



# Microphysiological systems for modeling gut-organ interaction

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**Key words:** Microfluidics, Gut-liver axis, Gut-brain axis, Microphysiological systems

**Abstract:** The gut is a digestive organ that absorbs nutrients but also plays a vital role in immune response and defense against external compounds. The complex interaction between the gut microbiota and other organs including the immune system of the host has been known in various contexts, yielding the notion of 'axes' between the gut and other organs. While the presence of various gut-organ axes has been reported, the lack of adequate *in vitro* model systems for studying this interaction has restricted a deeper insight into these phenomena. Recently developed microphysiological systems (MPS), also known as organ-on-a-chip, allow researchers to study complex interactions between diverse organs, and here we provide a review of how recently developed gut-on-a-chip systems are used for building models of various diseases that were difficult to study.

## Introduction

One of the key steps for orally administered drugs is the process of absorption in the gut and subsequent metabolism, which happens mainly in the liver but also occurs in the intestine, known as first-pass metabolism [1]. In addition, the gut has a close relationship with the whole body, acting as the first layer of defense against external compounds. The human intestine is a highly complex organ with a unique 3D architecture and diverse components comprising the system. It consists of different cell types, such as epithelial (enterocytes), endocrine, immune cells, as well as microbiota, making it a difficult organ to model [2].

Developing models of the human gut that faithfully reproduce the structure and the functions is important in terms of studying drug absorption and metabolism, as well as studying gut-related diseases. Currently, there is a wide range of *in vitro* models that aim to reproduce the intestinal functions with varying degrees of complexity, for example, a relatively simple, parallel artificial membrane permeability assay (PAMPA) to simulate the gut barrier function on one end [3], and intestinal membrane segments using tissue explants, which conserve the structure and functions of the native tissue [4]. Cell culture models are also widely used, such as Caco-2, which is a colorectal cancer-derived cell

line, but have significant limitations in terms of physiological relevance to the native tissues.

Most importantly, these models only aim to reproduce the gut microenvironment and neglect the important aspect of its interaction with other organs in the body. Being the first barrier to orally ingested compounds, an immunological organ, and a host to the gut microbiota implies that the gut has a complex mechanism of action and diverse roles within the body. This has led to the notion of various gut-organ axes. These gut-organ axes play crucial roles in maintaining the body's homeostasis, with organs such as the brain, liver, and skin being involved [5]. Despite the importance of this concept, it could only be observed in clinical settings or limited animal studies, due to the complex nature of the interaction between different organs in the body. One should also note that the mechanisms of the interaction are diverse, including molecular interaction by systemic circulation as well as neurological pathways between nervous systems [6].

Microphysiological systems (MPS), also known as organ-on-a-chip, are based on microfabrication and microfluidics technology, aimed to reproduce the microenvironment of human tissues to improve the physiological relevance of the models [7]. Initially, organ-on-a-chip technology has been developed to reproduce the microenvironment of specific organs or tissues. For example, a gut-on-a-chip attempts to recapitulate the important aspects of the human intestine, such as the 3D structural architecture [8], mechanical movements of the intestine such as peristalsis [9], and commensal gut microbiome residing in the intestine [10]. Several excellent review papers have been published for

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Received: 04 February 2024; Accepted: 10 May 2024;

Published: 02 August 2024



readers who are interested in recent developments in organ-on-a-chip technology [11,12].

Particularly, MPS comprising multiple organ components, often known as multi-organ-on-a-chip, can not only reproduce individual organ functions but also recapitulate the interactions between the organs [13]. While most up-to-date research on gut-on-a-chip systems has focused on reproducing the gut microenvironment, a few studies are emerging that capture the crosstalk between organs, although in a primitive manner. In particular, microfluidics-based systems are ideal for capturing the interaction between tissues via a vascular network [14]. Earlier, proof-of-concept studies have shown that reproducing the molecular interaction between organ components is feasible by implementing fluidic connections between them [15], which were followed by more advanced systems enabling more accurate recapitulations of molecular interactions in the body [16]. Although still at a preliminary stage, several important papers have been published showing prominent and interesting findings using such multi-organ-on-a-chip systems. In this review, we report on the recent progress in the development of multi-organ MPS, which we will name as gut-organ-axis-on-a-chip systems, aimed at reproducing the complex interactions of the gut with the whole body. The key element in achieving this goal is a faithful reproduction of the gut physiology and functions.

### Gut-Liver-on-a-Chip

Orally taken substances are absorbed through the gut epithelium, which is followed by hepatic metabolism in the liver. Known as the first-pass metabolism, this sequential action by the gut and the liver exerts a significant effect on the fate of orally administered drugs [17]. Due to the importance of the first-pass metabolism, several early attempts have been made to reproduce the gut-liver crosstalk, particularly in the context of drugs processed in the body. More recently, the pathological communication between the gut and the liver, for example, non-alcoholic fatty liver disease (NAFLD) or gut inflammation, has been studied, which we will discuss in more detail in the following sections. Here we summarize the recent progress in developing gut-liver axis-on-a-chip systems (Fig. 1).

The interactions of oral drugs in the gut and the liver have a profound effect on the pharmacokinetic (PK) profiles of the drugs. Therefore, an accurate reproduction of the dynamic interactions between the gut and the liver holds a key to successful drug development, as well as a better understanding of many gut and liver-related diseases. Conventionally, the absorption of drugs in the gut and the metabolism in the liver were assessed in separate processes and then combined to predict the fate of drugs, often with the help of mathematical models such as PK models.

Early primitive experiments to reproduce the first-pass metabolism of drugs by the gut and the liver in a single system were made, although in a very primitive form of co-culturing gut and liver cells in the apical and basal compartments of a Transwell insert, respectively [18]. This early attempt was followed by a more advanced format utilizing a fluidic connection between the gut and liver

compartment, where authors observed improved Cytochrome P450 (CYP) metabolic enzyme activity, possibly due to the stimulation by the perfusion of liquids [19]. With the advent of microfluidics technology in the early 2000s, more advanced microfluidic-based gut-liver systems using cell lines have appeared. A micro total bioassay system, developed by Yoshimura et al., was designed to work as a microfluidic system that sequentially assesses intestinal absorption, hepatic metabolism, and bioactivity [20,21]. Cell lines representing the respective organs were used; Caco-2 for the gut, HepG2 for the liver, and MCF-7 for breast carcinoma.

Shuler research group published several pioneering papers on developing multi-organ microfluidic systems [15,22]. Based on these early attempts, a microfluidic chip for studying the effect of orally taken nanoparticles on liver injury was published [23], which was further advanced by utilizing the integration of single-organ chips with modular design [24], and also expanded to accommodate up to 14 compartments [25]. Another study demonstrated a modified chip design mimicking the liver micro-lobe structure while co-culturing primary intestinal and hepatocarcinoma cells for up to 14 days [26].

Bricks et al. published several papers on what they called the Integrated Insert Dynamic Microfluidic Platform (IIDMP), a microfluidic chip for co-culturing gut and liver cells with fluidic connections [27–29]. Liver cells from different origins were compared for their metabolic activity and physiological relevance to humans. In particular, it is notable that a mathematical PK model was employed to calculate key parameters such as intrinsic clearance, which made it easier for authors to compare their results with clinical data. Another research group attempted a four-organ co-culture in a microfluidic setting, including the intestine, liver, skin, and kidney [30]. Although a quantitative study on the pharmacokinetics of drugs is lacking in this study, it is noteworthy that a long-term co-culture was demonstrated successfully. More recently, Choe et al. developed a gut-liver chip with gravity-induced passive flow for reproducing the oral drug's first-pass metabolism [31]. A simple, but useful two-layer design employed by the authors allowed easy and efficient co-culture of gut and liver cells in a microfluidic device while maintaining proximity between the two cells.

As illustrated by the early example by Prot [29], PK models are useful tools for analyzing the dynamic interaction between different modules in a chipset. The first-pass metabolism of acetaminophen was analyzed using a PK model, with parameters extracted from experimental data using a gut-liver-on-a-chip [32]. In this study, optimal design parameters were suggested that could theoretically enable more accurate reproduction of PK profiles of a model drug. This is an excellent example of utilizing a mathematical and numerical framework for interpreting the experimental data from the chip, as well as exploring various chip design parameters for improved predictions. Since organ-on-a-chip are essentially miniaturized systems of the human body, it inevitably carries the limitation of deviating from the original, due to the disproportionate scaling of different organs. Some researchers suggested

theoretical methodologies to approach this issue [33], with some pioneering, proof-of-concept works using model drugs [34–36]. In these papers, mechanistic model-based analysis was used to predict intrinsic PK parameters of diclofenac and hydrocortisone with some success. Although still at a preliminary stage, it has been demonstrated that prediction of key PK parameters using chip systems may be possible [37]. However, more robust and expandable mathematical principles are needed for wider acceptance of such systems.

The gut-liver communication plays important roles in diseased states as well. For example, the gut-liver axis has important implications in terms of NAFLD, with accumulating evidence pointing to a close relationship between the gut microbiome and inflammation [38]. One of the well-known examples is the short-chain fatty acids (SCFAs) produced by the gut commensal microbes suppressing inflammation. The gut-liver axis is also thought to affect the progression of cancer as well as metastasis. Lipopolysaccharides (LPS), which is the component of the outer membrane of Gram-negative bacteria are a ligand of innate immune receptors that can induce inflammatory responses [39]. The difficulty of recapitulating such complex interactions lies not only in the inherent complexity of the interaction, but also in the need to incorporate diverse components, such as the gut epithelium, hepatocytes as well as other supporting cells in the liver, and gut microbiota. Multi-organ-on-a-chip have demonstrated their usefulness for recapitulating the key interactions between different components. Gut and liver cell cultures connected with fluidic circulation recapitulated the migration of colon carcinoma, by reproducing the entry of disseminated cancer cells into circulation leading to the liver [40]. Another critical pathological aspect of interest is an inflammatory response. An integrative gut and liver platform was used to recapitulate the inflammation process [41]. Notably, cells were co-cultured with Kupffer cells and goblet cells improved the physiological relevance. In a subsequent study, the immune component was added to the system with T cells to model ulcerative colitis [42]. This paper is noteworthy in that a detailed study of the mechanism of T-cell-mediated inflammation and the role of SCFAs in this context. Lee et al. developed an *in vitro* model of gut liver in the context of NAFLD [43,44]. In this work, free fatty acids are absorbed across the epithelium before exerting any effect on the liver cells. Simply by reproducing this sequential action of the free fatty acids, it was possible to demonstrate the antisteatotic activity of different compounds was demonstrated. A subsequent study by the same group further included an immune component by adding macrophages to the system [45]. A gut-liver chip for modeling NAFLD was developed by co-culturing Caco-2 and HepG2 cell lines in a closed-circulation loop fluidic chip [46]. Notably, treating with fatty acids changed the pattern related to endoplasmic reticulum stress, implying that studying the mechanism of NAFLD in a more physiologically relevant context was possible.

The effect of ethanol on hyperpermeability and stromal injury in the intestine was studied using intestine-liver-on-chip, revealing the protective role of the intestine [47]. A gut-liver-on-a-chip was exposed to fine particulate matter

(PM<sub>2.5</sub>) to reproduce the metabolic dysregulation induced by the particles [48]. A significant dysregulation in cholesterol and bile acid metabolism was observed. The gut microbiome is also a critical part of the gut-liver crosstalk. A gut-liver chip with gut epithelial cells, hepatocarcinoma cells in the form of 3D spheroids, was used to examine the effect of microbe-derived metabolites [49]. Interestingly, adding the microbe-derived metabolites seemed to enhance various liver-specific functions, demonstrating their beneficial effect.

Implementation of high-throughput analysis, possibly with robotic liquid handling and chip operation is also an important research area. Satoh et al. published a paper on what authors termed a multi-throughput system, for up to 16 systems in parallel [50]. In a similar study by a different group, the effect of combinations of drugs was evaluated using the gut-liver system [51]. An enterohepatic system was used to examine the fate of triazolam after phase I and II metabolism [52]. Such studies show promise that eventually multi-throughput implementation and automatic analysis of drugs' action in the body will be possible.

Due to their anatomical proximity, the gut and the liver interact closely during many physiological processes, including digestion, absorption, and metabolism. One of the prominent examples is the role of bile, which is synthesized by the liver, in the digestion and absorption in the intestine. Bile is produced by the liver, stored in the gallbladder, and released through the bile duct to the duodenum. Bile has multiple functions, including the breakdown of fats so that they can be absorbed, the absorption of fat-soluble vitamins, and the removal of metabolic wastes [53]. The majority of bile acids are reabsorbed in the distal ileum and returned to the liver via the portal vein, known as the enterohepatic circulation of bile acids [54]. Several intestinal and hepatic disorders are related to dysregulation in the signaling mediated by the bile, such as the case of bile acid-induced diarrhea caused by a decrease in the bile acid-induced ileal hormone Fibroblast Growth Factor 15/19 (FGF15/19) [55]. Midwood et al. attempted to recapitulate the ileal hormone signaling using a chip platform, by co-culturing the gut and liver tissue slices and exposing the slices to the primary bile acids, which induced the expression of FGF15 in the intestinal slice, resulting in the down-regulation of cytochrome enzyme in the liver [56]. Given the importance of bile metabolism, it is expected that there will be more attempts to develop models of the gut liver involving bile metabolism.

### Gut-Brain-on-a-Chip

There has been accumulating evidence on the link between the gut and the brain, the gut environment affecting the neurocognitive functions, or vice versa [57]. An interesting feature of the gut-brain axis is that there exist multiple pathways of interaction between the gut and the brain, including biomolecular signaling through blood circulation [58], as well as neurological pathways [59]. It is also noteworthy that extracellular vesicles play important roles in communication between the two organs [60,61] (Fig. 2A).

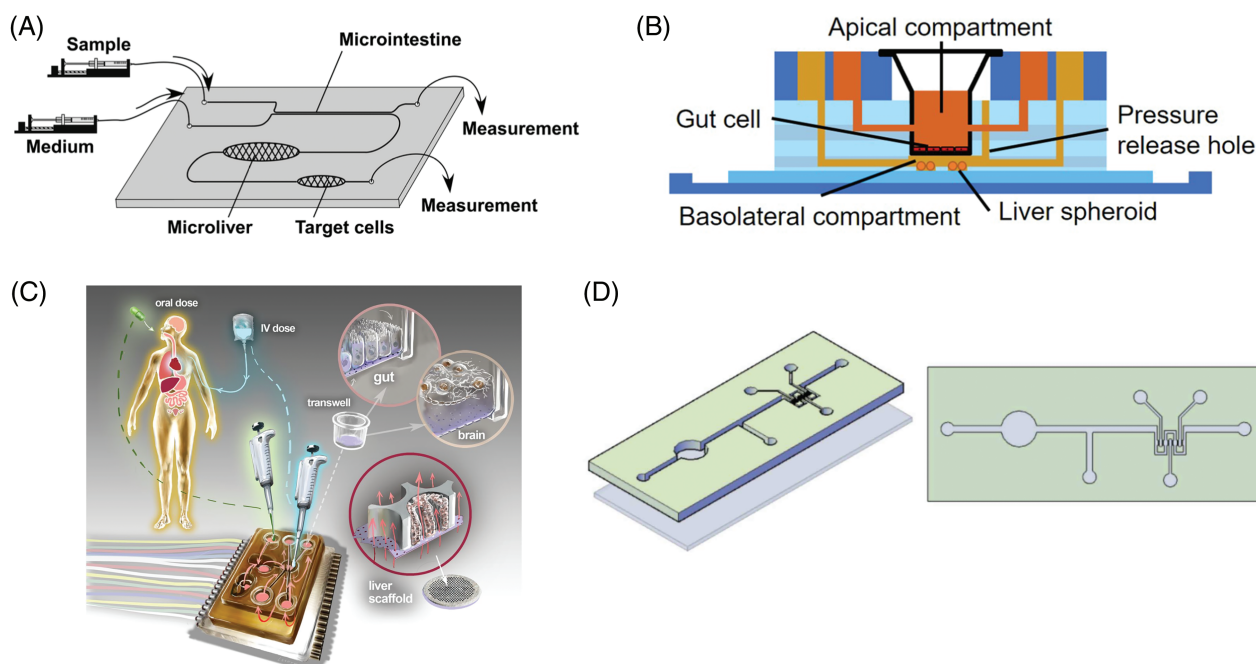
Due to the complex nature of the communication between the gut and the brain, an *in vitro* system that recapitulates all of the key mechanisms is yet to be developed. However, there are a few examples of preliminary systems that attempted to partially reproduce the gut-brain interaction.

One of the key components of the gut-brain axis is the presence of a robust and selective barrier between the systemic circulation and the brain, known as the blood-brain-barrier (BBB), and many chip-based systems have delved into reproducing the structure and the key functions of this barrier [62]. Several prominent examples of miniaturized, microfluidics-based models of BBB have been developed, which are reviewed elsewhere [63]. Based on this recent development, there have been attempts to integrate the 'gut barrier' model with the 'brain barrier' model. For example, a gut-brain-on-chip was developed with a two-layer microfluidic chip for gut cells and brain microvascular endothelial cells (hBMECs) nearby [64]. The formation of both gut epithelium and BBB inside the chip-enabled the study of the migration of exosomes across the BBB. The model of the gut-brain axis was constructed for Parkinson's disease, where the gut, liver, and cerebral MPS were connected with containing CD4<sup>+</sup> T and T helper cells [65]. One of the superior features of this model was that it contained multiple cell types including the brain, such as neurons, astrocytes, and microglia, containing mutations causing Parkinson's disease. Short-chain fatty acids (SCFAs) secreted from gut microbes induced several transcriptomic changes, indicating that such a model can be a useful platform for studying the mechanism of gut-brain interaction.

An important player in the context of any organ-organ interaction is the immune component. The gut-brain-immune axis was studied by using a multi-organ chip for co-culturing tissue slices [66]. To represent the immune component, the tissue slices of the Peyer's patch and mesenteric lymph node were used, and cell migration and cytokine secretion were examined. Another interesting example used cortical neurons and dendritic cells to study neuro-immunological communication [67]. This study is unique in that the authors studied the electrophysiological signal between the two components, linked by synapses. These platforms successfully demonstrate that at least some parts of the features of the gut-brain axis can be recapitulated, which should provide valuable platforms for studying related diseases.

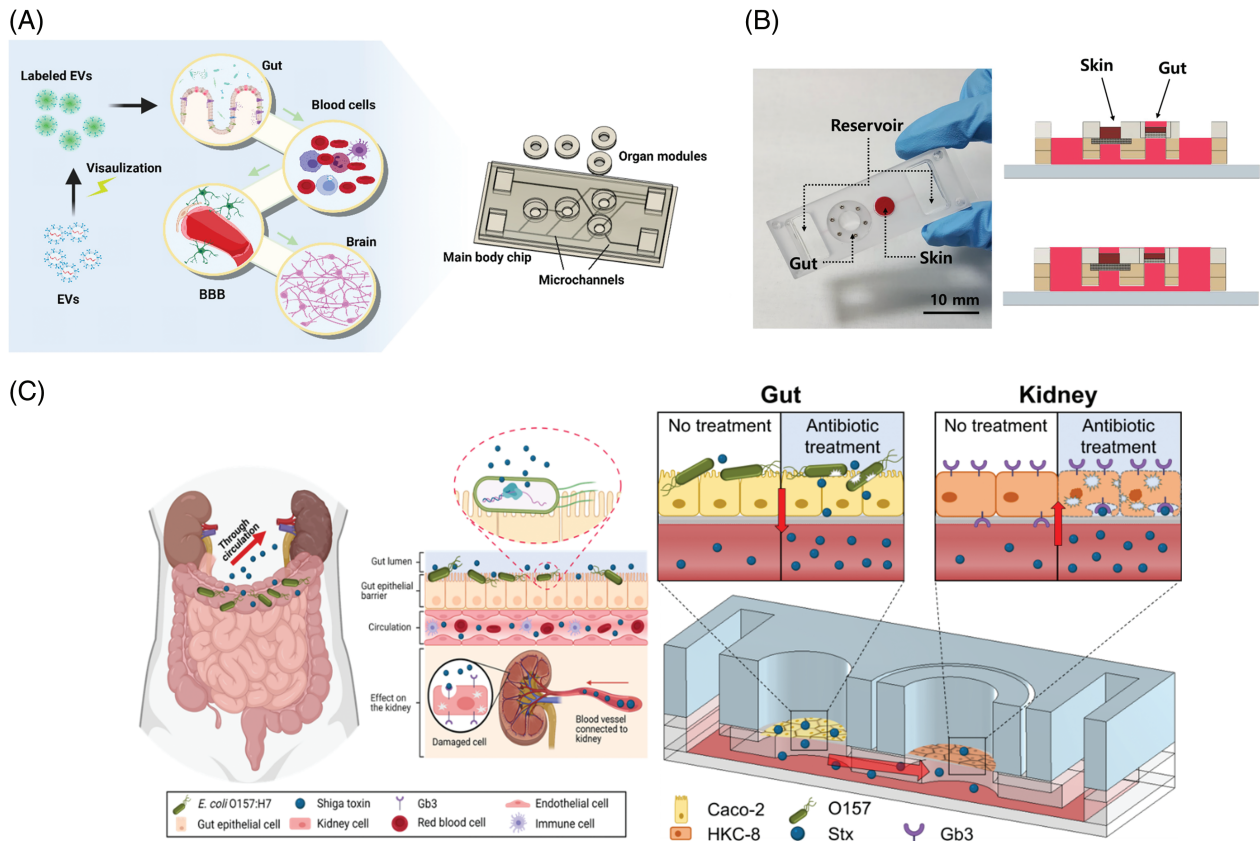
### Other Gut-Organ-on-a-Chip

The gut-skin axis is also an active area of research [68]. The gut and the skin have many features in common, including the epithelial barrier, the presence of commensal bacteria, and immune components. Inflammatory responses are deeply involved in pathological conditions in both organs, often observed simultaneously. A preliminary attempt was made to fluidically connect the gut and the skin tissue in a microfluidic chip [69] (Fig. 2B). Although several important components were absent, such as immune cells, challenging the gut compartment with inflammatory signals resulted in skin inflammation, as well as increased expression of a dermal disease marker. Integration of the skin vasculature with this model system is thought to further improve the physiological relevance of the model [70].



**FIGURE 1.** Examples of gut-liver-axis-on-a-chip systems (A) Microfluidic chip connecting the gut, liver, and the target organ, reproduced with permission from [21]. (B) A gut-liver chip for reproducing the PK of drugs, reproduced with permission from [32] (C) An interconnected microphysiological system, containing the gut, liver, and brain, reproduced from [34] with permission under the terms of the Creative Commons CC BY license (D) Intestine-liver axis on-chip for emulating ethanol first-pass metabolism and hepatic damage, reproduced from [47] with permission under the terms of the Creative Commons CC BY license.





**FIGURE 2.** Various gut-organ axis on a chip system (A) Modular multi-organ-on-a-chip system for reproducing the gut-brain axis, reproduced from [60], with permission under the terms of the Creative Commons CC BY license (B) A microfluidic gut-skin-on-a-chip for reproducing the gut-skin interaction, reproduced from [69] with permission (C) Gut-Kidney-on-a-chip platform for studying Hemolytic Uremia Syndrome, reproduced from [76] under an open access Creative Common CC BY license.

It has also been known that inflammatory gastrointestinal diseases have close associations with pulmonary complications [71]. Recent studies also suggest that Severe Acute Respiratory Syndrome Coronavirus-2 (SARS-CoV-2), which causes COVID-19, can also affect the gastrointestinal tract and the gut microbiome [72]. An attempt to develop a model of gut infection by SARS-CoV-2 was made [73]. Although this work did not contain the lung compartment and therefore not exactly a model of the gut-lung axis, it is not difficult to imagine the integration of such a platform with already established lung-on-a-chip platforms [74].

The hemolytic-uremic syndrome (HUS) is an acute disorder characterized by the triad of microangiopathic hemolytic anemia, nephropathy, and thrombocytopenia [75]. One of the major causes of HUS is Shiga-toxin-producing bacteria, such as *Escherichia coli* (*E. coli*), often transmitted through ground beef and unpasteurized milk. This pathological interaction between the gut microbe and the kidney failure was reproduced using a microfluidic gut-kidney chip [76] (Fig. 2C). When gut cells were infected by *E. coli* and treated with antibiotics, Shiga toxins released from the microbes in the gut compartment migrated to the kidney cells via fluidic channels and caused cell death. It is interesting to note that different antibiotics showed different toxic effects on the kidney cells. Ciprofloxacin was more detrimental than Gentamycin, which was consistent with previously reported clinical observations [77].

## Conclusion and Outlook

As a summary of our previous discussion, we included a table of major examples of gut-organ axis models within the past five years (Table 1). Although organ-on-a-chip systems for reproducing various gut-organ axes are still in their preliminary stages, recent studies show great promise in this direction. Because *in vitro* model systems that can model such interactions between different organs are still lacking, these platforms should find uses in many areas, such as the drug development process and the medical science field by enabling *in vitro* experiments on complex biological processes in the body. For example, studying the effect of drugs on gut-liver interaction involving bile metabolism would be possible. Biochemical and neurological communication between the gut and the brain consists of complex and multi-faceted mechanisms that are difficult to reproduce using conventional *in vitro* models, and multi-organ-on-a-chip systems are platforms that can complement traditional systems.

However, there are still many challenges that need to be overcome. In many cases, connecting two organ components alone would not be sufficient to model the whole process. For example, the immune component plays an important role in the progression of many inflammatory diseases involving the gut, skin, liver, etc. Incorporation of immune cells into the organ-on-a-chip systems, or even integration with immunological organs such as the lymph node should yield

TABLE 1

Prominent examples of gut-organ axis model-on-a-chip. Key papers were selected among papers published within the last 5 years

Organ-axis	Cells	Key features	Reference
Gut-liver	<ul style="list-style-type: none"> <li>Gut (Caco-2)</li> <li>Blood vessel (HUVEC)</li> <li>Liver (primary hepatocytes, Primary liver sinusoidal microvascular endothelial cells)</li> <li>Kidney (primary human renal proximal tubule epithelial cells, primary human glomerular microvascular endothelial cells)</li> </ul>	<ul style="list-style-type: none"> <li>Key human PK parameters for oral nicotine and cisplatin were predicted</li> <li>Three organ-on-a-chip modules were fluidically connected via tubing</li> </ul>	[37]
	<ul style="list-style-type: none"> <li>Gut (Caco-2)</li> <li>Liver (HepG2)</li> <li>Immune (RAW264.7)</li> </ul>	<ul style="list-style-type: none"> <li>Fluidically connected, three cell types co-cultured in a single chip</li> <li>The inflammatory response was observed</li> </ul>	[45]
Gut-brain	<ul style="list-style-type: none"> <li>Gut (primary gut cells, macrophages, dendritic cells)</li> <li>Liver (primary hepatocytes, Kupffer cells)</li> <li>Immune (T cells)</li> <li>Brain (neurons, astrocytes, microglia)</li> </ul>	<ul style="list-style-type: none"> <li>Gut-liver interaction in the context of Parkinson's disease (PD)</li> <li>Effect of short-chain fatty acids enhancing expression of PD-related proteins</li> </ul>	[65]
Gut-skin	<ul style="list-style-type: none"> <li>Gut (Caco-2)</li> <li>Skin (primary fibroblasts, keratinocytes HaCaT)</li> <li>Immune (RAW 264.7)</li> </ul>	<ul style="list-style-type: none"> <li>Gut epithelium fluidically connected with 3D skin tissue construct</li> <li>Impairment of gut barrier by fatty acids inducing inflammatory dermal disease marker hBD-2</li> </ul>	[69]
Gut-kidney	<ul style="list-style-type: none"> <li>Gut (Caco-2)</li> <li>Kidney (renal tubule cell HKC-8)</li> </ul>	<ul style="list-style-type: none"> <li>Infection of gut epithelium with <i>E. coli</i> and treatment with antibiotics released toxins that damaged kidney cells</li> </ul>	[76]

significantly advanced *in vitro* models compared to current ones. The presence of proper vascular networks is also important. Co-culturing such multiple components in a single system will inevitably give rise to many problems, such as media compatibility. This will likely require the development of new, universal cell culture media that can support multiple cell types. Novel methodologies for maintaining tissue microenvironment niches for each tissue in a microfluidic system may also be possible, by utilizing the unique advantages of microfluidics [78].

The issue of scalability, mass production, and cost is also important. Since organ-on-a-chip technology has been gaining attention and expanding in the last few years, most of the reported systems are still at the research level and not ready for wider application in the industry. The use of PDMS (polydimethylsiloxane) has contributed significantly to this field by providing a biocompatible, flexible, transparent, and gas-permeable material for fast design and fabrication of microfluidic devices, it is not suitable for mass production, compared to more conventional plastics. More work needs to be done in terms of standardization, automation, and mass production for high-throughput applications in the future.

Another important aspect of organ-on-a-chip technology is how fast it will be accepted by relevant

research communities and pharmaceutical companies. This will require support from the regulatory agencies. The recent launching of the Complement-ARIE (Animal Research in Experimentation) program by the NIH (National Institute of Health) council, which aims to catalyze the development, standardization, validation, and use of human-based new approach methodologies is encouraging in this aspect [79]. A wider acceptance in the field and application in the industry will require more thorough validation of the new models, as well as standardization for mass production [11]. Given the recent achievement in this field, it is likely that shortly, we will see more robust and physiologically relevant models being developed and employed in real-world applications.

**Acknowledgement:** None.

**Funding Statement:** This work was supported by Korea Technology and Information Promotion Agency (S3316767), National Research Foundation of Korea (2022R1A4A2000748 and 2022M3A9B6018217), Alchemist Project (KEIT 20018560, NTIS 1415184668) by of the Korea Evaluation Institute of Industrial Technology, Korea Institute of Marine Science & Technology Promotion (KIMST, RS-2024-00402200), and Hongik University Research Fund.

**Author Contributions:** J. H. Sung carried out all of the work to prepare the draft and write this paper.

**Availability of Data and Materials:** Data sharing is not applicable to this article as no datasets were generated or analyzed during the current study.

**Ethics Approval:** Not applicable.

**Conflicts of Interest:** The author declares that they have no conflicts of interest to report regarding the present study.

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