DEVICE FOR AUTOMATED SELECTION AND PLACEMENT OF CELL CLUSTERS WITHIN BIOFABRICATED TISSUE CONSTRUCTS

IMPACT OF DECEASED DONOR PUBLIC POLICY ON ORGAN TRANSPLANT OPPORTUNITIES

A Thesis Prospectus
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Bachelor of Science in Biomedical Engineering

By
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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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Organ transplants can be a critical component in an individual's recovery from chronic disease; however, treatment remains unavailable even in the developed world. According to statistics published by the Health Resources and Services Administration, over 100,000 patients reside on the transplant waiting list, but only 39,000 procedures were performed in 2020. Additionally, it is estimated that on average, 17 individuals die every day while waiting for a life-saving donation (*Organ Donation Statistics*, 2021).

The complications that give rise to this issue appear on multiple fronts, both sociological and biological. The chief limiting factor to organ donation is supply. Transplants in many cases cannot be obtained from live individuals, and even in the exceptional cases, such as kidney transplants, willing living donors are scarce and further complicate the procedure. As for the biological facet, a degree of transplant compatibility must exist between the donor and recipient's bodies. This further limits the availability of transplants for patients. Even in successful cases, patients typically find themselves requiring immunosuppressant drugs for the remainder of their lives. Immunocompromise results in previously ill patients becoming further susceptible to disease.

Bioprinting seeks to combat the enumerated issues plaguing organ transplantation by manufacturing *de novo* tissues and organs using a patient's own cells. However, the field is still emerging and requires a laundry list of technological innovations in order to address the underlying issues of organ scarcity. The technical project seeks to create an automated micromanipulator that can aspirate and place cell clusters within bioprinted constructs. This seeks to further the field of bioprinting by creating new research tools and methods to increase throughput and repeatability. The end goal of this work is to elevate the bioprinting field closer to the level of widespread, reproducible usage in *de novo* organ creation. The intended timeline of this project is to have assembled a physical prototype device by December 2021 and to incorporate coding and verification by May of 2022.

The STS research topic intends to analyze the underlying reasons behind why deceased donor programs have not been implemented and if it would be possible to design a system that would be more readily adopted worldwide. Kidney transplants comprise the majority of transplant surgeries worldwide, thus it makes an apt model organ to analyze. This STS topic seeks to analyze the organ availability given varying levels of robustness in deceased donor programs worldwide. This will be accomplished by analyzing per capita data on chronic kidney failure and transplant rates. Countries with lower transplant frequencies and more restrictive deceased donor programs will then be studied further for the underlying sociological reasons. Then, this data will be incorporated into potential modifications to current model deceased donor programs to create a more holistic approach to implementing these systems in hesitant societies worldwide. The two topics are tightly coupled, as innovations to bioprinting and organ availability will show positive impacts on donor scarcity worldwide. However, if bioprinting technology faces the same barriers to implementation as deceased donor programs, benefits will only be localized to already successful countries. Thus, in order to successfully implement future technology, it is necessary to understand the shortcomings of modern systems and design improvements that place public policy and social acceptance at its core.

AN AUTOMATIC MICROMANIPULATOR

A major limiting factor for the creation of *de novo* organs via bioprinting is the incorporation of cells into fabricated constructs. Cells are the primary living components of functional organs. Current bioprinting methods include inkjet, extrusion, and laser-assisted, but each method carries critical flaws that prevent them from filling the needs of organ fabrication. Both inkjet and extrusion techniques force cells through a nozzle in order to control their distribution. While this method is efficient, it places potentially damaging mechanical forces onto the cells. These stresses have the potential to reduce cell viability within the organ. Laser-assisted bioprinting by contrast use a system of droplets to fall onto the desired substrate. While the cell viability in laser-assisted methods is higher, it is too expensive to be used on a larger scale (Dey & Ozbolat, 2020; Mandrycky et al., 2016 pp. 2-4). Additionally, popular extant bioprinting methods use a homogenous ink. While this ink may be useful for creating a living framework for an organ, it prevents the fine-tuning of the construct (Gungor-Ozkerim et al., 2018 pp. 2-3). Multiple cell lines cannot be incorporated, which obstructs organ creation, as a single organ may be composed of numerous tissues including vasculature, structural, and functional cell lines. Other alternatives for bioprinting serve more as proof of concepts and are resource and manpower-intensive. A reconstructed pancreas has gained traction due to the possibility of recycling deceased tissues, but their production methods currently prevent widespread usage (Napierala et al., 2017, pp. 8-11). Bioprinting serves as one of the best methods forward in *de novo* organ manufacturing, as it is both high throughput and can be automated. However, the field requires a great deal of research advancements to fulfill these promises, which will only be possible by the creation of new tools and methods.

These shortcomings of current bioprinting methods prevent the elevation of the field to the next stage with the end goal of printing a complete and functional organ. The objective of this technical project is to develop a micromanipulator that is able to automatically aspirate and place cell clusters in predetermined positions. This micromanipulator will be capable of transferring cell clusters as small as 500 microns from a culture to a bioprinted construct. This project will also be compatible with artificial polymer-based bioprinting techniques as well, potentially relieving need for sourcing medical-grade organic material (Wang, 2019 pp. 1-4). The creation of the device will be accomplished by building upon previous work done by Xu et al., who created a low-cost, open-source micromanipulator able to work on the single-cell level. The key components and a description of the device is available in Figure 1. It consists of 3 linear actuators that control the device's position in space and a manually adjustable and operable aspirator capable of picking up individual cells. Though their device overperforms in its precision, it is largely manual, requiring human input in order to aspirate and place cells (Xu et al., 2021). Since the Xu micromanipulator meets or exceeds the baseline requirements of the proposed device, it acts as an ideal physical foundation upon which layers of automation and computer control can be built.

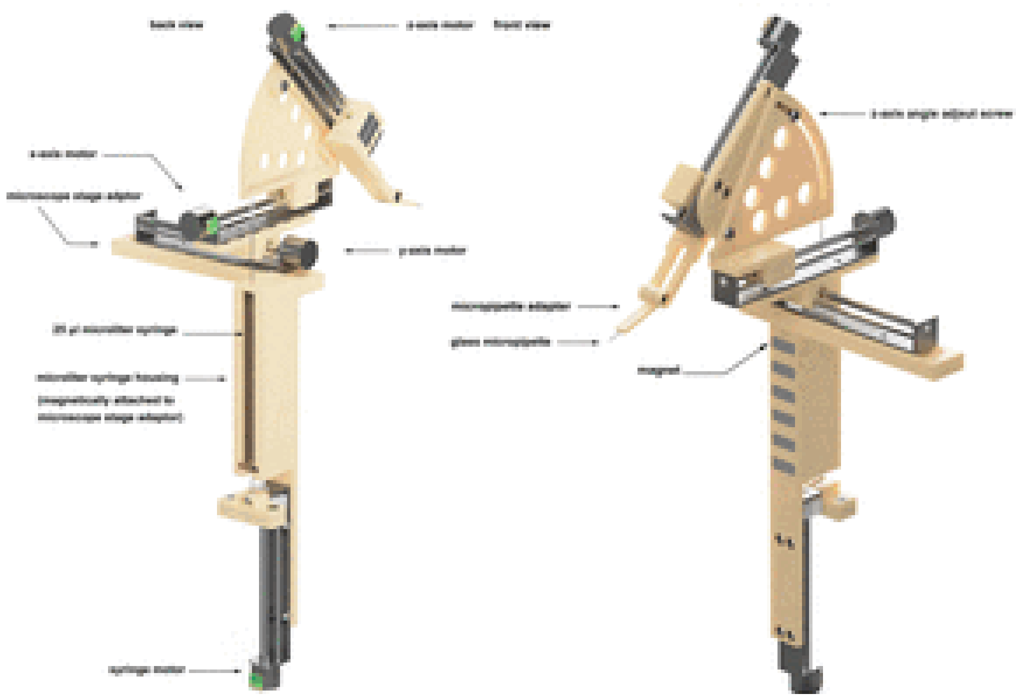

Figure 1: The Xu et al. Micromanipulator. The illustration shows front and back views of a manually operated aspirator whose position is controlled by three linear actuators. (Adapted by Luu (2022) from Xu et al. (2021)).

The technical project will innovate on Xu et al.'s design by incorporating automatic movement driven by motors attached to linear actuators capable of both positioning the device and aspirating model cell clusters. The success of the device will be evaluated based upon the accuracy to which it can aspirate cell clusters as well as the viability of the aspirated clusters, to be measured by the number of polymer microsphere stand-ins that are left undamaged. This project will be accomplished by a team consisting of biomedical engineering students Timothy Luu, Garrett McQuain, and Matthew Runyan under the advisory of Chris Highley from the Department of Biomedical Engineering. The resources available to accomplish this come from Highley Lab, which will have ownership of the device upon conclusion of the technical project. The preferred outcome of this project is the creation of a micromanipulator device that is capable of aspirating cell clusters with perfect accuracy without damaging them. The recreation of the Xu device would be in itself a large breakthrough in the project, and the incorporation of automating inputs given a simple location would be the ideal outcome. Finally, the results of this study will be presented in the form of a scholarly article.

BARRIERS TO IMPLEMENTING ORGAN DONOR PROGRAMS

Worldwide, there is an inequality in the availability of organ transplants. An individual nation's public policy can shape the prevalence of obtaining transplants from deceased individuals. Even within deceased donor programs, contention can arise based on the classification of death and whether transplants can be effectively used. United States standard organ donors include both braindead patients and those who have suffered cardiac death; however, the same systems are not in place worldwide (Girlanda, 2016; *Public Policy*, 2014 p. 452). In Figure 2, Dominguez-Gil et al. demonstrate that even within European countries, there are variations in the willingness to establish deceased donation programs after circulatory death. This distribution demonstrates that policymakers can control a great deal about which methods may be implemented. Nations with hesitant lawmakers will produce less robust deceased donor programs by restricting organ sourcing. A follow-up study on this data corroborates this expectation. In 2020, only 1 nation that had no plans to implement a cardiac death program, Lithuania, adopted some form of it in the 9 years since Dominguez-Gil et al.'s study, while 5 of the nations that intended to incorporate such a program did so (Lomero et al., 2020 p. 79). Lawmakers' willingness to adopt programs and create permissive public policy can shape the success of deceased donor programs, thus a holistic approach to designing organ donation systems would include key policy changes on top of recommending infrastructure and procedures.

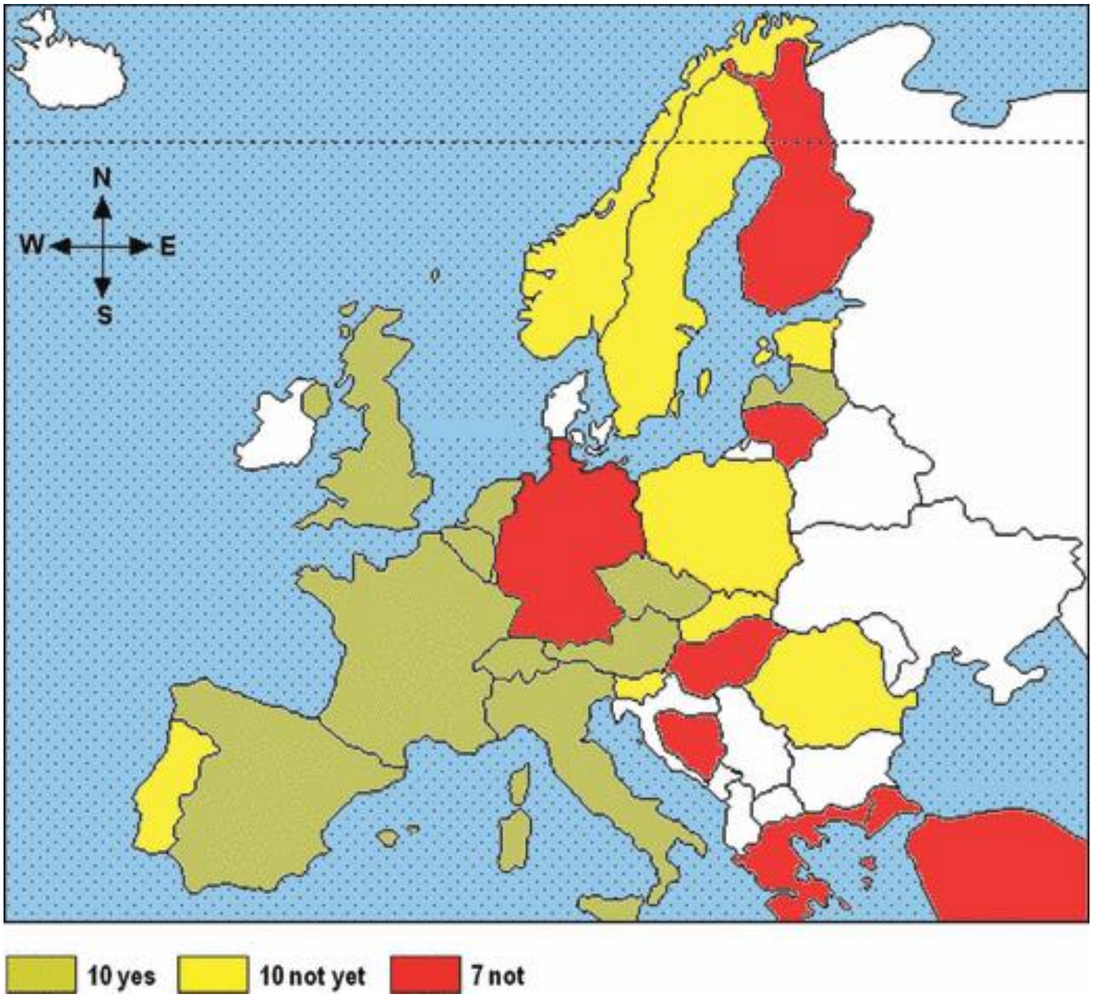

Figure 2: Willingness in European Countries to Adopt Donation After Cardiac Death Policies. Green indicates a program is present, yellow indicates intent to establish one, and red indicates that there is no plan to do so. (Adapted by Luu (2022) from Domínguez-Gil et al. (2011)).

Within developing nations, there is a distinct lack of deceased donor programs. As of 2019, Pakistan lacks any deceased donor programs and instead transplants could only be sourced from live, willing donors. Ahmad et al. (2021) writes, “The deceased donor program is the ultimate requirement of developing countries for providing enough supply of kidney for patients” (p. 1090 para. 5). However, support for deceased donor programs throughout the country are not so ardent. In a study on healthcare professionals in Pakistan, only 51 percent would be supportive of an established deceased donor program, many citing religious objections or distrust for the government (Siddiqui et al., 2012 para. 17). The data illustrates that obstructions to the establishment of deceased donor programs are gated not only by policymakers, but also the collective consciousness of society at large. Thus, in order to create a more widely accepted deceased donor program, there must be means to improve its perception among the general populous as well.

The STS topic seeks to look deeper into countries with lower rates of transplantation and more restrictive donor programs and understand the underlying reasons. Because kidneys are the most frequently transplanted organ, they will serve as a model organ for transplants on a whole. Thus, the STS topic will gather per capita chronic kidney failure death rates and kidney transplant rates on countries with deceased donor programs including circulatory and brain death, those to include only brain death, and those with no established deceased donor programs. Then, where available, the reasons behind this difference will be examined. This analysis will then be applied as a means to improve upon preexisting organ transplant system models. Methods such as the Spanish model are already exemplary within their field (Miranda et al., 1999). However, the method remains unimplemented worldwide due to differences in both public policy and social values. By incorporating mechanisms of legal and social change into preexisting models, they may be adapted to be better accepted worldwide.

This topic will examine the data in the framework of diffusion of technology. In nations that have yet to adopt a widespread deceased donor program, it can be observed that societal forces prevent it from reaching the general populous. According to Rogers (2003), the technology is being arrested at the early adopter phase when it has reached only 16% of the population. By reevaluating organ transplant methods through a sociological lens, the technology may be able to reach the early majority phase according to the theory of diffusion of technology.

From there, the benefits of the technology will reach approximately 50% of individuals within society, and public opinion will shift once the procedure has become commonplace and its full benefits are elucidated. The remaining 50% of the population is anticipated to change opinions once the method has reached critical mass alongside evidence of safety and efficacy. The study will analyze how the implementation of a robust deceased donor program is either accepted or curtailed by both public policy and cultural values.

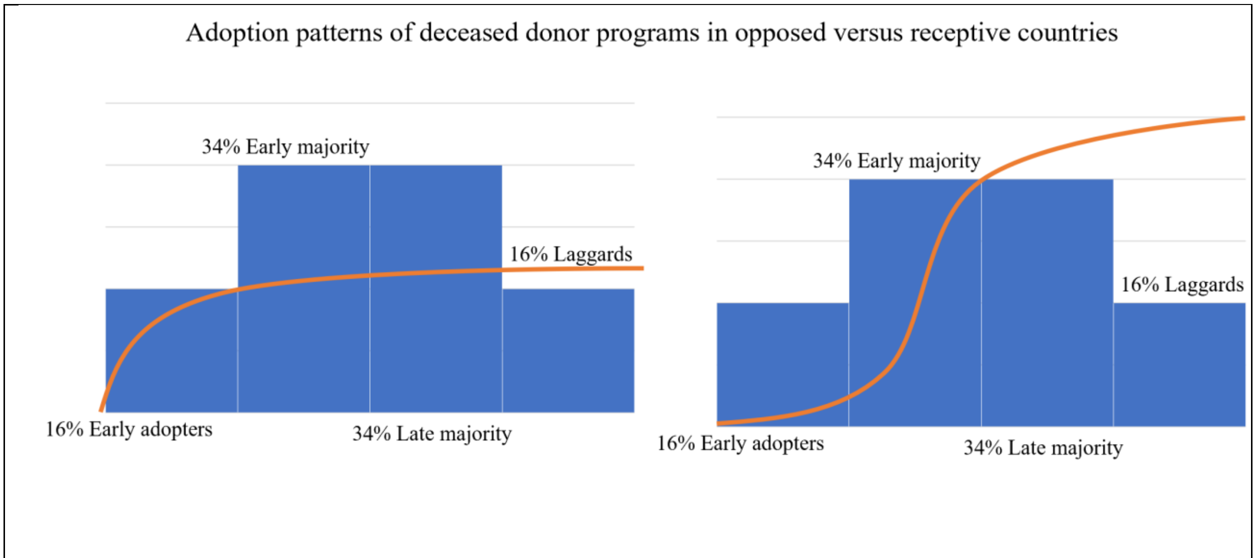

Figure 3: Adoption patterns of deceased donor programs in opposed versus receptive countries. The left graph demonstrates how due to arresting the diffusion of technology at early adopter phases, technology reaches a significantly lower proportion of the population. The figure on the graph on the right details a more receptive population with reduced barriers to deceased donor implementation. The technology is able to reach saturation unlike in opposed countries. (Adapted by Luu (2022) from Rogers (2003)).

Figure 4 proposes a means to determine how public policy acts as a contextual factor in shaping the system of organ transplantation. Engineers who create organ transplant systems are gated by both public policy and a society's values that determine their willingness to uptake it. Policymakers act as a concrete gatekeeper for their society and control whether the system can be attempted. By shaping designed deceased donor programs to societal values and influencing public policy, the system can reach the hands of healthcare professionals (C. Baritaud, B.

Carlson, personal communication, July 26 2009). Finally, this project will analyze trends in the boundaries established for deceased donor programs to determine if there is correlation among countries experiencing varying success with their transplant programs.

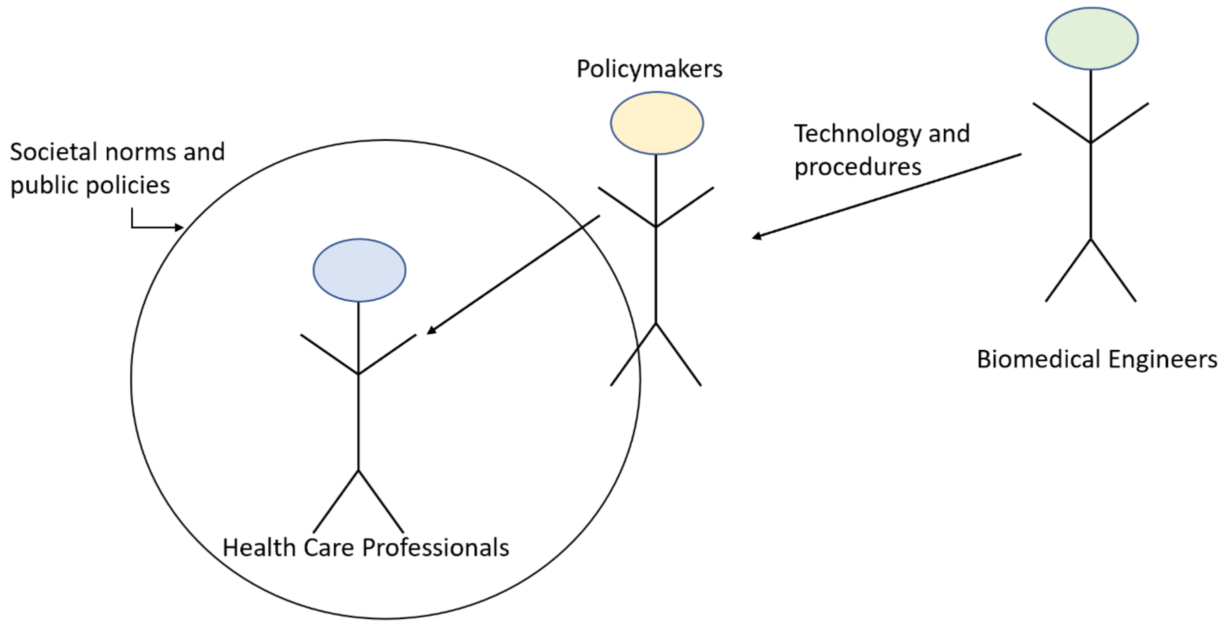

Figure 4: Permission and Acceptance of Deceased Donor Programs. An evaluation of how obstruction to the spread of organ transplant systems and technology can prevent it from being adopted by healthcare professionals. (Adapted by Luu (2022) from Rogers (2003)).

The anticipated outcome of this research is that nations with more robust deceased donor programs with fewer restrictions on which donors may be selected will present lower rates of per capita death as a result of chronic kidney failure and higher per capita transplants. Nations with more restrictive programs are expected to have roots in their public policy, and in accordance, the prevailing public opinion would reject the system. The fruits of this research will be presented in the format of a scholarly article to properly address the underlying factors contributing to organ scarcity worldwide.

SIGNIFICANCE AND IMPACT

The focus of this project is to analyze organ transplant scarcity worldwide and to address one of its underlying causes. The STS research project seeks to determine the degree of not only organ scarcity worldwide but also inequality born out of shortcomings within public policy and opinion. The technical project seeks to propose a mechanism through which transplant scarcity might be alleviated by proposing a method of automatically constructing printed organs. The technical project promises new tools to further bioprinting research and bring *de novo* organs to the populace at large. Thus, it is critical to have a solid grasp on not only the societal context this technology will be introduced into, but also the impacts that extant innovations and systems have had upon organ paucity. Together, the projects seek to analyze the root and extent of the issue and propose a solution to it. The proposed thesis will pair these two topics in order to design a new technological innovation in bioprinting and to understand how to best encourage its acceptance by society.

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