Regenerative Medicine

Activity 4.1.3. Can a plant be a scaffold for a human organ?

The strongest item in this pack, by a wide margin. A real wet lab with a scalpel, a detergent, a 24 hour incubation, and a negative control that you have to design yourself and get approved before you set it up. Nobody is going to tell you the answer and nobody is going to tell you the control.

WORTH 8 POINTS | HELPS ON THE WEBXAM. THE MOST OF ANYTHING HERE | WET LAB, IN SCHOOL, SUPERVISED | TWO SESSIONS, 24 HOURS APART

What this is and why it is worth doing

You carve a slice of apple into the shape of an organ, strip the apple cells out of it with detergent, and see whether what is left over could serve as a frame for human cells to grow on. That is not a metaphor. It is a simplified version of what a regenerative medicine laboratory actually does.

Over 100,000 people are on the national transplant waiting list in the United States and the number rises every year. An average of 17 of them die each day waiting. One donor can save up to eight lives and improve fifty more through eye and tissue donation, and the supply still does not come close to the demand. Regenerative medicine is one of the few routes out of that arithmetic: instead of waiting for a donor, build the part.

DOES THIS HELP ON THE WEBXAM? YES, MORE THAN ANYTHING ELSE IN THIS PACK

PBS is assessed by WebXam 072110, which is 53.93 percent **Handling, Preparation, Storage and Disposal** and 46.07 percent **Biotechnology Research and Experiments**. This activity is the only item in the pack that puts you inside the first domain properly: you handle a chemical, wear the right protective equipment, use a sharp, store an experiment for 24 hours, and dispose of everything correctly. It also lands squarely in the second domain, because the negative control is yours to design.

It is not on the class calendar for one reason only. The 24 hour incubation costs a class day, and the December calendar does not have one to give.

AND THE PART THAT IS WORTH DOING FOR ITS OWN SAKE

Scientists have grown beating human heart tissue on spinach leaves. They removed the plant cells from the leaves, added human heart muscle stem cells, and within a few days the heart cells were contracting. When blood was introduced to the system, it flowed, because a leaf's vein structure and a heart's vessel structure solve the same problem in similar ways.

That result is about eight years old and you can run the first half of it with an apple, a scalpel and some soap. Not many high school labs are one step removed from a live research frontier. This one is.

How long it takes and what you need first

SESSION	HOW LONG	WHAT HAPPENS
Session one	About 45 minutes	Read the background, do the opacity test on both slices, carve both slices, set up the experimental slice in detergent, **get your control approved**, set up the control, label and store both.
The wait	24 hours	Nothing. Both slices sit. On a shaker or rocker if one is available.
Session two	About 25 minutes	Repeat the opacity test on both slices, record general observations, answer the written questions, clean up.

ARRANGE ALL OF THIS BEFORE SESSION ONE

- **See Mr. Mendoza at least a day ahead.** The detergent, the scalpel and the bench have to be set out for you, and a supervised slot has to exist. Turning up unannounced means turning around.
- **Book both sessions at once,** 24 hours apart. Session two cannot slide, because the incubation time is part of the experiment. If it has to slide, write down the actual elapsed time and report that instead of writing 24.
- **Bring:** your lab notebook, a pen, a phone or camera for the opacity photographs, and a printed page of ordinary text. If you can, bring the same printed page to both sessions.
- **The lab supplies:** 0.5 percent sodium dodecyl sulfate, apple slices, a beaker, a scalpel, a ruler, tweezers, gloves, safety glasses and a lab coat.
- **Wear closed shoes and clothing you do not mind.** Detergent marks fabric.

Safety. Read all of it before you touch anything

This is the most hazardous item in the pack. Three separate things on this bench can hurt you and one of them is the one people take least seriously.

THE SCALPEL

- **A scalpel is not a knife.** It is sharper than anything in your kitchen and it cuts without your noticing, which is exactly why scalpel cuts are usually deep before anyone reacts.
- **Cut on the board, never in your hand.** The apple stays flat on the cutting surface. Your other hand stays behind the blade and behind the apple, never in front of it and never wrapped around it.
- **Cut away from your body,** in short controlled strokes. If you find yourself pushing hard, stop. Force is how the blade skips.
- **One person cuts at a time** and everyone else stays back from the board. Do not talk to the person cutting.
- **Never pass a scalpel blade first, and never catch a dropped one.** Put it down on the board and let the other person pick it up. A dropped scalpel is allowed to hit the floor. Step back, let it land, then pick it up by the handle.
- **Blade down between cuts.** Not in your hand, not in a pocket, not resting on a paper towel where somebody will sweep it up.
- **Blades go in the sharps container** when you are done. Never in the regular bin.
- **If you cut yourself,** however small: stop, tell Mr. Mendoza immediately, rinse under running water, and let him deal with it. Do not carry on and mention it later.

THE DETERGENT, 0.5 PERCENT SODIUM DODECYL SULFATE

- **What it is.** SDS is a strong detergent. It destroys cell membranes, which is precisely why it strips the cells out of the apple. Your own cells are made of the same kind of membrane. It does not care whose cells they are.
- **It is a skin and eye irritant.** Gloves, safety glasses and a lab coat go on before the container is opened and stay on until it is closed and the bench is wiped.
- **Do not shake it, do not splash it, do not make foam.** Foam carries the detergent into the air. Pour slowly down the inside wall of the beaker.
- **Skin contact:** rinse with running water for a full minute and tell Mr. Mendoza.
- **Eye contact:** go straight to the eyewash, hold the eyelid open, rinse for fifteen minutes, and have someone else tell Mr. Mendoza while you rinse. Fifteen minutes is longer than it feels. Do not stop early.
- **Do not touch your face, your phone, or your eyes with gloves on.** The phone is the one people forget, and you will be using it for the photographs. Take the gloves off, then take the photograph.
- **Wash your hands with soap and water afterwards even though you wore gloves.**

THE APPLE, WHICH IS THE ONE PEOPLE TAKE LEAST SERIOUSLY

- **Nothing on this bench is food.** It looks like food, it smells like food, and it has been in a laboratory. Nobody eats anything, tastes anything, or licks a finger. Not before the detergent, not after.
- Apple slices that have been in SDS are chemical waste, not compost and not rubbish. They go where Mr. Mendoza directs.
- Tell Mr. Mendoza in advance about any allergy, including latex if gloves are latex.

THE 24 HOUR STORAGE, AND CLEANUP

- **Label both containers before you leave:** your name, your period, the date, the time you set them up, and what is in each one. An unlabelled beaker of unknown liquid is a hazard for whoever finds it.
- Both containers stay where Mr. Mendoza puts them. Not in a locker, not in a bag, not on a windowsill.
- **Do not open either container during the 24 hours.** Full protective equipment goes back on before you open anything in session two.
- Liquid waste goes where you are told. Ask before you pour anything down a drain.
- Wipe the bench. Wash your hands. Return the ruler, the tweezers and the board.

The science you need

WHY MATCHING IS HARD

To receive an organ from a donor you have to match, and matching has two parts. The first is **blood type**. Red blood cells carry surface antigens that label them as type A, B, AB or O.

DONOR BLOOD TYPE	CAN DONATE TO
Type O	O, A, B, AB
Type A	A, AB
Type B	B, AB
Type AB	AB

The second part is **tissue typing**, also called HLA typing. HLA stands for human leukocyte antigen, and six of them matter for a match. Every cell in your body carries surface antigens that are yours specifically. Your immune system reads those antigens, recognises them as self, and leaves them alone. Anything it does not recognise it marks as non-self and attacks. That is exactly what protects you from infection, and it is exactly what attacks a transplant. The more HLA matches between donor and recipient, the better the odds the transplant is read as self. Even a good match is not a guarantee.

In the United States the **Organ Procurement and Transplantation Network**, or OPTN, runs the national database of everyone waiting, holds their blood and tissue types, and matches donated organs to recipients.

THE TWO ESCAPE ROUTES FROM THE MATCHING PROBLEM

Xenotransplantation is transplanting non-human cells, tissues or organs into humans. A pig heart is about the size of a human heart, and with gene editing a pig can be made to express human HLA antigens. That is a real and current line of work with real objections attached to it.

Regenerative medicine builds the part instead. Two leading strategies both use a **scaffold**, a frame in the shape of the body part that cells are applied to and grow on:

- **Ghost organs.** Take a donated organ, remove all of its cells, and keep the protein scaffold left behind. Add the patient's own cells and grow it in culture.
- **Bioprinting.** 3D print the scaffold, then add the patient's cells and grow it in culture.

Both end with an organ built from the patient's own cells, which the patient's immune system should read as self. Both need **stem cells**, which are cells that have not yet been assigned a job. A skin cell is a skin cell for good. A stem cell, found in bone marrow, teeth, brain and most tissues, can be pushed with hormones and chemicals into becoming a particular cell type, and it is young enough to grow over a scaffold.

Both routes have a problem. Donor organs are rare, which is the problem you started with, and bioprinting is expensive. That is why the Regenerative Medicine Lab wants to know whether plants will work. Plants are plentiful, cheap, and easy to grow.

The steps

Read all of them before you begin. Steps 1 to 9 are session one. Steps 10 and 11 are session two.

1. **Put on your safety glasses, gloves and lab coat.** All three. Before anything is opened.

   Done when: all three are on and your glasses are over your eyes.

2. **Get or cut two slices of apple, each about 1 mm thick and about 35 mm across.** They need to be as close to identical as you can make them, because you are about to compare them.

   Done when: two slices exist, you have measured both with the ruler, and you have written both measurements in your notebook.

3. **Set up your opacity test so that it is repeatable.** This is the measurement the whole experiment rests on and it is the step most people rush. Take a page of ordinary printed text and hold it upright, the way you would hold it to read. Hold one slice in front of the text and try to read through it. Repeat with the second slice.

   Before you record anything, fix these so you can do it identically tomorrow: which page and which paragraph, how far the slice sits from the paper, how far your eye sits from the slice, which light you are under, and which person is looking. Write all six down.

   Done when: your six conditions are written in your notebook and a photograph of each slice in front of the text exists.

4. **Record the result of the first opacity test for both slices.** Describe what you can and cannot make out. *How well can you see through the apple?* Write what you actually observe, not what you expect to observe later.

   Done when: both slices have a written description and a photograph, taken under your six fixed conditions.

5. **Choose a body part and carve both slices into that shape.** An ear, a nose, a patch of skin, a heart, anything you like. Both slices get carved into approximately the same shape, because they have to stay comparable. Cut on the board, away from you, one person at a time.

   Done when: two slices of the same shape and roughly the same size exist, and the scalpel is put down.

6. **Place one slice in a beaker and pour in just enough 0.5 percent SDS to cover it.** Pour slowly down the inside wall so you do not make foam. Several groups may be directed to share one beaker; if so, use enough SDS to cover every slice in it. This slice is your **experimental** slice.

   Done when: the experimental slice is covered, a lid or cover is on the container, and no foam was made.

   SDS is essentially a strong soap. It destroys the apple cells and lifts them off the scaffold underneath. That is the entire mechanism of this experiment.

7. **Design the treatment for your second slice. This is your negative control.** Write down what you are going to do to it and, in one sentence, why that treatment makes it a control. This is the graded thinking in this activity and it is not printed anywhere. Two questions to reason from: what exactly is the one thing you are testing, and what treatment would let you tell whether that one thing caused any change you see tomorrow?

   Done when: a written treatment and a written one sentence justification exist in your notebook, before you set anything up.

8. **STOP. Get your control approved by Mr. Mendoza before you set it up.** Show him what you wrote in step 7. Do not skip this and do not set the control up first and ask afterwards. A control that is not a control makes the entire 24 hour wait worthless, and there is no way to find that out until it is too late to redo it.

   Done when: Mr. Mendoza has read your written control and said yes.

9. **Set up your approved control, then label and store both containers.** Name, period, date, set-up time, and contents on each one. Leave both for 24 hours, on a shaker or rocker if one is available. Remove your protective equipment in the right order, wipe the bench, and wash your hands.

   Done when: two labelled containers are where Mr. Mendoza put them and your hands are washed.

10. **Session two. Protective equipment back on, then repeat the opacity test on both slices** using the six conditions you fixed in step 3. Same page, same paragraph, same distances, same light, same person. Photograph both.

    Done when: both slices have a second written description and a second photograph under identical conditions, and the actual elapsed time is written down.

11. **Record general observations on both slices and answer the written questions** in the next section. Then dispose of everything: liquid waste into the container Mr. Mendoza set out for it, never down a drain without asking him first; both apple slices into the solid chemical waste container, not the bin and not the sink. Wipe the bench and wash your hands.

    Done when: every question in the next section has a written answer and the bench is clear.

What finished looks like

Your submission is these seven things.

1. The opacity record: both slices, before and after, described in words, with the four photographs and your six fixed conditions written out.
2. Your written control treatment and the one sentence justification, exactly as you wrote it in step 7 before approval.
3. General observations on both slices after 24 hours: colour, texture, structural integrity, and anything else you can see or feel.
4. How effective was the decellularization, and **how do you know**. The second half is the graded half.
5. What factors could have affected how well decellularization worked.

6. When the cells were removed, what is the scaffold that was left behind made of? Research this and cite where you found it.
7. Your recommendation to the Regenerative Medicine Lab: should apples be used as a scaffold for replacement parts? Explain, from your own data.

AND THE QUESTION THAT IS REALLY BEING ASKED

Why was it important to create a negative control in this experiment, and if you had not had one, how might your conclusions have been different? Answer that specifically, about your control and your data, not in general terms about controls.

WORKED EXAMPLE OF THE FORMAT

This shows you the **shape** of a good write-up. The scenario is invented, the material is not apple, and the numbers are made up. It is deliberately an experiment that did not work, because that is the version most people need to see.

PRACTICE EXAMPLE PRACTICE-DECELL-1. INVENTED MATERIAL, INVENTED DATA, NOT THIS LAB

Opacity record. Conditions fixed: page 4 of my chemistry textbook, second paragraph, slice held 2 cm from the page, eye 25 cm from the slice, under the room's ceiling lights with the blind down, and I did all four readings myself. Before, experimental slice: could not make out any letters, only a change in brightness where the paragraph sat. Before, control slice: the same. After 26 hours, experimental slice: could make out the shapes of the largest words but not read them. After 26 hours, control slice: could also make out word shapes, slightly less clearly than the experimental one. Elapsed time was 26 hours, not 24, because session two was pushed by a fire drill.

Control and justification, as written before approval. We left the control slice sitting in the open air on the bench for 24 hours. We thought that would show us what happens to a slice that is not in detergent.

General observations. Experimental slice: paler, softer, floppy, tore when lifted with tweezers. Control slice: browned at the edges, noticeably shrunken, dry and stiff, curled at one corner.

How effective was it, and how do I know. I cannot tell, and the reason is the control. Both slices changed. My control changed through drying and browning in open air, which is a different process from the one I was testing, so I have no way to separate the change caused by the detergent from the change caused by simply sitting somewhere for a day. The control I chose changed more than one thing at once, which is the exact thing a control is supposed not to do.

What I would change. The control has to differ from the experimental slice in one way only, the thing I am testing, and match it in every other way: same container, same volume of liquid, same place, same temperature, same movement. Mine matched none of those. If I ran it again I would fix that first, and I would also weigh both slices before and after, because I only described texture and could not measure the difference I was describing.

Recommendation. I cannot make one from this run. Saying so is the accurate answer. Making up a recommendation my data does not support would be worse than having no data at all.

WHAT THAT EXAMPLE DOES THAT YOU SHOULD COPY

- The six conditions are written out, so a reader can tell the two readings are comparable.
- The real elapsed time is reported, not the planned one.
- The control is quoted exactly as written before approval, including the reasoning that turned out to be wrong.
- Observations are separated from interpretation. The colour and texture notes say what was seen. The judgement comes afterwards, in its own paragraph.
- The conclusion is *I cannot tell, and here is precisely why*. That earns full credit. It is a correct reading of the evidence.
- The last paragraph names a second weakness the writer was not asked about.

Where people get stuck

Waiting to be told what the control is.

It is not in this handout, it is not on the portal, and it is not in the PLTW student material. Designing it is the graded part of the activity. Answer the two questions in step 7 and write something down.

Doing the first opacity test casually.

If tomorrow's test is done at a different distance, in different light, or by a different person, the comparison is worthless and there is no way to redo yesterday. Fix the six conditions and write them down before you look through anything.

Cutting the two slices to different thicknesses.

A 1 mm slice and a 2 mm slice have different opacity before you do anything to either of them, so any difference tomorrow could be thickness rather than treatment. Measure both. Write both down.

Setting up the control before getting it approved.

The approval gate exists because a bad control cannot be detected until 24 hours later, when there is no time to start again. Two minutes of somebody reading your sentence saves the whole experiment.

Making the control differ in more than one way.

A control that sits somewhere else, in a different container, with different liquid, at a different temperature is not a control. It is a second experiment. Change exactly one thing.

Making foam when pouring the detergent.

Foam carries SDS into the air and into your face. Pour slowly down the inside wall of the beaker.

Touching a phone with gloves on.

You will be taking photographs. Gloves off, then photograph, then gloves on. Detergent on a phone screen goes straight to a face.

Writing the observations from memory in the corridor afterwards.

Texture and colour detail are gone within ten minutes. Write at the bench, with the slice in front of you.

Recording 24 hours when it was actually 26 or 21.

Write the real elapsed time. It is a variable and a reader needs it.

Concluding what you were expecting instead of what you saw.

If your data does not support a conclusion, say so and say why. An honest *I cannot tell from this* with reasoning attached is worth full points. A confident conclusion the data does not carry is worth none.

How to submit and how it is credited

THIS ITEM IS WORTH 8 POINTS

Two work sessions, four photographs, a designed control, and seven written pieces. It is the largest hands-on item in the pack and the one that feeds the WebXam hardest. It is paid above the two hour items because booking a supervised slot and committing to a 24 hour incubation cost you more than the bench time does. Remember the 20 point per semester cap across all extra credit.

- **Where it goes.** The same place as everything else. Your class form on the portal, or handed to Mr. Mendoza in person. Nothing about extra credit needs a different route.
- **What to hand in.** Photographs of your work where the handout asks for them, plus your written pages. Photographs of handwritten pages are fine. A photograph you cannot read is not.
- **Put your name and period on every page and every photograph filename.** Unlabelled work cannot be credited and it is the single most common way points get lost.
- **When.** Any day after the pack opens, right up to the last week of the semester. There is no deadline inside that window and no penalty for going slowly.
- **Feedback.** You get it back with a note. If a piece is short of full credit you may fix the named thing and resubmit once.

Words this activity uses

Regenerative medicine

Building or regrowing replacement tissues and organs rather than waiting for a donor.

Decellularization

The process of removing cells from a tissue, leaving the structural scaffold behind.

Scaffold

A frame in the shape of a body part that cells are applied to and grow on. It can come from a donated organ, a 3D printer, or, possibly, a plant.

Ghost organ

A donated organ with all its cells removed, leaving only the protein scaffold. Patient cells are then added and grown in culture.

Bioprinting

3D printing an organ scaffold, then adding patient cells and growing them in culture.

Stem cell

A cell that has not been assigned a job yet and can be stimulated with hormones and chemicals to become a specific cell type. Found in bone marrow, teeth, brain and most tissues.

Opacity

How much a material blocks light. The opposite of translucency. In this lab you are using it as a rough stand-in for how many cells are still present.

Antigen

A surface marker on a cell that the immune system reads. Your own are read as self and left alone. Others are read as non-self and attacked.

HLA typing

Human leukocyte antigen typing. Tissue typing. Six HLA antigens are compared when matching a donor to a recipient.

OPTN

The Organ Procurement and Transplantation Network, which runs the national United States database of transplant candidates and matches donated organs to them.

Xenotransplantation

Transplanting non-human cells, tissues or organs into a human.

Sodium dodecyl sulfate

SDS. A strong detergent that destroys cell membranes. Used here at 0.5 percent to strip cells off a scaffold. A skin and eye irritant.

Negative control

A sample set up so that the thing you are testing is absent, and everything else is the same. It tells you what would have happened anyway, so that you can tell whether your treatment caused the change you observed.

Variable

Anything in an experiment that can change. The one you change on purpose, the one you measure, and the ones you hold still.