3D Voxelated Bioprinting Highly Organized Yet Heterogeneous Tissue Constructs
(Technical Report)

The Classification of Bioprinted Organs and Its Legal and Societal Consequences
(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

Though 3D bioprinting is a promising method for the construction of synthetic tissues and organs, current techniques are still unable to fully mimic the functions of native tissue. Our capstone project will be testing a novel 3D bioprinter that utilizes spherical hydrogels particles, known as 0D voxels, to assemble tightly controlled heterogeneous tissue constructs that address some of the shortcomings of conventional 3D bioprinting methods. This method could have far-reaching applications in modelling tissues for pharmaceutical testing, eliminated the need for animal testing, and in regenerative medicine. However, the ultimate motivation for 3D bioprinting is the biomanufacturing of viable organs for the use of organ transplantation.

This merge of autologous cells and additive manufacturing is beginning to blur the line between artificial and natural as bioprinting techniques continue to advance. As the possibility of wide-spread use in the coming decades becomes more visible, questions are raised about how legal bodies will interpret this new technology. This is closely intertwined with social and cultural meaning of the human body, and what constitutes a human. One issue that has become evident as the concept of biofabricated organs emerges is how this technology will be regulated, and this is where the classification of biofabricated tissues and organs becomes an important aspect of this sociotechnical system. It is human nature to classify various aspects of life, such as technoscientific artifacts, into distinctly defined and mutually exclusive categories. However, with the innovation of these artifacts comes greater complexity, which makes it even more difficult to classify them into a clear category that meets the needs and views of legal and cultural entities.

For this paper, the framework of classifications and standard will be discussed in the scope of legal language and regulatory bodies to discuss how it affects social norms and perspectives on synthetic organs.

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The demand for organ transplantation has rapidly increased in the past decade with the rise of organ failures and the advancements in transplantation procedures. However, the shortage of adequate organs from donors and the increasing number of patients on transplant waiting lists have created an organ shortage crisis (Abouna, 2008). This past year, nearly 40,000 organ transplants were performed and there are currently over 100,000 patients on the national transplant list in the United States, with an average of 17 people dying each day waiting (*Organ Donation Statistics | Organ Donor*, 2020). With this growing shortage, along with the prevalence of chronic immune rejection of transplanted organs, 3D bioprinting is emerging as a novel and promising method for the bio-fabrication of complex constructs that can mimic native tissue. This method as the potential in creating viable, mass-produced organs made of the patient's own cells, that can mitigate the shortage in donor organs.

Current conventional 3D bioprinting techniques, which include extrusion-based, inkjet, stereolithography, and laser-assisted printing, have had success in constructing more simplified structures for tissue regeneration (Li et al., 2016). However, these methods have limitations in the level of complexity and precise positioning of cells within the construct, that prevent greater advances in fabricating highly functioning organs. Through our capstone project, two of these shortcomings will be addressed; the lack of controlled porosity and the lack of heterogeneous organization. Conventional bioprinting methods employ a layer-by-layer technique using 1D filaments, where the tight packing of these filaments causes a lack of porosity, which prevents

key nutrients from facilitating into the deeper, middle regions of the construct (Dey & Ozbolat, 2020; Heinrich et al., 2019). Although tissues may seem like a large, random arrangement of cells, they are in fact a highly organized structure with varying levels of heterogeneity. Current methods have had difficulty recreating this precise arrangement of different cell-types to form a functioning construct that fully mimics native tissue (Ashammakhi et al., 2019; Xu et al., 2013).

Dr. Liheng Cai, Ph.D., Assistant Professor of Material Science, Chemical Engineering, and Biomedical Engineering (by courtesy), and his lab has developed an innovative method for 3D bioprinting heterogeneous tissue constructs with the use of spherical hydrogel microparticles, called 0D voxels, by modifying an extrusion-based microfluidic printhead. Under his guidance, our capstone team, consisting of fourth year biomedical engineering students, Shannon Grady and Leander Nguyen, will be conducting further research into the capabilities of this design. Whereas 1D filaments allow for the construction of discrete zones of different cell types, the variable size and greater degrees of freedom of voxel particles enables the design of tissue constructs with higher printing resolutions, down to variations between single microparticles (Placone et al., 2020). As this is a novel technology, printing specifications are yet to be defined, so as part of our Capstone Project, the team will be characterizing the chemical and physical properties for the hydrogel bioink through three specific aims.

The first aim will to test and model the time it takes for the hydrogel bioink droplets to crosslink and coalesce. Since the scaffold is created by multiple droplets interconnecting, it is important to determine this time to allow the printing of hundreds of droplets before they solidify. This is quantified by the diffusion of Ca^{+} ions from the support bath into the alginate hydrogel. The second aim will be to then model the release profile of large biomolecules through the hydrogel. This is critical to determine the necessary hydrogel formulation that will allow for

a mesh size great enough for molecules necessary for cell and tissue growth to freely diffuse throughout the construct. These biomolecules will be represented by fluorescently-labeled dextran molecules and the diffusion of these molecules will be modeled through MATLAB. Finally, the third aim is to determine the relationship between polymer entanglement and mesh size to be able to fine-tune the porosity of the hydrogel bioink. The addition of PEO polymers had affected the mesh size of the hydrogel, and we will be creating a mathematical model to explain this phenomenon.

Overall, our team will be creating accurate models in order to optimize printing parameters for the 3D bioprinter for further use on different tissue constructs. This study will aid in furthering the lab's understanding of this bioprinting method in hopes of facilitating biomedical applications, such as organ biomanufacturing, in the future.

The Classification of Bioprinted Organs and Its Legal and Societal Consequences

As 3D bioprinting is edging closer to fully realizing its capabilities for manufacturing synthetic organs at a large scale, it is becoming evident that regulatory bodies are not prepared for its testing and introduction to the public. Scientific innovations are constantly changing and morphing into different or larger versions of itself, and it the regulatory body's job to keep up with such changes and produce up-to-date standards for the public. However, legislature and regulations cannot keep up with the evolving definition of 3D bioprinted body parts. Once considered purely medical devices, due to its more synthetic polymeric material, and mainly used for regenerative medicine, bioprinting is now becoming more naturally derived with the goals of tissue engineering complete replacements for an entire body part (Gebelein, 1984). As regulations deal with classification and standardizations, the language in which it describes synthetic organs are no longer conforming with the current state of the innovation. As legal

bodies, whether in professional organizations, manufacturers, or the government, adapt to these rapid changes, they may need to reassess how they have classified and categorized synthetic organs because it will play an important part in the decisions and views that society will have on this novel medicine.

The framework for this discussion will be derived from the book, *Sorting Things Out: Classifications and Its Consequences* (Bowker & Star, 1999). However, since the classification of synthetic organs is going to be analyzed in the context of legal bodies, standardization is inevitably an important sub-framework. Two works will be utilized to transition from the notion of classifications to its use in regulations and society, which are *Standards and Their Stories: How Quantifying, Classifying, and Formalizing Practices Shape Everyday Life* (Lampland & Star, 2009) and *Standards: Recipes for Reality* (Busch, 2011). These three resources will provide the scholarly definitions of classification and standards, which will aid in explaining the complexities of regulating synthetic organs and what they represent to society.

As defined by Bowker and Star (1999), classification is a spatio-temporal segmentation of the world, and an ideal classification system should exhibit three requirements. First, they are consistent and unique, which means origin and descent are often used to classify things. Second, the categories are mutually exclusive and third, these systems are complete. Standards, on the other hand, are any set of agreed-upon rules for the production of objects. They also have a temporal reach that persists over time and span multiple communities. And most importantly, legal bodies often enforce these standards. It is clearly stated by the authors, that real-world classification systems never meet these three ideal requirements, which will be evident when applying them to bioprinted organs. With the infinite complexities of the world,

classifications cannot encompass all the aspects of a certain object. There's overlap and a mix of different principles that produce contradictions. Lampland and Star (2009) and Busch (2011) further discuss the implications of these boundary objects by stating that standards are never completely adequate and are relative to different sociotechnical landscapes. They bring some aspects into view, while making others invisible. While certain standards and classification can be benign or irrelevant to an individual or group, it can be life-threatening to others. However, standards still represent and embody the morals and values of society. And the legal procedures that standardize these objects directly embeds itself and integrates into many layers of social and cultural infrastructure.

The fundamental question surrounding current negotiations about the classification of 3D bioprinted organs is whether they are actual biological tissue or a medical device. Currently regulators for the FDA, and regulatory bodies in other countries, are posed with the difficult decision of categorizing 3D bioprinting under medical devices or as biologics (Mendis & Rutschman, 2020) Most legal entities still consider bioprinting as a medical device, but as more cellular and bioactive factors are added into the manufacturing process, these two classifications are starting to overlap. 3D bioprinting no longer fits neatly into current regulatory frameworks, and regulatory agencies around the globe are trying to address these uncertainties and lack of uniformity in dealing with bioprinting (Gilbert et al., 2018). The article by Mendis and Rutschman (2020) also introduces the possibility of creating a completely new framework for which bioprinting would be regulated by, if neither categories are well-suited to encompass the major aspects 3D bioprinting. Whether through existing or new regulatory frameworks, this will shape the way the public can interact with the products. As mentioned earlier, classifications and standards embody moral and ethical values of society. So, another major area of contention

in regulation is if manufacturers are allowed to patent bioprinted material (Mendis & Rutschman, 2020). As 3D bioprinted products increasingly resemble human organs and tissues, the legality of patenting these materials would become a great moral issue. As the innovations in 3D bioprinting are evolving, so are the conversations pertaining to its regulation. In the process of defining synthetic organs, certain voices in society will be heard, while others silenced, so it's important to analyze how these classifications come about and how they are changing with the emergence of new information and ethical questions.

Conclusion

The next steps of this research will be to find literature pertaining to the legal language in which previous and current classifications of synthetic organs. This would include resources from legal experts, as well as regulatory bodies and their guidelines. It is also important to understand how different stakeholders view and define synthetic organs, so more journal articles from bioethicists, doctors, researchers, manufactures, companies, and laymen would also be included in the final paper. This will help create a more wholistic body of work on how classification of synthetic organs is evolving within the scientific community and in society.

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