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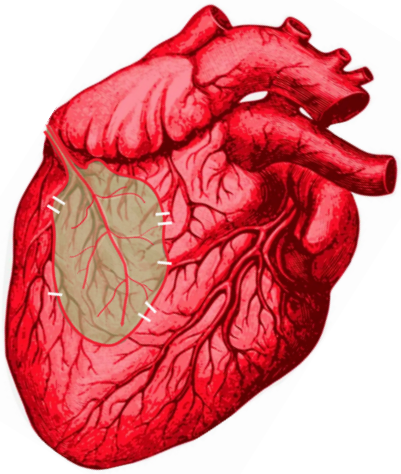
Spinach Hearts and Apple Ears: From Plant Tissue to Human Tissue



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Part I – Introduction to Decellularized Plant Tissue Scaffolds

Congratulations! You have been hired as a researcher at the Demetrian Research Institute, an organization with labs that contribute to both biological engineering and medical research. On your first day, you overhear some colleagues talking about researchers at the Worcester Polytechnic Institute using spinach to make human heart tissue. While you cannot catch all of what they are saying, you also hear something about human ears made from apples and tendons made out of green onions. What fascinating science! Turning fruits and vegetables into human tissue sounds like the kind of study you would want to lead. As any researcher at the Demetrian Institute can create a proposal for a research project, you decide to look into this more. You approach the group and ask what they are discussing. Quickly, one of them steps forward and excitedly shows you a video on their phone titled *Scientists Are Turning Spinach Leaves into Heart Tissue* (running time: 1:08 min, produced by Insider Tech, 2017). They explain that they are discussing decellularized plant tissue scaffolds. Watch the video here: <https://youtu.be/oDSUqoGFHz8>.

After watching the video, your new colleagues suggest you read the following short review article:

- Zhu, Y., Q. Zhang, S. Wang, J. Zhang, S. Fan, & X. Lin. (2021). Current advances in the development of decellularized plant extracellular matrix. *Frontiers in Bioengineering and Biotechnology* 9: 712262. <https://doi.org/10.3389/fbioe.2021.712262>

You realize that all the best science is done with the help of others, so after you finish reading the article you decide to meet with a group of your fellow scientists to discuss the questions below.

Questions

1. What is the main purpose of this review article? What are three (or more) main conclusions of the authors?

2. In terms of decellularized plant tissue scaffolds, explain the following terms in your own words: *decellularization, tissue,* and *extracellular matrix*.

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3. According to Zhu et al. (2021), what is a decellularized plant tissue scaffold? How can this technology be used? (You can draw a diagram showing the process if preferred.)

4. What are three limitations and three advantages of decellularized plant tissue scaffolds?

5. Referring to the specific limitations and advantages you outlined, how do you personally think decellularized plant tissue scaffolds should be used? Should they be used for medicine, tissue research, or something else?

6. Was there anything that surprised you about this review article?

7. Are there any questions you have about decellularized plant tissue scaffolds that remain unanswered?

Part II – Decellularizing a Plant Tissue

To further bolster your understanding of how decellularized plant tissue scaffolds work, you need to understand the structures that hold plant tissues together. Analyze the figures and answer the questions below about how plant tissue is constructed and can be decellularized. After you complete this section, you should have a strong understanding of plant cell wall structures and how to make decellularized plant tissue scaffolds.

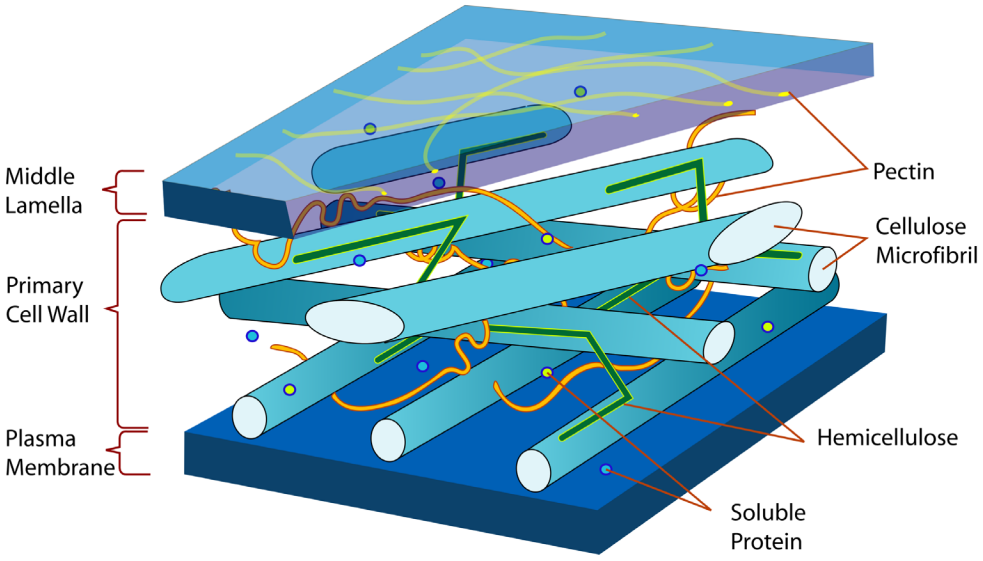

Figure 1. Basic plant cell wall structure with main sections and carbohydrate structures (pectin, cellulose, and hemicellulose) labeled. Pectin is important for cell adhesion and wall hydration in addition to determining wall porosity and structure (Houstin et al., 2016). Hemicellulose provides strength to the cell wall due to its physical connections to cellulose and in some cases lignin (Houstin et al., 2016). Cellulose is the primary source of tension resistance in the primary cell wall through its structure, which is stabilized by hydrogen bonds (Houstin et al., 2016). Lignin (not pictured) is an organic polymer that provides a barrier from pathogens and pests in addition to contributing to cell wall rigidity (Liu et al., 2018). *Image credit:* LadyofHats | Wikimedia Commons, PD.

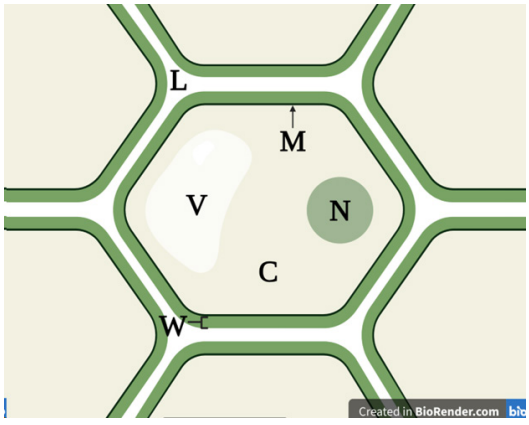

Figure 2. Depiction of a plant tissue with a single plant cell surrounded by other cells that are not shown in full. Middle lamella (L) is shown as the white between cells. Primary cell wall (W) shown in green. Plasma membrane (M) shown as a thin black line. Also shown are a vacuole (V), nucleus (N), and cytoplasm (C).

Questions

1. For each of the structures listed below, use Figure 1 and Figure 2 to describe the potential impacts on plant tissue integrity that would result from the loss of each structure. Please explain your reasoning. Read both the figure captions and assess the information within the figures to inform your conclusions.

   Cellulose:

   Hemicellulose:

   Pectin:

   Lignin:

   Middle lamella:

   Primary cell wall:

2. The caption of Figure 1 categorizes pectin, hemicellulose, and cellulose as carbohydrates (one of the four categories of biological macromolecules). What are the other three main biological macromolecule categories that compose plant cells? Where are these macromolecules normally found in plant cells?

Macromolecule	*Cellular Location(s)*
1) Carbohydrates	Plant cell walls
2)	
3)	
4)	

3. Are the macromolecules in plant cell walls and plant cells different or the same? After reaching a conclusion about their molecular similarities, explain how this might influence techniques of decellularization.

4. Looking at the "Chemical" decellularization row of Table 1 on the following page, why might certain plant tissues take longer to decellularize than others? Which plant species listed in column four ("Plants") would you expect to take longer? Which would you expect to take shorter?

Table 1. Methodologies for plant tissue decellularization. Information for this table was sourced from Harris et al., 2021.

Treatment	*Compounds*	*Time*	*Plants*	*Advantages*	*Limitations*
Chemical	SDS (0.1–10%); Iriton A-1UU; bleach (10%); hexane pre-treatment when wax cuticle present	12 hrs to 3 wks, depending on plant material	Celery, apple, bamboo, basil, leek, tomato, spinach, potato	- Gold standard - Well characterized - Works for many plant tissues and structures	- Harsh chemicals are environmentally toxic - Time consuming as toxic residue requires intense washing
Detergent-free	Heated bleach and $NaHCO_3$ solutions or bleach with surfactant	Few min to hrs, depending on plant material	Bamboo stem, *Ficus hispida,* garcinia	- Oxidation may enhance cellulose breakdown	- Strong chemicals - Scaffold can degrade when heated
Freeze/enzyme	Lyophilization DNAse I	24 hrs	Transgenic plant cultured cell lines; e.g., hairy root, tobacco bright yellow (BY-2), monocot rice cells (*Oryza sativa* L.)	- Retains native proteins	- Additional clearing with surfactant may be needed to remove debris
Supercritical fluid ($scCO_2$)	$scCO_2$ (2500 psi at 33 °C); PAA as cosolvent (2%); bleach if scaffold clearing required; hexane pre-treatment for wax cuticle	3 hrs (+ 6 hrs if clearing required)	Celery, parsley stem, spinach leaf, sweet mint	- Fast - Soft approach with minimal amount of chemicals - Sterilization step included	- Needs to be used on more plants still - Specialized equipment required

5. In the "Freeze / enzyme" decellularization row of Table 1, why might it be important to retain native cell wall proteins while constructing a plant tissue scaffold?

Part III – Assigning Different Decellularized Plant Tissue Scaffolds as Human Tissue Analogs

Now that you have an understanding of how plant decellularization works, you can move on to possible applications of decellularized plant tissue scaffolds. You decide to construct a decellularized plant tissue and use it to either treat or research a human tissue disorder, but first you need to figure out what plants are good candidates for different human tissue types. Luckily, your co-researchers have compiled a list of different plants and their properties once they are decellularized. Your job now is to determine which plants would be the best scaffolds for which tissue.

While many factors contribute to the physical properties of decellularized plant tissue scaffolds, one of the most important is the Young's modulus (YM). The YM is a measurement of a material's linear elastic limit in pascals (kPa), and your fellow researchers have gathered the YMs for various plants and human tissues (shown in Figures 3 and 4).

Question

1. Compare the YMs in Figures 3 and 4 and assign each plant species as an analog to at least one type of human tissue. When selecting a plant tissue analog for a human tissue, both the YM and the structure of the plant tissue should be considered. For each plant analog you designate, give a brief explanation of why you believe it is a good candidate for the chosen tissue. (Keep in mind that some plants will apply to more than one tissue type.)

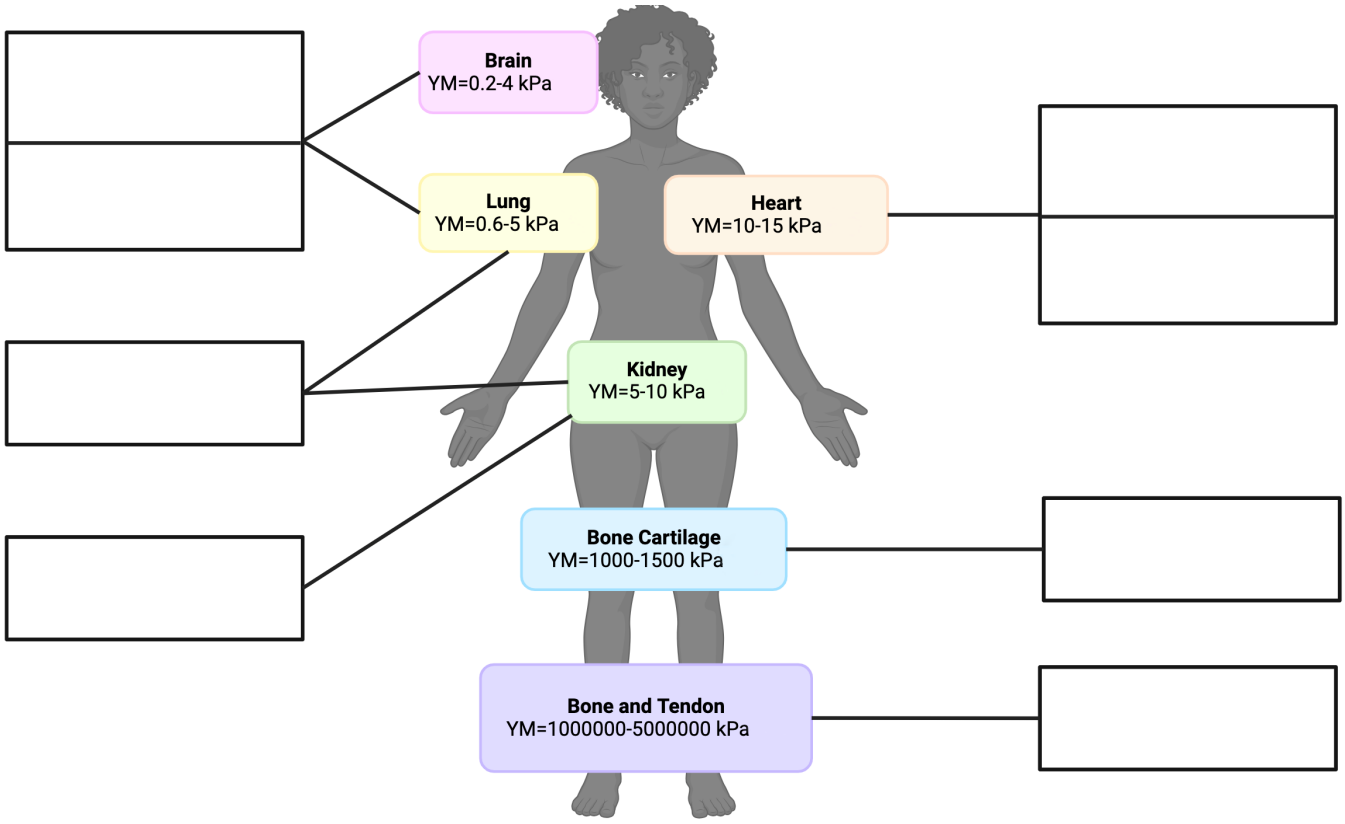

Figure 3. Different human organs and their Young's modulus (YM) when decellularized. The YM describes the linear elastic limit of a material measured in pascals (Pa) (Harris et al., 2021).

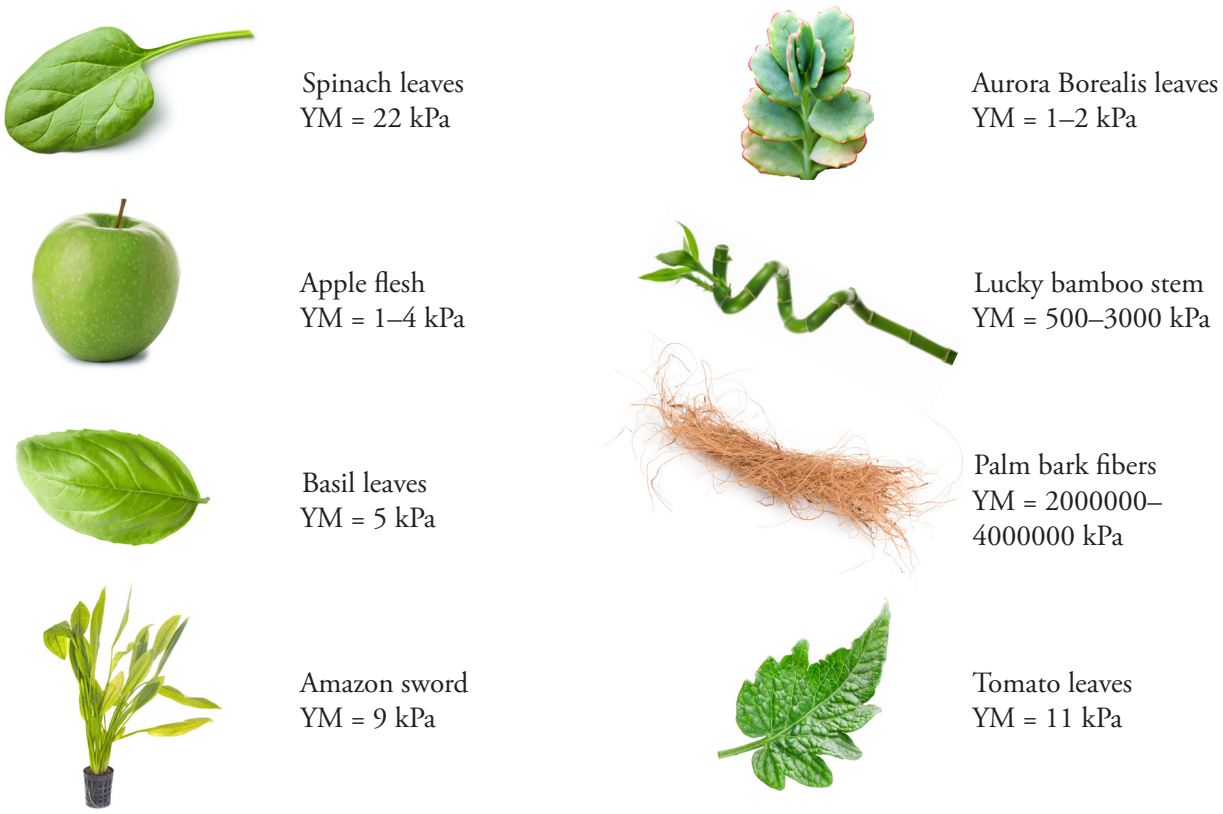

Figure 4. Images of various plants and their Young' modulus (YM) when decellularized. The YM describes the linear elastic limit of a material measured in pascals (Pa) (Harris et al., 2021).

Part IV – Constructing Your Research Proposal

Now that you have a thorough understanding of decellularized plant tissue scaffolds, you can construct a methodology for your research proposal. Using the questions below you will construct a preliminary methodology and research purpose statement to present to your supervisor at the Demetrian Institute. You are well on your way to establishing yourself as an invaluable member of the scientific community!

Questions

1. Choose a condition (this can range anywhere from a developmental disorder to an injury or disease) that impacts one of the human tissues labeled in the diagram you filled out for Part III, and state it below.

2. Returning to the original article that you read for this case study, and applying the knowledge that you now have, how would you use this scaffolding technology to help treat the disorder that you stated above? This will function as your research purpose statement.

3. Construct a methodology to decellularize a plant tissue of your choosing, and then describe how you would use this decellularized plant tissue scaffold in medicine or scientific research. Feel free to use any resources you would like that are academic in nature (go beyond Wikipedia). For this section, please provide at least one primary scientific literature article as a basis for the construction of your decellularization technique.

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