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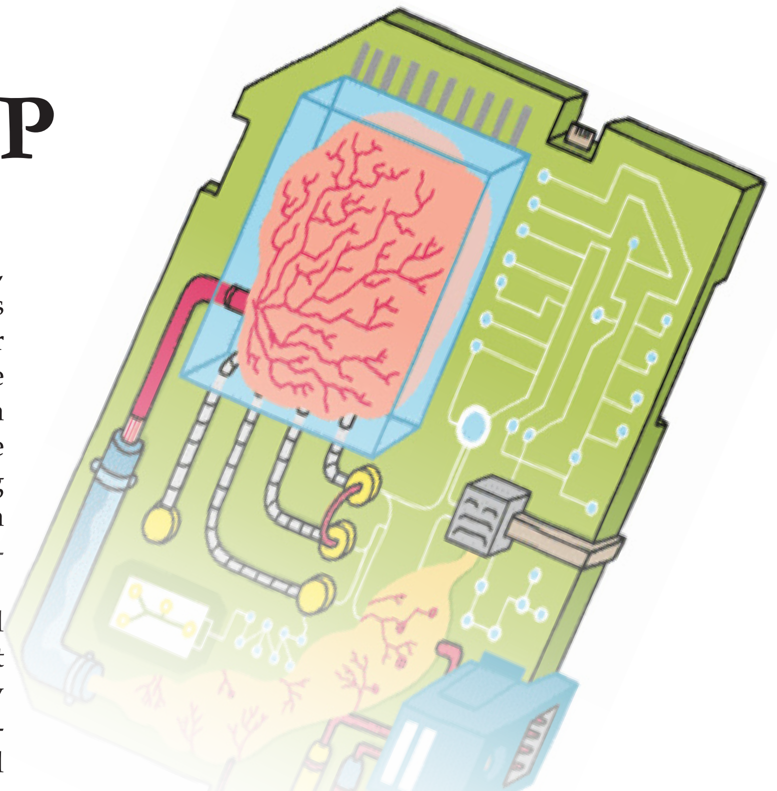
LAB-ON-A-CHIP

Ann Heslin



Picture the length of two centimeters. Now, picture a synthetic chip that can mimic all functions of an entire human lung within this two-centimeter length. Pretty remarkable, right? What is even more remarkable is that this in vitro (existing outside a living organism) device is precise on a micro-scale (10-6) and has been shown to be a reliable human lung model. This lung-on-a-chip device is one example of a broader category of synthetic devices known as 'lab-on-a chip' or 'microfluidic' devices.

Lab-on-a-chip is what it implies: miniaturized science. Though the theory of miniaturization is not hard to conceptualize, the process of turning theory into practice has proven to be much harder. Nevertheless, meticulous engineering has led to the rapid advance of these devices and has paved an auspicious path for their contribution to organ modeling.

One challenge the lung model faced was how to recreate a functional alveolar-capillary interface that is present in all human lungs. To understand this model, let us briefly revisit the physiology of a lung. During respiration, air passes down through the lungs into alveolar sacs where gas exchange occurs (Fig 1). But before oxygen can enter the bloodstream, it first diffuses through alveolar epithelial cells and then passes through a fused basement membrane, where it encounters the capillary endothelial cells.

In the lung model, a 10 μm porous membrane composed of poly(dimethylsiloxane) (PDMS) represented the fused basement membrane, which had epithelial and endothelial cells growing as monolayers on opposing side (Fig 2). Extracellular matrix (ECM), which can be thought of as a gluing substance, was coated on both sides of the PDMS membrane to allow the two cell lines to firmly adhere. Once attached to the material, both cell lines grew to 100 percent confluence (the whole PDMS membrane was covered with cells) and sustained themselves for a prolonged period of over two weeks.

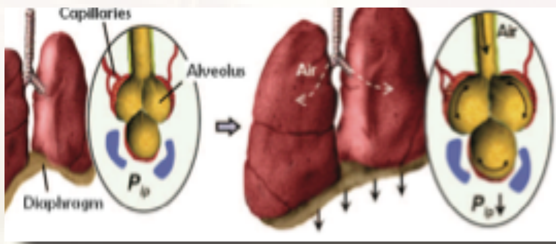


Figure 1.

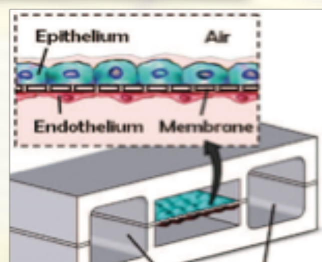


Figure 2.

Next, this thin PDMS membrane was clamped between two thicker layers to form two smaller side chambers and one larger central cavity (Fig 3). An etching solution was pumped through the two side microchannels so that only the larger, central cavity had an upper and lower compartment.

This engineering allowed for air to enter the upper compartment, mimicking the air-liquid interface within alveoli. A vascular medium (blood-like substance) was also allowed to run simultaneously through the lower compartment to emulate capillary blood-flow.

The lung model also accounted for the physical movements—expansion and contraction—that occur during respiration. During inspiration, the diaphragm contracts (moves downwards) to decrease the intrapleural pressure inside the alveolar sacs (sacs expand in volume), which increases the efficiency of gas exchange (Fig 1). A vacuum was applied to the two side micro chambers to create a pressure-driven stretching of the thin PDMS membrane (Fig 4). This vacuum-induced stretching distorted the cultured epithelial and endothelial cells, forcing them to survive a physical environment similar to one that occurs in vivo (inside a living organism).

Not only did the cells withstand the physical strain, but the top epithelium cells also responded by producing surfactant, a detergent-like substance that reduces surface tension. This biological response was significant because it further validated this lung-on-a-chip as a reliable model that could imitate a real lung.

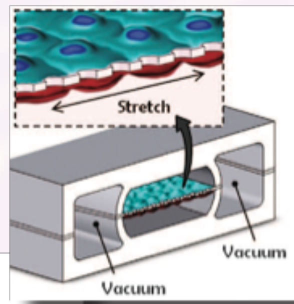


Figure 3.

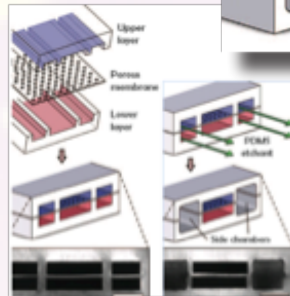


Figure 4.

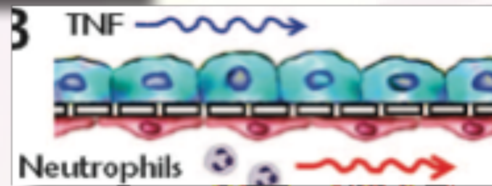


Figure 5.

Pulmonary inflammation was assessed in order to determine the model's ability to produce a whole-organ response. To start, TNF-α , a potent proinflammatory mediator, was applied to the top epithelial cells to induce an inflammatory response. Blood-borne immune cells (neutrophils) were simultaneously channeled through the lower (vascular) compartment of the middle cavity (Fig 5). The bottom epithelial cells that faced the vascular component responded to the pulmonary inflammation by recruiting neutrophils from the stream of blood. The fluorescently labeled neutrophils were visualized to physically adhere to the bottom endothelial cells, transmigrate across the thin PDMS membrane, and finally reemerge onto the top epithelium subsurface—all within minutes! More elaborate and extensive inflammatory response tests were conducted and all reconfirmed the reliability of this in vitro lung-on-a-chip.

Despite the remarkable accomplishment of this device, lab-on-a-chip needs to do more than merely reconfirm what biologists already know. Microfluidics is a tool for scientists to gather novel insight into to how, where, or why an organ might react in a given circumstance.

The lung-on-a-chip also led to these types of novel findings. Specifically, when silica nanoparticles (a known pulmonary irritant) were introduced to the system, mechanical strain was shown to exacerbate pulmonary inflammation. Silica nanoparticles were first introduced in the absence of the vacuum-induced strain, which resulted in the top epithelial cells producing the expected inflammatory response. However, when vacuum-induced strain was applied, silica nanoparticle absorption into the bloodstream increased. This in vitro lung apparatus observed the toxic effects of silica nanoparticles, a new mechanism of toxicity that had never been previously visualized. Despite the inherent differences between the in vitro lung-on-a-chip and an in vivo human lung, this microfluidics device has been established as the most reliable human lung model.

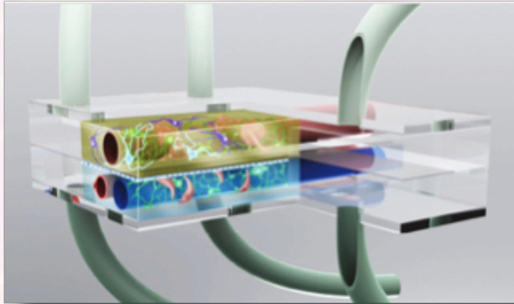
There is a need to extend models beyond that of just the lung. Insomuch as the heart and liver are the two organs most associated with idiosyncratic and adverse drug reactions, microfluidic developments in these areas are of particular priority. The reason for the slower development of reliable heart and liver models is that these models necessitate another component: a third dimension. Note that the lung model did not necessitate cells to exist beyond that of monolayers because the alveolar-capillary interface is, in fact, composed of single cell layers. 3-D cell culture has been shown to act differently than conventional 2-D ones, so 3-D models become essential for the proper and accurate modeling of a heart and liver.

3-D heart models have been less successful because they fail to incorporate both the electrophysiological properties and contractile organization of heart tissue. Even though heart tissues can physically contract in vitro, they lack either contractile alignment or 3-D structure. In vitro liver models must support hepatic polarity (diversity) and cannot disturb cell-matrix interactions. Advanced 2-D models were shown to improve cellular polarity, function, and viability, but past 3-D models have failed to provide the required quantities and qualities of oxygen and nutrients. In summary, in vitro cardiac and hepatic models cannot yet accommodate the high-throughput drug screenings necessary for generating reliable data that can predict heart and liver toxicities.

Nevertheless, efforts to develop these 3-D models have been accelerated with the integration of stem cell research. In 2012, Shinya Yamanaka and John Gurdon shared the Nobel Prize when they discovered how to reprogram human adult fibroblasts (a specific type of cell) into pluripotent stem cells. Specifically, the Yamanaka lab performed a retroviral transduction of four factors (inserted specific reprograming genes)

into human adult cells, which induced those mature cells to become pluripotent cells, stem cells capable of regenerating into any type of body cell.

Induced pluripotent stem (iPS) cells are advantageous because this means any adult tissue can be harvested and transformed into any type of body cell. One advantage to this is that patient- and disease-specific cells could be cultured to provide scientists with a personalized and regenerative 3-D model. Implementing iPS cell technology would also bypass the use of embryonic stem (ES) cells, which sparked a controversy about embryo interference. iPS cell technology has revitalized the potential for the coupling of stem cells with lab-on-a-chip to build better 3-D human models.



The National Institute of Health (NIH) has recognized the importance of developing more accurate 3-D in vitro models and has allocated various awards that support tissue chip technologies. In 2012 the NIH issued grants that supported the development of 3-D tissue and cell source models. One of these awards was presented to the Healy research group at UC Berkeley. Prof. Kevin Healy's lab in collaboration with Prof. Luke Lee, Prof. Bruce Conklin, Prof. Holger Willenbring at Berkeley, Gladstone Institutes, and UCSF respectively, are working on in vitro human cardiac and liver tissue based models that employ normal and patient-specific human iPS cells.

Anurag Mathur and Peter Loskill, postdoctoral fellows in the Healy lab are currently developing a microfluidic device that can support the function of cardiomyoctes (CMs) (cardiac muscle cells) derived from iPS cells. They insert CMs into a custom microfluidic device and then wait for the cells to attach and spread in vitro. These CMs form a 3-D tissue, which exhibits essential electro-physiological and contractile properties of the heart tissue.

Despite the advancements Mathur and coworkers have made, there are challenges concerning accurate reproduction and replication. The regeneration of CMs requires "meticulous documentation of every small molecule and/or growth factor that is introduced," he says. In other words, a slight deviation might cause lower yield or inconsistent data. Nevertheless these problems will most likely be resolved in the near future and continual development is worthwhile. The other NIH awards support the development other organ models, which are all rooted in one principle: that the integration of microfluidics and iPS cells will culminate in the best 3-D in vitro models.

The future of microfluidic and iPS cell integration is fascinating. Consider, for example, the layering of multiple in vitro organ models to generate an apparatus that can analyze multiple organ-organ responses. The heart and liver were previously mentioned as primary targets for organ toxicity. Imagine an apparatus that coupled both heart and liver organs—such a model would be indispensible to the field of predictive toxicology, which is currently inefficient, costly, and unreliable.

This inadequate drug discovery and development process can be attributed to insufficient toxicity models and dependency on animal testing. Don Ingber, head of the human-on-a-chip project at Wyss Institute at Harvard, labels this inefficiency as Eroom's Law, which states "that the number of medicines invented halves each year, while the prices seem to go up continually." Eroom's Law opposes the well-known Moore's Law (Eroom spelled backwards) that observes increasing price and product efficiency. The development of better predictive models that integrate iPS technology will make the drug discovery and development process more affordable, efficient and reliable.

"iPS cell technology has revitalized the potential for the coupling of stem cells with lab-on-a-chip to build better 3-D human models"

Coupling iPS cell technology with microfluidics will culminate in 3-D human models that offer a range of advantages: there will be faster higher-throughput analysis and screening of cellular responses to drugs, chemicals, particulates, toxins, and pathogens; assays will be reproducible and parallelized; miniaturization will decrease the amount of experiment material needed; biologists will have a cheaper tool to investigate mechanisms of toxicity and metabolic processes; patient-specific cell lines will be generated for personalized medicine; and, portable

technologies will be used in surveillance and detection of pathogens in public health.

The aforementioned examples do not encompass all the potential applications for lab-on-a-chip, and the established success within this field is a harbinger of the success that will to continue to emerge. It is also important to understand that the biology, chemistry, engineering and physics must continue to be interdisciplinary. The integration of multiple fields will enable microfluidic models to be more accurate—albeit complex—and will improve in vitro testing.

Abbreviations

PDMS : poly(dimethylsiloxane)
ECM: extracellular matrix (ECM)
TNF-α : tumor necrosis factor alpha
iPS: Induced pluripotent stem (iPS)
CMs: cardiomyoctes (CMs)

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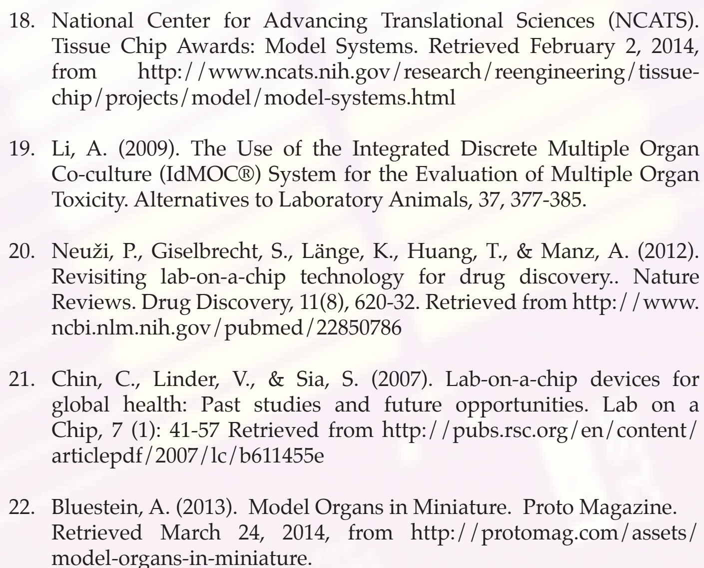

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