EMERGING SCIENCE FOR ENVIRONMENTAL HEALTH DECISIONS

DECEMBER 2014 ISSUE 11 ISSN 2376-16790

You-on-a-Chip

Is it possible to mimic the human body on the equivalent of a computer chip? Scientists in a wide array of disciplines are collaborating to develop technology that imitates organ function on devices that are smaller than your thumb. Why are these "tissue chips" of interest to the environmental community? As discussed in previous meetings of the National Academies Standing Committee on Use of Emerging Science for Environmental Health Decisions (ESEH), there is a pressing need for rapid assessment of the effects of human exposure to environmental stressors. Preliminary data on tissue chips suggest that some of these devices have the potential to predict the effects of exposure to environmental chemicals more accurately than do some of the gold-standard animal and in vitro testing models.

In June 2010, the ESEH committee convened a meeting, "Stem Cell Models for Environmental Health" (http://nas-sites.org/emergingscience/meetings/stem-cells/). Research has vastly expanded since then, and human stem cells are now being developed and tested with tissue-chip technologies. The merger of

continued on page 2

The Potential of the Tissue Chip for Environmental Health Studies

On July 21–22, 2014, the National Academies Standing Committee on Emerging Science for Environmental Health Decisions (ESEH) brought together experts in cell biology, engineering, and toxicology to discuss the development and application of tissue-chip technology in environmental health research. Tissue chips capitalize on recent advances in nanotechnology, microengineering, and systems biology, noted William Farland, of Colorado State University, the chair of the ESEH Committee. Efforts to build portions of human organs on chips to recreate attributes of organ-level functioning have had impressive success in the last few years, and pharmaceutical researchers have begun to investigate how these three-dimensional tissue assemblages may aid in drug screening. They are "truly the cutting edge" of research in developing methods and technology to get "the best information about the effects of exposures on the human system," said John Balbus, of the National Institute of Environmental Health Sciences (NIEHS). Toxicologists are beginning to take note of the developments and to envision how tissue chips may one day be used in environmental health research to complement the efforts already under way in in vitro chemical screening. Several workshop speakers emphasized that the goal of developing tissue chips is to reduce, if not replace, animal testing.

Whether you call them tissue chips or something else, this technology may help us to realize the vision of the future of toxicity testing presented in the 2007 National Research Council report *Toxicity Testing in the 21st Century,* noted Ivan Rusyn, of the University of North Carolina at Chapel Hill. In line with that vision, regulators and scientists in the United States, the European Union, China, and elsewhere are increasingly embracing alternatives to animal testing for evaluating the safety of drugs and other chemicals. Rusyn emphasized that a key goal of the present meeting is to bridge

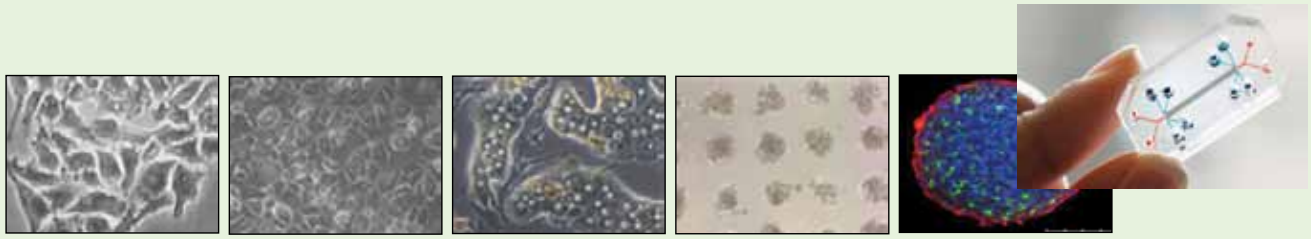
continued on page 2

IN THIS ISSUE

This newsletter and additional information about the committee and its activities can be found at **http://nas-sites.org/emergingscience/.** The newsletter is prepared by National Research Council staff to keep you informed of activities of the Standing Committee on Emerging Science for Environmental Health Decisions. The views expressed in the newsletter are those of the meeting presenters and participants. The newsletter does not represent either formal consensus conclusions of the attendees or positions necessarily endorsed by the National Research Council.

THE NATIONAL ACADEMIES
Advisers to the Nation on Science, Engineering, and Medicine

What Is a Tissue Chip?



Source: Modified from Francisca Boess

Tissue chips are engineered microchips that contain living human cells that replicate organ-level functions. They have many names, including organ-on-a-chip, biomimetic microsystems, and three-dimensional microphysiologic platforms. By any name, tissue-chip development is the result of collaboration of multiple disciplines, including engineering, materials science, nanotechnology, cell biology, and systems biology.

Potential applications of tissue chips include toxicology, drug screening, chemical diagnostics, and therapeutics. The image above depicts a range of in vitro testing platforms in order of increasing complexity. Cell lines, the least complex in vitro system, rank high in ease of use, but low in physiologic relevance. Tissue chips, a more complex in vitro system, rank low in ease of use, but high in physiologic relevance.

POTENTIAL, *cont. from page 1*

the gap between the scientists developing tissue chips and the policy-makers, risk assessors, and others who are seeking to apply the technology for different human health assessment scenarios.

What Makes a Tissue Chip?

Anthony Bahinski, lead senior staff scientist working in the lab of Dr. Donald Ingber at the Wyss Institute for Biologically Inspired Engineering at Harvard University, outlined key considerations and needs for developing tissue chips. He emphasized that tissue chips are *not* a recreation of a whole organ, like the heart or liver, but rather an attempt to design "the simplest system possible" that reconstructs key attributes of an organ's biochemistry and physiology. An "underlying challenge in developing these microsystems… is to replicate human organ-level functions in vitro," said Bahinski.

How are tissue chips made? Photolithography, a technique used to make microchips in computers, is combined with other microengineering techniques to make tissue chips. Through microengineering, researchers are able to design chips that recreate tissue–tissue interfaces, that provide the mechanical cues necessary to mimic human physiology (such as fluid flow and the air–liquid interface), that orient cells precisely for high-resolution real-time imaging, that place cells in separate channels so that different cell populations can be studied, and that create endothelium-lined vascular channels to enable real-time analysis of immune response or link different tissue chips together. Bahinski also noted that microengineering enables researchers to control fluid flow through the tissue chips to support long-term cell cultures (up to 2 months), and permits co-culture with the microbiome (important for gut tissue chips).

continued on page 2

YOU-ON-A-CHIP, *cont. from page 1*

avenues of research increases the potential for tissue chips to reflect human composition and physiology accurately. Perhaps one day soon, tissue chips can be applied to understanding of a wide array of environmental health topics that have been explored in previous ESEH meetings, such as assessing human variability and the role of genomic plasticity or the human microbiome in response to environmental stressors.

POTENTIAL, *cont. from page 2*

The Wyss Institute is developing not only tissue chips for specific organs—such as the lung, gut, and bone marrow—but chips that can serve as models of disease. Work by Bahinski has also shown that individual tissue chips can be successfully linked to create a tissue-chip organ system. Wyss has a prototype system that can link 12 tissue chips to one another.

Many of the tissue chips that have been developed are based on normal human cells. Primary human cells are somewhat limiting, said Kyle Kolaja, of Cellular Dynamics, in that one needs to be able to isolate and access them from a human, and they can lose function and phenotype when placed into culture. Kolaja believes that the convergence of induced pluripotent stem (iPS) cells with tissue-chip technology would be a "dramatic improvement" because "from a small amount of blood one can reprogram such a cell to make any tissue." iPS cells make it possible to individualize tissue chips and thus conduct research on a set of tissue chips that reflect the genetic diversity of human populations. "We can potentially make a human organ system based on you or me or populations of people," Bahinski remarked. Kolaja noted that another key advantage of stem-cell–derived tissues is the ability to genetically engineer them—to insert or delete specific genes to permit research on, for example, disease. Cellular Dynamics offers a wide variety of cell types and has already created cell lines for almost 50 diseases, including autism, Alzheimer disease, and hepatitis. Such modified cell types may eventually help researchers to evaluate genetic susceptibility. Some companies, including Cellular Dynamics, are also creating banks of iPS cells from a variety of healthy and diseased donors. The banks may eventually help researchers to evaluate the role played by genetic variability in response to environmental chemicals.

Key Components of Organ Composition and Function

What are the key attributes that a tissue chip needs to mimic a human organ's composition and function? Bahinski described seven characteristics that define a mammalian organ. He emphasized that if the goal of a tissue chip is to replicate human organ-level functions, researchers must identify and understand key components of organ composition and function. Organs are:

- Composed of two or more types of tissue that function as a unit when they are brought together.
- Perfused by blood flowing through endothelium-lined vessels.
- Controlled by chemical and molecular factors that are produced by constituent cells or delivered through blood.
- Regulated by such mechanical forces as the motion associated with breathing, blood flow, and the movement of food through the body.
- Structured to secrete or transport molecular signals such as ones involving growth and inflammation.
- Infiltrated by immune cells during inflammatory responses.
- Physiologically linked to other organs by the action of biochemical factors transported in blood.

Promise in Environmental Health

Why is there so much excitement about tissue chips? Danilo Tagle, of the Food and Drug Administration (FDA), outlined a broad array of future applications and benefits of tissue-chip technology, including the ability to advance disease research (particularly research on rare diseases), to understand genetic variation, to advance personalized medicine, to evaluate drug efficacy, and to conduct toxicity testing. He emphasized that the ability to capture the genotypes of people who have rare diseases is a particular benefit in that it will enable FDA and others to conduct risk assessment of candidate therapeutics for subpopulations that are particularly hard to study with traditional clinical-trial methods.

Weihsueh Chiu, of the Environmental Protection Agency (EPA), outlined a variety of challenges in environmental health. Four dominant challenges are conducting human health assessments to keep pace with the number of chemicals in commerce; quantifying uncertainty in health assessments

> ***This is all about convergent science. We are taking the best of physics, material science, bioengineering, microfluidics, and stem-cell technology and coming up with a human-on-a-chip.***
>
> **—Danilo Tagle**

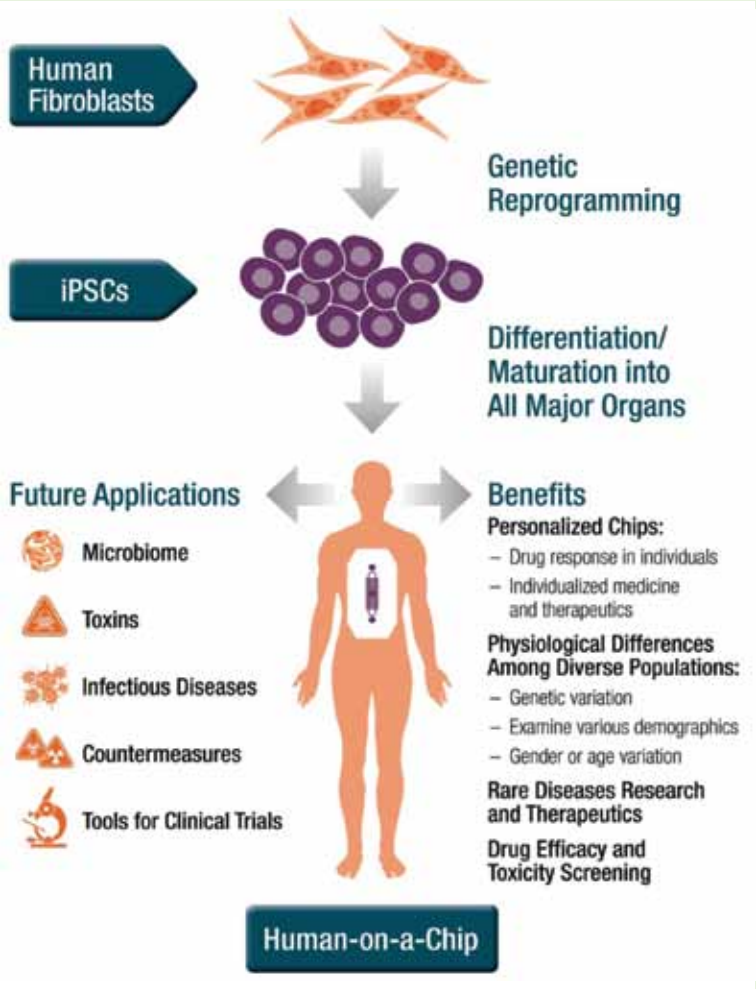
continued on page 4

POTENTIAL, *cont. from page 3*

due to animal-to-human extrapolation, human variability, and other factors; estimating human variability and susceptibility; and supporting economic benefit–cost analyses of regulatory actions. Chiu shared some ideas of how tissue chips might be applied to address each of those challenges. For example, tissue chips might decrease cost while increasing throughput for human health assessment compared with in vivo animal research. Joyce Tsuji, principal scientist with Exponent Engineering and Scientific Consulting, noted that tissue chips may even begin to replace animal testing. And they may offer a way to test effects for which models are lacking.

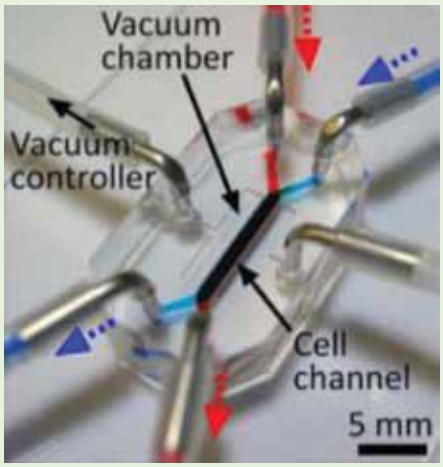
continued on page 5

The Potential of the Tissue Chip



Danilo Tagle summarized potential applications and benefits of tissue chips for disease research. He emphasized that the integration of iPS cells with tissue-chip technology is where we should expect to reap many benefits. Tagle, Anthony Bahinski, and other meeting participants envision that one day it might be possible to conduct human clinical trials with iPS-cell–based tissue chips.

Should Tissue Chips Replace Animal Testing?



Source: Anthony Bahinski

Meeting discussions reflected some disagreement in the research community about whether pharmaceutical development and toxicity testing should move away from animal testing. One attendee pointed out that the use of mouse embryonic stem cells has increased the accuracy of chemical testing. Is the extra sophistication of tissue chips really needed when there are animal tests that have more accurate predictions of outcomes?

"It would be a mistake to let the different types of in vitro technologies be evaluated independently," observed John Bucher, of NIEHS. The better option would be to evaluate in vitro methods as a collection of alternatives that potentially can be used to replace animal studies. "If you put together a cohesive battery of information, including data from tissues on a chip, you can present a larger set of approaches that could collectively have a better predictive value than animal studies or what we know in humans," Bucher said.

It is incumbent on regulatory agencies to strike and define a balance, commented Frank Weichold, of FDA. He emphasized that in the end we are all involved in defining pathways to the future, and holding a social conversation about animal testing may help.

POTENTIAL, *cont. from page 4*

Tissue chips might also be able to provide direct estimates of human variability, a major challenge in environmental health research, noted Chiu. The tools offer an opportunity to create a set of scenarios for assessing end points to which some groups are known to be more sensitive as opposed to addressing the full range of disparity, which would be a huge problem, said Lauren Zeise, deputy director of scientific affairs for California's Office of Environmental Health Hazard Assessment.

One factor to consider is where tissue chips fit into the source-to-outcome paradigm that guides current practices in environmental health—data-gathering, risk assessment and characterization, and ultimately regulatory and nonregulatory decisions (for example, setting priorities among chemicals for toxicity screening, setting of exposure limits, and risk comparisons). Chiu believes that tissue chips will most likely be useful for identifying pollutant hazards and elucidating dose–response mechanisms in people who are exposed to pollutants. Similarly, tissue chips are promising tools for helping scientists model exposure more effectively and to base decisions on molecular and pathway perturbations, said Russell ("Rusty") Thomas, director of the EPA National Center for Computational Toxicology. The chips potentially can help to define the circumstances and the degree to which toxicant exposures initiate biochemical pathways that result in harmful outcomes, he explained. On the basis of what we know about their cost and throughput, tissue chips appear well positioned to serve as secondary screens of toxicity, said Thomas. Chiu agreed and added that such secondary screening can help to elucidate what disease pathways may be perturbed by chemical exposure. Data banks of human tissue and serum samples from individuals with differing states of health and disease are becoming available. Chiu and Thomas both noted that tissue chips may serve as a screening tool of those data-banked samples, and potentially could elucidate human variability and susceptibility to disease.

> ***Tissue chips will not be used in isolation as the only piece of evidence; they will always be part of a larger body of evidence [that] drives a risk assessment.***
>
> **—Weisueh Chiu**

Chiu and Thomas both emphasized that regulators need help with modelling the effects of chemicals at the tissue level, and modelling the variability in individual susceptibility to the effects is needed. Using tissue-chip data in concert with other data and computational approaches may ultimately help with economic benefit–cost analyses that EPA must conduct to support regulations, particularly regarding noncancer end points. The technology may also prove useful in assessing the effects of exposure to mixtures of chemicals, Chiu said.

A key requirement for this to happen, Chiu stressed, is a much better understanding of the ability to use tissue chips to evaluate different types of chemicals and health effects and of their cost and throughput in comparison with in vivo data and alternative in vitro systems. In addition, he said, case studies of known toxicants would be useful as a proof of concept. William Farland, of Colorado State University, pointed out that testing with failed drugs might also prove helpful. The predictive assays that the Society of Toxicology has developed for different levels of exposure could also be useful added John Bucher, of the NIEHS National Toxicology Program.

D. Lansing (Lance) Taylor, of the University of Pittsburgh, noted that all the groups that have received federal funding to develop tissue chips are collecting and sharing their experimental data to produce a database that contains a wealth of information, including details of tests analyzing genetic variations.

Don't Oversell the Potential

Although tissue chips clearly have a great deal of potential, it is important not to oversell the potential, cautioned Thomas. Industry is using chips very differently from how EPA might use them; EPA is not trying to test large numbers of chemicals at one time, he stressed. And many drugs have a linear dose–response relationship, whereas the effects of exposure to environmental chemicals can be non–linear, as pointed out by Shuk-mei-Ho, of the University of Cincinnati.

Edward LeCluyse, of the Hamner Institutes for Health Sciences, has been investigating how tissue chips can be used for chemical testing. LeCluyse said that he and his colleagues

continued on page 6

POTENTIAL, *cont. from page 5*

are getting closer from a biologic standpoint to recapitulating the types of events that lead to pathway-based changes in organs, tissues, or cells. "Where we still need to bridge the gaps is in beginning to take a holistic or systems-biology approach to tying things together," he noted, adding that he still perceives some disjointedness and disconnections.

Taking into consideration the natural compartmentalization of human organs and the blood flow to the different compartments, LeCluyse raised a few questions about how tissue-chips can be used to address issues brought to light by animal testing: "From a toxicity point of view, what dose or exposure is the poison? How can we put it into perspective by tying compartments together?" Toxicologists are also interested in the potential effects of major metabolites generated by environmental chemicals to which people can be exposed, LeCluyse pointed out. In the body, metabolites generated by one organ system can go downstream and affect other organ systems.

The promise of tissue chips for drug testing does not necessarily mean that they will be effective for evaluating environmental chemicals, cautioned Helmet Zarbl, of the Environmental and Occupational Health Sciences Institute of the Robert Wood Johnson Medical School. From a testing standpoint, drugs and environmental chemicals are quite different. Drugs tend to be taken in relatively high doses and have many detectable effects; people are exposed to low doses of environmental chemicals, and the exposure is often chronic. "Low-dose responses are often different from high-dose responses," Zarbl said.

LeCluyse applauded efforts of researchers who are developing tissue chips for different organs to communicate with one another, saying that it is especially important for metabolites produced from an organ-on-a-chip to be made available for testing other tissue chips to study the effects of downstream exposure. For example, LeCluyse raised the question of how liver-on-a-chip developers account for the size of the liver and its ability to clear and metabolize compounds.

continued on page 7

The Design of Tissue Chips for Environmental Health Research: Practical Considerations

Christie Sayes, of RTI International, is working to develop lung- and gut-on-a-chip technologies for environmental-exposure studies. Her work with these model systems has demonstrated that more research by engineers, chemists, toxicologists, mathematicians, and others is needed to improve tissue-chip design for environmental research. Sayes briefly described four major issues that she believes need to be addressed:

Physiologic Dimensions. *The ratio of cells (for example, the number of macrophages per epithelial cell), the order in which new cells are added to the system, and clearance are three physiologic measures that need to be addressed. Tissue-chip systems need to be modular enough to add cells, such as polymorphic neutrophils or dendritic cells that arrive after injury, and need to be able to handle clearance of chemicals or their metabolites after the tissue chips are exposed.*

Sample Preparation. *"It's very important not to just take the raw material as is and expose it to the cells" in a tissue chip. Test materials need to be processed as they would be in a body. We need exposure and validation studies on prereacted materials and metabolites. It is common practice for the materials that will be tested on a gut-on-a-chip to be artificially digested first by being exposed to saliva and stomach and gastrointestinal fluids. Similarly, materials need to be aerosolized for lung-on-a-chip studies, and exposure chambers need to be designed to match deposition patterns (gravitational or gentle impaction). Tissue chips need to be modular enough for a variety of exposure scenarios with different sample materials.*

Dosimetry. *Selecting the right exposure dose is critical for environmental health research. "We have great models that have been developed for two-dimensional monolayers, but do those need to be re-evaluated for three-dimensional models?"*

Validation. *Validation assays are important both to calibrate the accuracy of test results and to eliminate false positives and false negatives. "Not having enough material to do the assay at the end of the analysis" is a major problem. Both the cell population and the well size in the tissue chips need to be large enough to run post-test validation assays.*

POTENTIAL, *cont. from page 6*

It will be important to establish relevant end points that can be compared with historical end points, such as an AC_{50}, the concentration of a chemical exposure at which activity is 50% of its maximum, LeCluyse said. "When most of the historical data have been generated in a two-dimensional static culture, such as the human hepatocellular carcinoma (HepG2) cell-line model, how can one standardize, benchmark, or compare data on a 3-D tissue chip? In addition to establishing criteria for making comparisons, criteria for controlling the conditions used in the tissue chips themselves must be established so that the results will be comparable. "In the end, all the data will be funneled through the same path to the risk assessors," he said.

To allow users of tissue chips to obtain regulatory approval for their work, details about their design must be made available. "Black boxes aren't going to work," stressed Rodger Curren, of the Institute for In Vitro Sciences. Industry scientists need to be convinced that the model and approach being used reflect what happens in the real world. A convincing argument will require an ability to describe what makes the test system physiologically relevant and, ideally, what historical data qualify the tissue system's use for testing chemicals.

In contrast Lois Lehman-McKeeman, of Bristol-Myers Squib, cautioned the audience not to undervalue the progress that has been made. "What I heard today was a lot of fundamentally strong science," she said. Lehman-McKeeman and others at the workshop noted that these are "advanced but still prototype systems." The complexity makes tissue-chip development challenging. Among the needs to advance the use of these tools in a consistent way are the availability of pluripotent stem cells, modeling, and databases of results that can be shared and evaluated, but, Lehman-McKeeman emphasized, the current state of the science is "not a trivial place to be, in any way, shape, or form."

> ***We're trying to replicate a very complex system, but we have to start simple.***
> **—Louis Lehman-McKeeman**

What's Next?

Zeise raised the question of whether tissue chips have been developed sufficiently for experts in high-throughput toxicology testing to work collaboratively with tissue-chip developers toward implementing the vision of the 2007 National Research Council report *Toxicity Testing in the 21st Century.*

In response, Tagle said that National Institutes of Health National Center for Advancing Translational Sciences has the infrastructure to enable it to use the same iPS cell lines for both tissue chips and high-throughput microarray platforms. That infrastructure will allow consistency in readouts and in what is expected at the level of molecular signatures from cell screening. One of the two main goals for the program is to use the chips for assessments of chemical safety. Tagle expects that some of the standalone tissue chip technologies being developed may be ready within a year for validation tests—tests of the tissue chips' ability to predict the activity of 50–100 compounds with known toxicity. A few years from now, Tagle expects researchers to begin assessing integrated tissue chips, and a program goal is to conduct clinical trials of humans-on-a-chip in the next 5–10 years.

Thomas is contemplating ways to link data from high-throughput testing with targeted testing by using tissue chips to serve as a proof of concept. That would include identifying a set of compounds that have been shown by high-throughput testing to target basic cell processes and other processes that have organ-specific targets.

"By looking at these systems a little more tangentially than we're used to, we may be able to ask complex questions in a way that we're not used to," said Rosemarie Hunziger, director of tissue engineering and regenerative medicine at the National Institute of Biomedical Imaging and Bioengineering. She pointed out that before scientists had the tools of microarray technologies, many questions that are now routine could not be asked, because nothing had prompted researchers to consider them. "When tools become available, we see many ways to use them that were not conceived of before," Hunziger stated.

Jonathan Himmelfarb of the University of Washington, who leads a group developing a kidney tissue chip, agreed: "If you put new tools into the hands of creative scientists, you can't predict what they will come up with. We're in the early stages of this, but we see a lot of exciting scientific possibilities."

In summarizing the diverse perspectives shared at the workshop, Rusyn outlined six topics that he believes need to be addressed as the next steps toward application

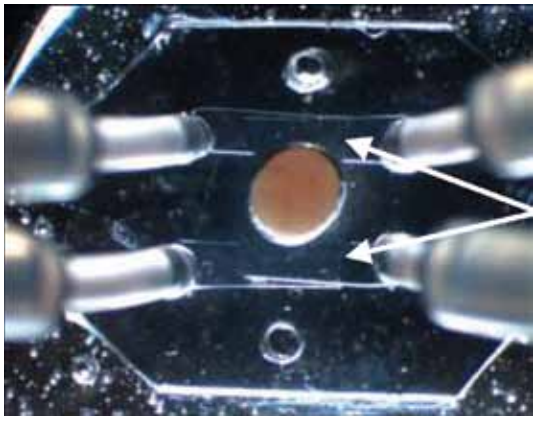
continued on page 8

Tissue Chips: Around the Corner and Down the Road

A variety of tissue chips are under development; some are more advanced than others. A key hurdle is identifying or developing alternative materials to prevent such problems as the adsorption of small hydrophobic molecules. "You don't want to be at the end stage with a cool technology that isn't usable," pointed out Anthony Bahinski of the Wyss Institute. Bahinski, Jonathan Himmelfarb of the University of Washington, Julie Kim of Northwestern University, and Lance Taylor of the University of Pittsburgh described in detail some of the tissue chips that they are developing and goals for their use.

Bone marrow-on-a-chip

The bone marrow chip is a small polydimethylsiloxane (PDMS; a silicon-based organic polymer) device. Researchers place demineralized bone powder and bone morphogenetic protein into a cylindric chamber and then implant the device under the skin of a rodent for 8 weeks to induce the formation of bone marrow, Bahinski explained. They can then remove the device and "put it into a microfluidic device and maintain it in culture for up to 7 days." The engineered marrow is similar in structure to, although larger than, the mouse's femur. In preliminary studies, "the engineered bone marrow mimics the in vivo response much better than in vitro cell cultures," said Bahinski. Research thus far suggests that bone marrow-on-a -chip has potential applications for bone marrow transplantation, in vitro blood-cell manufacturing, and as a stem-cell niche model.



Bone marrow-on-a-chip

Credit: Anthony Bahinski

> ***This is an exciting time to evolve in vitro systems into more powerful platforms.***
>
> **—Lance Taylor**

Gut-on-a-chip

The gut-on-a-chip, in simplest terms, is a model of the human intestines on a three-dimensional microfluidic platform. The gut chip design allows for peristaltic movement (muscular contractions in the gut) and two independent flow regimes: vascular flow (blood flow) and the "slow trickling flow that you normally see moving through your gut," explained Bahinski. Because of the peristaltic stretch and the trickling flow, it takes only 3 days to differentiate cells in a gut chip fully. That is 7 times faster than the process of differentiation in in vitro transwells (membrane inserts used for cell cultures between agar wells), "the gold standard in the pharmaceutical industry," Bahinski said. Bahinski pointed out that although the gut chip does not yet imitate intestinal physiology perfectly, it is an improvement over in vitro transwells with respect to cell differentiation and barrier function, drug-metabolizing enzymes, and mucus production. A topic area of research for Wyss is how to co-culture the human microbiome in a gut chip. Bahinski and

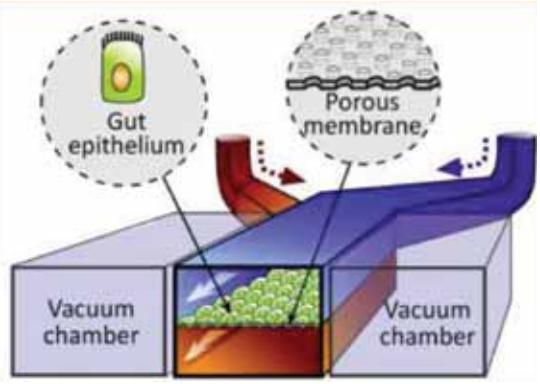
continued on page 9

POTENTIAL, *cont. from page 7*

of tissue-chip technologies in environmental health decisions:

- Validation to assess the qualification of tissue-chip systems in light of field practice and standards.
- Extrapolation from animal to human and in vitro to human as a part of laboratory investigations.
- Integration of tissue-chip data with in vitro and computational model data.
- Transparency about materials and methods used to develop the technologies, despite a "highly competitive state" of biotechnology industries, to bring tissue-chip technologies to market.
- Collective evaluation with the set of newer, alternative in vitro technologies available.
- Availability of tissue chips to a wide array of researchers to permit development of a broad and robust database

"The future is bright; we just need to get there," Rusyn concluded.



Gut-on-a-chip

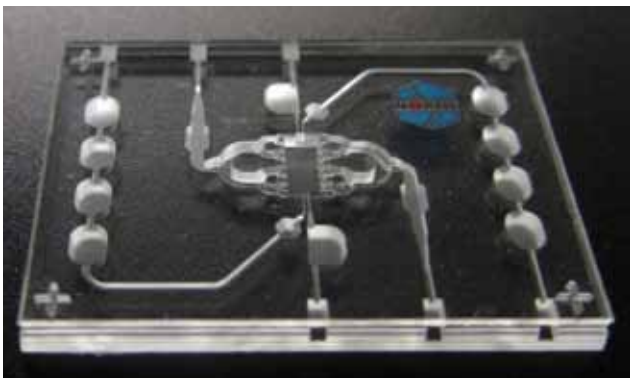
Credit: Anthony Bahinski

his colleagues envision that the gut-on-a-chip models will be useful for drug and nutrient transport studies and in developing models for such diseases as Crohn disease and inflammatory bowel disease.

Kidney-on-a-chip

Developing a kidney chip was an obvious choice for Himmelfarb given that "kidney function plays a critical role in the elimination of drugs and their metabolites and the kidney is highly susceptible to injury from drugs and environmental toxins." The kidney is a complex organ that has over 30 cell types (more than any other organ), and Himmelfarb focused on developing a chip for the kidney tubule, the part of the kidney that is most "critical for understanding drug and toxin elimination and most susceptible to drug- and toxin-mediated injury." The researchers have "established a robust, reproducible validation of physiologic function," said Himmelfarb. For example, they have successfully and repeatedly maintained the cells in the 3-D tissue chips for at least 28 days, a dramatic improvement compared with standard 2-D cell cultures.

The researchers have also demonstrated use of the kidney chip for accurate prediction of the toxicity of, for example, cyclosporin A, a chemical that has known kidney toxicity. Over the next year, Himmelfarb's research group is planning to test the systems with 12 known nephrotoxins to see whether they can generate molecular signatures. The team is also planning to integrate the kidney chip with liver and gut chips. Himmelfarb expects that the kidney chip will be sufficiently robust for broad use within 1–3 years.



Kidney-on-a-chip

Credit: Jonathan Himelfarb

Liver-on-a-chip

Taylor and colleagues have focused their research on developing a tissue chip that replicates the liver's acinus and sinusoids, where nutrients, fats, toxins, and bacteria that enter the liver from the gut are processed. They were able to develop two liver acinus chips. In one, they painstakingly layer the cells into position on the chip. In the second, they allow cells in the right proportions to self-assemble into position, explained Taylor. Both chips contain all the essential cell types found in the liver, including Kupffer cells, stellate cells, endothelial cells, and hepatocytes. Taylor and his colleagues have documented that the chips mimic key liver functions—including metabolism, clearance, protein synthesis, and urea detoxification—over a 28-day period. The chips also produce physiologically relevant concentrations of the liver proteins albumin and urea, Taylor said. Another of his group's achievements is the capture of clinically relevant toxicity information via real-time biosensors and microclinical analyzers. They can measure such things as mitochondrial calcium update and apoptosis. The researchers have been able to show how liver fibrosis is initiated and to show immune-mediated toxicity. Taylor noted that his work with a liver tissue chip is generating "reasonable" results in studies of exposure to 10–20 compounds that have known effects. Taylor predicts that within a year the liver-on-a-chip that his group is developing will be sufficiently robust to generate reproducible results.

> ***The key is to have 3-D organ constructs that integrate flow between organ systems. That is the ultimate goal.***
>
> **—Lance Taylor**

Lung-on-a-chip

The biodesign principles that guided Wyss scientists to develop a lung-on-a-chip are tissue-to-tissue interface, dynamic flow, and mechanical movement. Those principles are important because they guide the cellular composition and interaction, air and

continued on page 10

Tissue Chips in Use

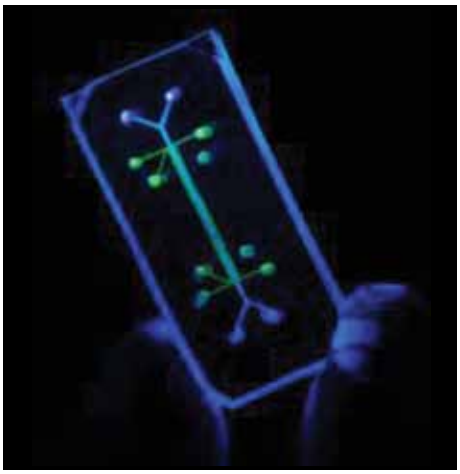
Pharmaceutical companies are beginning to use tissue chips to investigate drug efficacy and toxicity and to explain mechanisms of drug action when animal tests yield conflicting results. Franziska Boess, of Roche Pharmaceutical Sciences, outlined some of the promising approaches and applications that Roche is investigating with liver chips.

"In vitro methods in toxicology are being applied mostly in early phases of drug development," Boess said. In vitro tests are an inexpensive way to screen a drug candidate before committing more extensive resources (such as time, animals, and money) to in vivo testing. Some specific end points—such as metabolism and induction, genetic toxicity, cardiac function, and embryotoxicity—can

continued on page 11

CHIPS AROUND THE CORNER, *cont. from page 9*

blood flow, and breathing motion needed to mimic a lung, explained Bahinski. The Wyss lung-on-a-chip is see-through, to facilitate high-resolution microscopy; biocompatible, so cells can grow; and flexible, to enable mechanical activation (breathing motion). "In this little plastic system, we can recreate the whole human inflammatory response," said Bahinski. Their work showed that the cyclic mechanical strain of breathing accentuates the inflammatory response—"something you would not have seen in a static in vitro culture," Bahinski stated. Researchers have also successfully modeled pulmonary edema and fibrin clot formation in the lung chip and have confirmed their observed physiologic responses in a mouse whole-lung model. Wyss researchers are now testing to determine whether the lung chip can be used to predict drug efficacy.



Lung-on-a-chip Credit: Anthony Bahinski

Reproductive tract-on-a-chip

Kim is part of a research team that is working to develop tissue chips for the female and male reproductive tracts. In contrast with other organs, there is "no good animal model for human reproduction," because of vast species differences, Kim emphasized. The female reproductive tract is "an integrated organ system that comprises distinct functional units," said Kim, and this necessitates the development of tissue chips (which they refer to as "KUBES") for each unit: uterus, ovary, fallopian tube, and cervix. The successes that Kim and colleagues have achieved thus far include their OvaryKUBE's production of mature ova and induction of mouse follicles to mimic the human menstrual cycle. The researchers' TubeKUBE demonstrates that estrogen and progesterone regulate the movement of the cilia that push the mature ovum along the fallopian tube and that adding testosterone can stop that movement. In their CervixKUBE, the presence of estrogen and progesterone induces the production of mucus. Their goal is to integrate all the reproductive chips in a platform that they call the FemKUBE. They hope to use the FemKUBE components to study such diseases as ovarian and breast cancer and such benign hormonally mediated ailments as leiomyoma. The components also may prove helpful in studying HIV transmission and vaccines. A major next step toward their goal is to use iPS cells to develop reproductive tract tissues. "This has never been done before in the reproductive tract, and it will be quite a challenge," Kim emphasized. Eventually, the work will extend into developing a tissue chip model of the male reproductive tract, which will be called "DudeKUBE." Although the reproductive tract chips are still under development, Kim's research group is ready to pass along hormones and follicles to be tested in the chip systems that others are developing.

> ***We can all strongly agree that without the female reproductive tract, mankind will cease to exist.***
>
> **—Julie Kim**

CHIPS IN USE, *cont. from page 10*

be determined with in vitro testing. Yet Roche still struggles with how to use the available in vitro testing tools in the best way to predict organ toxicity. Boess explained that organ toxicities are often difficult to detect because organs are complex, toxicity may develop over a long period, and the events leading to toxicity may involve a multitude of factors and the interplay of different cell types. Another serious challenge is that organ toxicities are often species-dependent.

For example, when Roche used a 26-week in vivo test in rats for a new drug candidate, the study results showed liver proliferation and later neoplastic changes. But those effects were not seen in tests in dogs. The drug's project team requested that Boess's mechanistic-toxicology group conduct additional testing t o elucidate the mechanism of action and use in vitro tools to compare liver proliferation in rats, dogs, and humans. "We were not able to recapitulate the proliferation seen in the rats with a simple monolayer hepatocyte culture system," said Boess. They also were unable to demonstrate proliferation with cocultures of the rat hepatocytes and nonparenchymal cells, which previous work had shown can demonstrate more pronounced proliferation.

It was only when Boess's group used a liver-on-a-chip developed by Regenemed that they were able to observe a reproducible proliferative response to both the candidate drug and a positive control. The testing showed that the human system's reaction to the positive control was more pronounced than that of the rat cells. There was no increase in response to the new drug candidate in human cells although there clearly was in rat cells.

Research by Boess's group with the liver-on-a-chip devices shows that the 3-D system responds appropriately to insulin treatment with an increase in glycogen production, and also responds appropriately in tests for inflammatory reaction and drug metabolism. The system also responds as expected to enzyme inducers and inhibitors, Boess said. However, her group's efforts to measure the metabolic activity of the system with inert compounds yielded disappointing results. The researchers were not able to retrieve metabolites, which indicates that "nonspecific binding, most probably to the materials that hold the scaffold, may cause a considerable problem in this culture system," she explained.

Boess' team used the liver chip technology to evaluate drugs that had known effects and compared results with those in conventional 2-D in vitro culture systems. One of the drugs, troglitazone, was removed from the market in 1999 because of its link to acute liver failure in some patients. The tests in 2-D cell culture indicated liver toxicity in rat but not human hepatocytes. However, the findings in liver chips were reversed, she said. Cytotoxicity was observed beginning on the first day of treatment in the human liver chip, but no toxicity was seen in rat liver chip at the highest concentration.

Many technical challenges remain before tissue chips can be used on a regular basis, including ease of use, throughput, nonspecific compound binding, and reproducibility of results. The biggest challenge, in Boess's opinion, is "prospective, quantitative prediction of liver toxicity." However, Boess emphasized that "the performance of the 3-D culture system is very promising."

Presentations and Discussions

from the **Tissue Chips meeting** are available at

http://nas-sites.org/emergingscience/meetings/bioplatform/

MEETING INFORMATION

Meeting Presentations

Would you like more details about individual variability or other Emerging Science for Environmental Health meeting topics? Descriptions, agendas, presentations, and newsletters for our meeting topics are available through our Web site. We invite you to subscribe to our Listserv for the latest information about upcoming meetings, available newsletters, and other Emerging Science for Environmental Health activities. For more information, visit:

http://nas-sites.org/emergingscience/

Do you have an idea for a meeting topic? We would love to hear it. Please send us an email with your suggestion at eseh@nas.edu.

Previous Meetings

Integrating Environmental Health Data to Advance Discovery, January 10-11, 2013

Exploring Human Genomic Plasticity and Environmental Stressors: Emerging Evidence on Telomeres, Copy Number Variation, and Transposons, October 4-5, 2012

Systems Biology-Informed Risk Assessment, June 14-15, 2012

Biological Factors that Underlie Individual Susceptibility to Environmental Stressors and Their Implications for Decision-Making, April 18-29, 2012

Emerging Technologies for Measuring Individual Exposomes, December 8-9, 2011

Green Chemistry: Applying 21st Century Toxicology to Green Chemical and Material Design, Sept 20-21, 2011

Mixtures and Cumulative Risk Assessment: New Approaches Using the Latest Science and Thinking about Pathways, July 27-28, 2011

The Microbiome: Interplay of the Microbiome, Environmental Stressors, and Human Health, April 27-28, 2011

Early Indicators: The Use of In Utero and Post-natal Indicators to Predict Health Outcomes Later in Life, Oct 14-15, 2010

Stem Cell Models for Environmental Health, June 3-4, 2010

The Exposome: A Powerful Approach for Evaluating Environmental Exposures and Their Influences on Human Disease, Feb 25-26, 2010

Computational Toxicology: From Data to Analyses to Applications, September 21-22, 2009

Epigenetics: Use of Emerging Science and Technologies to Explore Epigenetic Mechanisms Underlying the Developmental Basis for Disease, July 30-31, 2009

About the Committee

At the request of the National Institute of Environmental Health Sciences, the National Academies forms the Standing Committee on Use of Emerging Science for Environmental Health Decisions to facilitate communication among government agencies, industry, environmental groups, and the academic community about scientific advances that may be used in the identification, quantification, and control of environmental impacts on human health.

The National Academies
500 Fifth Street, NW
Washington, DC 20001