Device for Automated Selection and Placement of Cell Clusters Within Biofabricated Tissue Constructs

Social, Legal, and Ethical Factors Considered in the Clinical Implementation of Islet Xenotransplantation

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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

Ex vivo research involves subjecting patient-derived samples to specific conditions and environments to evaluate proposed therapies and drugs. Research of this kind is pivotal in treating and understanding rare cancers and other diseases as there is a limited number of patients. Patient samples are most commonly integrated into two-dimensional (2D) monolayer cultures for *ex vivo* research despite lacking the properties of functional tumors. Three-dimensional (3D) cell cultures are a new approach to *ex vivo* research that yields a sample more similar to a functional tumor allowing for advanced targeted research on drug penetration and tumor development. However, difficulties in 3D cell cultures arise from a lack of standardization of methods and procedures, resulting in variations of spheroid shape and sizes contributing to irreproducible experimental results. Another challenge of research involving 3D cultures is that sample preparation, specifically the preselection phase, requires human input and is time-consuming. My team will design and fabricate a micromanipulator based upon an open-source design controllable within a computational framework to address these challenges that accompany 3D cell cultures. The creation of such a device will allow for the generation of multicellular spheroids of uniform morphology, microenvironment, and cellular physiology by eliminating the need for human assistance in the process of spheroid generation.

The STS topic of this thesis is related to the technical topic as it focuses on technology with similar applications and end therapeutic goals to work with 3D cell cultures. This technology is xenotransplantation, which entails transplanting either cells, tissues, or organs from one species to another and has been proposed and researched as a potential solution to the organ crisis for decades. Genetic Engineering advancements, improvements in animal husbandry methods, and an increased wealth of knowledge relating to cross-species immunology have

reignited the enthusiasm and potential for xenotransplantation as a potential therapy for late-stage organ therapy and eventually serve as the solution for ample supply of transplantable organs. Despite the potential health benefits and life prolongation promised to potential patients, xenotransplantation is accompanied by risk to the recipient and the wider public. Furthermore, xenotransplantation as a developing biotechnology does not fall within social, ethical, or legal norms. Thus I will utilize the Normative process theory to establish a network of social patterns through the interactions of objects, actors, and contexts involved in developing xenotransplantation as a norm of clinical practice.

Technical Topic

The limitations of two-dimensional (2D) monolayer cell culture and animal models to effectively recapitulate human drug interactions results in high pharmaceutical development attrition rates (Horvath, 2016). Three-dimensional cellular models have gained enthusiasm from the scientific community as they have been promised to recapitulate *in vivo* tissue microenvironments more reliably than 2D models. Three-dimensional (3D) cell cultures have been shown t “o be more physiologically relevant models for drug testing (Carragher, 2018) as well as for cancer research owing to the ability to facilitate analysis of drug penetration and tumor development (Szade, 2015; Sawant-Basak, 2018). Multicellular tumor spheroids are constructed from tumor cells which aggregate to form sphere-like structures with various morphologies depending on the cell lines and culture conditions used (Nath, 2016). The physiological tumor microenvironment can be more accurately reproduced in tumor spheroids due to the three-dimensional structure that creates pH, nutrient, and oxygen gradients characteristic of in vivo solid tumors (Brüningk, 2020; Nath, 2016). Currently, there is no unified spheroid generation protocol leading to spheroids of various shapes and sizes (Castillo, 2016).

Nevertheless, to ensure the reliability of spheroid assays, a homogeneous and uniform spheroid morphology, microenvironment, and cellular physiology is required (Friedrich, 2009). Therefore, spheroids are generated in various shapes and sizes and are subsequently selected based on spheroid morphology. However, this process requires human assistance resulting in a time-consuming and inconsistent process. Furthermore, significant morphological variations arise from current spheroid generation methods as lab technicians observe spheroids with the naked eye or a simple light microscope and are limited in this aspect.

Our team aims to design and construct a micromanipulator controllable within a computational framework to address these challenges. Based upon the work of Xu et al., we aim to recreate an open-source manual micromanipulator capable of aspirating polymer microspheres, representative of a multicellular spheroid. Following successful recreation of this device, we will develop a computational framework capable of positioning the manipulator precisely to the sample using steppers motors. Additionally, this computational framework will also operate the aspiration mechanism of the micromanipulator and its positioning. Following the successful development of the micromanipulator and corresponding computational framework, we will implement them in unison to successfully aspirate and move polymer microspheres to a predetermined target location while intact.

This device will enable more consistent and rapid development of engineered tissues *in vitro* by reducing the need for human inputs. In addition, this project lays the foundation for future designs that incorporate computer vision to automatically identify, select, and move target cell clusters.

STS Topic

On average, 17 people die every day from a lack of available organ transplants, and over 100,000 people in the US are currently on the waiting list for a lifesaving organ transplant(American Transplant Foundation, 2021). The lack of transplantable donor organs thrust forward research and development of alternative therapeutic strategies, including xenotransplantation, mechanical support devices, and cell transfer/tissue engineering protocols(Garry, 2005; Cooper, 2015). As discussed throughout the technical topic, many three-dimensional culture techniques aim to grow organs *ex vivo* for drug testing and transplantation. Although many organoids currently developed are used to model and study diseases, these engineered tissues are thought to serve as organ transplants in the future potentially. An alternative and long-lasting strategy to supplement non-functional organs is xenotransplantation, in which organs are generated in animals then transplanted to a human host. One of the most famous xenotransplantations performed was on an infant child with hypoplastic left heart syndrome, and a baboon heart was used in the transplantation at the Loma Linda University Medical Center. Unfortunately, the infant died 21 days after birth. Xenotransplantation has failed to become a commonly used therapy as immunological barriers have hindered the growth of the therapy mainly due to host rejection of donor tissues. Norman Shumaway, a pioneer of cardiac transplants, often said, “The future of transplantation is xenotransplantation, and it will always be the future,” implying that Shumaway does not believe xenotransplantation will ever be a viable option as an alternative therapeutic strategy (Cooper, 2012). The use of xenotransplantation, in which the donor and recipient are of distinct species, raises various issues, including the unavoidable harm done to the donor and the potential harm to the recipients.

For decades, xenotransplantation, the transplantation of cells, tissues, and whole organs from one species to another(Wang, 2019), has been thought to be the future solution for an unlimited supply of customizable organs. For many, xenotransplantation was always to be the future. However, recent advancements in genetic engineering (e.g., CRISPR-Cas9) and xenoimmunobiolgy and establishing protocols for donor-animal husbandry, patient follow-ups, and others have shown results that suggest clinical trials are likely to be a reality soon.

Risk and benefits are present in all decisions in life, and we all consider and assign weight to these factors in an individual and unique decision-making process before we act. The risks and benefits that accompany biotechnologies, such as xenotransplantation, should be considered in a decision-making process that considers the interest of relevant actors in the network of clinical xenotransplantation and includes both individual beneficiaries and the public in the dialogue of discussions.

One risk that has been identified since the introduction of xenotransplantation is the potential for transmission of zoonotic infection. Pigs provide organs of approximately the correct shape and size and are relatively easy to raise and breed. However, pigs carry specific porcine pathogens such as porcine CMV (pCMV), porcine lymphotropic herpesviruses (pLHV), and porcine endogenous retroviruses (PERVs) and must be considered among other infectious risks to the individual recipient and public (Wang, 2019). In addition, the recent COVID-19 pandemic has highlighted and drawn public attention toward the potential public health risk of zoonotic-driven viral infection.

The choice to engage in the practice of xenotransplantation is not solely an individual decision. It is also, and more importantly, a public decision concerning the governing bodies enforcing the legislation. The possible risk of xenotransplant recipients being infected with a

zoonotic disease manufactures a risk for the general public. This is the primary risk associated with xenotransplantation. Thus, appropriate analyses and establishing a respective actor-network theory will allow for improved trust in the scientific process and create a more informative and productive public dialogue on this developing biotechnology that does not fit within the categories established by current legal, social, and ethical norms.

Possible infection and human-human spread subjects not only the intended beneficiary of xenotransplant but also individuals frequently in contact with the beneficiary and the larger population as a whole. The risk created by this biotechnology challenges the established norms for informed consent in current medical practices as the risk is burdened by more than just the patient. Furthermore, ethical questions are raised regarding the appropriate protocol for informed consent in clinical xenotransplantation because the patient is asked to commit to life-long medical surveillance methods to reduce the risk of zoonotic disease transmission, which may be unreasonable to faithfully comprehend in a physical and mental state of a patient in late-stage organ failure. The scientific community and bioethics agree that established human rights must be infringed upon to some extent to protect the general public's safety while also providing a solution for an unlimited and fully customizable supply of transplantable organs. Xenotransplantation is currently associated with uncertainties that pose risks to the patient and global community as a zoonotic disease can pass through borders as easily as humans can in modern society. The global risk presents challenges for current informed consent norms and protocols. It highlights the necessity for establishing a global governing body that can unite efforts and establish standard protocols to protect the public while providing therapy to late-stage organ failure.

Despite the patient and public health risks posed by xenotransplantation, research continues to push forward and has provided positive results suggesting that clinical trials of human xenotransplantation will be a reality shortly. Using the principles of actor-network theory and concepts derived from Theodore M. Porters, “Trust in Numbers” to evaluate and communicate the relevant human and non-human actors that form the network of social relationships surrounding the translational progress of xenotransplantation. Effective communication of the nature of confined disciplines of expertise and the broader community is critical due to the foreign nature of such biotechnology and the broader risk it manufactures. In addition to placing the public-health at risk of infection from zoonotic diseases, the public investment required to develop the technology for xenotransplantation and overcome the barrier and reach clinical application is significant. Accordingly, the decision to engage in the idea and practice of xenotransplantation is an inherent risk both from a financial and public health perspective. The recent advances in genetic engineering and a deeper understanding of xenoimmunbioligy have created great hope for and incentive to develop xenotransplantation therapies and fully transplantable organs. With that said, I am not searching for unethical laboratory practices or malintent (i.e., falsifying data), but to establish the relevant actors, their respective interests, the social network they build, and the numbers they use to gain trust by minimizing risk. I aim to determine the relevant objects, actors, and contexts, as established by the Normative process theory, within the development of clinical islet xenotransplantation that interacts in social patterns to establish norms of the regulation and implementation of developing biotechnologies.

Key Texts

Theodore M. Porters, “Trust in Numbers (The Pursuit of Objectivity in Science and Public Life)” investigates how a better understanding of quantification leads to a better cultural understanding of objectivity. This book is relevant to my research as the quantification of risk to the transplant recipient, and the whole community is essential in gaining public trust.

The book “Healthcare and Big Data,” authored by Mary F.E Ebeling, discusses the digitization of the medical clinic and the shift to a data-based approach within healthcare. This piece is relevant to my discussion of xenotransplantation primarily due to the discussions linking the rise of institutions and healthcare institutions to the collection and surveillance of population data. The rise of big data within healthcare systems embedded a norm within society that gave rise to biopower and surveillance medicine implications.

A recent research article published in BMC Medical Ethics titled “What does it take to consent to islet cell xenotransplantation?: Insights from an interview study with type 1 diabetes patients and review of the literature” that conducts seven qualitative interviews with type one diabetes patients to understand the factors that were of utmost priority in their decision to undergo and consent to islet cell xenotransplantation. This research is related to my discussion as it serves as a basis to understand relevant (published in 2021) and qualitative aspects that affect what population of people xenotransplantation is tested on, developed for, and tailored to.

The second edition of the book “Xenotransplantation: methods and protocols” covers the technology and methods for generating the fundamental knowledge that can drive and ensure the improvement and clinical application of xenotransplantation. Specialists author chapters of this book and focus on the current research of their respective sub-disciplines that amalgamate into a wealth of knowledge that determines the progress of xenotransplantation as a clinical practice.

This book provides technical knowledge and potential evidence of where numbers may carry more judgment than they appear to.

Sara Fovargue is a lecturer at Lancaster University specializing in health care law and ethics, and her 2011 book, "Xenotransplantation and risk : Regulating a developing biotechnology" discusses how xenotransplantation will and does fall between or outside of ethical and legal norms. This applies to my discussion as she dedicates chapters to the ethical and legal aspects that arise from a novel biotechnology and what actions regulatory bodies should take to maintain the most significant interest of public health.

Methods

I will evaluate the transplantation of porcine islets as a treatment for type 1 diabetes using the Normalization process theory to trace the social network of xenotransplantation and the actions taken to implement new biotechnology while interacting with kinetic elements of their environments. Understanding the dynamics of how novel biotechnology is implemented or an existing one is modified is complex. Thus the Normalization process theory simplifies this problem by focusing on aspects or mechanisms that actors may invest and contribute. The various tasks and interactions carried out by an actor that carries new ideas become routine. Using concepts from "Trust in Numbers", I will evaluate the use of numbers concerning the objects, agents, and contexts of xenotransplantation as quantification can manufacture impersonality granting authority to scientific pronouncements. Porcine islet transplantation is considered the most likely form of xenotransplantation to reach clinical stages. Thus it is relevant and valuable as more technical details are presently established, allowing for a more extensive network to be built that will, if successful, provide a framework for establishing ethical, legal, and social norms for future forms of xenotransplantation. I will apply the Normalization

principle theory to biomedical and medical research literature relating to pre-clinical and clinical trials of xenotransplantation to determine the objects, agents, and contexts that determine the actualization of xenotransplantation as the norm for clinical solutions to late stage-organ failure and organ transplantation.

Conclusion

The technical portion of this thesis outlines the design and fabrication of an automated device for the selection and transfer of three-dimensional cell spheroids. The work completed in this process will be crucial to the building and testing of engineered tissues *in vivo*. I hope that applying the Normative process theory to the implementation of islet xenotransplantation as a clinical treatment for type diabetes will reveal the network of social patterns that embed normative ideologies with regard to the clinical application of this specific technology and technologies of similar nature.

References

Brüningk, S. C., Rivens, I., Box, C., Oelfke, U., & ter Haar, G. (2020). 3D tumour spheroids for the prediction of the effects of radiation and hyperthermia treatments. *Scientific Reports*, *10*(1), 1653. https://doi.org/10.1038/s41598-020-58569-4

Carragher, N., Piccinini, F., Tesei, A., Jr, O. J. T., Bickle, M., & Horvath, P. (2018). Concerns, challenges and promises of high-content analysis of 3D cellular models. *Nature Reviews Drug Discovery*, *17*(8), 606–606. https://doi.org/10.1038/nrd.2018.99

Cooper, D. K. C. (2012). A brief history of cross-species organ transplantation. *Proceedings (Baylor University. Medical Center)*, *25*(1), 49–57. https://doi.org/10.1080/08998280.2012.11928783

Cooper, D. K. C., Ekser, B., & Tector, A. J. (2015). A brief history of clinical xenotransplantation. *International Journal of Surgery (London, England)*, *23*(Pt B), 205–210. https://doi.org/10.1016/j.ijsu.2015.06.060

Cooper, D. K. C., Gollackner, B., & Sachs, D. H. (2002). Will the pig solve the transplantation backlog? *Annual Review of Medicine*, *53*, 133–147. https://doi.org/10.1146/annurev.med.53.082901.103900

Costa, C. (Ed.). (2020). *Xenotransplantation: Methods and Protocols* (Vol. 2110). Springer US. https://doi.org/10.1007/978-1-0716-0255-3

Cozzi, E., Schneeberger, S., Bellini, M. I., Berglund, E., Böhmig, G., Fowler, K., Hoogduijn, M., Jochmans, I., Marckmann, G., Marson, L., Neuberger, J., Oberbauer, R., Pierson, R. N., Reichart, B., Scobie, L., White, C., Naesens, M., & for ESOT Workstream 1 of the TLJ (Transplantation Learning Journey) Project. (2021). Organ transplants of the

future: Planning for innovations including xenotransplantation. *Transplant International*, tri.14031. https://doi.org/10.1111/tri.14031

Do Cyborgs Have Politics? (n.d.). Pax Solaria. Retrieved October 26, 2021, from http://www.paxsolaria.net/monthly/do-cyborgs-have-politics

Ebeling, M. F. E. (2016). *Healthcare and Big Data*. Palgrave Macmillan US. https://doi.org/10.1057/978-1-137-50221-6

Facts and Myths about Transplant. (n.d.). *American Transplant Foundation*. Retrieved October 19, 2021, from https://www.americantransplantfoundation.org/about-transplant/facts-and-myths/

French, M., & Monahan, T. (2020). Dis-ease Surveillance: How Might Surveillance Studies Address COVID-19? *Surveillance & Society*, *18*(1), 1–11. https://doi.org/10.24908/ss.v18i1.13985

Friedrich, J., Seidel, C., Ebner, R., & Kunz-Schughart, L. A. (2009). Spheroid-based drug screen: Considerations and practical approach. *Nature Protocols*, *4*(3), 309–324. https://doi.org/10.1038/nprot.2008.226

Garry, D. J., Goetsch, S. C., McGrath, A. J., & Mammen, P. P. A. (2005). Alternative therapies for orthotopic heart transplantation. *The American Journal of the Medical Sciences*, *330*(2), 88–101. https://doi.org/10.1097/00000441-200508000-00006

Horvath, P., Aulner, N., Bickle, M., Davies, A. M., Nery, E. D., Ebner, D., Montoya, M. C., Östling, P., Pietiäinen, V., Price, L. S., Shorte, S. L., Turcatti, G., von Schantz, C., & Carragher, N. O. (2016). Screening out irrelevant cell-based models of disease. *Nature Reviews Drug Discovery*, *15*(11), 751–769. https://doi.org/10.1038/nrd.2016.175

Howell, A., & Richter-Montpetit, M. (2019). Racism in Foucauldian Security Studies: Biopolitics, Liberal War, and the Whitewashing of Colonial and Racial Violence. *International Political Sociology*, *13*(1), 2–19. https://doi.org/10.1093/ips/oly031

Jagdale, A., Kumar, V., Anderson, D. J., Locke, J. E., Hanaway, M. J., Eckhoff, D. E., Iwase, H., & Cooper, D. K. C. (2021). Suggested Patient Selection Criteria for Initial Clinical Trials of Pig Kidney Xenotransplantation in the United States. *Transplantation*, *105*(9), 1904–1908. https://doi.org/10.1097/TP.0000000000003632

Kögel, J., Thiersch, S., Ludwig, B., Seissler, J., & Marckmann, G. (2021). What does it take to consent to islet cell xenotransplantation?: Insights from an interview study with type 1 diabetes patients and review of the literature. *BMC Medical Ethics*, *22*(1), 37. https://doi.org/10.1186/s12910-021-00607-5

MacKay, M., Colangeli, T., Thaivalappil, A., Del Bianco, A., McWhirter, J., & Papadopoulos, A. (2021). A Review and Analysis of the Literature on Public Health Emergency Communication Practices. *Journal of Community Health*. https://doi.org/10.1007/s10900-021-01032-w

Nath, S., & Devi, G. R. (2016). Three-dimensional culture systems in cancer research: Focus on tumor spheroid model. *Pharmacology & Therapeutics*, *163*, 94–108. https://doi.org/10.1016/j.pharmthera.2016.03.013

Porter, T. M. (1995). *Trust in numbers: The pursuit of objectivity in science and public life*. Princeton University Press.

Sawant-Basak, A., & Obach, R. S. (2018). Emerging Models of Drug Metabolism, Transporters, and Toxicity. *Drug Metabolism and Disposition: The Biological Fate of Chemicals*, *46*(11), 1556–1561. https://doi.org/10.1124/dmd.118.084293

Schoenrath, F., Falk, V., & Emmert, M. Y. (2021). Xenotransplantation in the era of a zoonotic pandemic. *European Heart Journal*, *42*(14), 1283–1285. https://doi.org/10.1093/eurheartj/ehaa1101

Sharma, A., Sebastiano, V., Scott, C. T., Magnus, D., Koyano-Nakagawa, N., Garry, D. J., Witte, O. N., Nakauchi, H., Wu, J. C., Weissman, I. L., & Wu, S. M. (2015). Lift NIH restrictions on chimera research. *Science (New York, N.Y.)*, *350*(6261), 640. https://doi.org/10.1126/science.350.6261.640-a

Szade, K., Zukowska, M., Szade, A., Collet, G., Kloska, D., Kieda, C., Jozkowicz, A., & Dulak, J. (2016). Spheroid-plug model as a tool to study tumor development, angiogenesis, and heterogeneity in vivo. *Tumor Biology*, *37*(2), 2481–2496. https://doi.org/10.1007/s13277-015-4065-z

The rise of surveillance medicine. (n.d.). 13.

Wang, W., Liang, Q., Nie, W., Zhang, J., & Chen, C. (2020). Biosafety Barrier to Xenotransplantation. In S. Miyagawa (Ed.), *Xenotransplantation—Comprehensive Study*. IntechOpen. https://doi.org/10.5772/intechopen.89134

Xenotransplantation: Science, Ethics, and Public Policy (p. 5365). (1996). National Academies Press. https://doi.org/10.17226/5365

Xenotransplantation_and_Risk_Regulating_a_Developi..._—_(Xenotransplantation_and_Risk).pdf. (n.d.).