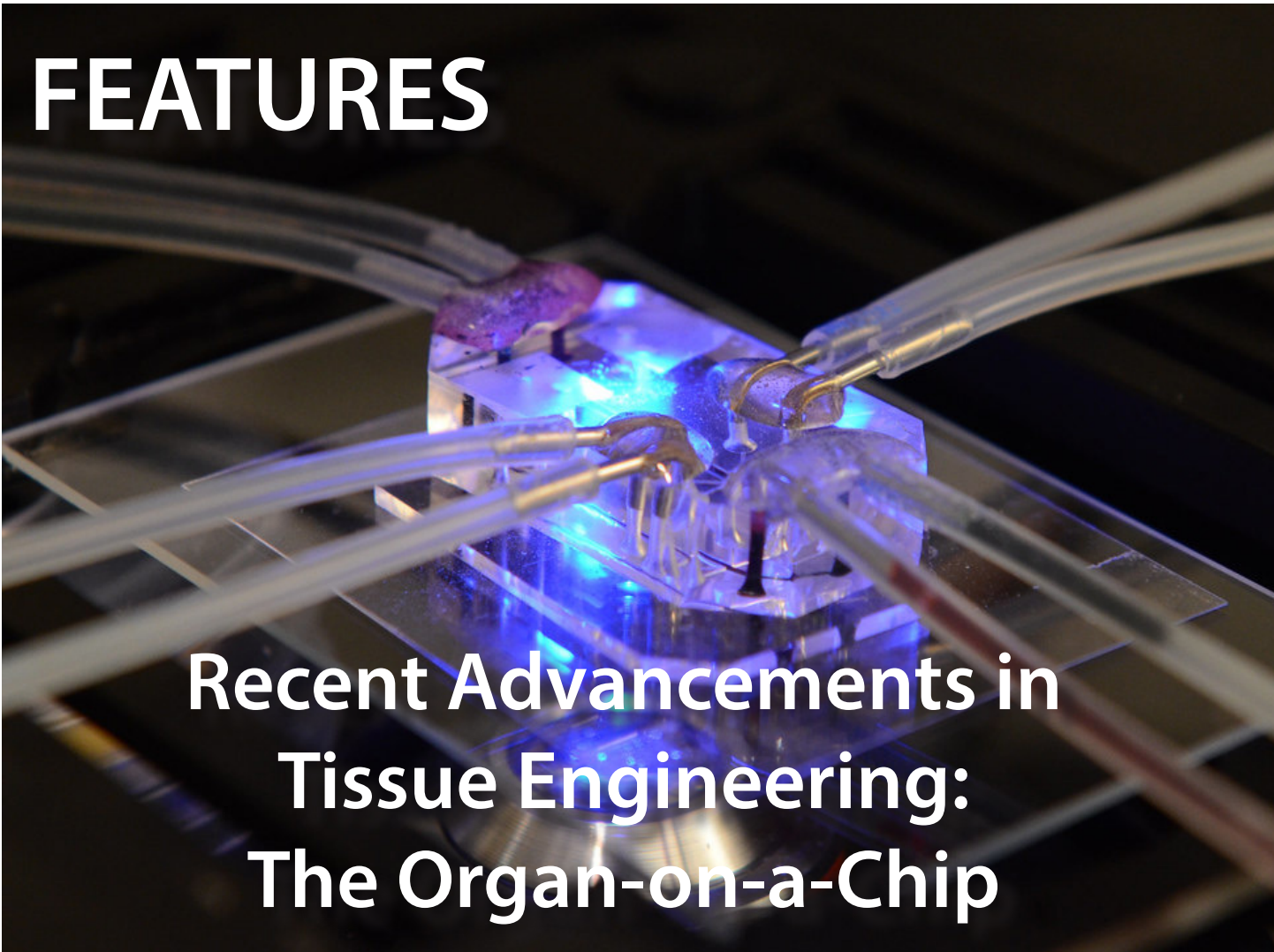


FEATURES



Recent Advancements in Tissue Engineering: The Organ-on-a-Chip

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From pipetting biological solutions within a vented biosafety cabinet to collecting epidemiology data on the ground at a disease hotspot, medical research is a fast-growing and extremely expensive industry. The National Institutes of Health (NIH), a federally funded organization for “all things health” in the U.S., spends almost \$48 billion annually on healthcare-related research and clinical trials, dispersed among universities, medical schools, hospitals, professional laboratories, and NIH’s own labs (National Institute of Health, 2023). Along with these foundational labs, private research facilities, large pharmaceutical companies, and biotech startups all compete within the biological innovation and medical therapeutics space. As a result, some biomedical researchers, many within the subfield of clinical medicine, publish more than 60 papers a year, or once about every 6 days (Ioannidis et al., 2023).

The Drawbacks of Traditional Biomedical Research

As the field undergoes rapid advancements, limitations of traditional

biomedical research models are brought to the forefront: (1) it is hard to find a balance between the application-based drawbacks of in vitro studies and ethically challenging in vivo studies; (2) animal studies do not always translate well to human physiology; and (3) animal experimentation is monetarily and temporally expensive.

In vitro research, which mimics biological processes outside living organisms, often fails to capture the complex mechanisms involved within the original species. For example, many cells grown in the lab adhere to the bottom of Petri dishes and grow in a single layer, while cells in the human body grow in multiple layers and are found in a 3D extracellular matrix. Thus, in vitro research is commonly used in preliminary studies of a therapeutic to test initial effects and to ensure its safety for use in living organisms. On the other hand, in vivo experimentation is conducted on non-human animals, including mice, rats, dogs, monkeys, and pigs. More than 110 million animals are sacrificed each year in American laboratories, and even more are intentionally induced with debilitating diseases, caged in small spaces, and exposed to harmful side effects of potential drug candidates (PETA Staff, n.d.). Moreover, a systematic review found that animal experimentation for human

application has historically led to many cases of misleading data and human harm due to a combination of laboratory-specific variables, variance between humans and other species, and differences in disease-related effects on humans versus animals (Akhtar, 2015). Furthermore, animal experimentation is time-consuming and expensive: a single cancer therapy study using rodents costs about \$2 to \$4 million and spans 4 to 5 years. Compared to “petri dish” in vitro testing, in vivo experiments are between 50% and 3000% more expensive (Van Norman, 2019).

If in vitro testing is not fully effective and in vivo testing is both detrimental to animals and costly, then what is the solution? Many researchers are working to mitigate these concerns using different methods. Some are turning to artificial intelligence to narrow down drug compounds from millions of potential candidates, effectively saving a considerable amount of time and resources. Others are seeking to develop novel solutions by leveraging the benefits of both in vitro and in vivo models while minimizing each’s drawbacks.

The Organ-on-a-Chip

One particularly robust solution is the organ-on-a-chip (OoC), a small chip that contains lab-grown tissues, natural tissues, or clusters of cells immersed in a controlled environment of nutrients, fluids, and essential molecules (Leung et al., 2022). They can be used to mimic microenvironments within the human body, test cell-cell interactions, and assess the impact of drugs. OoCs—a culmination of advances in tissue engineering, microfabrication, 3D bioprinting, microsystems, and microfluidics (the study of fluid flow through micro-sized channels)—bridge the gap between in vivo and in vitro studies by more accurately mimicking complex human physiolog while decreasing experimental costs and animal harm (Leung et al., 2022). In one of its original forms as a lung-on-a-chip, lung airway cells are grown in fluid-filled nanosized channels (colored lines) and separated by porous membranes within a PDMS chip (made of a clear, semi-flexible rubber material). This unassuming device was used to study the effects of respiratory diseases like pulmonary edema and COVID-19, guiding further research and therapeutic development (Fig. 1) (Folch, 2022).

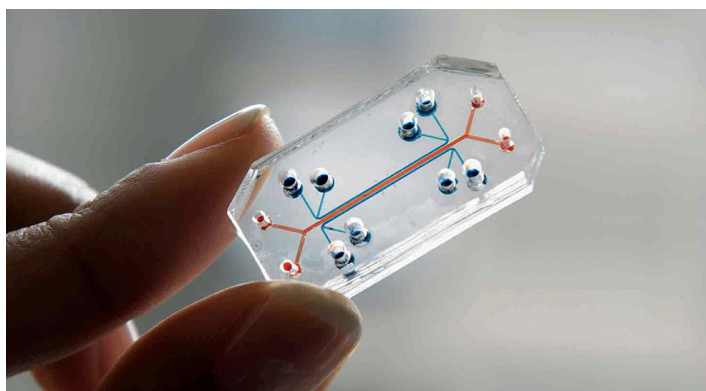


Fig. 1. Lung-on-a-chip (Folch, 2022).

The Heart-on-a-Chip

One famous example of the use of the OoC concept is the 3D-printed cardiac devices with fully integrated sensors from the Disease Biophysics Group at Harvard University. In the human body, cardiac muscle cells contract to pump blood into and out of the heart. The force these cells exert when they contract is called tissue contractile stress and can vary in response to different stimuli (e.g., minerals, hormones, neural signals, disease, drugs) (Burrows, 2016).

In 2016, Lind et al. improved existing methods to measure this useful contractile stress parameter with the development of a heart muscle organ-on-a-chip, only 75 millimeters wide and made of eight individual wells (Fig. 2). The device is printed in a continuous process and utilizes four individual printing nozzles and six different ink materials (determined via optimization testing to better mimic cardiac tissue stress and contractility). It includes grooved microstructures so that when rat heart muscle cells, or neonatal rat ventricular myocytes, are introduced into the device, these cells are guided to assemble into self-organized tissue (Lind et al., 2016).

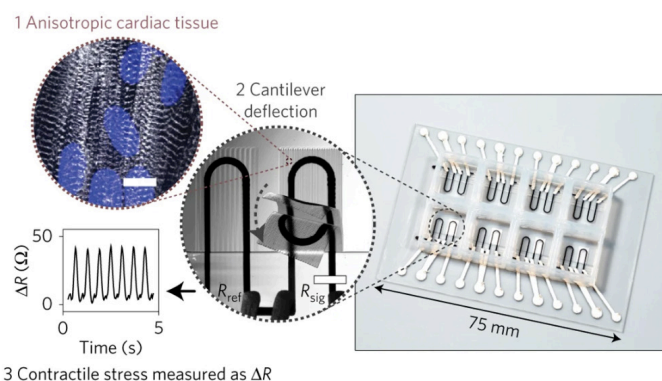


Fig. 2. Device to measure contractile stress of cardiac tissue (Lind et al, 2016).

This device is superior to previous measurement methods because embedded OoC sensors are direct, noninvasive, and enable electronic data collection (rather than optical collection via a microscope). As a result, scientists can store the device in a cell incubator—a closed box kept at about 99 ° and constant humidity levels—to better mimic the human body temperature. This enabled researchers in the Disease Biophysics Group to study the impact of common hypertension drugs, thicker cardiac tissue models, and even time on tissue contractile stress (Lind et al., 2016).

The Tumor-on-a-Chip

OoCs can do more than just mimic normal human body microenvironments—they can also mimic pathological states, including cancer. Cancer cells can proliferate rapidly, which is detrimental inside the human body but makes it much easier to keep tumors alive for in vitro studies. This benefit was evident when a biotechnology company in the Netherlands suspended

breast cancer cells in their company's high throughput organ-on-a-chip platform. Each device featured up to 96 iterations of a microfluidic chamber (**Fig. 3**), allowing them to simultaneously test multiple conditions across multiple trials (Lanz et al., 2017).

This approach differs from a 2D Petri dish or well-plate culture, as it involves suspending cancer cells in a liquid medium (e.g., collagen I) and inserting them into a chamber of the OoC device. Changes in both pH and time facilitate the gelation of collagen, successfully creating a 3D extracellular matrix (ECM) for the cancer cells to grow and proliferate in. This ECM environment, along with constant perfusion of cell nutrients, improves cell health and more closely mimics the *in vivo* tumor microenvironment, allowing researchers to observe distinct cellular functions and properties that cannot be observed in 2D cultures (Lanz et al., 2017). Thus, chemotherapy drug screening in 3D OoC cultures is more useful and applicable than screening in 2D cultures.



Fig. 3. Device with wells to hold ECM-embedded cancer tissue (Lanz et al., 2017).

The Human-on-a-Chip

The human-on-a-chip or body-on-a-chip boasts several tissue types within one device. At an industrial lab in Florida, a team of researchers has constructed a multi-organ system—that includes the liver, the spleen, the endothelium, and circulating red blood cells (RBCs)—to examine the pathological effects of malaria, particularly the effects caused by the protozoan parasite *P. falciparum*. Corresponding cell types are seeded within the liver, spleen, and endothelium compartments (**Fig. 4**), while RBCs and nutrients follow a gravity-driven flow between these compartments with sinusoidal rocking. Once the protozoa strains are introduced into the RBC culture in the device, various parameters of each cell type are tested along with the effects of chloroquine, an antimalarial medication (Rupar et al., 2023).

The body-on-a-chip can be a meaningful approach for many reasons. Conditions like malaria systematically affect more than one organ or area of the body (via infected RBC circulation), which is often hard to recapitulate with traditional *in vitro* methods. This OoC construct is a close replica of the

infected RBCs' interactions, focusing on organs related to the fundamental properties of malarial infection: the spleen assists the immune system in clearing the parasite, endothelial cells contain receptors to which infected RBCs bind, and the liver is responsible for drug metabolism. As a result, Rupar et al. were able to create a controlled, yet connected environment to study their pathology of choice (2023).

Organ-on-a-Chip Limitations

While OoCs offer promising advantages and opportunities, the organ-on-a-chip system is not flawless. Although their small size allows for the design of cheaper and easier-to-recapitulate experiments, it also presents disadvantages, as the simulated microenvironment often cannot completely replicate the full complexity of the environment within an organ. For example, a liver tissue microenvironment may not be able to establish sinusoids, which are tiny blood vessels found in an actual liver (Reif, 2014). This omits essential organ functions and may lead to discrepancies in experimental results in comparison to actual clinical trials, but it generally results in fewer discrepancies than conventional *in vitro* studies. Still, many labs are working to overcome this gap by leveraging strategies from adjacent fields (i.e. novel 3D bioprinting techniques and digitally controlled dynamic environments to induce vascularization). There are also technical challenges—since the quantity of fluid flow is very small, unwanted properties may appear due to surface tension and improper mixing of fluids (Danku et al. 2022).

Moreover, OoCs are a relatively new technology, lacking standardization of design between various labs and applications. Thus, even if the design of an OoC is well-elaborated within research papers, its many moving parts may lead to less reproducible results between different experiments. Ethical concerns include the process of gaining permission to use a person's cells once OoCs are personalized to patient groups or

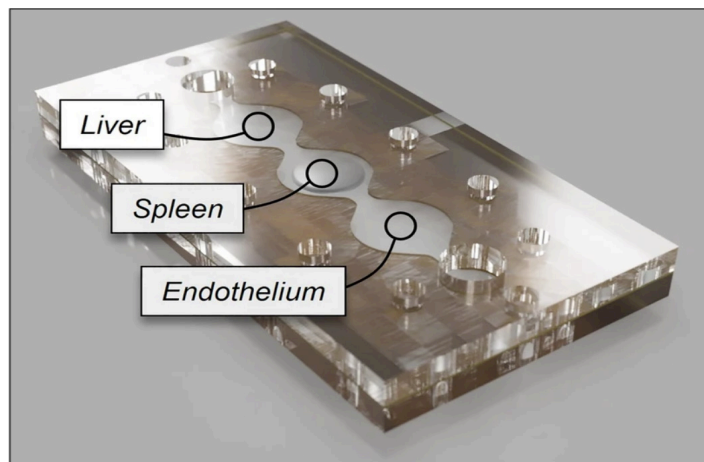


Fig. 4. Body-on-a-chip combining liver, spleen, and endothelium 3D cell cultures (Rupar et al., 2023). 2017).

even individual patients. As the field grows rapidly, question emerge: what protections would a fetus-on-a-chip deserve? What if a brain-on-a-chip develops some measurable consciousness property (Thakar, 2023)? At present, the state of OoC design means these questions are not yet pressing, yet considering rapid advancement of the biomedical research community, they stand none the less.

Conclusion

Regardless of the current limitations surrounding organ-on-a-chip technology, this field of biomedical research is here to stay. Many biotech companies from the Netherlands-based Mimetas to the Texas-based Systemic Bio are working to streamline and industrially manufacture these microfluidic chips. Additionally, OoC systems are being more broadly integrated into the biomedical research enterprise. For example, the incorporation of deep learning, an area of artificial intelligence, enables the instant screening of massive databases to narrow down experimental groups and facilitates rapid analysis of the large amounts of data generated by high-throughput OoC systems (Li et al., 2022). The organ-on-a-chip provides a platform for seemingly endless possibilities and will undoubtedly continue to change the face of conventional biomedical research, one recapitulated tissue at a time.

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