“Good Fences Make Good Neighbors”: How does the Human Gut Microchip Unravel Mechanism of Intestinal Inflammation?

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ABSTRACT
A microengineered human gut-on-a-chip has demonstrated intestinal physiology, three-dimensional (3D) epithelial morphogenesis, and longitudinal host-microbiome interactions in vitro. The modular accessibility and modularity of the microphysiological gut-on-a-chip can lead to the identification of the seminal trigger in intestinal inflammation. By coupling microbial and immune cells in a spatiotemporal manner, we discovered that the maintenance of healthy epithelial barrier function is necessary and sufficient to demonstrate the homeostatic tolerance of the gut. Here, we highlight the breakthrough of our new disease model and discuss the future impact of investigating the etiology and therapeutic targets in the multifactorial inflammatory bowel disease.

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Introduction
Intestinal inflammation is caused by complicated intercellular crosstalk between the gut microbiome, intestinal epithelium, and immune cells. Due to the complexity of the inflammatory microenvironment, there has been a lack of clear understanding of the etiology and progression of the disease. Various experimental models, including in vitro, ex vivo, and in vivo surrogates, have been extensively used to elucidate the disease causality. However, all these models come with several limitations. In vitro models, including a static dish culture and a 3D organoid culture, not only lack physiological legitimacies, such as multi-cellular interactions, shear stress, and mechanical motions but also cannot support stable co-culture of living host and microbial cells. In vivo models prove a difficulty in one-at-a-time disease dissection because all comprising elements are interconnected systemically. More importantly, different physiological conditions and pathophysiological responses significantly compromise cogency when using animal models. These existing challenges in conventional models have led to the development of a human gut inflammation-on-a-chip model.

The human gut inflammation-on-a-chip was inspired by the dextran sodium sulfate (DSS)-induced colitis model in mice and adapted from the original human gut-on-a-chip model. In the human gut inflammation-on-a-chip model, DSS is administrated to intestinal villous epithelium lined within a human gut-on-a-chip microdevice. The gut-on-a-chip is a microfluidic human intestine model that precisely emulates the epithelial barrier function and intercellular host-microbiome interactions using controlled fluid flow and mechanical deformations. Intestinal epithelium cultured in a gut-on-a-chip develops into 3D villous microstructures, demonstrating spatially differentiated epithelial phenotypes reminiscent of in vivo characteristics. Furthermore, physiological functions of the intestine such as mucus production, drug metabolizing activity, and glucose reuptake are demonstrated and validated through previous studies. The gut inflammation-on-a-chip model harnesses the basic functionality of the gut-on-a-chip model by providing strong spatiotemporal manipulability. The model comprises of elements of the microenvironment that can be precisely...
modulated to dissect the spatial and temporal manifestation in the human inflammatory disease.

**Innovation of the gut-on-a-chip to study disease mechanism**

Upon the emergence of the microfluidic human organ-on-a-chip technology, various microphysiological systems have been developed to model the human intestine. To our knowledge, our gut inflammation-on-a-chip is the first model that leverages the human gut-on-a-chip to mechanistically understand the complex disease etiology and pathophysiology of inflammatory bowel diseases (IBD). A majority of the reported tissue-engineered intestine models have primarily focused on the structural mimicry or the display of tissue-specific functions. For instance, some studies cultured human intestinal epithelial cells on the surface of the pre-formed 3D hydrogel scaffold that mimics structural characteristics of the intestinal villi; however, the static nature failed to exert mechanically dynamic milieu in the intestine. The human gut-on-a-chip, on the other hand, supports to spontaneously reform 3D intestinal epithelial microarchitecture from the initial planar cell monolayer. Furthermore, we identified the mechanism of 3D villous morphogenesis, using both Caco-2 cells and organoid-derived primary intestinal epithelium, that occurs in the gut-on-a-chip. It is evident that the microfluidic flow contributed to removing morphogen antagonists (e.g., Dickkopf 1) and induces upregulation of morphogen receptor (e.g., Frizzled 9) that synergistically enhance villous growth of epithelial cells in vitro. Pathophysiological features such as pathogenic infection, bacterial overgrowth under a cessation of intestinal bowel movements, and inflammatory responses have been demonstrated as well by involving the luminal (e.g., gut microbiome) and abluminal components (e.g., peripheral blood mononuclear cells, PBMC). In the study that harnessed the gut inflammation-on-a-chip, we not only reconstituted physiological structure and function of the gut but also leveraged a microengineering approach to dissect the disease elements to find the trigger of pathological cascades. The method of spatiotemporal decoupling and recoupling the disease contributing factors can be applied to other organ and disease models that can potentially solve challenging problems in the in vivo and clinical studies.

**New findings in human intestinal inflammation**

It has been unclear how DSS exactly causes gut inflammation in mice and rats, although histological analyses have shown shortened colon length and loss of goblet cells after DSS exposure. We found that DSS does not have cytotoxic effects on intestinal epithelium nor does it compromise cell viability, but rather exclusively disrupts barrier integrity. Intestinal barrier function was also recovered when DSS treatment was ceased, demonstrating intestinal barrier integrity could be accurately manipulated in the gut inflammation-on-a-chip model. This finding was also unprecedented because we could exclude the effects of other influencing factors in the intestinal microenvironment, unlike animal studies. Knowing that we could now manipulate the intestinal barrier integrity experimentally using our new model, we were able to perform several studies in terms of the etiology of gut inflammation.

We postulated that investigation of the effects of each inflammatory component independently would enable us to identify the trigger of intestinal inflammation. We first decoupled all the complex intercellular crosstalk mechanisms from the gut inflammation-on-a-chip model, then combinato rially recoupled the contributing elements spatiotemporally. Using this approach, we discovered that barrier dysfunction could cause the onset of intestinal inflammation. Under conditions involving the disruption of the intestinal barrier, immune elements (e.g., PBMC) and intestinal epithelium showed elevated levels of oxidative stress; PBMCs responded even to the physiological levels of luminal components such as lipopolysaccharide (LPS, 10 ng/mL) and non-pathogenic *Escherichia coli*. This finding and approach are unique because no other studies could investigate the effects of barrier dysfunction independently in a physiological experimental setup. Another discovery was that oxidative stress does not always lead to inflammation, where involvement of luminal contents is a crucial element that elicits inflammatory responses. It has been
known that inflammation and oxidative stress are inter-dependent. However, in the gut inflammation-on-a-chip model, we discovered that the presence of luminal components such as lipopolysaccharide (LPS) or gut bacteria in the barrier dysfunctional-gut is necessary to develop the inflammatory response.

**Potential impacts on clinical studies and pharmaceutical applications**

The human gut inflammation-on-a-chip allowed us to remarkably improve several issues in current mouse models, including the discrepancy between human and mouse physiology and microbial populations, as well as the variability that stems from different mouse strains. While the current DSS-induced colitis model in mice is robust and has pathological outcomes similar to that of IBD patients, there are critical limitations. Responses to DSS treatment widely vary upon mouse strains, housing conditions, or sex. Physiological characteristics and microbial population in mice are different from humans as well. On the contrary, the gut inflammation-on-a-chip model not only closely recapitulates pathophysiological features of gut inflammation but also has less variability arising from the factors in animal studies. As a result, the gut inflammation-on-a-chip model can be considered as a reliable platform that can potentially replace the DSS-induced colitis model in mice and minimize the gap between animal and clinical studies for IBD researches.

The gut inflammation-on-a-chip also successfully demonstrated that the disrupted barrier function directly elicits intestinal inflammatory responses. It has been implicated that IBD patients have impaired barrier function. For example, autophagy-associated genes such as ATG16L1 and IRGM are known to be mutated in Crohn’s disease patients. Defects in autophagy compromise the ability to degrade undesired components, such as bacteria, that can potentially cause inflammation. Barrier dysfunction in the leaky epithelial barrier increases the chances of interactions between luminal components and infiltrated immune cells, which substantially increases the manifestation of chronic inflammation. Furthermore, a compromised barrier function also allows higher oxygen levels in the lumen, which considerably perturbs the anaerobic microbial population in the commensal gut microbiota and induces higher oxidative stress to the tissue. An altered microbial population, due to the compromised anoxic microenvironment, substantially leads to the compromised short-chain fatty acid (SCFA) production. All of these perturbations of barrier function were proven to lead to inflammation of the intestinal tract.

The gut inflammation-on-a-chip model can additionally be used to examine the efficacy of probiotic therapy for IBD. Probiotic therapy is a promising approach to treat IBD, but most of the clinical trials have failed to validate its therapeutic efficacy. In contrast, both in vitro and in vivo assessments showed beneficial effects of various probiotics. In the gut inflammation-on-a-chip, administration of an over-the-counter probiotic formulation VSL#3 successfully augmented intestinal barrier function as well as showed protective effects from DSS. However, we found that the barrier-dysfunctional epithelium allows probiotics to migrate toward the vascular microchannel and induce a substantial production of pro-inflammatory cytokines in the basolateral side, suggesting that “good fences make good neighbors.” This finding is striking, but there already have been several reports that probiotic bacteria can even induce sepsis when an IBD patient has severe luminal injury. These reports show a good agreement with our experimental observation in the gut inflammation-on-a-chip model. The most effective strategy of probiotic administration, such as dosage and administration timing, has also been challenging to determine. The gut inflammation-on-a-chip can be used to solve this issue by enabling us to screen working probiotic strains or combinations of prebiotic and probiotic strains (e.g., synbiotics) to assure the efficacy and safety of probiotic therapy in various gastrointestinal diseases.

**Possible limitations**

From a “reverse engineering” standpoint, reductio of biological complexity in the design of a model is necessary. The biomimetic design principle needs to verify if this “reductionist’s approach” has a rationale to recapitulate the target structure and function in the bioinspired model. Although our gut inflammation-on-a-chip
successfully recapitulated the inflammatory pathophysiology and expounded the disease mechanism, there are notable limitations that may need to be improved in future studies. For instance, our model does not contain other tissue-specific cell types such as connective tissue components (e.g., fibroblasts), submucosal and myenteric plexus (e.g., enteric sensory neurons), muscularis (e.g., smooth muscle cells), or capillary and lymphatic microvasculature (e.g., endothelium) in the device. However, a lack of these elements does not necessarily induce limited functionality in the model because we validated the intercellular crosstalk in response to each experimental variable under the precise control of luminal, mucosal, and submucosal components in a defined space and time. We anticipate that increasing the biological complexity one element at a time will help to clarify the role of each factor on the overall disease outcome.

Tissue-specific resident immune cells including dendritic cells, macrophages, intraepithelial lymphocytes (IEL), or innate lymphoid cells (ILC) surveil and induce inflammatory responses in the intestinal submucosal microenvironment. However, because we used the blood-derived PBMC, our study might not fully demonstrate the role of specific immune subsets to induce the onset of pathogenesis in the inflammatory milieu. Thus, reflecting these components in the gut inflammation-on-a-chip can potentially allow for a more meticulous investigation of the disease mechanism.

In the gut inflammation-on-a-chip study, we used a Caco-2 immortalized cell line as an intestinal epithelial source. Although Caco-2 epithelium has successfully demonstrated in vivo relevant villous morphogenesis and physiological intestinal functions in the gut-on-a-chip microdevice, the use of patient-derived cells including intestinal epithelium, immune cells, and gut microbiome will more closely reflect the patient’s microenvironment to study patient-specific disease ecosystems.

**Future directions and perspectives**

We envision that the approach that leverages patient-derived cells will contribute to validating a patient-specific therapeutic strategy as the Precision Medicine perspective. One of the advantages of this approach is that we can reflect the genotypic and phenotypic background of individual patients, where the heterogeneity in various patient cohorts can also be contemplated. In this standpoint, adapting the single-cell analysis (e.g., single-cell genomics and transcriptomics) with patient-specific IBD pathomimetic models can potentially contribute to building the human gut cell atlas, which is a collaborative effort worldwide to define all cell populations in the human gut that are interacting to the microenvironmental factors.

Also, the gut inflammation-on-a-chip device can be connected to additional in situ detection modules to potentially detect secretomes from the host cells and microbiomes such as SCFAs, inflammatory cytokines, or reactive oxygen species. It is noted that the secretomes released by the cells can be collected directionally. This approach will enable faster and more effective detection of inflammatory responses during the host-microbiome crosstalk in the disease model.

The gut inflammation-on-a-chip system can be considered as a reliable platform to evaluate bacteria-based therapeutic interventions. In addition to probiotic therapy, our model can be used to validate the efficacy and safety of fecal microbiota transplantation (FMT). Although FMT is valid for the therapeutic control of *Clostridium difficile* infection, no other efficacy studies have been conducted with success. Because the therapeutic efficacy of FMT to IBD is still very elusive, we envision that the enabling technology in the patient-specific gut inflammation-on-a-chip will be useful for evaluating the potential of FMT in IBD and potentially other GI diseases such an irritable bowel syndrome, infection, colitis, or celiac disease. This experimental strategy is enabled by our recent progress to reconstitute the precise oxygen gradient in the anoxic-oxic interface (AOI) in the gut. We have recently demonstrated that creating an anoxic-oxic transepithelial gradient in a human AOI-on-a-chip can support stable coculture with anaerobic commensal gut bacteria, such as *Bifidobacterium adolescentis* and *Eubacterium hallii*, for up to a week. Because most of the gut microbiota are strict anaerobes, this new technology will be compelling to grow and stabilize the fecal gut microbiome in the device.

Altogether, the human gut inflammation-on-a-chip demonstrated an innovative approach to
study etiology and development of intestinal inflammation and proposes that microphysiological organ and disease models can be used to solve unanswered questions in clinical research. Further advancement of the system will allow the investigation of more patient-specific and precise pathophysiological responses, which could lead to the development of new, individualistic therapies.

**Disclosure of Potential Conflicts of Interest**

No potential conflicts of interest were disclosed.

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