Designing, Manufacturing, and Validating Cell Culture Inserts with Biodegradable Electrospun Nanofiber Membranes
(Technical Report)

Tissue-Engineered Organs and Its Relevance to Society
(STS Research Paper)

A Thesis Prospectus
In STS 4500-017
Presented to
The Faculty of the
School of Engineering and Applied Science
University of Virginia
In Partial Fulfillment of the Requirements for the Degree
Bachelor of Science in Biomedical Engineering

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November 1, 2021

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On my honor as a University student, I have neither given nor received unauthorized aid on this assignment as defined by the Honor Guidelines for Thesis-Related Assignments.

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Introduction

The study of cell biology investigates basis of nearly all aspects of life. Through the advancement of this field, along with increasing technological developments, researchers and engineers have been able to develop and produce solutions that better the quality of life for individuals with varying health complications. Tissue engineering emerged from this intersection of medicine, biology, and engineering - with the primary intention of achieving the manufacturing of a wide variety of functional organs that restore, improve, or replace damaged tissues to combat patient organ failure (National Institute of Biomedical Imaging and Bioengineering, 2019). There has been a major worldwide crisis in the shortage of organ supply, observed in the increasing number of waiting patients on the transplant list and also patients dying whilst on the waitlist (Abouna, 2008). Lab-manufactured organs and tissues could be the solution to this crisis, but foundational improvements to biological research techniques need to be made first.

Cell culturing, a laboratory technique in which cells cultivated from a living organism are grown under controlled conditions outside the organism, has become the foundational method for most experimental research and has historically been used in a wide variety of applications such as physiological studies, toxin or drug screening, or other cell-based assays (Moo-Young, 2011). The most important aspect of culturing cells that are intended to be used for treatment within the body is to maintain an environment most similar to the in vivo state. However, current culturing methods today are not always fit for animal cells to survive when isolated as they require a complex environment to maintain their existence once independent from their organism (Arango, Quintero-Ronderos, Castiblanco, & Montoya-Ortíz, 2013). Due to this limitation, the Department of Health and Human services put out a solicitation for the development of

alternative cell culture insert membranes to provide the proper conditions for more complex, 3-dimension cellular growth and tissue models, in addition to controlled biodegradability so that cells may produce their own supportive structure (America's Seed Fund, 2019). Alongside Luna Innovations, who worked on the first phase of this grant, and the Christ Lab at the University of Virginia, the objective of this project is to develop a cell well insert that includes a biodegradable membrane and can be used to increase the viability of cell culture growth through providing a more biomimetic environment. My thesis on this topic will be loosely related to my technical project, which is to design and manufacture cell culture well inserts with biodegradable membranes that promote better growth for cells and their corresponding extracellular matrix, so they can form into tissue scaffolds. Instead, it will analyze the societal and technological impact of developing tissue-engineered organs for the purpose of organ transplantation.

Technical Dimensions

The basis of many biomedical engineering research topics, which include tissue growth, genetics, and disease development, stems from a better understanding of the cellular mechanisms occurring within the body. Cell culture experiments have allowed for researchers to investigate these concepts through cellular reproduction, mimicking the proliferation of these biological building blocks and their corresponding systems. One fundamental aspect of successful cell culturing is to mimic *in vivo* conditions that provide cells with the most sufficient physiological environment for healthy growth and survival, promoting behavior that closely imitates natural biological processes. Current laboratory methods begin with sourcing a cell line and selecting a format for cell growth, either using plated (adherent) forms or suspension (Segeritz & Vallier, 2017). Although suspension-grown cells have their benefits for some fields of bioengineering, plated techniques promote cell proliferation on plates or in flasks that are coated with an

adhesion-supporting artificial substrate, allowing for the formation of monolayers. This method is beneficial as cell adhesion is essential in cell communication and regulation, and it is of fundamental importance in the development and maintenance of issues (Ahmad Khalili & Ahmad, 2015). This adhesion and the ability for intercellular communication creates an environment for organic cell conditions, thus making them the primary culture choice for most conducted research in a variety of industries. While they may be considered the most effective method on the market today, plated cultures still lack other essential components of *in vivo* cells, such as having 3-dimensional growth and extracellular matrixes (ECM), which allow for further complex interactions between cells and influence their behaviors. The ECM plays many roles in the growth of healthy cells and tissues due to its organized multidomain macromolecules that link together to form a stable network, contributing to physical and mechanical properties of tissues, and continuous supply of factors that support cell proliferation (Yue, 2014). To offset these limitations to some extent, cell culture inserts were developed and have become commercially available. These inserts allow for cell seeding to occur on the top and bottom of a porous membrane and for cells to be submerged within the growth media comprehensively. Using this innovation as a basis, our technical project focuses on the development of cell culture inserts with biodegradable membranes that support the growth of a self-withstanding extracellular matrix. By creating the conditions for multiple layers of cells to grow upon an anchoring layer of adhesive ECM with effective cell inhibition and communication, more doors are opened in the race to manufacture functional tissues.

The inserts we are creating are composed of two main parts: the snap-fit insert chamber and the nanofiber membranes. Both of these parts have prior designs that have been manufactured and tested on by Luna Innovations; our team will be optimizing upon the prior

research that they have done. The membrane will be created using sheets of electrospun nanofibers that will be fitted into the circular walls of the plastic cell culture inserts. The composition of this membrane is important as biomaterials should "replicate the biological and mechanical function of the native ECM found in tissues in the body…[providing] a three-dimensional space in which cell can attach, grow, and form new tissues with appropriate structure and function" (Olson, Atala, & Yoo, 2011, p. 1). These nanofibers will be composed of mixed polymer substrate chemistries that mimic that of *in vivo* conditions and have varying biodegradability rates. With these engrained properties, the cells should maintain viability while producing the necessary components of a sustaining ECM as the membrane structure degrades. The cell culture insert prototypes will be optimized by designing a better seal between the membrane and insert clip, as prior experiments suggested minimal leaking of media. To understand the effects of the varying membrane variables we may try in our efforts to select the most efficient design, we intend on running cell culture experiments on the inserts using mice myocytes. The goal of this project is to design and manufacture a cell culture insert with a degradable electrospun nanofiber membrane that is customized to promote myoblast infiltration, cell viability, and extracellular matrix deposition.

Artificial Organs, Ethical Debate, Funding, and National Policy

While efficient cell culturing is beneficial to a wide range of studies, the innovation of cell culture plating is just one step into creating better methods to grow the cells needed for tissue engineering. Tissue engineering is defined as an interdisciplinary field that utilizes aspects of both the life sciences and engineering towards the research and production of biological material that can be implemented within an organism to substitute or improve tissue or organ function (Langer & Vacanti, 1993). Along with scaffolds and growth factors, the selection of

cells that are used to populate the matrices are responsible for integrating functionality into developed tissues and organs and are an essential component to lab-grown organs (Ikada, 2006). The main objective of these artificial organs is to create an alternative source for organ transplantation surgeries, hence the heavy efforts in developing better cell culturing methods, scaffolding, and tissue manufacturing.

Despite advances in pharmacological and surgical therapies, organ failure is the leading cause of mortality all over the world (Wang, 2019). The list for patients in need of donation is over 113,000 people as of January 2019, with another person being added every 10 minutes and 20 people on the waiting list passing away as there is much difficulty in obtaining organs from the current list of donors (LifeCenter, 2019). This societal need has driven the focus behind tissue engineering and the demand for a new method of obtaining organs through their bioartificial creation. To explain the extensiveness at which the development of this technological solution to the organ crisis and diverse communities within society are interdependent and collaborative, I will use the theory of co-production.

Sheila Jasanoff (2004) defines the theory of co-production within society and technology as one in which the two are interlinked to influence the dynamics and growth of one another. It's the framework that provides the mindset to think about relationships, communities, institutions, and politics drive the development of technological knowledge, while technology embeds itself within to change the conditions of people. She states, "work in the co-productionist idiom stresses the constant intertwining of the cognitive, the material, the social and the normative". These bridges are held by what she labels as four recurrent themes: "the emergence and stabilization of new technoscientific objects and framing…the resolution of scientific and technical controversies; the processes by which technoscience are made intelligible and portable

across boundaries; and the adjustment of science's cultural practices in response to contexts in which science is done" (Jasanoff, 2004 p. 6, 38). Jasanoff outlines the four most common instruments of co-production as identities, institutions, discourses, and representations. Identities account for the resources from which individuals grasp sanity and reason during perceived mayhem. The identity can be human/non-human or individual/collective, but its redefinition of roles provides people with stabilization. Bruno Latour, summarized by Jasanoff, best defined institutions "as society's inscription devices…vehicles through which the validity of new knowledge can be accredited, the safety of new technological systems acknowledged, and accepted rules of behavior written into the as-yet-unordered domains that have become accessible through knowledge-making," along with serving "as sites for the testing and reaffirmation of political culture" (Jasanoff, 2004, p. 40). Discourses solves problems through selective language to manipulate audiences. Representations has many attributes but for the purpose of expressing co-productionism, representation is informed by human agency and behavior as means for politics and culture to influence scientific practices (Jasanoff, 2004). These instruments will be used in direct relation to this topic to display the co-production that is occurring.

One of the greatest challenges currently observed in this field is that the most preferred cell lines, human embryonic and cord-derived mesenchymal stems cells, is constrained by legality and ethical oppositions (Moreno-Borchart, 2004). These two lines are considered the most promising for success since embryonic stem cells can be differentiated to match tissue types of each patient and can be sustainable for long-term production, and umbilical cord blood also contains stem cells of similar ability, making both resourceful to an expansive population patient (Howard, Buttery, Shakesheff, & Roberts, 2008, p. 67-68). While scientific and

educational institutions maintain support for the use of cells, opposition has been enforced by the religious and moral identities of some individuals. Using discourse, religious leaders have used their sacred literature to sway their communities against supporting this new technology. Elsewhere, researchers have used specific language to promote government and private funding of stem cell research. Lab-made organs will also create its own shifts in society as regulation of whom has access to the technology will have to be decided, and there will have to be discourse on how its implemented alongside the current waiting list and within healthcare institutions. There are also other questions that cannot only be scientifically answered and have to keep normative principles in mind, such as discourses regarding the "the commercialization of human biological material…firms who want to make a profit – and there are issues of donation and control of human biological material" (Welin, 2008, p. 686). These normative principles are established as a representation of public opinion (determined by individual identities) and driven by legislative propaganda to be institutionalized within U.S. policy, thus making it an example of all four of Jasanoff's essential instruments at play in sociotechnical co-production.

Research Question and Methods

Following the framework of co-production, my research will explore the following question: how does society drive the research and development process of tissue-engineered organs and how may these organs shift the dynamics of organ transplant procedures? Understanding how engrained societal influence is on the progression of the technology will allow for targeted campaigning to gain the public and government support needed to ensure developmental and integrative success. Meanwhile, full cognizance of how the technology may influence industries and future patients allows for better preparation in advance of incorporating

these artificial organs into common healthcare to guarantee that discrimination does not occur and normative ethical standards are maintained.

When analyzing this relationship, I will refer to both legislative policy and past scientific studies. I will utilize readings and reports from government sources, such as presidential executive orders, speeches, and funding. Government policy drives a large portion of research funding for biomedical technology and is essential to a better understanding of the co-production relationship between lab-grown tissues and the communities it affects. One specific policy I will analyze is Former President George W. Bush's executive orders in which he "prohibited NIH funding for the derivation or use of additional embryonic stem cells" due to his pro-life stance but allowed for NIH funding of "stem cell research using embryonic stem cell lines already in existence" as a response to the promise held for treating degenerative diseases (Lo & Parham, 2009). I will also be analyzing findings from the primary biomedical and health research agency for the United States, the National Institutes of Health. The agency has conducted many studies to support their continuous need for utilization of stem cells due to their ability to be reprogrammed back into a pluripotent state, then used to later generate specified cultures of differentiated cells, and the accessibility they maintain due to high yield, long storage, and durability (National Institutes of Health, 2021). Lastly, I may refer to any other academic sources I come across that speak towards the current applications of tissue engineering or artificial organs and the public perception after their implementations.

Conclusion

The technical portion of this thesis describes the work my team is doing to develop a plating method more sufficient in maintaining cell viability and ECM structural components that are not as manageable with current culturing techniques. This innovation is just one way that

more efforts are being driven towards to final goal of producing artificial tissues and organs for future healthcare treatments, which is the technology discussed in the STS portion. Using the STS framework of co-production to analyze varying interviews and literature, I will determine the identities, institutions, discourses, and representations that play a role in the development of artificial organs and its present and future implementation within society. These identifications will answer the research question: how does society drive the research and development process of tissue-engineered organs and how may these organs shift the dynamics of organ transplant procedures? What is learned through analysis will be beneficial in determining the best way to go about initial artificial organ studies and experiments so that it will help the most people, and it will also aid in future pushes for policy and protocols within private, healthcare, and government agencies.

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