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OrChID-Bio: Organs-on-a-chip with Integrated Detection of Bioluminescence

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Organ-on-a-chip (OOAC) is an emerging technology that recreates human physiology and aims to surpass current in vitro models in accuracy and complexity. OOAC mimics the dynamic forces and mechanical stresses of tissues and organs in the human body using microfluidic devices that allow researchers to manipulate features of the OOAC environment. OOAC models often use bioluminescence analysis for data collection, but current methods require the chips to be removed from their microfluidic devices, disrupting data processing and additional post-experimental analysis, while also increasing risks of contamination. This research aims to create a novel method for in situ bioluminescence through OrChID-Bio, which stands for Organs-on-a-Chip with Integrated Detection of Bioluminescence. This device uses micro-photomultiplier tubes (µPMT) for real-time measurement of bioluminescence. Additionally, an integrated Python program for the OrChID-Bio system for data analysis was developed to analyze the raw data collected. To demonstrate the relevance and validate this method of using OOAC, experimental testing of the system measured bioluminescence and circadian rhythms as changes in flow rate and mechanical stretch were applied to the chips in different complexities of intestinal cells. Rotavirus and drug treatments were also separately applied to the system to observe differences from the control.

Organ-on-a-Chip | bio-luminescence

Introduction

Organ-on-chips (OOAC) are 3D microfluidic devices that model the structure, functionality, and behavior of specific human tissues or organs. OOAC incorporates microfabricated fluidic channels and microelectronics (Thakar et al., 2023). OOAC devices are currently used to replicate human organ functions in a controlled laboratory setting for drug testing and disease research. OOAC recreates the physiology of the human body as they contain networks of hair-fine microchannels for manipulating minute volumes and aim to surpass current in vitro models in accuracy and complexity. The organ serves as miniature tissues grown to reside in microfluidic chips, which can recapitulate one or more tissue-specific functions (Leung et al., 2022).

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 capture the complexity of human intestinal biology and diseases. OOAC microfluidic systems take these models one step further by integrating complex factors such as mechanical force and fluid flow to better mimic actual physiology. Our laboratory's intestine-on-a-chip system includes various epithelial cell types lining the gut, adjustable nutrient flow, an artificial blood supply, and mechanical stretch to replicate gut tissue features. These enhancements enable more accurate modeling of health and disease, speeding up medical treatment testing and development while reducing reliance on laboratory animals.

Bioluminescence is the production and emission of light by living organisms, typically because of a chemical reaction involving a light-emitting molecule that can be added to any tissue of interest (Adams Miller, 2020). 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. Unlike fluorescence-based methods, bioluminescent analysis does not require using an excitation light and has no risk of phototoxicity. In our laboratory, the bioluminescence is produced in the chips through luciferase, which is added to a rhythmically expressed gene of interest, such as the Per2 "clock gene", a core circadian clock gene. As the gene is expressed, the emitted light can be detected and recorded using a luminometer or in our case, the µPMT. Additionally, our laboratory most recently used furimazine to produce bioluminescence in chips.

Despite the benefits of using bioluminescence with OOAC, they require tissue removal from experiments 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 OOAC technologies allow for in situ bioluminescence analysis. At present, all measurements require the removal of the chips from the OOAC instrument and a separate device for post-processing measurement. Not only is this method inefficient, it also increases the likelihood of contamination during the transferring process. This project will address the current limitations in organoid technology by enabling real-time, in situ bioluminescence analysis within OOAC systems to enhance the efficiency and accuracy of measurements.

Last year's Capstone team constructed a functional circuit system that utilized a µPMT to count the photons generated by bioluminescent cells cultured in the OOAC. The emitted

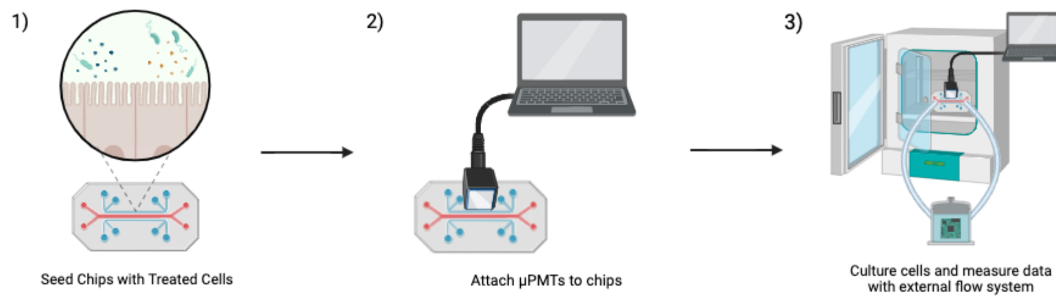

Fig. 1. OrChID-Bio experimental setup diagram, chip seeding followed by PMT application, followed by data collection and incubation.

photons have low levels of light intensity, requiring a high voltage (1000V) input into the µPMT and a low current input of approximately 0.6mA. A custom power supply system was designed, manufactured, and assembled to accommodate these requirements. It was decided that printed circuit boards (PCBs) would be the best foundation for the power supply. Two iterations of PCBs were designed using the software KiCad, both with the aim of delivering 1000V to the OrChID-Bio system. Iteration 1 contains a Bellnix OHV12-1.5K100N high voltage supply unit, paired with a RAC10-12SK AC/DC converter. The 1287-ST connectors and TB002 terminal blocks were all assembled and shipped by a manufacturer. Iteration 2 contains the same components as iteration 1, but with an added C10940-04 high voltage supply in lieu of the Bellnix Unit to fit the specifications of the circuit board.

Calibration of the µPMT was conducted via a 900V oscilloscope at 50 Ohm impedance and 1M Ohm termination. A reading of 0.48nA was recorded from the oscilloscope, indicating that the µPMT is fully functional while connected to the circuit systems. Spatial incompatibility was solved by hand sawing the plastic housing of the OOAC portable pod the plastic housing of the OOAC portable pod which allowed for the µPMT to be fully secured to the module with its photosensitive window positioned directly above the upper passage of the chip. The use and analysis of this data were intended to develop a baseline to which future work with integrated bioluminescence monitoring can be compared. The current Capstone team will develop the OrChID-Bio system to be fully functional and able to capture real-time bioluminescence data from different cell complexities.

The first aim of this project was to integrate and optimize the isolated functional components of OrChID-Bio into a cohesive protocol through several key tasks. First, the development of an integrated Python program for the OrChID-Bio system was developed to facilitate efficient data analysis. Additionally, the optimization of the power supply and electronics was carried out to enhance overall system performance as data was gathered. The second aimed to gather bioluminescence data on a circadian rhythm timescale from cells via the Emulate-integrated OrChID-Bio, Kronos-Dio, and Mimetas bioluminescence detection systems. This included utilizing a µPMT system to track bioluminescent oscillations of PER2:LUC over time and measuring bioluminescence and circadian rhythms while applying changes in flow rate and mechanical stretch to a cultured tissue model. Furthermore, the project will demonstrate the relevance of real-time bioluminescence monitoring across various cellular complexities, including Caco2 Per2:Luc cells, Per2:Luc mouse enteroids, Rotavirus samples, and human samples transfected with a luciferase reporter.

Results

The project had several different components associated with it, including a myriad of mediums through which data was collected. One of the main goals of this project was to see what was possible to accomplish with OrChID-Bio, so several different approaches were taken to push the system and determine its capabilities

Kronos-Dio System Analysis. In order to develop OrChID-Bio into an industry-leading technology, a comparable system had to be analyzed to determine data representation and analysis methods. Since the OrChID-Bio system is built from scratch, comparable data filtering and processing techniques would have to be developed to allow researchers to interpret the data. To accomplish this, experiments were set up on an existing bioluminescence collecting system, known as Kronos-Dio. Kronos Dio allows for cell cultures to be placed in a system in which bulk bioluminescence is captured over a time interval. A photomultiplier tube is present at the top of the pressure controlled chamber and collects data from dishes that move under the PMT in intervals.

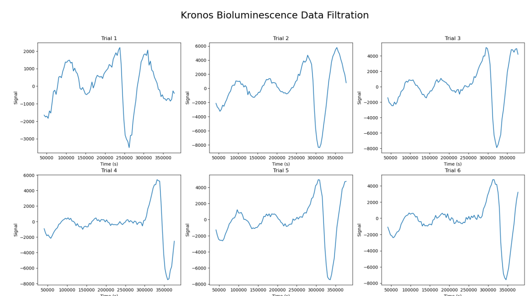

Fig. 2. Kronos Dio Caco-2 Cell culture data analysis, filtered through custom script and compared to commercial software

Data was collected with 6 different trials of Caco -2 cells transfected with Per2-LUC. In Figure 2 it can be seen that similar patterns can be seen in the signal data. The graphs

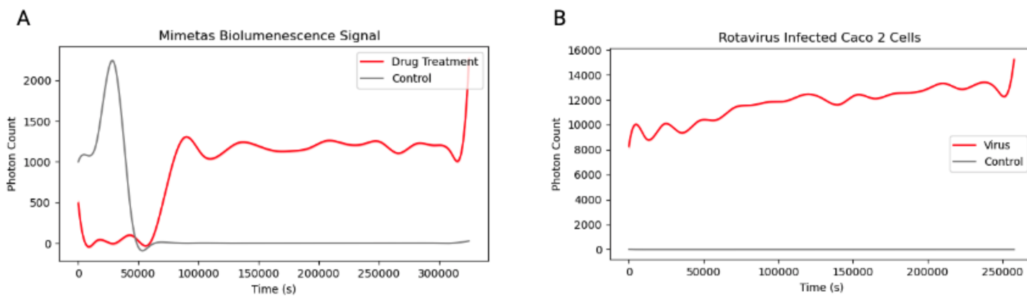

Fig. 3. A) Drug treated Caco-2 cells bioluminescence signal compared to the control (uninfected cells) cultured in Mimetas OOAC system B) Rotavirus bioluminescence compared to control cultured in Emulate system

produced were developed using a Python script that filtered and transformed the data to make it continuous and remove noise. A custom bidirectional rolling filter was utilized to smooth and connect the data. This allows for specific rhythmic trends to be found in the data that were comparable to the commercial filtering accomplished by the Kronos-Dio software package. This script allows for comparable results to be outputted by OrChID-Bio.

OrChID-Bio Data Collection. Testing the OrChID-Bio system required a significant amount of setup. The Emulate flow system was utilized for the initial data collection as the system was the most consistent in providing flow and had been tested previously. Two primary sets of data were collected, one set of Caco-2 cells that were treated with a GSK-3 inhibiting drug: CHIR99021, and a sample that collected data from rotavirus samples.

The purpose of the drug treatment was to see if any changes could be seen in the circadian rhythms between control and drug-treated enteroids. After applying the analysis software developed, it could be seen that drug treatment had a significant signal where the control did not. This made it difficult to draw specific conclusions regarding phase shifts, signal amplitude, and other changes. The initial spike seen in the control data was attributed to the opening and closing of the incubator. Due to the current set up, light pollution was a significant issue in the system and caused spikes in the data. The reasoning behind the lack of continued signal in the control sample could be attributed to overstimulation of the sensor from the spiking, causing the lower amounts of light to not be collected, requiring calibration of the system. This will have to be further investigated to determine the exact cause. When looking at the drug treatment data, a pattern is distinguishable, indicating that circadian rhythms can be measured using the system, and with a control trial comparisons would be able to be made. The chosen drug, a GSK-3 inhibitor, has been found to affect clock genes which directly relate to circadian rhythms. With further development, the exact changes to clock gene expression could be determined by mapping the data.

The second test that occurred involved a rotavirus culture, which is also expected to affect circadian rhythm expression. As seen in Figure 3 B, a similar issue occurred in which the control did not seem to pick up the signal. The signal is still expected in the control sample, and no signal indicates a problem with the placement of the µPMT or transfection of the luciferase. When it comes to the rotavirus signal, bioluminescence was detected immediately and steadily increased. A comparison would be needed to draw any meaningful conclusions in terms of trends but the signal strength is present which is promising.

Mimetas Chip Testing. As OrChID-Bio is meant to be a new data collection method, it should ultimately be able to be applied in many different scenarios. Originally, Emulate system compatible chips were used to measure data, but other organ-on-a-chip systems exist as well. They have several differences, such as size, fluid mechanic systems, and number of samples in one system. Another common system that is used in the field is the Mimetas OOAC system. Mimetas has significantly smaller chips, but still preserves the two-channel system, as shown in Figure 4, found in Emulate as well. The smaller chip size allows the over all form factor to be reduced, fitting more chips into the same form factor. Another difference in the Mimetas system compared to Emulate is the application of fluid motion and mechanics. In the Emulate system, fluid is pushed through the chip constantly at a speed on the scale of 30 µL/hr, but Mimetas implements a rocker based system that tilts the entire platform to move the fluid from one side to the other in the channel. This is not as optimal as Emulate, since physical forces cannot be tested as easily, and media cannot be changed within the chips without taking the system out of incubation, but the entire system allows for a 10-fold increase in potential samples.

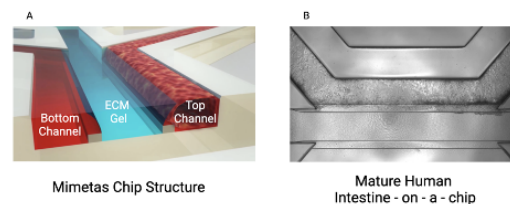

Fig. 4. A) Mimetas chip internal structure with two channels and ECM gel in the center B) Day 9 of human intestinal enteroid growth in Mimetas chip

As seen in Figure 4 B, the chip is able to grow intestinal organoids the same was that is possible in Emulate chips. When attempting to apply OrChID-Bio to this system, several problems were brought up. The chip size is significantly

smaller, so it is more difficult to place the µPMT on the correct channel and be sure that a signal can be collected. Since the system is placed in an incubator, and the entire plate is tilting in time intervals, the system had to be set up in a way that the cables would not get caught due to the motion, which was not something that had to be taken into consideration with the Emulate system. An advantage over Emulate was that more samples could be cultured with relative ease, meaning better samples could be chosen when choosing which chips to use for data collection. When performing data collection, unfortunately no samples were found to have positive results, due to the loss of signal in trials and issues with the software. However, the system was able to run correctly, and with proper setup, viable data can be collected showing the OrChID-Bio can be applied to other organ-on-a-chip systems

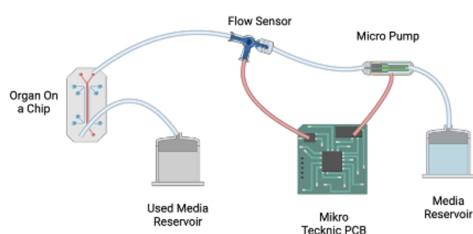

Fig. 5. Microfluidic system developed using the MikroTechnik Multi board with micro pumps capable of pushing liquid or gas at differing rates on the uL/s scale and a flow sensor able to capture speed and temperature of the fluid in system

MicroFluidic System Development. Organ-on-a-chip microfluidic systems can be expensive and proprietary, meaning any issues in the system can delay data collection and processing. The experiments performed can be expensive and resource-intensive, so losing trials can be very harmful to the research being conducted. To solve this, a low-cost microfluidics system was assessed and built to see if it could replace the current Emulate system fluidics system. A barebones microfluidics microcontroller was found called the MP-Multiboard II from a company known as Bartels MikroTechnic. The board has the capability of driving 6 pumps with variations in speed and intensity. Several drivers are provided with the board for certain pump requirements, a high driver, low driver, and multi-driver. The drivers determine the speed and number of pumps that can be connected. The pumps chosen were also provided by the same company and worked easily with the board. The Sensirion SLF3s flow sensor was connected to the system as recommended by the manufacturers. The Bartels software was used to adjust pump speed and flow, through two primary metrics, amplitude and frequency of the pump. Both of these parameters had to be tested to determine how they affect pump speed, and it was seen that increasing amplitude played a larger role than increasing the frequency of the pump. Further testing would have to be performed to determine the exact relationship between the two parameters. The flow sensor was connected to Sensirion software to get the most accurate results. At high speeds, flow reading were accurate along with temperature readings, but at lower speeds, the resolution of the sensor was not optimal. The lowest that could be measured was on the scale of 90 µL/min which is significantly higher than the needed µL/hr resolution. The sensor would show negative values when brought to a lower speed which is not ideal. Calibration will be needed to make the sensor fill the needs of the system. A diagram can be seen in Figure 5.

Discussion

OOAC testing results. The real-time testing of enteroids successfully demonstrated the detection of circadian rhythms using bioluminescence detection with µPMTs, confirming the potential viability of the OrChID-Bio system for monitoring OOAC cultures. OrChID-Bio serves as a platform-agnostic add-on capable of integration with various OOAC platforms and this project demonstrated its validity as a proof of concept. The study also observed changes in signal detection between samples under drug treatment. It was noted that viral infections could also impact circadian rhythms, emphasizing the need for a more robust baseline to draw specific conclusions. Custom fluidic systems could be developed to replace Emulate and offer increased flexibility, as was done with the microfluidic system employed in this project. Future efforts will focus on expanding data collection to enable comprehensive statistical analysis.

Impact. The successful real-time testing of enteroids demonstrated the detection of circadian rhythms using bioluminescence detection with uPMTs and affirmed the potential viability of the OrChID-Bio system for monitoring OOAC cultures as well as reducing the risk of contamination. Specifically, OrChID-Bio showed changes in signal detection between the control and experimental group(s) for drug and Rotavirus treatments that affect baseline circadian rhythm. Additionally, the custom fluidic system we developed to replace Emulate allows for more flexibility at a lower cost for the lab. Future work can be conducted using this cheaper setup. All these features have wide implications for translational discovery and can bring about more robust organ models, ultimately driving the development of new disease treatments.

Limitations and Future Work. A major limitation of this project was the long supply chain delays for important components. There were several instances where work could not be completed due to running out of media in the lab. Media took around one to two weeks to restock. Additionally, older µPMTs had to be replaced, and these replacements took weeks to arrive. This meant that the already low throughput from the experimental trials was further limited. Licensing issues arose while using LabVIEW, so obtaining a licensing key delayed lab work as well. The computer used to run the Hamamatsu software and analyze luminescence data would constantly crash, resulting in the loss of days worth of data. Ordering a new laptop capable of handling the experiments took several weeks. Despite the various sources of delays, the OrChID-Bio system was successfully tested with various treatments like flow rate changes, mechanical stretching, the Rotavirus, and drugs to show the system's ability to detect bi-

oluminescence. However, further testing of the system is required to confirm these initial results as throughput was low. The next steps for OrChID-Bio is to begin full-scale experimental testing and validation at a higher throughput. Currently, the experiment can only accommodate two chips at once, the control and the experimental group, meaning it is difficult to gather data with this system for more quantifiable analysis. By expanding the number of chips from two to four, data being collected can be doubled for better result validation. Another future goal is to integrate a custom microfluidic system into OrChID-Bio. This system, developed using the MikroTechnik Multi board, features micro-pumps that can handle liquid or gas flow at various rates on the µL/s scale. It also includes a flow sensor that captures both the speed and temperature of the fluid in the system. Finally, OrChID-Bio can eventually be used to monitor real-time bioluminescence in other types of organ-on-chips—such as lung, heart, etc—once the system has been further tested on intestine-on-a-chip.

Materials and Methods

Equipment. For this project, OOAC from the EmulateTM Human Emulation System, which involves the individual organ-chips, portable pod module, Zoë-CM2™ Culture Module, and an Orb Hub Module, were used as the foundation for prototype design and system construction. The µPMT chosen for this project was the Hamamatsu model H12400-00-01. This µPMT features a bialkali photocathode optimized for the detection of light between 300 nm and 650 nm in wavelength and outputs photon counts via a cable unit. A Hamamatsu Photon Count Unit C9744 was used to register the µPMT readings, and the outputs of that photon count unit are processed by a Hamamatsu Count Unit C8855-01. The use of the C8855-01 unit led to the use of the Hamamatsu C8855-17 computer software for system control and count recording. The pump system was developed primarily using materials from Bartels MikroTechnik.The MP-Multiboard II was used along with their MP6 micropumps. Tubing was purchased from them along with the utilization of their software to control the pumps. The flow sensor used was the Sensirion SLF3s flow sensor which was purchased from Sensirion.

Culturing. Crypts, which are invaginations in the intestinal epithelium, were extracted from the jejunum of Lewis rats. These isolated cells were then embedded in Matrigel, a medium that supports their growth and differentiation, and cultured for five days. After this period, they were transferred to a microfluidic device. To preserve the integrity of the enteroids, a cell recovery solution was used before their suspension was injected into the top channel of the Emulate device. Murine media were added to the bottom channel to promote confluency within the microfluidic system.

Data Processing. Data processing was primarily accomplished using Python 3.13. Packages utilized include Scipy.signal and Pygam. A bidirectional rolling window filter was developed to filter data and connect continuous values. The detrend method from scipy along with the savgol filter were also used to filter data and find appropriate trends for circadian rhythms.

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