The microbiome has been shown to play roles in many different disease processes and is implicated in many more, from infectious and gastrointestinal diseases to autoimmune and neurological disorders. Advanced models of the gut leveraging intestinal and colonic stem cells are enabling the development of more reliable and robust systems for evaluating the impact of changes in the microbiome on gut health and screening potential drug candidates, including small molecules, biologics, and live biotherapeutics.

EVALUATING MICROBIOME THERAPIES WITH INTESTINAL CELL MODELS

Scott Magness, Ph.D., Altis Biosystems

ALTIS BIOSYSTEMS

Intestinal and Colonic Stem Cells and Disease

Stem cells are implicated in a variety of disease process. Before it is possible to understand what those roles are, it is necessary to understand the basic biology of stem cells: how they work and how they renew themselves and differentiate. With that information in hand, it is possible to investigate the differences between normal physiology and disease states to determine what goes

wrong with stem cells during disease processes and ultimately to evaluate how different treatments for disease can impact stem cells.

For instance, radiation or chemotherapy treatments for cancer can have significant off-target effects involving intestinal and colonic stem cells, because the gut is one of the first areas of the body negatively affected by these therapies. With sufficient understanding of the normal physiology of these stem cells, there is considerable potential to preempt toxicities that occur as the result of various therapeutic regimens.

The obvious first step in this endeavor was the development of platforms based on primary human epithelial stem cells that can be used for drug screening. Historically, colonic cancer cells (Caco-2) have been used for decades to study drug absorption in the small intestine, but these models do not always mimic normal physiology, and many failures of Caco-2 as a preclinincal model have resulted. By contrast, new methods using gut epithelial tissue cultured from patient stem cells could be used not only to investigate their physiology but also construct a more effective platform for screening drug candidates.

Why Gut Microbes Matter

Beyond the epithelial lining itself, there are millions of microbes living in the gut lumen, many of which are vital to human health, and some that can cause serious illness; and indeed, the microbiome has been shown to be involved in a vast array of disease processes. In addition, microbial species present in the gut differ not only by disease states, but also from person to person, which can even change over the lifetime of the individual.

Researchers have isolated different gut microbes from healthy and sick people and sequenced their genomes, but, since much of this research is only correlative, their remains a lack of direct experimental evidence regarding the functional roles these gut microbes play. Understanding the direct and indirect interactions between intestinal and colonic epithelial cells and gut microbes could enable more effective drug development.

Another of Altis's cofounders, Nancy Albritton, developed the "SIMPLE" platform, a self-sustaining, intestinal microbiome platform in her laboratory at the University of North Carolina at Chapel Hill. This device allows the basal surface

One of the most important functions of the human gut is preventing components from within the gut from entering the bloodstream, and the RepliGut® system provides this barrier function.

Solving an Engineering Challenge

The question then becomes one of developing a method to empirically test — in a dish — some of the assumptions being made between microbiome sequencing data and human health. The bottleneck to doing so has been the fact that the majority of bacteria that grow in the gut are intolerant to oxygen, but gut cells require oxygen. If oxygen is removed to study the bacteria, the host cells are killed. If oxygen is used to maintain growth of healthy host cells, the bacteria die.

Altis Biosystems tackled this challenge one step at a time. The first goal was to culture bacteria tolerant to oxygen on the RepliGut® system, which comprises mammalian cells grown on a two-dimensional surface separated by two accessible compartments — commonly known as a Transwell system, here cells are cultured on a permeable membrane with a proprietary hydrogel suspended in a tissue culture well — one compartment on top (facing the apical surface) and the bottom compartment (facing the basal surface).

Bacteria interact with cells on the top (luminal) side of the system. By compartmentalizing the tissue, we are able to apply bacterial compounds, microbiota, or their metabolites to the appropriate sides of the cells as in the human body. Using proprietary methodologies developed at Altis to recapitulate the lining of the gut in all ways possible, the bacteria are cultured on the 2D surface to form a mucous layer.

of the cells in the RepliGut® system to receive oxygen while oxygen is removed from the luminal side. This is possible because the cells exist in a polarized monolayer that grows across the entire well from edge to edge to form a barrier.

One of the most important functions of the human gut is preventing components from within the gut from entering the bloodstream, and the RepliGut® system provides this barrier function. The oxygen on the basal surface of the cells does not readily move to the top. It has to diffuse through the layer of cells, where it is mostly metabolized. The fraction that reaches the top is scavenged by transporter proteins.

As a result, the cells that require oxygen and the anaerobic bacteria can survive in the same environment. With this approach, we were then able to culture anaerobic cells on the RepliGut® to investigate the impact of the microbiome on intestinal and colonic stem cells.

A More Physiologically Relevant Model

Of course, the human gut comprises not just one cell type, but multiple differentiated cells that each have a specific function. The vast majority of cells in the gut are absorptive; they absorb things from the gut lumen, including food, vitamins, water, and salts. A small minority of cells are secretory cells, and there are approximately four different classes.

These differentiated cells must be present in a model system in order to fully investigate how

bacteria interact with the gut. Screening a prebiotic, probiotic, or a compound or drug against only one type of cell will not provide the entire picture of what occurs in the human intestine, therefore, a complex and complete in vitro tissue model comprising all the different cell types found in the human intestine and colon is desirable and more physiologic.

Colonic cancer cells (Caco-2) traditionally used to investigate gut function do not fully differentiate into all the different cells found in the small intestine and colon, and they divide indefinitely, unlike the vast majority of cells in the gut, which do not divide at all. Additionally, Caco-2 cells are derived from mutated colon cells and not from the small intestine. As such, Caco-2 many times fail to accurately recapitulate normal physiology of small intestinal biology.

When combined, all the features of the RepliGut® system are likely going to much more closely replicate what takes place in the normal human being.

Considering the Microarchitecture

A question remains about the extent to which increasing the sophistication of the microarchitecture is truly beneficial in an in vitro culture system. The small intestine has protrusions (villi) that increase the epithelial surface area that absorb nutrients from the food, which must occur efficiently within the first 15 feet of the gut. We have been able to recapitulate this microarchitecture in the lab using microfabrication techniques, however, translating microfabricated villi to a commercial product is worth considering from a drug screening and valdation standpoint. Currently, it remains unclear whether incorporating this type of microarchitecture is important for the vast majority of drug screening and validation. Altis is exploring whether there is biological and practical merit for including the villus microarchitecture based on whether it provides more accurate readouts, and whether the added sophistication is necessary for the majority of drug screening and validation assays.

Assay Applications

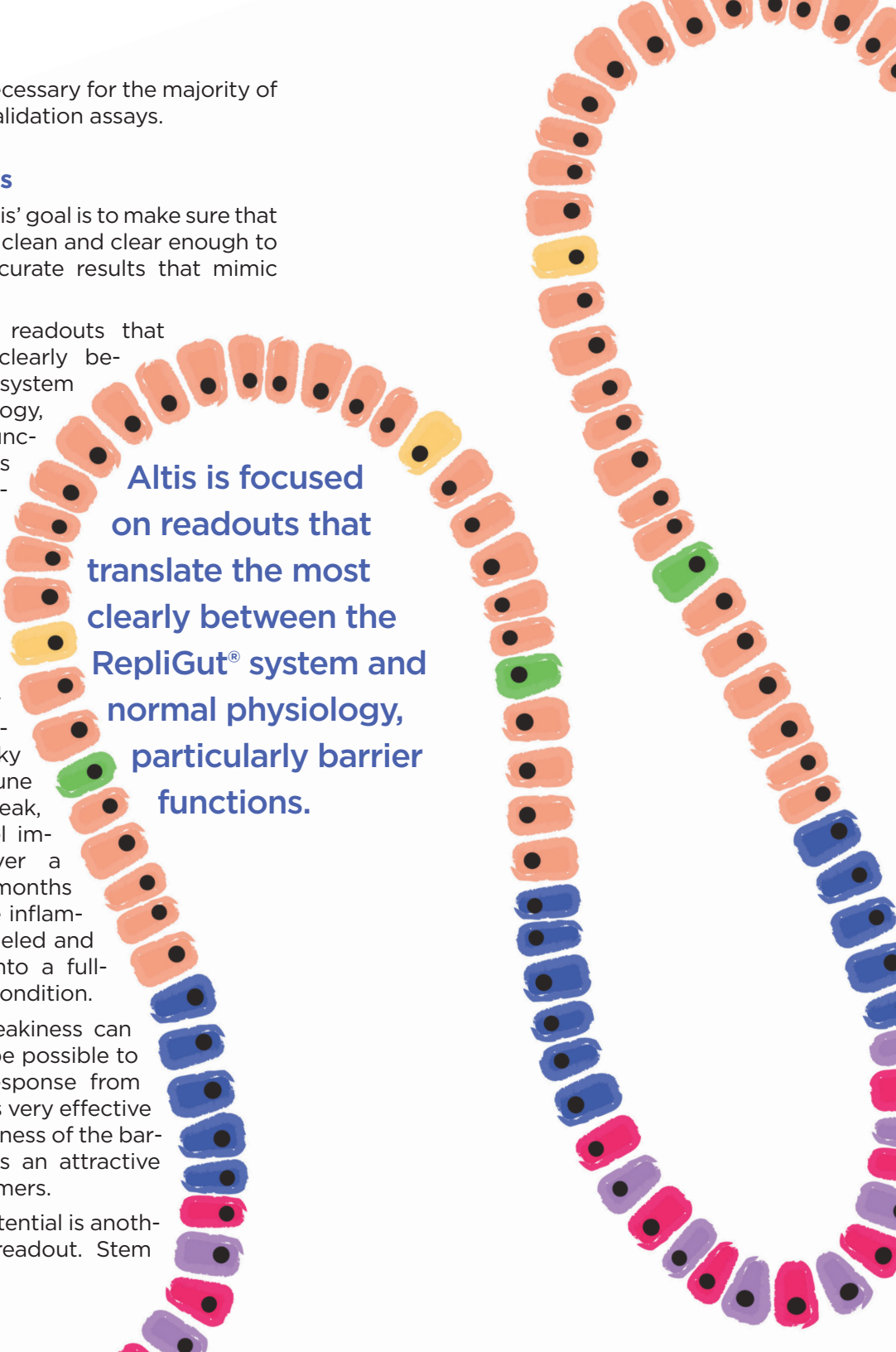
For the time being, Altis' goal is to make sure that all assay readouts are clean and clear enough to provide the most accurate results that mimic normal physiology.

Altis is focused on readouts that translate the most clearly between the RepliGut® system and normal physiology, particularly barrier functions. Many diseases — inflammatory bowel disease, and even liver disease — can result from a loss of epithelial barrier function — sometimes called a 'leaky gut'. Patients often do not realize they have a leaky gut, because it is just leaky enough that immune cells detect the break, leading to a low-level immune response. Over a long period of time (months to even decades), the inflammatory response is fueled and eventually matures into a full-blown inflammatory condition.

Altis is focused on readouts that translate the most clearly between the RepliGut® system and normal physiology, particularly barrier functions.

If the cause of the leakiness can be identified, it may be possible to keep the immune response from occurring. RepliGut® is very effective at measuring the leakiness of the barrier function, which is an attractive use for existing customers.

The differentiation potential is another important assay readout. Stem



cells must differentiate to establish different cells types that perform different functions. If a chemotherapeutic compound is toxic to normal stem cells in the gut, it will kill rapidly dividing stem cells, preventing sufficient differentiation and renewal of the gut lining, thereby reducing the barrier function causing a leaky gut. RepliGut® provides the ability to look for cell types that reflect off-target toxicity of different cell types found in the gut lining, including those that provide absorptive and secretory properties.

In addition to these discrete physiological readouts that have been vetted for RepliGut®, Altis has a number of others under development. For instance, we are looking at hormone release upon exposure to certain dietary compounds, microbiome products, or other materials. A robust assay for hormone release from enteroendocrine cells could be used to determine if an anti-hunger probiotic causes hormone-producing cells to tell the brain the stomach is satiated — a simpler and more natural approach than what is available today.

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Tackling the Question of Recapitulation

Recreating the full microbiome ecosystem in a gut model is difficult, but it is an area of active research for Altis. It requires populating RepliGut® to mimic the normal gut microbiome and the complexity of the various microbial communities. Even the best of the microbiologists in the world do not know how to do that yet, so developing a commercialized product is very challenging.

Altis is deconstructing components of the microbiome to enable testing of monocultures (single microbial strains) to see how they perform on RepliGut® and what specific conditions they need to survive and function. Those questions have to be answered before we can determine whether it will be possible to populate the system with normal human complex microbial communities and maintain the populated model in a dish over the length of the experiment.

It has been shown in conventional organoid culture systems where full fecal bacterial communities are injected into the organoids lumen that changes in the microbial communities occur during the first 24 hours, but then the complex community remains stable for the next 72 hours. The question to be addressed now is what needs to be changed in RepliGut® to enable this type of behavior and ideally even better performance that mimics the lumen in the human intestine and colon.

For instance, in the RepliGut® system, we can create a thicker mucous layer that allows mucophilic microbial strains to populate in the same manner they do in the gut. There also may be unknown compounds secreted from differentiated cells present in RepliGut® but not in the organoids that could inform the behavior of microbial communities. There is clearly significant work still to be done in this area.

Seeking Answers with RepliGut®

One high-profile goal in the scientific community is to understand what direct differences in the microbiomes between healthy and sick people lead to common diseases. Possible solutions to human health conditions related to the microbiome could be biological drugs that could target certain bacteria or inhibit enzymes that certain bacteria make. They could also be small molecules taken in pill form that change bacterial ratios, inhibit toxins, or promote bacterial secretion of healthy metabolites. These questions could be answered with a platform like RepliGut® once we can add the microbial species that cause a given disease on the platform, enabling us to perform large screens and targeted screens and validation.

Altis is using RepliGut® for the development of research tools using transgenesis and CRISPR-Cas9 gene editing. We have begun a new area of research to use gene editing to generate commercializable reporter gene cell types to evaluate the impacts of drugs on physiology and also to create genetic disease models for drug companies to test compounds for efficacy and off-target effects.

In one project, we are developing a toolbox of reporter genes in small intestine or colonic epithelial cells that indicate or "report" whether a specific physiologic condition exists, such as barrier defects, inflammation, absorption, or differentiation. The behavior of key genes involved in these health conditions and physiological processes are tracked using a fluorescent protein and can be readily detected in live cells under the microscope, allowing the high-throughput screening of thousands of cultures per hour. They will provide a nice tool for drug companies to screen compounds because they offer an easy, fast readout.

How microbes regulate nutrient handling along the GI tract is an emerging focus area for pharma. The Altis research and development team is also trying to recreate complex physiologic processes, such as fatty acid absorption, on the RepliGut® system to study the impact the microbiome has on them. The ultimate goal is to leverage what we know about the basic biology of fatty acid absorption, metabolism, and secretion and then establish the appropriate readouts from a platform-development perspective to ultimately create tools that can be commercialized.

Facilitating Microbiome-Based Therapy Development and Commercialization

At Altis, both our scientific and management teams understand how to efficiently and accurately translate the basic science into the commercial space. While Altis has a forward-looking vision for very sophisticated drug screening platforms, it really focuses on developing products based on where the vetted biology and science exist. In Altis' view, this approach provides a more predictable, accurate, and reliable product that is portable to many existing drug screening systems and can fit into a broad range of academic and industry research facilities without the requirement for expensive and complex peripheral instrumentation.

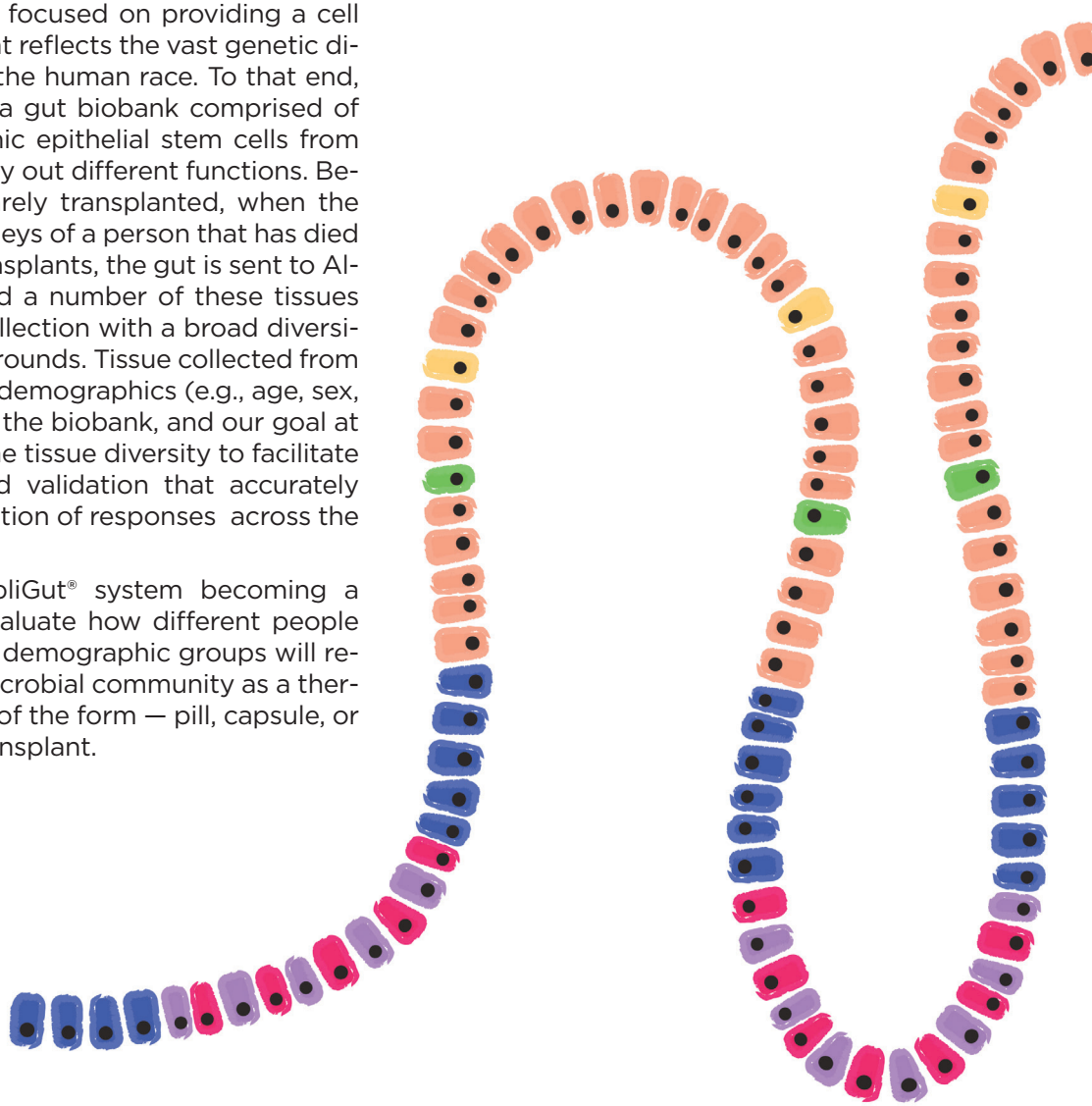
Going forward, Altis expects that some generalized probiotics will be determined to work in certain mechanistic ways and achieve certain responses. The result will be more refined definitions of what probiotics are doing to the microbiome from scientific and medical perspectives that can be vetted by the FDA and lead to approvals as drug products.

There is also a much more targeted approach in which microbiota are engineered specifically to treat certain diseases. Fecal microbiota from healthy donors have already been approved for transplant. However, this type of treatment is still in its infancy, questions remain about this technique, and some problems have occurred in the clinic. Establishing a platform to test the efficiency of FMT colonization and stability in an in vitro system is on the short list of assays under current consideration and could represent an additional level of safety monitoring for FMT.

Altis believes that, if better models are available for answering key questions about how the microbiome impacts the gut with respect to the development of disease conditions, it will be possible to develop therapeutics that offer more reliable and predictable outcomes for those targeted diseases.

That is why Altis is focused on providing a cell culture platform that reflects the vast genetic diversity comprising the human race. To that end, Altis is developing a gut biobank comprised of intestinal and colonic epithelial stem cells from six regions that carry out different functions. Because the gut is rarely transplanted, when the heart, liver, and kidneys of a person that has died are donated for transplants, the gut is sent to Altis. Altis has banked a number of these tissues and is building a collection with a broad diversity of genetic backgrounds. Tissue collected from donors of different demographics (e.g., age, sex, race, BMI) make up the biobank, and our goal at Altis is to expand the tissue diversity to facilitate drug screening and validation that accurately represents the variation of responses across the human population.

Altis sees the RepliGut® system becoming a routine assay to evaluate how different people across a number of demographic groups will respond to a given microbial community as a therapeutic, regardless of the form — pill, capsule, or fecal microbiota transplant.



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