Tissue Engineering: The Art of Growing Body Parts

a mini-course designed by

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Abstract

The main crux of the course is to familiarize the students with the general principles of Tissue Engineering. The main topics covered are the cells, the scaffold, and the signals used to create tissue engineered constructs. The course is advantageous for teachers looking to incorporate a broad range of biological topics covered into a review and present an example of these principles being applied in the "real world". Many interesting topics are touched in this course including transcription, translation, cell signaling, adult stem cells, embryonic stem cells, and ethical dilemmas.

Course Description

Introduction

The topic of teaching tissue engineering can sound daunting to those who have never had any experience in tissue engineering; however, the topic can be broken down into such a way that the main principles can be taught without an in depth technical knowledge of tissue engineering. Knowledge of high school biology and a little background reading should provide plenty information to teach the class. The overall course structure is two 75 minute sessions. The first is a general overview of tissue engineering and the three main components of tissue engineering. Once the student understands the concepts of the three main components the following session cover these components in more depth individually. For each session a project, worksheet, or demonstration was developed and performed. All of these can be carried out by anyone except for the scaffold biomaterials session, which required materials that won't be available to a high school teacher. The teacher can choose to teach both sessions, just the first session, or break the course down into four 35- 40 minute sessions by dividing the overview and the 3 main components of tissue engineering into separate sessions.

This course is predominantly designed for grades 9-12 with some background knowledge of biology. This course was originally taught at South Seneca High School to Janice Frossards' anatomy and physiology class.

Mini-Course Goals

Provide students with knowledge of the three main components of tissue engineering, which include the cells, scaffold, and signaling. This knowledge will include understanding of what these components are and how they are used in tissue engineering.

General Suggestions

One great use of this course would be to follow up learning about replication, transcription, and translation with this course. The cell and cell signaling session can really put all three together and help the student understand the big picture of what and why the cell is carrying out these processes.

Graduate Student Biography

I am a military brat that has lived in 5 states and attended 12 different schools over the course of my 24 year life so far. By the time you read this I will be married to my beautiful bride Rachel Allen. I graduated valedictorian from Airline High School in Bossier City, LA in 2000 and then magna cum laude from the University of Pennsylvania, where I majored in Bioengineering, in 2005. I worked in Dr. Dawn Elliott's orthopaedic biomechanics laboratory as an undergraduate for two years and then Dr. Steven Nicoll's tissue engineering laboratory for another year. Through this research experience I found my passion for orthopaedic tissue engineering and decided to pursue a PhD. I am currently and at the time of the course a first year PhD student in the Biomedical Engineering Department at Cornell University. My current research involves the study and development of a tissue engineered intervertebral disc. My non academic interests include Christianity, playing golf, playing softball/baseball, playing basketball and pretty much any athletic endeavor. I really do enjoy my research and hope through this course some students' interest will be sparked in science, engineering, and biotechnology.

Session 1: What is Tissue Engineering?

Learning Outcomes

Upon completion of this session, students will:

- be able to name the three main components of Tissue Engineering
- be able to state main function of each component
- be able to state one reason why tissue engineering holds an advantage over current implants.

Duration

Session lasts approximately 70 minutes including activity

Activities

Lecture

Toothpick project

Materials

- For each group of approximately four students you will need 12 toothpicks, 8 one inch diameter spheres of clay, 8 biodegradable peanuts, and one 4 inch wooden ball. Bucket that can hold at least a 5 x 5 x5 inch object. All these materials can be purchased at either A.C. Moore or Joanne Fabric.

Background Information

In this lesson, the students will learn the three main tools/concepts of tissue engineering and their role in the field. 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 method behind tissue engineering is that cells are placed on a biodegradable "scaffold". This scaffold is usually in the shape of the tissue that you want 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. The idea is that as this extracellular matrix is produced 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 repair themselves, giving them 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 a tissue engineered construct with the desired properties.

The scaffold provides the support and shape of the tissue. The scaffold provides a place on which the cells can attach and develop. One analogy that could be used is the scaffold is the framework of the 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. That is, they will be broken down inside the body. This is important because the main goal of tissue engineering is to create a tissue with nonsynthetic material: 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.

In the body, a variety of signals provides the instructions to the cells on how to build tissue. Once the cells are seeded in a scaffold they must produce the proteins that are appropriate to form the tissue desired. Therefore, tissue engineering attempts to mimic the signals that the cell sees in its natural environment. These signals can include hormones, growth factors, and mechanical signals. Through signaling the cells, one can have some control over what tissue is produced by the cell.

Procedures

1. Present slides 1-10 in powerpoint to the class (appendix)
2. Break class into groups of four
3. Pass out toothpicks , clay, wooden ball, bucket, biodegradable peanuts
4. Pass out worksheet (resource section)
5. Have students construct "tissue engineered construct" by first putting biodegradable peanut on each toothpick like a shishkabob. Then construct a cube by putting the ends of the toothpicks in the clay balls. Trap the wooden ball inside this cube. Now place this "tissue engineered construct" into the bucket filled with water. Biodegradable balls should come off and dissolve representing loss of biodegradable scaffold. Students should answer work sheet while performing this task
6. Go over worksheet with students

Suggestions

This seemed to be the most interesting lesson to the students. This session can be taught alone and probably provides as much information as a high school student will retain. The following sessions simply went in to too much depth I believe and the activities weren't as interesting. Really utilize the activity as it seems to drive home the main ideas. Also don't tell the students what will happen in the water because this surprised them and they seemed to enjoy it. Hopefully that will help the lesson stick.

Session 2: Cells, Scaffolds, and Signals

Learning Outcomes

Upon completion of this session, students will:

- State the importance of the cell
- State the difference between embryonic and adult stem cell
- State the importance of the scaffold
- State the properties one looks for in a scaffold
- State the importance of the signal
- State how cells interact with signals

Duration

Originally taught in 80 minutes including activities

Activities

Lecture

Protein Synthesis Review

Originally taught with a hydrogel scaffold demonstration (teacher won't be able to do this…skills specific to originator of the mini-course)

Materials

- Power Point
- Protein Synthesis handouts

Background Information

The cells as covered in the first session provide the living part of the tissue. You will select your cell based on what function you need your tissue to serve. If you are looking to make cartilage you will choose chondrocytes, which are the cells in cartilage. If you want to make bone you will choose osteoblasts, which are the cells in bone. You get the picture. We choose these cells because, despite the fact that all cells contain the complete genome, cells also have memory. That is, these cells have differentiated from some progenitor cell in the past and have something that either allows them to make certain proteins more often or to not be able make others at all. This can be due to a covalent modification of the actual DNA, a soluble signal floating around in the cytoplasm, or simply not having the receptors on the surface to listen to certain signals. As a result, you want to choose a cell that is going to most naturally produce the proteins and materials that you are looking to produce.

Despite the advantages that result from choosing the exact cell from the tissue you are trying to produce, there are some negative aspects to this approach as well. There are a limited number of these cells available. Also, you tend to kill the cells and damage the surrounding tissue from where you obtain these cells. Finally, the same reason that you are trying to obtain the cells may be why you cannot use these cells. If your cartilage is diseased then it is possible that these cells are causing it and therefore should not be obtained for making new tissue. The alternative solution to using the cells of the tissue that you are making is to use adult or embryonic stem cells and differentiate them into the cell you need. The difference between the two cell types is that adult stem cells are contained in all adults while embryonic stem cells are obtained from embryos as the name implies. They are usually found in bone marrow but have also been found in the umbilical chord and fat tissue. Adult stems cells have the advantage of being obtained in the adult and also not being subject to an immune response by the body if obtained from the individual needing the new tissue; however, there is worry that adult stem cells won't be able to differentiate into some types of cells. Embryonic stem cells have the ability to differentiate into any type of cell in the body opening up quite a range of possibilities; however, they have been subject to developing teratomas (tumor) when implanted into the body. Technical hurdles stand in the way of both types of cells and it is both a scientific and ethical question, as to which cell type will be utilized for treatments.

The scaffold provides the framework for the developing tissue. Many questions go into choosing the scaffold. First, we need to know if it's biocompatible. That is, if it will cause an immune response when implanted into the body. If the body reacts to the scaffold then the tissue will be rejected. The scaffold must also be biodegradable. This is so that the cell can replace the scaffold with extracellular matrix. The scaffold must also be chosen to have the correct mechanical and chemical characteristics. If the scaffold is not strong enough to support the forces exerted on it then the scaffold will not be able to develop before being destroyed. Finally, the scaffold must have the proper architecture to support tissue growth. This involves the optimized number of pores and pore size. If the pore size is too big then a solid tissue won't develop but if the pore size is too small then the cells won't fit inside.

Signals allow the cells to be told what to do. There are three types of signals, which are insoluble, soluble, and mechanical. The signals are often proteins that the cells actually can produce. They can be obtained and purified and then given to the cells either in a soluble form or insoluble form. The soluble forms come in a powder type form and can be added to the media (liquid with nutrients) used to feed the cells. The insoluble forms are usually attached to the scaffold. These proteins interact with receptors on the surface of the cells. Each cell type has different types of receptors on the surfaces. These receptors are specific to certain signals thus giving the cell the ability to respond to certain signals but not to others. Finally, cells respond to mechanical stimulation as well. When bone cells are mechanically loaded they produce more bone. This is the reason those who exercise more have higher bone density. Overall, these three signal types can control the proteins produced by these cells.

Procedures

1. Lecture on cell portion of the power point.
2. Hand out protein synthesis review and go over it with class (Resources)
3. Lecture scaffold portion of the power point
4. During the original class alginate was brought into the class and a hydrogel was made. This will not be available to the teacher. Alternatively, I suggest making jello, which is basically a hydrogel, with m and m's inside. This will demonstrate how scaffolds are made with cells trapped inside.
5. Lecture on signal portion of the scaffold

Suggestions

Keep the class as simple as possible and focus on the big picture. It may take some further brainstorming on this session as to some new projects to drive the ideas home. This session seemed to bore the students when I taught it because it was too in depth.

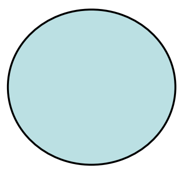
Resource Materials

For use with toothpick activity

Tissue Engineering Model

= ECM (structural protein)

= Scaffold



= Cell

= Body Environment (enzymes, signaling molecules, H20)

1. What three components were needed to produce the Tissue Engineered tissue?
   a. ____________Cells__________
   b. __________Scaffold____________
   c..___________Signals___________

2. What happened to the scaffold? What implication does that have on the body it is implanted into?
Scaffold degraded and was floating around in water (body environment). Degraded scaffold goes into the body and therefore must be biodegradable.

3. What is the scaffold replaced by? What importance do you think the cell has in this role?
Scaffold replaced by ECM. The cell produces the ECM as the scaffold breaks down.

4. What would be the advantage of having an implant made of living tissue produced by cells rather than a man-made material?

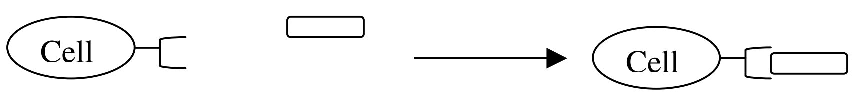
Living tissue implant can repair itself when damaged allowing it to last longer. Also, living tissue is more biocompatible than man made materials.

5. When would such an implant be required. What types of concerns would need to be considered in developing such an implant?

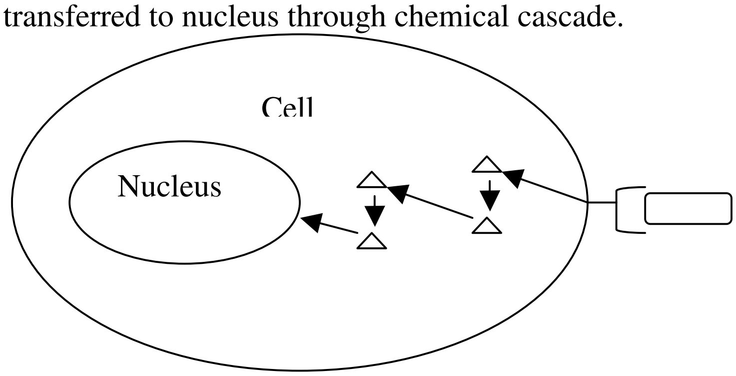
This type of implant is necessary when you need a long term implant. Man made material implants don't last long enough in many cases. You would need to consider what functions this tissue is going to carry out (mechanical properties, biocompatibility, metabolic needs, etc.)

Central Dogma of Protein Synthesis in TE

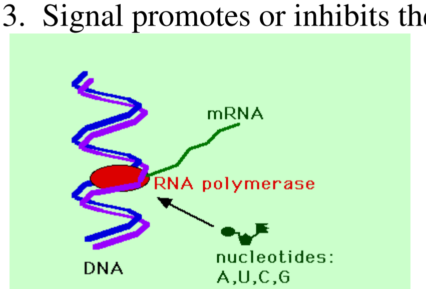
1. Cell is signaled



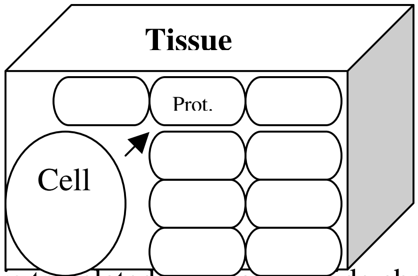
2. Signal is transferred to nucleus through chemical cascade.



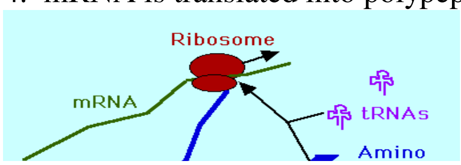
3. Signal promotes or inhibits the transcription of mRNA



5. Proteins are often secreted out of cell to build tissue or used as signaling molecules themselves.



4. mRNA is translated into polypeptide chain by ribosome (protein)



Q1. If you wanted a cell to produce a certain type of protein, as is the case in Tissue Engineering, what in this process could you use to make this happen?

Control the process at step 1 by introducing a signal that will produce the protein you desire in step 4

Appendix

POWERPOINT SLIDE CONTENT

Slide 1

Tissue Engineering: The art of growing body parts

Robby Bowles

Cornell University

Slide 2

What is Tissue Engineering?

- TE is an interdisciplinary field that applies the principles of engineering and the life sciences towards the development of biological substitutes that restore, maintain, or improve tissue function…blah blah blah
- Developing living tissue using cells, biomaterials, and signaling molecules

Slide 3

Some Fabricated Tissue Engineering Constructs

Slide 4

Need for Replacement

- Skin - 3 million procedures per year
- Bone - 1 million procedures per year
- Cartilage - 1 million procedures per year
- Blood Vessel - 1 million procedures per year
- Kidney - 600 thousand procedures per year
- Liver - 200 thousand procedures per year
- Nerve - 200 thousand procedures per year

Slide 5

Why Tissue Engineering?

- Synthetic materials

– Poor biocompatibility
– Mechanical Failure (undergo fatigue, wear, corrosion)
• Transplants
– Rejection
– Disease transmission
– Supply << Demand

Slide 6

3 Tools of Tissue Engineering
• Cells
– Living part of tissue
– Produces protein and provides function of cells
– Gives tissue reparative properties
• Scaffold
– Provides structural support and shape to construct
– Provides place for cell attachment and growth
– Usually biodegradable and biocompatible
• Cell Signaling
– Signals that tell the cell what to do
– Proteins or Mechanical Stimulation

Slide 7

Combinations of Tools
• Cells alone
– Carticel - commercially available product
• Purified Signaling molecules
– Bone Morphogenic Protein for osteoblasts
– Inject into tissue to encourage new tissue growth

- Cells in Scaffold
  – Chondrocytes (cartilage cells) in alginate hydrogel

Slide 8

What I do

The Intervertebral Disc

Slide 9

Strategy for Engineering IVD

- Scaffold
  – Alginate
    - Obtained from brown seaweed
    - Biodegradable
    - 3d printable
- Cells
  – Annulus Fibrosus Cells
  – Nucleus Pulposus Cells
- Signaling Molecules
  – Transforming Growth Factor- β

Mechanical Stimulation

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Toothpicks and Tissue Engineering

Slide 11

Cells

What is the importance of the cells?

- Living part of tissue

- Produces protein and provides function of cells
- Gives tissue reparative properties

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How do you select the cell?

- Need a cell that produces the function of desired tissue

– Load bearing tissues - produce proteins and molecules of the Extracellular Matrix (ECM)

– Other tissues - kidney - produce protein insulin

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What are our options for cells?

- Animal - Xenograft

– Rejection

– Immunosuppression necessary

– Who really wants animal cells in them?

- Other person - Allograft

– Rejection

– Requires immunosuppression

- From self - Autograft

– No immunosuppression necessary

– Limited source

– Donor site morbidity

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Autografts

- Exact cell type needed

– Cartilage - chondrocytes
– Bone - osteoblasts
– Kidney - islet cells
– Etc.
• Stem Cells
– Adult Stem Cells
– Embryonic Stem Cells

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What is a Stem Cell?
• Characteristics
– Undifferentiated
– Immortalized
– Ability to differentiate
• Potency
– Totipotent - can differentiate into any type of cell
– Pluripotent - can differentiate into any type of cell accept totipotent cells
– Multipotent - can differentiate into multiple but related cell types
– Unipotent - can differentiate into a single type of cell

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Embryonic Stem Cell
• Pluripotent
• Pros
– Has the ability to differentiate into any type of cell
– Could be used for numerous diseases
• Cons
– Develops into a teratoma (tumor) if implanted into adults
– Ethical concerns

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Adult Stem Cell

- Multipotent
- Pros
- – Can differentiate into many cell types
- – Easy to obtain
- – No rejection
- Cons
- – Unable to differentiate into all types of cells

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What is the main concern in choosing a cell source?

- Protein Synthesis
- – Cells must produce proteins to carry out tissue function
- Extracellular matrix for load bearing tissue
- Signaling molecules (ie hormones) for tissues such as the kidney
- Etc.

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Protein Synthesis Review

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Scaffold

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What do we want in a scaffold?

- 1. Biocompatible
- 2. Biodegradable
- 3. Chemical and Mechanical Properties
- 4. Proper architecture

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Types of Materials

- Metals (not degradable)
- Ceramics (stiff, brittle, long degradation)
- Polymers (wide range of properties)

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What is a polymer?

- Molecule made from a large number of repeatable units
- Advantages

– Control of architecture, reactivity, and degradation

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Polymers in TE

- Natural

– Derived from ECM

- + preprogrammed
- + Generally biocompatible
- +Biological degradation mechanism
- - not made, purified
- Synthetic

– Made by controlled process

- +/- Range of biological responses
- +/- Range of degradation
- Established production protocol

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Methods of Cell Delivery

- Cell Adhesion (solid/dry scaffolds)
- Cell Encapsulation
– Polymer solution to solid

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Architecture

- Pore size
– Average diameter of pores
- Porosity
– Porosity volume/total volume
- Interconnectivity
- Porogeneration (melt molding)

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Cell Signaling

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How do we control cell behavior?

- Insoluble signals
- Soluble signals
- Mechanical signals

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Insoluble signals

- How does this control cell behavior?

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How do cells interact with signals?

- Receptors
- Short amino acid sequences on protein
– RGD
- RGD peptide bound to scaffold

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Insoluble signals effects

- Adhesion
- Migration
- Apoptosis
- Proliferation
- Differentiation
- Mechanosensation

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Soluble Signals

- Interact in same way as insoluble signals
- Can be added to media to signal cells
- Easier to use than insoluble signals

Slide 33

Mechanotransduction

- Process by which cells turn mechanical signals into biochemical signals
- Mechanisms

– Stress sensitive ion channels

– Receptors bound to cytoskeleton bound to nucleus (works in combination with soluble signaling)

Slide 34

How can we use these signaling pathways in TE?

- Insoluble

– Use ECM scaffolds or bind signals to scaffold surface

- Soluble

– Put in media

- Mechanical signals

– Mechanically stimulate construct before implantation