Microdevice Enabling Long-term in Vitro Study of Biofabricated Constructs

(Technical Paper)

Comparing Animal Models and their Alternatives: Examining Ethical, Regulatory, and Technical Perspectives

(STS 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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Background

As research and innovation continue to push the boundaries of medicine and technology, the line between them has become blurred. People's lives have become dependent on these advancements. Approximately 200,000 people in the United States receive a pacemaker every year (Mond & Proclemer, 2011). Beyond implantable devices like pacemakers, hip replacements, and cochlear implants, synthetic tissues and models are also being developed. Artificial tissue models can be made from biomaterials, organoids, and organs-on-chips, which have been shown to be a viable option for modeling tissue and test drug therapies (Calandrini & Drost, 2022; Goh et al., 2015; Ma et al., 2021). Organoids are three-dimensional tissue cultures derived from stem cells (Kim, Koo, & Knoblich, 2020). Organs-on-chips are microfluidic devices that simulate the mechanics of natural tissue (Osório, Silva, & Mackay, 2021). Biomaterials are defined as any natural or synthetic material used to integrate, interface, or augment biological tissue (Nii & Katayama, 2021). One commonly used biomaterial in the field of tissue engineering is hydrogels, which are three-dimensional networks of hydrophilic polymer fibers. Within the field of biomedical engineering, hydrogels have various applications such as biosensors, drug delivery vectors, and matrices for cells within tissue engineering constructs (Chai, Jiao, & Yu, 2017).

Traditional hydrogels must be designed with precise dimensions depending on the application and have limited material properties (Hoare & Kohane, 2008). As a result, a new subset of hydrogels, called granular hydrogels, has been developed to overcome some of the pitfalls of traditional hydrogels. Granular hydrogels are composed of hydrogel microparticles (HMPs) that allow for injectable, self-assembling, shear-thinning, and self-healing properties (Riley, Schirmer, & Segura, 2019). These hydrogels are formed as the particles stack on top of one another. Their packing property leads to porosity, which promotes a more rapid exchange of reactants, nutrients, and waste (Loh & Choong, 2013). Granular hydrogels have also shown the

ability to improve cellular infiltration and subsequent tissue remodeling within living tissue, also known as in vivo (Riley, Schirmer, & Segura, 2019).

Due to the particles of the granular hydrogel not being crosslinked, meaning the particles are free-flowing rather than bonded together, they are dynamic in nature, making them difficult to study long-term in traditional cell culture environments. The hydrostatic pressures resulting from the exchange of media during cell culturing cause erosion of the HMPs, and as a result, the material becomes compromised and limits our ability to control its properties. This in turn limits our ability to integrate cells to create tissue and disease models. Cells have been successfully integrated into granular hydrogels, however, it has required very controlled and difficult-to-replicate environments (Muir et al., 2022). My capstone team will be working towards the creation of a microdevice that will enable researchers to harness the dynamic properties of granular hydrogels to mimic physiological environments.

Stabilizing Granular Hydrogels

The goal of my capstone is to create a device where small amounts, on the order of microliters, of a modified granular hydrogel can be kept stable, enabling long-term in vitro, or outside of living tissue, studies and integration of cells. My capstone project is a part of the BME 4063 curriculum, and I am working alongside fellow BME student Alex Burnside with Professor Highley as our advisor. The device will be composed of two parts: the main housing that will hold the granular hydrogel, and a mesh-integrated lid that will allow fresh media into the hydrogel. We will first design an initial prototype in Fusion 360 for the molds of the main housing and the lid. These designs will be 3D printed using stereolithography, which uses UV light to cure layers of the resin. To cast the device, we will be using a silicon polymer,

polydimethylsiloxane, more commonly known as PDMS. Beyond being inexpensive, PDMS is relatively easy to make, non-toxic, chemically inert, biocompatible, and clear, making it an ideal material for the needs of the project (Miranda et al., 2021). During the casting of the lid, a membrane will be placed over the mold. This membrane will then cure so that it is attached to the lid and creates a barrier between the main housing and the lid that will allow nutrients from cell culture media to pass through without displacement of the HMPs. Once the PDMS of the main housing is fully set, we will be using a plasma treatment to bond the device to a piece of glass, which will allow us to perform imaging techniques such as fluorescence microscopy to assess the structure of the material.

After the device is fully fabricated, the modified hyaluronic acid granular hydrogel will be injected into the well of the device. Then we will introduce cell culture media through the lid to ensure the device can withstand the pressures introduced during the swelling of the HMPs. As necessary, we will iterate on the design of the device to ensure the stability of the hydrogel. Once we have established that the hydrogel can be stabilized in the conditions of our device, we will introduce human vascular endothelial cells into the granular hydrogel which will be injected into the device. This will allow us to test the cell viability within the device and verify that it can be used to culture cells. If time allows, we plan to integrate additional fluidic channels and wells. Introducing such complexities would allow our model to represent more sophisticated interactions that are more representative of physiological environments. In the future, this technology may be used to help design and test in vitro tissues that could replace the use of some animal models in the development of therapeutic drugs.

Alternative to Animal Testing Models

Each year, approximately 50 million animals are used for research and development of therapeutic drugs in the United States alone (Animal Testing, n.d.). Due to their analogous physiological systems, biological similarities, and accessibility, animals have been the gold standard for testing. Additionally, because of their small size, shorter life cycle, and shorter gestation period, they offer an efficient means of testing drugs to see their potential adverse effects (Bryda, 2013). These animals are used to mitigate the health risks that humans face in clinical trials and to help prove a therapy's efficiency and safety.

While animals are still essential for the development of therapeutic drugs, there are promising in vitro technologies that have started to replace some animal tests (Doke & Dhawale, 2015). Toxicological tests are one area of interest for alternative, non-animal models and have seen success in preclinical screening because of their simplicity compared to other tests (Doke & Dhawale, 2015). Companies, such as Corning, have already developed systems that utilize in vitro technologies for high-throughput drug screening (Powell, 2018). Another company, StemoniX, has created microOrgan plates to test for drug neurotoxicity and cardiotoxicity (Powell, 2018). Furthermore, many large pharmaceutical companies, such as Johnson & Johnson and Merck, have adopted the 3Rs, replacement, reduction, and refinement, for animal use in research. The FDA also acknowledges the need for alternative testing models, with a separate division devoted to reducing animal testing (Erickson, 2022). Furthermore, there have been some alternative tests that the FDA has approved, mostly around skin irritations.

While the FDA has made some strides, the FDA still stands as one of the largest barriers to the mass adoption of alternative models. Due to regulations such as the FDA's Animal Rule, strict guidelines surrounding the use of animal models have further ingrained the systematic use

of animals in drug development (Singh & Seed, 2021). Even if the FDA completely shifted gears and wanted to transition from animal models to alternative ones, it would be an exceedingly slow and difficult task. For example, toxicology tests, which appear to be a relatively straightforward replacement, require 20 tests to assess any new substance (Festing & Wilkinson, 2007). As a result, even once these alternative tests are fully developed by engineers and researchers, they must go through the FDA or other regulatory agencies which must then write guidelines and protocols. Even if the FDA approves and creates the necessary protocols for alternative tests, large pharmaceutical companies are not likely to adopt them for years. Despite the fact that some alternative tests have been shown to be more economical, contract research organizations are most often used to take care of animal testing. This provides evidence that the systems already in place support the use of animal models. (Meigs et al., 2018). Large pharmaceutical companies have established protocols and methods for their drug development, and any transition will inevitably require more time, money, and result in hardships, even if it is beneficial in the long term. As a result of regulations, economics, established protocols, and a multitude of other factors, we can see the complexity and interconnectedness of animal testing in the scope of drug development.

Using actor-network theory, I will look into the relations and interplay between social groups like the FDA and manufacturing companies as well as technologies like alternative toxicology tests. The actor-network theory relies upon building a network comprised of nodes, that can either be human or nonhuman actors (Latour, 1992). Human actors are representative of individuals or social groups whereas nonhuman actors are composed of everything else, which can be artifacts, technologies, or concepts. While there is a clear distinction between human and nonhuman actors, the emphasis instead lies in that actors, regardless of their type, must impart

some influence on their system. By treating all actors as equals, you can strip away ideals from social or technological determinism and instead focus on the relations and effects between actors. These connections between nodes, which represent actors in the network, are defined as relationships. The true value in Latour's theory comes from the analysis of these relationships and how the actors impart their influence on the system. Latour defines several relationships between nodes that can be used in revealing the interconnections between actors. An artifact's *prescription* is what tells humans how they should act. For example, the *prescription* of a road is that the driver should steer the car in a particular direction. A *program of action* is the expected or intended action based on some artifact. The *program of action* of a road is to drive from one point to another. *Delegation* is the translation of some task or work from one actor to another. The action of moving from one place to another is *delegated* from the legs of a human to the car. *Discrimination* is a result of the physical form or other restrictions imposed on a human by an actor. For instance, the design of smart cars *discriminates* against the taller population who may not fit inside. Systems can be explored through these relations between nodes. It is important to acknowledge that systems are not static in nature. Instead, they are constantly evolving, shifting, and changing. Using Latour's theory, you can trace the effects of change and the influence of both social and technological factors over the development of a system. I will be using the actor-network theory to investigate how advancements in alternative models have affected the use of animals in the development of therapeutic drugs.

Methods

I will be using the actor-network theory to investigate how advancements in alternative models have affected the use of animals in the development of therapeutic drugs. In order to

gather data, I will conduct interviews to provide evidence of the relationships between various social groups as well as technologies. The primary interviews will focus on regulatory affairs specialists at large pharmaceutical companies such as Johnson & Johnson. I will ask them about the current processes regarding the development of therapeutic drugs and the role of animal testing. Additionally, I will ask them about any usage of alternative, in vitro technologies, and any plans to integrate other non-animal test models. My questions will also focus on asking about what the specific challenges are preventing a transition from animal models to in vitro models. This will allow me to determine what factors in the current system are reinforcing the use of animal models. Is it the lack of technical feasibility, the high cost of transitioning, regulations, or a combination of those and other factors?

Beyond large pharmaceutical companies, I will interview university researchers that focus on toxicology to gain a different perspective. The line of questioning will be similar, and my goal will be to get an understanding of the current usage of animal models for tests. I will ask about their implementation of in vitro tests rather than live animal ones. Furthermore, I will ask what is preventing them from transitioning to in vitro models. These interviews will provide information that I will be able to code for relationships like *prescription*, *delegation*, *program of action*, and *discrimination*, which can then be used to investigate the complex nature of the system surrounding the use of animal models and alternative models in therapeutic drug development.

Conclusion

Advancements in technologies have enabled researchers and engineers to create more physiologically accurate models of human tissue. However, the dynamic nature of these types of

materials makes them difficult to study under standard cell culture conditions and limits the impact of the technology. The goal of my capstone project is to create a device that will allow more complex and dynamic materials to be studied in vitro for long-term studies. The goal of my research is to explore the role of alternative models and how they have impacted the systematic use of in vivo models for testing therapeutic drugs.

References

Animal Testing and Experiments FAQs (n. d.). Humane Society of the United States. Retrieved October 26, 2022, from https://www.humanesociety.org/resources/animals-used-experiments-faq

Bryda E. C. (2013). The Mighty Mouse: the impact of rodents on advances in biomedical research. *Missouri medicine*, *110*(3), 207–211. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3987984/

Calandrini, C., & Drost, J. (2022). Normal and tumor-derived organoids as a drug screening platform for tumor-specific drug vulnerabilities. *STAR Protocols*, *3*(1), 101079. https://doi.org/10.1016/j.xpro.2021.101079

Chai, Q., Jiao, Y., & Yu, X. (2017). Hydrogels for Biomedical Applications: Their Characteristics and the Mechanisms behind Them. *Gels*, *3*(1), 6. https://doi.org/10.3390/gels3010006

Doke, S. K., & Dhawale, S. C. (2015). Alternatives to animal testing: A review. *Saudi Pharmaceutical Journal*, *23*(3), 223–229. https://doi.org/10.1016/j.jsps.2013.11.002

Erickson, B. E. (2022, August 2). US FDA seeks to slash animal testing. *American Chemical Society.* https://cen.acs.org/biological-chemistry/toxicology/US-FDA-seeks-slash-animal/100/i27

Festing, S., & Wilkinson, R. (2007b). The ethics of animal research. *EMBO Reports*, *8*(6), 526–530. https://doi.org/10.1038/sj.embor.7400993

Goh, J. Y., Weaver, R. J., Dixon, L., Platt, N. J., & Roberts, R. A. (2015). Development and use of in vitro alternatives to animal testing by the pharmaceutical industry 1980–2013. *Toxicology Research*, *4*(5), 1297–1307. https://doi.org/10.1039/c5tx00123d

Hoare, T. R., & Kohane, D. S. (2008). Hydrogels in drug delivery: Progress and challenges. *Polymer*, *49*(8), 1993–2007. https://doi.org/10.1016/j.polymer.2008.01.027

Latour, B. (1992). Where are the missing masses? The sociology of a few mundane artifacts. In W. E. Bijker & J. Law (ed.), *Shaping Technology / Building Society: Studies in Sociotechnical Change*, 225-258. The MIT Press.

Loh, Q. L., & Choong, C. (2013). Three-Dimensional Scaffolds for Tissue Engineering Applications: Role of Porosity and Pore Size. *Tissue Engineering Part B: Reviews*, *19*(6), 485–502. https://doi.org/10.1089/ten.teb.2012.0437

Kim, J., Koo, B. K., & Knoblich, J. A. (2020). Human organoids: model systems for human biology and medicine. *Nature Reviews Molecular Cell Biology*, *21*(10), 571–584. https://doi.org/10.1038/s41580-020-0259-3

Ma, C., Peng, Y., Li, H., & Chen, W. (2021). Organ-on-a-Chip: A New Paradigm for Drug Development. *Trends in Pharmacological Sciences*, *42*(2), 119–133. https://doi.org/10.1016/j.tips.2020.11.009

Meigs, L., Smirnova, L., Rovida, C., Leist, M., & Hartung, T. (2018). Animal testing and its alternatives – the most important omics is economics. *ALTEX - Alternatives to Animal Experimentation, 35*(3), 275–305. https://doi.org/10.14573/altex.1807041

Miranda, I., Souza, A., Sousa, P., Ribeiro, J., Castanheira, E. M. S., Lima, R., & Minas, G. (2021). Properties and Applications of PDMS for Biomedical Engineering: A Review. *Journal of Functional Biomaterials*, *13*(1), 2. https://doi.org/10.3390/jfb13010002

Mond, H. G., & Proclemer, A. (2011). The 11th World Survey of Cardiac Pacing and Implantable Cardioverter-Defibrillators: Calendar Year 2009-A World Society of

Arrhythmia's Project. *Pacing and Clinical Electrophysiology*, *34*(8), 1013–1027. https://doi.org/10.1111/j.1540-8159.2011.03150.x

Muir, V. G., Qazi, T. H., Weintraub, S., Torres Maldonado, B. O., Arratia, P. E., & Burdick, J. A. (2022). Sticking Together: Injectable Granular Hydrogels with Increased Functionality via Dynamic Covalent Inter‐Particle Crosslinking. *Small*, *18*(36), 2201115. https://doi.org/10.1002/smll.202201115

Nii, T., & Katayama, Y. (2021). Biomaterial-Assisted Regenerative Medicine. *International Journal of Molecular Sciences*, 22(16), 8657. https://doi.org/10.3390/ijms22168657

Osório, L. A., Silva, E., & Mackay, R. E. (2021). A Review of Biomaterials and Scaffold Fabrication for Organ-on-a-Chip (OOAC) Systems. *Bioengineering, 8*(8), 113. https://doi.org/10.3390/bioengineering8080113

Powell, K. (2018). Replacing the replacements: Animal model alternatives. *Science*, *362*(6411), 246–246. https://doi.org/10.1126/science.362.6411.246-b

Riley, L., Schirmer, L., & Segura, T. (2019). Granular hydrogels: emergent properties of jammed hydrogel microparticles and their applications in tissue repair and regeneration. *Current Opinion in Biotechnology*, *60*, 1–8. https://doi.org/10.1016/j.copbio.2018.11.001

Singh, V. K., & Seed, T. M. (2021). How necessary are animal models for modern drug discovery? *Expert Opinion on Drug Discovery*, *16*(12), 1391–1397. https://doi.org/10.1080/17460441.2021.1972255