

Key: Yellow highlight = required component

Ecology in Tissue Engineering

Subject Area(s) Biology

Associated Unit Ecology

Lesson Title Ecology in Tissue Engineering

Header

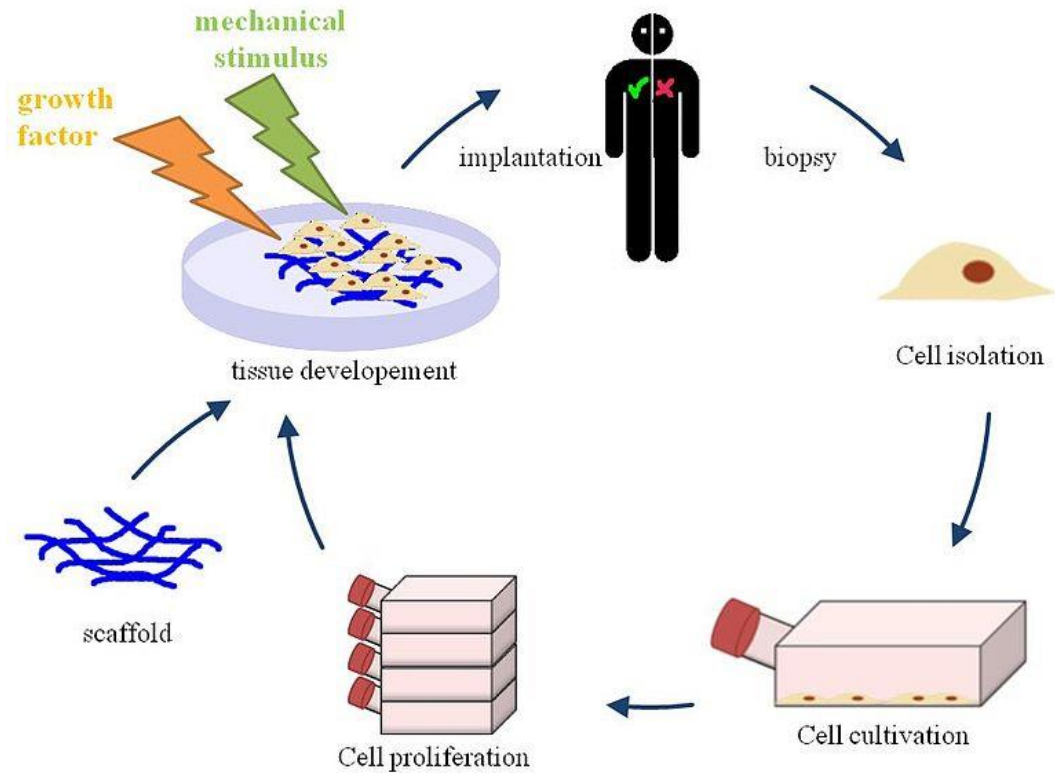


Image 1

Image file: ___?

ADA Description: This image shows a person's cells that were taken from a biopsy being cultured in a flask and then being grown on a nanofiber scaffold that simulates extracellular matrix. This tissue scaffold is then used to heal that person's wounds.

Source/Rights: https://en.wikipedia.org/wiki/Tissue_engineering

Caption: The process of creating a tissue scaffold.

Grade Level 9 (9-12)

Lesson # ___ of ___

Lesson Dependency

Time Required 3-4 50 minute class periods, or a total of 200 minutes

Summary

Students learn about exponential and logistic growth curves. They culture and passage cells using the aseptic technique. They take a daily count of their cells to create a growth curve for the cells that they are growing. Students then test how different variables affect the growth of their cells.

Engineering Connection

Students learn how engineers at USF are using these same procedures to grow cells on nanofiber membranes that are produced through the process of electrospinning in order to create tissue scaffolds for wound healing. Students will also learn about how an MTT assay is performed.

Engineering Category =

Choose the category that best describes this lesson's amount/depth of engineering content:

1. Relating science and/or math concept(s) to engineering

Keywords

Electrospinning, polymers, nanofibers, culture cells, passage cells, MTT assay, aseptic technique, engineering design process, hemocytometer.

Educational Standards (List 2-4)

State STEM Standard

Florida Next generation science standards, Life Science, 9-12, SC.912.L.17.5 Interdependence. Analyze how population size is determined by births, deaths, immigration, emigration, and limiting factors (biotic and abiotic) that determine carrying capacity.

Florida Next generation science standards, Life Science, 9-12, SC.912.L.16.10, Heredity and Reproduction, Evaluate the impact of biotechnology on the individual, society and the environment, including medical and ethical issues.

Florida Next Generation science standards, Nature of Science, 9-12, SC.912.N.1.1, The Practice of Science, Define a problem based on a specific body of knowledge, for example: biology, chemistry, physics, and earth/space science, and do the following:

1. pose questions about the natural world,
2. conduct systematic observations,
3. examine books and other sources of information to see what is already known,
4. review what is known in light of empirical evidence,
5. plan investigations,
6. use tools to gather, analyze, and interpret data (this includes the use of measurement in metric and other systems, and also the generation and interpretation of graphical representations of data, including data tables and graphs),
7. pose answers, explanations, or descriptions of events,
8. generate explanations that explicate or describe natural phenomena (inferences),
9. use appropriate evidence and reasoning to justify these explanations to others,
10. communicate results of scientific investigations, and evaluate the merits of the explanations produced by others

ITEEA Standard

The Nature of Technology

Students will develop an understanding of the relationships among technologies and the connections between technology and other fields of study

Technology and Society

Students will develop an understanding of the cultural, social, economic, and political effects of technology.

Design

Students will develop an understanding of the role of troubleshooting, research and development, invention and innovation, and experimentation in problem solving.

The Designed World

Students will develop an understanding of and be able to select and use medical technologies.

NGSS Standard

Science, Grades 9-12, HS-LS2-2, From Molecules to Organisms: Structures and Processes

Students who demonstrate understanding can:

Use mathematical representations to support and revise explanations based on evidence about factors affecting biodiversity and populations in ecosystems of different scales

CCSS Standard (strongly recommended)

Pre-Requisite Knowledge

Students must have an understanding of dehydration synthesis of polymers, mitochondria, spectrophotometry, eukaryotic cell structure

Learning Objectives

After this lesson, students should be able to:

- Compare and contrast exponential and logistic growth
- Contrast density-dependent and density-independent limiting factors
- Explain the process of electrospinning
- Perform research using the engineering design process
- Use the aseptic technique to culture cells and passage cells
- Count cells using a hemocytometer.

Introduction / Motivation (5E – Engage)

Today you will start class by watching a short video clip on human population growth. While watching the video, think about why the population has grown so rapidly in recent years. (Students watch the 2:58 minute video clip on human population growth at

<https://www.youtube.com/watch?v=sc4HxPxNrZ0>)

Now study the graph of human population growth. Discuss with your table partners possible explanations for the human population growth curve. (This graph is in the PPT. Allow students a few minutes to discuss with their table partners and then open up a short classroom discussion).

Do you think that the human population can continue growing in this trend? Why or why not? (Allow students a few minutes to discuss with their table partners and then open up a short classroom discussion).

Lesson Background & Concepts for Teachers (5E – Explain)

Exponential growth happens when a population exists in ideal conditions without any limiting factors. The population is growing very fast and there is plenty of food, water, habitat, etc. Most populations will have a decrease in the rate of growth when resources begin to become scarce. During logistic growth, the population will reach its carrying capacity and begin to plateau.

There are two types of limiting factors. Density-dependent limiting factors depend on population size and include competition, predation, disease, habitat and food sources. Density-independent factors do not depend on population size and usually involve abiotic factors such as severe weather, temperature and pollution.

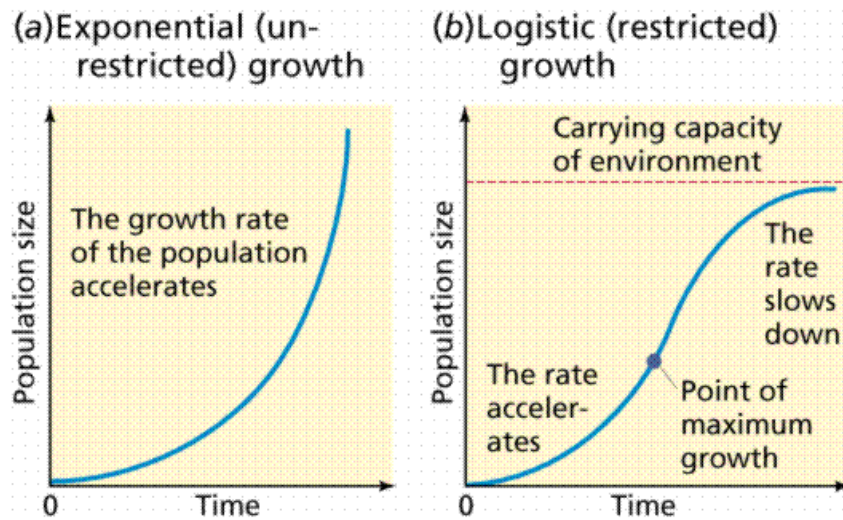


Figure 2

Image file: ___?

ADA Description: The graph of exponential growth shows a rapid acceleration in population growth. The graph of logistic growth shows a rapid acceleration in population growth that then slows down at the carrying capacity.

Source/Rights: <http://mhsaphuge1.wikispaces.com/Population+%26+Migration>

Caption: Exponential Vs. Logistic Growth Curves

Logistic growth can be witnessed when culturing cells. **Cell culturing** involves removing cells from a multicellular eukaryotic organism and growing them in a flask or petri dish with medium and other favorable environmental conditions, such as temperature and CO₂ levels. Eventually, cells will reproduce enough that they will reach their carrying capacity. Before this happens, cells should be passaged.

Cell passaging involves transferring some of the cells from the original flask to a new flask once the cells have reached 80-100% confluence. This is important for a few reasons: so that there are fresh nutrients in the new media, to avoid a buildup of toxic metabolites and to avoid contact inhibition by giving the cells more room for them to grow.

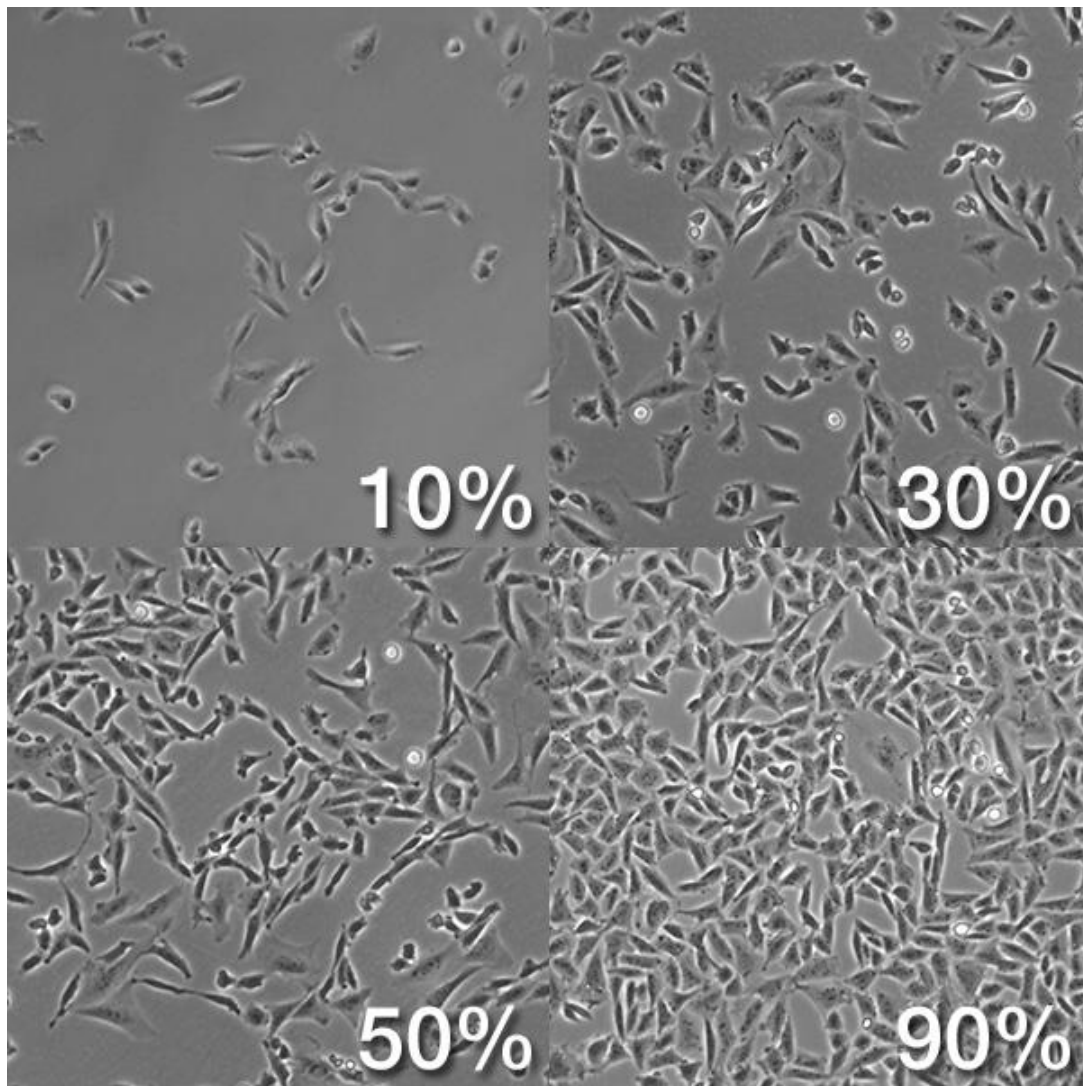


Figure 3

Image file: ___?

ADA Description: Cells are shown at 10%, 30%, 50%, and 90% confluency. The higher the confluency, the more dense the cell growth.

Source/Rights: <https://en.wikipedia.org/wiki/Hemocytometer>

Caption: Cell Confluency

Cell culturing and cell passaging must be performed with aseptic technique. Aseptic technique is a method to perform laboratory procedures in such a way as to avoid microbial contamination. Sterile gloves are worn. The biosafety hood and all equipment that goes inside is sprayed with 70% ethanol or 70% isopropyl alcohol. Movement of people, and thus air, is limited in the work area. Lids are kept on all containers until needed. Hands are not placed above open containers. Tips of pipettes must not touch the necks of the bottles or flasks.

Cell counting is performed using a hemocytometer in order to determine your total cell count, cell/mL, or percent viability. A hemocytometer is a slide that contains grids of 9 squares on each side of the slide. Each large square is 1 mm². Usually, when looking at one set of 9 squares, the four large corner squares and the large center square is used for counting live and dead cells. 200 total cells should be counted for your data to be statistically significant. Therefore, you may need to count all 18 squares on the hemocytometer if necessary. To visualize the cells, they must first be dyed using Trypan blue. Living cells will appear clear since the dye does not penetrate the cell membrane of living cells. The dye will penetrate the cell membrane of dead cells, making them appear blue. Students will perform the Edvotek lab and count their cells daily to create a logistic growth curve of their cells.

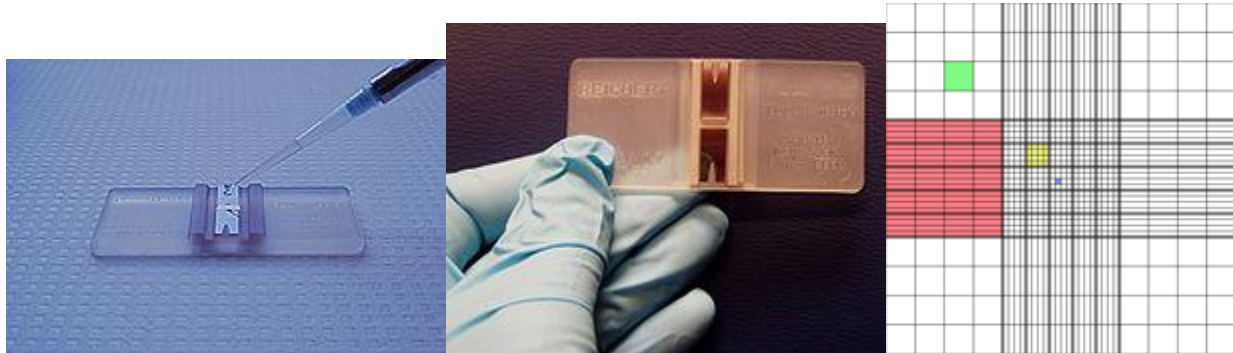


Figure 4

Image file: ____?

ADA Description: A micropipette is used to fill both sides of the hemocytometer slide with about 15-20ul of cell suspension. When placed under the microscope, the cells are counted using the squares in the grid on the slide.

Source/Rights: <https://en.wikipedia.org/wiki/Hemocytometer>

Caption: A hemocytometer slide.

Cells can be cultured in a flask and then transferred to a nanofiber membrane that can be used as a tissue scaffold. The nanofiber membranes are created through a process called electrospinning. This process involves dissolving polymer in a solvent to create a polymer solution that is placed inside of a syringe. An electric field is then applied as the positive terminal is connected to the tip of the syringe needle and the negative terminal is connected to a collector plate. The applied electric potential overcomes the surface tension of the polymer solution. The polymer solution forms a Taylor cone at the tip of the syringe. A polymer jet is then ejected from the syringe needle tip and is deposited onto the collector as a non-woven web. The repulsive electrostatic forces create bending instabilities that cause the jet to spiral as its traveling to the collector. To minimize the instability, the jet undergoes plastic stretching, which reduces its diameter, thus forming extremely thin fibers. The solvent evaporates and only the polymer fibers land on the collector. If one uses a low polymer concentration, or low molecular weight polymer, fibers may not form due to the lack of polymer chain entanglements and the jet breaking down into droplets as an electro-spray instead.



Figure 5

Image file: ___?

ADA Description: A polymer solution is being electrospun onto an aluminum foil collector. Fibers are evident on the tip of the syringe. The light allows the fibers to be more visible.

Source/Rights: Erica Wilkes

Caption: Electrospinning Apparatus

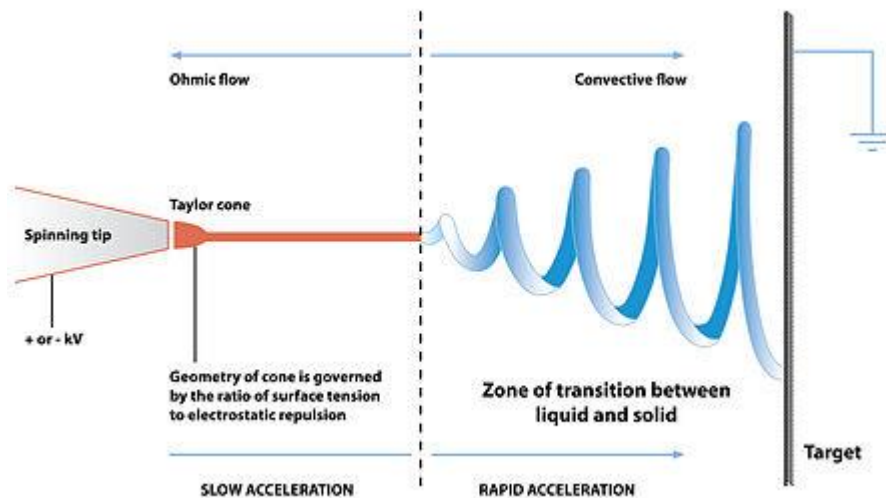


Figure 6

Image file: ___?

ADA Description: A polymer solution is being electrospun onto a collector. As the polymer solution leaves the tip of the syringe, the solvent evaporates and the polymer fibers following a spiral, spinning pattern towards the collector.

Source/Rights: <https://en.wikipedia.org/wiki/Electrospinning>

Caption: The path of the polymer during electrospinning.

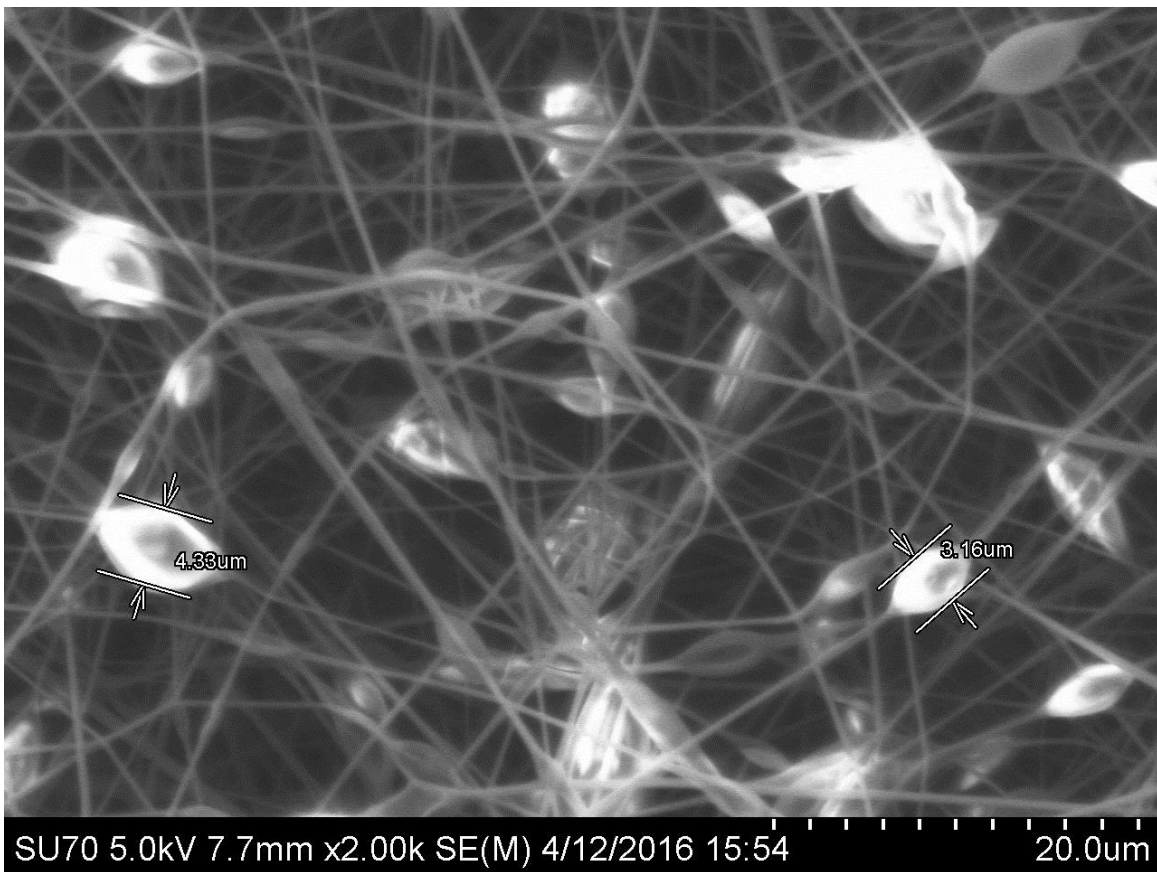


Figure 7

Image file: ___?

ADA Description: An SEM picture of nanofibers showing the high SA:V ratio of the fibers and the porosity of the membrane. The bright beads are due to the addition of cactus mucilage, which has been shown to increase cell proliferation.

Source/Rights: Venkatesh Eppili

Caption: Nanofibers made of 70:30 solution of 20% polystyrene in D-Limonene: 1% cactus mucilage in distilled water

When testing a variable on cell viability or proliferation, an MTT assay can be performed. Mitochondrial enzymes in metabolically active cells cleave the yellow tetrazolium salt MTT (3-(4, 5-dimethylthiazolyl-2)-2, 5-diphenyltetrazolium bromide) forming an intracellular purple punctate precipitate named formazan.

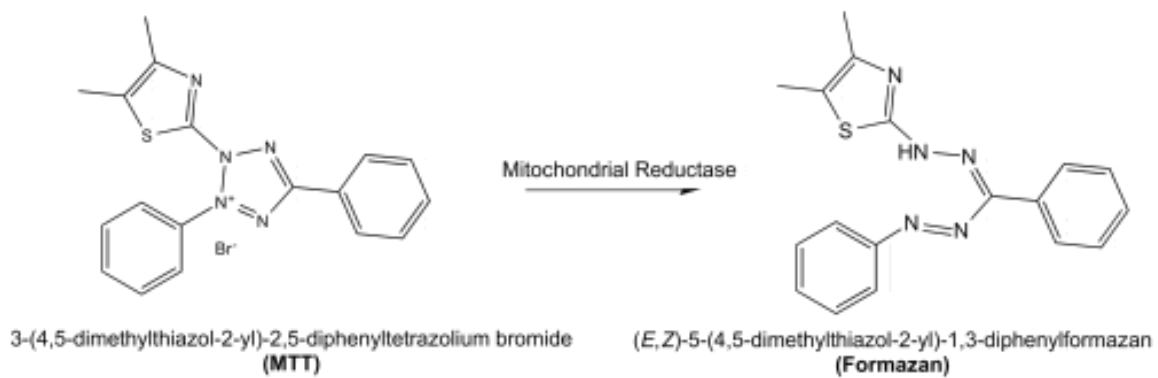


Figure 8

Image file: ___?

ADA Description: The enzyme mitochondrial reductase reduces MTT by cleaving its ring structure and forming formazan.

Source/Rights: https://en.wikipedia.org/wiki/MTT_assay

Caption: Reduction of MTT to Formazan

Dimethyl sulfoxide (DMSO) dissolves formazan into a colored solution, which can be quantified by a spectrophotometer. The more purple the solution, the greater the absorbance and the greater the number of cells.

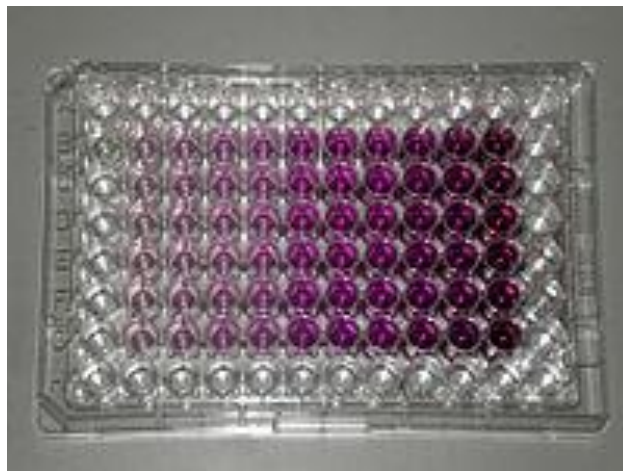


Figure 9

Image file: ___?

ADA Description: A 96 well plate shows wells increasing in the concentration of formazan, and thus the color purple, from left to right.

Source/Rights: https://en.wikipedia.org/wiki/MTT_assay

Caption: MTT assay

Vocabulary / Definitions

All definitions come from <http://www.merriam-webster.com/>

Word	Definition
Polymer	a chemical compound that is made of small molecules that are arranged in a simple repeating structure to form a larger molecule
Biodegradable	capable of being slowly destroyed and broken down into very small parts by natural processes, bacteria, etc
Aseptic	free from germs that cause disease

Associated Activities (5E – Explore)

Day 1:

After students discuss the human population growth curve in the introduction activity, they will learn about exponential growth curves and logistic growth curves. They will also learn about Carrying Capacity,

Students will work in groups to correctly label the graphs using the manipulatives that are provided. The teacher should copy these in color on card stock or laminate them. The teacher should give the student groups the laminated graphs and phrases already cut out.

Students will then learn about density-dependent limiting factors, and density-independent limiting factors. Lastly, they will learn about predator-prey graphs.

Students will work in pairs with their shoulder partner to categorize cards as either density-dependent or density in-dependent. They can play it as a game where each student gets 9 cards to quiz the other. If the other student answers incorrectly, they get to keep the card. If the other student answers correctly, the other student gets to keep the card. The teacher should copy these on card stock so that the card are not see through. The teacher should also laminate the cards.

Day 1-2

Students will relate carrying capacity to cell growth to prepare for the lab that they will perform.

Students will read over the Edotek lab handout to understand the tasks they will perform tomorrow. This includes cell passaging and cell counting.

For the Edvotek lab: <http://www.edvotek.com/1001>

For the Edvotek lab instructions: <http://www.edvotek.com/site/pdf/1001.pdf>

Students will watch the video on cell culturing. <http://www.jove.com/science-education/5052/passaging-cells>

Students will practice using the micropipette to learn this technique before the lab.

Day 3

Students will use the phase contrast microscope to view the cells in the flask that has been prepared by the teacher using the Edvotek guidelines. They will learn to passage the cells and count the cells using a hemocytometer.

Day 4

Teacher will introduce electrospinning by discussing the research being done at USF's Advanced Materials Bio and Integration Research Laboratory (AMBIR Lab) and showing the animation on electrospinning.

Teacher will introduce the concept of tissue engineering using scaffolds by showing the video on lab grown human organs for transplant.

Teacher will introduce the Engineering Design Process.

Teacher discusses the MTT assay.

Teacher can allow students to use the engineering design process to extend the lab by testing different variables on the cells.

Lesson Closure

In summary, you have learned about exponential and logistic growth, including limiting factors that affect the carrying capacity. You witness logistic growth through the culturing of cells. You have learned about the process of electrospinning and how the resulting nanofibers are being researched as tissue scaffolds. You have learned how adding different substances to the polymer solution before electrospinning creates fibers with different functions, allowing nanofibers to be classified as functional materials. For example, if we incorporate cactus mucilage into our fibers, cells are more likely to proliferate. You have been able to relate your knowledge of spectrophotometry to its use in the MTT assay as a measurement of cell proliferation. You have learned about the engineering design process and modeled the initial process of tissue engineering by using the aseptic technique to culture, passage, and count cells.

Assessment (5E – Evaluate)

Pre-Lesson Assessment

Bellwork Questions: Video and discussion of the human population growth graph

Post-Introduction Assessment

Teacher monitoring of graph manipulatives and card game. Teacher monitoring of lab procedures. Study Questions in the Edvotek lab instructions.

Lesson Summary Assessment

Quiz on density-dependent limiting factors, density-independent limiting factors, exponential growth and logistic growth.

Homework

Descriptive Title: Students finish the questions in the Edvotek lab instructions.

Lesson Extension Activities (5E – Extension)

1. Research real life situations of polymers used in tissue engineering. Report on
 - what type of tissue or organ was created
 - the properties of the polymers that make them an appropriate choice for use as a tissue scaffold
 - the necessary characteristics of a tissue scaffold that allow cell growth and proliferation.
 - obstacles that must be overcome to perform electrospinning on a large scale for industry purposes.

2. After learning how to culture and passage cells, students could extend their lab by testing how a certain variable affects cell growth. Some possible independent variables they could test are:
 - a. The effect of temperature on cell growth.
 - b. The effect of CO₂ levels on cell growth
 - c. The effect of pH on cell growth.
 - d. The effect of a substance on your cell culture.

Additional Multimedia Support

2:58 minute video on human population growth:

<https://www.youtube.com/watch?v=sc4HxPxNrZ0>)

10 minute video on cell culturing <http://www.jove.com/science-education/5052/passaging-cells>

Simulation on electrospinning <http://nano.mtu.edu/documents/Electrospinning.swf>

2:13 video on lab grown human organs for transplant <https://www.youtube.com/watch?v=bdLL0a79CsI>

References

Attachments

PPT for the lesson

Graph Manipulatives

Density-dependent and density-independent cards

Other

Redirect URL

Contributors

Erica Wilkes – teacher at King High School

Supporting Program

AMBIR Lab – Dr. Sylvia W. Thomas, Department of Electrical Engineering, University of South Florida

Acknowledgements

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Classroom Testing Information

A drought has
dried up all of
the
vegetation.

DI

It is hunting
season and
you are shot
by a hunter.

DD

Your habitat
has been
destroyed for
a new
shopping
mall.

DI

You caught a
disease.

DD

Too many
deer were born
last year and
you are
struggling to
find food to
eat.

DD

There has
been a
chemical spill
into the river
you normally
drink from.

DI

You are bit by
a tick.

DD

A severe
winter has left
you hungry in
search of
food.

DI

You were able
to hide in the
brush and
camouflage
yourself from
a predator.

DD

Extreme heat
has left you
suffering from
dehydration.

DI

You have
been caught
by a predator.

DD

A hurricane
has left severe
flooding and
killed many
of your fellow
deer.

DI

You were able
to travel to a
new habitat to
avoid
overcrowding

DD

Many new
fawn were
born this year.

DD

There was a
wildfire that
burned down
your entire
habitat.

DI

A new
neighborhood
was built with
many
gardens.

DD

You
experience
competition
for mates.

DD

An exotic
species was
introduced into
your habitat.

DD