineering. Alginate is an anionic polysaccharide obtained from brown algae with some very favorable properties for tissue engineering. (On a side note, alginate is used in a very prominent fast food restaurant’s shakes as a thickener. The reason they do not call them milkshakes is because they do not contain milk, but contain alginate instead. The students usually enjoy this anecdote.) Often you hope to be able to trap cells within your scaffold. Alginate makes this process very easy. First, alginate is non-cytotoxic (i.e. does not kill cells) and cells can be encapsulated into the alginate without deleteriously affecting the cells. The property of alginate that is most useful to the tissue engineer is its ability to gel. A solution of alginate can be mixed with a Ca++ solution and a gel will be formed. In addition, you can mix in cells before adding the Ca++ in order to encapsulate the cells within the scaffold. The key to alginate gel formation is the anionic nature of the alginate. By adding a divalent cation, ionic crosslinks are formed between the chains of alginate. As a result, the solution forms a gel after crosslinking. Of importance is the divalent nature of the cation. This divalent state allows ionic bonds to form between 2 chains of alginate to form the crosslinks, while a monovalent cation will only form an ionic bond with a single
chain and fail to form crosslinks (Figure 1). Thus, divalent cations produce gels when mixed with alginate and monovalent cations do not.

Figure 1 – (A.) Alginate chains crosslinked by Ca++. (B.) Alginate chains associated with Na+ but not crosslinked.

alginate reacts with monovalent and divalent cations to teach students how bonding affects material properties and how the valence state affects this bonding. The advantage of using alginate for this activity is that it gives students a visual and tactile representation of bond (ionic) formation when the solution turns into a solid. Secondly, it allows them to begin to understand the difference between monovalent and divalent ions and how that affects bond formation.
Preparation:

**Dry Activity:** A list of materials was provided on page 2 of this document (Figure 2). The teacher will need to distribute the materials at the appropriate number of workstations as well as provide the students with handout #1 (Tissue Engineering Handouts.pdf). The students will be working through the handout during the activity.

![Figure 2 – Dry activity supplies](image)

**Inquiry Activity:** A list of materials was provided on page 2 of this document (Figure 3). The teacher will need to mix 30 ml of alginate for each group at 2% (w/v) (20 mg/ml) in distilled water. This is best done with a stir bar and magnetic stirring plate. You will want to mix in the alginate slowly to avoid clumping. Mixing will often take over 2 hours and is usually best done overnight to ensure mixing before class. After mixing, add blue food dye to alginate solution. The teacher will also need to mix 30 ml of CaCl$_2$ (2% w/v) (20 mg/ml) in distilled water. The solution will be oversaturated so you will see particles in the solution. This is expected, students will just want to shake solution before using to evenly mix. After making solution, add yellow food dye to CaCl$_2$ solution. Finally, teacher will need to mix 2% w/v (20 mg/ml) NaCl solution in distilled water. After mixing add red food dye to NaCl solution.
30 ml of each solution should be distributed in 30 ml freestanding conical tubes. In addition, each group should have 5 additional freestanding 30 ml conical tubes. Also, distribute 3 transfer pipettes per group. Finally, provide students with Handout #2 and #3 (Tissue Engineering Handouts.pdf) (Bowles Handouts GK-12). The students will be working through the handout during the activity.

**Figure 3** – Inquiry activity supplies
Classroom Procedure:

DAY 1

Teacher should cover PowerPoint slides 1-8 (TissueEngineering_Bowles.ppt) through the “toothpicks and tissue engineering” slide. After completing slides, teacher should do “Dry Activity” discussed below.

Dry Activity: Teacher should briefly go over the key diagram at the top of handout #1. Explaining that the toothpick alone represents extracellular matrix, the toothpick with packing peanut on it represents the scaffold, the Styrofoam ball represents the cell, and the bucket full of water represents the body environment. The teacher will want to demonstrate the construction of the scaffold with cell as illustrated below in Figure 4. The students should be instructed to place the construct into the water and observe what happens and answer the questions on the handout accordingly. (What should happen is the packing peanuts will dissolve off of the toothpicks leaving only the toothpicks. This represents the scaffold degrading and being replaced by ECM.) At the completion of the class, if you have time, go over the handout with the students. (Answers are provided in red on the handout).

Figure 4 – Constructed dry activity scaffold with cell
DAY 2

Teacher should cover PowerPoint slides 9-16. These slides focus on scaffolds and their properties. The final slide asks “what properties do we want in a scaffold”, use this slide and time to engage in a discussion on what the students think would be important in the design of a scaffold. Try to encourage the students to engage with each other on the topic. *This day is the most boring for the students since it involves only lecturing. Attempt to engage the class as much as possible and feel free to adjust the lecture to make it as interactive as possible.

DAY 3

Inquiry Activity: This activity is inquiry based and limited instruction should be given to the students. Students are provided with the information that the red solution contains a monovalent cation, the yellow solution contains a divalent cation, and the blue solution is alginate. In addition, they are provided with the generalized structure of alginate with an “x” in place of the carboxyl - group. The students are told that they are going to be using the information and supplies provided to determine the properties of the “x” group by mixing the solutions.

The teacher should then cover the steps of model-based inquiry to the students that they are too follow in their experiments. (1.) Those steps first involve making observations about the solutions, which includes both visual observations and thinking about the information provided to them. (2.) Then the students should develop a model/hypothesis for what will happen when they mix the solutions. Force the students to be as specific as possible. (3.) Once a model is developed they should develop an experiment to test their model and (4.) The students then carry out their experiment while collecting whatever data they deem appropriate. (5.) Next they should interpret their data in terms of their model. (Is it correct, wrong, need adjustments?) (6.) Have them make those changes to the model and think about what would be next to test in their model. The important concept here is to emphasize the model-based and iterative nature of science. We make models, we test them, we adjust the models, we continue.

Most students decide to mix each possible combination of solutions to see what happens. It is advantageous to the students if they develop a specific model before doing this experiment. The provided alginate chain structure will help in the development of this model. It is important to note that students will be hesitant that their model is incorrect. This provides a great opportunity to explain to the students that scientific models are often wrong, and that is why the experiments are carried out to test each model. The students will observe during their experimentation that the blue and yellow solutions form a gel when mixed, the blue and red do nothing observable, as do the yellow and red. The students often note a color change, but may have to be redirected back to the model. “Does a color change support or disprove your model?”

There are two main conclusions we hope the students reach and can be lightly guided if need be (the less guidance the better). The first conclusion is that the alginate “x” group is negatively charged. The negative charge is why the yellow and blue
solutions gel when mixed by forming ionic bonds between the alginate chains. This conclusion provides a visual and tactile representation of how the formation of bonds can affect material properties. Most students come to this conclusion from their experimentation and analysis.

The second conclusion, based on the negative charge of alginate, is more advanced, but students are able to come to it if forced to think about it. Alginate forms a gel with the divalent cation solution because the divalent cation provides a plus two charge that can form ionic bonds with two minus one charges of the “x” groups of alginate and form bridges (crosslinks) between these chains of alginate (Figure 1a). On the other hand, the monovalent cation only has a plus one charge and therefore can’t bridge two chains of alginate (Figure 1b). The question you hope they ask is, “why does it gel with the divalent cation but not the monovalent cation?” Asking this question will start the students down the right path. If deemed appropriate, the teacher can provide this information to them after they struggle with it. Another hint that the teacher can provide late in this exercise is to draw the Lewis dot diagram for a divalent cation. This can help them to see that there are two positive charges available to form a bridge between 2 strands of alginate that have negative charges.

These two conclusions are the most useful in relating the scaffold formation and tissue engineering to ionic bonding. The student’s final model will ideally resemble figure 1. Most students will naturally move in this direction and the teacher will naturally nudge them in this direction; however, these are not the only directions the students can go. The important aspect is that the students develop a model, test it through experimentation, analyze it, adjust their model, and plan for future experiments. This allows them to experience science as it is carried out every day in the laboratory. It is important that the students fill out their handouts as they carry out their experiments. The teacher can explain to them the handout is like their “lab notebook” and discuss how these notebooks are used in the lab to write down everything they do so they have a record of their experiments and thinking.

DAY 4

The students should be asked to prepare and present their findings as a group to the class. Explain that this is an important aspect of science in order to educate others of your findings.
Assessment:

The following rubric can be used to assess students for each part of the inquiry activity. The term “expectations” here refers to the content, process and attitudinal goals for this activity. Evidence for understanding may be in the form of oral as well as written communication, both with the teacher as well as observed communication with other students. Specifics are listed in the table below.

1= exceeds expectations  
2= meets expectations consistently  
3= meets expectations occasionally  
4= not meeting expectations

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<thead>
<tr>
<th>Model</th>
<th>Experiment</th>
<th>Analyze and adjust model</th>
<th>Future Directions</th>
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<tr>
<td>1</td>
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In addition, the following 2 essay assignments can be assigned to the students. The first distributed after Day 1 and the second after the completion of Day 4. When distributing the second essay, ask them to expand on their answer from the original essay in context of what they have learned.

Essay #1
Name: _______________ Class: ____ Date:______

Using a **minimum** of 5 sentences, write about what you could do. Include how chemistry is involved, specifically bonding and tissue engineering…

You live in a small development in a small rural school district. Your neighbor, a real close friend of yours, was riding her bike without a helmet. She crashed and tore off most of her ear. In the mêlée of emergency crews, the pieces were lost or so badly shredded that they could not be reattached. She will have minimal scarring on the side of her face, thanks to the way she hit the pavement. However, that is nothing compared to the embarrassment of not having an ear. Her parents know you are a scientist, and the family is pleading with you to help her any way you can…
Essay #2
Name: _______________ Class: ____ Date: _______

Using a minimum of 5 sentences, respond to your neighbor informing them about what you have developed in your lab. You will be able to help their daughter! Include how chemistry is involved, specifically bonding and tissue engineering…

(**Note** - You know much more than you did at the start of the week. Do not think you can write the same answer that you did for the first assignment and get full credit. Try to be specific in your response.)

Supplemental Information:

Please download associated PowerPoint (TissueEngineering_Bowles.ppt) found on the website www.climb.bme.cornell.edu. The PowerPoint has notes on each slide and how to present them.

Acknowledgments:

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