ORCHID-BIO: ORGANS ON A CHIP WITH INTEGRATED DETECTION OF BIOLUMINESCENCE

A Technical Paper submitted to the Department of Biomedical Engineering
Presented to the Faculty of the School of Engineering and Applied Science
University of Virginia • Charlottesville, Virginia
In Partial Fulfillment of the Requirements for the Degree
Bachelor of Science, School of Engineering

By

Samir Muhammad

May 7, 2023

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.

ADVISOR
Sean R. Moore, Department of Pediatrics

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

Georgia Brousseau, Samir Muhammad, A Vinicios Alves Da Silva, Sean R. Moore

Word Count: 2752
Number of Figures: 7
Number of Equations: 0
Number of Supplements: 0
Number of References: 5

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

Georgia Brousseau[1], Samir Muhammad[1], Dr. A Vinicios Alves Da Silva[2], Dr. Sean Moore[2]

[1]Department of Biomedical Engineering, University of Virginia

[2]Department of Pediatrics, University of Virginia

Abstract

Organs-on-a-chip (OOAC) are an emerging modeling technology which recreate the dynamic forces and activities of the human body, emulating organ physiology in a realistic manner that surpasses conventional *in vitro* models, such as 2D cell culture and 3D organoids, in both accuracy and complexity. Bioluminescence monitoring is one method of using OOAC, but to gather such data the chips must be removed from their microfluidic device. This decreases the advantages of OOAC, renders real-time measurements impossible, and increases the risk of contamination. In response, this research investigates the combination of OOAC and micro-photomultiplier tubes (μPMTs) in order to create a novel device called OrChID-Bio, which stands for Organs-on-a-Chip with Integrated Detection of Bioluminescence. In pursuit of a functional prototype, the team developed multiple iterations of the printed circuit board required to power the μPMT and conducted verification and calibration testing on the power system. Alterations of the commercially available OOAC were made to enable compatibility between the μPMT and bioluminescent chips, and a final prototype connecting all necessary system components was developed. Additionally software options for processing recorded photon counts were investigated, culminating in the selection of the R package *Waveclock*. The team was not able to monitor bioluminescence in the cultured intestines-on-a-chip via the OrChID-Bio system due to time constraints, so future work will focus on performing full-scale experimental testing of the prototype.

Keywords: organ-on-a-chip, bioluminescence, microphysiological system

---

Introduction

Organs-on-a-chip (OOAC) are an emerging technology which recreate the dynamic forces and physiology of the human body to provide a detailed description of the behavior of the cells that comprise tissues and organs. For example, the intestine-on-a-chip system in our laboratory contains: 1) multiple types of epithelial cell that line the gut, 2) adjustable nutrient flow, 3) an artificial blood supply, and 4) mechanical stretch to induce structural features in the OOAC that mimic those found in living gut tissue. These features allow for more robust modeling of health function and disease, which, in turn, accelerates the testing and development of new medical treatments and reduces the need for laboratory animals. Citing their potential to improve lives and transform

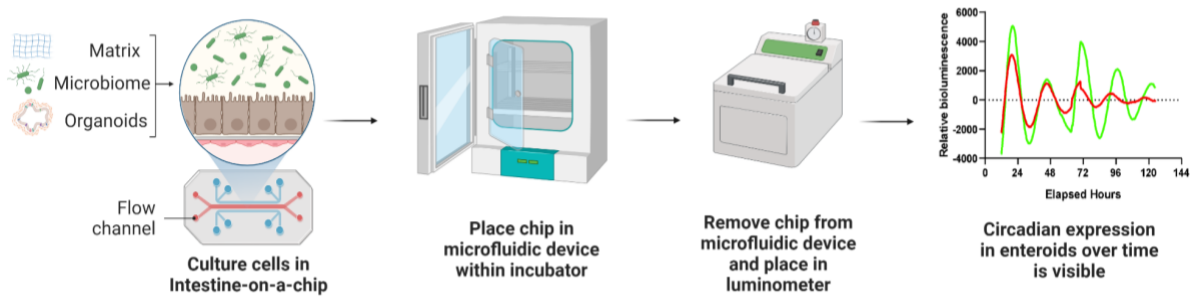

Fig. 1. Current Methodology. Intestines-on-a-chip are cultivated in a microfluidic device before being removed into a luminometer where bioluminescent oscillations are measured

medicine, OOAC were ranked 6th among the "top ten emerging technologies" by the World Economic Forum in 2016 [1]. According to Allied Market Research, the global organ-on-a-chip market was $103.4M in 2020 and is expected to reach $1.6B in 2030, a growth rate of 31% from 2021 to 2030 [2]. The vast potential of OOAC technologies has also recently been recognized by the U.S. Food and Drug Administration, as on December 29th 2022 the FDA Modernization Act 2.0 was signed and enacted into law by President Biden [3]. This new act amends the Federal Food, Drug, and Cosmetics Act and updates the FDA regulations to no longer mandate animal testing for drugs and biosimilars, and both allow and promote the use of alternative human-relevant testing methods — including OOAC microphysiological systems.

One method of obtaining and analyzing data from OOAC is via bioluminescence monitoring. Luciferase is an enzyme which emits light when exposed to the compound luciferin [3]. The type of bioluminescent analysis addressed by this project involves conjugating luciferase to a rhythmically expressed gene of interest, such as the *Per2* "clock gene" which is a core circadian clock gene. As the gene is expressed by the cells being monitored, the light emitted can be registered and recorded by a luminometer. Unlike fluorescence-based methods, bioluminescent analysis does not require the use of an excitation light and has no risk of phototoxicity. Bioluminescence is also a particularly attractive monitoring method because it has a high signal-to-noise ratio, is widely applicable, and is relatively efficient [4].

All these factors make bioluminescent analysis compatible with OOAC systems, and it has been utilized as an analysis method for various areas of physiology-on-a-chip research. While the rhythm-tracking benefits of integrated bioluminescence analysis was the primary interest for this project, it is an extremely versatile tool which can be used to monitor many biological processes of interest. The current process for bioluminescent analysis of OOAC is outlined in Figure 1, and has been used to verify that intestinal organoids cultivated in OOAC display robust bioluminescence and circadian rhythms. However, a major problem is that real-time, *in situ* measurement of bioluminescence in OOAC is currently impossible. This is because monitoring bioluminescence in OOAC requires the chips to be removed from their microfluidic device and placed in a separate photo-luminometer which lacks the dynamic factors which give OOAC their advantages. Such a transfer also increases the risk of contamination.

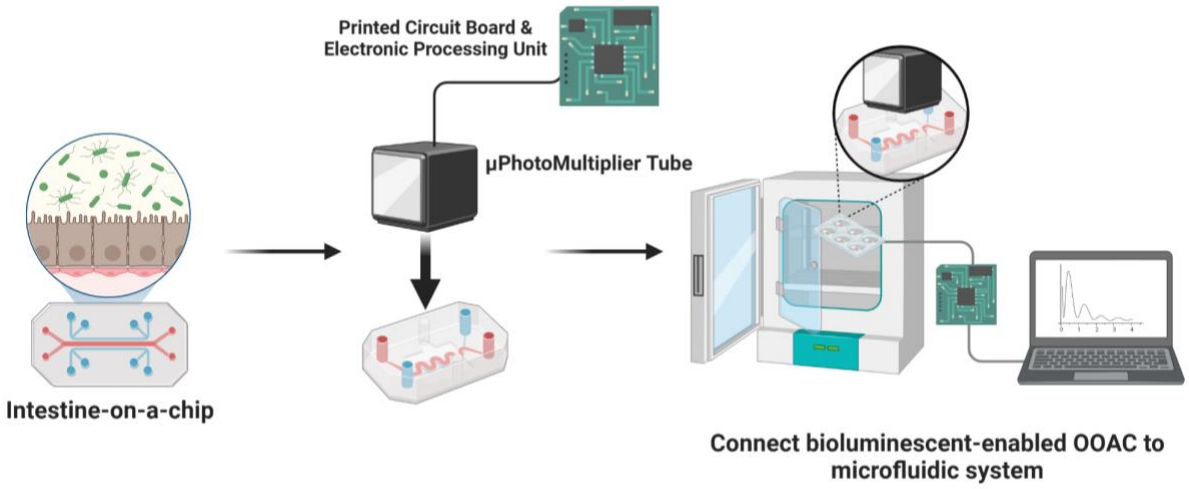

Fig. 2. Integrated Bioluminescent Analysis of OOAC. A µPMT is mounted on the OOAC system and bioluminescence monitoring occurs without removal from the microfluidic device to track oscillations in real-time.

In order to remediate those difficulties and improve the application of bioluminescent monitoring to OOAC technologies, this project aimed to create a novel, hybrid instrument which integrates a microscale photomultiplier tube onto an OOAC. Such a device enables real-time, *in situ* bioluminescent monitoring and analysis and layers on the "fourth dimension" of time that is absent from current methodologies. By adding integrative capabilities to OOAC systems, OrChiD-Bio will enable cost-, time-, and labor-effective clinical research with vast potential applications to medical and translational discovery. Figure 2, above, demonstrates the general conceptual set-up for OrChID-Bio, in which the bioluminescence-enabled OOAC system can be placed within an incubator where the data is collected and oscillations recorded over time.

The approach for this project was to combine a Hamamatsu™ microscale photomultiplier tube (µPMT) with the Emulate™ Human Emulation System, which involves the individual organ-chips, portable pod module, and a culture module [5]. In that system, the portable pod module houses the organ-chip, contains media and effluent, and allows for fluid control. The culture module controls the chips' microenvironment, enabling adjustments of fluid flow and mechanical stretch to recreate the dynamic forces which are present *in vivo*. In order for the µPMT to function properly, it must receive the correct power supply and connect to separate photon-counting and counting units which are also developed by Hamamatsu. The photon-counting unit directly receives the pulse output of the µPMT, while the counting unit processes the readouts from the photon-counting unit and sends the output to the graphical user interface of the Hamamatsu software.

With this approach, the µPMT is used as an embedded biosensing system at the small scale required of OOAC. This combination of elements will thus be compatible with existing OOAC devices and allow for real-time measurements and automated analysis of bioluminescence. In order to enable completion of the project, a list of specific aims was created.

The first aim of this project was the construction of a bioluminescence-enabled organ-on-a-chip microfluidic device. This aim was divided into two sub-aims, the first

of which was the construction and calibration of a functional circuit system compatible with the µPMT. We proposed to accomplish this by designing, manufacturing, and assembling a printed circuit board, to meet the specific power supply requirements of the µPMT. The second sub-aim was testing and calibration of the µPMT. Additionally, the constructed power supply system had to be tested and calibrated before being connected to the µPMT in order to verify outputs and ensure compatibility.

Our second aim was to use the integrated PMT system to bioluminescently monitor oscillations in PER2:LUC enteroids. One key element of this aim was to actually cultivate the PER2:LUC enteroids in Emulate OOAC to create bioluminescent intestines-on-a-chip. Another important consideration was determining a data-processing workflow which was compatible with both the current standard methodology and the readout format of the OrChID-Bio system.

The successful creation of a system for integrated detection of bioluminescence in OOAC as discussed in the outlined approach and specific aims will improve the ease and accuracy of measurements from microphysiological systems, enabling the application of these technologies to enhance medical and scientific research.

Results

µPMT Power Supply

The OrChID-Bio device utilizes a microPMT to count the photons generated by bioluminescent cells cultured in the OOAC. The emitted photons have low levels of light intensity, requiring a high voltage input into the µPMT. However, the µPMT also requires a very low current input of approximately 0.6mA. In order to satisfy these very specific requirements a custom power supply system was designed, manufactured, and assembled.

Based on the requirements of the intended OrChID-Bio system design, 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, as seen in Figure 3, contains a Bellnix OHV12-1.5K100N high voltage supply unit, paired with a RAC10-12SK AC/DC converter. Along with 1287-ST connectors and TB002 terminal blocks, all parts were assembled and shipped by the outside manufacturer Advanced Product Design & Manufacturing, Inc (APD&M).

Iteration 2 contains many of the same components as the first iteration, including the connectors and terminal blocks. The AC/DC converter was slightly changed to deliver an output voltage of 5V to fit the specifications of the circuit board. A main difference between the two iterations is that Iteration 2 contains a Hamamatsu C10940-04 high voltage supply in lieu of the Bellnix unit.

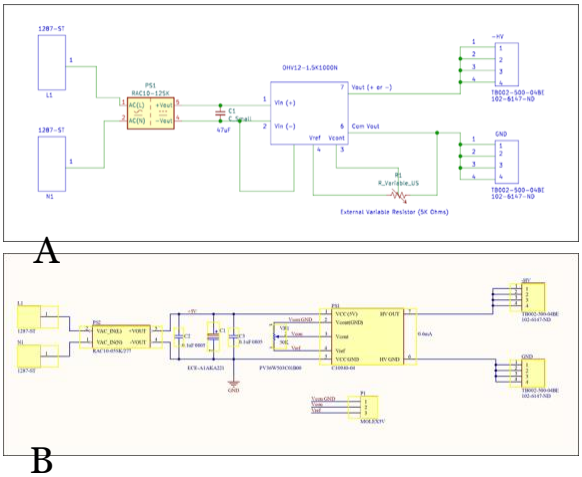

Fig. 3. PCB Schematics
(A) Iteration 1 (B) Iteration 2

Testing of the final PCBs was conducted to ensure proper voltage output and to calibrate

future readings from the µPMT. A high voltage multimeter recorded measurements from both iterations of the PCB, each confirmed to deliver ~1100V - exceeding the required amount - to the µPMT.

Calibration of the photomultiplier tube was conducted via a 900V oscilloscope at 50 Ohm impedance and 1M Ohm termination. At 900V, the dark current expected from an H12400 µPMT is 0.3nA per the Hamamatsu datasheet. A reading of 0.48nA was recorded from the oscilloscope, indicating that the µPMT is fully functional while connected to the circuit systems.

OOAC Modules

Another aspect of the project was modification of the OOAC module system for physical compatibility with the µPMT units. The photosensitive area on the Hamamatsu µPMT assembly is very small, only 1 x 3 mm, but the assembly itself is slightly larger. Indeed, the plastic housing of the assembly of the µPMT renders it slightly too large for the Emulate OOAC pod module with which our design combined it.

Fig. 4. Portable Pod Module with µPMT

In order to solve that spatial incompatibility, the plastic housing of the OOAC portable pod module was cut using a handsaw and associated equipment accessed via the BME Fabrication Lab in Stacey Hall. This adjustment did not compromise the integrity of the OOAC system but allowed for the µPMT to be fully secured to the module with its photosensitive window positioned directly above the upper passage of the chip.

In preparation for testing the OrChID-Bio system's ability to actually register and record bioluminescence from OOAC, murine enteroids were cultured in the Emulate OOAC. Testing with these enteroids-on-chips was ultimately not possible due to time constraints arising from PMT and supply chain issues, but images of the early stages of their development were obtained via brightfield microscopy, Figure 5.

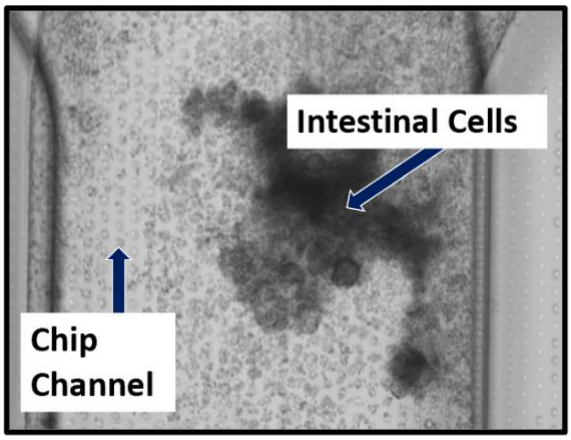

Fig. 5. Murine Enteroid-on-a-Chip

Data Processing

Another phase of the project that was performed in anticipation of full bioluminescence monitoring was the processing and analysis of photon count data. Since assembly delays and time constraints meant that luminescence data could not be collected from the OrChID-Bio assembly, data was collected from intestinal organoids monitored using the conventional methodology. The use and analysis of this data was intended to develop a baseline which future work with integrated bioluminescence monitoring can be

compared to. Thus, luminescence data points from a Kronos-Dio luminometer were processed using the *Waveclock* package in R. Continuous wavelet transforms were performed in order to quantify bioluminescent oscillations, and results were consistent with those established in the literature.

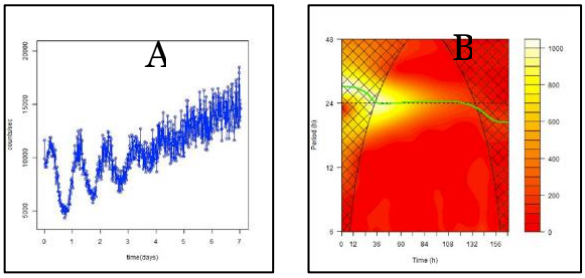

Fig. 6. Bioluminescence Plots
(A) Chart of enteroid luminescence, quantified as number of emitted photons, versus time. (B) Wavelet scalogram illustrating a modal frequency (green line) of 24 hours, which is consistent with the circadian rhythms expected in PER2:LUC enteroids.

Final System Construction

After each of the OrChID-Bio system's components was chosen, designed, and evaluated as appropriate, the final system's proof-of-concept was constructed, as is visible in Figure 7. In this setup, the PCB is connected to a conventional power outlet, and that power is multiplied via the circuit's high voltage module to provide the correct power supply to the µPMT. The µPMT is mounted on the OOAC pod module and uses a coaxial cable to deliver luminescence readings to the photon count unit. The photon counting unit is connected to and controlled by a counting unit which receives commands from the laptop's C8855-17 software.

Each individual element of this assembly was verified to be functional, and the cables and wires chosen for all points of attachment are compatible with use within an incubator or incubating luminometer. While Figure 7 as included here portrays the set up outside on a laboratory bench, in an experimental scenario the OOAC pod system with the µPMT would be placed within the microfluidic culture module located inside an incubator.

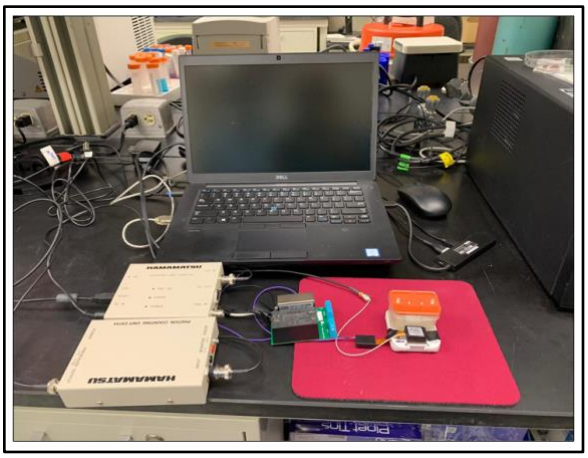

Fig. 7. OrChID-Bio Assembly

Discussion

PMT System Testing

Results gathered from the high voltage multimeter and oscilloscope suggest that the OrChID-Bio system is operational and can be used for *in situ* testing. As both the voltage and current exceeded the required values for a functional system, these results indicate that the integrity of both components, the Emulate organ-on-a-chip and µPMT, remain intact when combined. Due to supply chain delays in receiving components and time restraints, the OrChID-Bio system was not used to record bioluminescent oscillations from enteroids seeded onto the OOAC device as originally planned. However, system testing concludes that the device has the potential to measure photon emission from cultured cells inside the incubator.

Impact

The project provides a proof of concept indicating that microscale luminometers are compatible with commercially available organ-on-a-chip systems. Additionally, the integration of a µPMT onto OOAC technology was shown to not compromise

the functionality of either system, including the circuit boards. Following future testing with live cultures, the OrChID-Bio system could potentially monitor bioluminescent expression emitted by the cells seeded onto the chips. The significance of the device lies in the ability to record *in situ*, real-time, measurements, all while reducing the risk of contamination and the use of animal models. This has wide implications for translational discovery and can bring about more robust organ models, ultimately driving the development of new disease treatments.

Limitations & Future Work

A major limitation of this project was the unanticipatedly long lead times and supply chain delays for crucial components. The supplies from Hamamatsu are developed in and shipped from Japan, which resulted in several month periods between ordering and receipt. These delays meant that work with the full µPMT system was restricted to a limited period of time. The switch between iterations of the PCBs was necessitated by issues with obtaining components in a timely manner, but even with the alterations obtaining the power system took much longer than originally anticipated. Because of these constraints, there was simply not enough time to conduct full experimental testing of the completed system with active, bioluminescent enteroids-on-chips. Additionally, this meant that the suitability of the *Waveclock* data processing workflow could not be fully verified for use with OrChID-Bio.

The primary hindrance to the conclusions of this study is thus the lack of further testing with the system to show its ability in detecting bioluminescence. Results indicate that the device is capable of measuring photon emission, but future research is needed to prove this notion.

The next step for OrChID-Bio is thus to begin full-scale testing and experimental validation. In order to directly compare the photon counts registered by the µPMT to those recorded by a conventional incubating luminometer, the OrChID-Bio assembly should be placed within a conventional incubating luminometer and the OOAC monitored. This phase of testing should confirm that the photon detecting capabilities of OrChID-Bio are comparable to those of traditional luminometers. Once that is complete, OrChID-Bio can be used to monitor bioluminescence in real-time within the Emulate microfluidic instrument without the need for any conventional luminometer. This work can also be expanded upon to investigate real-time, *in situ* bioluminescence monitoring and analysis of other types of organoids-on-chips and to develop compatibility with OOAC manufacturers other than Emulate.

Materials and Methods

Equipment

For this project, OOAC from the Emulate™ 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.

Circuit Board Fabrication

The PCBs were assembled and manufactured to provide power to the µPMT. The schematics for each iteration of the were developed using the KiCad software suite,

which is freely available online. Once its schematic was created, Iteration 1 was sent to APD&M to be manufactured and assembled via their production facility. Iteration 2 of the PCB was manufactured via an on-demand PCB printer. Soldering and component assembly was performed with assistance in the UVA Link Lab.

Enteroid Cultivation

Crypts, invaginations of the intestinal epithelium, were isolated from the jejunum of Lewis rat models. The isolated cells were embedded into Matrigel to provide an environment that promotes differentiation and growth, and were cultured for five days before being seeded onto the microfluidic device. Following the use of cell recovery solution to ensure the integrity of the enteroids, the suspension was injected into the top channel of the Emulate device with murine media in the bottom channel to encourage confluency within the microfluidic device.

End Matter

Author Contributions and Notes

Brousseau, G., Muhammad, S., Moore, S. and Alves da Silva, A.V. designed research; Brousseau, G., Muhammad, S. and Alves da Silva, A.V. performed research; Brousseau, G. and Muhammad, S. analyzed data; Brousseau, G. and Muhammad, S. wrote the final report.

The authors declare no conflict of interest.

Acknowledgements

The Capstone team would like to thank Sean Moore, M.D. and Vincios Alves Da Silva, P.H.D for advising the research and development of this project. Additional thanks to Luisa Quesada and Victor Sobral for their help with the design and assembly of the PCBs, Olivia Luu and equipment provided by Stacey Hall for the help in adjusting the organ-on-a-chip device, as well as the Jefferson Trust for providing funding.

References

[1] "The computer chip that thinks it's a tiny human liver," *World Economic Forum*, Jun. 23, 2016. https://www.weforum.org/agenda/2016/06/organs-on-chips/ (accessed April 28, 2023).

[2] "Organ-on-Chip Market Size & Growth Statistics By 2030," *Allied Market Research*. https://www.alliedmarketresearch.com/organ-on-chip-market (accessed April 28, 2023).

[3] "Methods for detecting PER2::LUCIFERASE bioluminescence rhythms in freely moving mice - PMC." https://www-ncbi-nlm-nih-gov.proxy1.library.virginia.edu/pmc/articles/PMC9282071/ (accessed May 01, 2023).

[4] J. J. Han, "FDA Modernization Act 2.0 allows for alternatives to animal testing," *Artif Organs*, vol. 47, no. 3, pp. 449–450, Mar. 2023, doi: 10.1111/aor.14503.

[5] "Human Emulation System," *Emulate*. https://emulatebio.com/human-emulation-system/ (accessed May 01, 2023).