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Review

Microphysiological Systems to Recapitulate the Gut–Kidney Axis

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Chronic kidney disease (CKD) typically appears alongside other comorbidities, highlighting an underlying complex pathophysiology that is thought to be vastly modulated by the bidirectional gut–kidney crosstalk. By combining advances in tissue engineering, biofabrication, microfluidics, and biosensors, microphysiological systems (MPSs) have emerged as promising approaches for emulating the *in vitro* interconnection of multiple organs, while addressing the limitations of animal models. Mimicking the (patho)physiological states of the gut–kidney axis *in vitro* requires an MPS that can simulate not only this direct bidirectional crosstalk but also the contributions of other physiological participants such as the liver and the immune system. We discuss recent developments in the field that could potentially lead to *in vitro* modeling of the gut–kidney axis in CKD.

Highlights

Gut microbiota-derived metabolites are key molecular mediators of the microbiota–host axis.

The excretory capacity of the kidney is an essential part of human gut microbial symbiosis.

Chronic kidney disease (CKD) is a metabolic disease in which gut microbiota-derived metabolites accumulate in the blood and adversely affect host physiological functions.

Intestine-on-a-chip models have been developed that recapitulate the 3D epithelial barrier, the gut–microbiome interaction, and intercellular crosstalk with remote organs.

Components of the immune system are pivotal in remote communication between the gut and kidney.

The combination of engineered microphysiological systems with high-throughput multiomic analysis will provide novel insights into organ intercommunication in CKD.

Chronic Kidney Disease: A Metabolic Disorder with Disrupted Inter-Organ and Inter-Organismal Signaling

Chronic kidney disease (CKD) is the most widespread kidney disease and is characterized by the gradual loss of organ function over time, which impairs the ability to filter metabolic waste products from the blood (Box 1). The kidneys have many highly specialized functions, such as blood filtration and active secretion for the removal of metabolic waste, reabsorption of essential nutrients, maintenance of blood volume and electrolyte homeostasis, and metabolic and endocrine activity [1].

The complex and enigmatic pathophysiology of CKD is thought to be modulated by kidney crosstalk with multiple organs and systems, particularly via bidirectional inter-organ communication with the gastrointestinal tract, referred to as the gut–kidney axis [2]. The human gut accommodates a complex community of microbes that live in a commensal relationship with their host [3] and provide significant and unique contributions to the **human metabolome** (see Glossary). In **symbiosis**, intestinal absorption ensures the uptake of beneficial microbial metabolites, whereas the kidneys maintain homeostasis by excreting potentially toxic metabolic end-products. Conversely, kidney failure results in the accumulation of gut **microbiota**-derived metabolites (i.e., **uremic toxins**) leading to the development of **uremic syndrome**. This complication contributes to **gut dysbiosis** that adversely affects the inflammatory, endocrine, and neurological pathways involved in CKD onset and progression (Box 2 and Figure 1) [4]. Overall, CKD can be viewed as a metabolic disorder that reflects disrupted inter-organ and inter-organismal flow of metabolites and signaling molecules accompanied by overactivation of the immune system (Figure 2). Accordingly, the central role of gut–kidney remote signaling via uremic toxins [5] raises the need to further characterize the gut metabolome in CKD.

Traditionally, kidney disease research has largely relied on clinical [6] and animal studies [7] that offer limited control over experimental parameters and have high inter-species variability. Owing to the lack of suitable *in vitro* experimental models, there is currently an imperative need for cell culture systems that can capture the different aspects of *in vivo* organ function through the use

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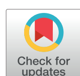

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Box 1. Chronic Kidney Disease: Mechanism of Disease

Epidemiology

CKD affects ~10% of the world population and has recently been classified as a major public health threat owing to its drastic rise in prevalence worldwide over recent decades. It often appears together with other comorbidities and, if left untreated, progresses to **end-stage kidney disease (ESKD)** [61,62]. At present, no effective treatment is at hand, and **dialysis** and kidney transplantation are the only interventions available for CKD management in ESKD [1]. Accordingly, the long-term effects on patient quality of life and associated comorbidities are predicted to impose a high burden on economies and healthcare systems worldwide [62,63]. These are associated with morbidity, productivity loss, increased hospitalization, and early mortality, and the annual societal and healthcare costs increase as CKD advances [60]. In addition, animal and clinical pharmacokinetic studies have demonstrated that the systemic deleterious effects of kidney failure can also alter the efficacy of hepatic and intestinal drug metabolism, thus increasing the risk of adverse drug reactions among patients [64].

Pathogenesis

The pathological hallmark of CKD is the progressive and irreversible loss of functional nephrons and the consequent decline in **glomerular filtration rate (GFR)**, features that are thought to be triggered by sustained or repeated injury to the organ, eventually leading to kidney fibrosis and scarring [63,65,66]. Because the kidney has only a limited ability to regenerate, kidney fibrosis is initiated as an attempt to preserve organ function [67]. However, upon sustained or repeated injury, this repair process is deregulated and leads to the replacement of **kidney parenchymal tissues** with ECM, causing irreversible damage to the tissue and nephron loss [66]. Such damage also triggers both kidney and systemic inflammation, leading to the production of reactive oxygen species and secretion of both proinflammatory and profibrotic mediators that further promote interstitial injury and fibrosis, perpetuating nephron damage [68,69].

Disease Classification

The Kidney Disease Improving Global Outcomes organization classifies CKD severity into five stages, where stage 5 represents ESKD, and recognizes a patient as having CKD if any abnormalities of kidney structure and function persist for more than 3 months. CKD severity is measured either by using a marker of kidney function, defined as a GFR of <60 ml/minute per 1.73 m^2, and/or by **albuminuria**, a marker of kidney barrier dysfunction, defined as a urinary albumin:creatinine ratio of >30 mg/g [68,70].

Box 2. The Gut–Kidney Axis in CKD: A Two-Way Interaction

Gut Dysbiosis Leading to Kidney Function Impairment

Diet plays a crucial role in regulating the composition of the gut microbiome. Following nutrient intake, fermentable dietary fibers are transformed through fermentation by saccharolytic bacteria into SCFAs, the primary energy source of the intestinal epithelium [2,4,71]. Undigested peptides are transformed into protein metabolites through fermentation by proteolytic bacteria [2,74]. Upon sufficient intake of fermentable fibers, protein fermentation metabolites are excreted through the stool. Conversely, reduced colon transit time, poor diet, or excess protein intake reduce the amount of fermentable fiber in the colon, leading to enhanced proteolytic fermentation activity. A prolonged shift towards proteolytic fermentation alters the gut microbiota and its metabolites, inducing gut dysbiosis [2,71,72]. This results in decreased production of SCFAs, that are known to have protective effects on both kidneys and gut, and a concomitant increase in the production of uremic nephrotoxins, end-products that result from hepatic handling of proteolytic bacteria metabolites [4,43,73]. Uremic toxins are normally excreted through the kidneys; however, kidney impairment reduces toxin elimination, leading to the development of uremic syndrome (Figure 1). Uremic toxins exert their nephrotoxic effects by increasing intracellular oxidative stress. This induces kidney tubule interstitium fibrosis which exacerbates kidney damage and hence hampers their excretion in a positive feedback loop [2,4,71]. Uremic syndrome overstimulates the immune system, leading to systemic inflammation that further damages the kidneys and other organs [74].

Kidney Function Impairment Leading to Gut Dysbiosis

Kidney clearance deficiency leads to the accumulation of metabolic waste products in plasma. Following nutrient intake, the amino acid catabolic end-product, ammonia, is converted into urea through liver metabolism and released into the circulation. Urea is predominantly excreted through the kidneys and partly through the colon. However, deterioration of kidney function shifts the primary site of excretion from the kidneys to the colon [71,74]. In turn, the sustained presence of urea in the colon triggers the proliferation of urease-producing bacteria, leading to gut dysbiosis. The shift in microbiota composition enhances gut ammonia production, thus raising the physiological pH of the gut lumen and leading to mucosal irritation and damaging the colonic epithelial barrier. This results in increased intestinal permeability, commonly referred to as 'leaky gut'. Consequently, endotoxins and bacterial products translocate into the circulation and induce both local inflammation, caused by immune cell activation and the release of proinflammatory cytokines and chemokines, and chronic systemic inflammation, further exacerbating the deterioration of kidney function [4].

Glossary

Albuminuria: the abnormal presence of the protein albumin in the urine.
Dialysis: a kidney replacement therapy in which the filtration function of the kidneys in ESKD is replaced by direct removal of waste and excess fluids from the body.
End-stage kidney disease (ESKD): the most advanced stage of CKD in which patients are dependent on kidney replacement therapies.
Glomerular filtration rate (GFR): a measure of kidney function: the rate at which plasma is filtered through the glomeruli, usually measured in ml/minute. GFR is used to determine the stage of kidney disease.
Gut dysbiosis: an imbalance in the composition and function of the gut microbiota and its metabolic end-products that compromises host homeostasis and host–microbiota/microbiome crosstalk within the gut.
Human metabolome: the complete set of metabolites within the human body.
Kidney parenchymal tissues: epithelial tissue of the kidney corpuscles and tubules, the structural units of the nephrons.
Microbiome/microbiota: the microbiome refers to the collection of microorganisms and their genomes. By contrast, the microbiota usually refers to specific microorganisms that are found within a specific environment.
Nephron: the functional unit of the kidneys that is responsible for maintaining the chemical balance of the body. Each kidney contains ~1 million nephrons.
Obligate anaerobes: bacteria that do not require oxygen for growth and function, and whose survival can be threatened in its presence.
Short-chain fatty acids (SCFAs): end-products of gut microbiota metabolism resulting from the fermentation of dietary fibers/indigestible carbohydrates.
Symbiosis: sustained physical and metabolic association between different organisms that can either be mutualistic, commensalistic, or parasitic.
Urea: a waste product produced by the metabolism of proteins and amino acids.
Uremic syndrome: the clinical term for the accumulation of waste products as a result of kidney impairment.

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of highly controlled and specialized culture microenvironments, including 3D scaffolds and microfluidics [8]. Given the advances in multicellular culturing and biomanufacturing, the integration of real-time monitoring features, and the independent control of experimental parameters, capturing the complexities of human physiology *in vitro* is certainly in sight. We provide a comprehensive overview of the most emblematic and recent advances in the field of 3D *in vitro* models, and highlight their relevance for the development of a 3D bidirectional gut–kidney axis system. We also discuss the major hurdles that these entail, how to overcome them, and offer new insights into the current direction of this field in the context of CKD.

Uremic toxins: solutes that accumulate in the plasma of patients with kidney disease and that have been shown to have deleterious biological effects. Research over recent decades has led to the compilation of a database by the European Uremic Toxin Work Group (EUTox, endorsed by the European Society of Artificial Organs, ESAO; and the European Renal Association–European Dialysis and Transplant Association, ERA-EDTA) that currently comprises >150 identified compounds (http://www.uremic-toxins.org/).

Microphysiological Models to Unravel Complex Organ Interconnections

The advent of microphysiological systems (MPSs), also referred to as organ-on-chips (OOCs), has created novel possibilities to study the physiological processes involved in individual organs and inter-organ crosstalk. A range of different multi-MPSs are emerging that underpin a 'physiome-on-a-chip' approach for simulating the functional units of organs, as well as crosstalk between them, instead of aiming to reproduce the complete organ(s). From a technical standpoint, MPSs often consist of single or multiple microfluidic channels with cross-sectional dimensions of hundreds of micrometers in which small volumes (nanoliters to microliters) are (re)circulated. By ensuring close contact between the cells, these volumes allow dynamic cell–cell interplay to be captured while ensuring minimal reagent consumption and compound dilution [9,10]. Added laminar flow can continuously supply cells with fresh nutrients and oxygen while simultaneously removing waste products, and can generate accurate spatiotemporal chemical and mechanical gradients in their

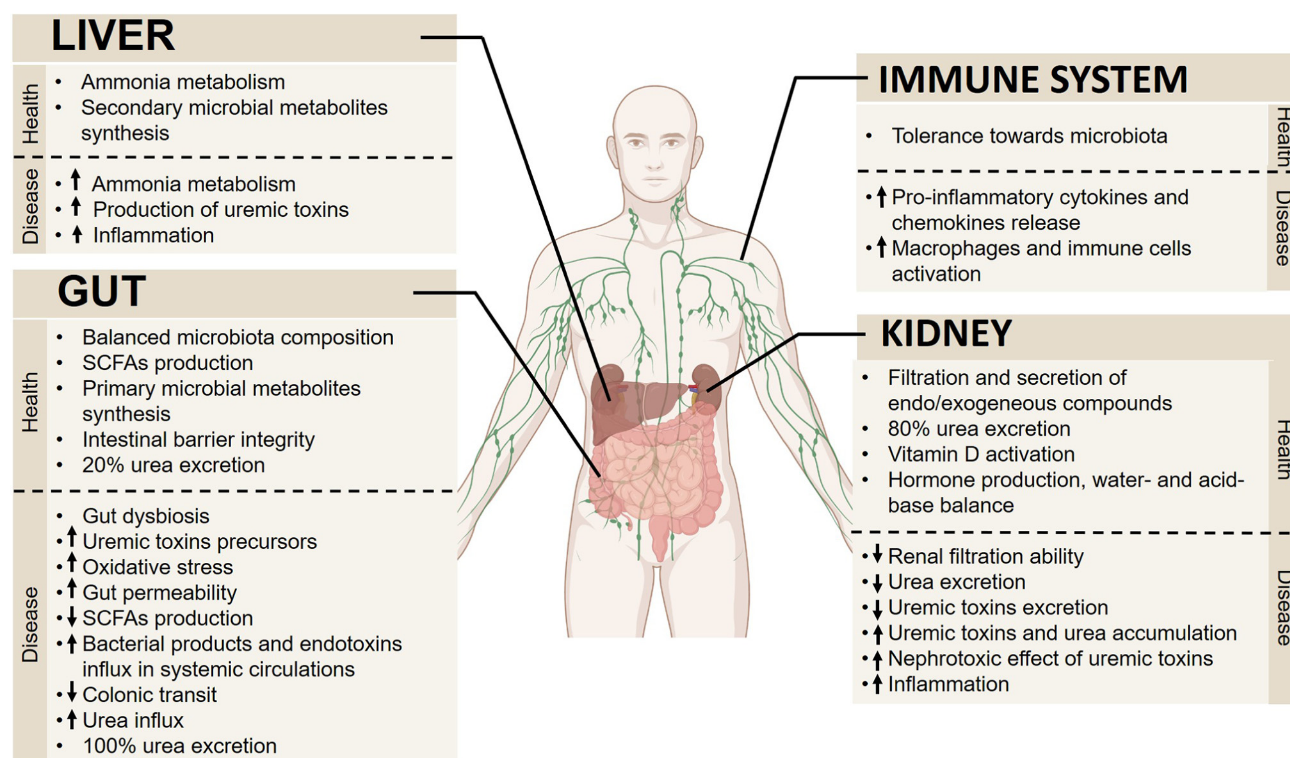

Figure 1. Gut–Kidney Axis Multiorgan Interactions in the Healthy State and in Chronic Kidney Disease (CKD). Illustration of the pivotal role of gut–kidney axis crosstalk with the liver and immune system. Figure created with BioRender.com. Abbreviation: SCFA, short-chain fatty acid.

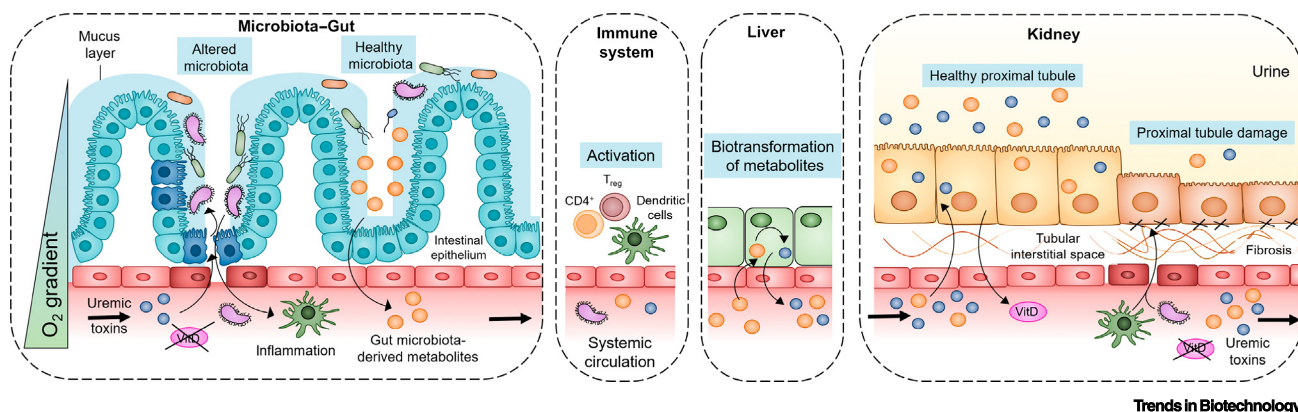

Figure 2. Overview of Microbiota–Gut–(Immune System–Liver)–Kidney Axis Interactions in the Development of Chronic Kidney Disease (CKD). Gut dysbiosis contributes to the production of the uremic toxin precursors that are metabolized into potential uremic nephrotoxins by the liver. In the kidney, damage induced by uremic toxins leads to kidney fibrosis and compromises renal filtration and reabsorption. This results in reduced excretion and concomitant systemic accumulation of the uremic toxins which can, in the gut, further alter microbiota composition and function. In addition, reduced production of active vitamin D by the damaged kidneys affects the integrity of the intestinal barrier, making it more prone to disruption. This results in leakage of bacterial products and endotoxins into the circulation, leading to both local and systemic overactivation of the immune system, that in turn further exacerbates the kidney and intestinal damage and affects other organs in the body. Abbreviations: Treg, regulatory T cell; VitD, vitamin D.

vicinity [11]. Physical isolation of different tissue analogs is achieved through compartmentalization into microchannels separated by thin porous membranes or layers of extracellular matrix (ECM) [12].

The creation of the lung-on-a-chip platform, in which mechanical strain and multiple cell types were combined to mimic the respiration of the lung, pioneered the development of biologically inspired MPSs [13]. Since then, progress in microfluidic handling has made it possible to connect different organ models and control their crosstalk within one or multiple devices [14,15]. The latest developments in the field prompt us to discuss these advances in relation to modeling the gut–kidney axis in CKD and explore the requirements of MPSs for supporting inter-organ communication, also taking into consideration the synergistic approach of combining them with *in silico* models.

En Route to Replicating the Gut–Kidney Axis Using MPSs

Several gut-on-a-chip systems have been established by integrating microfluidics, tissue engineering, and biomicroelectromechanical systems. Among the most representative configurations, the gut-on-a-chip from the Wyss Institute (USA) successfully emulated the dynamic human intestinal microenvironment through the application of physiologically relevant fluid flow and peristalsis-like mechanical forces, and these supported cell differentiation into villus- and crypt-like structures, the formation of a thick epithelial monolayer, and enhanced cellular function (Figure 3A) [16–19]. Recently, topological features have emerged as being pivotal in directing cell function, but only a few studies have attempted to replicate the crypt–villus architecture in microfluidic systems that can now be easily obtained through 3D high-resolution stereolithography [20], photolithography [21], and micromolding of crosslinked hydrogels [22]. At present, mimicking intestinal tubule-like structures has been addressed by culturing intestinal cells on the apical side of a perfusable hollow-fiber membrane system [23,24] or in the lumen of microchannels [25]. The addition of an ECM coating and unidirectional apical flow resulted in a mature intestinal tubule phenotype with villus-like structures. Exposure to *Clostridium difficile* secreted toxin A, a natural gut inhabitant virulence factor and intestinal barrier disruptor in dysbiosis, or to the gut microbiota-derived metabolite, *p*-cresol, resulted in enhanced barrier permeability [23,24]. Concomitantly, *p*-cresol was converted to *p*-cresyl sulfate and *p*-cresyl glucuronide, the end-metabolites that accumulate in plasma during CKD progression, likely via cytochrome P450-mediated metabolism

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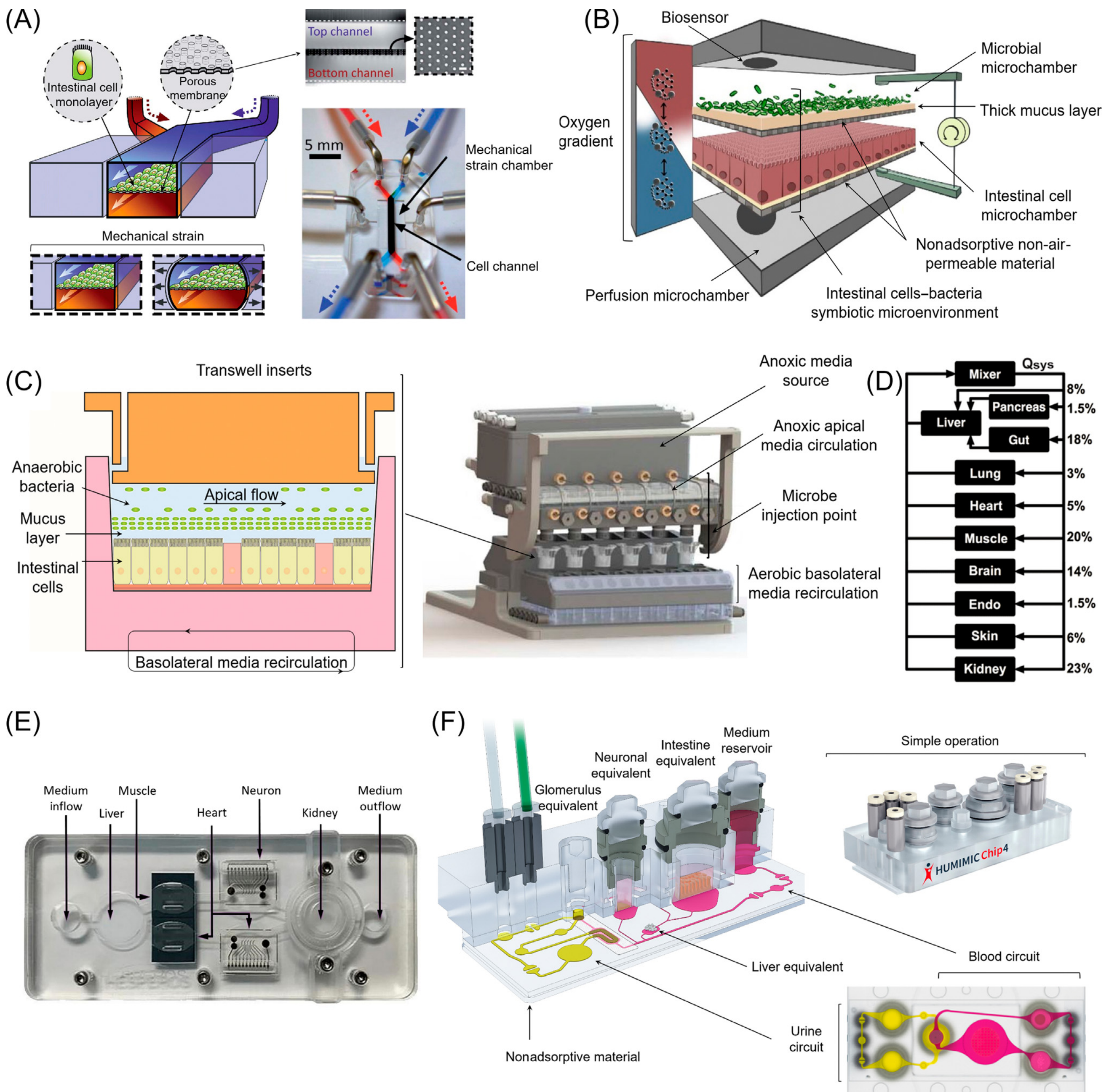

Figure 3. Representation of Emblematic Microphysiological Systems (MPSs) Developed by Different Research Groups for the Study of Inter-Organ and Inter-Organismal Interactions. (A) Illustration of the gut-on-a-chip developed by the Ingber laboratory (Wyss institute, USA). The upper and lower chambers are separated by a porous membrane to which mechanical strain can be applied (by using negative pressure) to recreate peristalsis-like movements. This design allows the integration of a broad bacterial consortium, together with intestinal, vasculature, and immune cells (image adapted, with permission, from [16]). (B) Conceptual diagram of the HuMix device that recreates the anaerobic microbiome–intestinal cell interaction in the presence of a thick mucus layer and the absence of apical flow (image adapted, with permission, from [34]). (C) Schematic representation of the GuMi physiome platform. Intestinal epithelial cells are cocultured in standardized Transwell® with anaerobic bacteria by using apical anoxic media and basolateral aerobic media applied to the system with individual flow control (image adapted, with permission, from [37]). (D) A Transwell® format MPS configuration allows the integration of up to 10 organ tissues (image reproduced, with permission, from [50]). (E) Physical representation of the pumpless Hesperos Inc. MPS system (panel provided by Hesperos Inc. and reproduced with permission; https://hesperosinc.com/). (F) Representation of the four-organ-on-a-chip TissUse® system representing the kidney glomerulus, liver, intestine, and neuronal tissue. By employing protocols compatible with conventional cell culture methods, this device ensures easy operation and high throughput (image adapted, with permission, from [75]; https://www.tissuse.com/en/).

followed by conjugation, thus highlighting the contribution of the intestine to the biotransformation of gut microbiota-derived metabolites into uremic toxins [23].

The complexity and diversity of the intestinal epithelium can be reliably recapitulated by using 3D human tissue organoids [26,27]. However, their use has proved challenging because their closed, outside-in configuration hampers transport studies and exposure to commensal and pathogenic bacteria. Nevertheless, Thorne and coworkers showed that, through enzymatic dissociation of organoids, primary intestinal cells were able to self-organize and *de novo* segregate into undifferentiated or differentiated regions, forming niche-like compartments [28]. By integrating a separate microvascular endothelium cultured under independent flow and cyclic deformation, the absorption properties of these cells were evaluated [29,30]. Recently, the inclusion of crypt–villus domains in a tube-shaped epithelium with perfusable lumen was demonstrated to sustain stereotypical cell patterning features with self-regenerative potential [31].

The *in vitro* study of host–microbiota interactions has been hampered by the inability of conventional models to sustain a viable complex microbiota for several days. Although the contribution of the mucus layer to host–**microbiome** interactions has often been overlooked, it was recently demonstrated that the integration of a thick mucus layer – that acts as a physiological barrier between the bacteria and the intestinal epithelium – could delay barrier damage and paracellular permeability [32,33]. Accordingly, the sophisticated microfluidic model, HuMiX, enabled direct coculture of anaerobic bacteria and intestinal cells by incorporating a functional mucus layer as well as pulsatile flow and mechanical stimulation (Figure 3B) [34].

The majority of the gut microbiota are **obligate anaerobes** that require <0.5% O_2 growth conditions that are difficult to represent *in vitro* [19,35]. This limitation was overcome by engineering MPSs that incorporate physiologic oxygen gradients and support the dynamic interaction between intestinal and vascular endothelial layers. The chip consisted of an upper anaerobic epithelial chamber and a lower aerobic endothelial chamber, separated by a polydimethylsiloxane (PDMS) membrane. Through a radial oxygen gradient generated by the system, intestinal cells were oxygenated whereas anaerobic conditions allowed microbiota growth, as assessed by real-time monitoring via integrated noninvasive oxygen sensors [19,36]. Similar physiological hypoxia conditions were achieved by Zhang and coworkers who cocultured oxygen super-sensitive bacterial species using a differently designed MPS, the GuMi (Figure 3C) [37]. This platform induced a steep oxygen gradient through the addition of a long-term continuous flow of anoxic apical medium and aerobic basal media. The use of polysulfone, which unlike PDMS is an oxygen-impermeable material, prevented any oxygen leakage.

The development of kidney-on-a-chip systems has also been challenging owing to the lack of functional cells to recapitulate *in vitro* the multicellular structure and functional complexity within the **nephron.** Accordingly, relative to the gut-on-a-chip device, the development of kidney-on-a-chip systems is to some extent lagging behind. To date, models of glomerular, proximal tubule, and distal tubule physiology have been developed, but the integration of all components into a complete nephron-on-a-chip remains to be achieved [38]. To be physiologically relevant, in addition to cellular complexity, a biomimetic kidney-on-a-chip should integrate: (i) cell–cell interactions such as those between podocytes or proximal tubule epithelial cells and the (micro)vascular endothelium, (ii) the transcellular electrochemical and osmotic pressure gradients that drive fluids and metabolites across the interstitial space, (iii) fluid flow, and (iv) the structural arrangement of the kidney tubules, as well as (v) cellular metabolic and endocrine functions [38].

The proximal tubule plays a crucial role in metabolic waste excretion and biomolecule reabsorption, and has therefore been a major focus of interest in the development of *in vitro* kidney-on-a-chip systems that recapitulate *in vivo* kidney tissue. The development of functional kidney tubules using proximal tubule cells with biofunctionalized hollow fibers enabled Jansen and coworkers to study the secretory clearance of gut microbiota-derived metabolites. This system enabled the researchers to demonstrate how, through remote sensing and signaling, proximal tubule cells sense elevated levels of indoxyl sulfate and accordingly adjust the expression of the transporters responsible for their excretion in an attempt to maintain stable metabolite levels and homeostasis [39].

Endothelium–interstitial space–epithelium interactions govern the continuous exchange of solutes between the circulatory and urine compartments. Lin and coworkers successfully developed a perfusable 3D vascularized proximal tubule that was able to simulate, via tubule–vasculature exchange of solutes, the active reabsorption function of the kidney [40]. This model allows quantification of kidney albumin uptake and glucose reabsorption over time, offering a promising tool for investigating kidney (patho)physiological functions and pharmacology. Other than solute exchange, the kidney interstitial space is also considered to be central to the development of kidney fibrosis, a hallmark of CKD. This is thought to be caused by scarring of the tubule interstitial space as a result of interstitial myofibroblast activation and subsequent ECM deposition. Nevertheless, only a few studies have reported its integration into 3D *in vitro* systems. The validation of a simple and highly reproducible 3D tubule/interstitium microenvironment model for the study of kidney fibrosis in a physiologically relevant *in vitro* system was reported by Moll and coworkers [41]. This study used cisplatin to successfully mimic acute tubular injury. The *in vitro* replication of the renal tubule/interstitium microenvironment was achieved by using human dermal fibroblasts instead of renal fibroblasts because the former express low levels of fibrotic markers under basal conditions. Nevertheless, despite this limitation, the system demonstrated that epithelial cells play a central role in triggering the activation and differentiation of myofibroblasts. Moll and coworkers attempted to repeat this study using primary renal fibroblasts, but encountered great variability in the results. Given the importance of the interstitial space in CKD, further 3D *in vitro* studies will be necessary to elucidate its role in disease onset and progression.

The kidneys also activate 25(OH)vitamin D by hydroxylation at the 1α position, resulting in 1,25 $(OH)_2$vitamin D, an essential hormone that is often deficient among CKD patients and that can affect gut microbiota composition and barrier integrity. Recently, an on-chip representation of hepatic metabolism and kidney activation of vitamin D was developed by perfusing vitamin D-containing medium into a microfluidic chip, suggesting that complex inter-organ metabolic interactions are highly attainable using MPS technologies [42].

In CKD, the reduction in **short-chain fatty acid (SCFA)** production, complemented by the simultaneous increase in uremic toxin production and their systemic accumulation [4], is assumed to drive the chronic inflammatory condition that is typical of CKD [4,43]. Indeed, SCFAs, particularly butyrate, have both nephroprotective and intestinal protective effects [4,44], and high levels of butyrate have been associated with gut barrier integrity and intestinal immunity improvements as a result of its anti-inflammatory properties [45]. Nevertheless, this was recently contradicted by Trapecar and coworkers who, in a physiomimetic approach, demonstrated that SCFAs can exacerbate an inflammatory response in a gut–liver model. By connecting two pneumatic plates separately representing the gut and the liver, $CD4^+$ T cells and inflammatory type 17 T helper (Th17) cells could circulate within and between the two compartments. The opposing effects of SCFAs might correlate with the degree of inflammation, with a heightened inflammatory state producing a more deleterious effect [46].

To our knowledge, there is currently no MPS that addresses the effects of gut-derived metabolites on the gut, kidney, or other organs, with concomitant tracking of their biotransformation, in the context of CKD. Tuning the chip to faithfully recapitulate the bidirectionality of production and removal fluxes of metabolites will be a challenge. Integrating microbiota derived from stool samples of CKD patients into an intestinal microfluidic system would enable us to study alterations in microbial metabolism and analyze their (in)direct effects on remote organs, a feature that is not attainable through *in vivo* experiments.

Technological Advances Enhancing the *In Vivo* Translational Value of MPSs

Designing an MPS is challenging and requires a multidisciplinary approach. It is important to note that no single MPS 'can do it all', and, depending on the application, different systems may be required. The advantages and limitations of available systems for tackling the gut–kidney axis are summarized in Table 1. One of the most common challenges in the field is to design a system that is biologically complex and sufficiently technically simple to be established in cell culture laboratories.

The Ingber group (Wyss Institute, USA) has established well-optimized protocols for cell culturing, connecting microfluidic components to the chip and sampling [16–18,47]. Although technologically advanced, their microfluidic system requires significant training of nontechnical operators, even for automatic microfluidic assays [47]. Pumpless multiorgan chips, pioneered by Shuler laboratory (Cornell University, USA), as well as by companies such as Hesperos Inc. (Figure 3E) and InSphero, increase the throughput at the expense of limited control over the flow and device complexity [48] (https://hesperosinc.com/). Although limited in replicating biophysical cues, the MPS developed by the Griffith laboratory (Massachusetts Institute of Technology, USA) makes use of more conventional protocols, for example, by enabling direct access to the tissue analog and by using modified standard Transwell® inserts (Figure 3C,D) [37,49].

Innovative companies have similarly developed multiorgan platforms, such as TissUse® (https://www.tissuse.com/en/), that are both user-friendly and compatible with conventional organ models (Figure 3F). Their on-chip pumps connect the organs and make the system less prone to bubble trapping and leakage. However, these devices offer limited microfluidic routing, for example, the apical flow in the gut model is lacking, and customization of the tissue models is difficult.

Another major challenge is the chip material. PDMS is among the most frequently used materials because of its excellent oxygen permeability, optical clarity, and prototyping properties. However, oxygen permeability is a downside when coculturing the obligate anaerobe microbiome with intestinal cells [20,37]. In testing hydrophobic compounds, for example, in drug toxicity or efficacy studies, PDMS is not recommended because it absorbs small hydrophobic molecules. Hence, MPSs composed of more inert materials, that prevent nonspecific binding of compounds, are the most reliable. For instance, Edington and coworkers developed a polystyrene-based microfluidic platform of interconnected MPSs in an attempt to recreate a physiome-on-a-chip that can generate complex molecular distribution profiles for advanced drug discovery applications [50].

The development of platforms with integrated sensors (oxygen, **urea**, lactate, or glucose), and/or optical transparency, have facilitated real-time noninvasive cellular analytics (Box 3) [51,52]. A platform with fully integrated modular sensing has recently been developed. This operates MPS units in a continual, dynamic, and automated manner, and includes physical sensors to monitor the extracellular microenvironment, biochemical sensors to measure soluble biomarkers, miniature microscopes to capture morphological changes, and a microfluidic-routing breadboard to route fluids in a timely manner [53].

Table 1. Emblematic MPS Designs for Recreating the Gut–Kidney Axis

Advantages	Disadvantages	Refs
Emulate (Ingber laboratory, Wyss Institute, USA)		
Mechanical strain Homogeneous apical shear stress Coculture with vasculature Optimized protocols Establishment of a symbiotic microenvironment between intestinal cells and the gut microbiome Mucus production Integration of immune system components Broad bacterial consortium Sensor integration possible	Absorptive and air-permeable material Protocols incompatible with conventional cell culture methods Lower throughput	[16,18,47]
GuMI (Griffith Laboratory, MIT, USA)		
Protocols more compatible with conventional cell culture methods Nonadsorptive, non-air-permeable material Higher throughput Anaerobic bacteria Establishment of a symbiotic microenvironment between intestinal cells and gut bacteria Bacterial fermentation activity	More difficult sensor integration Absence of a bacterial consortium Absence of immune system components	[37,49]
HuMiX (Wilmes laboratory, University of Luxembourg)		
Less-adsorptive material Sensor integration possible Nonadsorptive non-air-permeable material Anaerobic microbiome Establishment of a symbiotic microenvironment between intestinal cells and the gut microbiome Mucus production Integration of immune system components	No apical flow in gut model Protocols incompatible with conventional cell culture methods Complex design Separate microbiome compartment Lower throughput Limited bacterial consortium	[34]
TissUse (Technische Universität Berlin, Germany)		
Protocols more compatible with conventional cell culture methods Easier operation Less-adsorptive material Higher throughput Inducible pluripotent stem cell (iPSC)-derived tissue	No apical flow in gut model Not possible to add microbiome No sensor integration Incomplete tissue maturation Incomplete kidney tissue differentiation	[61,75] https://www.tissuse.com/en/

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Table 1. (continued)

Advantages	Disadvantages	Refs
Hesperos Inc. (Shuler laboratory, Cornell University, and Hickman laboratory, University of Central Florida, USA)		
Simple perfusion Less-adsorptive material Homogeneous apical shear stress	Complex chip assembly, lower throughput Protocols incompatible with conventional cell culture methods Not possible to add microbiome No sensor integration No simultaneous apical and basolateral compartments Absence of immune components	[76,77] https://hesperosinc.com/

Computational analysis is also necessary to establish whether MPS-derived experimental data can be extrapolated to *in vivo* performance [54]. Thus, the integration of machine learning algorithms (*in silico* modeling) should become a strategic component of MPSs [55]. The computational model can be adjusted to help to resolve the limitations of experimentally linked MPSs and bring the data within the range of animal studies and extrapolation methods [54]. Predictions obtained from *in silico* studies can provide feedback to further improve MPS models [55]. For instance, *in silico* studies could be employed to model immune cell motility following intestinal barrier damage and predict cell behavior upon exposure to specific parameters or biomolecules [56–59].

Box 3. The Paramount Importance of Integrating Biosensors into MPSs

Quantification of key factors in the microfluidic system microenvironment can provide valuable information for biological and biotechnical applications. Biosensors are powerful analytical devices that allow continuous real-time collection and readout of spatial and temporal data, and thus enable direct observation and manipulation of the microenvironment. This technology has enhanced analytical performance, reliability, and accuracy compared with conventional laboratory techniques, and also offers miniaturized alternatives that decrease analysis time, cost, and waste production. Accordingly, integrating this technology within microfluidic systems can provide information on the viability of the microenvironment and simultaneously further enhance the physiological relevance of the *in vitro* systems [78].

Numerous biosensors have been integrated into microfluidic systems, and have demonstrated great specificity/selectivity and reproducibility through online monitoring, data transmission, and multiplexing, but they are not widely used [79]. Diverse sensors have been developed over the past decades, and optical and electrochemical sensors are the most commonly integrated into microfluidic devices [79]. The suitability of one particular biosensor versus another depends on the physical and chemical characteristics of the microfluidic device as well as on the biochemical features of the microenvironment represented in the system. Biosensors can be optimized for specific applications and can be designed for single or multiparametric detection units [80].

When designing a representative gut–kidney axis MPS, it is paramount to integrate biosensors to measure the microenvironmental dynamics. Sensors monitoring pH, temperature, oxygen levels, metabolite production, and nutrient consumption can provide real-time information regarding cell viability following exposure to specific compounds such as SCFAs, urea, and uremic toxins. Likewise, monitoring gut microbiome metabolites through the integration of appropriate sensors would allow microbiome alterations, as well as the direct effects of metabolites on the microenvironment, and vice versa, to be studied in real time.

Although many sophisticated sensors have been developed, there is currently a lack of sensors for monitoring specific compounds, including small molecules, proteins, and biomarkers. A major advantage of this technology is that sensors can be integrated into differently sized systems, enabling cross-scale comparisons of analytical data [80]. The enormous possibilities brought about by sensor integration into microfluidic systems, including sensitive, automated, and real-time monitoring, will enhance the potential of these devices for clinical translation [79].

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Concluding Remarks and Future Perspectives

Over the next century the prevalence of CKD is predicted to drastically increase worldwide, posing significant economic and societal challenges. Regardless of the country of origin, the annual healthcare and societal costs have been found to increase in parallel with the progression of CKD [60], highlighting the urgent need for a disease model platform in which to study CKD pathophysiology and identify potential therapeutic targets.

It is now evident that interdisciplinary collaboration has led to the development of innovative MPSs that have successfully overcome the technical and biological challenges that for a long time hampered the study of organ interconnections *in vitro*. The efficacy of these systems in mimicking the (patho)physiological mechanisms operating *in vivo*, combined with the ability to control the experimental parameters, make these models a valuable breakthrough for biomedical applications in the pharmaceutical industry. Moreover, the combination with *in silico* models via computational modeling offers great potential to address the limitations of current systems and enhance their physiological relevance. These technological advances are bringing us rapidly closer to the development of a gut–kidney MPS that will eventually lead to an *in vitro* disease model of CKD. Such a model will be a valuable addition in the field of drug development by enabling us not only to reduce the use of costly animal models but also to generate models of greater physiological relevance. This will undoubtedly benefit the pharmaceutical industry by limiting the failure of many pipeline drugs that is often linked to large physiological differences between species.

Nevertheless, many challenges remain to be addressed, and several issues must be resolved before MPSs can be developed that accurately model CKD (see Outstanding Questions). For example, the initial events that drive the onset of CKD remain unknown, making the modeling of CKD onset in MPS challenging. Kidney injuries that lead to the development of CKD are diverse in nature and often involve a cardiovascular component, making their representation even more difficult. In addition, the composition of the gut microbiome is complex and challenging to reproduce; nonetheless, it is an essential requirement for a CKD disease model. The latest successful developments of MPSs that integrate anaerobic bacteria have been made possible by the integration of biosensors for oxygen sensing, as well as by the inclusion of controlled flows and a mucus layer that reduce bacteria overgrowth and limit intestinal cell damage (Table 1). However, broad anaerobic bacterial consortiums within the systems remain to be achieved, although this will be necessary for a physiological representation of the gut microbiome. Issues with absorptive and air-permeable materials also represent a major hurdle in the field, challenging the suitability of the systems for the growth of anaerobic bacteria or for testing lipophilic compounds. The relevance of organ interconnections has been strongly highlighted in this review; it is therefore of pivotal importance to integrate circulatory and immune systems within MPSs, but these have been incorporated into only a few models.

By enhancing interdisciplinarity, the integration of bioprinting, biomaterials, and biosensors for real-time monitoring of the microenvironment could address the anatomical and biochemical features, as well as the complexity, of the systems that are necessary to increase their physiological relevance. As MPS technology progresses, alongside the current trend towards enhanced multidisciplinary approaches, these unanswered questions will eventually be addressed.

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Outstanding Questions

How can we engineer MPSs to model gut dysbiosis induced by disease states, poor nutrition, or antibiotic use?

How can we address the diversity of the gut microbiome composition *in vitro* to faithfully recreate the spectrum of intestinal microbes in CKD patients?

Will technological advances in the field enable us to establish the bidirectionality of the axis using MPSs so as to replicate the effects of kidney injury on the gut and vice versa?

How long will we need to sustain a viable gut–kidney axis model before pathological changes typical of CKD can be observed?

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