Organs-on-a-Chip with Integrated Detection of Bioluminescence

(Technical Paper)

An Ethical Consideration of Organoid Technology

(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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Introduction

General Research Problem: Ensuring the Ethical use of Organoid Technology

How can organoid technology safely and ethically advance scientific research?

Organoids are emerging powerful technology systems that mimic the complexity of organs and provide an opportunity to accurately model human biology (Corrò et al., 2020). These three- dimensional (3D) systems were first expressed from intestinal stem cells and were shown to form 3D intestinal organoids by Sato et. Al in 2009. From there, organoids continued to evolve through the establishment of this technology in other biological systems. Organ-on-chips (OoCs) are 3D microdevices that model the structure, functionality, and behavior of specific tissues or organs using human cells. OoCs serve as a branch under the organoid technology field but have an added device fabrication component of incorporating microfabricated fluidic channels and microelectronics (Thakar et al., 2023). OoCs devices are currently used to replicate human organ functions in a controlled laboratory setting for drug testing and disease research.

The goal of the Technical Capstone project is to contribute to the development of OoCs in a clinical setting by enhancing the accuracy of bioluminescent monitoring instrumentation in the microfluidic device. The project will also develop computational tools to analyze circadian rhythms of intestines-on- a-chip over days to weeks while tracking gene expression of human tissues in micro-physiologic systems. At the conclusion of this project, the Capstone team will have developed an integrated OoC system for detection of bioluminescence for circadian rhythm analysis.

The STS research project will focus on the ethical consideration of organoid technology use, as the field is continuously changing and growing faster than regulations can manage. Organoid ownership and individual rights of organ donors must be considered when developing these products for commercialization. Reviewing the ideas of identification approaches in organoid use, commercialization considerations, and informed consent models of the OoCs, like what the technical project is developing, will help create an ethical framework to navigate this rapidly evolving landscape. The Capstone project enhances OoC technology for clinical applications, with a focus on bioluminescent monitoring and circadian rhythms, while the STS research project addresses emerging ethical challenges associated with organoid technology, offering a crucial outline for its responsible progression.

Technical Topic

Developing Organs-on-a-Chip for Real-Time Bioluminescence Detection to Measure Circadian Rhythms and Gene Expression of Human Tissues

How can an integrated organs-on-a-chip device provide a better physiological model for studying the structure and function of the intestinal epithelium?

Organs-on-a-chip is an emerging technology that recreates the physiology and activities of the human body. These microfluidic devices aim to surpass current *in vitro* models in accuracy and complexity as they contain networks of hair-fine microchannels for manipulating minute volumes. The organ serves to be miniature tissues grown to reside in the microfluidic chips, which can recapitulate one or more tissue-specific functions. These properties allow researchers to study these specific functions in a highly controlled environment and develop robust models that can potentially advance current medical treatments for a variety of pathophysiological diseases while maintaining cost efficiency and patient specificity. Current technology includes

intestinal organoids developed from human or mouse gut biopsies which provide a powerful 3-D model system for studying the structure and function of the intestinal epithelium and screening cellular responses to nutrients, microbes, and drugs. Human intestinal organoids, which are currently used for clinical research, lack the immune components needed to fully capture the complexity of human intestinal biology and diseases. Organ-on-a-chip microfluidic systems take these models one step further by integrating complex factors such as mechanical force and fluid flow to better recapitulate actual physiology (Leung et al., 2022).

Organ models often use bioluminescence analysis, via standalone incubating luminometers, due to its high signal-to-noise-ratio metrics, simple equipment integration, and broad applicability. Despite these benefits, they require tissue removal or additional biosensors. Specifically, biosensors are needed for continuous *in situ* monitoring of the status of the micro-physiological systems, over short or long-time frames and in an automated manner. Moreover, no current OoC technologies allow for *in situ* bioluminescence analysis (Leung et al., 2022). One of the goals of this project being to use OoC to measure circadian rhythms in tissues and cultures, real-time measurement of OoC is crucial. At present, all measurements require the removal of the chips from the OoC instrument and a separate device for post-processing measurement, resulting in an inefficient protocol. This project aims to address the current limitations in organoid technology by enabling real-time, in situ bioluminescence analysis within OoC systems to enhance the efficiency and accuracy of measurements.

There are two aims of this project: to integrate and optimize isolated functional components of the current OoC into a cohesive protocol and analyze bioluminescence data from a circadian timescale. For this project, the Caco-2 cell line is used to build the OoC

system and collect bioluminescence data. It is chosen for its ability to spontaneously differentiate into a monolayer of cells with many properties typical of absorptive enterocytes with brush border layer as found in the small intestine. This cell line is also modified to demonstrate luciferase activity for bioluminescence. Bioluminescence occurs when an enzyme, known as luciferase, oxidizes a small-molecule substrate, known as a luciferin (Adams & Miller, 2020). For the Caco-2 tissue cultures, a photomultiplier tube (PMT) system will be used to track bioluminescent oscillations of PER2:LUC, the gene modified to have luciferase activity, over a period of days to weeks. A PMT is an extremely sensitive light detection device that amplifies weak optical signals. It consists of a photosensitive cathode that generates electrons in response to incident photons, which are then multiplied through a series of dynodes, resulting in a highly amplified electrical signal proportional to the incoming light intensity. The addition of the PMT to the OoC is crucial for accurate bioluminescence detection. Due to the sensitivity of the PMT, all outside light must be blocked during experimentation (Kim et al., 2020). To optimize the function of the OoC, a light-blocker must be added to prevent light pollution of incubators and device from interfering with the *in-situ* monitoring of bioluminescence. Another subtask of this aim is to improve efficiency of power supply used to run OoC system by analyzing power requirements and consolidating electronics. After data is collected, another goal of this project is to focus on developing software for data analysis. A Python program can be written for data integration from the system that includes ease of analysis.

The listed aims will be implemented by testing the OoC system in the laboratory environment on human and mouse enteroids modified for bioluminescence and cultured on two different commercial Organs-on-a-chip systems. The data collected will be validated by data

collected from off-chip monitoring using a KronosDio luminometer, which is the device currently used for bioluminescence monitoring in this lab. The current OoC system used in the laboratory is functional but is still a network of complex components rather than an integrated tool, which is the primary goal of this project. By adding integrative capabilities to OOAC systems, the OoC system will enable cost-, time-, and labor-effective clinical research with vast potential applications to medical and translational discovery.

STS Research Project

An Ethical Consideration of Organoid Technology Use

How can organoid technology continue to be advanced while adhering to ethical principles and addressing privacy concerns?

Organoids are cells with stem cell potential that are incubated under 3D culture systems to aggregate by adhesion, self-organize, and differentiate into 3D cell masses with the corresponding organ tissue morphology. Organoids are remarkably similar parental cells that replicate and simulate their biological characteristics. Additionally, they can self-renew and self-organize, contain various cell types, perform some specific functions, and form spatial structures like those of in vivo organs. Organoids are effective models for studying the occurrence, development, and progression of diseases (Yang et al., 2020). Under this organoid technology umbrella, OoCs are generated from the combination of organoid technologies and organ-on-a-chip devices that hold potential for advancing disease research and drug testing. Overall, organoids hold promise for applications in disease modeling, drug testing, and personalized medicine. Bridging the discussion on the potential applications of organoids to the ethical considerations at the heart of this technology, it is important to recognize that at its core, all organoid technology is grown from cells and tissues obtained from human individuals (Shariati

et al., 2021). This connection between organoids and humans gives rise to concerns at the level of the organoids themselves and of the individual patient or donor.

Several informed consent models have been proposed and discussed for organoid research. Blanket consent, opt-out, broad consent, and specific consent represent distinct approaches to governing the future utilization of organoids in research. Specific consent involves donors agreeing to a particular research project, while blanket consent and opt-out entail open-ended consent for future tissue use. Broad consent falls in between, involving consent for a broader category of research without specific details, offering varying levels of control and information to participants while allowing them to refuse further scientific use of their biomaterial. Different consent models, such as specific, tiered, opt-in, and dynamic, typically require explicit participant consent for each scientific sample reuse. In contrast, broad, blanket, governance consent, and opt-out models allow samples to be reused for various research projects without recontacting the donor or providing specific project information. The various informed consent models proposed for organoid research offer diverse approaches to balance donor control and the scope of future research use, catering to different ethical considerations and levels of participant engagement (Lewis & Holm, 2022).

The commercialization of organoids also presents technologies become potentially patentable, third parties, aside from donors, might gain property rights over them. The commercial value of organoids is on the rise, attracting both public and private stakeholders, including pharmaceutical companies. This profit potential has the potential to drive scientific advancements, though it also raises concerns among patients about excessive commercial involvement. Making a profit from organoids raises concerns among patients, donors, and stakeholders as it creates tension between the altruistic motives of donors and the monetary

interests of commercial businesses. Donors provide tissue without personal benefit while commercial parties profit from organoids through property rights and sales. Therefore, fair benefit distribution must be developed address these concerns among commercial parties, researchers, donors, and other stakeholders. Profiting from patient-derived tissues is seen as ethically unfair due to their vulnerable position. Given these informed consent and commercialization concerns, it is imperative to revisit and refine current policies to create a more balanced approach that respects both the potential for scientific advancements and the ethical considerations tied to organoid technology development (de Jongh et al., 2022).

To begin creating a framework on the ethical consideration of organoid technology development, a review of existing literature on organoid technology and its applications is needed. Similarly, an analysis of the ethical frameworks and guidelines that currently exist in the field will be used to create ethical guidelines for the responsible advancement of organoid technology. Specifically, studying organoid development policy of major corporations and governments will be essential in understanding frameworks for privacy concerns and commercialization of this technology. Lastly, case studies from various patients and donor perspectives are required to include concerns that real stakeholders face as the field advances. This comprehensive approach will be facilitated through a systematic literature review, encompassing studies, reports, and documents related to organoid technology, its ethical considerations, and the policies of pharmaceutical and biotechnology companies, research laboratories and institutions, and governments.

Understanding the complexities of organoid technology is vital for stakeholders, shaping responsible progress and ethics in its development and use by researchers, donors, companies, and users. Through my STS research, I will create a framework that aims to bridge the gap

between innovation and ethical responsibility of organoids so that potential benefits of this technology are realized. A review of existing policies can identify potential areas for improvement. Similarly, engaging with the perspectives of diverse stakeholders will ensure the framework accounts for their concerns and needs.

Working at the intersection of medicine and engineering, Organs-on-a-chip present an enormous advancement in the field of biomedical research and therapeutic design. The OoC system will present a more efficient and streamlined method of analyzing intestinal tissue using real-time bioluminescence detection. As such, the technology of organoids and OoCs pose several ethical considerations. By carefully reviewing current policies and understanding the gaps in regulation of this technology, a framework can be created to responsible and transparent development, addressing potential risks, and promoting the ethical use of these innovative advancements in healthcare and research.

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