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Microfluidics' Watershed Moment

From our June 15 Issue: The Confluence of Multiple Inflows Is Building Toward an Irresistible Outflow, Refreshing Research and Diagnostics Along the Way

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Multiple subdisciplines are feeding into microfluidics, and the cascading confluences are bound to culminate in a surging outflow. At least, those closest to microfluidics feel that way. "It's a major interdisciplinary adventure," exclaims Steve Soper, Ph.D., a professor at the University of North Carolina.

Dr. Soper couples microfluidics with nanofluidics to build integrated fluidic systems for analysis of circulating tumor cells.

Much like a white water rafting team works together to negotiate the twists and turns of a challenging set of rapids, diverse specialists collaborate to realize microfluidic applications. "You need engineers, chemists, nanotechnologists, bioinformaticians, and of course cancer biologists to help home in on clinical indications," insists Dr. Soper.

Teamwork is also emphasized by Michael Roper, Ph.D., an analytical chemist at Florida State University. To build microfluidic systems that deliver different stimuli to living cells, Dr. Roper collaborates with mathematicians and other specialists. "We have to be able to speak the languages of several different scientific disciplines," he explains. Expecting that interdisciplinary work will characterize future science, Dr. Roper adds that delving into microfluidic technology is "great training for our students."

The microfluidic system shown above has been used by Florida State University researchers to deliver glucose to islets of Langerhans, the endocrine portion of the pancreas. The researchers used laser illumination to measure hormone release and assess the response to varying glucose levels.

Drs. Roper and Soper are among the presenters at an upcoming SelectBio event, the Lab-on-a-Chip, Microfluidics & Microarrays World Congress. This event, which will be held September 28–30 in San Diego, will showcase game-changing applications flowing from emerging microfluidic lab-on-a-chip (LOC) platforms.

Some of the events highlights are discussed in this article. They include predictive drug cytotoxicity assays, low-cost point-of-care (POC) diagnostics, hormone secretion profiling, glycoproteomic biomarker screening, and chemotherapeutic response tests.

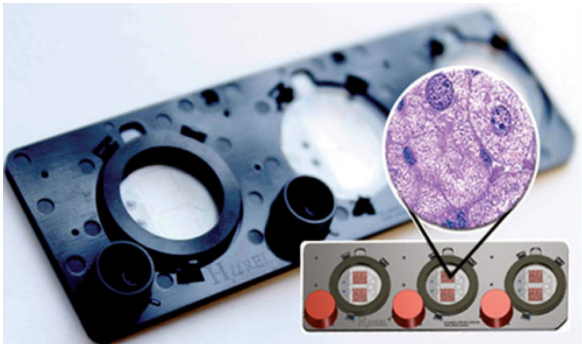
Back to the Headwaters

The H*μ*REL*flow*™ cell-based assay system has three microfluidic devices on a biochip. Each device incorporates two cell culture compartments interconnected by microscale canals. Integrated micropumps recirculate culture media within the device.

"[LOCs] are like little bionic devices because they include man-made elements as well as natural elements that are closely integrated together," says Robert Freedman, CEO, Hurel. The company's H*μ*REL® technology is designed to accomplish the preclinical prediction of drug safety. It improves the "predictivity of the experimental results scientists employ to determine the absorption, distribution, metabolism, and excretion (ADME) of drugs," asserts Freedman.

The bedrock of the two-tissue hepatic cell-based H*μ*REL*flow* LOC consists of high-functioning micropatterned, co-cultured liver cells. The device models the cytotoxic effects of reactive metabolites. "The significance of liver cells," explains Freedman, "is they are the biotransformation engine of the body, responsible for metabolism and detoxification." Hence, liver cells are valuable tools for predicting drug cytotoxicity *in vivo*.

Turning on the flow in the device brings oxygen and nutrients to the cells more efficiently. The flow sweeping away the cellular waste or crud produced by the liver is probably the "principle reason why cells perform so much better under flow," notes Freedman. "Otherwise, the cells are basically living and swimming in their own excretum."



*The H*μ*RELflow™ cell-based assay system has three microfluidic devices on a biochip. Each device incorporates two cell culture compartments interconnected by microscale canals. Integrated micropumps recirculate culture media within the device.*

Through microfluidic channels, the liver cocultures can be connected to a second culture such as cardiomyocytes, renal tubule cells, or disease model cells for simultaneous drug cytotoxicity and efficacy assays on a single LOC.

Fishing for Pathogens

"We've developed cheap, hybrid paper microfluidic devices for rapid POC infectious disease diagnosis," says presenter Xiujun James Li, Ph.D, an assistant professor at the University of Texas at El Paso. This includes detection of foodborne pathogens such as *staphylococcus* and *salmonella*, as well as meningitis, whooping cough, and tuberculosis associated pathogens.

Infectious diseases often occur in resource-constrained settings, and as Dr. Li underscores, "many developing nations cannot afford modern diagnostic techniques such as real-time PCR or associated expensive or bulky equipment."

From a historical perspective, microfluidics has been around since the 1990's. "Initially there were silicone and glass substrates, later

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polymers, and most recently paper," relates Dr. Li. "Each substrate has its own advantages and limitations. We developed the first paper and polymer hybrid device, which benefits from both substrates."

The polydimethylsiloxane (PDMS)/paper hybrid system can be integrated with aptamer-functionalized nanobiosensors for low-cost infectious disease diagnosis. Aptamers are short segments of nucleic acids or proteins that bind to specific targets. "The aptamer method is very simple," says Dr. Li. "We don't need DNA amplification or cell lysis, and microorganisms are directly detected."

Alternatively, to increase detection sensitivity, devices can be integrated with loop-mediated isothermal DNA amplification (LAMP) technology, which is also a simple, rapid, specific, and cost-effective approach. Dr. Li says that paper inside the LOC "enables stable results over a long period of time."

Islets in the Stream

Blood sugar regulation is a basic process; defects in insulin secretion are involved in metabolic diseases such as type 2 diabetes. "We develop microfluidic systems to investigate islets of Langerhans, the cells in the pancreas responsible for releasing peptide hormones such as insulin into the bloodstream to help regulate glucose," says Dr. Roper. Oscillations of insulin release from islets working in unison are critical to regulation. However, how the islets synchronize their behavior is still an open question.

The microfluidic systems are designed to deliver different stimuli to the living islets located on the LOC device. "The measurement of both intracellular and extracellular responses from the cells in real-time is possible with these systems," explains Dr. Roper, "allowing cellular responses to be measured with high sensitivity."

The liver is mathematically modeled in the systems to mimic *in vivo* feedback loops between the pancreas and the liver. "For example, we can take the insulin secretion amounts being released by glucose-stimulated islets and plug them into the model," Dr. Roper elaborates. "The faster we can measure secretion, the more rapidly we can update the model to give feedback to the cells by either lowering or increasing glucose levels."

"Our microfluidic system has shown pretty convincingly, in an *in vitro* fashion, that there could be a synchronization of multiple islets through in vivo feedback loop signals," concludes Dr. Roper. For example, insulin-glucose feedback loops could produce small oscillations in the glucose level that could synchronize islets in the pancreas to secrete insulin in unison.

Clearing Troublesome Logjams

"Microfluidics is a very powerful platform for sample preparation," comments Dr. Roper. It can, for example, overcome some of the limitations of classical sample preparation. According to Dr. Roper, the automation of sample preparation by means of microfluidics can relieve a bottleneck of many, many different techniques and disciplines: "If we can automate those processes using microfluidics, it would save time and money and as well as reduce errors."

Some of the limitations that HμREL can overcome affect traditional liver cell cultures, which have notoriously limited viability. "HμREL's technology provides a useful alternative to testing for hepatotoxicity in *in vivo* animal models," Freedman says with pride. "HμREL, human and relevant, won a People for the Ethical Treatment of Animals (PETA) award for best scientific achievement. Along with HμREL's PETA's award, the Humane Society of the United States is a venture capital investor in the company."

The infectious disease POC LOCs enable diagnosis within an hour and overcome the need for specialized equipment and expertise. They are "instrument-free detection devices," says Dr. Li. "Just use a cheap portable UV lamp to shine the sample and see the results. We are also developing cell phone apps; take a picture of your results and send them to the doctor for diagnosis or to a computer for quantitative analysis."

Circulating Biomarkers

Yong Zeng, Ph.D., an assistant professor at the University of Kansas, develops LOC technology that could transform the early diagnosis and management of some cancers. "Few protein biomarkers have been approved by the FDA for early detection of cancer," says Dr. Zeng. This is because protein levels per se aren't necessary correlated with the pathological aspects of the disease. Post-translational modifications may be of the essence.

"We aim to identify another dimension of molecular information that can actually be used for cancer diagnostics," Dr. Zeng continues. "The premise is free protein shed by the tumor into circulation may have different glycan structures than normal proteins."

Dr. Zeng will present new approaches for lectin-glycoprotein bioaffinity assays that reinvent certain aspects of classical technology into microfluidic formats for glycoproteomics profiling of tumor-associated proteins from liquid biopsies. Lectins, which constitute a class of proteins localizing to carbohydrate moieties of proteins, are established probes for detecting proteins containing carbohydrates as a post-translational modification.

"In conventional methods, most people just label the protein and then use the lectin to capture the protein because they can do direct fluorescence for quantification," informs Dr. Zeng. "That, however, is very, very limited if you actually want to move this technique into practical applications for analyzing clinical samples.

"It is difficult to economically and reliably fluorescently label clinical samples," he explains. "You need to be able to capture them first and then detect the targets."

Dr. Zeng aims to array a large library of lectins on the LOC to capture different types of glycan structures from a single liquid biopsy. "The lectins recognize the proteins through their glycans and capture them," he says. This should allow the identification of tumor-type or patient-specific molecular glycosylation signatures, which has potential applications in precision medicine.

"Generally speaking, quantitative analysis of biology is a challenge, " remarks Dr. Zeng. However, the on-chip reaction is much more sensitive and specific for detecting glycoproteins than past protocols. Such enhancements to detection, Dr. Zeng believes, are helping to "push the analytical performance of glycoproteomics to the next level."

Chemotherapeutic Flotsam and Jetsam

Dr. Soper will also be presenting LOC technology optimized for liquid biopsy samples. This technology, he says, straddles the microfluidics/nanofluidics divide: "It's simply the size of the different structures you use to process the information."

"Microfluidics is used to interface the real world, including the blood sample from which the circulating tumor cells are isolated to the microfluidic domain," he continues. "We process from 3–10 milliliters of whole blood using the microfluidic end of our system."

There are up to several hundred circulating tumor cells in this liquid biopsy sample, which then undergoes a purification step (which uses an antibody to target tumor surface antigen) and a volume-reduction step (which brings the volume down to about 2 microliters). Cells are lysed, and DNA is isolated and purified.

The purified DNA is assayed for damage. "We label the abasic sites, a location in the DNA that lacks a nucleotide," says Dr. Soper. "Abasic sites are a marker of DNA damage and an indicator of response to cancer chemotherapy."

The abasic sites are labeled with a fluorescent dye. Then the DNA is launched into another device, which consists of structures below 100 nanometers in size, and the DNA is stretched out in the structures.

"We physically count the number of abasic sites by measuring fluorescent signatures along the stretched out DNA chain," details Dr. Soper. "This is completely done under a microscope on a module resident within the LOC system. The number of abasic sites is a great indication of how the patient is responding to chemotherapy."

"There are several steps that need to be invoked on the sample," summarizes Dr. Roper. "What we do is build microfluidics systems that are very similar in architecture to your computer's motherboard. We take a fluid motherboard and connect to that. Task-specific modules perform different processing steps. An entire process, from sample 'in' to answer 'out,' is conducted without requiring any operator intervention. That's quite attractive from a clinical perspective."

It is crystal clear from the presenters and their topics that diverse platforms are bringing microfluidics into the mainstream, contributing to new approaches in research, clinical science, and medicine.