04

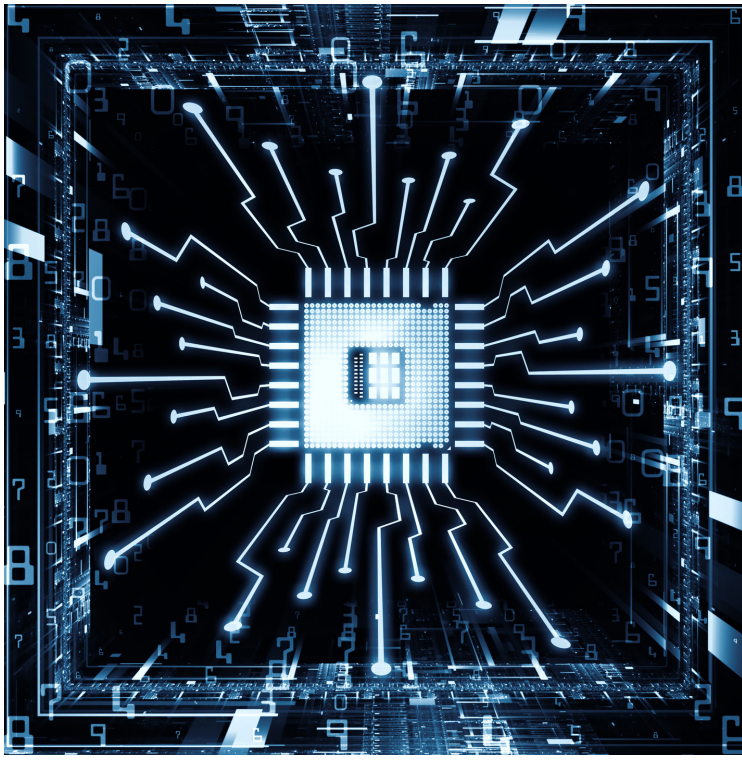
USHERING IN A NEW ERA OF MEDICINE WITH ANALYTICAL CHEMISTRY

4.1 MICROFLUIDICS: THE TINY TECHNOLOGY WITH A BIG FUTURE

Miniaturization is a key current trend in analytical chemistry, and, over recent years, there have been several developments to downsize three crucial elements – sample preparation, separation, and detection. Examples include both solid- and liquid-phase microextraction, chip-based gas chromatography, capillary electrophoresis, and miniaturized ion source methods based on electron spray ionization. There are many products already on the market that harness these technologies.

Microfluidics, the smallest endpoint of miniaturization, is emerging as a powerful technology. Although it has been around for many years, only now is the full potential of this science beginning to be realized.

In addition to developments in analytical sciences, microfluidics has been intertwined with progress in the consumer electronics industry, harnessing many of the same techniques and materials. The first microfluidic device was the inkjet printer. However, materials and micro components are now used to tailor the technology to specific analytic functions, overcoming obstacles to portability and cost-efficacy, and facilitating experiments not previously possible.



An entire lab-on-a-chip

One of the driving forces behind the development of microfluidics has been the desire to create lab-on-a-chip (LOC) devices. These are microfluidic devices that can perform single or multiple laboratory functions on a single chip. Examples include electrophoresis, DNA microarrays, biosensors, and flow cytometers. These devices are particularly suited to automation, making them incredibly useful in the fields of molecular biology, diagnostics, and drug development.

Another slant on this technology is organ-on-a-chip (OOC) devices. These seek to review the behavior of cells, organs, and whole organ systems to provide more reliable results than existing methods of cell culture and animal

testing. They may also assist a shift away from animal testing, driven by ethical and efficacy concerns.

Microfluidics, due to its small-scale and ease of manipulation, is uniquely suited to this aim. Cells can be cultured within an OOC device and their environment closely controlled, in terms of the nutrients, mechanics, and even the microbes they are exposed to. Such technology could ultimately give way to personalized medicine, with a person's cells used to create a model organ, as well as discoveries about fundamental human biology.

Microfluidics meets the body

As well as laboratory-based research, the future of microfluidic devices is likely to involve their direct integration with the human body. This poses several challenges, not least the fundamental incompatibility between solid electrical components, and the flexible, dynamic nature of tissue.

Researchers have recently developed microfluidic devices embedded in skin-like adhesives, similar to a transfer tattoo that can be worn on the skin. One such device, created by John Rogers' lab at Northwestern University, is a battery-free continuous monitor that absorbs sweat from the skin and provides instant information on the rate of sweating and electrolyte concentrations. It could have a wide range of applications, and the team has already explored its use in patients recovering from a stroke and professional athletes.

The technology is also likely to simplify the measurement of many external medical observations, such as heart rate, blood pressure, skin temperature, and respiration rate. This could be particularly important in neonatal care settings, eliminating the many sensors and wires currently required for continuous monitoring of premature newborns.

4.1a THE RISE AND REVOLUTION OF MICROFLUIDICS IN ANALYTICAL CHEMISTRY

Miniaturization is a significant trend within analytical science and, at its most extreme, lies microfluidics. These devices, which are capable of shrinking multiple functions onto a single chip, are poised on the edge of a revolution in biological and chemical science. At the same time, due to their efficiency, cost-savings, and improved accessibility, microfluidic devices have enabled experiments and observations that would not have been possible without the technology.

However, while the principles of microfluidics are long-established, it has not come close to fulfilling its potential. At Pittcon, we heard from leaders in the field about why that is beginning to change.

In the following sections, we will discuss the developments that have led to the emergence of more compact, portable devices in analytical sciences, including microfluidics, and the link between this and the consumer electronics industry. Stemming from advances in materials science, electronics, and engineering,

microfluidic devices have enabled the creation of lab-on-a-chip (LOC) and organ-on-a-chip (OOC) devices.

LOCs allows single or multiple laboratory functions to be scaled down onto a single chip that requires only minute samples and tiny reagent volumes. They facilitate numerous parallel experiments, something which has already been harnessed within genomic analysis.

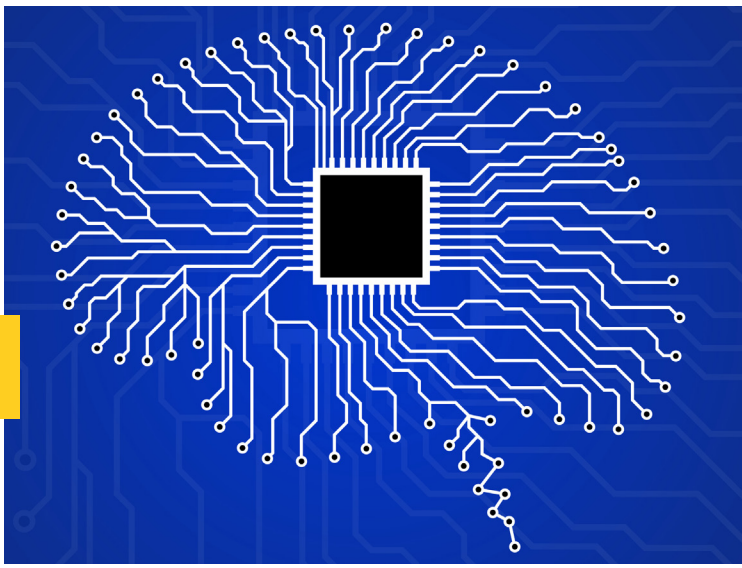
OOC devices could also help the pharmaceutical industry to tackle one of its most fundamental issues – the high failure rate of drug candidates.

LOC enables the mimicking and manipulation of human organs and organ systems on a tiny scale, which helps predict drug pharmacokinetics, toxicity, and efficacy more closely than existing cell culture or animal-testing approaches.

At Pittcon, we heard how microfluidic devices could be integrated with the human body itself. Devices with skin-like properties that adhere to the body and provide real-time data on its function, such as sweat production and electrolyte balance, have already been developed. In the future, such devices could even sit within the human body, interacting with organs such as the brain or heart to prevent or overcome disease.

The Pittcon conference was attended by leading manufacturers who have already brought miniaturized devices to the market. However, there were speakers at the conference who are pioneers in the field of microfluidics.

Among the distinguished speakers, John Rogers from Northwestern University presented the Wallace H Coulter lecture and described his lab's achievements in developing a wearable, microfluidic, continuous monitoring device. The team has tested its technology in stroke patients as well as professional athletes, demonstrating the broad scope for such devices in healthcare, human performance, and the consumer market.



The Rise and Revolution of Analytical

Miniaturization is one of the most prominent current trends in analytical chemistry. It is often associated with greater efficiency, ergonomics, cost reductions, and accessibility. Not only does miniaturization refer to analytical devices and their components, but also the sample size and volumes of reagents and organic solvents needed.

There has been much interest in creating downsized systems through the miniaturization of the different analytical process steps. In recent years, advances have been made in analytical chemistry to facilitate this miniaturization in three main areas.

Sample preparation

Sample preparation is a significant step in the analytical process but is prone to errors. Along with sample collection and preservation, it is also the most time-consuming.

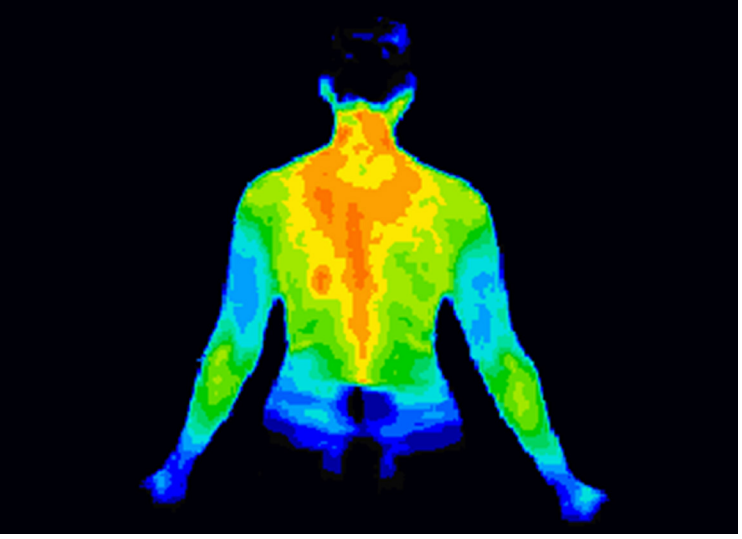
Solid-phase and solvent extraction are well-established and the most commonly used approaches. However, these classical techniques are associated with organic solvent use and wastage, as well as low-efficiency enrichment factors (EFs).

In the early 1990s, solid-phase micro-extraction became the first miniaturized form of sample preparation and is embraced by the research community. Other developments in solvent-free extraction have included stir bar sorptive extraction, which helped further improve extraction efficiency. Other methods developed include thin-film microextraction, solid-phase dynamic extraction, and microextraction via syringe.

Within solvent extraction, liquid-phase microextraction techniques have recently been developed to improve on conventional methods and the quantity of solvent required. They also help to obtain higher EFs and accelerate the speed of the process to facilitate high-throughput analyses. Examples include single-drop microextraction and dispersive liquid-liquid microextraction.

Analytical separation

The most commonly used separation techniques are liquid chromatography, gas chromatography, and capillary electrophoresis, which are applied before analysis to separate target analytes within a sample. As with sample preparation, the main goals of miniaturizing this process are to enhance speed and efficiency and reduce the amounts of sample, solvents, and reagents needed, as well as to reduce costs and provide portability. A major driving force behind the development of miniaturized separation methods has been the need to reduce the amount of space occupied in the laboratory.



There has been miniaturization of all three primary separation methods. In the 1970s, the first GC-on-a-chip was developed, but work is ongoing to improve performance to match conventional GC. Micro-GC systems have been developed thanks to advances in miniaturized components, including pumps, preconcentrators, columns, and sensors.

Likewise, in LC, miniaturization has been achieved through the reduction in column dimensions, as well as new stationary phase materials and improved detection technology.

Chip-based LC was first reported in the 1990s and has been facilitated by novel stationary phase materials and engineering advancements that enable microchannels on the chip surface with a high degree of accuracy.

Recent research has led to the commercial availability of miniaturized, portable electrophoresis instruments. In general, chip-based analysis systems have employed electrophoresis over LC or GC. As well as portability, chip-based CE benefits from a reduced sample, chemical, and energy requirements, as well as being quick. One battery-powered, portable chip-based CE described in the literature was able to perform separations in under 12 seconds.

Detection techniques

Advances in the fields of electrical engineering and materials science that have given way to micro-electro-mechanical systems have allowed the miniaturization of analytical detection systems. These systems maintain or improve upon the accuracy of full-size systems while being portable and requiring less power.

Examples include ultraviolet and visible absorption spectrometry, while there have also been advances in infrared (IR) spectroscopy. The development of quantum cascade lasers, interferometers and electronic circuits have improved IR spectroscopy.

Mass spectrometers have been successfully miniaturized, which has evolved from the instrumentation developments that gave rise to modern LC-MS systems. Miniaturization has been achieved primarily through reductions in the mass selective analyzers. The ion source has also been miniaturized using ambient ionization methods such as electron spray ionization, paper spray ionization, and miniaturized high vacuum pumps. These advances could allow bedside MS analysis, albeit with a reduced scope compared to the flexibility of full-size LC-MS systems.

Electrochemical detection techniques are also highly amenable to miniaturization and have been employed for several decades within glucose monitoring devices. Thanks to developments in microelectronics and microfabrication methods, electrochemical detection techniques have been downsized without impairing performance. They have been used in lab-on-a-chip systems and are particularly suited to pairing with capillary electrophoresis.

Several products that have helped to bring advanced analysis to the benchtop are currently available.

Fourier Transform IR

ThermoFisher offers the Nicolet Summit compact FTIR spectrometer, which is an easy-to-use device that sits within the multi-user lab, offering speed and accuracy to a whole range of users.

Shimadzu offers a line of IR instruments, including the IRTracer-100. This benchtop device is suited to basic IR analysis, but also more sophisticated experiments, such as high-sensitivity analysis of trace foreign matter and high-precision semi-conductor analyses.

NIR

Another Pittcon attendee, Malvern Panalytical, delivers near infrared-based devices, including its ASD FieldSpec range. These UV/Vis/NIR/SWIR spectroradiometers are fully portable and deliver the fastest and most accurate measurements available from any commercial field-portable spectroradiometer.

Raman Spectroscopy

Kaiser Optical offers a hybrid Raman analyzer that can be situated on the benchtop or an optional ergonomic trolley. The analyzer includes a built-in probe and optical storage, a routine-analysis sample compartment, fiber storage, and the analyzer control system.

Mass Spectrometry

ThermoFisher also attended Pittcon. Among its wide range of analytical instruments is the Orbitrap ID-X Tribrid Mass Spectrometer, which is specifically designed for the analysis of small molecules.

Microfluidics represents the most extreme endpoint of miniaturization. Although the concept has been around for more than 30 years, it is only recently that it has come to be a significant player and is set to play a transformative role in analytical chemistry and research.

References

» *Pena-Pereira F. From Conventional to Miniaturized Analytical Systems.*

» *Zhou X, Liu J, Cooks RG, et al. Development of miniature mass spectrometry systems for bioanalysis outside the conventional laboratories. Bioanalysis 2014; 6: 1497–1508. doi:10.4155/bio.14.100.*

» *Matysik F-M. Miniaturization of electroanalytical systems. Anal Bioanal Chem 2003; 375: 33-5. doi: 10.1007/s00216-002-1635-x.*

4.1b THE ERA OF MICROFLUIDICS

Microfluidics is not the newest of technologies. Many consider the inkjet printer to be the first microfluidic device, and that was back in 1965. However, microfluidics has not yet reached its full potential for various reasons. This looks set to change.

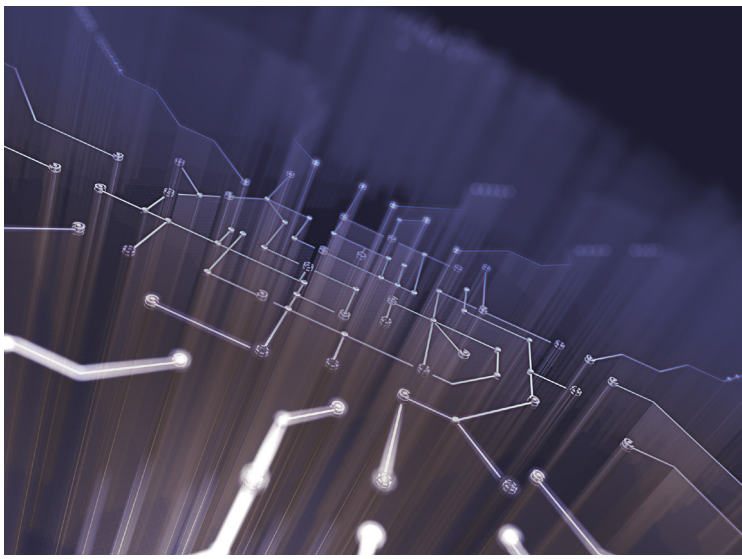
Microfluidics involves the control and manipulation of fluids on a microscopic scale within microchannels – typically defined as 100 nm to 100 µm. This technology has enabled small-scale devices that perform analytical functions, including multiple functions within a single device.

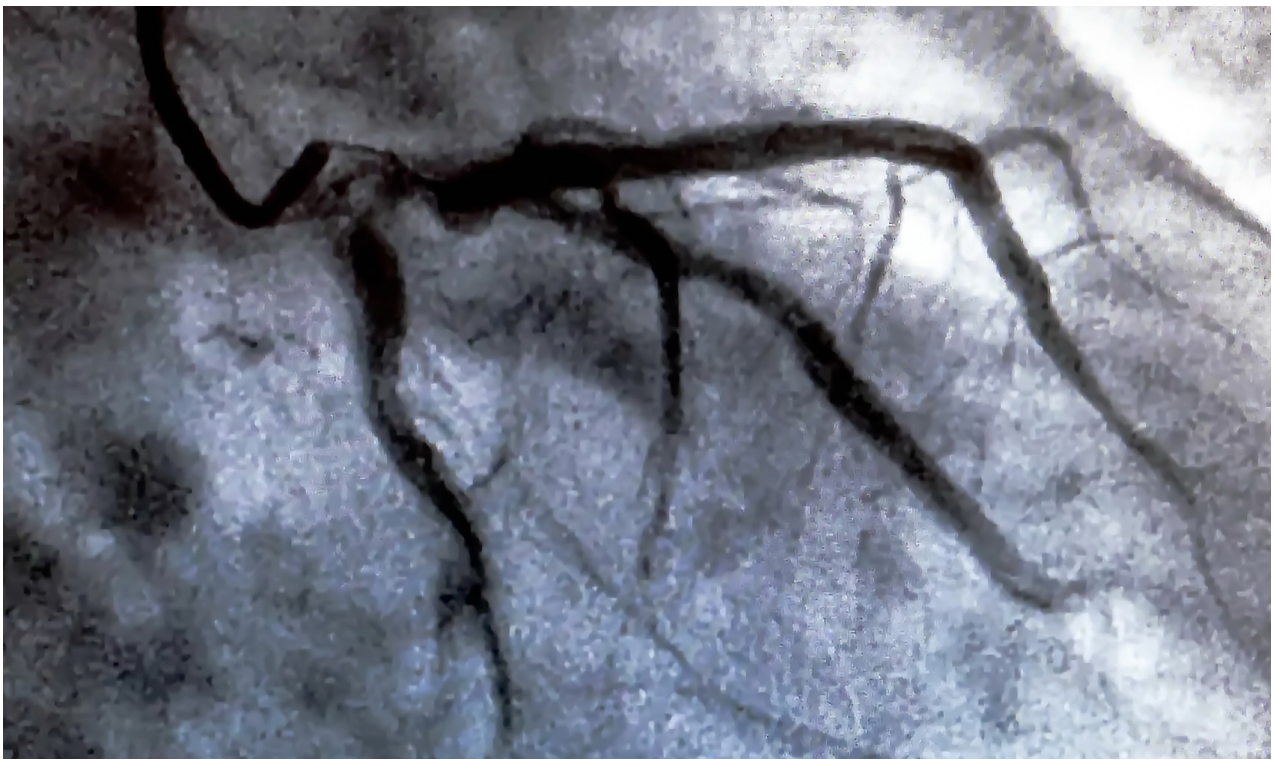
One of the most important drivers behind the most recent push to advance microfluidics comes from biological and chemical sciences, where the ability to compress the functions of an entire lab can be integrated into a single chip (lab-on-a-chip (LOC) devices). One of the main advantages of microfluidic devices is that they only require small samples and limited reagent quantities due to the volumes of fluid involved – something that has both financial and environmental benefits. They also help facilitate automation.

Microfluidics does not represent downsizing for the sake of downsizing. Miniaturization can be the byproduct of a desire to perform a new function that has not been achieved before, or to improve upon one that already exists, making it more accurate and efficient. In these instances, miniaturization is not an end-goal.

The development of microfluidics has co-evolved with or developed on advances made in consumer electronics. Photolithography, which is the foundation of microfluidic devices, was initially used for the creation of printed circuit boards in electronics.

Microfluidics was also heavily influenced by the 'silicon era'. However, the materials used have long-since moved on. In particular, poly(dimethylsiloxane), PDMS, is now the material of choice for microfluidic chips, thanks to its flexibility, low cost, and ease of molding.





At Pittcon, Andrew deMello discussed the use of microfluidics for ultra-high-throughput chemistry and biology. DeMello is a professor of biochemical engineering in the Department of Chemistry and Applied Biosciences at ETH Zurich and Head of the Institute for Chemical and Bioengineering.

DeMello's lab focuses on the development of microfluidic tools for high-throughput experiments and high-sensitivity optical detection methods. One significant aspect of their work has been the use of droplet-based microfluidic devices to perform chemical and biological experiments. Their future aims center around the development of ultra-high-throughput imaging flow cytometry and circulating biomarker detection for diagnostic purposes.

DeMello's lecture considered how droplet-based techniques can be exploited and combined with spectroscopy to perform high-throughput, information-rich experiments.

He also described how droplet-based microfluidic systems can be developed and applied to problems in chemistry and biology, before moving on to discuss the lab's advances in optical spectroscopies and how these relate to the development of label-free imaging flow cytometry.

References

» *Convery N, Gadegaard N. 30 years of microfluidics. Micro and Nano Engineering 2019; 2: 76-91. doi: 10.1016/j.mne.2019.01.003.*

4.1c MICROFLUIDICS AND BIOLOGICAL

Without even breaking the skin, there are parameters within the human body with the potential for monitoring. Devices help monitor the body's temperature, sound, mechanical movement, and electrical signals, revealing the body's processes. These are used every day in medicine to monitor patients and detect disease.

Using the advances of microfluidics, researchers have recently been asking if measurements such as these could be taken with wearable devices, simply adhered to the skin, or even embedded within the body. Not only could they be used in research, diagnostics, and monitoring, but also for treatment.

A major challenge is a fundamental mismatch between the nature of biological tissues and electronics. The human body is flexible, 3D, dynamic, and soft, while electronics are rigid and static.

How can electronics integrate seamlessly with biological tissues? John Rogers and his lab at Northwestern dedicate their time to the creation of biologically compatible 'soft' electronics to address this question.

The Rogers lab developed a biocompatible skin-like sensor for continuous monitoring. They have focused on devices that can be adhered to the skin, but in the future, envision them being used directly with organs such as the heart and the brain. His work is based on adapting the electronic engineering found within consumer devices to interface with the soft tissues of the human body.

The technology has a wide range of potential applications within and outside of clinical settings.

The team has explored the use of its sensor technology in the neonatal intensive care setting. This is an environment where the continuous recording and display of vital signs is critical. However, the standard ways of doing so require the infant to be connected to multiple monitoring systems with accompanying wiring.

This setup quickly becomes an obstruction to the baby's care, by complicating clinical tasks and potentially slowing down access in emergency procedures. The many adhesives required can damage the delicate skin of the newborn, producing bruising and scarring. The baby is also isolated from skin-to-skin contact, which prevents bonding opportunities between the parents and the infant.

To overcome these issues, Rogers and his team developed a wireless, battery-free sensor that sits on the baby's skin. They attached two, including one on the chest to capture electrocardiograms (ECGs), and a second on the foot to record their heart rate through photoplethysmograms (PPGs). Collectively, the devices provide data on skin temperature, heart rate, heart rate variability, respiration rate, blood oxygenation, and systolic blood pressure.

In a recent paper published in Science, the researchers carried out a pilot study of their technology in a neonatal intensive care unit (NICU). They showed that the system was able to be immersed in water, is compatible with the humidity of NICU incubators, and facilitates skin-to-skin contact with several alternate positions for the upper monitor. The device is also compatible with medical imaging techniques in the NICU, such as magnetic resonance imaging (MRI).

The team created an adhesive microfluidic device containing channels into which sweat can enter via a port. This allows the local sweat rate to be determined. It also contains four colorimetric dots in the center corresponding to chloride, glucose, pH, and lactate levels, as well as thermochromic liquid crystals. The disposable device does not contain electronics and can be read by the eye, or interpreted by a cell phone app using a camera.

The device can provide athletes with real-time performance data and inform them about hydration during competition. The team at Northwestern has explored how to make a fully waterproof version of the device that operates fully underwater. They hope that it will be a useful format for swimmers and triathletes, where existing sweat collection and sensing technologies are ineffective.

While this technology holds promise for use by professional athletes and consumers, Rogers and his team have also been testing it out in patients who have experienced a stroke. Differences in sweat rates after a stroke occur between the left and right sides of the body. These can then be measured to monitor progress during rehabilitation. The device could also be used as a simple screen for cystic fibrosis in infants by measuring electrolytes in sweat, something which is currently done with a bulky, inconvenient device.

References

- *Rogers JA. Materials for biointegrated electronic and microfluidic systems. MRS Bulletin 2019; 44: 195-202. doi: 10.1557/mrs.2019.46.*
- *Chung HU, Kim BH, Lee JY, et al. Binodal, wireless epidermal electronic systems with in-sensor analytics for neonatal intensive care. Science 2019; 363: eaau0780. doi: 10.1126/science.aau0780.*
- *Guinsburg R. Improving care of critically ill newborns. Science 2019; 363: 924-925. doi: 10.1126/science.aaw2085.*
- *Reeder JT, Choi J, Xue Y, et al. Waterproof, electronics-enabled, epidermal microfluidic devices for sweat collection, biomarker analysis, and thermography in aquatic settings. Science Advances 2019; 5: eeau6356. doi: 10.1126/sciadv.aau6356.*

4.1d MICROFLUIDIC TECHNOLOGY MEETS CELLS

Lab-on-a-chip

Lab-on-a-chip (LOC) technology involves scaling down individual or multiple laboratory functions into a chip-format, ranging from a few square millimeters to centimeters. This miniaturization offers many advantages, including portability, cost-effectiveness, speed, throughput, and automated analysis. The approach has been applied primarily in molecular biology and diagnostic and genomic analysis, but also in biochemical analysis, proteomics, cell research, and drug development.

Proteomics

LOC devices have been applied in proteomics to help overcome some of the challenges of proteome profiling. In particular, it can assist with protein separation and detection, while being quick, easy, and requiring lower amounts of reagents compared with alternative approaches.

Microarray

DNA microarrays allow for the fast and accurate characterization of DNA expression. Microarrays screen for infectious agents and genetic diseases and can be used in drug screening and forensic analysis.

Biosensors

There are two types of LOC biosensors. In bioaffinity devices, target analytes bind to a ligand partner, such as an antibody or oligonucleotide. This is used in DNA screening, where target nucleic acid fragments hybridize with a single-stranded DNA probe. In biocatalytic devices, an enzyme for which the analyte of interest is a substrate is immobilized. This approach is used in glucose monitoring where glucose oxidase, the enzyme, detects glucose, the substrate.

Cell research

Through the use of a microchannel, LOC performs experiments on single cells. With the cell positioned in the microchannel, there is then the opportunity to control the surrounding environment and expose the cell to analytes.

The use of LOC in flow cytometry is now well established and helps to overcome the limitations and restrictions in traditional flow cytometry through greater affordability and accessibility. It can even be used for point of care testing in clinical settings, removing the need to transport blood samples, and providing diagnostic and monitoring tools in remote areas.

Drug development

LOC has several applications within drug development, where experiments primarily seek to examine the interaction between a potential drug and a target such as a cell. LOC allows many of these experiments to take place in parallel, and is well suited to automation, allowing for high-throughput drug candidate screening. It has many applications at all stages of drug development, such as enzyme kinetics, toxicity screening, assessing drug-protein interactions, and the discovery of potential drug targets on cells.

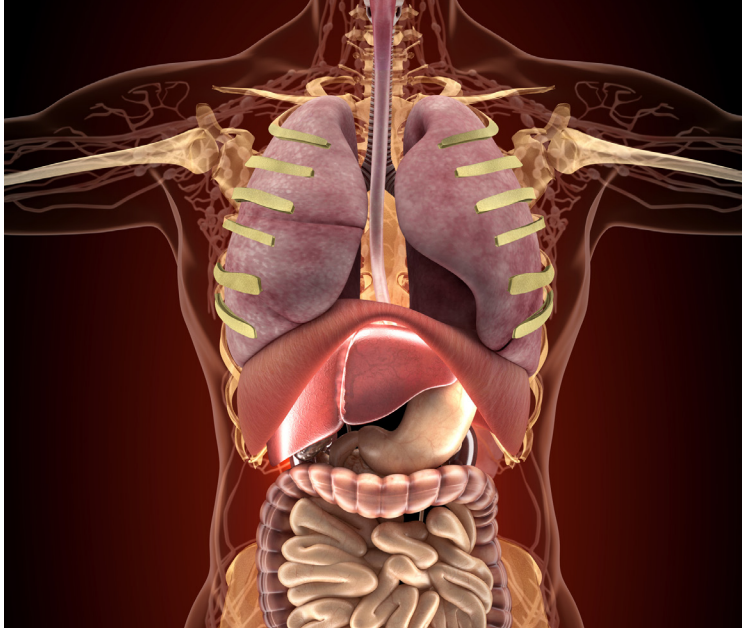
Organ-on-a-chip

As well as providing opportunities to scale down laboratory functions, microfluidic technology is also applied to the modeling of human organs and systems. This approach helps to overcome some of the limitations of traditional cell culture, which include a failure to replicate the complex, dynamic natural environment of real tissue and the subsequent unnatural behavior of the cells.

In vitro cell lines frequently die out or lose certain functions as they become deactivated. Organ-on-a-chip (OOC) also addresses concerns about both the ethics and utility of animal models for disease, which have not helped the high failure rate for drug candidates - a major source of financial losses in the pharmaceutical industry.

OOC technology recapitulates the natural tissue environment by mimicking the blood flow that delivers oxygen and nutrients to the cells, as well as the mechanical stimuli of stretching and shear stress.

Recent developments in materials science, cell engineering, and imaging have resulted in increased excitement and expectations of OOC technology and what it could deliver in the future.



Below are some examples of OOC technology that have been developed.

Lung-on-a-chip

Researchers at Harvard University have developed a lung-on-a-chip device. The design replicates the exchange surface of the lung by using a microporous membrane made of polydimethylsiloxane on which the team cultured alveolar endothelial cells on one side and vascular endothelial cells on the other.

As the device has two chambers, the cell culture on each side of the membrane can be exposed to separate media, in this case, airflow and culture medium, respectively. By applying a vacuum to channels on either side of the device, the researchers can recapitulate the mechanics of breathing that cells in the lung are exposed to. Research using their model has been able to demonstrate the lung's inflammatory response to bacterial infection and has also been used as a disease model to explore the use of a low-molecular-weight drug for pulmonary edema.

Liver-on-a-chip

The liver is a vital organ in drug discovery due to its critical role in drug metabolism, which relates not only to the efficacy of the drug, but also potential toxicity. However, the traditional cell culture of hepatocytes often leads to loss of activity. To overcome this, researchers have developed a liver-on-a-chip that cultures cells on a 3D scaffold to allow continuous perfusion while also subjecting them to the mechanical stresses observed in vivo. They demonstrated that the setup enabled the cells to form biologically reminiscent 3D structures that were sustained for at least two weeks in a bioreactor.

In other research, a team was able to recreate the microstructure of the hepatic cord, the smallest functional unit of liver tissue. The team did this by seeding hepatocytes in two lines, similar to what is seen in the liver. Culturing the cells under perfusion, they showed that the hepatocytes were self-organized and formed structures known as bile canaliculi along with the hepatic cord-like design. By exposing the model to a compound with a fluorescent metabolite, the team was able to demonstrate the metabolite transportation and that the canaliculi were functional. The researchers suggest that such a model could be used for analyzing the metabolism and excretion of drug candidates as an alternative to animal testing.

Intestine-on-a-chip

The small intestine, as a site of absorption, is another crucial organ to be considered in drug discovery. Researchers have developed a small intestine-on-a-chip model containing two compartments separated by a microporous membrane, on which small intestinal cells were cultured. Paired with an optical detection system, they were able to measure the transport of a fluorescent marker across the cell monolayer.

Other research has sought to go further to recreate the intestinal environment with a gut-on-a-chip device that mimics the peristaltic motions of the intestine. They were able to show that the cultured intestinal cells underwent configuration changes and differentiation in response to these mechanics. They also used the model to explore the effects of microbes co-cultured with the epithelial cells.

At Pittcon, Nancy Allbritton spoke about the work of her lab at the University of North Carolina. Her multidisciplinary team brings together expertise from chemistry, physics, engineering, and materials science to develop new biomedical technologies and assays. Its three main areas of focus are single-cell biochemical assays, microfabricated cell array platforms, and organ-on-a-chip systems.

Allbritton's talk covered in detail the progress her lab has made in these areas. Their current focus is organ-on-a-chip technology, developing a model of the large intestine. This work is particularly challenging due to the need to mimic not just the physical structure of the intestinal lining and the motion of the colon, but also the gut's microbial environment. These gut microbes interact with the intestinal cells and play essential roles in digestion and immunity. The microbes' presence also dictates the need for a steep oxygen gradient to keep both the anaerobic microbes and human cells functioning happily side-by-side.

Allbritton hopes that, in the future, patients will be able to have their own colon-on-a-chip that can be used to explore the effects of drugs and drug interactions, as well as environmental factors such as diet. More widely, the technology will be useful for regenerative medical research and understanding the fundamental biology of the intestine through the exploration of host-parasite interactions.

Nancy Allbritton is the Kenan Professor of Chemistry and Biomedical Engineering and Chair of the Joint Department of Biomedical Engineering at the University of North Carolina at Chapel Hill and North Carolina State University. She was recently appointed Frank & Julie Jungers Dean of the College of Engineering at the University of Washington.

References

- *Yilmaz B & Yilmaz F. Lab-on-a-chip technology and its applications. In: Omics Technologies and Bio-engineering: Towards Improving Quality of Life (2018). Barh D & Azevedo V (eds.) Academic Press; London, United Kingdom.*
- *Mohan A, Marshkole N, Nair AP, et al. Development of a Lab on a Chip Flow Cytometer Portable and Affordable Flowcytometer for Point of Care Diagnostics in Rural Areas. Proceedings of the 10th International Joint Conference on Biomedical Engineering Systems and Technologies (BIOSTEC 2017); 179-185. doi: 10.5220/000617301790185.*
- *Neuži P, Giselbrecht S, Länge K, Huang TJ, Manz A. Revisiting lab-on-a-chip technology for drug discovery. Nat Rev Drug Discov. 2012; 11: 620–632. doi:10.1038/nrd3799.*
- *Kimura H, Sakai Y & Fujii T. Organ/bosy-on-a-chip based on microfluidic technology for drug discovery. Drug Metabolism and Pharmacokinetics 2018; 33: 43-48. doi: 10.10106/j. dmpk.2017.11.003.*
- *Powers MJ, Domansky K, Kaazempur-Mofrad MR, et al. A Microfabricated Array Bioreactor for Perfused 3D Liver Culture. Biotechnol Bioeng 2002; 78:257-269. DOI: 10.1002/ bit.1014.*
- *Nakao Y, Kimura H, Sakai Y, et al. Bile canaliculi formation by aligning rat primary hepatocytes in a microfluidic device. Biomicrofluidics 2011; 5: 22212. doi:10.1063/1.3580753.*

CONCLUSION

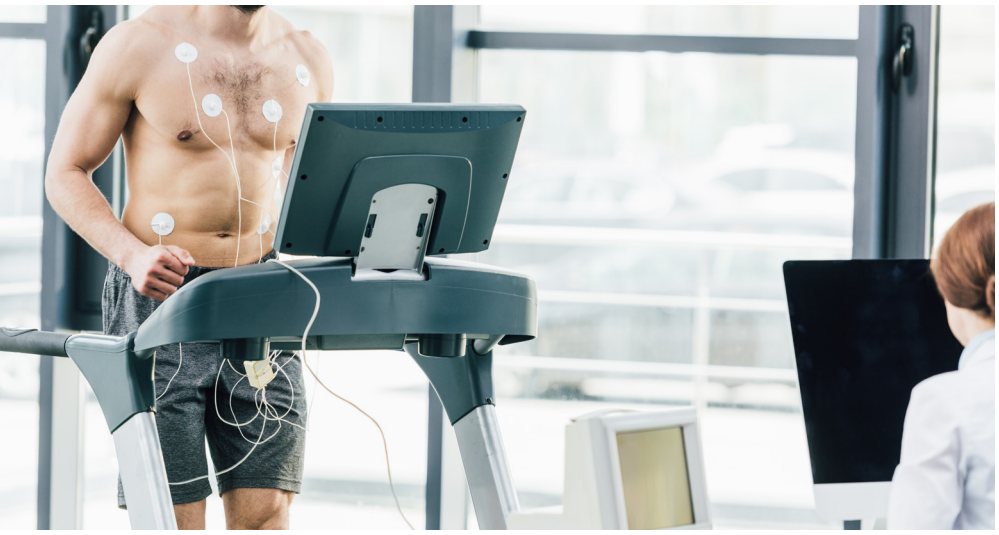
There have been progressive advancements that have facilitated the miniaturization of analytical systems. These have not just been within analytical chemistry itself but have also been heavily influenced by developments in consumer electronics, engineering, and microfabrication.

Microfluidics, the most extreme form of miniaturization, offers advantages that go beyond simple compactness. While there are many examples of the technology improving portability and efficiency, microfluidics is also permitting previously impossible experiments, bringing whole new dimensions to research.

Critical developments within microfluidics include LOC and OOC devices. LOC devices are already well established in fields such as genomics and proteomics. Meanwhile, OOC devices continue to evolve but show huge potential to model the behavior of the human body on a single chip.

Microfluidics is already giving rise to wearable technologies that could allow consumers, athletes, and doctors to monitor the body in ways not previously possible. In the future, microfluidic devices could integrate with the human body to deliver treatment or interact with the internal organs.

Microfluidics is no longer waiting in the wings – it is about to revolutionize analytical science, research, and healthcare. Pittcon was the ideal time and place to learn more, as we heard from some of the most prominent researchers in the field. There was also the opportunity to meet with companies that have brought pioneering, miniaturized technologies to the market.



4.2 THE ROLE OF MICROPHYSIOLOGICAL SYSTEMS FOR ONCOLOGY AND STEM CELL RESEARCH

In this interview, Dr. Nancy Allbritton from The Allbritton Lab talks about the revolutionary technology and techniques for the application of new technologies for oncology and stem cell research.

Dr. Nancy Allbritton provides insight into the organ-on-a-chip and how its ability to monitor and control the environment at the cellular and tissue level is one of the most promising applications for microengineered systems.

What are microphysiological systems (organs-on-chips) and why are they becoming increasingly important in biology and medicine?

Microphysical systems seek to replicate the smallest functioning unit of an organ. For an intestine-on-a-chip, it might be large or small intestinal crypts, while, for a heart, it may be a section of contractile muscle. For the liver, it may be a liver lobule or group of lobules. It is a group of interconnected cells so that they are not just behaving as a single cell or a small cluster, but they began to show higher-order behavior and functioning.

I think one of the reasons microphysical systems are becoming increasingly important is that we have had a lot of breakthroughs in stem cell technology. The ability to grow human stem cells from different organs is now present, as well as the ability to create a tissue of differentiated cell types from primary stem cells or organ-specific cells from induced pluripotent stem cells.

It is now the perfect time for this technology as we have all the enabling microfabrication methods and stem cell biology innovations coming together. Another reason organs-on-chips are becoming important is the ability to grow human tissue. It is very hard to carry out human experiments and get a good representation of the population, either because people are not volunteering or because there is a lack of people in that particular group of individuals. With the chip, you can begin to sample population-wide tissue variation.

Organs on chips can also be a better way to test drugs. Rather than testing cells in a dish, which usually include tumor cells that are significantly abnormal in their growth and other characteristics, organs-on-chips constructed from normal human cells can be used to get a more accurate representation of how drugs will affect humans. Additionally, they may outperform murine experiments in many ways since humans are simply not 70 kg mice.

We have been able to cure mice of all sorts of diseases, but much of this work has not translated to humans. Therefore, while mice offered good screening technology, a lot of drugs got through that were quite toxic to humans, and then a lot of drugs that may have worked well in humans but were harmful to mice got blocked.

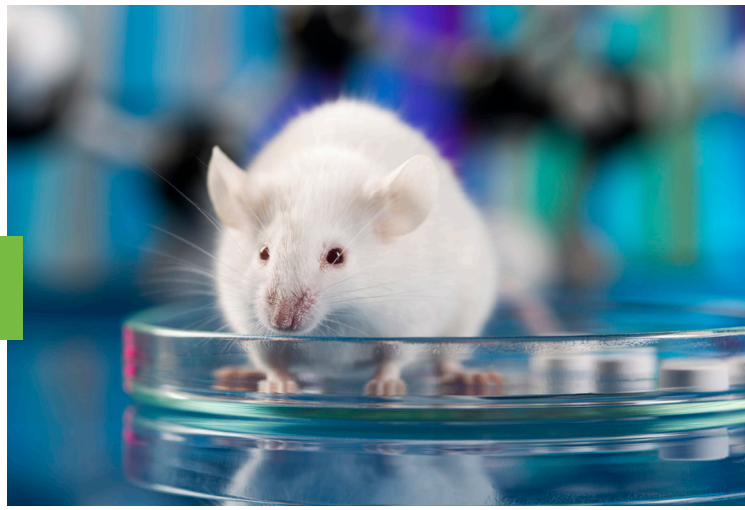
"Microphysiologic devices should maximize the number of useful drugs that could work well in humans. You can hopefully also get higher value information and create a much better drug pipeline."

Dr. Nancy Allbritton

I think doing a high-throughput screening on organ subunits using microfabricated devices can complement drug screening in humans. The idea is that you may be able to cut costs in several ways, including:

1. Reducing the number of animal studies
2. Removing ineffective drugs from the pipeline earlier (bad drugs fail earlier in the pipeline)
3. Minimizing the extent to which human studies are necessary

I believe you can also begin to develop human disease models. You can create mouse models of human disease, but they rarely completely recapitulate human disease. Even when it is a simple genetic mutation, the mice can often be asymptomatic or present different symptoms and outcomes to that mutation. With the organ-on-a-chip, you can have functioning human tissue that mimics many of the symptoms of human diseases. Even more exciting, we can begin to put, for example, an intestine-on-chip coupled with the human gut microbiome or the gut flora and begin to understand how the tissue and microbiota interact. This is important because our gut flora is very different from that of a mouse and other model organisms.



We now know that intestinal microbes have a tremendous physiologic impact throughout our body, including metabolism and mentation, our feeding behavior, and these are aspects of human behavior or physiology that cannot be recapitulated in an animal model or cells in a dish.

We will eventually be able to string human organs together as human organ-on-chip systems. For example, food is added to the gut model and absorbed. The nutrients then travel to the liver, which metabolizes the absorbed compounds, sending them out to the body, including the brain and heart. The organs must be tied together to see the full effect in which the behavior or functioning of one organ impacts other connected organs. That will never replace a fully functioning human, but I think the expectation is that it may end up being a lot more accurate and, in some ways, cheaper than mice or other model mammalian systems.

Organ-on-a-chip systems will help us understand basic biology and physiology of the human.

Why are new engineering methods needed for microphysiological systems?

Microphysiologic devices depend on many fields of chemistry for their advancement, including synthetic organic, polymer, and analytical chemistry. We need more synthetic matrices to support cells and tissues, including smart polymers and scaffolding materials that will support, direct, and shape these organ systems.

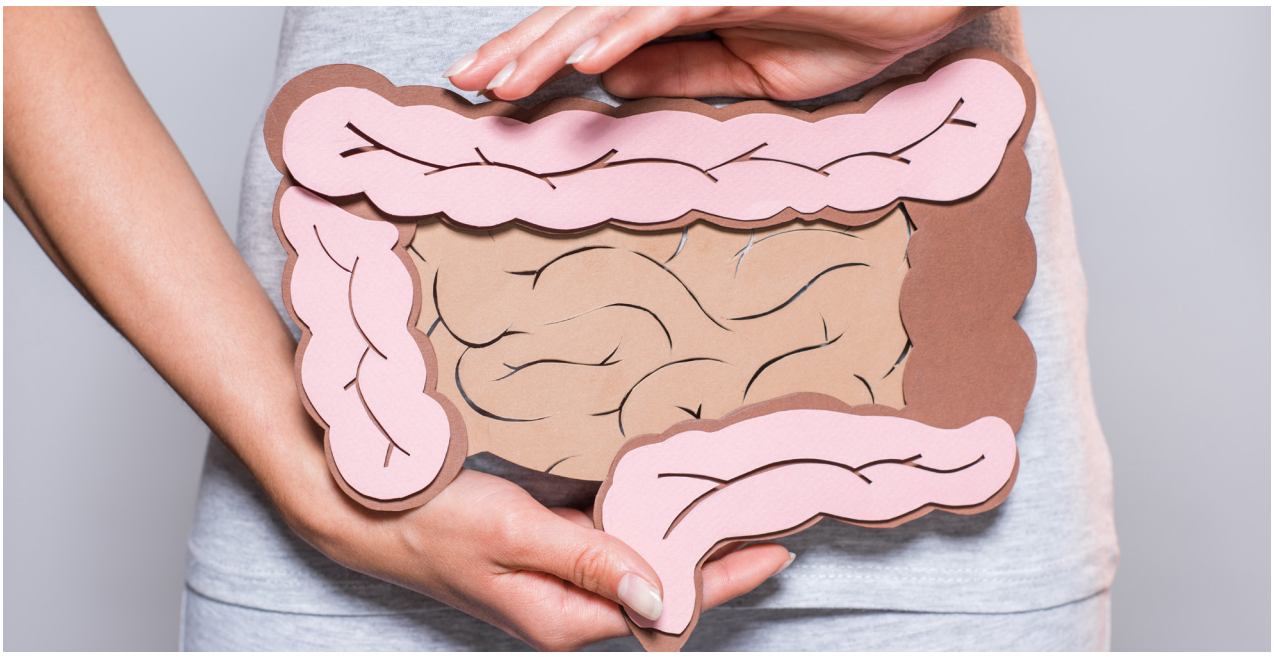
People often use matrices derived from native biologic materials such as collagen or matrigel, which are expensive and not fully defined.

Polymer and synthetic chemists are working hard to develop novel materials and matrices, while analytical chemists and engineers are developing materials and methods for microdevice fabrication, sensor enhancements, and other device-related innovations.

> "Microphysiologic systems will need an array of embedded and external sensors to monitor their health and well-being as well as pathophysiologic attributes."
>
> *Dr. Nancy Allbritton*

They will require robust, reliable, and often miniaturized embedded sensors for oxygen consumption, glucose concentration, CO2 production, pH, and other chemical and physical attributes. Importantly, the sensors should not perturb the system. These sensors will likely require external instrumentation or detection methods to monitor the embedded sensors, for example, RFID or optical readouts.

For engineering and chemistry, these are going to be huge areas where many can contribute to moving the field forward. For engineering, developing ways to make integrated systems efficient, low cost, manufacturable, shippable, self-contained, and talk to each other will be significant. This is an area where engineering and chemistry must work together in a team to move the field forward. It is going to take both disciplines to advance the field.



What is microfabricated technology and how can it be used to overcome these issues?

Microfabricated technology refers to devices developed with micron-sized features. Cells have diameters on the order of 10 microns, and many organ subunits span hundreds of microns so that micron-sized architectural features are required to recapitulate key features. For example, large intestinal crypts (the key physiologic subunit of the large intestine) is approximately 400 microns in length and 100 microns wide, with the stem cell niche spanning tens of microns. Therefore, microfabrication methods are perfect for rebuilding many of the key architectural features of the large intestine.

How were the microfabricated platforms used in your lab developed?

We do intestine-on-a-chip, which is mostly the large intestine and has several platforms of varying complexity. The simplest is the human intestinal epithelial monolayers that have a stem and/or differentiated cells. These assess how the intestine transports and metabolizes drugs and nutrients or how the stem cells differentiate into mucus-producing, hormone-producing, or absorptive cell types. These systems offer higher throughput models that are simple but do not possess the greatest possible information content. However, they are robust and reliable.

We also have complex 3D tissues that replicate a wide range of physiologic behaviors as well as the architectural features of the human intestine. Importantly, many of these systems will support the vast array of chemical and gas gradients found in the human intestine. They can also host the human microbiome so that a better understanding of the complex interplay between the human cells and microbes can be developed in both health and disease.

As with all of our model systems, we usually advise users to employ the most straightforward platform possible and then add in complexity as needed for the task at hand.

How have you used the platforms to create structures that resemble tissues in vivo?

We have more complex platforms that replicate a lot of the different features of the human intestine, such as the architecture, cell migration behaviors, and stem cell fate decisions. These are shaped three-dimensional systems that exist as arrays of crypts (or microwells) covered with a monolayer of intestinal epithelial cells. The crypt array has a basal surface for a nutrient diffusion, and a lumen, resembling the inside of the intestine.

You can imagine that the cells facing the waste or food on the inside of the intestine are very different from the cells that are at the base of the intestinal crypts. In these systems, the stem cells are found at the crypt or microwell base while the differentiated, mature cells such as absorptive cells face the luminal surface (waste side).

"These systems are chemically and architecturally much more faithful duplicates of the human intestine than the simpler systems, but that also means they are slightly harder to create, build, and maintain."

Dr. Nancy Allbritton

With some of these three-dimensional systems, we create chemical gradients across the tissues, i.e., the long axis of the intestinal crypt, just as stem cell factors and food/bacterial metabolites exist in a gradient across your intestinal crypts. This microdevice permits very sophisticated micro-environmental control of these complex tissues.

Living tissues and organs are highly dynamic. Are you able to replicate the extracellular matrix and extracellular signals that would typically exist within an organ?

We can do a lot of it, but we cannot do it all. For example, with the chemical gradients, the stem cells that sit at the base of these microwells are exposed to very high growth factors, but about 400 microns away, so a few hair diameters, the cells do not see much of these factors. This chemical gradient mimics the signaling microenvironment of the intestine very closely.

There are many types of bacterial metabolites and products that are at very high concentration for the cells at the luminal surface, but they are low where the stem cells are down at the base of the microwells or crypts. We can replicate these food and bacterial metabolite gradients by using purified compounds or actual bacteria on the luminal side of the intestine on a chip.

The 3D system begins to duplicate the human physiologic signals that control the cells' behavior, but there are still some things we cannot do. For example, we do not have a blood supply or blood vessels going into our chip. This is one of the next steps, but there is a lot of work to do to build it, yet still have a robust and reliable intestine-on-chip system.

Our tissue is growing in complexity, but we only have one to two tissues on the devices and a small subset of the bacteria found in the human intestine. One could imagine increasingly putting more and more tissue types into the system. The intestine has epithelial cells, fibroblasts, muscle cells, neural cells, immune cells, and other cell types. Therefore, adding in all of those cell types would create a more normal physiologic organ. I think those are all future goals to help us move forward in our research.

How could the intestinal monolayers you have developed in your laboratory be used for drug development?

Our intestinal-on-chip technologies help us to understand drug transport and metabolism in humans (as opposed to mice or tissue-cultured tumor cells). The microbes in the human intestine or the human intestinal epithelium can convert drugs into a toxic metabolite or their active form. Along that line, we could begin to do rapid screens looking at how drugs are modified, metabolized, and transported across the intestinal epithelium. Importantly, many drugs cause intestinal dysfunction or side effects. Therefore, screens can help us to look at the impact of drugs on intestinal cells and intestinal barrier function, for example, leakiness.

It is now also clear that in cancer chemotherapy, the gut and the bacteria within the gut play a huge role in how well chemotherapy is working. How this works is not well understood, so I think there is going to be a big push to use these systems to look at how we can make more effective anti-cancer drugs that are less toxic on the gut and other biological systems. We are only beginning to scratch the surface.

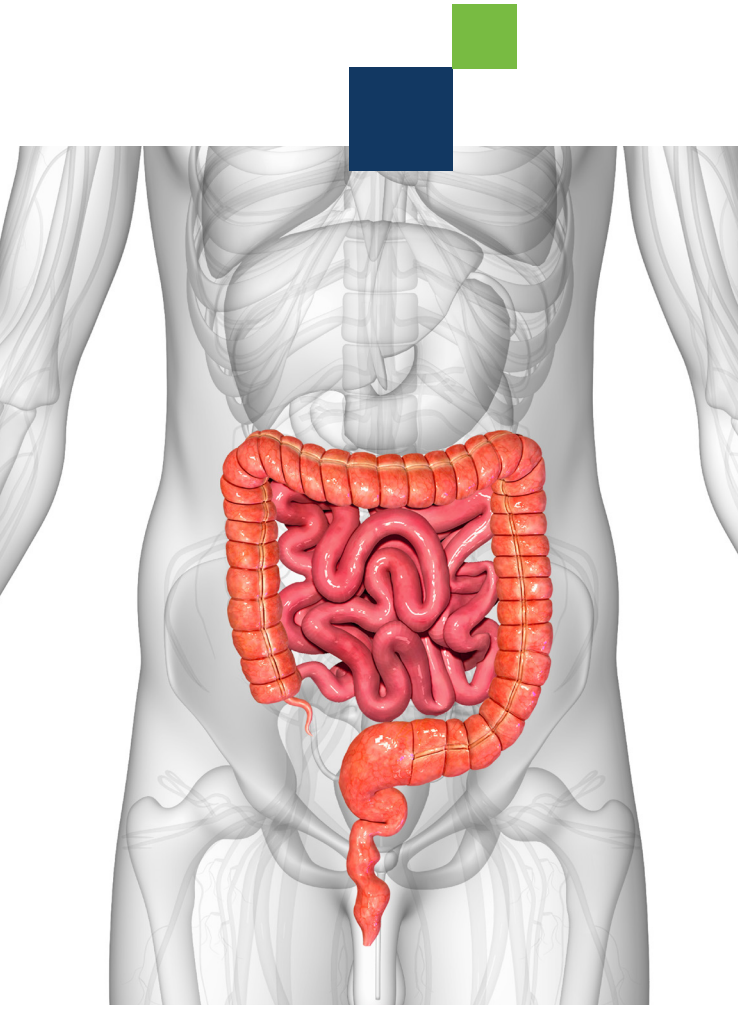
Do you think that microphysiological systems could one day replace animal models? What challenges will we need to overcome before this is possible?

I do not think microphysiological systems can ever totally replace animal models. It is the law in the US to test drugs on animals. We are also a long way off from having a full human-on-a-chip with all of the different organ systems in place and interconnected, whereas a mouse or other animal system is already there. I think it is more likely that the chips will reduce the number of animals used so that you can get higher value information, complementing rather than replacing animal models. The chips will also offer insights into how humans might ultimately respond differently from animal model systems.

On-going work in the organ-on-chip area helps demonstrate that these devices mimic and replicate human responses (and many have already mimicked human physiology where animal models have failed). Overall, the future for organ-on-chip technologies appears quite bright and will undoubtedly grow in future importance and impact.

How far are we away from building a microphysiological system that could be used to study complex diseases, such as cardiovascular disorders?

There are already devices that demonstrate many of the complex disease phenotypes. For example, blood vessels on-chip have replicated blood vessel diseases such as atherosclerosis and tumor metastases. There are some fantastic heart-on-chip devices with functioning cardiac tissue subunits that accurately recapitulate the impact of cardiovascular drugs, so I think we are making good progress. I believe that we will see a growing number of impressive and highly predictive disease models as time goes by.



Finally, what does the future hold for you and your research?

Near term, our big goal is to make an intestine-on-a-chip that fully replicates the human small and large intestines. We are working hard at putting a human microbiome or normal intestinal bacteria on our intestine-on-chip. In addition to chemical gradients, gas gradients such as oxygen exist across the intestinal crypts, which we are working hard at replicating. We also want to add in more tissue types, such as the immune system, fibroblasts, and nervous tissue.

We have some work to do to make a fully functioning replica, so we are also starting to team up with other teams, particularly those working on a liver-on-a-chip. This will mean that our intestine-on-a-chip can absorb food and then send it to their liver-on-a-chip to create a fully functioning liver-intestinal model to recapitulate food digestion, metabolism, and detoxification.

A big part of our work is to make the systems robust and reliable as well as easy to use for biologists and clinical investigators. A lot of great devices remain isolated in the inventors' lab due to their high degree of complexity. When biologists try to use these complex devices, there are just too many failure points. Even to make one of these systems shippable will be a challenge, because how do we ensure it arrives in good condition when we post it across the world?

There are a lot of challenges with scalability, manufacturing, robustness, and reliability that my lab, in particular, is interested in tackling to make sure that these organ-on-chips get out into the real world and fulfill their potential.

4.2b IMPROVING STEM CELL AND ONCOLOGY RESEARCH WITH MICROFLUIDICS

The below article is a summary of Nancy Albritton's talk at Pittcon titled 'Microengineered Analytical Platforms for Biomedical Research'.

A major problem for the pharmaceutical industry is that the cost of making drugs are ever-increasing, but fewer new drugs are coming to the market. If you look at some of the data from pharmaceutical companies, it takes around 12 years and almost $2 billion for just one drug to enter the market. Some even claim it is up to as much as $12 billion per drug. This will not be sustainable over the years as a way to develop new drugs to treat diseases.

You can look at the 12 years to bring a drug to market and compare it to the changes in other technologies over 12 years. We have gone from a flip phone to an iPhone, from just a mere search engine to Google Conglomerate, from a little floppy disk drive to portable drives that can hold an incredible amount of information. Other areas are progressing rapidly; how come a single drug is taking this long?

A lot of compounds are screened from large libraries where they get whittled down at a very slow rate. Researchers carry out cell cultures, animal-based screenings, and then human trials. Of the drugs that make it into human trials, only around 1 in 20 get through. This is where an incredible amount of money is being spent per drug, and the one drug that succeeds has to pay for the 19 failures.

Why do drugs fail so often?

Cells in a dish are not very representative of the human body, and studies are typically carried out in mice or other animals. Drugs do not currently get tested in people until the late stages. We need better screening tools, so that good drugs to make it through, and the bad ones need to fail very fast so we can exclude them early on.

This is where the origin of organ-on-a-chip for microphysiological systems lie. We can make flight simulators for big, sophisticated jets, so why can't we make a human simulator, where we put all the organs together, connect them all up and see how they handle drugs? This would give us greater predictive ability and lead to fewer bad outcomes.

The intestines-on-a-chip model

We are very interested in the large intestine because it plays a fundamental role in drug absorption and drug secretion, and a lot of cytotoxicity of drugs happens at the level of the large intestine. Therefore, we need better analytical tools for human intestines.

The large intestine is full of essential bacteria. There are more bacterial cells within the body than cells in the rest of the body. We co-evolved with a very diverse community of microbes that lives largely in our large intestine, and it has become abundantly clear that the microbes and large intestine work together to educate our immune system. They also regulate our metabolism and how we handle glucose and other energy supplies.

The large intestine is also called the second brain. It is the second-largest site of serotonin secretion, as well as a whole host of other hormones that regulate how we feel. These microbes secrete all sorts of compounds that regulate our satiety, feeding behavior, and cardiovascular health.

It is also now evident that some of the next significant drug advances will manipulate the microbial community in our large intestine, either through foodstuffs or through providing bacteria to re-populate the gut.

Building the model

"Our model, from an analytical chemist's point of view, is an array of microwells. The intestinal epithelium forms into long structures called crypts, which are a lining of a single layer of cells that form long skinny microwells."

Dr. Nancy Allbritton

The stem cells are down at the bottom of the crypt. They reproduce at a stunning rate because the epithelial cells appear to live for only a week. After a week, they die, so the intestinal lining is recovered every week, which is astonishing given the size of the organ and the surface area. The stem cells divide quickly, and their progeny migrate up to the crypts, where they differentiate. There is a luminal side where the food is, and a basal side where the blood is.

When we started this work, the state-of-the-art technology was organoids. These are not an ideal model. They have a lumen, but it is inaccessible. The stem cells end up everywhere in the model rather than on the basal side. It is simply not a high-throughput analytical tool. Therefore, we decided to replace this with a whole host of new systems, and to do so, we needed to rebuild the intestine.

Our model contains little arrays of microwells, where there is a basal surface and a luminal surface. We have a stem cell compartment and a differentiated cell zone, with the flow of cells in the proper direction. We also have a thick mucus layer protecting our cells from anaerobic bacteria in the lumen.

The large intestine contains a stunning array of gradients that span the length of these crypts. The crypts are only around 400 microns long, meaning the gradients are very steep. There are gradients in stem cell factors, differentiation factors, and cytokines. At the base, the cells are heavily, well-oxygenated, but there is no oxygen at the top of the crypt. There are also all sorts of food and bacterial metabolites that exist in gradients. This is a rich playground to build a micro-engineered device to replicate the human body.

We only use human cells now taken from biopsies. We spent three years trying to develop a culture system that would allow us to grow the cells as a monolayer. We also screened a considerable number of support systems and scaffolding before we could get a monolayer system that would support stem cells and differentiated cells in this human primary large intestinal epithelium.

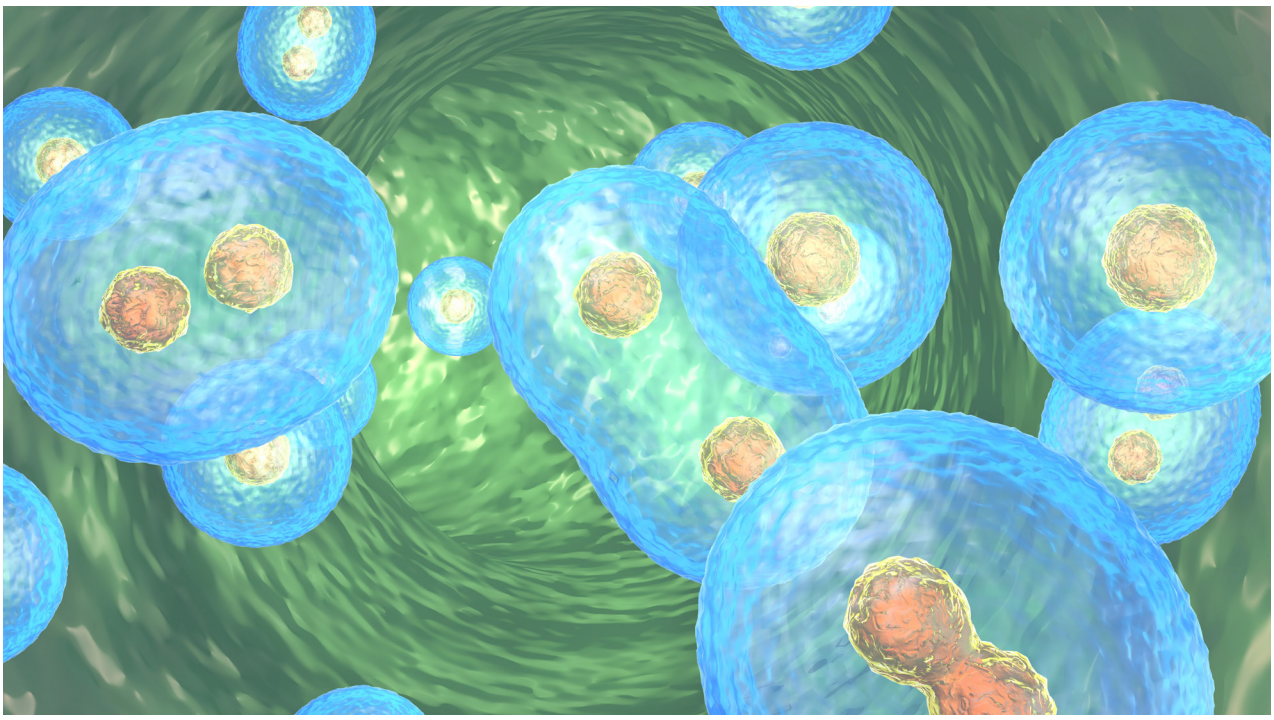
Our intestinal epithelium has every single cell type found in a living human. We have hormone-secreting cells that regulate feeding behavior, mucus-producing cells, stem cells, and absorptive cells. We now have these very complex systems that accurately represent the intestine.

Applications in biomedicine

It is very important to prevent inflammation within the intestine, which can be very unpleasant. As an experiment, we can artificially inflame the endothelium and then screen for compounds that diminish inflammation. This can be used, for example, for drug development for inflammatory bowel disease such as Crohn's disease, as well as other GI inflammatory states. We can also take a resting epithelium and see what kind of compounds inflame it.

We have also figured out how to increase the hormone-secreting cells, known as enteroendocrine cells. These cells secrete a whole array of hormones, and it is evident that these fundamentally control what we eat, how we eat, how much we eat, and impact our weight. We can make these enteroendocrine cell-rich systems, and then we can begin to screen for dietary compounds in drugs that modulate serotonin production or other hormone peptides.

As you might imagine, there is a considerable interest in the drug industry to treat obesity and diabetes. One advantage of this system is that these are all human cells, and it enables us to look at diverse human populations. We have also developed a system that mimics human drug uptake via transporters and metabolism, which is also of interest to pharmaceutical companies.



Building the structure

Our most sophisticated systems are our three-dimensional systems. We wanted to recapitulate the native architecture of the intestinal epithelium so that we could begin to reproduce complex disease states. This could be an excellent analytical and screening platform for drugs or to look at the origins of human disease. To do this, we microfabricated a scaffolding system, seeded stem progenitor cells from the human intestinal epithelium, and then applied gradients across it.

We used a transwell and removed the base to allow us to install a diffusion window with our membrane and impermeable film. We then placed our scaffolding material in, giving us an array of microwells made out of an extracellular matrix above the diffusion window. It represents a simple transwell system, but it is one that has been highly modified. We can then make this a 96- plate, 24-, or 12-well plate, and it fits into existing workflows.

Adding the gradients

If there is no stem cell factor gradient in the crypt, the stem and proliferative cells are all over the place. But as soon as we apply the gradient, the proliferative and stem cells are all pushed down to the bottom, as this is the only place where there is enough growth factor to support the stem cells. By contrast, the cells nearer the top of the crypt are all highly differentiated.

Next, we can begin to explore what happens when it is exposed to different metabolites. Microbes within the large intestine ferment fiber into short-chain fatty acids: acetate, propionate, and butyrate. These are at minimal concentrations in the large intestine and form very steep gradients across the crypt.

Using cell staining, we were able to see that acetate and propionate have minimal effect on our crypts. However, butyrate turns the cells pink, indicating alkaline phosphatase

accumulation. We can do a close-up look and see lots of alkaline phosphatases which marks the mature enterocytes. This shows how the addition of butyrate diminished stem cell proliferation and enhances differentiation. It is thought that the reason why eating fiber decreases the risk of colon cancer is because it reduces the rate of proliferation of these cells, which is where tumors typically form in the development of colon cancer.

To create an incredibly steep oxygen gradient across the length of the crypt, we made the luminal sides of our model highly oxygen impermeable, and the basal sides highly oxygen-permeable. This leads to the tops of the crypts being de-oxygenated while the base is still oxygenated.

We then experimented to see if we could support microbes in this model. We added aerobic bacteria to our system and saw that they were able to co-exist happily with the epithelial cells. Looking closely at the bacteria, they were positioned on top of the cells' microvilli.

"We also added a pathogenic obligate anaerobe, C. Difficile, a major problem in hospitals, and saw how they grew very well in the deoxygenated lumen."

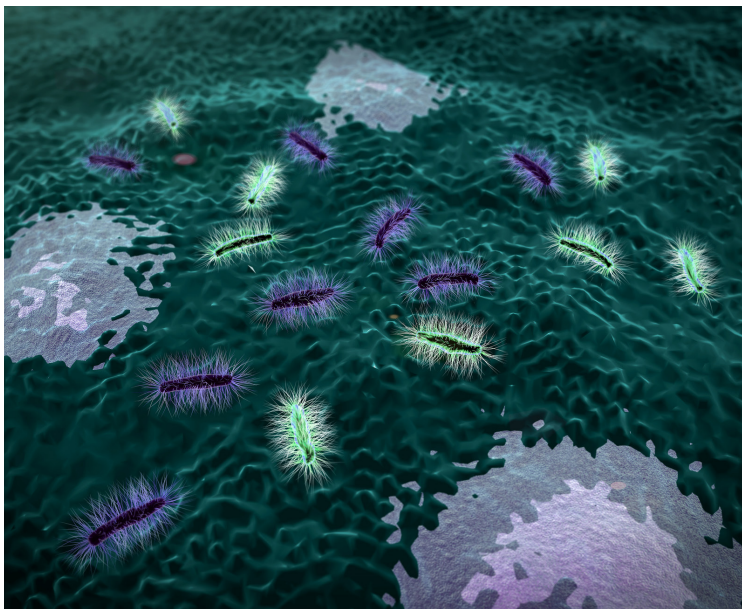
Dr. Nancy Allbritton

At zero hours with the C. Difficile, the cells looked pretty good. But as time goes by, C. Difficile makes toxins and destroys the intestinal epithelium. Using electron microscopy, we could see that the bacteria were quite happy existing on the dead and dying intestinal cells.

We explored the effect of playing around with the oxygen gradient and what it does to the stem cells. We discovered it pushes them farther down into the crypt base and diminishes their proliferation, demonstrating another mechanism by which stem cell proliferation is controlled.

The mucus layer

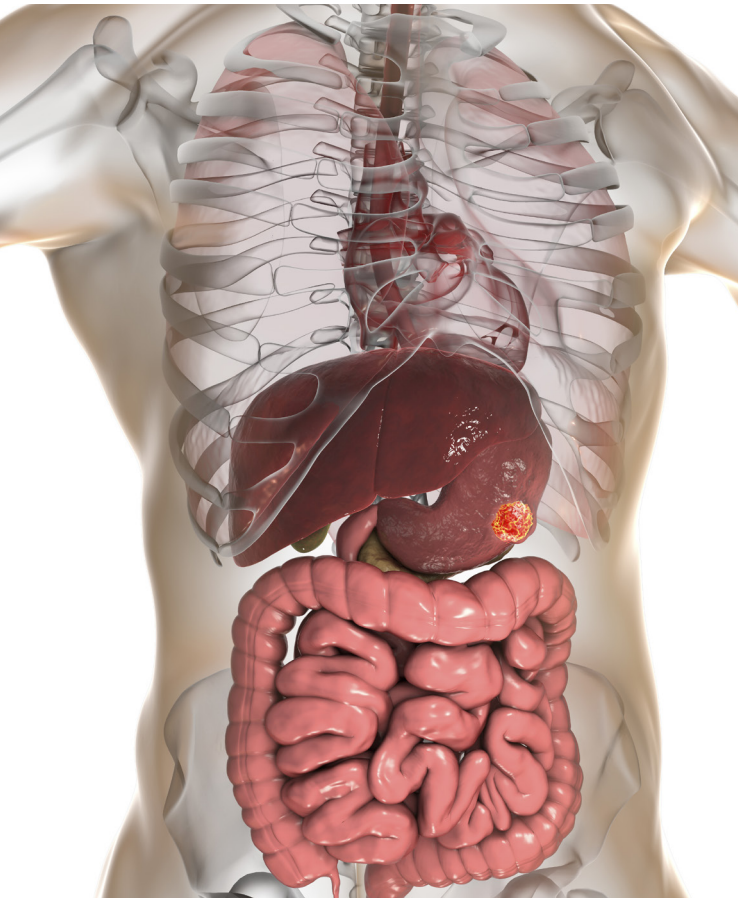
Our model also needed a fundamentally important mucus layer. The layer is impervious to bacteria, and when we take drugs, they have to migrate through it. To generate the mucus layer, we removed the air and basal intestinal peptides. This really increased the secretion of mucus and the number of goblet cells or mucus-producing cells.



The longer we form the mucus, the thicker it gets, and we can get mucus that is hundreds of microns thick, just like in the living human. We can show that our mucus is made of everything that the human mucus layer is. This is predominantly mucin 2 (MUC2), but it also contains many antimicrobial peptides and MUC5A, which is another mucin in human colonic mucus. We can show that it acts as a strong barrier like it is supposed to, with the use of beaded particles. We showed how small beads could diffuse through the thick mucus layer, but big beads became stuck. This gives us an analytical system for drug and particle delivery through a human mucus system.

We tested this again to show that the mucus layer is impermeable to bacteria. Using electron microscopy, we saw that although there is a diffuse layer at the top that the bacteria can partially penetrate, they then get stuck in their tracks.

To test whether our mucus is physiologic, we took our epithelium and put E.coli bacteria at the top and white blood cells down at the bottom. If there is no mucus and you do this, the epithelial cells get inflamed. The white blood cells can sense these E.coli, and they secrete a large number of cytokines and other inflammatory factors. However, if we add a mucus layer in, nothing happens with the bacteria. Everything stays quiescent because none of the microbial products make it down to the epithelium or the white blood cells. We have also shown that C.difficile toxin no longer penetrates this mucus layer.



CONCLUSION

Organ-on-a-chip systems are great for regenerative medicine and basic biology, but they are also effective analytical tools for drug discovery and testing the impact of food and metabolites. They are giving us very sophisticated sensor systems on the normal human epithelium. Using this approach has provided us with very fast high-throughput analytical assays as well as a more sophisticated complex system, which is lower in throughput but much higher in information content.

4.3 THE ROLE OF SKIN-INTERFACED MICROFLUIDIC DEVICES IN SPORT PERFORMANCE

The below article is a summary of Professor John Rogers Keynote presentation at Pittcon Titled 'Soft, Skin-Interfaced Microfluidic Systems for Capture and Analysis of Sweat'.

This research is part of a broader collection of programs where the focus is on the transformation of standard wafer-based platforms into biocompatible systems that can interface chronically with the soft tissues of the human body. These integrated circuit chips are divorced from the underlying plane or rigid semiconductor wafer support and rendered into tissue-like platforms. These can softly and intimately integrate with the curved linear-time dynamic surface of a bio organism or part of the body (such as the brain), which is biology's most sophisticated form of electronics.

Exploring the vast mismatch between the modules, shapes, and time dynamic curved linear surfaces found in the brain in comparison with an integrated circuit chip sets the stage for research around electronic materials and device design. If these issues can be addressed, we can develop new ways to use man-made systems for human health.

The skin offers an interface point for quantitative analysis of parameters related to health status and trajectory. Wearable technologies have already made some progress in this area, but all of these use the same basic approach - a clunky piece of rigid electronics loosely coupled with the body via a strap, typically at the wrist.

These devices do not have the intimate tissue interface needed to achieve ICU-grade monitoring of health status. Over the last decade, we have worked with materials and devices that allow us to build electronics with skin-like physical characteristics and wireless operational options. It is possible to construct sophisticated silicon-based electronic systems, biosensors, radio communication capabilities, and energy harvesting technologies in platforms that have physical characteristics matched to those of the skin.

We and others in this emerging community have developed a toolbox of measurement capabilities and analysis options that can be supported in these types of platforms. These range from precision thermal characteristics and measurements of the skin, to thermography with nano-kelvin precision and thermal transport measurements. We can also take electrical measurements such as ECG, EEG, and hydration readings.

We are focusing on sweat and the ability to build microfluidic handling capabilities into these platforms. This allows us to measure mechanical characteristics, the stiffness of the skin, motion, strain, pressure, optical characteristics, and the acoustic signatures of underlying processes.

"Sweat is an interesting but relatively under-explored class of biofluid that can be captured in a purely non-invasive way. Sweat can reveal a lot of health information, so we are developing fluid handling and biomarker analysis systems in the absence of electronics."

Prof. John Rogers

Our systems are microfluidic, lab-on-a-chip devices made from exceedingly soft elastomers in sufficiently thin geometries that can tolerate bending mechanics. This lets us maintain a robust, watertight seal to the surface of the skin, even in the presence of vigorous sweating.

Multilayer structures consist of an elastomer material that defines the network of channels, reservoirs, and valve-type components. Embedded within these channels and reservoirs are color-responsive chemistries allowing us to track sweat loss and quantitatively measure sweat chemistry by color change. With this approach, we bypass the need for power supplies or wireless communication hardware.

The device adheres to the skin using a double-sided adhesive layer, bounded to the skin on one side, with the base of the microfluidic platform on the other side, with openings aligned with inlet ports that exist on the backside of the device. Small volumes of sweat are collected at locations defined by openings in the adhesive layer, and these are typically large enough to capture 20 to 50 individual sweat pores or sweat glands.

The key is a set of mechanics that keep the device adhered to the skin in a non-invasive, non-irritating way, with a watertight seal. Our approach does not rely on super strong adhesives, but rather in the design of ultra-soft, ultra-thin, microfluidic platforms that minimize interface stresses that may develop deformation, driving the lamination of the device away from the skin. A range of color measure chemical reagents can be adapted for use in this platform, including reagents to measure chloride, glucose concentration, lactate concentration, pH, and even information around anaerobic muscle activity.

Gatorade has expressed interest in this technology, and we launched a development program with them to establish the accuracy, repeatability, practicality, and cost structure of these devices. Their goal was to give these devices away or sell them to their customer base to help athletes understand how best to utilize Gatorade to manage hydration electrolyte balance. We have also been contacted by many professional sports teams, including aquatic sports professionals.

There is momentum around the basic platform, and the possibility of added functionality and analytical chemistry has the potential to open all kinds of possibilities. We are looking to move beyond passive soft microfluidics to platforms that offer active functionality, such as being able to switch valves open and closed, pump fluids around, and perform controlled mixing operations. While this provides a range of opportunities in sports science, there are also exciting prospects in clinical medicine.

Sweat is already used as a clinical gold standard for specific screening processes, in particular screening for cystic fibrosis in pediatrics. Sweat chloride can be used as a screen, and if chloride levels are too high, this suggests a risk for CF. We are working with pediatricians at Lurie Children's Hospital to eliminate problems with their existing collection strategy, which is uncomfortable and has a 25% failure rate due to the insufficient sample collected. Addressing this involved a straightforward adaptation of the device mentioned earlier, and so far, we have screened around 100 infants with no failures.

Sweat analysis using our devices can offer a real alternative to more invasive procedures such as the analysis of blood or urine, and we predict a wide range of beneficial applications in their future.

4.4 MICROFLUIDIC SYSTEMS FOR SWEAT ANALYSIS AND NEONATAL CARE

In this interview, Professor John Rogers talks to News-Medical Life Sciences about his research and work in developing biocompatible electronics and microfluidic systems with skin-like properties.

Biological systems are traditionally mechanically soft, however, modern electronic and microfluidic technologies are rigid, meaning the layouts are completely different. Eliminating this mismatch will create huge opportunities in human-made systems that can be used for diagnostics, therapeutics, and in clinical and healthcare. Can you tell us about the new opportunities these human-made systems will create?

There are all kinds of exciting and compelling opportunities that could come from thinking about how to reformulate the types of systems that form the core foundations of devices that you see in consumer gadgetry, including computer chips, integrated circuit chips that are flat, rigid, and planar, into forms that are more naturally biocompatible with the soft surfaces of the human body. Integrated circuits are not the only kind of human-made technology that has those kinds of physical characteristics and geometrical shapes.

The same type of thing is seen in optoelectronic devices, lab-on-a-chip type technologies, and microelectromechanical systems. The goal behind our research and that of a growing community of researchers is to create new ideas in material science and manufacturing, mechanical engineering, and electrical engineering that will allow us to reformulate those sorts of technologies, without sacrificing the performance or capabilities, into platforms with geometries and mechanical properties that are inherently biocompatible and can be interfaced with soft tissue systems. These include the skin, brain, heart, peripheral nervous system, bladder, and kidneys. The idea is to develop those technologies into a system that can ultimately enhance human health and extend life.

Can you tell us more about these 'biocompatible' electronic and microfluidic systems with skin-like physical properties?

The skin-like devices that we have developed are designed to interface with the skin and to use the skin as a window for measuring clinical-grade physiological status parameters associated with the natural processes of the human body. For example, it looks at cardiac and respiratory activity, as well as flow properties associated with blood through near-surface arteries and veins to reproduce what is carried out in hospitals. However, natural processes are measured in platforms that can be worn continuously for wireless streaming of data outside of the hospital in the home setting to develop a deep foundational basis of information on health status.

This information can be used with artificial intelligence algorithms to assess a person's well-being at any given moment and to make predictive assessments of health trajectories over time. This kind of personalized, digitally oriented model for healthcare enabled by these kinds of skin-like platforms will be a compelling way that healthcare will evolve into the future for reduced costs and improved outcomes.

You presented the Wallace. H. Coulter Lecture this year at Pittcon. What did you discuss in your talk?

I focused on skin-interfaced systems, which provide electronic monitoring functionality and have tiny embedded networks of microchannels. The microchannels, along with very small reservoirs and valves, capture and analyze biomarkers in sweat. The system is designed to capture sweat that is pumped to the surface of the skin through the Eccrine glands and the connective ducts.

Sweat is a relatively under-explored but very potentially important class of bio-fluid that could provide information content to substitute for blood draws. The idea is to use and non-invasively collect sweat to carry out biochemical-based assessments of health status to complement the type of physics-based measurements that we can achieve with our electronic devices.

What sparked your interest in 'soft' materials?

My core expertise is in electronic materials. I like to think about novel electronic materials in the context of technologies with capabilities that go beyond what is currently supported with conventional sorts of electronic materials. We got our start in this area thinking about flexible displays, which are paper-like displays that could replace the kind of liquid crystal and organic light-emitting diode displays that you see in consumer devices today. We looked at thin paper-like systems that are lightweight, mechanically rugged, and capable of rolling up in storage when they are not being used. The systems have been interesting us for a while, and it remains a significant focus at most large display companies.

I happened to be giving a talk at the University of Pennsylvania on that kind of technology. It turned out that a couple of curious neuroscientists were in the audience, and they came up to me after the talk and asked whether we could take those kinds of flexible electronic devices and put them on the brain to study the electrical activity of the brain. That was the first suggestion that these kinds of devices could be used to investigate important problems in human health and for research around the fundamental mechanisms that govern the behavior of living systems. That interaction catalyzed a whole new set of research opportunities for us, and it has been a sustained area of activity in the group for the last 10 years.

What extra levels of functionality do soft electronics provide? How were they discovered?

Soft electronics allow you to intimately and persistently integrate advanced biosensors, radios, stimulators, microprocessors, and digital memory technologies with the human body, in ways that go far beyond what is possible with conventional wearable technologies that you see on the market today.

Commercial devices are dominated by bulky, clunky pieces of electronics, loosely strapped to the body, typically at the wrist. That kind of technology approach enables specific parameter measurements, qualitative assessments of health and wellbeing, step counting, and heart rate estimates. However, these parameters cannot be readily interpreted and acted on by physicians.

What we are thinking about is the next generation of wearable technology that integrates more intimately with the body, almost serving as a second skin that laminates in a physically imperceptible way on the surface of your skin. The technology would almost act as a temporary tattoo or a bandaid to provide continuous ICU grade measurements of health status to allow physicians to track health progression overtime at a very detailed level. Measurements would not be taken in an episodic way, as seen in current systems used when a patient comes to a hospital or a clinic. I feel that continuous measurements open up new frontiers to think about how to manage health conditions and promote healthy living.

What 'skin-like' physical properties do biocompatible electronic and microfluidic systems have?

"We target a set of physical properties that are precisely matched to the skin itself. The skin stretches somewhat and can flex, bend and wrinkle. It has certain thermal characteristics, as well as water permeability characteristics."

Prof. John Rogers

Over the last decade, our research focus has involved trying to embody those exact skin-like properties into electronic devices. The mechanical properties of a silicon-based integrated circuit are a million times different than those of the skin. Therefore, there is a vast chasm and a gap there. A silicon integrated circuit is perfectly flat and cannot conform to a natural curvature and the sort of sub-millimeter scale texture associated with the skin. To the best of our ability, we take a collection of materials and build them into an electronic system that has the same type of functionality you achieve with a silicon-based electronic platform, but with mechanical properties and geometrical features that precisely match the skin.

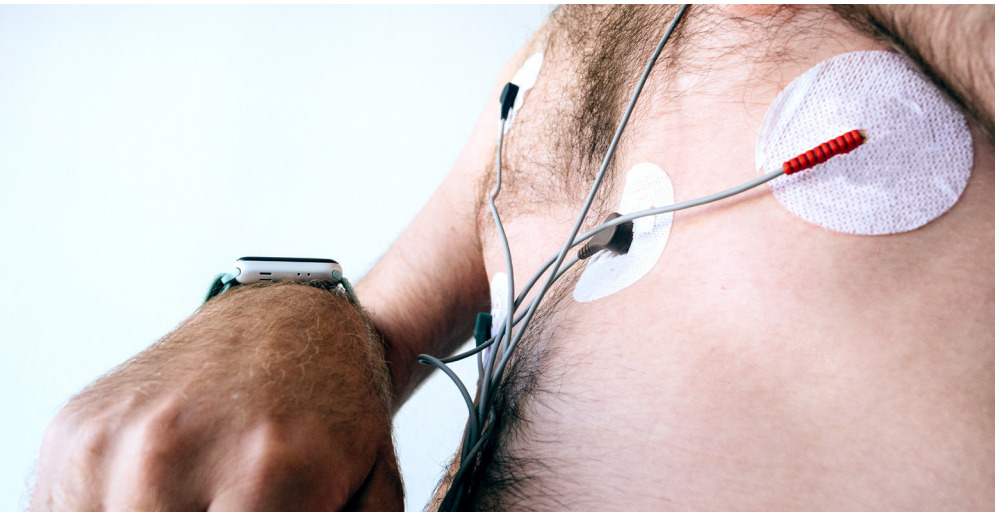
The goal is to develop something that resembles a second skin that interfaces directly and naturally with your natural skin so you can wear these devices for long periods without feeling any physical sensation. We think that this is not just a convenience, but an essential characteristic of the devices, as nobody will wear the devices and patient compliance will be unacceptably low if they irritate the surface of the skin.

That is the goal around engineering, and it turns out that with a few relatively simple ideas, we can get very close. The devices are thinner than the epidermis, the mechanical properties almost precisely matched, and the overall thermal mass is almost the same, with no thermal load as a result.

Could you name some of the main advantages of using soft, skin-like electronics over using conventional hospital apparatus?

In hospitals today, biosensors are primarily attached to the surface of the skin with adhesive tapes only. They connect via hard wires to external boxes of electronics that carry out all the data acquisition, storage, and processing. This works reasonably well for an adult patient who is in a hospital bed and not moving around a lot.

However, the wires, even in that kind of scenario, create a pretty serious inconvenience. In many cases, they frustrate basic operations in clinical care, and the presence of wires confounds surgical operations.



The idea is to develop a wireless platform, eliminating the forces imparted through the wires to the interface between the biosensors and the skin. We would use an adhesive strength with a factor of 10 or 100 times lower than that, which is required for the wired based devices. The consequence of that is that you end up with a much more comfortable interface with less skin irritation.

In the context of almost all sorts of hospital monitoring practices, these kinds of skin-like or band-aid-like devices represent a significant advantage, particularly in the most extreme scenarios such as neonatal intensive care units, where wires are even more problematic.

As they are in a fragile health status, premature babies have to be monitored 24/7 for all vital signs using clinical-grade quality equipment. However, their skin is also very delicate, and they do not effectively accommodate strong adhesive tapes. The wires frustrate the natural motions of the baby and are more than just a nuisance. They also hinder the ability of the parents to interact with the baby because they have to manage the wires.

A lot of the work that we have done so far focuses on neonatal intensive care as the most compelling opportunity for these kinds of technologies. We have done a lot of work in the NICU facility at Lurie Children's Hospital in Chicago, US. We tested out the devices on around 100 neonates who have come through the hospital, and we have shown equivalency in the measurements made with our wireless skin-like devices to those determined with the conventional wired-based devices and external boxes of electronics.

As there are no monitoring capabilities in the developing world, the platforms are now deployed at scale in Africa through funding from the Gates Foundation and the Save The Children Foundation. The idea is to leapfrog the traditional wire-based devices and go straight to wireless to provide improved capabilities in neonatal, pediatric, maternal, and fetal health contexts.

Another application of biocompatible electronic systems is in sport and fitness research. Why is sweat an important bodily fluid to research?

Sweat, in the context of athletics, athletic performance, fitness, and general well-being, is a sort of low-hanging fruit in terms of how to think about it as a biofluid that can characterize health. This is because it is evident that sweat loss can lead to dehydration.

Maintaining optimal sports performance requires optimal hydration management. If you enter a training scenario or athletic competition, you need to keep your body at an optimally hydrated state. Therefore, the ability of these skin-interfaced microfluidic devices to continuously monitor sweat loss

locally - and that local measurement correlates to full body sweat loss - can inform an athlete precisely how much water they need to drink to replenish lost water.

We can also measure the electrolyte concentration in sweat, a quantity that varies depending on the individual's genetic, racial, and dietary background. The devices measure not only sweat loss but electrolyte loss as well, allowing you to replenish lost water and electrolytes.

For competition at the highest levels, a few percentage improvements that can result from data-driven hydration management can be very important. We have a partnership with Gatorade to distribute these devices to pro and youth athletes to maintain better performance and help avoid cramping and injuries resulting from poor hydration management.

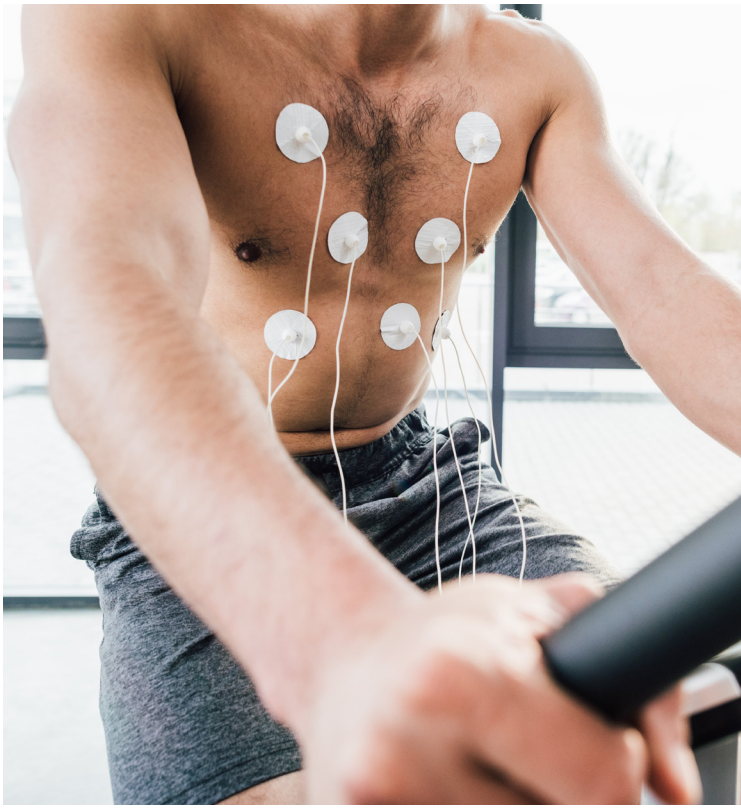
Nanotechnology has become an increasingly investigated area within the science industry, also having many applications within medicine. How does nanotechnology take part in your research?

"Nanotechnology is important for us, but it is not necessarily the end goal. We will use nanotechnology where it makes sense."

Prof. John Rogers

However, we are focused at the system level, and how you can achieve novelty in devices and construction to yield data streams that are a direct benefit for health or fitness or sports.

But nanotechnology specifically does come into play in a pretty simple way. A silicon wafer has a certain set of mechanical properties that are defined by the silicon itself, but also by the geometry of the wafer. It is fairly thick, about a millimeter in thickness or half a millimeter in thickness, and it is partly because of that thickness that the silicon wafer cannot be bent without fracturing the material. Nanotechnology comes into play then because reducing the thickness of the silicon imparts flexibility to the silicon due to elementary bending mechanics. You cannot bend a 2 x 4 structure, but you can bend a sheet of paper. Using nanotechnology uses the same materials but transforms it into a bendable paper thickness.



The same principles apply to silicon. We deploy silicon in nano-scale forms rather than in wafer-based forms. If you take the thickness of a wafer (half a millimeter) and shrink that down to a hundred nanometers, the flexibility improves by a factor of around 1012. It is transformative in terms of the way you think about the material. That is how nanotechnology enters the systems that we are interested in - it enables a straightforward route to ultimately making material such as silicon flexible and skin-compatible.

If this continued research is carried out into the field of biocompatible electronic and microfluidic systems, where could this take us?

The skin-interfaced devices represent the most immediate opportunity because they are minimally invasive, and it easy to get approvals for using these devices on human patients. They can be easily removed if any kind of adverse effect develops, although this has not been seen. The systems are a straightforward and natural starting point for bio-integrated electronics such as a human skin interface. However, we have carried out work on implantable systems used primarily in animal model studies with the aim of using them in humans in the future.

The frontier for us is in taking the proven design principles for skin interface devices and deploying the same technology on the brain or the heart to allow similar types of functionality but in the context of internal organs. Electronically enhanced organ health is the way you can think about it. An advanced type of pacemaker is distributed around the outside of the heart to monitor basic cardiac function and deliver therapeutic stimulus. The same kinds of possibilities are present in the context of brain disorders as well. Therefore, I think moving these devices from the skin to internal organs in the body is a huge area of opportunity.

What is next in your research into soft, skin-like electronics?

We are more deeply exploring the value and information content embedded in sweat. Sweat has not been nearly as thoroughly explored as blood or interstitial fluid as a biofluid that contains biomarkers of relevance to health status.

There is some work to be done there, but the area is opening up because we now have microfluidic devices that allow us to capture tiny but pristine quantities of sweat that can be used for exact chemical analysis and correlation to blood.

It is a technology-enabled opportunity in studies of human physiology and basic biological questions around how sweat relates to blood. If you can establish those correlations, then I think sweat becomes a compelling way to make a biochemical assessment of health that avoids the need draw blood.

Can you tell us why you come to Pittcon?

There is a fantastic collection of people who are interested in topics very similar to those that represent core activities in my research group. There is a vast synergy and resonance between my interests and the issues covered at Pittcon. It is also comprehensive.

Pittcon is an extensive meeting with all the key experts and represents a one-stop-shop for work in this area. I think it is a fantastic event and I have been to this meeting many times in the past.

What do you expect to achieve at Pittcon?

I will be delivering this special lecture at Pittcon, and I expect, as occurs many times, that I will be able to strike up some conversations and seed some areas for collaboration. Conferences for me are successful if I make new connections, meet new people, and maybe open up new research opportunities.

Why are events like Pittcon important for your research but also important for the analytical chemistry industry?

The exchange of ideas is incredibly important as a catalyzing aspect of how science works. It is essential to share insights and ideas. A conference of this type provides an excellent platform for doing that, and so I think it helps everyone. It helps the whole community and society in a sense because it just accelerates

4.5 HOW SPECTROSCOPIC TECHNOLOGIES ARE CHANGING PRECISION MEDICINE

Achievements in medicine continue to astound as increasingly complex surgeries and treatments successfully improve the quality of life and life expectancy of patients struck by devasting illness or trauma. However, the greater technical and medical capabilities available to improve patient outcomes are placing an increasing financial burden on healthcare systems. Novel innovative medical advances are expensive, and the cost of the latest digital technologies continues to increase.

Naturally, every patient believes that they are entitled to the latest, most effective treatment. However, since available funding is limited, difficult decisions must be made. The demands on healthcare expenditures are further increased by the steadily rising average age of growing populations and the greater prevalence of chronic diseases, such as diabetes.

As the global population ages and grows, sedentary diseases and diseases of aging are becoming more prevalent, and the increase in population size is stretching healthcare

systems to the limit. Healthcare budgets are now also affected by higher labor costs amidst workforce shortages and the development of new or redesigned healthcare models.

A more significant number of people requiring more costly treatments is a key driver of escalating costs, and available budgets would go further if fewer people required treatment. Consequently, disease prevention and early intervention to reduce the onset and severity of chronic disease are receiving increasing attention. More targeted use of resources could help improve the return on healthcare expenditure.

Population health management is being used to ensure that healthcare services and resources are being used most effectively by assessing specific healthcare needs and offering services accordingly. Cost-effective identification of those patients most susceptible to a particular disease, and therefore most likely to benefit from prevention strategies, enables efforts to be concentrated where they are needed most. Similarly, prediction of the treatment from which a given patient will gain most benefit can spare the cost of trial and error with several different treatments before the desired efficacy is achieved. Such tailoring of preventative and therapeutic interventions according to the specific individual characteristics of a patient is often referred to as precision or targeted medicine.

Molecular characterization of disease is fundamental to precision medicine and the individual tailoring of treatment strategies. Advances in molecular biology have enabled rapid, comprehensive, and cost-effective analysis of clinical samples.

The resultant explosion in disease-relevant molecular data has the potential to dramatically increase the accuracy of disease diagnosis and the effectiveness of treatment, improving patient outcomes.

This article will highlight recent advances in spectroscopic techniques that could be applied to the rapid screening, diagnosis, and treatment of patients in the era of personalized, targeted medicine.

Companies dedicated to the development of quality spectroscopy instrumentation, including **BioRad, Bruker, GenTech Scientific, Malvern Panalytical, SCIEX,** and **Zeiss**, are providing an invaluable opportunity to discuss the application of such equipment in precision medicine.

4.5a THE IMPORTANCE OF EARLY DIAGNOSIS

Alzheimer's disease

Alzheimer's disease is a chronic progressive neurodegenerative disease characterized by cognitive decline and behavioral issues. It is the most common form of dementia in the elderly population and was the sixth biggest killer in the USA in 2017 [Center for Disease Control and Prevention 2017].

The underlying pathology of Alzheimer's disease is known to involve cerebral deposits of soluble amyloid-β peptide oligomers and neurofibrillary tangles as well as inflammation by glial cell activation [Forloni and Balducci 2017].

As the evidence base supporting a role of oligomerization of the ubiquitous amyloid-β protein in the aetiology of Alzheimer's disease grows, the need increases for the elucidation of the pathways through which such oligomer formation occurs [Bernstein et al 2009]. Understanding the process by which amyloid-β oligomers are produced may lead to the identification of therapeutic targets.

Furthermore, these amyloid oligomers also appear to play a role in the development of several other chronic diseases, including type 2 diabetes that was the seventh leading cause of death in the USA in 2017 [Center for Disease Control and Prevention 2017]. Therefore, the study of the oligomerization of the amyloid peptide to inform the development of potential novel treatments that can control it may provide wide-reaching benefits [Bernstein et al 2009].

Importance of early diagnosis

Neuropathological changes are apparent in the brains of patients with Alzheimer's disease for up to 20 years before symptoms become apparent [Chu LW 2012; Langa and Burke 2019]. Therefore, early identification of these changes provides the opportunity to potentially defer or reduce the onset of life-changing cognitive decline. Techniques to identify these changes are readily available and include:

- Magnetic resonance brain imaging
- Cerebrospinal fluid biomarkers
- Fluorodeoxyglucose positron emission tomography

The techniques facilitate accurate diagnosis of Alzheimer's disease at an early stage when only mild cognitive impairment has occurred.

Companies specializing in the production of high-quality equipment that facilitates the implementation of such techniques, including **Bruker, GenTech Scientific, Malvern Panalytical,** and **Waters**, attended Pittcon. They provided an invaluable opportunity to discuss technological research requirements and technical specifications of the latest products directly with the manufacturers.

Clinical evidence suggests that implementing appropriate treatment strategies at the preclinical stages of Alzheimer's disease may prevent the onset of symptoms. However, the only drugs approved for the management of Alzheimer's disease—cholinesterase inhibitors and memantine—provide symptom reduction rather than a disease-modifying effect [Dou et al. 2018].

There has been much research into the development of treatments able to slow neurodegeneration and disease progression by reducing the amyloid plaques that develop in the brain of patients with Alzheimer's disease. Preclinical data indicated that the reduction in the number of amyloid plaques was associated with a delay in the onset of cognitive decline, prompting the development of anti-amyloid immunizations and monoclonal antibodies (MAb) specific for amyloid oligomers.

Results from phase 1 clinical trials of the anti-amyloid oligomer MAb aducanumab (NCT01397539, NCT01677572) were indeed promising and showed a significant reduction in cerebral amyloid plaques and the rate of cognitive decline compared with a placebo that was sustained over three years [Ferrero et al. 2016; Castrillo-Viguera et al. 2019]. However, subsequent phase 2 (NCT03639987) and phase 3 trials (NCT02477800, NCT02484547) were stopped early due to a lack of efficacy. A phase 3 trial of another MAb, solanezumab, is ongoing in patients with evidence of amyloid plaque build-up (NCT02008357).

Anti-inflammatory treatments have shown effective prevention of cognitive deficit developments on exposure to amyloid oligomers [Forloni and Balducci 2017] and some indications that reducing prescriptions of anticholinergic medications in patients showing signs of Alzheimer-like morphological changes in the brain can reverse the development of the disease [Campbell et al 2019].

Although the data for disease-modifying treatments for Alzheimer's disease have not been entirely promising so far, there is still hope that anti-amyloid immunotherapy holds promise for providing clinical benefit [Rosenberg and Lambracht-Washington 2019]. Since amyloid accumulation in the brain can be detected decades before the onset of Alzheimer's disease symptoms, and its reduction can prevent many downstream pathologies, such as loss of neuronal plasticity, it holds unprecedented potential for targeted preventive treatments. Researchers hope that refining the methodologies and analytical technologies used in the assessment of anti-amyloid immunotherapy will ultimately lead to the identification of targeted disease-modifying treatment options for Alzheimer's disease.

Analytical techniques applied to the study of Alzheimer's disease: ion mobility separation

A variety of analytical techniques is being used to further study the processes involved in the development of Alzheimer's disease and the involvement of these pathways in other diseases. Such analysis is fundamental to fully understand the molecular mechanisms that lead to the disease pathology, which, in turn, is essential to inform the development of effective diagnostic tools and treatments.

Liquid chromatography separations coupled to mass spectrometry (LC-MS) continues to be the usual choice for achieving the separation, identification, and quantification of biological molecules, including amyloid-β peptides [Garimella et al 2019]. Separation using liquid chromatography utilizes several different stationary phases (eg, C18, porous graphitic carbon) and chromatographic modes (eg, reversed-phase, normal phase), making it suitable for widespread application across numerous research projects. However, several separations requiring an hour or more each to complete are often needed, which drastically limits throughput. The speed of separation

was reduced to less than a second with ion mobility separations that detach analytes in gas based on their structure/shape and mass-to-charge ratio.

However, the attractive time gains achieved with this technique were offset by reduced resolution and sensitivity. Further methodological variations have since increased the resolution by extending the device path length for separation and the sensitivity by increasing ion utilization and transmission efficiencies. Significant gains in ion mobility resolution have been achieved with structures for lossless ion manipulations (SLIM)-based ion mobility separations [Garimella et al 2019]. As with liquid chromatography, ion mobility separations can be coupled to mass spectrometry. Such ion mobility-mass spectrometry (IM-MS) techniques are being increasingly explored to enhance performance for biological tissue imaging. In IM-MS approaches, the ion mobility separation reduces background and isobaric interferences enabling greater specificity and accuracy in mass spectrometry imaging of small molecules [Sans et al. 2018]. The key outcome is a more accurate determination of the spatial distribution of molecular ions.

At Pittcon, the **31st James L Waters Symposium** focused on IM-MS and included a presentation by Dr. Michael T Bowers, University of California, that explores its application in the study of amyloid oligomer assembly. The presentation, entitled 'Amyloid Assembly: The Early Oligomer States' discussed results from IM-MS experiments, often complemented by other biophysical techniques, exploring the oligomeric distributions and structures of several amyloid systems. It is well established that early soluble amyloid oligomers are implicated as the primary disease agents in several diseases, such as Alzheimer's

disease and type 2 diabetes [Bernstein et al. 2009]. Dr. Bowers presented data confirming that there is crosstalk between the oligomeric states of different conditions.

Several companies with expertise in producing IM-MS instrumentation were present at Pittcon to discuss the capabilities of these technologies. These included Extrel, the premier supplier of **quadrupole-based mass spectrometry systems** and components, SCIEX who produces IM instrumentation using **SelexION® technology**, Waters who produces the **SELECT SERIES Cyclic IMS, and Vion IMS** systems that combines ion mobility separations with high-resolution mass spectroscopy.

The technique may also be used to monitor disease progression by analyzing the levels of amyloid peptide in biological fluids. Immunoprecipitation, combined with MALDI-TOF mass spectrometry, is a powerful tool for the measurement of amyloid peptides [Pekov SI et al. 2019].

Pilot experiments with artificial cerebrospinal fluid and mouse brain tissue indicated that accurate quantification of specific amyloid peptides in biological fluids and tissues could be achieved using the IP-MALDI-TOF/TOF approach.

Glycoproteins are also implicated in the etiology of Alzheimer's disease. The clearance of amyloid-β peptides involves transport across the blood-brain barrier via P-glycoprotein efflux transporters. This efflux pump is highly expressed on endothelial cells of the blood-brain barrier and has been shown to have decreased function in patients with Alzheimer's disease [van Assema et al. 2011]. Although several peptide and protein biomarkers for Alzheimer's disease have been identified in cerebrospinal fluid, the ability to make an unequivocal diagnosis during the early phases of the disease is lacking.

In his presentation at Pittcon entitled 'Chemical Tags Enabled Quantitative (Glyco)proteomic Analysis of Cerebrospinal Fluids in Alzheimer's Disease', Dr. Lingjun Li from the University of Wisconsin-Madison highlighted the use of hydrophilic interaction chromatography for the detection of glycoprotein biomarkers in cerebrospinal fluid for the diagnosis of Alzheimer's disease.

Dr. Lingjun and his team have developed multiplexed isobaric and isotopic tagging strategies to discover, identify, and evaluate candidate biomarkers for the reliable diagnosis and monitoring of Alzheimer's disease. The presentation detailed their global glycoproteomics approach that combines enhanced N-glycopeptide sequential enrichment by hydrophilic interaction chromatography (HILIC) and boronic acid enrichment with electron transfer and higher-energy collision dissociation (EThcD) for large-scale intact glycopeptide analysis. Using this technique, the team has revealed distinct glycosylation patterns and dynamic changes of certain glycoforms in Alzheimer's disease [Wang et al, 2016].

There were opportunities to explore the latest HILIC instrumentation at Pittcon, where numerous companies, including **Shinwa Chemical Industries** and **GenTech** were on hand to discuss their products.

References

- *Bernstein SL, et al. Nat Chem 2009;1(4):326–331. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2918915/pdf/nihms-201926.pdf*
- *Campbell NL, et al. AMA Intern Med. 2019;179(8):1093-1094. https://jamanetwork.com/journals/jamainternalmedicine/article-abstract/2736349?widget=personalizedcontent&previousarticle=2737753*
- *Castrillo-Viguera C, et al. Neurology 2019;92(15 Supplement):S9.006; https://n.neurology.org/content/92/15_Supplement/S9.006*
- *Centers for Disease Control and Prevention. Leading Causes of Death 2017. https://www.cdc.gov/nchs/fastats/leading-causes-of-death.htm*
- *Dou K-X, et al. Alzheimers Res Ther. 2018;10:126. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6309083/*
- *Ferrero J, et al. Alzheimers Dement (N Y) 2016;2(3):169 176. https://www.ncbi.nlm.nih.gov/pubmed/29067304*
- *Forloni G and Baldacci C. Journal of Alzheimer's Disease 2018;62:1261–1276. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5869993/pdf/jad-62-jad170819.pdf*
- *Garimella SVB, et al. Trends in Analytical Chemistry 2019;116:300 307. https://www.sciencedirect.com/science/article/abs/pii/S016599361830685X*
- *Langa KM, Burke JF. AMA Intern Med. 2019;179(9):1161-1162. https://jamanetwork.com/journals/jamainternalmedicine/fullarticle/2737753*
- *Pekov SI, et al. J. Am. Soc. Mass Spectrom. 2019;30:1325–1329. https://link.springer.com/article/10.1007/s13361-019-02199-2*
- *Rosenberg RN, Lambracht-Washington D. JAMA Neurol. Published online December 9, 2019. doi:10.1001/jamaneurol.2019.4182. https://jamanetwork.com/journals/jamaneurology/article-abstract/2756406?widget=personalizedcontent&previousarticle=2737753*
- *Sans M, et al. Current Opinion in Chemical Biology 2018;42:138 146. https://www.sciencedirect.com/science/article/pii/S1367593117301643?via%3Dihub*
- *Van Assema DM, et al. Brain. 2012;135(Pt 1):181_189. https://www.ncbi.nlm.nih.gov/pubmed/22120145*
- *Wang J, et al. Proteomics Clin Appl. 2016;10(12):1225-1241. https://onlinelibrary.wiley.com/doi/abs/10.1002/prca.201600009*
- *Wyttenbach T, Bowers M.T. (2003) Gas-Phase Conformations: The Ion Mobility/Ion Chromatography Method. In: Schalley C.A. (eds) Modern Mass Spectrometry. Topics in Current Chemistry, vol 225. Springer, Berlin, Heidelberg. https://link.springer.com/chapter/10.1007/3-540-36113-8_6*

4.5b USING RAMAN SPECTROSCOPY AS A SCREENING TOOL FOR ALZHEIMER'S DISEASE

Raman spectroscopy is a sensitive, label-free, and non-destructive optical analytical tool that measures the interaction between light and matter where light is inelastically scattered. The light of a single wavelength focuses on the sample, and the scattered photons then generate the spectrum. This provides detailed information about the molecular and biochemical composition of the sample.

The application of spontaneous Raman spectroscopy in medical diagnostics using biofluid samples and biopsies is becoming increasingly popular due to its high chemical specificity and capacity to reveal molecular information.

The technique is useful as a diagnostic tool for several neurological disorders, including Huntington's disease [Huefner et al 2020], and it is hoped that Raman spectroscopy may also be a useful diagnostic tool for Alzheimer's disease.

The differentiation of Alzheimer's disease from other types of dementia is challenging, and its early diagnosis is complicated since the available biomarkers lack the necessary sensitivity and specificity. It is only currently possible to definitively diagnose Alzheimer's disease during a post-mortem examination. Furthermore, the detection of existing biomarkers of Alzheimer's disease necessitates the invasive collection of cerebrospinal fluid.

Identification of biomarkers for Alzheimer's disease in peripheral blood samples is highly desirable, and much research is underway to achieve this. Raman spectroscopy has already been used to successfully detect early-stage Alzheimer's disease and discriminate between serum samples taken from healthy volunteers and those with varying stages of Alzheimer's disease [Paraskevaidi et al, 2018]. This method has revealed changes in metabolites found in blood plasma in patients with Alzheimer's disease [Habartová et al 2019].

Dr. Igor Lednev from the University at Albany, New York, gave a presentation at Pittcon entitled 'Raman Hyperspectroscopy and Machine Learning for Medical Diagnostics and Forensic Purposes' in which he described a new patented approach for Alzheimer's disease diagnostics.

The novel methodology, which combines Raman hyperspectroscopy with machine learning, probes the total biochemical composition of bodily fluid and has great potential for becoming a universal tool for a variety of biomedical diagnostic applications.

It is hoped that this inexpensive non-invasive analysis technique will ultimately enable the screening of at-risk patient populations for the development and progression of Alzheimer's disease. It has already discriminated between different types of dementia and healthy control subjects with more than 95% sensitivity and specificity.

Dr. Lednev and his team previously demonstrated that hyperspectral Raman spectroscopy could be a useful tool for the reliable diagnosis of Alzheimer's disease [Ralbovsky and Lednev 2018]. Hyperspectral imaging obtains a spectrum for each pixel of an image taken of a sample enabling determination of the spatial distribution of its components.

The exceptional chemical specificity of Raman spectroscopy makes it a perfect candidate for hyperspectral imaging. Combining this with machine learning allowed a specific spectroscopic signature to be determined for Alzheimer's disease, which can then be used for diagnostic purposes.

Producers of quality near-infrared spectrometers, including **Malvern Panalytical** and **ZEISS Microscopy**, were on-site at Pittcon to discuss how they could support Raman spectroscopy research requirements.

References

» *Habartová L, et al. Clinical Biochemistry 2019;72:58-63. https://www.sciencedirect.com/science/article/pii/S0009912019303406*

» *Huefner A, et al. Chem. Sci. 2020;11:525 533. https://pubs.rsc.org/en/content/articlehtml/2020/sc/c9sc03711j*

» *Paraskevaidi M, et al. ACS Chem. Neurosci. 2018;9:2786–2794. https://www.ncbi.nlm.nih.gov/pubmed/?term=29865787%5Buid%5D*

» *Ralbovsky N, Lednev IK. Biophotonics 2018. https://www.photonics.com/Articles/Raman_Hyperspectroscopy_Shows_Promise_for/a63331*

4.5c IMAGING MASS SPECTROMETRY IN ONCOLOGY

Cancer is a generic term for a large group of diseases characterized by uncontrolled cell growth. Precision treatment has proved invaluable in the provision of optimal therapy for many cancer types. Different tumors and neoplasms have different drug susceptibilities and can differ markedly in their growth rate and prognosis. Therefore, effective diagnosis and monitoring of cancer are critical to decisions regarding treatment strategy.

Cancer is the second leading cause of death globally, and it has been estimated that there will be 27.5 million new cases of cancer each year by 2040 [World Health Organization]. In 2018, there were 17 million new cases of cancer and 9.6 million deaths [Cancer Research UK; Centers for Disease Control and Prevention].

With increasing cancer prevalence, optimizing the management of this disease is of particular importance for enhancing patient outcomes, and maximizing the value of healthcare resources.

Solid tumor cancers are typically diagnosed by analysis of a biopsy sample. This can be obtained using a needle or an endoscope to withdraw tissue or fluid, or surgical excision of cancerous tissue. The removed cancerous cells are then usually scrutinized under the microscope by a pathologist, who will describe the size, shape, and appearance of a specimen as it looks to the naked eye. This information can help determine the type of cancer and how advanced the disease is [National Cancer Institute].

Light microscopy is the gold standard for the histopathological diagnosis of breast cancer [Chang et al 2019]. The removed tissue undergoes formalin processing and is embedded in paraffin. The sample is then sliced using a high-precision instrument and mounted on glass slides to enable visual inspection of histological sections under the microscope. The slides are also stained with hematoxylin and eosin dyes to make the nuclei and cytoplasm visible.

Such histological evaluation can be used to check that surgical excision has successfully removed the entire tumor by confirming that all margins of the excised tumor are surrounded by healthy tissue. This is of the most significant importance for maximizing the potential for prolonged disease-free survival. However, histopathological analysis is labor- and time-intensive, which can delay decision-making during diagnostic and therapeutic procedures and impact patient outcomes. The determination of the delicate boundary between cancerous and normal tissues during surgery can be extremely challenging, especially during lumpectomy surgery for breast cancer removal, and requires extensive experience.

Such challenges create a high risk of human error. Even among pathologists with a strong professional background and rich experience, error rates in histopathology are high, which can have a devastating impact on the patient [Chuang, et al 2019].

Mass spectrometry imaging holds the potential to reduce misclassification during histopathological analysis [Sans et al. 2108]. Hundreds of molecular species in a tissue sample can be simultaneously analyzed in an untargeted manner and related to histological features using mass spectrometry.

Molecular species are chemically identified based on high-accuracy measurements of the mass-to-charge ratios, isotopic distributions, and tandem fragmentation patterns.

The value of mass spectrometry imaging in cancer diagnosis has been further increased by gas-phase ion mobility spectrometry techniques. These enable a greater separation of isomeric molecular ions before mass analysis, which, in turn, increases the accuracy of spatial distribution assessments.

Practical examples of the use of ambient ionization MS techniques to facilitate cancer diagnosis were presented at Pittcon in a talk by Dr. Livia Schiavinato Eberlin, the University of Texas at Austin, entitled ‘Improving Treatment and Clinical Outcomes for Patients with Mass Spectrometry Technologies’. Ambient ionization mass spectrometry techniques provide the specificity and sensitivity

necessary to perform in situ analysis of tissue samples for near real-time assessment of their molecular signatures.

Mass spectrometry imaging is also showing great potential in the analysis of fine-needle aspiration thyroid biopsies [DeHoog RJ, et al, 2019]. Histologic discrimination between malignant and benign thyroid nodules can be challenging, and inconclusive results can necessitate diagnostic surgery, which commonly leads to a benign diagnosis. Imaging fine-needle aspiration thyroid biopsies using desorption electrospray ionization mass spectrometry (DESI-MS) has successfully classified malignant thyroid carcinomas and benign thyroid tissues. This technique demonstrated a high potential for reducing the number of unnecessary diagnostic thyroid surgeries.

Mass spectroscopy has also been utilized to facilitate the identification of the boundary of cancerous tissue. Researchers developed a handheld pen-like mass spectrometry system that rapidly identifies the molecular profile of tissues using a small volume water droplet and mass spectrometry analysis [Zhang et al., 2017]. Requiring only three seconds of contact with the tissue surface, the pen can rapidly distinguish tumors from healthy tissue by characterizing diagnostic proteins, lipids, and metabolites.

The mass spectrometry pen, used in conjunction with machine learning, has also been shown to provide robust molecular models for predicting serous ovarian cancer that could enable rapid and accurate ovarian cancer diagnosis [Sans et al. 2019].

Companies supplying instrumentation and software solutions for mass spectrometry imaging attended Pittcon and were available to discuss specific mass spectrometry analytical requirements. For example, Bruker showcased its **rapifleX**, the most advanced MALDI-Time-of-Flight (TOF) imaging system. JEOL was on-hand to provide more details on its unique SpiralTOF™ ion optic system as used in the

MALDI SpiralTOF™ mass spectrometer, and representatives from the Bio-Rad informatics division demonstrated its world-leading **KnowItAll**™ Mass Spectral Library that offers access to over one million mass spectra.

The use of fingerprint spectroscopic signals in chemical microscopy was discussed at Pittcon in a presentation by Dr. Ji-Xin Cheng of Boston University Photonics Center entitled 'Chemical Microscopy: A New Platform for Life Science and Translational Medicine'. Dr. Cheng presented innovations in developing advanced chemical imaging modalities covering the entire optical window from visible to mid-infrared, and illustrated their potential as techniques for precision diagnosis and treatment.

The team has already demonstrated the value of the technique in the non-invasive diagnosis of prostate cancer [Wu et al, 2019].

Prostate cancer screening is commonly conducted by measuring levels of serum prostate-specific antigen. However, the results of such analysis can be complicated by common conditions, such as benign prostatic hyperplasia and prostatitis, giving rise to false positives and numerous unnecessary prostate biopsies.

Delegates at Pittcon were able to explore the potential of chemical microscopy in a discussion with the producers of spectrophotometry and microscopy instrumentation. Hanna Instruments was on-site to discuss the **Iris portable spectrophotometer**, which enables measurement in the spectrum of all wavelengths of visible light and not just pre-specified wavelengths. Hitachi High Technologies America, Inc presented its range of specialized analyzers, and ZEISS Microscopy was available to discuss its portfolio of **complementary microscopy technologies.**

References

- *Cancer Research UK. https://www.cancerresearchuk.org/health-professional/cancer-statistics/worldwide-cancer. Accessed January 2020.*
- *Centers for Disease Control and Prevention. https://www.cdc.gov/nchs/fastats/leading-causes-of-death.htm Accessed January 2020.*
- *Chang Z, et al. BMC Medical Informatics and Decision Making. 2019;19:article 198. https://bmcmedinformdecismak.biomedcentral.com/articles/10.1186/s12911-019-0913-x*
- *DeHoog RJ, et al. PNAS 2019;116(43):21401-21408. https://www.pnas.org/content/116/43/21401*
- *National Cancer Institute. https://www.cancer.gov/about-cancer/diagnosis-staging/diagnosis/pathology-reports-fact-sheet Accessed January 2020.*
- *Sans M, et al. Current Opinion in Chemical Biology 2018;42:138-146. https://www.sciencedirect.com/science/article/pii/S1367593117301643?via%3Dihub*
- *Sans M, et al. Clinical Chemistry 2019;65(5): 674-683. http://clinchem.aaccjnls.org/content/65/5/674*
- *World Health Organization. https://www.who.int/en/news-room/fact-sheets/detail/cancer Accessed January 2020.*
- *Wu J, et al. Chemrxiv 2019. doi.org/10.26434/chemrxiv.11426994.v1. https://s3-eu-west-1.amazonaws.com/itempdf74155353254prod/11426994/Functionalized_NIR-II_Semiconducting_Polymer_Nanoparticles_for_Single-Cell_to_Whole-Organ_Imaging_of_PSMA-Positive_Prost_v1.pdf*
- *Zhang J, et al. Science Translational Medicine 2017:9(406):3968 https://stm.sciencemag.org/content/9/406/eaan3968*

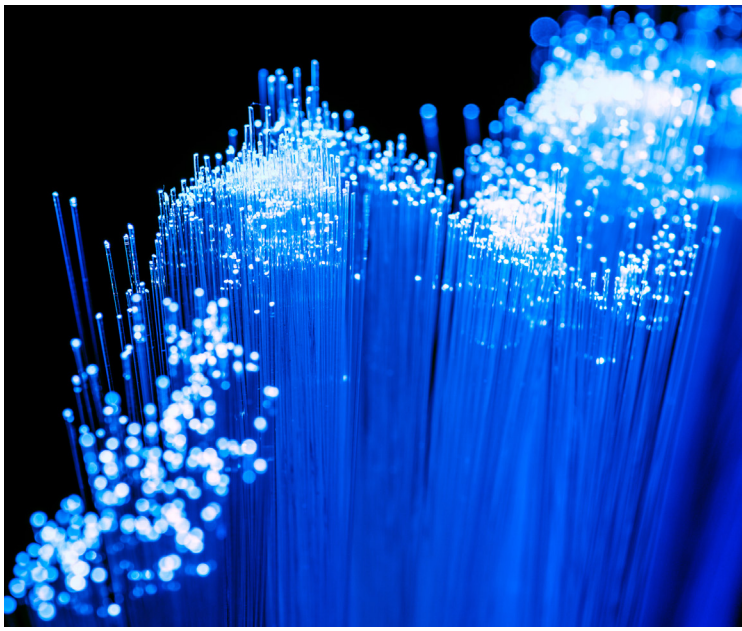
4.5d PLASMONICS AND PERSONALIZED MEDICINE

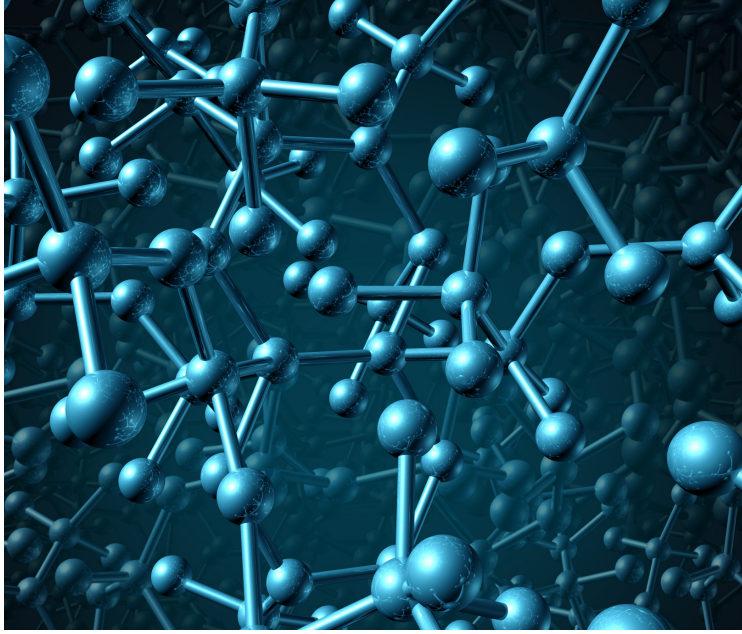
Plasmonics is a combination of optical and electronic data transfer that utilizes the strongest features of each. It provides the ability to transmit data extremely rapidly along the surface of a tiny metal wire at optical frequencies.

The use of optical data allows high-bandwidth transfer but usually requires bulky "wires", as in fiber optics. Electronic data transfer can only occur at frequencies substantially inferior to those possible with fiber optics but has the benefit of being achievable with only tiny wires.

Plasmons are density waves of electrons, created when light hits the surface of metal under precise circumstances. The tiny and rapid density waves are generated at optical frequencies and can encode a lot more information than is possible with conventional electronics [Niedziółka-Jönsson J and Mackowski S, 2019].

Plasmonic sensors are now being developed for the early detection of disease biomarkers. The application of plasmonics in the diagnosis of cancer had promising initial results [Ameen et al, 2017]. Although the immunoassays currently used to diagnose cancer are effective, they do have several limitations, such as poor availability of high-specificity antibodies and limited stability of biological reagents [Tu et al. 2016]. Sensitive detection schemes, such as colorimetry, radiometry, fluorimetry, are required to detect trace targets and necessitate the use of the corresponding antibody- or antigen-conjugated labels, including enzymes, DNA and RNA reporters, radioisotopes, chemiluminescent, and redox probes. These labels for assisting detection carry problems that introduce storage and usage concerns. For example, DNA and RNA reporters have limited stability, radioisotopes may cause health hazards, and chemiluminescent and redox probes are disturbing and susceptible to the effects of the surrounding environment. Consequently, there has been much research into the development of antibody-free and enzyme-free immunoassays with novel detection schemes that can overcome the drawbacks mentioned above.





Molecularly imprinted polymers

One alternative to antibodies that have been extensively investigated is the use of molecularly imprinted polymers (MIPs). These are synthetic receptors that possess antibody-like binding properties or enzyme-like activities.

MIPs are easier to prepare, more cost-efficient, and more stable than antibodies. A gold-based boronate affinity MIP array was successfully used to precisely extract a target glycoprotein from complex samples [Tu et al. 2016].

The captured glycoprotein was labeled with boronate affinity silver-based Raman nanotag. Exposure to laser caused the gold-based MIP array to generate a surface plasmon wave, enabling ultra-high sensitivity by enhancing the Raman scattering (SERS) signal of the silver-based Raman nanotags.

The technique has recently been modified to form a dual MIP-based plasmonic immunosandwich assay [Rongrong X, et al, 2019]. With the powerful combination of high MIP specificity and ultra-sensitive detection by chemiluminescence and SERS, MIP-based sandwich assays have emerged as promising analytical tools for the detection of disease biomarkers.

The potential value of dual MIP-based immunoassay in the diagnosis of disease was demonstrated in the detection of neuron-specific enolase (NSE) in human serum, where it successfully differentiated patients with small-cell lung cancer from healthy individuals [Rongrong X, et al, 2019]. The dual MIP-based plasmonic immunosandwich assay exhibited numerous benefits over a standard enzyme-linked immunosorbent assay (ELISA), including being a more straightforward procedure that could be conducted more rapidly, requiring a smaller sample volume and having a more comprehensive linear range.

A further modification of the technique has also recently been successfully used for the quantitation of a carcinoembryonic antigen (a routinely used marker for colon cancer) in human serum [Zhou et al., 2019]. This latest methodology, described as orthogonal dual molecularly imprinted polymer-based plasmonic immunosandwich assay (odMIP-PISA), includes two different types of epitope-imprinted gold nanoparticle MIPs to provide double recognition of a target glycoprotein and glycans-imprinted Raman-active silver nanoparticles as labeling nanotags for detection.

Gold nanoparticles (AuNPs) significantly increase imaging power, with the light scattered by the AuNPs being up to a million-fold more powerful than the intensity of light released from a fluorescent color [Sharifi et al 2019]. They have also demonstrated tremendous e□ciency in laser-based drug delivery. However, their use for medical applications will require a greater understanding of the risk for cytotoxicity and the depth of light penetration in the body for practical imaging.

Precision medicine represents an important new healthcare approach that aims to increase the quality of life and improve patient-specific and individualized diagnoses, medical decisions, medication, therapies, and prognoses. An in-depth understanding of molecular mechanisms of disease will enable the integration of treatment strategies with an individual's pharmacogenomics profile that defines their response to drugs. It was made possible by advances in molecular biology and omic technologies, but the development of more powerful techniques, such as PISA, has further widened its potential [Popa et al., 2018]. The value of precision medicine has been highlighted for a range of diseases. Triple-negative breast cancer (TNBC) is a prime example. Although the tumors are highly sensitive to cytotoxic chemotherapy, treatment achieves only relatively low rates of pathological response. A recent study evaluated previously published data for five potentially carcinogenic signaling pathways in TNBC. The review showed that concurrent inhibition of tumorigenic pathways might inhibit the cancer process [Wu et al., 2018], indicating that the identification of key signaling pathways in TNBC could enable the development of precise, highly targeted medicines that improve clinical outcomes.

Novel analytical techniques, such as the plasmonic immunosandwich assay, will help maximize the potential benefits in patient outcomes that are achievable by facilitating the identification of potential targets and enabling the widespread implementation of precision medicine.

The role of the plasmonic immunosandwich assay in precision medicine was explored at Pittcon by Professor Zhen Liu of Nanjing University, one of the Advances in Measurement Science Lectureship winners. Professor Liu's team utilized the plasmonic immunosandwich assay for probing low-copy-number proteins in single cells [Liu et al., 2016]. During his presentation in the session entitled 'Probing Signaling Proteins and Protein-protein Complexes in Single Living Cells via Plasmonic Immunosandwich Assay', Professor Liu illustrated how the plasmonic immunosandwich assay approach is a powerful tool for facile probing of proteins and protein-protein complexes in the signaling pathways of apoptosis in single living cells. Liu also suggested that it is an efficient tool for precise anti-cancer efficacy evaluation.

Companies specializing in the development of powerful instrumentation suitable for furthering our understanding of signaling pathways and enabling rapid identification of biomarkers were present at Pittcon and available to discuss the application of their products in precision medicine. Malvern Panalytical was present, providing the opportunity to learn more about using its **morphologically-directed Raman spectroscopy** (MDRS) for rapid, automated chemical and morphological characterization of the individual components in a multi-component sample.

Integrated Optics, UAB, was present to discuss the available **laser options** for conducting Raman spectroscopy, and Ibsen Photonics was also there to discuss its range of **Raman spectroscopy platforms.**

References

- *Ameen A, et al. Advanced Optical Materials 2017;5(11): 1601051. https://onlinelibrary.wiley.com/doi/full/10.1002/adom.201601051*
- *Liu J, et al. Angew Chem Int Ed Engl. 2016;55(42):13215-13218. https://www.ncbi.nlm.nih.gov/pubmed/?term=Liu%2C+J.%3B+Liu%2C+Z.%2C+et+al.+Angew.+Chem.+Int.+Ed.+2016%2C+55%2C+13215.*
- *Niedziółka-Jönsson J, Mackowski S. Materials (Basel) 2019;12(9):E1418. https://www.ncbi.nlm.nih.gov/pubmed/31052366*
- *Popa M-L, et al. Journal of Immunoassay and Immunochemistry 2019;40:1–23. https://www.tandfonline.com/doi/abs/10.1080/15321819.2018.1562940?journalCode=ljii20*
- *Rongrong X, et al. Anal. Chem. 2019;91(15):9993-10000. https://pubs.acs.org/doi/10.1021/acs.analchem.9b01826*
- *Sharifi M, et al. Journal of Controlled Release 2019;311–312:Pages 170-189.https://www.sciencedirect.com/science/article/pii/S0168365919305164?via%3Dihub*
- *Tu X, et al. Anal. Chem. 2016;88(24):12363-12370. https://pubs.acs.org/doi/abs/10.1021/acs.analchem.6b03597*
- *Wu N, et al. Oncol Lett. 2018;16(4):4984-4996. https://www.ncbi.nlm.nih.gov/pubmed/30250564*
- *Zhou L, et al. Biosensors and Bioelectronics 2019;145:111729. https://www.sciencedirect.com/science/article/pii/S0956566319308085#!*

CONCLUSION

Healthcare resources are being stretched to the limit as populations continue to grow and life expectancies increase. The steadily aging population and changes in lifestyle have also given rise to a greater prevalence of chronic diseases. The costs of providing healthcare are increasing as treatments become more expensive and labor costs increase. To maximize the return on healthcare expenditure, fundamental changes are being made to the way diseases are managed, and advances in spectroscopic techniques have been key in facilitating the shift to more cost-effective healthcare.

As the sensitivity and resolution achievable with analytical technologies have been extended, so has our understanding of disease processes. The heterogeneity of diseases is now evident, and one treatment does not necessarily suit all patients with a given condition. Treatment strategies should be based on an individual's specific pharmacogenomic profile to achieve the best patient outcomes. In this way, the treatment most likely to be efficacious can be predicted rather than wasting resources in a trial and error approach.

The availability of rapid and reliable spectroscopic analyses has made such a system feasible. The vital role that continuing advances in spectroscopic techniques will play in shaping a robust future for healthcare providers cannot be overstated.

Sensitive detection using imaging techniques has also demonstrated its value in facilitating rapid and reliable early diagnosis to enable treatment to be initiated during the early stages of the disease. Ion mobility-mass spectrometry and Raman hyperspectroscopy methodologies are showing huge potential for facilitating the early diagnosis of Alzheimer's disease, which can currently only be accurately diagnosed during post-mortem investigations.

Novel non-invasive, label-free, antibody-free, and enzyme-free techniques for the early detection of disease biomarkers are also showing great promise in providing high-specificity detection while overcoming some of the limitations associated with the immunoassays currently used. Of particular interest are the gold-based molecularly imprinted polymers (MIPS), which generate a surface plasmon wave to provide ultra-high detection sensitivity by enhancing the Raman scattering signal of the silver-based Raman nanotags. MIP assays used in conjunction with plasmonic sensors have already proved capable of differentiating patients with early-stage cancers from healthy individuals.

Developments in mass spectrometry imaging are showing great potential for the management of cancer.

Gas-phase ion mobility mass spectrometry and desorption electrospray ionization mass spectrometry techniques have increased the accuracy of non-invasive cancer diagnosis and the development of a handheld pen-like mass spectrometry system reduces the risk of misclassification during the histopathological analysis of tumor biopsies.

These novel analytical techniques hold significant potential for optimizing patient outcomes through the widespread implementation of precision medicine.

4.6 ACCELERATING THE DIAGNOSIS AND TREATMENT OF CANCER WITH MASS-SPECTROMETRY BASED TECHNIQUES

An interview with Livia Eberlin, Assistant Professor at the University of Texas at Austin, discussing the development of mass-spectrometry-based techniques for cancer diagnosis and improved clinical outcomes.

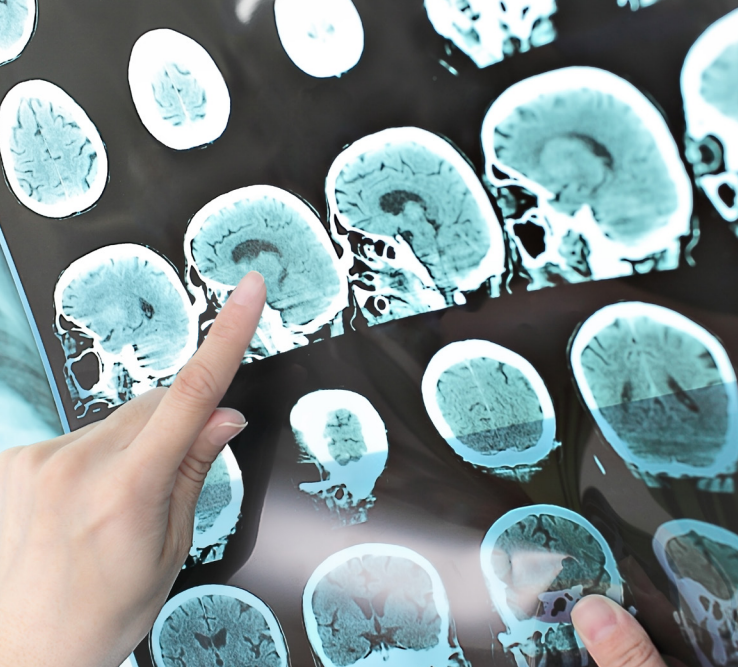
Why is there a need for alternatives to cytological analysis in the field of oncology?

Within the context of cancer surgeries, histopathology is a clinical method for analyzing tissues that are being removed from the patient to make sure that all cancer has been removed.

Histopathology includes the analysis of frozen sections. These are pieces of tissue that are sent to a laboratory and frozen, sectioned, and stained. The pathologist then looks at the stained tissue section under a microscope to determine if all the cancer has been removed during the surgical procedure.

The problem is that this procedure can take a long time. Sometimes the surgeries are extended by 35 to 40 minutes because you have to wait for the frozen section analysis to be carried out. It can also be entirely subjective because this process of freezing the tissue quickly during surgery to be able to get a section for the pathologist to look at using a microscope can cause some changes to the tissue histology and cytology. It can be challenging for a pathologist to precisely evaluate if there is cancer or not in that region of the tissue section.

There is a great need for new technologies that could be applied in the clinic, preferably in the operating room, to help the surgeons by providing them with information on the presence or absence of cancer at specific positions of the body.



This would guide the surgical resection so that they would know, at specific positions, when they are removing cancer, if all the cancer has been removed or not, and if that is a region of healthy tissue that can remain or diseased tissue that still needs to be removed.

How can mass-spectrometry based techniques be used to improve the diagnosis and monitoring of patients with cancer?

Mass spectrometry is an analytical technology that can provide the highest level of sensitivity and specificity for chemical analysis. With mass spectrometry, we can dig deep into the molecular composition of complex samples and say, "These are the specific metabolites that are present in this sample. These are the lipids that are present, and the proteins as well".

With that kind of detailed molecular information, we can then evaluate if a sample, let's say a clinical sample such as a piece of tissue, is diseased or if it is healthy tissue in a manner of seconds. This is because the profiles of these molecules are very characteristic of cells that are healthy or unhealthy cells i.e. cells that have cancer.

If we can adapt mass spectrometry technology, which is still mostly a research and development technology that requires relatively complex instrumentation, to translate it to the clinic and put these technologies in the hands of the clinicians, let's say the surgeons or the pathologists who are the clinical professionals making these decisions, we can empower them with molecular information that can be highly accurate in regards to the diagnosis of a clinical specimen. In that way, we can provide new information currently unavailable to help clinicians make better treatment decisions for their patients.

How do these techniques compare to gene and RNA sequencing?

Mass spectrometry techniques are normally used to analyze molecules such as metabolites, lipids, and proteins. In particular, for the technology that I have been developing, we have mainly focused on small molecules, which would be metabolites, fatty acids, and lipids. When you compare to DNA and RNA-seq, you are looking at entirely different types of molecules. Furthermore, what I think is appealing about metabolic information is that it is providing a real-time picture of the processes that are going on in the tissue.

With DNA and RNA, you are looking at overall mutations or expression patterns of genes and the transcription processes that are going on in cells. With metabolites, you are looking at the end products of these reactions and things that are happening in real-time in the cell related to metabolism. Both approaches have incredible value.

It is remarkable how DNA and RNA sequencing technologies are increasingly incorporated within the clinic. For example, it can provide information on the likelihood that someone will develop a particular type of breast cancer, and that has incredible value in managing patients.

We are trying to help improve clinical decisions in nearly real-time using mass spectrometry. My lab uses a type of mass spectrometry that provides analysis in a few seconds. If you compare this with DNA and RNA sequencing, that normally takes a longer time. With mass spec, we could be providing this information with high throughput to hopefully expedite and improve the treatment decision for the patient.

Please can you tell us about your recent research into thyroid neoplasia?

"Our thyroid cancer research focuses on helping patients that come into the clinic with a thyroid nodule to know if the nodule is a cancerous nodule that needs to be removed, or if it is a benign nodule that does not necessarily require surgery."

Livia Eberlin

Thyroid cancer incidence is rapidly increasing in the USA and around the world, and 50% of people by the age of 60 will find a nodule in their thyroid. The good news is that the majority of thyroid nodules are benign, so these are not cancerous lesions that need to be removed.

The problem is that current cytology methods that are used to evaluate cells from a thyroid biopsy under a microscope are often inconclusive. It can be challenging for a clinician to say if a nodule is benign or malignant. Therefore, patients often go into surgery without even knowing if they have cancer. You can determine if it is cancer during surgery, but, in a majority of cases, for the follicular type of neoplasms, the patient does not have cancer, so the surgery was likely unnecessary.

What we are trying to do is use mass spectrometry before surgery to analyze these cells from a minimally invasive biopsy of the nodule to accurately determine if the individual does have cancer and needs surgery. The approach identifies if the nodule is benign and helps prevent unnecessary surgeries, which are terrible for the patient but are also costly and a burden to the healthcare system.

What were the conclusions of this study, and were the findings significant?

We started this study in thyroid cancer using banked tissues collected from patients and available as resources for researchers. We analyzed 178 tissues and acquired over 100,000 mass spectra. We used this extensive data set to build statistical classifiers that could determine if a nodule was cancer or if it was just a benign tumor. We tested that, and we did well for a specific type of thyroid cancer, papillary thyroid carcinoma, with over 90% accuracy. For follicular types, we had 83% accuracy, which is reasonable considering that this type of thyroid nodule cannot be determined with cytology alone.

Then we started a prospective trial study in the clinic where patients were coming in for a routine biopsy. We then got an additional biopsy for our research. We have carried this out with over 100 patients so far and have kept accuracy in diagnosis at just about 90%.

These results are really exciting because we had information that could have prevented patients from going into potentially unnecessary surgery in a lot of these cases. However, we need to do a much larger validation study that we are planning to do as a multi-center project to validate these findings and prove its value for patient care.

You also developed a 'MassSpec Pen' to improve the accuracy of diagnoses. What is this device and how does it work?

The vision in developing the mass spec pen was to provide a handheld, easy-to-use device based on mass spectrometry analysis that could be routinely used by surgeons and pathologists. We wanted it to be something that they could handle to empower them to do the research and take advantage of the high accuracy and sensitivity of mass spectrometry analysis in helping them make clinical decisions.

When looking at the MassSpec Pen device, it seems pretty simple, and that was the intention. It is a handheld tool, and, although we called it a pen, it does not function as one. It works by providing a single droplet of solvent to extract molecules from a tissue. We use water for the pen tip most of the time, and we have automated the process so that once you touch the tissue and trigger the device with a foot paddle, everything happens without any more user input.

"Water is an excellent solvent. Once that water droplet interacts with the tissue, it extracts metabolites, lipids, and even small proteins from the tissue. We then have a tubing system that transfers the droplet to the mass spectrometer."

Livia Eberlin

Using this setup, we receive a molecular analysis of these molecules in nearly real-time. Based on the pattern of these molecules, we can then tell the surgeon or the clinician whether the tissue region is normal or cancerous. We do that in a timeframe of about 10-15 seconds.

The time can be a little shorter or longer, mostly depending on how far your mass spectrometer is from the tissue site and the tubing system that we use. We have looked at over 1000 tissues in the lab, and our accuracies for cancer diagnosis are pretty exciting at around 96%. We have been in the clinic testing this device in the operating room in vivo and on freshly excised tissues with over 100 surgeries now, and the intraoperative results are promising as well.

We are in the process of publishing this pilot study, which shows that mass spectrometry technology has incredible value in guiding clinical decisions. With the mass spec pen, I think the simplicity and ease of use in the way that we designed the technology is appealing. It can be well incorporated into a clinical workflow with minimal training requirements.

Why did you choose to focus on ovarian cancer?

We have been working on ovarian cancer in my lab for a few years since I started my laboratory, and the focus on ovarian cancer was driven by our desire to help patients and women that are suffering from this disease.

We also had an ongoing project looking at the various aspects of the clinical diagnosis of ovarian cancer and treatment outcomes. When we developed the mass spec pen, we knew that is tough to identify regions of metastasis in ovarian cancer surgery, which is very common for high-grade ovarian cancer.

You usually find ovarian cancer throughout the abdominal cavity of a patient. For a surgeon, it can be imperative to have a tool that will help them to identify these potential regions of metastasis and remove all of the cancer.

We know from clinical data that there have been extensive studies that show that removing all of the cancer from the patient will give them a higher chance of disease-free survival. It was a scenario where we had access to these patients and tissues, and there was a significant clinical need. We are incredibly passionate about helping ovarian cancer patients.

The technique involves the use of machine learning. Do you think AI and machine learning will form a vital part of the future for clinical diagnostics?

I believe that AI and machine learning will be essential parts of the clinical decision, and that will happen sooner than we expect. They are crucial technologies, especially as we are constantly moving towards big data and incorporating molecular data with clinical and imaging information.

Once you start to get to these complex data sets and try to make decisions based on this complex information, there is a limit to what the human brain can do rapidly and automatically. Incorporating AI and machine learning will be critical to achieving that level of throughput and certainty.

However, there is a lot of hype in this field as well, and it is essential for researchers like myself, and I'm constantly trying to learn more about AI and machine learning, to select the right tools to make sure that our models are not over-fitting.

Validating your models, testing your models, and the use of independent data sets and clinical samples will be crucial to demonstrate the value and robustness of this type of technology in helping the development of clinical care.

Do you think that these techniques will one day overtake histopathology, or work alongside it?

I think that new technologies such as mass spectrometry and other modalities will be complementary to what pathologists are already doing. The human aspect of the evaluation of tissue for diagnosis is crucial to patient care.

The thought, or at least my goal, is to empower clinicians with newer technologies that can help them to make decisions. Not to replace them, but to enable them to make more informed and better decisions, primarily based on the molecular information that is reliable and can be highly predictive of the disease state.

Why did you feel it was important to share your work at Pittcon?

I have been to Pittcon several times. It is a conference that I enjoy very much because it combines several areas of chemistry and analytical chemistry, showcasing the new technologies launched during the conference every year.

I think that seeing the scientific talks and getting this new information about what people are working on and developing is exciting and motivates me to also pursue new areas of research.

Being able to see the vendors and the new products that are being launched to help researchers in academia and industry is special and unique to Pittcon. It is an excellent avenue for networking as well.

Events such as Pittcon are really important for me to talk to other scientists and researchers, and to share ideas and see if they have any input. How can we better develop what we are doing? How can we think of new ways to reach our goals and help patients?

That interaction with other people who are leading in the field is essential for researchers and students. Seeing the technology and products that are being launched by the industry in the companies is appealing and can help our research and instrumentation.

4.7 STUDYING THE PROTEOME AND HUMAN DISEASES USING MASS SPECTROMETRY

An interview with Amanda Hummon Ph.D., Associate Professor from Ohio State University, discussing how mass spectrometry can be used to analyze the proteome and how this can help with the study of human disease.

How is mass spectrometry used to study the proteome?

Mass spectrometers are essentially costly weighing machines or balances. They have been beautifully applied to the study of proteins and the global proteome because proteins are composed of repeating amino acids that all have distinct masses. We can use mass spectrometry to distinguish individual proteins, figure out which ones are present, and how much is present.

The beautiful thing about this is that we think of proteins as the action molecules of the cells. Therefore, they carry out a lot of the work, and studying the proteome of healthy tissue versus diseased tissue, for example, can give you a lot of information as to why disease happens and also what could potentially be done to treat it. For this reason, mass spectrometry and proteomics come together really beautifully.

How crucial are studies into the proteome to advance our understanding of human diseases?

"It is incredibly important to study the proteome to understand human diseases. There are two different ways in which looking at the proteome can be advantageous: the first is looking at diagnostics, and the second is understanding treatment options."

Amanda Hummon Ph.D

For example, if you have a patient who is developing a disease, often that disease will be triggered by the expression of proteins that either would not be present in the healthy state or potentially would be present in different amounts in a healthy state versus a diseased state.

By studying the proteome and looking at what proteins are present and the quantity present, we can figure out, for example, if a patient has a particular disease. That is the first huge application.

The second application in which proteome studies can be very useful is looking at treatment options. Again, for example, if you have different drugs that can be used to treat a disease, you can

use the proteins as a readout to see whether or not your drug is working, and if a different drug needs to be used for that patient. We think of proteomics as a very good readout on the health and viability of human cells.

What are some of the advances that have been made in mass spectrometry methods?

Over the last five to ten years, we have seen enormous advances in the area of mass spectrometry instrumentation, as evidenced by the growing popularity of the technique. If you attend the American Society for Mass Spectrometry Meeting, it just gets bigger and bigger every year. This is in large measure because anything can be studied by looking at its molecular weight.

Some of the fascinating advances that have occurred in the last few years are the introduction of techniques such as ion mobility, which allows you to discriminate between molecules that have the same molecular weight but different shapes.

With mass spectrometry, you are usually studying things and looking at differences in weight. When we add ion mobility at the front end of a mass spectrometer, we can look at things that have the same weight but different shapes. We are reaching an exciting point where we can look at almost anything.

In my area of research, I do a lot of proteomics and a lot of imaging mass spectrometry. There have been many developments in the world of imaging as well that allow us to look at, with really exquisite spatial resolution, just a few cells at a time in a spatially discrete manner by mass spectrometry. I am also very excited by those advances.

How does this type of innovation enable research that goes beyond a standard analysis?

With these new types of methods, we can start asking questions that we could not get at before. To go back to the question of ion mobility, you are using ion mobility in different types of research. Let us say, for example, you had two molecules that were precisely the same mass.

A lot of smaller molecules, such as lipids, will often have the same mass, so we could not discriminate between these various species previously. But now, with these new advances, we can start figuring out these subtle differences.

Human cells have been doing this for thousands of years; they are really good at discriminating and using these different molecules. We are now just catching up with the instrumentation and being able to discriminate and use these is very advantageous.

Why do you choose to focus on this research and what are the benefits of the research?

I was trained as an analytical chemist. I worked in an analytical chemistry group as a graduate student, and I love how fundamental and widely applicable analytical chemistry is. All science is driven by measurement. If you do not know what you have, you really cannot do research. Analytical chemistry is the area of research that makes all that possible. I love that aspect of it.

I also love method development and being able to develop these rigorous robust methods. I like doing analytical chemistry in the area of biomedical research because I fundamentally want to do research that will help people. That is the overarching goal of research in general.

My family has a history of certain types of cancers. Therefore, we have focused all the efforts of my research group in applying analytical chemistry to cancer biology and trying to use these measurement strategies to explore compelling questions in cancer biology.

What is in store for the future of your research in cancer biology?

My research group does a lot of work using different types of cancer model systems. More specifically, the work over the last ten years has been using immortalized cell lines to form three-dimensional cell cultures, which are mimics of human tumors.

We have developed a suite of mass spectrometric methods that are very robust and rigorous, and we spent ten years developing these methods so that we could then translate them from the cell culture models into actual patient tissues. We are now making this transition and moving our analytical methods from cell lines into patient-derived organoids.

These are samples that are grown from patient biopsies, and we can use our mass spectrometric methods, for example, to figure out whether or not a patient will respond to a specific drug. We can take one of these biopsies, culture it, treat it with the drug, and use mass spectrometry to see whether or not the patient can metabolize that drug. Having that information can help guide clinical decisions.

What other research focuses are at Pittcon?

I have given a talk on using the proteome to look at ways to increase chemotherapeutic efficacy. My lab has been investigating different nutritional modifications to examine the question of whether or not we can make chemotherapy work better. We have some very promising preliminary data indicating that eating less can increase chemotherapeutic efficacy in colon cancer cells. This is based on both phenotypic and proteomic data.

I have also given a talk on my research group's imaging mass spectrometry work, using our three-dimensional spheroid models and organoids to look at both pharmacokinetics and pharmacodynamics of different compounds that are available for colorectal cancer patients.

With both of these talks, we were looking to apply mass spectrometry to what I consider significant problems in colorectal cancer research, and hopefully come up with better options for patients.

What do you hope to gain from Pittcon with these research presentations?

It is always wonderful to come here because you get to see all your friends and colleagues as well as meet new people. During one of my talks, I met a bunch of people I had never met before and got to hear about their research, and some of the ideas and concepts that were introduced in the symposium were things I had not thought of.

That changes my perspective a little bit on the research, and sometimes it forces you to focus on other aspects or hone your hypotheses a little bit. I enjoy being introduced to these new ideas.

If you get really good questions after you give a talk, it can sometimes be hard to answer them, but it also can make your research that much better if it forces you to think about why you are doing what you are doing. I find Pittcon to be a very rich scientific environment, and there are a lot of good discussions going on here.

Why do you think Pittcon benefits the analytical science community?

I have been coming to Pittcon since I was a grad student; I think this is my 15th year here, perhaps. It is always so important to interact with your friends and colleagues to find out what is going on in the field and to get a sense of where the community is moving.

It has also been positive to see changes in the focus of analytical chemistry over the years and to see different topics becoming emphasized and more critical.

4.8 BIOTHERAPEUTICS ARE JUST THE BEGINNING

Over the past few decades, we have seen the beginning of a new era of therapeutics. While traditional drugs consist of small molecules produced by chemical synthesis, half of the new drugs approved by the FDA each year are now biotherapeutics (aka biologics). As a result, the global biologics market was worth $221 billion in 2017 and will grow to $399.5 Billion by 2025.[1-3]

Biotherapeutics are complex biological molecules such as proteins, hormones, antibodies, growth hormones, and cytokines, to name just a few. As they are so complicated, we cannot produce biologics using traditional chemical synthesis. Instead, biotherapeutics are produced using genetically engineered bacteria, yeast, fungi, or cells.[4,5]

Biological macromolecules make effective therapeutics because they mimic the molecules found naturally in biological systems and can bind to biological targets with high specificity. As a result, biologics can perform highly sophisticated and specific functions, which would be impossible for small drug molecules. Biologics are also much less likely to elicit immune responses and undesired side effects in patients.[4,5]

The first biotherapeutic to hit the market was insulin, which is a hormone that is essential for people with diabetes to maintain their blood sugar levels. Historically, insulin was extracted from animal pancreases. Thanks to the invention of recombinant DNA technology, human insulin is now produced in bacteria.[4]

The number of recombinant biotherapeutics has expanded continuously since the introduction of recombinant human insulin in 1982. Biologics are now routinely used to treat conditions such as cancer, heart disease, multiple sclerosis, anemia, and rheumatoid arthritis. The world's best-selling drug, Humira, is a biological therapeutic that has bought relief to millions of people with colitis and psoriasis.[4,5]

CHALLENGES FACED BY BIOTHERAPEUTICS

Despite their successes, discovering and producing biologics remains difficult. To reduce the cost of new therapies, we need to address the following challenges.

1. **Biologics often have complex 3D structures, and their modes of action are frequently unknown**

Determining the full structure of biologics can be extremely difficult because biological macromolecules are so complex and sensitive to their environment. As a result, the complete design and function of biotherapies often remain unknown, even after the FDA has approved them. An incomplete understanding of the drug's structure and mode of action makes it difficult to ensure the drug is given to the right patient. [6]

2. **Manufacturing procedures for biologics are highly complex and sensitive**

Designing production methods that provide high yields of a biologic can be extremely challenging. Because manufacturing biologics relies on living organisms, their yields are often much lower than small drug molecules that can be made by chemical synthesis. As a result, manufacturing biotherapeutics is an expensive, complicated, and energy-intensive balancing act.[7]

The living systems used to manufacture biologics are very sensitive to environmental conditions. Small changes in synthesis conditions can result in changes to the structure of biotherapeutics that render them ineffective or even toxic. Therefore, process and quality control systems are vital to ensuring the safety and efficacy of biotherapeutic products.[7]

3. **There is a high risk of contamination during production**

Proteins and other substances from host organisms can contaminate biotherapeutics and cause an immune response in patients. Bacterial endotoxins are one potential contaminant. They are molecules from bacterial cell walls that can cause severe immune reactions in patients, resulting in sepsis and even death. Another example is proteins from host organisms, which could interfere with or counteract the therapeutic molecule, rendering the drug ineffective.[7,8]

All biopharmaceuticals must be contamination-free to ensure the safety of patients. Analyzing biologics for contaminant molecules can be challenging because biotherapeutics are often complex mixtures that mask the presence of undesirable contaminant molecules. We need highly sensitive and reliable analytical techniques to ensure our biotherapeutics are contamination-free.[7,8]

4. Biologics can be unstable, and delivery to their target site may be non-trivial

Delivery of biotherapeutics can be challenging. They cannot be delivered orally as they are usually broken down by digestion. Other methods must be used to ensure that they reach their target.[9]

Biological molecules are often susceptible to environmental conditions. Manufacturers must ensure that their products are stable and survive production, packaging, storage, and delivery to reach their target site in their intended form.[10]

5. Biosimilars bring new challenges to manufacturers and regulatory authorities

Some of the first biologics are now beginning to come 'off-patent'. As a result, manufacturers are interested in producing generic biotherapeutics, also known as 'biosimilars.' However, it is difficult, if not impossible, to recreate the production process of a biotherapeutic without intimate knowledge of its manufacturing process. How can we be sure that biosimilars are safe and effective?[11]

New regulations have been introduced for manufacturers producing biosimilars. The regulations require manufacturers to compare their biosimilar with the corresponding reference product. Analytical technologies play a crucial role in these comparisons and ensure the safety and efficacy of biosimilars. [11]

References

1. *'Biotherapeutics: Challenges and Opportunities for Predictive Toxicology of Monoclonal Antibodies' – Johnson DE, International Journal of Molecular Sciences, 2018.*
2. *'Biologics Global Market Opportunities and Strategies To 2021' https://www.marketwatch.com/press-release/biologics-global-market-opportunities-and-strategies-to-2021-2018-08-14*
3. *'Biologics Market Size Worth $399.5 Billion By 2025 | Growth Rate: 3.9%' https://www.grandviewresearch.com/press-release/global-biologics-market*
4. *'Biosimilars and Biologics' – Lucio S, American Society of Health-System Pharmacists, 2018.*
5. *'Biotherapeutic products' https://www.who.int/biologicals/vaccines/biotherapeutic-products/en/*
6. *'Proteomics for Biological Discovery' – Veenstra TD, Yates JR, John Wiley & Sons, 2006.*
7. *'Defining the difference: What Makes Biologics Unique' – Morrow T, Felcone LH, Biotechnology Healthcare, 2004.*
8. *'Case Studies of Microbial Contamination in Biologic Product Manufacturing' – Suvarna K, Lolas A, Hughes P, RL Friedman, American Pharmaceutical Review, 2011.*
9. *'Biologics: the role of delivery systems in improved therapy' – Škalko-Basnet N, Biologics: Targets & Therapy, 2014.*
10. *'Stability Studies for Biologics' in 'Handbook of Stability Testing in Pharmaceutical Development' – Mazzeo A, Carpenter P, Springer, 2009.*
11. *'Analytical tools for characterizing biopharmaceuticals and the implications for biosimilars' –Berkowitz SA, Engen JR, Mazzeo JR, Jones GB, Nature Reviews: Drug Discovery, 2012.*

4.8a THE ANALYTICAL CHALLENGES OF CURRENT TECHNIQUES FOR BIOTHERAPEUTICS

Analytical science has always been a vital part of drug discovery and manufacturing. Biotherapeutics are no exception to this rule. Identifying potential biological drugs, developing them, and ultimately manufacturing them is heavily reliant on analytical science. Analytical chemistry is a critical part of the biotherapeutic industry from early development to final production.[1]

Analytical techniques that are typically relied on for small molecule pharmaceuticals are not suitable for biotherapeutics because they are large, complex molecules. The analysis of biotherapeutics is often more challenging compared with other therapeutics.[1]

We need analysis techniques that are specifically designed for analyzing biotherapeutics and can answer the following questions about our products:

- Did I make what I intended to?
- How does it behave?
- Does it do what it is supposed to do?
- Is it a robust product?

X-ray crystallography and nuclear magnetic resonance (NMR) can give detailed information about the structure of biotherapeutics, but these techniques can be complicated, slow, and expensive. Instead, we use combinations of other methods to give a detailed analysis of biotherapeutics.[1]

Pittcon featured a symposium called 'Introducing the Analytical Challenges of New Biotherapeutic Drug Modalities. The symposium focused on the role of analytical science in biotherapeutics and outlined some of the state-of-the-art techniques for characterizing new biotherapeutic drugs.[2]

CURRENT TECHNIQUES FOR ANALYSIS OF BIOLOGICS

Dynamic Light Scattering (DLS)

One primary concern in developing biotherapeutic formulations is aggregation. Aggregated biotherapeutic molecules can reduce efficacy and pose an immunogenic risk to the patient. DLS is a popular technique for detecting aggregation.[3]

DLS involves illuminating a sample with monochromatic light. The light is scattered by the molecules, particles, or aggregates in the sample solution. The diffracted light is detected and used to determine the size distribution of the molecules or aggregates in the solution. As a result, DLS can be used to detect undesired aggregation, evaluate stability, and measure the viscosity of biotherapeutic formulations. [3,4]

Limitations of DLS include that it is a low-resolution technique, so it is not suitable for analyzing smaller molecules. It is also sensitive to temperature and solvent, so these must be kept constant during measurements. DLS can only be used with transparent, liquid samples.[3,4]

Several high-throughput DLS technologies are available, including the Wyatt Technology Dyna Pro Reader III. The high-throughput capabilities make DLS particularly suited to early-stage development, where the stability of many drug candidates, formulations, and conditions can be screened quickly.[5]

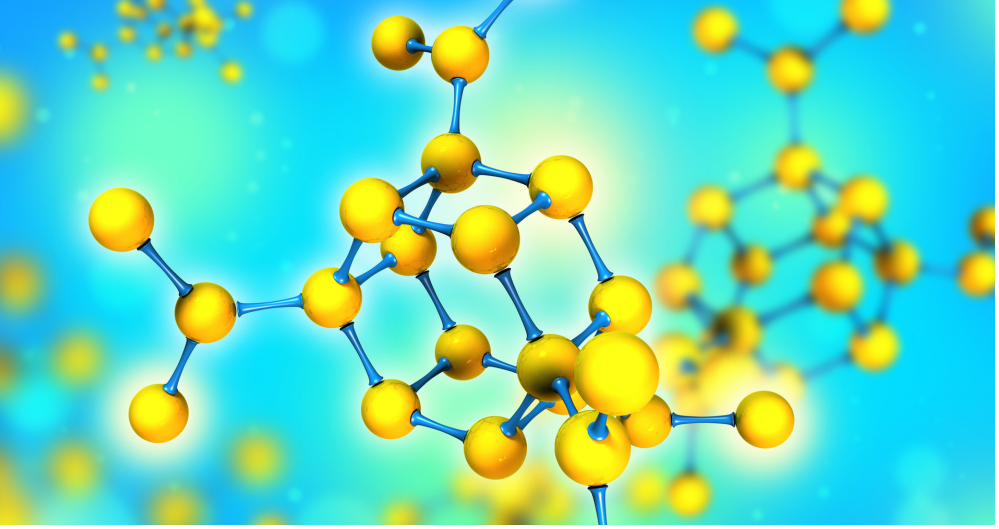
Electrophoretic Light Scattering (ELS)

ELS is based on combining DLS with electrophoresis. A sample of particles or molecules in solution is exposed to an electric field in a cell with two electrodes. The particles or molecules with a net charge migrate towards the electrode with the opposite charge.[6]

The sample is illuminated with a laser, and the scattered light is used to determine the mobility of the dispersed particles or molecules. The Zeta potential and isoelectric point of molecules can also be determined. For protein biotherapeutics, ELS data can be used to determine protein charge, which can be related to factors such as activity and reaction kinetics.[6,7]

The advantages of ELS include its speed and the fact that it only requires a small sample volume. ELS measurements can be made over a wide range of concentrations. Disadvantages include the fact that ELS requires molecules or particles with an overall charge. Furthermore, like DLS, it requires transparent solutions.[6,7]

The Pittcon expo featured the Mobius from Wyatt Technology, providing an opportunity to learn more about light scattering techniques for biotherapeutics.[5]



Circular Dichroism

Circular dichroism relies on differences in the absorption of polarized light due to structural asymmetry to detect changes in molecular structure. The technique is useful for comparing biologic systems and studying its changes depending on its source or conditions.[3,8]

Circular dichroism can detect small changes in the secondary structure motifs of proteins such as helixes, beta-sheets, or random coils. A limitation of the technique is that it cannot make meaning information about mixed motifs.[3,8]

Bioassays and Immunoassays

Bioassays determine its concentration, purity, or biological activity using the effect of a molecule on cells or animals. They are usually not particularly precise or accurate, but they can provide valuable information about biological activity.[3]

Immunoassays use antibodies to determine the concentration of an antigen. Immunoassays offer high specificity and low detection limits, making them very powerful in biotherapeutic analysis. They can be used in high throughput analysis, however, immunoassays rely on the availability of a suitable antigen, which can limit their applications.[3]

Ultraviolet/Visible, and Vibrational Spectroscopy

Spectroscopic techniques provide information about a molecule based on its ability to absorb light. In UV/Vis spectrometry, the sample is illuminated by UV/Vis light, and the absorbance is measured. Particular bonds in the molecule absorb light at specific wavelengths depending on their identity and environment. [3]

UV/Vis can also indicate the environments that surround an amino acid within a protein. As a result, changes in absorption peaks can be used to measure conformational changes of macromolecules, including folding, unfolding, and degradation. Unfortunately, UV/Vis cannot give useful information about the secondary structure and is susceptible to interference.[3]

Vibrational spectroscopy, on the other hand, can be used to study the secondary and tertiary structure of macromolecules. Vibrational spectroscopic techniques are sensitive to chemical composition and molecular architecture, making them valuable tools for studying biologics. Both Fourier transform infrared (FTIR) and Raman spectroscopy are widely used to study the interactions of amino acid side chains, which contribute to the formation of secondary structure motifs in proteins.[3,9]

Vibrational spectroscopy works well with a wide range of biological samples, including liquids and solids. It also offers excellent time resolution, requires only a small amount of sample, is non-destructive, and uses relatively low-cost equipment.

A disadvantage of vibrational spectroscopy is that the data can be challenging to interpret and susceptible to interference.[3,9]

A vast range of companies in the various fields of spectroscopy was present at Pittcon. For those looking to learn more about protein spectroscopy, the Pittcon expo was a great place to start.

Light scattering, spectroscopy, and biological assays are all vital tools in studying biotherapeutics. In this chapter, we have discussed a few of the relevant techniques that were featured at Pittcon. There are, of course, a myriad of other applicable analytical methods that were featured at the conference, including chromatographic methods and mass spectrometry.

References

1. *Analytical tools for characterizing biopharmaceuticals and the implications for biosimilars' – Berkowitz SA, Engen JR, Mazzeo JR, Jones GB, Nature Reviews: Drug Discovery, 2012.*
2. *'Addressing the Analytical Challenges of New Biotherapeutic Drug Modalities' https://s23.a2zinc.net/clients/pittcon/pit20/Public/SessionDetails.aspx?FromPage=Sessions.aspx&SessionID=35&SessionDateID=2*
3. *'Introduction to Biologic and Biosimilar Product Development and Analysis' – Nagel KM, Springer 2018.*
4. *'Dynamic light scattering: a practical guide and applications in biomedical sciences' –Stetefeld J, McKenna SA, Patel TR, Biophysical Reviews, 2016.*
5. *'Dynamic Light Scattering Instruments' https://www.wyatt.com/products/instruments/dynamic-electrophoretic-light-scattering-detectors.html*
6. *'Challenges of Nanoparticle Characterization and How to Overcome them with the Light Scattering Toolkit' https://www.azom.com/article.aspx?ArticleID=13104*
7. *'Recent applications of light scattering measurement in the biological and biopharmaceutical sciences' – Minton AP, Analytical Biochemistry, 2016.*
8. *'CD spectroscopy has intrinsic limitations for protein secondary structure analysis' – Khrapunov S, Analytical Biochemistry, 2009.*
9. *'Infrared spectroscopy of proteins' – Barth A, Biochimica et Biophysica Acta (BBA) – Bioenergetics, 2007.*

4.8b PROTEIN PURIFICATION AND CHROMATOGRAPHY

The number of protein therapeutics has grown substantially in recent years. This is not surprising as proteins are considered as the most important class of biological compounds, and the molecular machines that do almost all of the 'work' in our cells.[1]

Proteins have a massive array of functions. They include enzymes that carry out chemical reactions, antibodies that bind to foreign substances, messenger molecules, transport molecules, and structural components.[1]

Over the past few decades, we have begun to exploit the properties of proteins and use them to treat a wide range of conditions such as cancer, autoimmune diseases, kidney failure, infectious diseases, eye conditions, and arthritis, to name just a few.

Therapeutic proteins can replace proteins in the body that are deficient or abnormal, augment an existing biological pathway, provide a new function, interfere with a molecule or organism, or deliver other compounds to targets in the body.[1,2]

Proteins are large, complex biological molecules made up of long chains of amino acid building blocks. The amino acid sequence of a protein, also known as its primary structure, allows it to fold into secondary structure motifs and ultimately into the unique 3D or tertiary structure, which gives it its specific function within a biological system.[1]

Separating and purifying proteins from complex biological solutions is a critical step in their characterization. Techniques that separate proteins often rely on differences in the solubility, size, charge, or absorption characteristics. There are several popular methods for separating and purifying proteins from complex mixtures, each with their own advantages and disadvantages. Several separation techniques are often used in sequence to fully separate all the proteins in a mixture.[3]

Most protein purification techniques rely on chromatography. In the past, chromatography techniques were limited by throughput, but now a wide range of technologies make high throughput chromatography an affordable reality. Teledyne was at the Pittcon expo, providing the opportunity to learn more about high throughput chromatography.[4]

TECHNIQUES FOR PROTEIN PURIFICATION AND SEPARATION

Salting Out and Dialysis

Salting out and dialysis is the most straightforward way to purify a protein. Salting out involves precipitating a protein using a highly concentrated salt solution, such as ammonium sulfate. Once the protein salt is formed, it can be separated from the solution by dialysis, which involves passing the solution through a semipermeable membrane.

> Protein salts are large, so they are retained on one side of the membrane. Ultrafiltration can also be used, where pressure is applied to the membrane filtration process.[3]

While salting out has the advantage of being relatively cheap and straightforward, it does not separate proteins based on molecular weight and is typically only useful for solutions that contain one protein. To provide more information about dialysis, Thermo Fischer was present at the Pittcon expo, where it exhibited its Zeba desalting products.[3,5,6]

Electrophoresis

Electrophoresis uses an electromagnetic field to separate macromolecules. Gel electrophoresis is the most common form of electrophoresis used in protein analysis. An electromagnetic field is applied to the gel, and macromolecules move from one side to the other. The mobility of the proteins depends on their size, change, and 3D structure.

Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) is usually used for proteins because the negatively charged SDS detergents denature the proteins, causing them to unfold. The unfolded proteins can then migrate through the gel, and their measured mobility can determine their molecular weight.[6]

Proteins can also be separated using electrophoresis based on their isoelectric point, using a technique called isoelectric focusing. Another useful method is capillary electrophoresis, which separates molecules based on their electrophoretic mobility, a characteristic that is dependent upon the proteins charge, viscosity, and size.[6]

Electrophoresis can be used to measure protein purity and determine molecular weight. It is highly sensitive and can separate proteins with a 2% difference in mass. The disadvantage of electrophoresis is that proteins may be denatured in the process of separation. Gel preparation can be slow and costly.[6]

Field-Flow Fractionation (FFF)

FFF is a technique that separates particles as they flow through a narrow channel using a force field orthogonal to the flow. FFF techniques are very flexible and versatile, separating analytes from macromolecules to particles.

They provide high resolution, gentle separation of molecules that cannot be separated by conventional chromatography methods. A limitation of FFF techniques is that developing optimum separation parameters can be time-consuming and require expert knowledge.[3,6-8]

There is a wide range of FFF sub techniques, which all use different separation fields such as thermal FFF, flow FFF, centrifugal FFF, and gravitational FFF. The most commonly applied FFF technology today is the asymmetric flow field-flow fractionation technology, also known as AF4.[3,6-8]

AF4 technology can separate a wide variety of analytes, including synthetic polymers as well as biopolymers, polysaccharides, proteins, viruses, antibodies, and nanoparticles, both in aqueous and organic solvents. AF4 is often called a "Universal Separator." The Pittcon expo featured Postnova and its AF2000 MultiFlow FFF Series.[6-9]

Ion-exchange chromatography

Ion-exchange chromatography separates molecules by adsorption based on charge. A mixture of impure proteins is introduced to the ion-exchange column at a particular pH. The charged protein molecules in the solution are adsorbed to a solid support matrix in the column via electrostatic interactions. Gradually changing the ionic strength or pH of the eluent releases the proteins from the column according to their charge, resulting in separated, pure proteins.[6,10]

Key advantages of using ion-exchange chromatography include that it does not denature the proteins during separation, and it provides excellent resolution for separating proteins with only small differences in charge. It also provides very predictable eluent patterns. However, the major limitation of ion-exchange chromatography is that it requires charged proteins.[6,10]

Affinity chromatography

In affinity chromatography, a mixture of impure proteins is introduced to a column that has ligands with an affinity for the desired protein. A mobile phase moves the proteins through the column. Proteins with a high affinity for the ligand will move more slowly than proteins with a low affinity of the stationary phase. The result is that the proteins are separated based on their affinity for ligands.[6,11]

The advantages of affinity chromatography include that it does not require charged proteins, and it is extremely specific. Very high purity proteins can be obtained using affinity chromatography, and the process is highly reproducible. Affinity chromatography can also provide information about a protein's binding sites. Disadvantages include the use of expensive ligands, and a relatively complicated method development process.[6,11]

Size-exclusion chromatography

Size-exclusion chromatography uses porous beads to separate proteins based on size. Small proteins enter the pores of the beads and pass through the column slowly, while large proteins cannot enter the pores and pass through the column quickly.

This method offers excellent separation of large and small proteins from a low volume of sample. It uses a mild mobile phase, meaning that proteins retain their native structure and characteristics. However, proteins must have at least a 10% difference in molecular mass to achieve excellent resolution.[6,12]

High-Performance Liquid Chromatography (HPLC) and Ultra-High-performance Liquid Chromatography (UHPLC)

HPLC is probably the most widely used form of chromatography in biotherapeutic analysis. The liquid sample is injected into a narrow column containing small, packed particles. Pressure is applied to a mobile phase, which moves the molecules through the column. The molecules are separated by retention time and typically detected using another technique such as mass spectrometry or UV/Vis spectroscopy.[6,13,14]

UHPLC is similar to HPLC but uses smaller columns, smaller particles, higher pressures, and lower flow rates, providing speed, higher throughput, and better resolution compared with HPLC. Compared with other chromatography techniques, HPLC and UHPLC are quick and efficient. However, they are still slower than other analytical methods such as light scattering and spectroscopy.[6,13,14]

The Pittcon Expo featured all the major HPLC and UHPLC suppliers, including Shimadzu with their biocompatible Nexera Bio UHPLC, Advion with their Avant UHPLC, and Walters with their UHPLC Peptide Analysis Solutions.[15,16]

OVERCOMING POOR SELECTIVITY AND RESOLUTION IN PROTEIN CHROMATOGRAPHY

Chromatography of proteins is frequently limited by poor selectivity and resolution, resulting in peak overlaps. This is because the proteins are not sufficiently different for complete separation based on their size, charge, or absorption using a one-column system and linear mobile phase gradient.

For those interested in creating their own 'infinite selectivity liquid chromatography' system, Thermo Fischer was available to provide information on this at the Pittcon expo. They have a broad range of liquid chromatography columns that could be ideal for protein separations.[18]

References

1. *'Proteins' in 'Pharmacognosy' – Murray JE, Laurieri N, Delgoda R, Academic Press, 2017.*
2. *'Therapeutic proteins' – Dimitrov DS, Methods in Molecular Biology, 2012.*
3. *'Protein Purification Techniques' https://www.news-medical.net/life-sciences/Protein-Purification-Techniques.aspx*
4. *'Chromatography' https://www.teledyneisco.com/en-us/chromatography*
5. *'Zeba Desalting Products' https://www.thermofisher.com/uk/en/home/life-science/protein-biology/protein-purification-isolation/protein-dialysis-desalting-concentration/zeba-desalting-products.html*
6. *'Protein Separation and Characterization Procedures' in 'Food Analysis' – Smith DM, Springer, 2017.*
7. *'Application of flow field-flow fractionation for the characterization of macromolecules of biological interest: a review' – Qureshi RN, Kok WT, Analytical and Bioanalytical Chemistry, 2011.*
8. *'The use of asymmetrical flow field-flow fractionation in pharmaceutics and biopharmaceutics' – Fraunhofer W, Winter G, European Journal of Pharmaceutics and Biopharmaceutics, 2004.*
9. *AF2000 MultiFlow FFF - Universal Separator https://www.postnova.com/af2000-applications.html*
10. *'How Does Ion Exchange Chromatography Work?' https://www.news-medical.net/life-sciences/How-Does-Ion-Exchange-Chromatography-Work.aspx*
11. *'Affinity Chromatography – How Does it Work?' https://www.news-medical.net/life-sciences/Affinity-Chromatography-e28093-How-Does-it-Work.aspx*
12. *'Size Exclusion Chromatography of Protein Biopharmaceuticals: Past, Present and Future' – Fekete S, Goyon A, Veuthey JL, Guillarme D, American Pharmaceutical Review, 2018.*
13. *'HPLC and UHPLC for Biopharmaceutical Analysis' in 'HPLC and UHPLC for Practicing Scientists, Second Edition' – Rea J, Zhang T, Wiley, 2019.*
14. *'High-Performance Liquid Chromatography vs. Ultra-High Performance Liquid Chromatography' https://www.azom.com/article.aspx?ArticleID=15555*
15. *'Nexera Bio' https://www.ssi.shimadzu.com/products/liquid-chromatography/nexera-bio.html*
16. *'Peptide Mapping & Peptide Analysis' https://www.waters.com/waters/en_US/Peptide-Mapping-%26-Peptide-Analysis/nav.htm?locale=en_US&cid=514535*
17. *'Approaching Infinite Selectivity in Liquid Chromatography for Protein Separations' https://s23.a2zinc.net/clients/pittcon/pit20/Public/SessionDetails.aspx?FromPage=Sessions.aspx&SessionID=264&SessionDateID=3*
18. *'Intact Protein Analysis Workflows' https://www.thermofisher.com/uk/en/home/industrial/pharma-biopharma/biopharmaceutical-analytical-testing/intact-protein-analysis-workflows.html*

4.8c MASS SPECTROMETRY IN PROTEIN SCIENCE

Mass spectrometry is a core analytical technique for protein science. Measuring the mass-to-charge ratio of proteins allows their identification and quantification, even in complex mixtures. Mass spectrometry provides high specificity and can detect post-translational modifications and protein degradation.[1]

Mass spectrometry is typically coupled with other techniques, such as liquid chromatography (LC-MS), to provide resolved information about individual proteins within a solution. LC-MS can provide intact mass analysis, peptide mapping, and protein de novo sequencing, for a complete overview of your proteins.[2,3]

For those interested in LC-MS, Advion was present at the Pittcon expo exhibiting its Avant LC-MS solutions, and Agilent and ThermoFisher were present with their range of LC-MS technologies.[4-6]

Historically, mass spectrometry techniques for protein analysis have been limited by throughput and resolution in complex mixtures. However, the past two decades have been a time of rapid advancements in the field of protein mass spectrometry, with the development of a range of high-throughput, quantitative techniques. An on-going challenge in protein mass spectrometry is processing and interpreting the vast amount of data it can provide.[1,7]

Ion mobility spectrometry-mass spectrometry (IMS-MS)

IMS-MS is a relatively new tool in the field of protein analysis that combines both ion mobility spectrometry and mass spectrometry. First, the sample is ionized, and an ion mobility spectrometer separates molecules based on their mobility through a buffer gas. The mass to charge ratio of the separated ions are then determined by mass spectrometry. [8,9]

The combination of techniques means that proteins are separated by size, shape, charge, and mass. IMS-MS allows separation of molecules that may not be sufficiently resolved by LC-MS and provides information about the conformation, flexibility, and folding mechanisms of the proteins.[8,9]

The folding mechanisms of proteins is a challenging topic in the field of biochemistry. How a protein's amino acid structure dictates its final 3D structure is not yet fully understood because of the complex combination of thermodynamic and kinetic processes involved. Fully understanding how and why proteins fold, to the point where we can predict a protein's 3D structure from its amino acid sequence, would open the door to designing new therapeutic proteins.[10]

This year at Pittcon, Professor David Clemmer from Indiana University gave a presentation during the Thirty-First James L. Waters Symposium – Ion Mobility Spectrometry-Mass Spectrometry (IMS-MS) symposium.[11]

The talk entitled 'Mass-spectrometry based Strategies for Characterizing Native and Non-native Protein Structures,' outlined his work studying protein folding mechanisms with IMS-MS. Professor Clemmer presented data from IMS-MS analysis of simple proteins, giving detailed information about the folding and unfolding process.[11]

While IMS-MS is beginning to be recognized as a critical tool for studying proteins thanks to the conformational information and supplies, commercially available IMS-MS instruments do not yet offer sufficient mobility or mass resolution to study intact proteins.

Professor David H. Russell of Texas A&M University also gave a presentation at the James L. Waters Symposium entitled 'Native Ion Mobility-Mass Spectrometry for Studies of Membrane Complexes.' His talk outlined his work developing next-generation, high-resolution IM-MS machines. He presented the application of his techniques to studying protein complexes.[12,13]

The Pittcon Thirty-First James L. Waters Symposium – Ion Mobility Spectrometry-Mass Spectrometry (IMS-MS) was an excellent opportunity to learn more about protein mass spectrometry. These new techniques are improving our understanding of protein folding and confirmation, meaning we may be able to design our own proteins in the future precisely.

References

1. *'Mass Spectrometry for Proteomics' – Han X, Aslanian A, Yates JR, Current Opinion in Chemical Biology, 2008.*
2. *'Review: Antibody Protein Sequence Analysis Using Mass Spectrometry' https://www.news-medical.net/whitepaper/20180618/Review-Antibody-Protein-Sequence-Analysis-Using-Mass-Spectrometry.aspx*
3. *'Separation of biological proteins by liquid chromatography' – Ali I, Aboul-Enein HY, Singh P, Singh R, Sharmaa B, Saudi Pharmaceutical Journal,2010.*
4. *'About Advion' https://www.advion.com/about/*
5. *'Liquid Chromatography/Mass Spectrometry (LC/MS)' https://www.agilent.com/en/products/liquid-chromatography-mass-spectrometry-lc-ms?gclid=EAIaIQobChMIkZznwfCA5wIVA7DtCh2dyQ3HEAAYAyAAEgL4EfD_BwE&gclsrc=aw.ds*
6. *'Orbitrap Exploris 480 Mass Spectrometer' https://www.thermofisher.com/uk/en/home/industrial/mass-spectrometry/liquid-chromatography-mass-spectrometry-lc-ms/lc-ms-systems/orbitrap-lc-ms/orbitrap-exploris-mass-spectrometers.html*
7. *'Protein analysis: key to the future' – Boodhun N, BioTechniques, 2018.*
8. *'Native ion mobility-mass spectrometry and related methods in structural biology' – Konijnenberg A, Butterer A, Sobott F, Biochimica et Biophysica Acta (BBA) - Proteins and Proteomics, 2013.*
9. *'Ion Mobility-Mass Spectrometry' in 'Mass Spectrometry, Encyclopaedia of Analytical Chemistry' – Jiang W, Robinson RAS, Wiley, 2013. https://www.nature.com/articles/nchem.1889*
10. *'The power of ion mobility-mass spectrometry for structural characterization and the study of conformational dynamics' – Lanucara F, Holman SW, Gray CJ, Eyers CE, Nature Chemistry, 2014.*
11. *'Mass-spectrometry based Strategies for Characterizing Native and Non-native Protein Structures' https://s23.a2zinc.net/clients/pittcon/pit20/Public/SessionDetails.aspx?FromPage=Sessions.aspx&SessionID=620&SessionDateID=2*
12. *'Native Ion Mobility-Mass Spectrometry for Studies of Membrane Protein Complexes' https://s23.a2zinc.net/clients/pittcon/pit20/Public/SessionDetails.aspx?FromPage=Sessions.aspx&SessionID=274&SessionDateID=2*
13. *'The Protein Folding Problem' – Dill KA, Ozkan SB, Shell MS, Weikl TR, Annual Review of Biophysics, 2008.*

4.8d THE KEY TO THE FUTURE IS PROTEIN ANALYSIS

Proteins are the machinery of our cells, controlling virtually every cellular process. They play a vital role in maintaining our health. As a result, protein analysis is a critical part of the pharmaceutical industry, from drug discovery to final manufacturing and release.[1,2]

Identifying proteins in cells, determining their structures, and gathering information about their functions can help us understand why things go wrong and how diseases develop.

In this way, we can discover useful biomarkers and design effective therapies for patients.

Protein analysis and analytical chemistry are entwined topics that have the power to affect us all now and in the future.

At Pittcon, Professor Daniel Armstrong from the University of Texas at Arlington received the LCGC Lifetime Achievement in Chromatography Award for his work advancing analytical techniques that are vital to the future of biotherapeutics.

His presentation entitled 'From Micelles, Chiral and Ionic Liquids to Ultrafast and Molecular Rotational Resonance Detection,' gave an overview of the separation techniques he has developed during his career so far, including enantiomeric separations, pseudo phase separations, the 3-phase model, and theory of separations. He discussed his work developing ultrafast separation that led to the peak processing revolution in chromatography.[3]

THE NEXT GENERATION OF ANALYSIS IN BIOTHERAPEUTICS

The last two decades have seen rapid advances in technologies for biologic analysis, particularly in mass spectroscopy. New data acquisition techniques, machine learning, and de-convolution have been the focus of much research in the field of mass spectrometry in recent years, resulting in new methods for protein profiling and analysis.

As outlined earlier, IMS-MS is one of the techniques that has been enhancing the field of protein analysis. Other technologies, such as data-independent acquisition mass spectrometry (DIA-MS) are also impacting the field.

DIA-MS fragments and analyses every protein in a sample in parallel, without the need for separation or purification. DIA-MS can also provide quantitative, reproducible, comprehensive protein profiles with high throughput. The data produced by DIA can be extraordinarily complex and require deconvolution to identify and quantify individual proteins.

Most DIA methods currently refer to a database to identify proteins, which can be limiting if a protein is not in the database. It can also be challenging to identify proteins that are present in tiny concentrations. For more information about DIA-MS, Sciex, Waters, and Bruker were present at the Pittcon expo.[4-6]

THE FUTURE OF MEDICINE DEPENDS ON PROTEIN ANALYSIS

The future of medicine is personalized, and protein analysis has a vital role to play. Quantification of relevant proteins can help a health care professional select the best treatment for their patient, which could, in turn, be a biotherapeutic that has been specifically designed for them. Understanding how proteins fold and adopt unique 3D structure could enable us to design and engineer new proteins for therapeutics.[7]

A technique that simultaneously profiles all the proteins in a sample could revolutionize the drug development process by speeding up the identification of biomarkers, drug candidates, and drug targets.

Perhaps one day, we can expect that all our medical samples will be routinely protein-profiled so our health care providers can find us the best treatment quickly and easily. Biologics have been described as the 'sniper' of the drug world, but they are only useful if they are targeting the correct biological pathway for each patient.

Biotherapeutics, protein science, and analytical science will undoubtedly play a central role in the future of medicine. Therefore, we must continue to develop and improve our techniques for studying biological macromolecules.

References

1. *'Proteins' in 'Pharmacognosy' – Murray JE, Laurieri N, Delgoda R, Academic Press, 2017.*
2. *'Protein analysis: key to the future' – Boodhun N, BioTechniques, 2018.*
3. *'From Micelles, Chiral and Ionic Liquids to Ultrafast and Molecular Rotational Resonance Detection' https://s23.a2zinc.net/clients/pittcon/pit20/Public/SessionDetails.aspx?FromPage=Sessions.aspx&SessionID=656&SessionDateID=3*
4. *'See It All with SWATH® Acquisition' https://sciex.com/technology/swath-acquisition*
5. *'Proteomics and Protein Analysis: Ushering in the 4D revolution' https://www.bruker.com/applications/life-sciences/proteomics.html*
6. *'SONAR: Stop Looking, Start Seeing' https://www.waters.com/waters/en_US/SONAR/nav.htm?cid=134908444&locale=en_US*
7. *'Protein function in precision medicine: deep understanding with machine learning' –Rost B, Radivojac P, Bromberg Y, FEBS Letters, 2016.*

4.9 DEVELOPING VACCINES AND THERAPEUTICS BY EXPLOITING STRUCTURE IN SPHERICAL NUCLEIC ACIDS

Dr. Chad Mirkin, Director of International Institute for Nanotechnology talks to News-Medical Life Science about the use of spherical nucleic acids (SNAs) in vaccinations and treatments for disease, and what they show about the importance of structure in pharmaceutical development.

What are spherical nucleic acids (SNAs)?

Spherical nucleic acids (SNAs) are structures that are made by taking nanoparticles and synthesizing short snippets of DNA or RNA that are terminated with groups that can chemically bond to the particles. Under appropriate conditions, you can load up the short snippets of DNA or RNA onto the surface of the particles so that they adopt the shape of the central particle core.

What properties make SNAs useful for medical research and development?

What is interesting about an SNA is that, on a sequence basis, it naturally has completely different properties to its linear cousin. It interacts with biological systems e ntirely differently.

For example, if you feed linear nucleic acids to human cells, they will reject them. The reason behind this is that the cell membrane is negatively charged and the DNA is also negatively charged.

However, if you take that same set of DNA strands and arrange them in spherical nucleic acid form, not only do they go in, they go in better than anything known to man.

This is an interesting observation. The reason for it is that there are receptors on the surface of the cells that recognize the clustered oligonucleotides in the form of spherical nucleic acid. These receptors, called scavenger receptors, localize them on the cell membrane and then facilitate a process called caveolae-mediated endocytosis.

Once internalized, you can then use those structures to make measurements inside cells. You can also use them to regulate what cells do, in gene regulation approaches, as immunostimulatory agents, and to develop many analytical tools and possible therapeutics.

How can these properties be exploited in the creation of SNA-based vaccines?

With a vaccine, you have two essential components. You have something called an adjuvant, which is a molecule that stimulates your immune system, and you have something called an antigen, typically a peptide signature that helps train the immune system.

With a spherical nucleic acid vaccine, you take advantage of the fact that this type of architecture can enter cells such as dendritic cells and antigen-presenting cells, which are essential in the immune system. Specific sequences of DNA or RNA can be used to stimulate those cells selectively.

They can also carry things in with them if you put something in their cores or load upon them a particular type of antigen, for example, structures that train the immune system by training T cells to give a very selective killer response.

For example, if you are trying to develop a cancer vaccine, you take a spherical nucleic acid made of the appropriate adjuvant molecules, and you load into or onto them, the appropriate peptide signatures that are unique to those cancer cells. You then locally stimulate, for example, the subcutaneous injection. You train those cells to train T cells to selectively go out into the lymphatic system, find cells that have those signatures, the cancer cells, and selectively lyse them.

You recently demonstrated the ability of SNAs to deliver DNA to different regions of the body. Can you tell us more about this research?

In addition to getting into cells well, spherical nucleic acids have very different biodistribution profiles than linear nucleic acids. So, when they are systemically administered, they go to all regions of the body. They can enter the lung, epithelial cells, liver, and heart. They can cross the blood-brain barrier.

The SNA structure makes a difference on a basic level, but it also means that you can then use this understanding to begin to create new approaches to therapies. Very recently, we learned how to take spherical nucleic acids deep into the brain by intrathecally injecting them into the spinal cord, which gives them access to all parts of the brain.

This is exciting for the development of drugs to treat neurodegenerative disorders. As a result, we are beginning to look at this first in the area of Friedreich's ataxia. This genetic disease affects a significant number of people, but also in spinal muscular atrophy, a condition that affects young children. We are also aiming to investigate a wide range of other types of disorders, such as Huntington disease, Parkinson's disease, and Alzheimer's disease.

At Pittcon 2018, you presented three structural SNAs that had the differing anti-tumor efficiencies. How do these structures differ and how did this change their ability to target the tumors?

One of the exciting things about vaccine development is that in some respects, it is fantastic vaccines even work at all. When you get a vaccine, the adjuvant and antigen are often just coinjected into a person, and then you let nature take its course. You do not control the activity or biodistribution properties by presenting them in a particular structure.

We have learned how to control the amount of adjuvant in spherical nucleic acid vaccines. We have also discovered how to control the amount of antigen and how to present them in different ways.

I think the conventional way that people develop vaccines is the blender approach, which consists of taking the actions of components, putting them in, and hoping they work. If they work, you can run with it. If they do not work, you move on to the next set of components.

We have asked the fundamental question: does structure make a difference? Say you have something that works, can you make it better by controlling the structure in the way those components are presented in the context of a spherical nucleic acid?

The answer is that structure makes a huge difference. We have been able to look at three different structures systematically across many different animal models for cancer, and we have shown consistently that one of the three structures outperforms the others substantially. On one end of the spectrum, you have entirely ineffective vaccines, and in the other case, you have curative vaccines, which is remarkable. That has many implications. One implication is that it says we are probably misdesigning vaccines and we can do a lot better.

A second implication is that it makes you wonder about where people have looked for possible components for adjuvants and antigens and decided they did not work in a vaccine. Did they have the wrong components, or did they have the wrong structure? It could have been that they had perfectly fine components, but they presented them in the incorrect way for the type of response they wanted.

How far are vaccines based on spherical nucleic acids from routine clinical use?

"They are on the horizon. There are a whole series of drugs based on spherical nucleic acids that are now in human clinical trials, such as drugs for psoriasis and atopic dermatitis, and also for glioblastoma multiforme, a deadly form of brain cancer."

Dr. Chad Mirkin

There are even drugs in clinical trials for treating a wide variety of cancers where you use spherical nucleic acids as a stimulant for the immune system. There are also ones where you couple that stimulation with drugs called checkpoint inhibitors, which can give you a combined type of response.

We are systematically pushing the development of spherical nucleic acids from the benchtop at a lab to the clinic. There are now many shots at making this happen and many ongoing major trials that are going to tell the story going forward. Hopefully, it is going to be a very bright story for spherical nucleic acids, and they will be broadly used for treating many different diseases.

What do the next few years look like for you in your research?

We want to push this concept of structure making a difference. I am a chemist by training. I believe that if you look at the history of pharmaceutical development, the structure has always been important. Sometimes very subtle changes in the design of a drug can have a considerable impact on its performance.

The same has to be true with vaccines; we just have not had a platform to look at how structure makes a difference there systematically. However, now we do. We are going to push this concept of rational vaccinology, the idea that design can be carried out through an understanding of how structure translates to activity differences and can lead to increased performance in terms of therapeutics.

I think this is a fundamental concept, not just for spherical nucleic acids, but for nanomedicines and macromolecular approaches to developing medicines in general, because you tend to move away from the discrete architecture.

The bigger you go, the more complex you get. However, I think that also creates an opportunity in that the structural considerations there could be an essential knob that you can turn to adjust efficacy.

Why did you come to Pittcon?

Pittcon is great. They do a phenomenal job of attracting super smart people to whatever area they are in. They display state-of-the-art equipment and capabilities, and they put together a scientific program that is truly spectacular and attracts some of the best and brightest from all over the world.
It is a great way to learn about everything important in terms of science, instrumentation, and the science being conducted with that instrumentation.

Why do you think Pittcon is important for the analytical chemistry industry and the overall science industry?

Pittcon is central to the whole analytical science industry. I do not think there is any other venue that puts together a better show and puts together a better platform of the state-of-the-art, both on the instrument side and the science side. If I think of one show that I want to go to, it is Pittcon.