THE DEVELOPMENT AND TESTING OF A NOVEL AUTOMATIC ORGANOID/MICROSPHERE MOVEMENT DEVICE

EXAMINATION OF THE ETHICAL IMPACTS ON RESOURCE ALLOCATION TO ORGANOID AND ADULT STEM CELL RESEARCH

A Thesis Prospectus
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Bachelors of Science in Biomedical Engineering

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Organoids are three-dimensional tissue constructs derived from stem cells that have the ability to replicate both the functional and structural properties of *in vivo* human organs (De Souza, 2018). Human stem cells were first successfully collected in 1998, but the first derivation of these stem cells into organoids did not occur until 2009 when Sato et al. (2009) was able to to derive an organoid by seeding a human stem cell into Matrigel, which is a gel made of proteins, laminin and other growth factors. This research displayed a key factor in stem cell differentiation and therefore organoid derivation, that being the ability of hydrogels containing decellularized extracellular matrices to promote the differentiation of stem cells (Magno et al., 2020). As a result of this discovery, the field of organoid research has grown, with many different types of organoids being developed to mimic the function of different human organs. Since organoids have this ability to mimic *in vivo* organs, they are used to study the effects of diseases, drug testing and development, molecular medicine, and even organ transplantation (De Souza, 2018).

Despite the benefits of organoids in medical research, two issues hinder the growth of this technology; the lack of an automated method to seed organoids into biomaterials for growth and ethical concerns. Organoids are currently seeded into hydrogels manually, a method that is both imprecise and excessively time consuming, thus leading to organoid research progressing slower than if an automated method of organoid seeding was possible. The ethical concerns involving organoids stem from the fact that many organoids are derived from embryonic stem cells (ESCs). The harvesting of ESCs is seen by some groups, particularly religious organizations, as the ending of a human life, which leads to the controversy surrounding organoid research (Mollaki, 2021). One possible result from this controversy is that organoid research does not receive as much funding as it would if it did not involve ethically concerning methods.

The technical project and tightly coupled STS research project discussed in this paper will attempt to address both of these issues hindering the progress of organoids as a medical technology. The goal of the technical project is to create an automated device that can recognize the presence of organoids and then precisely seed them into a hydrogel for differentiation. This will attempt to address the current issues of imprecision and excessive time consumption in organoid research as an automated system will be more precise than the human hand and the user can work on other tasks as the system seeds the organoids. The STS research project will investigate the differences in resource allocation between organoids and adult stem cell (ASC) research, which is a field with significantly less ethical concerns than organoid research, in order to determine the impact of the societal factor of ethics on technological development. These projects will be completed in the Fall 2022 and Spring 2023 semesters, with the distribution of work being displayed in Figure 1.

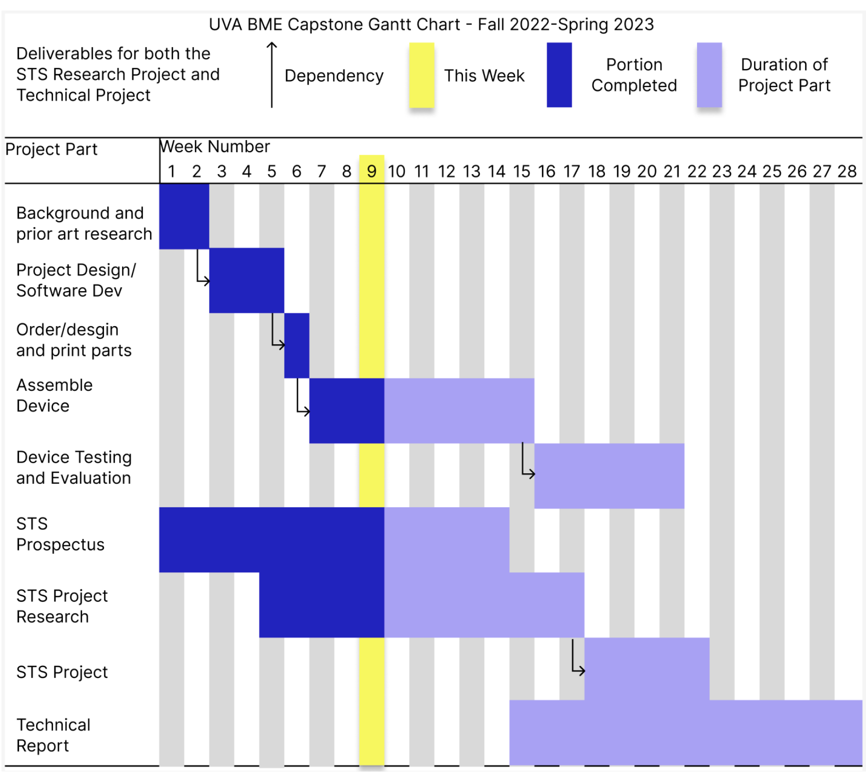

Figure 1: UVA BME capstone gantt chart. This figure provides a visual representation of the timeline for the major deliverables of the STS and technical projects. (Hoffman, 2022).

THE SOFTWARE AND HARDWARE DEVELOPMENT OF AN AUTOMATED ORGANOID SEEDING DEVICE

One current problem holding back the development of organoids as a medical technology is the process by which they are seeded into hydrogels for differentiation. In the current method of seeding, stem cells are placed into biomaterials by hand using a pipette and vacuum aspiration (Daly et al., 2021). Although effective for small-scale studies where precision is less of an important variable, the current organoid movement method is time-consuming, imprecise, and limits the scale and scope of potential studies (Ren et al., 2021). As with any procedure that relies heavily on human manual dexterity, the placement of organoids into specific locations within biomaterials is imprecise, thus introducing error and limiting study conclusions (Ren et al., 2021). Vacuum aspiration itself also introduces error through the accompaniment of media with organoid deposition. The extra media deposited with the organoid alters the desired extracellular environment, again limiting conclusions that can be made (Vonk et al., 2020). By improving the current movement method of stem cells and organoids, new research could be conducted with higher degrees of accuracy with a faster turnaround time. Additionally, a more precise placement method would open the possibility for more abstract organoid arrangements to better mimic *in-vivo* conditions (Yin et al., 2016). These improvements could have a major effect on the rate at which significant advancements in organoid technology are made. This advancement is essential because of the unique ability of organoids to mimic the cell-signaling pathways of human organs, which allows for accurate *in vitro* testing of disease mechanisms in these organs, leading to new cures and treatments that were not previously possible (Kapałczyńska et al., 2018).

Under the advisement of Chris Highly, a professor in the Department of Biomedical Engineering at the University of Virginia, a team of biomedical engineering undergraduate students at the University of Virginia, including Remington Martinez, Jack Maschler, Josh Sanderson, and myself, aim to design a device that precisely places organoids into a user-designated position in a permissive biomaterial using an automated set-it and forget-it approach. There are two major user needs the team intends to meet with the development of this device: the ability to designate organoid placement locations, and for the pick-up and placement to be done without human intervention. All materials used in this research were supplied by and paid for by the Chris Highly lab and all research was done in said lab.

METHOD OF DESIGNING AN AUTOMATED ORGANOID SEEDING DEVICE

To allow the user to designate organoid placement locations, a GUI will be created using Python that allows the user to input the desired placement location(s). Next, we will use this input to move both the 96-well plate containing the organoid(s) and the plate containing the permissive biomaterial using a premade multiaxis sled movement system powered by NEMA 23 motors controlled by the same Python program. Then, to pick up and place the organoids, a 2020 Nano-liter injector, also controlled by Python, will be attached to the vertical axis of the multi-axis sled movement system as displayed in Figure 2. To attach the Nano-liter injector, new components will be designed and fabricated using 3D printing and/or laser-cutting which can also be seen in Figure 2. In conjunction, the aforementioned components will move plates and Nano-liter injector to pick up and place the organoid(s) precisely in the user-inputted location. The precision of the automated system is meant to limit the variability that is currently present in organoid models as a result of manual seeding (De Souza, 2018).

To fully automate the pick-up and placement of the organoids, a Basler Ace Camera will be attached to the vertical axis of the multi-axis sled movement system as specified by Figure 2. Using image detection in Python, this camera will be able to detect the presence of organoids within wells in the 96-well plate allowing for the aforementioned components to work seamlessly without user interaction.

Finally, to verify the device is working as intended, nanospheres and organoids will be loaded randomly into a 96-well plate and various placement patterns and locations will be input into the GUI. The resulting biomaterial containing organoids and nanospheres will be analyzed to determine if the organoids were placed in the desired locations. Additionally, the biomaterial will be compared to a biomaterial containing human-placed organoids to quantify the improvement in precision, scalability, and time consumption.

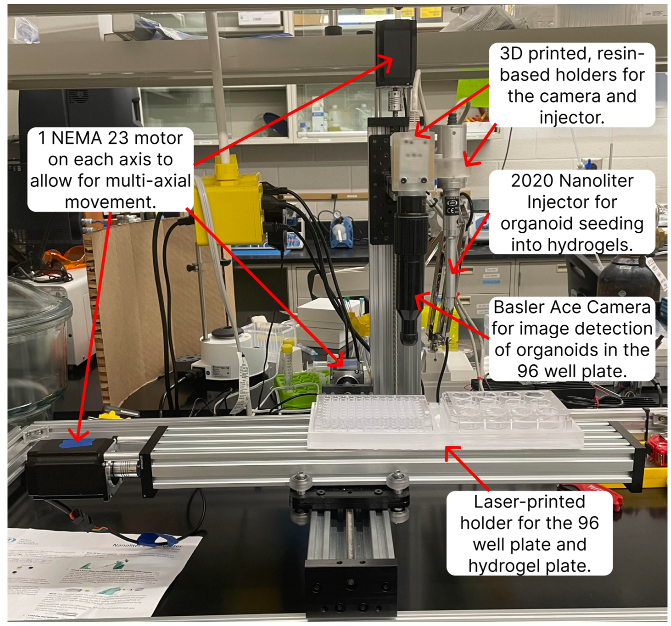

Figure 2: Hardware components of an automated organoid seeding device. This figure depicts the assembled hardware components of the technical project, including the motors, injector, camera, and printing components. (Hoffman, 2022).

The anticipated outcomes from this project is an automated method of organoid seeding that is able to place organoids within at least 100 microns of a user selected location, transfer less than 5 microliters of media from the well plate to the biomaterial, and can locate, pick up, and seed a 96 well plate full of organoids 200% faster than can be done manually by a trained researcher. These design specifications were determined by the team advisor Chirs Highly. The entirety of this project will be documented in the form of a journal article, in which the research, results, and potential impact of this project on the future of organoid technology and medicine will be detailed.

EXAMINATION OF RESOURCE ALLOCATION TO ORGANOID AND ADULT STEM CELL RESEARCH

Stem cell research is a rapidly growing field in the medical science community with a global market size of around $11.89 billion in 2021 (Grand View Research, 2021, p. 1). However, despite the growth of this industry, it continues to be dominated by research involving adult stem cells (ASCs), with 82.5% of the market being allocated to research of ASCs, in comparison to the around 10% allocated to studies involving embryonic stem cells (ESCs) (Grand View Research, 2021, p. 2). Embryonic stem cells are one of the primary cell types used for differentiation into organoids (Azar et al., 2021). While this difference in resource allocation may be the result of the differences in the uses of the cell types, one factor that must be considered as a possible reason for this discrepancy is the presence of ethical concerns in embryonic stem cell harvesting. The collection of ESCs requires the destruction of a human embryo and thus can be seen as trading a human life in exchange for scientific benefit (Mollaki, 2021).

These ethical concerns surrounding ESCs and therefore organoids could be having significant effects on the development of organoids as a medical technology. This is an important problem to consider because of the potential benefits that organoids have in the field of medicine, from new disease cures to artificial organ transplants that could potentially save countless lives in the future. More funding for research based on ESCs could result in greater progression towards new treatments but it also requires what some consider a human life to be ended, begging the question is it worth it to sacrifice lives now for the potential of saving more lives later?

This problem requires more research because of the impact that it has on the future of modern medicine. There are two distinct viewpoints on the ethical concerns of organoid use in medical research: those who believe that organoids result in the death of an unborn human and thus should not be a viable research option, and those who believe that the medical benefits of organoids warrant their use in research (Mollaki, 2021). These views are depicted in Figure 3, along with the relationships between technical and social aspects of this issue. Society's view on organoids as a technology impacts its technological development through the amount of funding it receives, which in turn impacts society as it determines the rate at which new treatments are developed, thus affecting the health of society members. This illustrates Johnson's (2005) depiction of social constructivism, which she describes as the idea that technology and society do not develop independently, but instead impact the development of each other (p. 1792). In accordance with this idea, the objective of the research work done on this topic will be to investigate the effect that the societal factor of ethics has on the success of technological development in biomedical engineering. This will be done by comparing the resource allocation between organoid research, a field with major ethical concerns, and ASC research, a field with minor ethical concerns, as well as their respective rates of development that result from the amount of investment they receive.

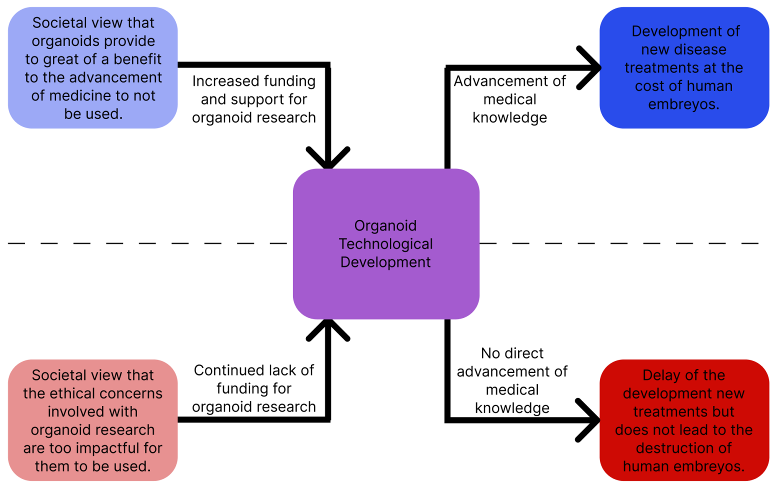

Figure 3: Impacts of opposing views on organoid technology. This figure illustrates the potential pathways that result from pro-organoid and anti-organoid viewpoints. (Hoffman, 2020).

THE POWER OF MONEY IN TECHNOLOGY

As described in Johnson (2005), investment and resource allocation are some of the most recognizable and direct methods by which societal values can influence the development of a technology (p. 1793). When a developing technology is endorsed by society it tends to receive a greater amount of investment from both private organizations and federal agencies. This increase in funding typically leads to greater rates of development of technology, as well as a higher chance of successful results (Johnson, 2005, p. 1793). An increasingly prominent example of this idea is exemplified by the increase in popularity of ethical investment in the United States, with 13% of all investment dollars being allocated to ethical investment funds in 2001, a number that has likely increased since then (Michelson et al., 2004, p. 1). Ethical technologies are more likely to be accepted and supported by society. As a result, investors are starting to take ethics into account more when deciding on what technologies to invest in. This indicates that technologies with ethical concerns, such as organoids, may be receiving less funding and thus the development of such technologies are being delayed despite their potential benefits to society.

This lack of funding is not just a result of individual firm's decisions of what to invest in, but also federal legislation. As described in Andrews (2003), pro-life protests resulted in the passing of an appropriations rider that banned any federal funding to research that involves the destruction or discarding of a human embryo, which is a category that organoids are a part of (p. 113). Although this amendment, known as the Dickey-Wicker amendment, has since been adjusted, it remains an obstacle for federally funded research on embryos (Kearl, 2010). This example exemplifies the ability of society to impact the development of technology, as research involving embryos was not accepted by a portion of the community and thus their protests of this research led to legislation banning the funding of such technology, which has slowed the progress of its development.

The difference in resource allocation between ASC research and organoid research and its effect on each technology's development will be investigated through the use of the social construction of technology (SCOT) framework, initially pioneered by Trevor Pinch and Wiebe Bijker in 1984 (Bijker & Pinch, 1984). In this framework, as displayed in Figure 4, each group acts as a social factor that influences the engineer's design and therefore the development of the technology. The engineer and technology also offer something to each group, representing the impact that technology has on the development of society. This mutual relationship between the engineer, technology, and social factors involved in organoid research will be investigated through the research done in this project with the hope of providing a better understanding of how ethical concerns can influence resource allocation to technological research and therefore the development of technology. This research project, in the form of a scholarly article, will hope to not only provide a better understanding of this relationship and its effect on the development of organoids as a technology,

Figure 4: Organoid research SCOT model. This figure displays the influence that societal factors such as investment have on the development of organoid technology. (Adapted by Hoffman (2022) from Carlson, 2008).

but also how the lack of investment in organoid technology has negatively affected society in the form of slower rates of cure and treatment development.

ORGANOIDS: UNREALIZED POTENTIAL

The potential of organoids as a medical technology are seemingly endless because of their ability to imitate the functions of human organs outside of the body. This allows for research to be conducted on how organs respond to different diseases, how to cure diseases, and even how to replace faulty organs. Despite the potential benefits of this technology, their potential has still not been fully realized because of ethical concerns involved with the creation of organoids and the lack of an automated seeding method for organoid differentiation. The technical project and tightly coupled STS research project described in this prospectus aim to address these two issues currently holding back organoids from realizing their potential. The technical project will attempt to create an automated organoid seeding device that will not only speed up the process of seeding but also increase the precision at which it is done. The STS research project will investigate how the ethical concerns involved with organoid technology lead to less resource allocation towards research involving the topic and the effects that this has on the development of new cures and treatments. Together these projects aim to allow the potential of organoids as a medical technology to finally be fully realized.

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