Socio-technical Synthesis: Improved Organoid Movement and Moral Responsibility for Clinical Trial Deaths

My technical work and my STS research are connected primarily through the idea of improved disease treatment, and exploring how new social and technical developments may achieve that. My technical work revolves around the development of a novel device to automatically move organoids free from human intervention. Organoids are clusters of stem cells that are differentiated into different tissues and can be used to study disease, create drug models, and more, thus improving the organoid movement method dramatically increases the scalability of organoid studies to improve disease treatment. My research investigates the moral responsibility of the deaths involved in the Audentes Therapeutics gene therapy trial to gain insight into which actors may hold moral responsibility. Although my technical work approaches improved disease treatment from an upstream manner and my STS research from a downstream manner, the concept of improved disease treatment is central across both projects.

My technical work aims to help overcome the time barrier in organoid research. In any study involving organoids, at some point, a researcher will have to move organoids between different well-plates or centrifuge tubes completely by hand. This dramatically decreases the scale at which organoid studies can be conducted purely due to the increased time required and decreased precision due to human manual dexterity limitations. My capstone team developed a device utilizing commercially available and CAD-created components and a machine-learning algorithm to identify, pick up, and place organoids in user-specified locations. The goal of our project is to allow researchers to improve their organoid study design and throughput by utilizing our device in hopes of eventually improving disease treatment.

My STS research aims to identify different actors that may hold moral responsibility for the deaths in the Audentes Therapeutics gene therapy trials. Michel Callon's actor-network theory and Van de Poel and Royakkers' moral fairness requirement are employed sequentially to first identify the key actors involved in the trial deaths and then which may be considered morally responsible due to their foreseeability and freedom of action. My claim is that Audentes Therapeutics and the FDA can be held morally responsible for the trial deaths through their violations of the moral fairness requirement. My paper explores their violations in hopes of developing a more comprehensive understanding of the root cause of the Audentes Therapeutics trial deaths and other gene therapy deaths alike. Through understanding the causes of gene therapy deaths, future severe adverse events can be mitigated, improving the treatment of disease.

Simultaneously approaching improved disease treatment from an upstream approach with my technical project and a downstream approach with my STS research helped me develop a more comprehensive understanding of how each step in the process is equally important. Working on my technical project gave me a better understanding of how important study trial design is on trial outcomes, which helped me identify clinical trial protocol shortcomings that contributed to the fatalities. Similarly, the research I conducted for my STS paper helped me understand future obstacles the applications of my technical project will have to overcome, such as FDA approval, which helped design device testing protocols. In summary, working on the STS research paper and my technical project in tandem has allowed me to explore improved disease treatment from multiple angles while also improving the quality of each project.

The Development and Testing of a Novel Automatic Organoid/Microsphere Movement Device

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Word Count: 4288
Number of Figures: 7
Number of Tables:0
Number of Equations:0
Number of Supplements:1
Number of References: 18

The Development and Testing of a Novel Automatic Organoid/Microsphere Movement Device

Abstract

Organoid research has exploded in the past decade going from 84 publications to over 3000 in 2022. With exponential growth like that streamlining the process of setting up organoid studies becomes increasingly important. Here we present a proof-of-concept study of our novel device that automatically withdraws and deposits hydrogel microparticles in user-defined locations. This device uses commercially available products and CAD-created components paired in a Python-programmed embedded system to identify microparticles within a 96-well plate, pick them up, and place them into a specified location in a 12-well plate. In a 50-sample movement trial, the cumulative success rate of the device was 45.97% or 23 out of 50 particles successfully detected, withdrawn, and deposited in the desired location. When the speed of moving 10 particles was compared to the manual alternative, the device was initially slower before the fourth particle but then surpassed the human subject likely due to fatigue. Over the movement of 10 microparticles, the device was significantly faster than the manual alternative ($p = 0.0094$). These results suggest that our device is a viable option and a swifter alternative to the current standard for moving organoids/microparticles: manual vacuum aspiration. If further iteration of the pick-up and placement modality increases the cumulative success rate, it is likely that this device would dramatically increase the scalability and scope of organoid studies by decreasing active and training time necessary to move organoids and improving the accuracy and precision of organoid placement.

Keywords: Organoid, Spheroid, Automatic Movement

Introduction

Overview

Organoids are three-dimensional cell cultures constructed from stem cells that function to mimic human organs in-vitro. Human stem cells were first successfully harvested in 1998, however, the first true organoid was not developed until 2009 when Sato et al. derived the first organoid from a single adult stem cell (ASC) seeded in Matrigel, a hydrogel material[1]. The landmark study in 2009 demonstrated that the key factor in the induction of ASC differentiation, and thus organoid formation was the biomaterial and environment in which it was seeded[1]. Since then, many different organoids have been developed and used to study diseases, develop drugs, and even the preliminary development of transplantable organs[2]. Although the range of organ tissues that can be mimicked in-vitro using organoids has increased dramatically, their use in research remains limited by their lack of freedom of mobility [3].

ASCs are placed into biomaterials by hand using a pipette and once differentiated into organoids, moved to imprecise locations via vacuum aspiration[4]. Although effective for small-scale studies where precision is less of a critical variable, the current organoid movement method is time-consuming, imprecise, and limits the scale and scope of potential studies[3]. As with any procedure that relies heavily on human manual dexterity, the placement of organoids into specific locations within biomaterials is imprecise, thus introducing error and limiting study conclusions[3]. Vacuum aspiration itself also introduces error through the accompaniment of media with organoid deposition. The extra media deposited with the organoid alters the desired extracellular environment, again limiting conclusions that can be made[5]. By improving the current

movement method of stem cells and organoids, new research could be conducted with higher degrees of accuracy and a faster turnaround time. Additionally, a more precise placement method would open the possibility for more abstract organoid arrangements to better mimic in-vivo conditions[6].

Here, we aim to design and construct a device that precisely places organoids into a designated position in a permissive biomaterial using an automated set-it and forget-it approach. As aforementioned, there is currently no automatic way to withdraw and deposit organoids, thus this paper presents a novel device that may potentially remedy that shortcoming. Given the novel nature of our device and the two-semester timeline, the scope of this study is limited to that of a proof-of-concept to show that our device (1) successfully withdraws and deposits hydrogel beads and (2) does so faster than if it was done manually.

More specifically, we have designated two aims for our project which have been accomplished. **Aim 1** consisted of designing and constructing a device with multiaxial movement capabilities that can hold all the necessary components for image detection and organoid movement using existing or CAD-created components. In order to accomplish Aim 1 we utilized Fusion 360 and laser printing technology to develop 3D components capable of holding well plates, a high-resolution camera, and a nano-injector (Figure 1). Second, we integrated linear guide rails and NEMA-23 motors that are capable of moving the components on all three axes. In conjunction, these components allowed for automated movement of the well plates, the camera, and the nanoinjector, which is necessary for organoid withdrawal and deposition. To integrate the aforementioned components **Aim 2** sought to develop a Python program to automate the system in which organoids are moved into a biomaterial. This was achieved by creating a unified communications network between all components and designing a system that requires minimal human input in order to remove human error and reduce the operational hours of manual labor.

By accomplishing the aforementioned aims and subjecting the device to proof-of-concept trials, we show that our novel device has the potential to be both more efficient and effective than the current manual alternative. Thus, future iterations of our device will save countless hours for researchers while also increasing the reliability and scalability of their organoid-reliant studies.

Materials and Methods

Multiaxial movement and device holding capabilities

The base of the device is derived from a prebuilt multi-axial sled movement system containing a system of linear guide rails as depicted in Figure 1. The movement in the device is powered by NEMA 23 motors that are controlled by a Python system and connected to a GUI. A 12V AC to DC converter is used to power these motors. Three of these motors are used, one on each axis, to allow for the multiaxial movement necessary for this device to serve its purpose. The two motors on the horizontal axes motors are used to move both a 96-well plate containing the organoid(s) and a plate containing the permissive biomaterial into which the organoids will be seeded, which are both held in a purchased holding device as shown in Figure 1. The motor on the vertical axis of the device allows for the vertical movement of the Basler Ace camera and 2020 Nanoliter injector to allow for both the image detection of organoids by the camera and the withdrawal and deposition of organoids by the injector. Both the

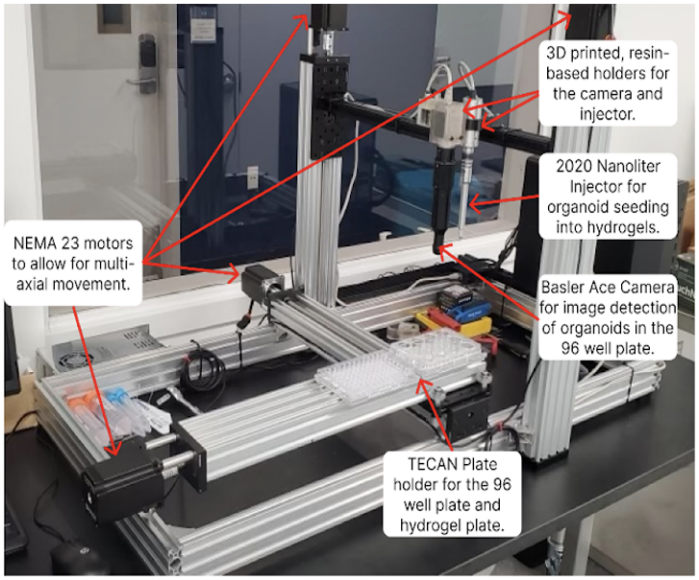
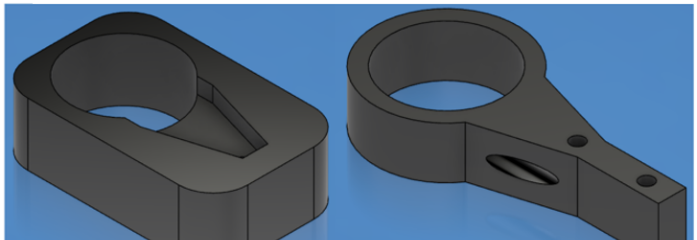


Figure 1: Hardware components of an automated organoid seeding device.

camera and injector are held in place on one of the linear guide rails using 3D-printed holders that allow for minimal shaking in these devices. These components required a few rounds of iteration in order to fit the needs of this device. For the camera holder, the initial design placed the camera too close to the well plates, resulting in contact and inaccurate image detection. To remedy this, a buffer component was created to increase the height at which the camera sat in the device, allowing for more accurate image detection. For the injector component, the initial design was built to allow the attachment component of the injector to sit in the holder and then be screwed into the



guide rails. While effective in holding the injector, this design required all of the holding force to be put on one end of the design, ultimately leading to the deformation of the device upon repeated use. This deformation caused crooked and imprecise movement of the injector. To remedy this issue, we altered the design so that the attachment component of the injector was actually removed and instead built into the holder, the design change can be seen in Figure 2. This allowed us to not only screw the holder into the guide rails but also directly into the injector, leading to a better dispersion of holding force and thus less deformation in the holder. The final configuration of these devices in the system can be seen in Figure 1. For the image detection done by the camera to be accurate, there must be uniform lighting of the target, and as such, the device must be used in an area containing uniform lighting[7].

Python-Controlled Automation of the entire Device

Embedded systems are specially designed computer systems meant to function as a part of a larger machine with the purpose of controlling services to the system. The Universal Serial Bus (USB) standard has been implemented to allow for serial communication across the computer's serial ports to allow for ease of debugging between the stepper motors, camera, and injector. This USB standard functions by creating a logical connection between the host and device endpoints in a method known as piping which transfers data bytes known as packets across the USB communication system. Token packets allow for OUT or IN token references in which the data is either written to or read from the device with expected data packets being sent or received, respectively[8]. Through this, data from the camera can be read while bit transfers are sent to the stepper motor and injector to move the device within the triaxial system and allow for more precise microsphere/organoid pickup. Once the user has inputted an organoid formation into the GUI and placed a 96-well plate of organoids into the plate holder, the camera will begin image detection of the 96-well plate. Using the Python system, the camera will systematically determine if an organoid is present in each well. When new cells are created, the machine inherently does not know where they are. As such, images are saved to a training diagram in which an 80%, 20%, and 10% split is created (Figure 3). Permutations are made around the user's inserted data to allow for the correlation between new positions, differing saturation and brightness levels, and with repositioning to generate synthetic data. This allows for a higher training amount as compared to the standard method of hand labeling although it may not show as good of recall (reference the original fluorophore data that was made by hand over 300 images with the new hydrogel synthetic 300 image amount).

After completing this process, the organoid's location in each well will be communicated to the motors and Nanoliter injector through the Python communication network as described previously. The motors will then move the Nanoliter injector to the location of the first organoid to be seeded, which will be the bottom leftmost well. The injector will then be moved down into the well where the suction system of the injector will be activated to capture the organoid. Next, the motors will move the injector up and over to the designated location in the permissive biomaterial, where the injector will again be lowered to the desired depth in the biomaterial. The injector will then be told by the Python system to release the organoid. This process will be repeated until all of the user-selected formations have been completed. The entirety of this process will be completely controlled by the Python communication network and thus fully automated outside of the user selecting their desired organoid formation[9].

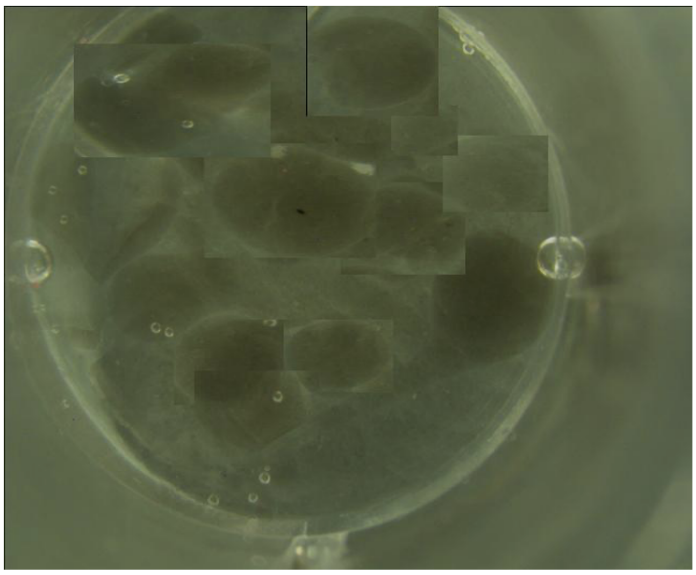


Figure 3: Illustration of the microparticles being imaged for image detection using a machine learning algorithm.

Testing Methods

The calibration of the image detection software was determined by having the program predict the coordinates of the bounding box around the spheroid when a spheroid is present in the image, as seen in Figure 4, where the algorithm has created a bounding box around a fluorophore. The coordinates of ground truth and predicted bounding boxes are then compared and the mean-square

error is calculated. The actual precision of the image detection software was measured as the percentage of predictions from the previous data that are correct as a

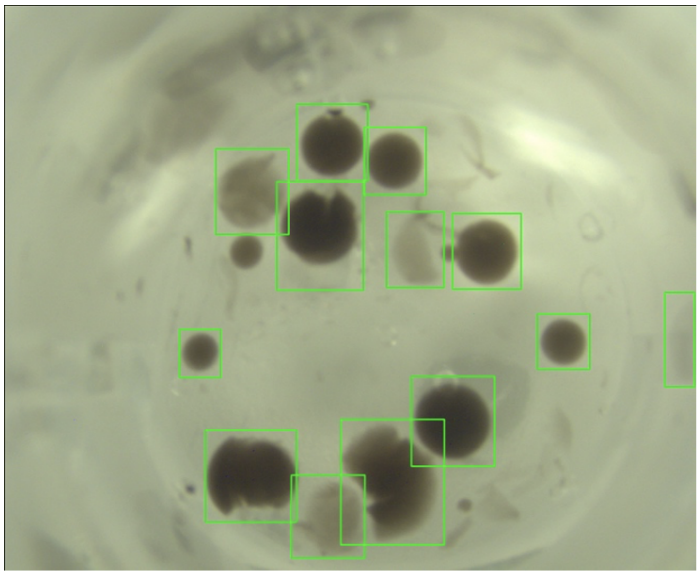


Figure 4: Image of a single well containing microparticles that have gone through image detection and have been traced by the software.

measure of True Positive (TP)/ (TP + False Positive). To test the image detection and pickup/placement capabilities of this device, microparticles and fluorophores were suspended separately into an aqueous solution and randomly dispersed into wells of a 96-well plate[10]. For our initial testing, we treated hydrophobic fluorophores using a tween-based method involving the boiling of deionized water and the use of an immersion blender as described by Cospheric LLC[11]. This method effectively "coats" the hydrophobic fluorophores so that are able to interact with a hydrophilic environment, in this case, water. This method did not end up showing promising results as elaborated upon in the discussion section, thus, hydrophilic hydrogel beads were constructed for the remainder of the testing. Once the algorithm is trained using the aforementioned method, the image detection portion then detects and traces what it believes to be particles in the well. An image of the traced particles is presented and then we recorded how many particles were actually in the well, how many the program recognized, and how many false positives the program produced. Next, the program chooses a particle and begins to pick the particle and transfer it to the 12-well plate where it places the particle. For this portion, we recorded whether or not the injector hit the targeted particle or fluorophore, whether it displaced the particle or fluorophore, whether it picked up the particle or fluorophore, and whether the injector successfully placed the particle or fluorophore. This was repeated for a total of 50 trials for both the microparticles and fluorophores. In comparison to seeding done by hand, the effectiveness of this device in terms of speed was tested by having a trained, unbiased individual pick up and place 10 microparticles consecutively[12]. The same process was completed by our device. Additionally, these trials were timed and the split for each individual particle was recorded to determine the effect of fatigue on the manual trials.

Results

Design Constraints

It is possible that the motors used for the multiaxial movement do not receive the full 12V from the power source, which may lead to inaccurate movement of the devices by the motor. This is accounted for by testing the voltage across the motor using a multimeter so this is unlikely to affect the performance of the device[13]. The 12V power source is also a constraint as this power cap limits the speed at which the components can be moved and thus the speed at which the device can work. However, we believe that the speed at which the motors can move the guide rails with a 12V power source (0.023 mm/s) is sufficient to meet the aims and goals of this project. An additional design constraint is that the horizontal linear rail is slightly elevated which causes the injector to touch the bottom of the well too closely. This could result in the organoid not being released properly; however, we have not seen any limitations to this with the microparticles. Correspondingly, the depth from the camera to the bottom of the well was measured by hand, which limits our precision of the distance needed to lower the injector in order to pick up a particle.

The step size of the NEMA 23 stepper motors could limit the accuracy of organoid seeding as this determines the minimum distance the injector can move at a time. However, we have not seen this significantly affect the performance of the device as the step size in this device is 14 microns, which is small enough to place organoids within the 100 micron threshold for success. There is also the possibility of misclassification within the dataset where the user erroneously classifies an image which could degrade the accuracy of the training model or have an organoid that can not be trained through the neural network using the priori model and would require a data-dependent method and stringent testing to view the model classification to ensure limited false discoveries within the dataset[14].

A potential constraint of the image detection capabilities of the device is that despite efforts to create uniform lighting around the plates, there are still variations in light levels. This could lead to inaccuracies in detecting the presence of organoids, which would limit the ability of the device to seed said organoids into the biomaterial[7]. A constraint involving the ability of the device to pick up and move the organoids is that since organoids vary in size, the capillary tube of the Nanoliter injector may not be able to pick up all types of organoids. The capillary tube has a diameter of 1.5 mm, meaning that organoids with significantly larger diameters will likely be unable to be picked up by the device. However, organoids are typically 0.4-0.7 mm in diameter, so this is not likely to be an issue[15] Additionally, the processing power of the hardware is a further constraint as not having enough computational power could lead to prolonged periods of computer calculations and an inability for the device to perform under 60 minutes. Ideally, the device and hardware required to run the software would be low with regards to the computational power, having an idealized minimum requirement of a Raspberry Pi, which would allow for large-scale deployment and more potential refinement of the final device design. Having limited processing power directly affects the system's ability to run the convolutional neural network on training data, create prediction sets, and implement serial bus commands to interact with the attached devices to move the spheroids. An i7-7500U can train, on average, 115 samples per second, while a Raspberry Pi can expect to take around three samples per second[16]. As such, our current device will have the limitations of requiring a minimum CPU boost speed comparable to an i7-6850k@3.60 GHz with 16 GB of memory to allow for rapid computation of the neural network[17].

Device Testing Iterations

As mentioned in the materials/methods section, our first test involved attempting to move fluorophore particles between the 96-well and 12-well plates. Fluorophore particles were originally chosen for ease of sight and cost-effectiveness, however, the withdrawal and deposition rates were poor due to the particles having a tendency to stick to the glass of the capillary connected to the nano-injector. Out of 50 trials, only one trial was considered successful, that is, the fluorophore was correctly detected, picked up, and placed in the 12-well plate (Figure 5).

Given the unfruitful nature of the testing with the fluorophores, we retrained the algorithm to detect black-dyed hydrogel beads, a nonliving particle of similar diameter and hydrophilicity to organoids and cell spheroids[18]. In the preliminary testing with these particles, particle displacement occurred as the nano-injector broke the surface of the water in the 96-well plate. This caused the nano-injector to fail in picking up the particle the algorithm had detected as it had moved after the completion of the image detection. To circumvent this issue, we implemented a delay right after the nano-injector broke the surface of the water which dramatically decreased the frequency of the particle displacement. After being able to successfully pick up and place the hydrogel beads more reliably, we conducted 50 trials to assess the algorithm's image detection success rate, the number of false positives per trial, whether the injector moved properly to the algorithm detected location (hit), and

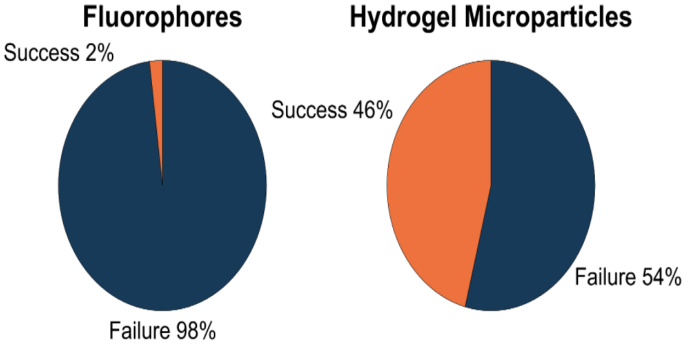


Figure 5: Pie chart displaying the success rate of pick up and placement of fluorophores and hydrogel microparticles by the device.

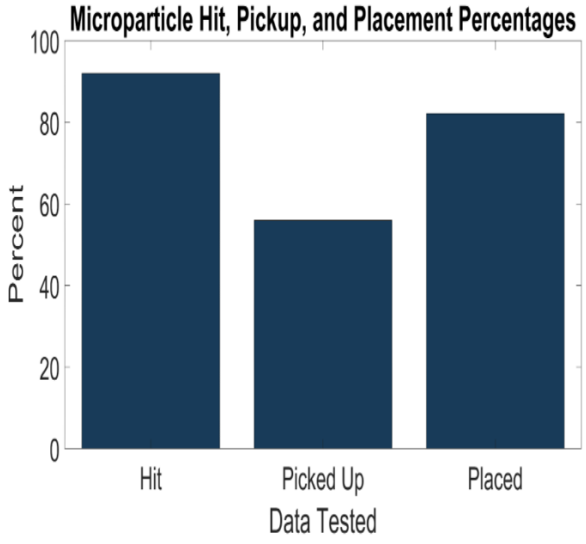


Figure 6: Bar graph displaying the hit, pickup, and place percentages of the device when testing with microparticles.

finally the withdrawal and deposition rate. The algorithm successfully identified 100% of the particles in 42 out of the 50 trials and had a cumulative success rate of 96.33% (367 detected out of 381 present). With respect to false positives, defined as a particle detected that is not actually a particle, the average was 0.7 ± 0.76 beads (average ± standard deviation) per trial. As illustrated in Figure 6 the particles were successfully picked up 56.0% of the time and successfully placed 82.1% of the time. Together this gives a cumulative success rate of 45.97% which indicates that out of the 50 trials, 23 out of 50 particles were successfully detected, withdrawn from the 96-well plate, and deposited into the 12-well plate (Supplementary Video 1).

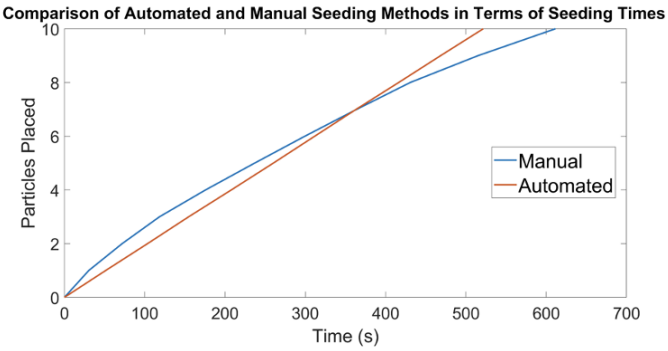


Figure 7: Line graph comparing the seeding times between automated and manual methods.

In addition to testing the effectiveness of the device, we tested its efficiency as compared to the manual alternative. As shown in Figure 7 the device maintained a relatively constant withdrawal and deposition rate while the subject took more time with each additional particle withdrawn and deposited. Although the subject was initially quicker at moving the particles, by the fourth particle moved the device had already caught and surpassed the subject. Over the movement of just 10 particles, the device took statistically significantly less time to pick up and place the microparticles in comparison to the manual method. The difference between the time splits for the automated and manual methods was determined using a Student's T-test, which resulted in a p-value of 0.0094, indicating statistical significance.

Discussion

Interpretation of Testing

Fluorophore Test

As discussed in the previous section, the movement testing with the fluorophore particles did not produce ideal results. However, the results suggest that despite being treated with tween-20 to become hydrophilic, the inherent hydrophobic nature of the particles remains prominent in their behavior in water. Additionally, this test highlighted a key aspect of our device that is important to note. Given the hydrophobic nature of glass and our use of capillaries in our movement method, it is likely that strongly hydrophobic particles will have a lower success rate due to the nano-injector supplying insufficient force to break the adhesion between the hydrophobic particle and the glass capillary.

Hydrogel Bead Test

Although the cumulative success rate of the device was only 45.97%, the relatively high identification rate of 96.33%, and the 0.7 average false positives per trial suggest that neither the camera, the motors/track, nor the algorithm is the main source of the lower-than-ideal cumulative success rate. This leaves the nano-injector as the final variable that might be limiting the success rate. This is in line with our expectations as the nano-injector is not specifically designed for this application and has a limited volume that can assist in the withdrawal and deposition of the particles. Despite the low success rate, the results still show that our device can indeed accomplish the task of moving hydrogel microparticles with a set-it-and-forget-it approach.

Machine versus Man

As mentioned in the results section, the device could withdraw and deposit hydrogel microparticles significantly faster than the subject after only the seventh bead moved ($p=0.0094$). This is in line with our expectations as humans are susceptible to distractions, fatigue, and boredom when it comes to monotonous tasks like picking up and placing hydrogel beads. Given that such a robust difference was present in a small-scale trial of only 10 beads, it is reasonable to assume the result would be drastically more profound in a trial that is an order of magnitude larger. Thus, these results suggest that our device is an effective alternative to the manual method with respect to speed, especially for sample sizes larger than four beads.

Limitations

Although the results of our testing show the proof-of-concept that our device can withdraw and deposit hydrogel beads and do so faster than the manual alternative, there are still some limitations with respect to the scope of our testing and results. First, the amount of time provided to construct and test the device was limited to two semesters with all team members having other

additional scholarly obligations. This decreased the amount of time available to iterate on the design and potentially ideate a better movement modality than the nano-injector. Second, in order to both save time and mitigate costs, all testing was done with non-living particles rather than organoids themselves. This limits the conclusions that can be drawn from our results as it is possible that the hydrogel microparticles interact with the movement system differently than living organoids or cell spheroids. Finally, the aforementioned time constraint decreased the number of trials where we could test both the effectiveness of the device and the speed compared to a human subject.

Impact

Despite the limitations to our testing and the device itself, our results are promising as preliminary efforts to increase the scalability of organoid and cell spheroid studies while also decreasing the amount of active and training time required to conduct them. With continued iterations of our design and further testing, it is likely that the cumulative success rate can dramatically increase, making it a viable option for research labs across the globe.

Future Work

By continuing to iterate on the design while consistently testing with hydrogel microparticles we believe that we can achieve a higher cumulative success rate. Additionally, we believe iteration efforts should be focused on either improving the ability of the nano-injector to withdraw and deposit the beads or developing a new pickup and placement method altogether as that was likely the source of the low success rate in the trials above. After achieving a success rate of 85%, testing with organoids and cell spheroids should be conducted to see if the success rate stands despite using living cells. It is likely that the success rate will stand given the similar external properties between the hydrogel beads and the organoids, but if it does not, further iteration should involve switching the 96-well plate from a flat bottom to a curved bottom and adding an additional camera so that the algorithm can detect the organoid's position in three dimensions rather than just two. Once the organoid tests are successful, the device can move from the prototype phase to the design for the manufacturing (DFM) phase while a patent for the movement method is filed. During the DFM phase, the device aesthetic and size footprint should be significantly streamlined so that there is no erroneous material and that it can fit in a traditional lab space. Additionally, software developers can be brought on to improve the user experience with the GUI and to add more placement options for the user to input into the GUI. Finally, after these iterations have been made, the device will be ready for the market.

End Matter

Author Contributions and Notes

Hoffman, K., Maschler, J., Martinez, R., and Sanderson, J., created CAD designs, constructed prototypes, developed the algorithm, designed and conducted trials, and wrote the final report. Highley, C. created the hydrogel beads, supplied funding, and advised when issues arose. The authors declare no conflict of interest.

Acknowledgments

The capstone team would like to thank Dr. Christopher Higley from the UVA Department of Biomedical Engineering for advising the research and development of the device. The team would also like to thank Dr. Timothy Allen and Dr. Shannon Barker from the capstone teaching staff in the UVA Department of Biomedical Engineering for advising throughout the project.

Supplementary Video:

a) Supplementary Video 1
https://youtu.be/v_N2w09sM6E

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Who is Morally Responsible for the Deaths Involved in the Audentes Therapeutics Gene Therapy Clinical Trial?

STS Research Paper
Presented to the Faculty of the
School of Engineering and Applied Science
University of Virginia

By

Jack Maschler

May 12, 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

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Introduction

On May 5th, 2020, the first patient fatality from the clinical trials investigating an Audentes Therapeutics adenovirus-associated vector (AAV) gene therapy treatment, AT132, for X-linked myotubular myopathy (XLMTM) occurred. In the subsequent months, another two patients receiving the same dose of the treatment tragically passed away before the Food and Drug Administration (FDA) put the clinical trial on hold. On December 24, 2020, the FDA lifted the clinical trial hold in response to trial protocol modifications such as decreasing the maximum treatment dose ("RECENSUS / INCEPTUS / ASPIRO Studies", 2021). Despite this, another patient died from the new maximum dose treatment in September 2021 (Philippidis, 2021). Currently, scholars investigating the deaths from the AT132 clinical trials have focused on their broader implications in the future of gene therapy treatments. However, this analysis fails to consider the underlying human and non-human actors who played a role in the tragedies and which actors are morally responsible. In absence of the determination of the actors who were morally responsible for the tragedies, scientists and regulatory bodies will remain inadequately informed on the root cause of the AT132 tragedies and other gene therapy tragedies alike.

Through the case of the AT132 clinical trials, I will utilize actor-network theory (ANT) and the distribution of responsibility to argue that Audentes Therapeutics and regulatory bodies such as the FDA are morally responsible for the deaths involved. ANT is a science technology and society (STS) framework that investigates an actor-network that is generated by a network builder who recruits both human and non-human actors to accomplish a specific goal (Callon, 1987). The distribution of responsibility is a framework that utilizes four tenants, wrong-doing, causal contribution, foreseeability, and freedom of action, to assign moral responsibility for particular actions (Van de Poel & Royakkers, 2011). I will begin by using publicly released

statements by Audentes Therapeutics and established data regarding AAVs to map both the human and non-human actors involved in the AT132 trial tragedy. I will then use two tenants of the moral fairness requirement, foreseeability and freedom of action, to show the aforementioned human actors are morally responsible for deaths in the AT132 clinical trials.

Background

AT132 is an adenovirus-associated vector (AAV) gene therapy treatment for X-linked myotubular myopathy, a rare genetic disease primarily affecting newborns and infants. XLMTM is characterized by progressive muscle weakness and decreased muscle tone which impairs the development of necessary motor skills (Annoussamy et al., 2019). AAVs have been used to treat genetic diseases since 1995, and work by switching the mutated gene causing the disease with a gene that will express the wild phenotype (Wang et al., 2019). Although AAV gene therapy shows great promise in treating disease, they have consistently been shown to be hepatotoxic (Chand et al., 2021; Ramamurthy et al., 2022)**.**

Literature Review

Currently, no scholarly sources have directly investigated the moral responsibility of the deaths involved in the Audentes Therapeutics gene therapy clinical trial. However, Morales et. al has analyzed the severe adverse events (SAEs) involved in an attempt to define a path forward to prevent SAEs in future gene therapy constructs. Additionally, other scholars have investigated the factors involved in the death of Jesse Gilsinger, a teenager who died receiving a gene therapy treatment for a different genetic disease called ornithine transcarbamylase deficiency (OTC). The following analyses provide insight into the plausible scientific mechanisms involved in the AT132 trial tragedies and the factors involved in a similar gene therapy death but do not evaluate actors involved in the AT132 trial to determine moral responsibility.

In *Broader Implications of Progressive Liver Dysfunction and Lethal Sepsis in Two Boys following Systemic High-Dose AAV,* Morales et. al begins by summarizing XLMTM and the clinical milestones leading up to the hold the FDA placed on the clinical trial. They then go on to suggest "plausible mechanisms for lethal toxicity must emphasize hepatotoxicity from the vector capsid or transgene product," and state that "interpretation[of the primary etiology] is further complicated by an aspect of the disease that is poorly understood[a significant proportion of XLMTM patients have underlying liver disease]." This is followed by further speculation on methods to mitigate patient risks and a call to action: "as soon as possible the findings of clinical toxicology must be made public to accelerate the development of novel vectors with the expectation of substantially improved therapeutic index,"(Morales et al., 2020). While Morales et. al does note that inclusion/exclusion criteria, informed consent, and data monitoring must be overhauled, there is no discussion on the moral responsibility of the actors who played a role in the deaths involved in the trial (Morales et al., 2020).

In *Smoke and Mirrors: Jesse Gilsinger, Human Experimentation, and Gene Therapy*, Abeel analyzes a similar gene therapy death involving Jesse Gilsinger and argues that ego, financial conflict of interest, and human errors were the contributors to his death. He begins by describing the timeline and series of events that lead to Gilsinger's death before supporting his argument with primary sources that demonstrate egotistical action, financial conflict of interest, and human errors:

> Ego: "Batshaw diverted the conversation [about risk] away from Jesse and to the results they had achieved in laboratory mice and human subjects."

Financial Conflict of Interest: “it was later disclosed that he [one of the lead researchers in the trial] was the founder of a biotech company, Genovo, and held a 30% stake in the company's non-voting stock.”

Human Errors: “Left out of the consent document were reservations about the procedure within the RAC committee and adverse events associated with trials of OTCD treatment on animals and human beings.”

Abeel then concludes with a call to action stating, “one potentially positive development…would be the implementation of clear federal regulations…[where] patients will be fully aware of the context, potential complications, and conflicts of interest surrounding these treatments,”(Abeel, 2010). Although this article highlights key actors that played a role in Gilsinger’s death, the actors do not overlap in the Audentes Therapeutics case nor does Abeel discuss moral responsibility.

The insight provided by Morales et. al and Abeel into the mechanisms behind the deaths of the AT132 patients and the factors that played a role in a similar tragedy is tremendously valuable. However, further investigation into both the human and non-human actors involved in the Audentes Therapeutics case to elucidate moral responsibility will dramatically expand the understanding of the root cause of the trial’s four tragic deaths.

Conceptual Framework

In order to determine the moral responsibility for the deaths in the AT132 clinical trial, I will first draw on Actor-Network Theory (ANT) to identify both human and non-human actors within the Audentes Therapeutics network and then draw on the distribution of responsibility to elucidate moral responsibility. ANT is a science, technology, and society (STS) framework fundamentally characterized by its use in deconstructing and analyzing the function of intricate

sociotechnical systems. ANT identifies a network builder in an attempt to trace back the complex heterogenous relationships that exist between the various relevant human and non-human actors (Callon, 1987). The network builder is an entity that recruits both human and non-human actors to achieve a common goal in which no single actor has greater power than another. The power of a particular actor-network is not created by the individual strength or contribution of actors within the network, but rather it is defined by the relationships the relevant actors have within the network itself (Latour, 1986). Although using ANT to identify a network builder will allow me to deconstruct the actor-network to identify the relevant human and non-human actors involved in the deaths of the AT132 trial, I will need to draw on the distribution of responsibility to determine moral responsibility.

To describe the distribution of responsibility I must first define what constitutes an individual as morally responsible. According to Van de Poel and Royakkers, an individual can be held morally responsible for an outcome if all of the following were present:

1. Wrong-Doing
2. Causal Contribution
3. Foreseeability
4. Freedom of Action

Figure 1: Van de Poel and Royakkers' elements of the moral fairness requirement.

By utilizing the elements in this list one can ascribe individual responsibility to the various actors that participated in a given action, hence distributing the responsibility (Van de Poel & Royakkers, 2011). In my analysis of the moral responsibility of the actors who played a role in the AT132 trial deaths, I will focus on elements three and four as wrong-doing and causal contribution are prerequisites for any violation of foreseeability or freedom of action. Foreseeability is defined as how likely it was that a person or group could have anticipated the

actual consequences of their actions (Zimmerman, 1986), and freedom of action is characterized by the absence of an entity that coerced the action in question (Van de Poel & Royakkers, 2011).

In the following analysis, I will begin by utilizing ANT to identify a network builder and deconstruct the actor-network to illustrate both the relevant human and non-human actors. I will then utilize elements three and four of the moral fairness requirement to elucidate the distribution of moral responsibility among the identified human actors.

Analysis

Audentes Therapeutics Network

Reconstruction of the Audentes Therapeutics network will be the foundation on which the following analysis is built. In order to reconstruct the network, I will first define the network builder and the desired goal, then the relevant heterogeneous actors, and finally the interaction and association between them (Figure 2). The goal of Audentes Therapeutics is to make AT132 publicly available for the treatment of XLMTM, thus, it acts as a network builder to recruit the subsequently mentioned heterogeneous actors to accomplish its goal. By utilizing the FDA drug approval step-by-step guidelines, I have traced back the relevant human actors who have contributed to the approval and hold placed on the clinical trial (Food and Drug Administration, 2020). To bring a drug to clinical trials, a drug sponsor, in this case, Audentes Therapeutics, submits an investigational new drug (IND) application to the FDA including results from animal trials, drug composition, manufacturing protocols, and planned clinical trial protocols. Once the FDA critically reviews and approves the IND, the drug sponsor is cleared to initiate the planned clinical trials (Food and Drug Administration, 2020). Thus, the relevant human actors in bringing a drug to clinical trials are (i) the drug sponsor, *Audentes Therapeutics*, (ii) the *FDA*, and (iii) the trial participants, *XLMTM patients* (Figure 2). To determine the relevant non-human actors, I will

analyze the various technical and social components required in the carrying out of a clinical trial. To initiate a clinical trial there must be an overarching medical condition to be treated, an intervention to treat the condition and a written clinical trial protocol within an IND that contains informed consent documents ("Learn About Clinical Studies," 2019). Thus, the relevant non-human actors are (iv) the medical conditions, *XLMTM*, and *underlying liver disease*, (v) the treatment, *AT132*, and (vi) the *IND* (Figure 2).

In the Audentes Therapeutics network, the interactions between the aforementioned heterogeneous actors are again governed by the FDA drug approval guidelines, but also the underlying physiological mechanisms involved in XLMTM. For example, hepatobiliary disease is a very common comorbidity of XLMTM, and thus two separate conditions directly affect XLMTM clinical trial participants (D'Amico et al., 2021). By again tracing the FDA drug approval timeline and analyzing the conditions which affect trial participants, I have reconstructed the Audentes Therapeutics actor-network in Figure 2. The use of uni- and bidirectional arrows characterize which actors have a one or two-way interaction.

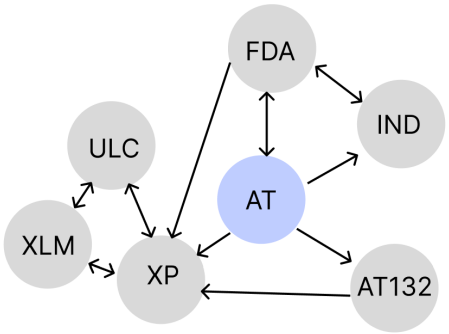

Figure 2: The Audentes Therapeutics Network. AT is Audentes Therapeutics, XP is XLMTM patients, XLM is XLMTM, and ULC is underlying liver conditions.

Can Non-human Actors be Morally Responsible?

To determine if non-human actors can be morally responsible for the deaths due to the AT132 clinical trial I will draw on element four of Van de Poel and Royakkers' moral fairness requirement: freedom of action. The freedom of action principle immediately extinguishes the moral responsibility of any non-human actor. Each of the non-human actors in the Audentes Therapeutics actor-network lacks the ability to control their actions and thus does not violate the

freedom of action principle, removing them from the pool of moral responsibility. In the following section, I will utilize elements three and four of the moral fairness requirement to show that Audentes Therapeutics and the FDA are morally responsible for the four deaths due to the AT132 treatment.

Foreseeability

Through the lens of foreseeability, a potential moral actor is considered accountable for the consequences of an action if the actor could have reasonably anticipated the said consequences (Zimmerman, 1986). Audentes Therapeutics shows reasonable foreseeability for the deaths due to treatment with AT132. On June 23, 2020, Audentes released a statement regarding the second death of a patient in the XLMTM trial: "Notable features among the three patients with these SAEs include older age, heavier weight, *evidence of pre-existing hepatobiliary disease,* and dosing with the higher dose of $3x10^{14}$ vg/kg," ("RECENSUS / INCEPTUS / ASPIRO Studies", 2021). Taken as a stand-alone statement, Audentes would not have violated the principle of foreseeability as it is just reporting the notable similarities between the patients who succumbed to liver complications as a result of the treatment. However, when considering the clinical trial exclusion criteria first posted on June 27, 2017, the foreseeability violation becomes evident. "Subject has a clinically significant underlying liver disease," is clearly written under the "Key Exclusion Criteria" section of the clinical trial protocol (Astellas Gene Therapies, 2023). The inclusion of the aforementioned exclusion criteria demonstrates that Audentes had knowledge that the treatment may have unfavorable interactions with patients who have underlying liver conditions, yet it still enrolled patients that, "demonstrated evidence of pre-existing hepatobiliary disease," directly resulting in three out of the four deaths ("RECENSUS / INCEPTUS / ASPIRO Studies," 2021).

As I have argued, Audentes Therapeutics can be labeled morally responsible for the deaths in the AT132 clinical trial due to a violation of the foreseeability principle. However, some may contend this by stating that the preclinical trials in multiple animal models (mouse, dog, and non-human primate (NHPs)) showed no significant SAEs following AT132 treatments up to $8*10^{14}$ vg/kg (Shieh et al., 2020). While it is important to consider the preclinical animal toxicology studies when determining foreseeability, they alone cannot justify a lack of foreseeability when similar AAV treatments have been shown to be severely toxic in other animal models. For example, a 2018 study investigating an AAV treatment in NHPs and piglets found that "[an] observation from the NHP study that is of relevance to clinical applications of high-dose systemic AAV is the significant variation in toxicity between animals,"(Hinderer et al., 2018). This finding is of particular relevance as it suggests that there is a high likelihood of discrepancy between safety data regarding AAVs used in NHPs and AAVs used in humans, as clearly demonstrated by the deaths in the AT132 clinical trial. Given that this data was published prior to the initiation of the clinical trial, it is reasonable to assume Audentes Therapeutics had knowledge that there may be significant differences in toxicity between humans and NHPs treated with an AAV, thus further demonstrating foreseeability of the consequences of its actions.

Freedom of Action

Using the freedom of action principle, one can be considered morally responsible for the consequences of their actions if the actor was acting on free will, meaning that there is no entity governing their course of action. Both the FDA and Audentes Therapeutics can be held morally responsible for the deaths in the AT132 trial through the lens of freedom of action. Although the FDA is a government organization, the only instance in which other bodies of the government can dictate its actions is in an emergency use authorization of medical products (EAU), which

occurs in instances of large threats to global or domestic public health such as the COVID-19 pandemic ("Emergency Use Authorization of Medical Products", 2023). In the case of the IND clearance for AT132, there was no such EAU, thus the FDA acted on its own accord. Furthermore, the presence of IND guidance documents does not undermine the FDA's ability to act of free will. The FDA states "because guidances are not regulations or laws, they are not enforceable, either through administrative actions or through the courts," (Food and Drug Administration, 2022). This quote not only removes FDA liability from not acting in accordance with previously established guidance documents but further supports the FDA's ability to act free of other entities. Given the FDA's ability to act of free will, it can be held morally responsible for clearing the AT132 IND despite unclear trial exclusion criteria. As aforementioned, one of the exclusion criteria in the clinical trial stated "subject has clinically significant underlying liver condition," (Astellas Gene Therapies, 2023). In high-quality clinical research, inclusion and exclusion criteria should be consistent, reliable, uniform, and objective in nature (Garg, 2016). The lack of objectivity in the verbiage utilized in the exclusion criteria directly resulted in over 50% of the subjects enrolled in the study having evidence of pre-existing hepatobiliary disease (Shieh et al., 2020). This is particularly important as all four patients who died were of the 50% with pre-existing hepatobiliary disease (Philippidis, 2021). The role of the FDA in reviewing an IND is to assure the research subjects will not be subjected to unreasonable risk, yet it still cleared the AT132 IND despite poor exclusion criteria completely free of coercion. Through this action, the FDA can be held morally responsible for the deaths in the AT132 clinical trial via the freedom of action principle.

In addition to the moral responsibility held by the FDA for clearing the IND resulting in the first three deaths, both Audentes Therapeutics and the FDA can be held morally responsible

for the fourth death, which was after the maximum dose had been lowered from $3.5*10^{14}$ to $1.3*10^{14}$ vg/kg. Given that Audentes Therapeutics is a private sector company, no governing body besides the FDA regulates its actions with respect to a particular drug trial. In response to the formal clinical trial hold that the FDA placed on May 7th, 2020, the FDA cleared Audentes Therapeutics to continue the trial at a lower maximum dose of $1.3*10^{14}$vg/kg (Audentes Therapeutics, Inc., 2021). This may at first seem like a justified action given that the new dose is less than half of the previous maximum dose, however, a member of the *Human Gene Therapy* editorial board and gene therapy expert, Nicole Paulk stated that "This new dose of $1.3*10^{14}$ vg/kg is still a very high dose, particularly for intravenous infusion, so the serious adverse events (SAEs) will still be the same as we've seen previously with 'high-dose IV administered' AAVs," (Philippidis, 2021). This is particularly noteworthy as it suggests that SAEs are likely to continue so long as the trial continued with a high dose, which she classifies as roughly $1*10^{14}$ vg/kg or more. Despite both the FDA and Audentes Therapeutics having the ability to continue the trial with moderate or low doses free from coercion, they continued the trial with doses still considered to be very high by gene therapy experts. Through their roles in continuing the trial, the FDA and Audentes Therapeutics can be held morally responsible for the fourth death in the trial via the freedom of action principle.

Conclusion

In this paper, I have used both the sociotechnical concept of ANT and the ethical framework of moral responsibility to first identify the relevant human and nonhuman actors and then determine which actors can be held morally responsible for the deaths involved in the AT132 clinical trial. Through the lenses of foreseeability and freedom of action, it is evident that both Audentes Therapeutics and the FDA can be held morally responsible for the fatal

consequences of their respective actions: enrolling patients into the trial with pre-existing hepatobiliary disease and clearing the AT132 IND despite exclusion criteria ambiguity. By investigating the moral responsibility of the four fatalities in the AT132 clinical trial, the general reader will have developed a more comprehensive knowledge of the root cause of the AT132 fatalities and other gene therapy fatalities alike.

Although gene therapy has the potential to be life-changing for individuals plagued with many different conditions, it is important for engineers to be more meticulous in future trial designs to further mitigate the risk of SAEs. While it is difficult to predict the outcome of a clinical trial, utilizing frameworks such as ANT and moral responsibility to investigate the root cause of critical errors in previous trials can help ignite exploratory thought to develop better guidelines for future gene therapy trials.

Word Count: 3427

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The Software and Hardware Development of an Automated Microsphere/Cell Movement Device
Analysis of the Success of In-vitro Human Kidney Organoid Models

A Thesis Prospectus
In STS 4500
Presented to
The Faculty of the
School of Engineering and Applied Science
University of Virginia
In Partial Fulfillment of the Requirements for the Degree
Bachelor of Science in Biomedical Engineering

By
Jack Maschler

October 15, 2022

Technical Project Team Members:
Kaden Hoffman, Remington Martinez, Joshua Sanderson,

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.

ADVISORS

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Introduction

Prior to the development of 3D organoid structures in 2009 by Sato et al., the study of disease in-vitro (outside of a living organism) was greatly limited by the absence of the ability to mimic the in-vivo (inside a living organism) environment. However, the development of 3D organoid structures, cell structures constructed from stem cells, has allowed for the rapid improvement and success of in-vitro organ models used to study disease and drug efficacy. A clinician's ability to treat once untreatable diseases vastly improves as the disease and drug models become more personalized and better at mimicking in-vivo conditions. Therefore, researchers and companies would benefit greatly from an improved method for developing more complex organoid structures to model in-vivo conditions.

To develop organ models utilizing organoids, they are typically placed into a biomaterial to allow for vascularization to best mimic the in-vivo conditions (Hoang & Ma, 2021). Currently, the deposition of organoids into permissive biomaterials is done entirely manually through the use of pipettes and vacuum aspiration (Daly et al., 2021). The current approach limits the progression of organoid structures through the large degree of manual dexterity it employs, the monotonous and time-consuming nature of the method, and the pure lack of scalability and precision (Ren et al., 2021). To circumvent the limitations in the current approach, I will propose the development of a device that can automatically pick up and place organoids in user-defined orientations within a permissive biomaterial.

Although the efficacy and efficiency improvements to the current organoid structure development protocols are paramount to the continued success of in-vitro organ model research, the non-technical factors play an equally important role. The researchers, research funders, food marketers, and corporate America all function to permit the success of in-vitro organ model

research through different mechanisms that are highlighted by evaluating the success of in-vitro organoid-based kidney models. Failure to understand both the technical and non-technical actors will limit the success and future of in-vitro organ model research, ultimately hindering the development of treatments for devastating diseases.

To effectively develop a novel technology to automatically pick up and place organoids in a permissive biomaterial both the social and technical aspects of the issue must be considered. I will use both hardware and software to address the technical challenge of moving organoids into permissive biomaterials automatically while applying Actor-Network theory to address the human and non-human actors in the success of in-vitro 3D organoid-based human kidney models.

Technical Project Proposal

Organoids are three-dimensional cell cultures constructed from stem cells that function to mimic human organs in-vitro. Human stem cells were first successfully harvested in 1998, however, the first true organoid was not developed until 2009 when Sato et al. derived the first organoid from a single adult stem cell (ASC) seeded in Matrigel, a hydrogel material (Sato et al., 2009). The landmark study in 2009 demonstrated that the key factor in the induction of ASC differentiation, and thus organoid formation was the biomaterial and environment in which it was seeded (Sato et al., 2009). Since then, many different organoids have been developed and used to study diseases, develop drugs, and even the preliminary development of transplantable organs (Magno et al., 2020). Although the range of organ tissues that can be mimicked in-vitro using organoids has increased dramatically, their use in research remains limited by their lack of freedom of mobility (Ren et al., 2021).

ASCs are placed into biomaterials by hand using a pipette and once differentiated into organoids, moved to imprecise locations via vacuum aspiration (Daly et al., 2021). Although effective for small-scale studies where precision is less of a critical variable, the current organoid movement method is time-consuming, imprecise, and limits the scale and scope of potential studies (Ren et al., 2021). As with any procedure that relies heavily on human manual dexterity, the placement of organoids into specific locations within biomaterials is imprecise, thus introducing error and limiting study conclusions (Ren et al., 2021). Vacuum aspiration itself also introduces error through the accompaniment of media with organoid deposition. The extra media deposited with the organoid alters the desired extracellular environment, again limiting conclusions that can be made (Vonk et al., 2020). By improving the current movement method of stem cells and organoids, new research could be conducted with higher degrees of accuracy and a faster turnaround time. Additionally, a more precise placement method would open the possibility for more abstract organoid arrangements to better mimic in-vivo conditions (Yin et al., 2016).

This technical project aims to design a device that precisely places organoids into a designated position in a permissive biomaterial using an automated set-it and forget-it approach. There are two major user needs the team intends to meet with the development of this device: the ability to designate organoid placement locations, and for the pick-up and placement to be done without human intervention.

To allow the user to designate organoid placement locations, a GUI will be created using Python that allows the user to input the desired placement location(s). Next, we will use this input to move both the 96-well plate containing the organoid(s) and the plate containing the permissive biomaterial using a premade multiaxis sled movement system powered by NEMA 23

motors controlled by the same Python program. Then, to pick up and place the organoids, a 2020 Nano-liter injector, also controlled by Python, will be attached to the vertical axis of the multiaxis sled movement system. To attach the Nano-liter injector, new components will be designed and fabricated using 3D printing and/or laser-cutting. In conjunction, the aforementioned components will move plates and the Nano-liter injector to pick up and place the organoid(s) in the user-inputted location.

To fully automate the pick-up and placement of the organoids, a Basler Ace Camera will be attached to the vertical axis of the multiaxis sled movement system. Using image detection in Python, this camera will be able to detect the presence of organoids within wells in the 96-well plate allowing for the aforementioned components to work seamlessly without user interaction.

Finally, to verify the device is working as intended, nanospheres and organoids will be loaded randomly into a 96-well plate and various placement patterns and locations will be input into the GUI. The resulting biomaterial containing organoids and nanospheres will be analyzed to determine if the organoids were placed in the desired locations. Additionally, the biomaterial will be compared to a biomaterial containing human-placed organoids to quantify the improvement in precision, scalability, and time consumption.

STS Project Proposal

In November of 2015, researchers from Harvard University published the first instance of the creation of nephron organoids derived from human pluripotent stem cells, cells that can differentiate into any body tissue type. (Morizane et al., 2015). While the original findings were limited to studying mechanisms of human kidney development and toxicity, they were pivotal in the recent development of personalized 3D human kidney models to study disease progression and drug efficacy which directly support the goal of the NIH (National Institue of Health) to

enhance health and reduce illness. Chronic Kidney Disease (CKD), a disease treated only by kidney transplantation or life-long dialysis, plagues over 37 million Americans and is one of the leading causes of death in the US (*Chronic kidney disease in the United States, 2021*, 2022). The recent success in the improvement of in-vitro human kidney models using organoids has improved the understanding of specific kidney diseases, ultimately opening the door for the development of more accessible and effective treatments for CKD.

The success of the recapitulation of in-vivo human kidney conditions with in-vitro 3D organoid models is typically associated with the direct technical actors recruited by the NIH: research funding groups, researchers, and companies building on prior art. However, this outlook fails to consider how food marketers and corporate America have indirectly contributed considerably to the success. The most important factor in the permission of specific research to progress is funding (Aagaard et al., 2021). While funding can be raised through multiple modalities, in the medical research field it is the conditions with the highest prevalence and disability-adjusted life years (DALYs) lost to the disease that get funded the most (Kyu et al., 2018). The prevalence of CKD has increased from 13.2% to 14.4% over the past decade, which likely is a major contributor to the over 120 million dollar increase in CKD research funding from the NIH since 2010 ("National Institute of Health Research Portfolio Online Reporting Tools," 2022). This increase in prevalence can be tied to the increase in the prevalence of diabetes and hypertension, which are major, but controllable risk factors for CKD development. Both diabetes and hypertension are direct byproducts of obesity and thus have increased in prevalence as a result of the obesity epidemic (Hall et al., 2015). The continued progression of the obesity epidemic is often attributed to marketing the consumption of highly processed and palatable "junk" food and decreased physical activity due to the inherent sedentary nature of jobs

in corporate America (Cizza & Rother, 2012). Thus, both food marketers and corporate America have indirectly played a significant role in the improvement of in-vitro organoid-based human kidney models through their direct impact on the obesity epidemic which increased the prevalence and thus funding, for CKD.

The analysis of the success and progression of in-vitro organ modeling based solely on direct actors such as researchers and research funders fails to encapsulate the broader picture of how non-technical actors can indirectly influence research funding and ultimately research success and progression. Without these considerations, emerging research in organoid models for non-kidney organs may not be able to raise sufficient funding for success.

I argue that both the direct actors—the research funders and researchers—and the indirect actors—the food marketers and corporate America—led to the success of the recapitulation of in-vivo human kidney conditions with in-vitro 3D organoid models. To support my argument I will draw on Actor-network theory, which investigates a network builder that recruits both human and non-human actors to collaborate to accomplish a specific goal (Callon, 1987). I will use both Actor-Network theory and Michel Callon's concept of translation, the process of forming and maintaining an actor network, to examine the roles of human and non-human actors within the in-vitro 3D-organoid-based human kidney model network. Through this analysis, I will determine both who and what must be considered when aiming for success in future in-vitro organoid-based human organ model research. To accomplish this analysis, I will utilize research regarding the progression of 3D-organoid based human kidney models, epidemiological data regarding CKD, hypertension, diabetes, and obesity, and research regarding the effect of food marketers and corporate America on the obesity epidemic.

Conclusion

The deliverable for the technical problem discussed in this paper will be the development of a device that automatically picks up and places organoids into user-defined formations into a permissive biomaterial. The STS research paper will seek to determine why the development of in-vitro 3D organoid-based human kidney models was successful, which will provide insight into future non-technical considerations in the development of other organoid-based organ models. This will be accomplished by applying Actor-Network theory to identify how relevant human and non-human actors played a role in the success of in-vitro 3D organoid-based human kidney models. The combined results of this prospectus will serve to address the issue regarding the success and development of improved in-vitro organoid-based organ models from a socio-technical perspective, highlighting key considerations for researchers seeking both funding and improved methods for the development of de novo organoid-based organ models.

Word Count without citations:128 + 626 + 635 + 380 = 1,769

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