Developing Microphysiological Systems for Use as Regulatory Tools – Challenges and Opportunities

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In the last few years, scientists have made important progress in developing systems using human cells to test the effects of drugs and other substances. These systems have the potential to improve toxicity testing beyond currently available tools. The innovative new tools, which are known as microsystems, microphysiological systems, or organs on a chip, can aid in the development of medical products so that toxicity may be identified earlier in product development. This may lower costs and speed new treatments to patients. Experts believe that these systems may eventually enable scientists to test more environmental compounds more efficiently.

On May 10, 2013 more than 220 scientists in academia, industry and regulatory agencies met in person and virtually to discuss the essential elements needed to develop these systems for use as regulatory tools, as well as pathways to their qualification as regulatory tools. The one-day workshop was co-sponsored by the Food and Drug Administration, National Institutes of Health, National Institute for Environmental Health Sciences, National Center for Advancing Translational Science, Environmental Protection Agency, Johns Hopkins School of Public Health's Center for Alternatives to Animal Testing and the International Consortium for Innovation and Quality in Pharmaceutical Development.

Jesse L. Goodman, the chief scientist of the Food and Drug Administration (FDA), opened the workshop by predicting that microphysiological systems may impact both the specificity of toxicology and how it is modeled. "We have many compounds and interventions that we have to reject now that if we can improve specificity we may not have to reject." The tools may help illuminate the reasons for toxicity, as well as how genetics may come into play, he says. The tools also hold promise for furthering the study of disease models, Goodman said. "Being able to have 3-dimensional complex models using human cells offers tremendous potential."

Donald E. Ingber, the director of the Wyss Institute for Biologically Inspired Engineering at Harvard University described the potential of microphysiological systems from the academic perspective. In 2010 Ingber and colleagues produced what they call a lung-on-a-chip and described it in *Science* (Huh et al., 2010). The chip recreates the alveolar-capillary interface, one of the major functional units in the lung and the site where oxygen enters the body. This same interface is where aerosol-based drugs are delivered, where some cancers can metastasize (Huh et al., 2012), and it is a major site where pneumonias develop, among other things. The lung-on-a-chip is the size of a computer memory stick, Ingber said. It was inspired in part by recent advances in microfluidics, which involves use of computer microfabrication techniques to construct networks of hollow channels that can control and manipulate fluids at sub-milliliter scales to take advantage of changes in how the fluids behave at these microscopic dimensions. The researchers were able to use the lung on a chip to mimic common lung functions and generate predictions about previously unknown functions that were confirmed in studies with whole mouse lungs. For example, they showed that exposure to airborne particulates in the form of colloidal silica nanoparticles can create an inflammatory response in the lung on a chip, and they discovered that the cyclic mechanical strain of breathing accentuates the toxic and inflammatory responses. "Breathing alone does not achieve this, and the nanoparticles alone do not do it. Only together does this happen," Ingber explained. The Wyss researchers have also successfully mimicked pulmonary edema,

a deadly condition in which the lungs fill with fluid and blood clots (Huh et al., 2012).

The pharmaceutical industry's view of microphysiological systems was provided by James L. Stevens, a distinguished research fellow at Lilly Research Laboratories, the research and development arm of Eli Lilly and Company, one of the world's largest pharmaceutical corporations. He shared his thoughts on how microphysiological systems can help improve safety assessment by avoiding toxicity, predicting target organ toxicity, and managing risks in clinical trials. He said that Lilly tends to use microphysiological systems "on the back side" after a negative outcome has been brought to light to replicate the pharmacology and biology to see if the problems can be avoided or managed. For example, Lilly scientists have used human cardiomyocyte heart muscle cells derived from induced pluripotent stem (iPS) cells to identify pharmacology-based impacts on cardiovascular function. He says that when pharmacologists know a target organ, microphysiological systems can also focus screening.

Douglas Throckmorton, deputy director for regulatory programs for the Food and Drug Administration's (FDA) Center for Drug Evaluation and Research, discussed microphysiological systems from a regulatory perspective. He reminded the audience that new drug success rates are not as good as they could be (Kola and Landis, 2004; Arrowsmith et al., 2011). To address these problems, FDA needs to support the development of predictive physiological biomarkers, Throckmorton continued. The process through which biomarkers are currently generated, which tends to be on a case-by-case basis driven by drug manufacturers' needs with a slow movement towards general use as scientific experience accumulates, is inefficient. In this context, Throckmorton perceives that microphysiological systems offer "profound opportunities." In addition to being non-animal-based, they can be highly efficient in terms of the number of products that can be screened. They also have the potential to be integrative, in that they offer the potential to answer more than one question about a drug's effects.

Thomas Hartung, the Doerenkamp-Zbinden Professor and Chair for Evidence-based Toxicology at Johns Hopkins University, gave a talk on the topic of Good Cell Culture Practices (GCCP) and quality control. He told the audience that he believes the human on a chip microphysiology approach can help overcome important shortcomings associated with using *in vitro* models. The fact that they have the potential to mimic the differentiation of organs is a positive, as is their potential to supply oxygen to the cultured cells. Another plus is that microphysiological systems are likely to overcome problems associated with the tumor origin of many cell lines as they mainly make use of induced pluripotent stem cells. 3-dimensional cultures offer some improvements over 2-D cultures, Hartung continued. These include increased cell survival and differentiation. The 3-D cultures also allow for increased cell-to-cell interaction, and they do a better job of reproducing the complexity of human organs. However, scientists don't yet have a plan to stress the human-on-a-chip cells continuously, so they are likely to be as "bored" as conventional *in vitro* cells, which Hartung sees as a key reason for dedifferentiation *in vitro*. There is as yet no way to mimic metabolism or stimulate defense, either. Thus far there also are no plans for introducing analytics to determine the fate of test compounds in culture. Validating organs-on-a-chip presents a new challenge, Hartung told the audience. There's a dramatic difference between a model and a test, he explained. Just as liver cells are a model, microphysiological systems are models, not tests, he continued. A given test is defined by its purpose, Hartung stated. It is defined by a very precise protocol. For example, hepatocytes can be cultured in different ways to produce an endless number of different tests depending on the intended goal and how the parameters are set up. While animal models have been mostly accepted based on their "face validity" because they use a healthy living organism, microphysiological systems are essentially devices with a variety of different elements. As models, they must be defined by precise protocols based on the phenomenological similarity to the organisms and/or current scientific understanding.

Kyle Kolaja of Cellular Dynamics International (CDI), a company that manufactures human iPS tissues, made a case for why stem cell-derived tissues can help researchers develop microphysiological systems. Stem cell-derived cells are likely the most ideal format available for generating the cells and tissues used in microphysiological systems, Kolaja said. From the perspective of toxicology, the limitations of primary cell culture have hindered the potential of replacing animal and human experiments, Kolaja continued. The challenges associated with making the shift include consistent access to primary human and animal cells, variability due to how cells can be isolated, and the degeneration of the *in vivo* phenotype once in culture. By addressing these issues, stem cell-derived tissues can also help further the development of the 3-dimensional models needed to produce microphysiological systems. A key advantage of stem cells results from how they can be derived via genetic engineering, Kolaja told the audience. "The iPSC field has moved past the early days of integrated viruses," he said. "Now methods that do not require integration in the genome are used predominantly to reprogram cells. Small amounts of peripheral blood or other tissue can be used as starting material. The iPS cells are grown in defined media and on well characterized matrices, two improvements that have helped provide consistency to stem cell culture," he explained.

Danilo Tagle, the associate director for special initiatives of the National Institutes of Health's National Center for Advancing Translational Science, chaired a session highlighting efforts to build a representative microphysiological organ system.

D. Lansing Taylor, the director of the University of Pittsburgh's Drug Discovery Institute, talked about his institute's collaboration with the Massachusetts General Hospital's Center for Engineering in Medicine to create a liver on a chip. Specifically, their 3-D system replicates the liver's acinus and sinusoids. These are the areas where nutrients, fats, toxins and bacteria that enter the liver via venous blood from the gut are processed. The 3-D liver chip that Taylor's group has created has a grooved design intended to mimic the hepatic chords in the acinus. The channels created by the microgrooves represent sinusoids. Each sinusoid groove contains all the essential cell types found in the liver, including Kupffer cells, stellate cells, endothelial cells and hepatocytes. The chip uses microfluidics to control the environ-

ment and the flow rates of medium continuously bathing the liver chip. The group's goals for the 3-D liver platform include reducing drug attrition rates by recapitulating the human liver acinus physiology and making the optimal measurements to characterize it. A key element of this is building a predictive database, Taylor explained. "We also want to use all of the data we're collecting for a variety of systems biology modeling tasks," he said.

Donald E. Ingber gave his second talk of the day on the efforts of his group at Harvard's Wyss Institute for Biologically Inspired Engineering to build representative microphysiological organ systems. He focused mainly on his group's successes in constructing models of the human gastrointestinal system, including the microbiome, but he also discussed research underway toward recreating a human kidney proximal tubule, a small airway, and bone marrow on chips. The Wyss researchers have produced what they call a peristaltic human gut on a chip, Ingber said. They started with the lung organ and modified it to mimic the human intestine with its microbiome. They made the organ higher and wider and set it up to produce a trickling type of a flow similar to the one in the human gut. Rather than exerting breathing motions, it was engineered to have the cyclic deformations associated with the wave-like contractions of peristalsis. They used human CaCo-2 colon cancer cells, which are known to not be well-differentiated and in existing static culture systems appear more like skin cells than gut cells. However, after just three days of experiencing the flow and strain in the artificial gut on a chip, they began to look columnar, like gut cells. Over time, they spontaneously reorganized to form villi, which are the cell-lined finger-like projections that are normally found lining the human gut. Just like the villi in the human intestine, these structures have tight junctions and are covered with mucus. The structures also include the crypts containing proliferative cells found in the human gut, which include four different types of differentiated epithelial cells (absorptive, mucus-secretory (Goblet), enteroendocrine, and Paneth) that take characteristic positions similar to those observed in the living human small intestine. Ingber's group has also used primary human intestinal epithelial cells to recreate the same structure with the gut on the chip, as recently described in articles in *Lab on a Chip* (Kim et al., 2012) and *Integrative Biology* (Kim and Ingber, 2013). The fact that colon tumor cells work so well to reproduce the intestinal physiology and even produce mucous suggests that the cells are "rebootable," especially since CaCo-2 cells are known normally not to produce mucous in static cultures. Transcriptome profiling of the gut epithelium revealed that the expression of about 10% of 22,203 human genes was significantly altered in mechanically active environments including flow and/or strain. Just trickling flow can alter the phenotype of these cells, and trickling flow plus cyclic strain change them in an entirely different way, Ingber said.

Jonathan Himmelfarb, the director of the University of Washington's Kidney Research Institute, talked about his group's efforts to create a kidney on a chip. The formula for how the kidneys clear drugs and toxins was first laid out almost 100 years ago. The rate of filtration and the ability to reabsorb through the kidney's nephron and tubules is well-understood and both can be easily modeled clinically. "But to this day we cannot effectively model tubular secretion of drugs and toxins," Himmelfarb said. To the pharmaceutical companies, tubular secretion remains a black box in terms of understanding either if any compound is secreted or the extent to which it is going to be secreted, he explained. The functional unit that Himmelfarb's group set out to model includes the vasculature of the peritubular capillaries with the pericytes, which communicate with the blood vessels' endothelial cells, and the proximal tubule. This unit is critical to kidney toxicity and how the kidney eliminates drugs. The proximal convoluted tubule cells are full of mitochondria and are highly active metabolically. "The work of the proximal tubule is all about transport, whether it's reab-sorption or secretion. It's really the factory in the kidney for the transport of solutes," he said. Its functions also include the generation of ammonia from glutamine, or ammoniagenesis, and the 1-α hydroxylation of vitamin D. It is also the cell in the kidney that is most subject to injury because it is so metabolically active and is exposed to such high concentrations of the kidney filtrate, Himmelfarb said. By including three types of cells, the proximal tubules, the pericytes, and the microvascular epithelium, Himmelfarb said his group's tubular interstitium on a chip should be able to effectively model the kidney's secretory process. The chip they are designing includes a parallel tubule and a parallel microvessel.

The final session of the conference focused on the integration of microphysiological organ systems. **Robert Kavlock**, the Deputy Assistant Administrator for Science within the Environmental Protection Agency's (EPA) Office of Research and Development, told the audience that he was "absolutely amazed by the progress" in creating the microphysiological organ systems that the previous speakers described. Both scientists in the pharmaceutical research area and in the environmental health research area are motivated by the new technologies' ability to "allow us to get inside the black box" between the exposure to a chemical and the responses that can be detected by animal testing, Kavlock said. For EPA, the driver is the need to collect toxicity data. In the long run "how we integrate all of these systems together and how we share knowledge and share information… will help us to move forward – whether you're interested in drug development or whether you're interested in the effect of chemicals in the environment," he said.

Melvin Andersen, the Charles E. Hamner Distinguished Fellow at the Hamner Institute, discussed what the new systems do that current systems are incapable of achieving. He also asked the audience to consider where the successes achieved at this early stage of development will lead us. The new platform may enable scientists to test more environmental compounds more efficiently and it may also help speed the testing of new drugs and biological products for which human efficacy studies are neither ethical nor feasible (products which fall under the FDA's animal rule which allows approval based on the proof of efficacy in animals) by allowing studies to be conducted on a limited human platform. In order for the platforms to have value, scientists need to be confident that they are capturing the likely toxic responses, Andersen stressed. A key issue that Andersen said he would like to see addressed is how microphysiological systems can be used mechanistically with human tissue aggregates to get a better understanding of modes and mechanisms of action. He pointed out that "no matter how we hook these tissues together, they don't

add up to a human on a chip. Each one of them lacks critical components to be a full tissue, but it still can be tremendously useful," he said. If the goal is to improve evaluation of the effects of chemical exposures, Andersen suggested that it may make sense to consider more carefully which tissue systems should be included in the first test systems. Rather than rushing to combine all of the organ systems to produce a "truncated human on a chip," Andersen pointed out that the collaborators may do well to show that a more limited platform can faithfully represent expected tissue exposures in an intact organism.

Suzanne Fitzpatrick, Senior Science Advisor of the FDA's Office of the Commissioner's Office of the Chief Scientist closed the meeting. "In putting together this program with the FDA and CAAT, we wanted to create a community of people who were all working toward a common purpose," she said. She credited the National Research Council's *Toxicity Testing in the 21st Century* report for helping to catalyze the creation of such a community. "To move innovation, we really need a whole community of people… to look at these new toxicology models," she concluded.

A more detailed summary of the workshop is available at: http://www.altex-edition.org

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