Tissue Engineering using Polymers

Subject Areas: Biology, Science and Technology

Associated Unit: Cell biology and biotechnology

Lesson Title: Tissue Engineering using Polymers

Grade Level: 9-12

Time Required: 2-3 class periods

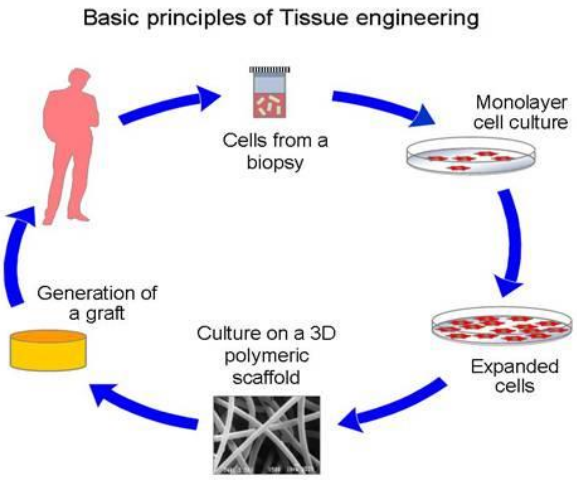


Image 1: Cells of interest can be removed from the patient, grown in culture, grown on a polymer scaffold to regenerate the necessary tissue.

Source: http://textile.iitd.ac.in/highlights/fol8/01.htm

Summary

Students will begin the lesson with a quick video from www.youtube.com called "Growing Noses, Ears, and Windpipes." After a brief discussion on tissue regeneration, the students will brainstorm possible problems with the process that could occur. The class will then read the article "How to Build a Heart" using the "Read and Say Something" CRISS strategy. The instructor will then take the class through the engineering cycle on how to solve a problem. The students will then be introduced to aseptic techniques and how to culture cells and will perform an activity where they culture hamster ovarian cells and study them under a microscope. The instructor will then lead them through a lesson on the use of polymers in the field of biomedical engineering, focusing on pNIPAAm, a hydrogel that is responsive to temperature changes. The students will perform an activity where they will hydrate this polymer pNIPAAm at different temperatures and measure the changes in the polymer and graph the changes that occur. A discussion on how cells are seeded onto the polymers and the properties of the polymer allow the cells to be released from the polymer concludes the lesson, with a possible option of a field trip to a bioengineering lab using polymers to observe the entire process and possibly participate in the process.

Engineering Connection
Students will learn about the engineering cycle to solve a problem and apply this process within the context of biology.

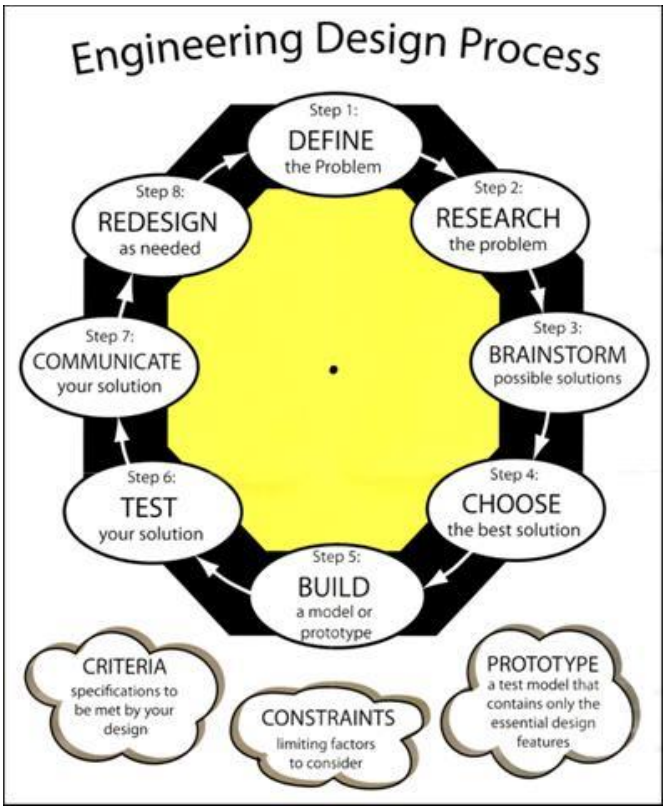


Image 2: The Engineering Design Process. Source: http://home.catholicweb.com/math/index.cfm/NewsItem?ID=323063&From=Home

Engineering Connection: 1-3
1. Relating science and/or math concept(s) to engineering
2. Engineering analysis or partial design
3. Engineering design process

Keywords: Tissue engineering, polymers, thermo-responsive, cell culture, adherent

Educational Standards:

SC.912.L.16.10
Evaluate the impact of biotechnology on the individual, society and the environment, including medical and ethical issues.

SC.4.N.1.8
Recognize that science involves creativity in designing experiments.

ITEEA: Standard 14. Students will develop an understanding of and be able to select and use medical technologies. (Grades K - 12)

Learning Objectives: (Students should be able to.....)

* Apply their understanding of aseptic techniques in laboratory

* Be able to culture cells using proper cell culturing techniques

* Be able to observe, identify, and sketch cells under a light microscope

* Be able to hydrate PNIPAAm polymer while collecting data on change in mass and then graphing the changes in the polymer

Introduction/Motivation (5E Engage):
Students will begin the lesson with a quick video from www.youtube.com called "Growing Noses, Ears, and Windpipes." The class will then have a discussion on what a scientist would need to know and what they would need to be able to do in order to grow tissues in a laboratory.

Lesson Background & Concepts (5E-Explain):
Tissue regeneration is an exciting field of research that is showing advances from the traditional methods of scaffolding, where immune system responses have been a problem, to using functional materials to create viable three-dimensional tissues. The use of a thermally responsive hydrogel, poly N-isopropylacrylamide (PNIPAAM) as a cell culture platform allows the release of intact cells in defined geometries or sheets without the damage to the extracellular matrix (ECM) that traditional release techniques, such as the use of enzymes, have caused.

PNIPAAM has a lower critical solution temperature (LCST) of 32*C; below this temperature, the polymer becomes hydrophilic and swells while above this temperature, the polymer becomes hydrophobic and collapses.

As cell release platform, when PNIPAAM swells, the strain between the polymer and the cells causes the cells to detach intactly from the polymer due to disruptions of the cell matrix caused by the expansion of the polymer.

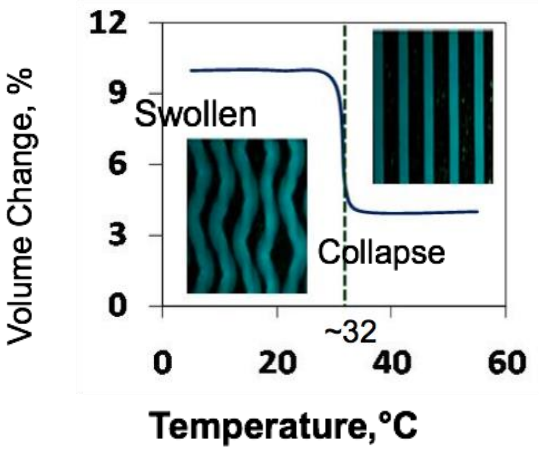
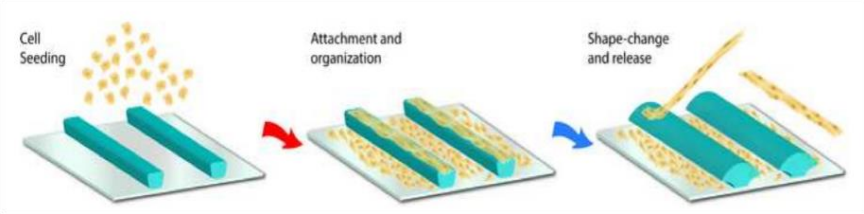


Figure 1 Microstructural change of PNiPAAm around its LCST and exposure to water [1].



Vocabulary/Definitions:

Word	Definition
PNIPAAm	thermo-responsive polymer that can swell and/or collapse at 32*C
Thermo-responsive	the ability to respond to temperature changes
Cell culture	to grow cells in a controlled condition outside of their natural environment
Aseptic	technique used in laboratory that is used to perform a technique under sterile conditions

Associated Activities (5E- Explore):

❖ Students will work in groups of three to read the article called “How to Build a Heart” using the “Read and Say Something” CRISS strategy. Students will read a paragraph or small section of text (as much as they are comfortable with) and then say something, which could be a comment, a question for the group, or something they found interesting or want to know more about. Then the next person in the group will do the same thing until the group finishes the article. The teacher will circulate and monitor on-task conversations and discussions.

❖ Once students have an understanding of the basics of tissue engineering, the concept of cell culturing and what cells need to grow can be introduced. The teacher can ask their class “What does a cell need?”. Hopefully students will contribute some answers, which will lead into the Ward’s Cell Culture Kit, where the students have an opportunity to culture and grow cells in special chambers that allow anchorage and growth of adherent cells on internal surfaces. Students will then observe their cells under the microscope.

❖ After students have an understanding of the cell biology aspect of tissue engineering, students will be introduced to a thermo-responsive polymer PNIPAAm. The students will be given vials of the polymer and will fill their vials with water after they have taken an initial mass measurement. As the students hydrate their polymer, they will take measurements over the course of five days and graph their data. Students will test three different densities of polymer based on the amount of cross-linkages inside of the polymer (2%, 3%, and 4% cross-linked). When the data has been collected, the teacher will lead the discussion on how cross-linking affects the hydration and swelling of the polymer and why that would be important to know. The teacher will play the video showing cell being released from a cooled polymer.

Assessment (5E- Evaluate):

- Lab Report for Cell Culturing Lab and for the Hydration of a Polymer Activity

Lab Report must include the following sections:
1. Objective
2. Background
3. Procedure
4. Results/Data with drawings and graph
5. Conclusion with possible future research

Lesson Extension Activities (5E-Extension):

*Interested students will have the opportunity to take a field trip to USF for a lab tour and an opportunity to see the entire cell printing process.

Additional Multimedia Support:

Cell release from a polymer video: Use external link

How to Build a Heart article: http://www.nature.com/news/tissue-engineering-how-to-build-a-heart-1.13327

Growing Ears, Noses, and Windpipes videoclip:
http://www.youtube.com/watch?v=e8QXxeWKLbo

Wards Cell Culture Lab:
https://www.wardsci.com/store/catalog/product.jsp?catalog_number=366100

References:

[1] DuPont, S.; Cates, R.; Stroot, P.; Toomey, R.; Swelling-induced Instabilities in Microscale, Surface-confined Poly(N-isopropylacrylamide) Hydrogels. Soft Matter 2010, 6, 3876-3882.

Attachment:

“Tissue Engineering Using Polymers” Worksheet

Name _____

Tissue Engineering Using Polymers

Watch the video “Growing Ears, Nose, and Windpipes” from http://www.youtube.com/watch?v=e8QXxeWKLbo.

What would a scientist need to know about in order to grow tissues?

What would a scientist be able to need to know how to do in order to grow tissues?

What problems could occur in the growing of organs?

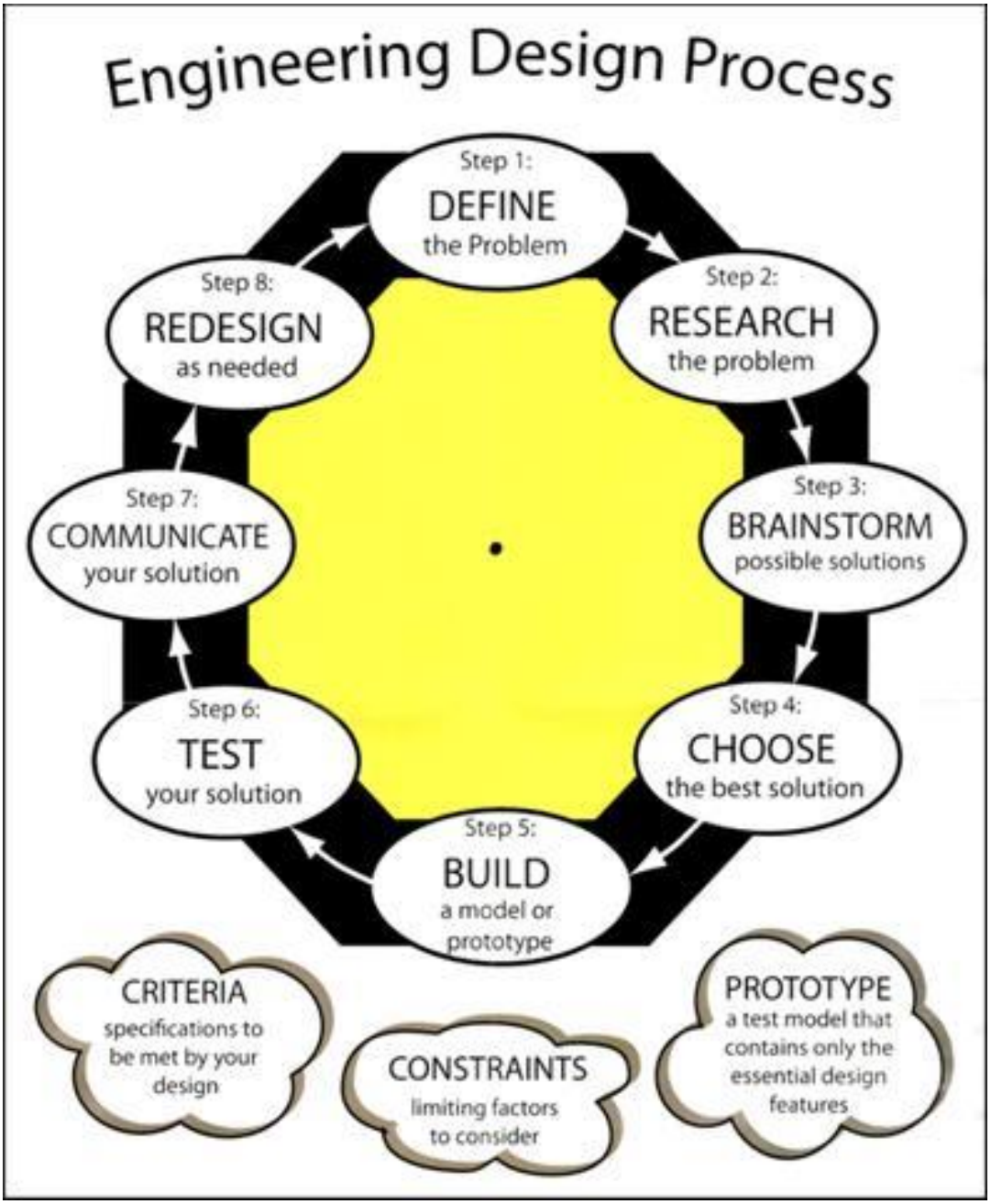
How to Build a Heart- Read and Say Something! activity

Each of you will read a small section of text (as much as you are comfortable with) and then say something, which could be a comment, a question for the group, or something you found interesting or want to know more about. Then the next person in the group will do the same thing until the group finishes the article.

Record your group comments here.

Name _____

The Engineering Design Process



Read the following article about aseptic techniques, highlighting sentences that are particularly important.

Name _____

Aseptic Techniques

Notes based on 'Basic practical microbiology' © Society for General Microbiology.

Aseptic techniques underpin all work in microbiology. Sterile equipment and media should be used in the transfer and culture of microorganisms. Aseptic technique should be observed whenever microorganisms are transferred from one container to another.

It is wise to treat all cultures as potentially pathogenic, because cultures may have been contaminated, and because mutations to disease-causing forms may occur. The aseptic techniques described here control the opportunities for contamination of cultures by microorganisms from the environment, or contamination of the environment by the microorganisms being handled.

There are some general rules to follow for any aseptic technique.

* Close windows and doors to reduce draughts and prevent sudden movements which might disturb the air.

* Make transfers over a disinfected surface. Ethanol disinfection is recommended because of its rapid action. If the bench surface is difficult to clean, cover the bench with a sheet of tough material which is more easily disinfected.

* Start the operations only when all apparatus and materials are within immediate reach.

* Complete all operations as quickly as possible, but without any hurry.

* Vessels must be open for the minimum amount of time possible.

* While vessels are open, all work must be done close to a Bunsen burner flame where air currents are drawn upwards.

* On opening a test tube or bottle, the neck must be immediately warmed by flaming (see below) with the vessel held as near to horizontal as possible and so that any movement of air is outwards from the vessel.

* During manipulations involving a Petri dish, limit exposure of the sterile inner surfaces to contamination from the air.

* The parts of sterile pipettes which will be put into cultures or sterile vessels must not be touched or allowed to come into contact with other non-sterile surfaces, such as clothing, the surface of the working area, or the outside of bottles/ test tubes.

Name _____

* All items which come into contact with microorganisms must be sterilized before and after each such exposure. This could be either by the technical team preparing for and clearing up after a piece of practical work (for example, in the case of glassware to be used), or by the worker during the course of the practical (for example, in flaming a wire loop).

Introduction to Cell Culturing

Cell culture refers to the removal of cells from an animal or plant and their subsequent growth in a favorable artificial environment. The cells may be removed from the tissue directly and disaggregated by enzymatic or mechanical means before cultivation, or they may be derived from a cell line or cell strain that has already been established.

Primary culture refers to the stage of the culture after the cells are isolated from the tissue and proliferated under the appropriate conditions until they occupy all of the available substrate (i.e., reach confluence). At this stage, the cells have to be subcultured (i.e., passaged) by transferring them to a new vessel with fresh growth medium to provide more room for continued growth.

Why are aseptic techniques necessary when culturing cells?

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What Do Cells Need to Grow In-Vitro?

Culture conditions vary widely for each cell type, but the artificial environment in which the cells are cultured invariably consists of a suitable vessel containing the following:

* a substrate or medium that supplies the essential nutrients (amino acids, carbohydrates, vitamins, minerals)
* growth factors
* hormones
* gases (O2, CO2)
* a regulated physico-chemical environment (pH, osmotic pressure, temperature)

Most cells are anchorage-dependent and must be cultured while attached to a solid or semi-solid substrate, while others can be grown floating in the culture medium (suspension culture).

What is the Major Purpose for Culturing Cells?

Cell culture is one of the major tools used in cellular and molecular biology, providing excellent model systems for studying the normal physiology and biochemistry of cells (e.g., metabolic studies, aging), the effects of drugs and toxic compounds on the cells, and mutagenesis and carcinogenesis. It is also used in drug screening and development, and large scale manufacturing of biological compounds (e.g., vaccines, therapeutic proteins). The major advantage of using cell culture for any of these applications is the consistency and reproducibility of results that can be obtained from using a batch of clonal cells.

***Using this information, you will now learn the techniques of cell culturing. Your lab write-up for this lab will be due on _____.

Hydrating a Polymer and its Usage for Tissue Engineering

Tissue regeneration is an exciting field of research that is showing advances from the traditional methods of scaffolding, where immune system responses have been a problem, to using functional materials to create viable three-dimensional tissues. The use of a thermally responsive hydrogel, poly N-isopropylacrylamide (PNIPAAM) as a

Name _____

cell culture platform allows the release of intact cells in defined geometries or sheets without the damage to the extracellular matrix (ECM) that traditional release techniques, such as the use of enzymes, have caused.

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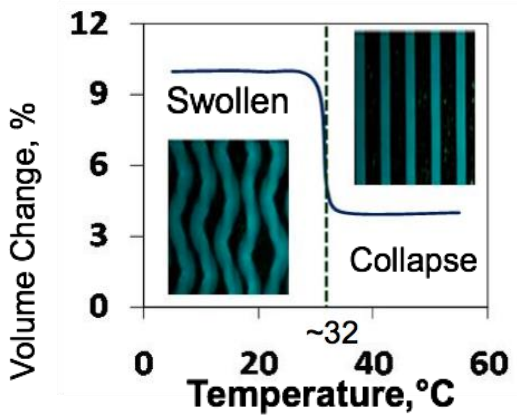
What does thermo-responsive mean?



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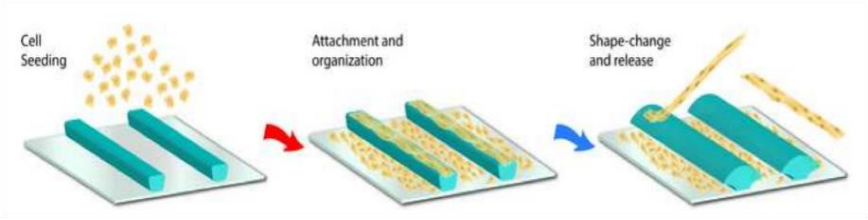

Your group will be given given vials of the polymer and will hydrate your polymer samples with water after you have taken an initial mass measurement. As your group hydrates your polymer, you will take measurements over the course of five days and graph your data. You will test three different densities of polymer based on the amount of cross-linkages inside of the polymer (2%, 3%, and 4% cross-linked). Your goal is to determine the hydration rates for the different densities of polymer.