Evaluating and Quantifying Viability Of 3D-Printed Cells with Mathematical Modeling Across Different Bioprinting Methods
(Technical Report)

How Past Biomedical Interventions Inform the Emerging Ethical Concerns Surrounding Artificial Organs
(STS Research Paper)

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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

Three-dimensional bioprinting is an emerging technology in the field of tissue engineering (TE) and regenerative medicine (RM) that has shown potential as a tool for automated biofabrication of synthetic tissue constructs.("An Overview of Extrusion-Based Bioprinting with a Focus on Induced Shear Stress and Its Effect on Cell Viability," 2020; Bour et al., 2020; Cidonio et al., 2019; Derakhshanfar et al., 2018; Murphy & Atala, 2014) However, during the printing process, cells undergo shear forces which damage cell integrity and can lead to cell death thereby diminishing the functionality of the final tissue construct. Therefore, the successful integration of this technology in the biomanufacturing process for clinically successful synthetic tissue constructs relies on the ability to ensure sufficiently high cell viability during the bioprinting process. I will conduct a quantitative analysis of cell viability across two printing methods and develop a mathematical model relating printing parameters to cell viability. If successful, the results would enable a larger degree of control and predictability over shear stress and cell viability when biofabricating therapeutic tissue constructs.

The emergence and fast-paced development of 3D bioprinting technology in recent years is a crucial step towards realizing the ultimate goal of creating fully functioning artificial organs. This impending reality of organ printing drove me to consider what ethical concerns will arise as a result of this technology. In order to better understand how this technology will be implemented and regulated in the future, I think it is helpful to draw upon past technological breakthroughs in the medical field. I think the way in which these past interventions were adopted by experts can serve as an example for what to expect in the future with organ printing. Through my research, I want to explore these ethics and understand how experts involved in the future implementation of 3D-printed artificial organs and tissues are discussing this technology and the potential issues surrounding it.

Technical Topic

Extrusion-based bioprinting is the most widely used method of bioprinting in the field of TE and RM because of its simplicity.("An Overview of Extrusion-Based Bioprinting with a Focus on Induced

Shear Stress and Its Effect on Cell Viability," 2020; Fisch et al., 2020) This method involves the extrusion of cells embedded in a hydrogel bioink through a nozzle in specific pre-determined patterns. There are two popular mechanisms of extrusion with this method: pneumatic extrusion and piston-driven extrusion.("An Overview of Extrusion-Based Bioprinting with a Focus on Induced Shear Stress and Its Effect on Cell Viability," 2020; Derakhshanfar et al., 2018) Tissue-engineered muscle repair (TEMR) constructs are a prime example of the potential this technology has to achieve automation, cost and time reduction, and reproducibility in the manufacturing process.(Bour et al., 2020; Cidonio et al., 2019) These constructs are being explored as novel treatment options for volumetric muscle loss (VML) as current therapies involve multiple reconstructive surgical procedures which often fail to fully restore muscle volume and function, especially in more severe cases often seen in combat veterans.(Bour et al., 2020) TEMR constructs are typically manufactured by integrating decellularized extracellular matrix (dECM) scaffolds with muscle progenitor cells (MPCs) to promote skeletal muscle regeneration once implanted.(Bour et al., 2020; Cidonio et al., 2019) When constructed manually, the process can take approximately 15-17 days, including a 10-day proliferation period, and requires high cell densities when seeding the MPCs onto the dECM scaffold.(Bour et al., 2020) However, with bioprinting, confluence on both sides of the dECM sheet is achieved after 24 hours and a lower cell density is required to achieve sufficient cell coverage.(Bour et al., 2020)

When designing novel therapeutics such as these for human implantation, it is necessary to understand all aspects of the manufacturing process for the sake of reproducibility and safety. It is known that cells are subjected to many mechanical forces during the extrusion printing process.("An Overview of Extrusion-Based Bioprinting with a Focus on Induced Shear Stress and Its Effect on Cell Viability," 2020; Fisch et al., 2020; Lepowsky et al., 2018; Nair et al., 2009; Ning et al., 2018; Zhao et al., 2015) These compressive, tensile, and shear forces can drive many biological signals and processes such as growth, proliferation, and differentiation. Of particular importance in the field of TE and RM medicine is the effect of bioprinting-induced shear stress on cell viability. The bioprinting process inherently results

in some percentage of damaged or dead cells due to the induced mechanical stress upon the cells during extrusion.("An Overview of Extrusion-Based Bioprinting with a Focus on Induced Shear Stress and Its Effect on Cell Viability," 2020; Fisch et al., 2020; Lepowsky et al., 2018; Nair et al., 2009; Ning et al., 2018; Zhao et al., 2015) Implantation of any number of dead cells needs to be accounted for and have a strong rationale in order to ensure success of clinical translation of TEMR constructs.(Cidonio et al., 2019) Additionally, the functionality of the tissue construct is highly dependent on the viability of the cells which comprise it.(Cidonio et al., 2019; Lepowsky et al., 2018; Murphy & Atala, 2014) Therefore, maintaining the highest possible cell viability through the printing process is an essential consideration for biomanufacturing TEMR constructs for autologous skeletal muscle regeneration following VML. I believe reproducibility and greater user control, are essential to the clinical success of bioprinted tissue constructs and the integration of bioprinting into the biofabrication process in TE and RM.

Under the guidance of Dr. George Christ, Ph.D., Professor of Biomedical Engineering and Orthopedic Surgery, my goal is to not only elucidate how bioprinting affects cell viability but to ultimately develop a mathematical model which can be applied to different extrusion-based printers to reliably quantify and predict cell viability of the processed cells. This model will be developed in the context of VML and the biomanufacturing of tissue constructs to aid muscular regeneration. As such, I will be printing C2C12 cells – an immortalized murine skeletal muscle myoblast – and human muscle progenitor cells, which are more clinically relevant in the context of VML, in a hyaluronic acid hydrogel using pneumatic and piston-driven extrusion bioprinters. Once initial cell viability data has been gathered, I will then develop the mathematical model which will be validated with further experimentation and adjusted as needed. Accomplishing these aims will allow for reliable prediction of bioprinting results and control over printing-induced shear stress in order to achieve consistently high cell viability and ensure the success of future bioprinted tissue constructs.

STS Topic

According to the United Network for Organ Sharing (UNOS) and the Health Resources & Services Administration, there are currently more than 109,000 people in the U.S. on the waiting list for a lifesaving organ transplant. One person is added to this list every minute and 17 people waiting for an organ transplant die every day (*Organ Donation Statistics*, 2018). I believe the future solution to the devastating shortage of organ donors will be the advent of on-demand organ printing. It is important to note that scientists are still far from being able to print fully functioning complex organs that are biocompatible and have proper vasculature (Yasinski, 2020). However, the successful development and implementation of organoid models, organ on a chip models, and smaller tissue constructs point to the tremendous potential for 3D bioprinting. Therefore, I think the research focus and direction of the field of tissue engineering make organ printing an inevitable reality one day. With all of the hype and promise in the media surrounding the idea of 3D printed organs, it is important to start envisioning and preparing for what the future of this groundbreaking technology will truly look like.

Despite this hype that is present in the media and news headlines, there is a lack of literature addressing the ethical implications of 3D bioprinting artificial organs (Vermeulen et al., 2017). The absence of this conversation about social and ethical concerns is made apparent by the lack of clear, standardized regulations or guidance over how this kind of research with synthetic tissues should be applied to humans and translated to the clinic. A recent publication in the Journal of Medical Ethics revealed these gaps in regulation by examining the current practices when testing artificial organs on brain-dead patients (Truong, 2019). They noted that of particular concern were the questions of candidate selection, transparency, and sensitivity. This initial effort by key actors including researchers and bioethicists is the beginning of a larger conversation that needs to happen. The available social scientific literature on this subject will provide insight into the language, priorities, and opinions of technology developers, policymakers, and the various institutions involved (healthcare, government, academia, review boards, etc.) Additionally, when anticipating the societal impact of a new technology it is useful to look at previous technologies that share similarities. In doing so, it is important to establish the

justifications for such comparisons that allow predictions to be made about future ethical concerns. I consider the successes and failures of past implantable biomedical interventions and how they inform the future of this emerging technology of synthetic tissues and artificial organs. In doing so I will evaluate the most pressing ethical concerns from the perspective of researchers, bioethicists, and other experts involved in this field.

Implanting biomedical devices in the human body to replace impaired or missing biological functions dates back to 1960 when the first clinically successful cardiac pacemaker was implanted in patients (Greatbatch & Holmes, 1991). Since then, numerous implantable devices have been successfully developed including implantable defibrillators for the heart, targeted drug delivery systems to assist specific tissues and organs afflicted by disease (e.g. insulin pumps), intraocular lenses, and coronary stents. Furthermore, the use of artificial joints to replace damaged joints such as hips and knees predates the cardiac pacemaker by roughly 10 years and is now a relatively common procedure (Gomez & Morcuende, 2005). All of these have seen varying successes and failures throughout their development, clinical implementation, and widespread adoption. By drawing comparisons between these implantable medical interventions and artificial organs, I can begin to establish an anticipatory framework for the problems that will arise from artificial organs. Hutchsion & Sparrow (2016) conduct a similar analysis by comparing artificial organs to pacemakers and identifying five key features of the pacemaker and its implementation that gave rise to many ethical concerns. My focus will be on comparing the aforementioned implantable biomedical devices, specifically cardiac pacemakers, insulin pumps, and artificial joints, to artificial organs. I first identify the key features these technologies share that create the contexts in which the various ethical questions arise. Drawing from Hutchison & Sparrow, I first look at the ethical concerns arising from the invasive nature of implantation and the complexity of these technologies which no doubt require surveillance and maintenance by different specialists (Hutchison & Sparrow, 2016). Once implanted, these technologies then give rise to questions of privacy, patient autonomy, and proprietorship as well. Who owns the device, the patient or the manufacturer? Are these

implants considered “life support” that can be withdrawn or do they become part of the patient’s body? The third common feature that justifies the comparisons upon which I explore the ethics surrounding artificial organs is the natural tendency for these technologies to improve upon themselves quite rapidly. Researchers in the field suggest these technologies will no doubt create a multi-billion-dollar market (Vermeulen et al., 2017). This already raises ethical questions which will also be discussed. However, as new iterations and competing brands of these technologies are developed it creates difficulties for patients and can lead to the potential stratification and discrimination (Hutchison & Sparrow, 2016; Vermeulen et al., 2017). Socioeconomic factors often play a role in determining access to the best medical care and cutting-edge therapies.

I will be applying this framework from the perspective of biomedical researchers developing this technology of artificial organs and bioethicists discussing the societal implications. As such, I will be drawing from a recent publication which I previously mentioned from the Journal of Medical Ethics which provides insight into the bioethics perspective on artificial organs (Parent et al., 2020). Additionally, I will be examining the language, priorities, questions and opinions presented at the International Conference on Artificial Organs, Biomaterials and Tissue Engineering to be held in Rome, Italy on December 10-11, 2020 which is sponsored by the World Academy of Science, Engineering, and Technology. Using the insight from these two sources I will then be able to evaluate how the ethical questions from the previously chosen biomedical technologies inform the discussion surrounding the emerging technology.

Next Steps

The next steps of this research endeavor will require a thorough literature review of how cardiac pacemakers, insulin pumps, and artificial joints transitioned from research & development to clinical application and what the successes and failures were along the way. Further, I will be thoroughly examining the selected authors and submitted papers from the Conference on Artificial Organs, Biomaterials and Tissue Engineering.

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