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CELLULAR AND MOLECULAR GASTROENTEROLOGY AND HEPATOLOGY

COMMENTARY

Organoids and Engineered Organ Systems

This introduction to the special *Cellular and Molecular Gastroenterology and Hepatology* series *Organoids and Engineered Organ Systems* summarizes original research articles, reviews, and commentaries published within the journal over the past year. A few studies remain to be published, but the series is nearly complete and can be accessed at cmghjournal.org/content/collection_organoids.

The revolution initiated by the availability of organoids and engineered organ systems has transformed the research landscape for gastrointestinal, liver, and pancreatobiliary scientists. These powerful new in vitro cellular models to study mechanisms of human gastrointestinal (GI) physiology and pathophysiology have advanced rapidly as a result of collaborative interactions between bioengineers and cell biologists; some of these astonishing advances are included in the special article series. Other contributions highlight the breadth of the topic and show the broad impact that this technology is having on scientific discovery and therapeutic developments in our field.

Organoid cell culture technologies take advantage of basic discoveries relevant to stem cell biology and GI tissue development to design culture strategies to sustain the growth of human organ-specific stem cells or to instruct pluripotent stem cells to follow developmental cascades and generate organotypic structures. In parallel, bioengineers and materials scientists have explored novel microfabrication and microfluidic strategies that, when combined with these primary human stem cell cultures, have led to innovative culture models of digestive organs. These dynamic culture systems, commonly containing both organ-specific stem/progenitor and differentiated cells, have been established for human intestine, colon, stomach, esophagus, liver, biliary tissue, and pancreas, providing a major improvement over traditional transformed cell lines derived from GI cancers that have served as the dominant in vitro cell model for many decades.

Cultured GI organ models are being used to advance our understanding of a wide array of physiological functions and diseases, providing insights into stem cell biology, mechanisms of cellular differentiation, transport physiology, disease pathogenesis, drug discovery, cancer genetics, inflammatory mechanisms, and more. It is particularly encouraging that bioengineers, biologists, and clinicians are working together to advance this emerging area, each bringing their specialized perspectives and expertise to work toward engineering physiologically relevant human GI organ cell culture systems to model aspects of human GI biology and disease. We are especially pleased that this special series of *Cellular and Molecular Gastroenterology and Hepatology* captures this diversity in thought and approach, including articles from different expert viewpoints studying a variety of GI organ systems and describing new technological platforms and materials, which together have contributed to several basic scientific discoveries.

This topical collection succeeds at multiple levels by providing both in-depth subject reviews for those seeking to learn more about this quickly developing area as well as cutting edge original research articles from leading investigators. Two review articles in particular provide key summaries of GI organoid technology from the bioengineering and developmental biology perspectives. Gural et al[1] provide a comprehensive view of 2-dimensional (2D) and 3-dimensional culture platforms used to study human liver-dependent infectious diseases in their article entitled "Engineered Livers for Infectious Diseases." This review discusses how these various human liver culture platforms serve as essential tools for biological and therapeutic discovery. The investigators highlight advances in the study of hepatitis and malarial parasites, using these novel culture systems to model human liver cell responses to these important human pathogens.

Wells' laboratory has used insights from developmental biology and pluripotent stem cells to develop novel culture systems of human luminal organs (stomach, intestine, and colon). With co-authors, Wells discusses mechanisms of stomach development and examines how developmental signaling gradients can be manipulated in culture to induce human pluripotent stem cells to differentiate into human gastric tissue in culture.[2] The review, entitled "Translating Developmental Principles to Generate Human Gastric Organoids," highlights the importance that knowledge of basic developmental mechanisms and cell biology plays in developing strategies to generate complex organotypic cultures. Importantly, organoids generated from human pluripotent stem cells include organ-specific stromal and epithelial cells and thereby provides a potential system for exploration of epithelial-mesenchymal cross-talk during organogenesis and in disease.

From the primary research publications it was difficult to select which ones to highlight for this commentary. These primary research studies show the exciting breadth of this maturing and critically important field. Various contributions explore tissue-engineering advances as well as basic and translational discoveries made possible through the use of cultured GI organ systems representing human colon, liver, intestine, and esophagus. Microfabricated organoid culture systems often are designed to allow high-throughput analysis for small-molecule screens, toxicology studies, or drug development. Thus, these in vitro systems show great promise for in vitro reproduction of in vivo human cellular responses, which will be an important component of future drug discovery pipelines.

One of these studies that best represents the intersection of basic cell biology with tissue microfabrication and bioengineering was provided by the Magness and Allbritton

laboratories. The work, entitled "Formation of Human Colonic Crypt Array by Application of Chemical Gradients Across a Shaped Epithelial Monolayer," describes a new bioengineered model of the human colonic epithelium.[3] Together these 2 groups, with strengths in stem cell biology and microfabricated platforms, engineered a human colon culture system designed to mimic fundamental aspects of tissue structure. Collagen scaffolds, molded to the shape of human crypts, were seeded with human colonic epithelial stem cells. Microfluidic methodologies were used to establish opposing Wnt and bone morphogenetic protein (BMP) signaling gradients that supported stem/progenitor cells at the base and differentiated cells at the top, thereby replicating in vivo morphology in vitro. To further validate this in vitro system, the investigators assessed responses to short-chain fatty acids and inflammatory mediators and found that they were similar to those observed in vivo.

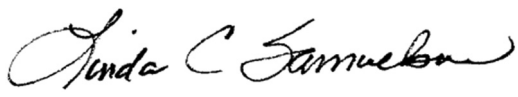
Equally exciting is the adaptability of the primary tissue organoids into diverse research platforms, including 3-dimensional, 2D, and tissue chip approaches. An advance in human intestinal tissue chip technology is reported in an article from Barrett's laboratory entitled, "Enhanced Utilization of Induced Pluripotent Stem Cell-Derived Human Intestinal Organoids Using Microengineered Chips."[4] To improve the accessibility of human intestinal cells for high-throughput drug and toxicology screens, Barrett's group seeded chips with intestinal epithelial cells isolated from human pluripotent stem cell–derived intestinal organoids. This would be a reliable and uniform cell source for the mass production of human intestinal chips. In contrast, the report from Donowitz's group entitled, "Molecular Basis and Differentiation-Associated Alterations of Anion Secretion in Human Duodenal Enteroid Monolayers," used a different approach and plated human intestinal organoids in 2D monolayers (which are more convenient for electrophysiological analysis) to define key mechanisms of anion secretion associated with cellular differentiation.[5] Both of these studies afford new opportunities not available with transformed human colon cancer cell lines.

Another cell biological advance is reported by the Khetani group in "A Cell Culture Platform to Maintain Long-Term Phenotype of Primary Human Hepatocytes and Endothelial Cells."[6] This study highlighted a current theme to develop better organoid models by including other relevant cell types in co-culture with GI epithelial cells. Here, they created cultures that included primary human hepatocytes, primary human liver sinusoidal endothelial cells, and mouse 3T3-J2 fibroblast cells to build a more physiologically relevant organotypic system. The tricultures were stable for weeks, showing greater differentiated cellular function than prior cultures. The complexity of cell–cell interactions in these cultures may provide a platform to explore how these contacts support hepatocyte survival and differentiated cell function. Co-culture approaches such as these are currently being used widely in different cell and organ contexts to uncover mechanisms of cell–cell or microbe–cell signaling.

Last, but by no means least, the Spence group reported on a novel method of organoid production that greatly impacts clinical and translational research. In "A Method for Cryogenic Preservation of Human Biopsy Specimens and Subsequent Organ Culture,"[7] they described a practical method to cryopreserve live human biopsy tissue, which is simple enough to be adopted in the clinics. These frozen human specimens then can be stored or shipped frozen and later thawed to generate new cultures of gastrointestinal epithelial organoids (stomach, intestine, colon). They show that this methodology is robust and efficient for biopsy specimens from human beings aged 2 to 70 years, yields organoids no different from those grown from fresh nonfrozen biopsy specimens, and show that frozen biopsy specimens can be shipped on dry ice with no loss in culture efficiencies. We anticipate this approach will be rapidly assimilated and widely used in clinical centers, further driving interest in human organoid research and allowing study of patients around the world.

Together, this collection highlights some of the important contributions that GI organoids and engineered organ systems have had on our field, and their great potential for highly impactful contributions in the future. Organotypic GI culture models are fueling breakthroughs in the study of human GI development, physiology, pathophysiology, therapeutics, and toxicology. These technologies establish experimentally tractable and physiologically relevant systems to understand human cellular responses, model human diseases, and provide important alternatives to approaches using human cancer cell lines and animal models.

It is exciting to see bioengineers and GI scientists working in close collaboration to advance these model systems. Through this shared effort, the future of GI research is bright and made even more powerful when combined with Clustered Regularly Interspersed Short Palindromic Repeat (CRISPR)/CRISPR-associated protein 9 gene editing approaches to engineer organoid models of human genetic diseases and to tease out the molecular and cellular mechanisms of GI function by manipulation of specific genes and signaling pathways. The next generation of novel human GI organoids along with enhanced microfabrication techniques will yield new insights and provide innovative laboratory platforms to continue the advancement of our understanding of human disease pathogenesis and therapeutics.



LINDA C. SAMUELSON, PhD
Department of Molecular and Integrative Physiology
The University of Michigan
Ann Arbor, Michigan

JOHN P. LYNCH, MD
Janssen Research and Development
Spring House, Pennsylvania

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Correspondence
Address correspondence to: Linda C. Samuelson, PhD, Department of Molecular and Integrative Physiology, The University of Michigan, Ann Arbor, Michigan 48104. e-mail: lcsam@umich.edu.

Conflicts of interest
The authors disclose no conflicts.

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