If Frankenstein Used His Knowledge For Good: Examining Lab-Grown Organ Systems as a Viable Solution to Animal Testing

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The darkened glass of the gas chamber betrays the panic inside. A mouse is suffocating on carbon monoxide filling the space, scrambling around the enclosure in mortal anguish as the chemical burns its eyes and lungs before losing consciousness. A week ago, it began showing symptoms of glioblastoma it was genetically engineered to develop, huddling away in the corner of its cage in pain and refusing food. Its body will be dissected, subjected to cold, impassive analysis, and then incinerated, erasing the traces of its short life, suffering, and death.

Years later, a twelve-year-old boy is sleeping on a hospital bed, IV tubes dripping fluid into his blood, a plush rabbit reclining in the gentle embrace of his arms. His body is frail and emaciated, thinner than any child his age should be. His mother is there with him, lulled into uneasy sleep by the monotonous beeping of his vital signs monitor, her weary face a history of seemingly unending angst-filled days and sleepless nights. She wakes up at once at the sound of the surgeon coming into the room. "Good news," he says, smiling warmly. "The new treatment appears to be working. We are observing complete remission."

Twentieth-century science had us believe that you could not have one without the other – to develop effective treatments, compounds must be tested on animals. Face it or get out of biomedical research. This realisation had a profound impact on me – as an ethical vegan, the thought of experimenting on animals was unbearable, no matter the moral benefits it would bring. However, the same scientific

field that spawned this ethical dilemma also gave birth to its solution, as recent breakthroughs in molecular biology, bioinformatics, and bioengineering resulted in the development of viable alternatives to animal research. Soon, organs-on-chips - structured colonies of tissue cells in carefully controlled microenvironments – and lab-grown organ surrogates may replace animals in investigations of biological drug effects, eliminating the need for sentient creatures to experience fear, pain, and anguish as a stepping stone to making life-saving biological discoveries.

It is futile to deny the importance of animal testing, a bedrock of biological research that has yielded innumerable cures that continue to save millions of patients around the world, and yet it is equally impossible to deny the inherent problems of this approach. While ethical concerns were a personal motivating factor for me in researching this topic, they carry a significant degree of subjectivity and therefore present a poor ground for arguing against animal testing, especially when most people (including myself) would allow animals to die if that would yield a treatment to save a loved one. Nonetheless, there are concrete, objective reasons for replacing or minimising this practice that go beyond moral consideration.

Specifically, while common animal models such as mice share a vast number of important physiological features with humans, there are certain biological and evolutionary differences that may cause the results obtained from these organisms to be inapplicable in biomedical research; for instance, a drug that appears to be effective and safe in animal models may prove to be toxic once administered to patients. Such was the case of TGN1412, a therapeutic antibody developed as a treatment for B-cell lymphoma and rheumatoid arthritis. In his 2010 review "TGN1412: From Discovery to Disaster," Husain Attarwalla, a researcher at Northeastern University's Department of Pharmaceutical Sciences, details the catastrophic conclusion of the drug's development. While preclinical trials in cynomolgus and rhesus monkeys showed no adverse effects to the drug at doses as high as 50 mg/kg, the phase I trial in human volunteers ended disastrously: within an hour of receiving a therapeutic dose of 0.1 mg/kg, all six test subjects

developed cytokine release syndrome, a life-threatening immune response that resulted in multiorgan failure and hospitalisation (Attarwalla 332-5).

Cases like this contribute to the erosion of trust that the scientific community puts in animal testing. According to Dr. Aysha Akthar, a neurologist and a fellow at the Oxford Centre for Animal Ethics, "as medical research has explored the complexities and subtle nuances of biological systems, problems have arisen because the differences among species along these subtler biological dimensions far outweigh the similarities, as a growing body of evidence attests. These profoundly important—and often undetected—differences are likely one of the main reasons human clinical trials fail" (Akthar 413, emphasis in original). Therefore, while the use of animals for drug testing has historically been the foundation of the pharmaceutical industry and has given rise to many successful medications, it is clear that the significant challenge posed by the species gap cannot be left unaddressed as the field evolves, prompting the search for non-animal alternatives that can model the human organism more accurately.

While this challenge may have appeared unsolvable several decades ago, the tremendous advances in a range of scientific disciplines over the last few decades resulted in technological developments that hold new promise for replacing or minimising animal testing. The first such technology, dubbed "organ-on-a-chip" (OoC), consists of an artificial cell system in a highly controlled environment that can be used to study disease development or test novel therapeutics. These devices reveal a complex internal microenvironment that "reflects the structural and functional characteristics of human tissue and can predict response to an array of stimuli including drug responses and environmental effects," write Qirui Wu and colleagues in their 2020 review "Organ-On-a-Chip: Recent Breakthroughs and Future Prospects" (Wu et al. 1). Organs-on-chips typically feature a small chamber engineered to create the desired biophysical conditions and filled with cells of interest. The internal state of the chip is regulated through tubes connected to external pumps that control the flow of media

through the chamber and monitored through arrays of sensors mounted into the casing (Wu et al. 3-5). Together, these features grant the researchers the "ability to regulate key parameters including concentration gradients, shear force, cell patterning, tissue-boundaries, and tissue–organ interactions" (Wu et al. 1-2).

In a 2012 study that serves as a prime example of the possibilities offered by this technology, Dongeun Hu and his co-workers at the Wyss Institute for Biologically Inspired Engineering used the OoC approach to investigate drug toxicity-induced pulmonary oedema (a severe lung inflammation characterised by accumulation of fluid in alveoli). The researchers used chemical etching to create microcapillaries in a polymer substrate, which were subsequently seeded with pulmonary epithelial cells to mimic the oxygen-exchange surface in human lungs. Lung motion was simulated with periodic mechanical stretching of the cell layer by applying vacuum to both sides of the chamber. This lifelike recreation of a human lung enabled the researchers to simulate a dangerous side-effect of the cancer immunotherapy agent interleukin-2 without the need for animal models: "this on-chip pulmonary edema model effectively reproduces the intra-alveolar fluid accumulation, fibrin deposition, and impaired gas exchange that have been observed in living edematous lungs following 2 to 8 days of IL-2 therapy in humans." (Huh et al. 4). As such, despite their seemingly trivial operation principle, organs-on-chips deserve serious consideration as the next-generation biomedical modelling technology.

While OoCs serve as an excellent way to simulate the environment of a human organ, they are by no means the only one. While this may seem like pure science fiction, researchers have devised a way to grow organoids – simpler replicas of human organs – in a laboratory setting. This concept harnesses the ability of a certain kind of stem cell to differentiate into any other cell type in the body – a quality known as pluripotency. To take advantage of this trait for the process of growing organoids, researchers use induced pluripotent stem cells (iPSCs) – regular stem cells treated with particular signalling molecules that revert them to a "blank slate" state – or pluripotent stem

cells harvested from human embryos. At first glance, organoid development seems like a long, complicated process. Surprisingly, this is not the case. Edward Kessler, a PhD student at Harvard Medical School studying cortical organoids, reveals that the procedure is not particularly difficult: "it's essentially a sequence of media changes," he told me in a personal interview, albeit noting that the composition of each media is strictly controlled. He explains that, in the process of creating an organoid, a monolayer of stem cells grown in a Petri dish is reaggregated into a clump called an embryoid body, which is then placed into growth media containing factors that induce desired differentiation in the cells. "You can imagine a stem cell as sitting on top of a hill, with many different paths it can take. By combining different signal factors in the media, you can push it in the right direction, and then just let the cells self-organise" (Kessler).

This technology has given rise to stunning biotechnological achievements. In a landmark 2013 project, Madeline Lancaster and her colleagues at the Institute of Molecular Biotechnology at the Austrian Academy of Science developed the first iPSC-derived cerebral organoid with separate brain regions. The researchers cultured stem cells in media containing fibroblast growth factor (a signaling protein inducing cell division), followed by a transfer to neural induction media to facilitate cell differentiation into neurons. Next, neural embryoid bodies were injected into gel droplets that acted as semi-solid scaffolds for more complex 3D tissue formation, after which the maturation process in stirring liquid media was completed in just 20-30 days. While the organoids grown in this experiment hardly resemble human brains – at most 4 mm in diameter and looking more like misshapen berries than real cortices – they nonetheless represent a monumental breakthrough in this field. Using fluorescent staining for biomarker proteins expressed by cells in different brain regions, Lancaster's team was able to identify complex cell organisation and the development of several distinct areas, bearing close similarity to the development of real human cortical tissue (Lancaster 373-80). Scientific advances such as this provide fertile ground for the exploration of organoids as suitable models for study-

ing disease pathways and drug toxicity.

Both organs-on-chips and organoids possess significant advantages over other drug testing methods such as animal models or in vitro human cell cultures. Specifically, the use of human cells in both OoCs and organoids bridges the species gap and makes data obtained from drug experiments more applicable to human patients. Furthermore, the complex internal structure of organs-on-chips make it possible to "control cellular and specific tissue architecture to emulate chemical gradients and biomechanical forces," according to Lucie Low and co-workers at the National Institutes of Health (Low et al. 347). It is also possible to re-create blood vessel networks in OoCs by introducing microscopic channels into the chip, which enables researchers to supply the cells with nutrients in a lifelike manner. The high degree of control over the cellular architecture present in the OoC technology makes it possible to repeat the same experiment numerous times with a high degree of fidelity. Meanwhile, the construction of the chip allows extensive monitoring of the experimental set-up through optical microscopy, microelectrodes, and other sensors, facilitating massive biochemical data harvesting (Low et al. 347-50).

Organoids approach the question of mimicking a living system from a different angle, and therefore present a set of completely different benefits. Firstly, the use of pluripotent stem cells that self-organise under exposure to different growth factors closely mirrors the natural developmental process of organs. This allows for a much more realistic cellular environment than OoCs, which are entirely artificial and feature extensive human intervention in natural biological processes ("Dan Huh's Organs-on-Chips and Organoids: Best of Both Worlds" 0:45). Furthermore, allowing cells to form structures by themselves results in a much more complex arrangement of cell types and tissue structures than could be obtained by a simple 2D culture of cells in vitro, which is a key advantage for research on organs with highly specific structures such as kidneys or the brain (Hofer and Lutolf, 402).

While it may seem like organoids and organs-on-chips are perfect models for biological experimentation,

it is important to recognise a number of shortcomings associated with both. Crucially, the main approach to creating OoCs includes "reverse-engineering" human organs by considering the elements that comprise them and re-creating them on a chip. Therefore, a lack of understanding about certain biological processes, as well as financial and logistical constraints, place a limit on how accurately these systems can represent anatomical structures. Furthermore, while the combination of several discrete organs-on-chips into one unified "human-on-a-chip" opens new opportunities for research on compound biological systems, such as considering the effects of a drug on the organism as a whole, the new level of complexity inherent in this technology brings with it challenges that are absent in single OoCs. For instance, different tissue types in a multi-organ system need to be supplied with different nutrients and signal factors, which presents difficulties when a single universal medium is circulated through the system (Low et al. 349-50).

On the other hand, the organoid technology suffers from a high degree of variability. In a 2020 review of organoids that replicate brain tissue, Silvia Velasco and co-workers at Harvard University's Department of Stem Cell and Regenerative Biology recognise that allowing cells to self-organise is a process that inherently carries a high degree of unpredictability and randomness, though noting that several projects have attained promising levels of reproducibility (Velasco et al. 380). Similar to OoCs, the process of inducing cell differentiation is largely a result of reverse engineering the corresponding natural processes and attempting to recreate them in vitro. As such, gaps in existing knowledge about organ formation inevitably result in organoids that lack some biological features of full organs: "...noticeable omissions include the organization of cells into defined anatomical structures (e.g., cortical lamina, thalamic nuclei), regional patterning, and the formation of cell type–specific long- distance and local connectivity" (Velasco et al. 382).

The aforementioned issues are a testament to the novelty of the organoid and organ-on-chip technologies, as we are witnessing a new field of research unfold before our very eyes. While animal

testing will continue to dominate the field of biological research in the observable future due to problems associated with artificial models and the established nature of animal experimentation, there is a significant push for greater exploration and development of alternatives in the scientific community. In an interview for Nature Reviews Materials, Dr. Donald Ingber, the founding director of the Wyss Institute for Bioinspired Engineering, explains the need for raising greater awareness of the existing non-animal testing technology: "There is … a proposal to start a new institute in the National Institutes of Health (NIH) focusing on in vitro models, which may help to build up the number of people familiar with this opportunity … Engaging more researchers may help to push the technology over the top, such that people start looking beyond animal models" (Horejs 373).

As more scientists are drawn into this field, one can imagine further breakthroughs. Merging organoids and organs-on-chips into one technology, combining several organs-on-chips into a unified "human-on-a-chip", improving methods for real-time data collection in OoCs, and developing more complex differentiation and growth protocols for creating more lifelike organs are all targets of ongoing research. On a personal note, I can say that in the course of writing this work, I have regained my faith in biotechnology as a way of harnessing human knowledge to do good. I hope that this review has also sparked some measure of interest in my reader, perhaps serving as an inspiration to look deeper into this topic or share it with others, thereby answering Dr. Ingber's call for greater involvement in the field and continuing to drive a revolution in biomedicine.

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