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Organoids and organ-on-chip for investigating host-microorganism interactions

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Short abstract

Recent advances in organoid and organ-on-chip (OoC) technologies offer an unprecedented level of tissue mimicry. These models can recapitulate the diversity of cellular composition, 3D-organization, and mechanical stimulation. These approaches are intensively used to understand complex diseases. This review focuses on the latest advances in this field to study host-microorganism interactions.

Introduction

Each organ or tissue delivers biological function through the coordinated perception and integration of instructive cues from the surrounding microenvironment, such as physical parameters including rigidity and tissue tension, oxygen level, and biochemical parameters like metabolites and hormonal signaling. Cell specification within these tissues ensures the elaboration of adapted responses that all together lead to tissue homeostasis. Events such as microbial infection, carcinogenic processes, or drastic metabolic changes perturb this finely regulated equilibrium state. Until recently, investigating these processes was essentially based on either *in vitro* 2D cell culture or on *in vivo* models. However, while the former is clearly oversimplified, the latter renders live-imaging of deeply buried organs very difficult, and often animals lack at recapitulating human responses due to wide species divergences. In addition, the usage of animal models raises several ethical issues pushing the community to apply the 3R principle: reducing, refining, and replacing animal experimentation. In this respect, biomedical researchers opted for other models using non-animal tissue surrogates.

Organoids are 3D structures that are generated through the self-organization and differentiation of stem cells or tissue-specific progenitor cells¹. They exhibit tissue-like characteristics of their organ of origins, including cellular heterogeneity and tissue architecture. The methods to derive organoids vary depending on the source of cells being used: either Pluripotent Stem Cells (PSCs) or Adult Stem Cells (ASCs) and Tissue-Specific Progenitor Cells (TSPCs). Importantly some types of organoids can only be generated from PSCs since there is no source of tissue containing ASCs/TSPCs. Also, the generation of PSC-derived organoids is more challenging than ASC/TSPC-derived ones and so far, these organoids

are closer to fetal-stage tissues whereas ASC/TSPC-derived organoids recapitulate more accurately the adult tissues¹. Another advantage of ASC/TSPC-derived organoids is to use them to develop precision medicine, particularly interesting in the field of cancer by isolating malignant tissues to generate patient-specific tumor organoids (tumoroids) and perform drug tests.

Pluripotent Stem Cells (PSCs), such as embryonic stem cells (ESCs) or induced pluripotent stem cells (iPSCs), can differentiate into organoids representing various organs. For this, PSCs are first expanded and maintained in 2D, then organ-specific differentiation is induced by the addition of appropriate growth factors and morphogens, and cultured in 3D by embedding them in a gel composed of extracellular matrix (ECM), which promotes the self-organization of organoids. Adult Stem Cells (ASCs) and Tissue-Specific Progenitor Cells (TSPCs) are present in specific tissues and can be isolated and expanded in 3D, provided with the specific media supporting the self-renewal and differentiation to generate organoids that closely mimic the native tissue (Figure 1).

Organ-on-chip (OoC) systems are microfluidic devices, generally produced by using microfabrication techniques, where mammalian cells can be cultured for multiple days. They aim at recapitulating one or multiple physiological functions of a selected tissue or organ *in vitro*². Today OoCs are included in a larger family of *in vitro* approaches, termed microphysiological systems (MPS), that could also include organoid-based approaches. OoC devices are made of clear and biocompatible polymers (e.g. Polydimethylsiloxane or PET) containing perfusable chambers or channels, that can receive specific cell types (either cell line or primary cells derived from organoids) for long-term culture. Today multiple OoC/MPS have been described and some are commercially available, they differ in the material they use, their ability to recapitulate physical cues, or the 3D architecture they produce. These OoC/MPS have been originally developed to address questions intractable while using animal models or standard 2D cell culture. Today the initial development phase is giving way to broader employment in various contexts including host-pathogen interactions (Figure 1).

Organoids and OoC present specific advantages or disadvantages (Figure 1). Although organoids reproduce well the 3D organisation and cellular diversity of a given organ, they can neither recapitulate the mechanical forces applied to the tissue nor an air liquid interface. Furthermore, as organoids are closed structures, it is difficult to access the apical side from which most microorganisms, pathogenic or not, originate. Therefore, studying host-pathogen interactions with organoids needs either microinjecting the microorganism into the lumen, or generating inverted polarity organoids. On the other hand, OoC enables access to the apical side of a tissue and some devices can recapitulate flow-induced shear stress and physiological strain.

This review will focus on examples of organoids and OoC employment to investigate host-microorganism interactions, in the digestive, respiratory, urinary, nervous systems, and other tissues, and how these new technologies have changed the way these investigations are performed.

1-The digestive system

The main role of the digestive system is to extract energy and nutrients from ingested food. It is composed of a group of major organs that constitute the gastrointestinal (GI) tract, divided anatomically into two parts: the upper part composed of the oral cavity, esophagus, stomach, and small intestine (duodenum, jejunum, and ileum); the lower part composed of the large intestine (colon, rectum, and anus)³. The efficiency of the digestive process is carried out through complex and coordinated neuronal and hormonal control and the action of mechanical forces. In addition, mechanical forces (fluid shear stress (FSS) and peristalsis) constantly applied to the GI tract are necessary to maintain tissue homeostasis and renewal⁴. Another important part of the digestive system is the microbiota, a group of microorganisms in a symbiotic relationship with the host that promotes digestion and regulates the immune response. The gastrointestinal interface is heavily burdened by pathogens from food and water, hence many gastrointestinal models have been developed to study their interaction with microorganisms⁵.

1-1-Stomach

The stomach produces gastric acid, which creates a microenvironment that is not favorable for the proliferation of microorganisms ingested with food. *Helicobacter pylori* (*Hp*) can nevertheless, survive and colonize the stomach lumen, infect the tissue, and ultimately cause gastric ulcers and cancer if left untreated. Important steps of *Hp* pathogenesis are bacterial attachment to epithelium and translocation of the CagA protein, which leads to impaired cell polarity. However, it remains unclear which cell subtype *Hp* preferentially targets. Using the gastric organoid-derived 2D monolayers, Aguilar *et al.*, showed that *Hp* preferentially targets most differentiated pit cells, the tropism of which is independent of MUC5AC and prostate stem cell antigen (PSGA), but requires bacterial chemotaxis towards urea produced by pit cells⁶. Intraluminal microinjection of *Hp* in gastric organoids derived from tissue biopsy revealed that *Hp* infection induces epithelial cell proliferation and exhibits a high innate immune response in the proliferating gland domain^{7 8}. It would be interesting to investigate differences in *Helicobacter* infection and host immune response between healthy and cancer contexts by comparing bacterial infection in control and gastric tumor organoids.

1-2-Intestine

The human intestine is a complex environment organized in a 3D multilayer topology, with differences between the small and the large intestine. The epithelial layer is organized such that crypts and villi alternate in the small intestine where only crypts are found in the large intestine. Diverse cellular subtypes are present throughout the intestine. Self-renewing LGR5+ stem cells are located in the deepest part of the crypts, alternatively surrounded by the Paneth cells⁹. Proliferating progenitor cells are pushed toward the villi top. While migrating, these cells differentiate into absorptive enterocytes/colonocytes or into secretory cells such as the goblet cells and enteroendocrine cells. Goblet cells are specialized in the production of mucins that, when excreted, form the mucus layer. This barrier covers the epithelium and is colonized by the intestinal microbiota.

The intestinal tissue undergoes constant mechanical stimulation. First, it is exposed to peristaltic motion caused by the cyclic stretching of the muscles under the mucosa. Second, the flow of the bolus generates shear stress (FSS) in the range of 0.02-0.08 dyn.cm⁻² on the apical face of the epithelial cells¹⁰. Using an OoC device, these mechanical forces were shown to recreate spontaneously a 3D topology with villi structures using the human colonic cell line Caco-2¹¹. Furthermore, using the same OoC device this time seeded with human duodenal organoids, Kasendra et al. were able to show that combining organoid and OoC under mechanical stimulation allow at mimicking a highly physiological intestinal barrier very close to those found in the human duodenum *in vivo* as revealed by transcriptomic analysis¹². The intestine is by far the most highly used surrogate organ. There are many examples in the literature describing the interaction of pathogens or microbiota with the gut using organoids and OoCs^{13, 14, 15}, which will be summarized in this section per type of microorganism.

1-2-1-Bacterial pathogens

Listeria monocytogenes is a foodborne pathogen causing human listeriosis. Internalin (InIA), a *Listeria* surface protein, interacts with its host species-specific receptor E-cadherin (Ecad), allowing it to traverse the intestinal epithelium through goblet cells where Ecad is lumenally accessible¹⁶. Using intestinal organoids from the small intestine of mice expressing humanized Ecad, Kim et al developed an *ex vivo* minigut system where *Listeria* internalization and translocation are recapitulated with a natural route of bacteria entry after intraluminal bacteria microinjections^{17,18}. They have demonstrated that this bacteria transit through goblet cells in a microtubule-dependent manner, as they do *in vivo*¹⁸,¹⁶, and that Rab11 is necessary for the release of *Listeria* at the basolateral pole of goblet cells, demonstrating that *Listeria* hijacks the E-cadherin recycling pathway to cross the intestinal barrier. Furthermore, this innovative *ex vivo* approaches of infection and the possibility to have real-time images offer a wide range of options to directly explore pathogen interactions with host barriers¹.

When understanding infection in the context of varying mechanical forces is important, OoC approaches are favored. *Shigella flexneri*, is an enteric bacterium that causes bacillary dysentery in the human colon. Its intracellular lifestyle has been extensively investigated, but the corresponding mechanisms of invasion remain to be clarified. This lack of knowledge is primarily caused by the bacterium's notorious poor ability to infect polarized epithelial monolayers *in vitro*, both in Transwell systems and, more recently, in human colorectal biopsy-derived enteroids^{19, 20}. The pioneering work of Grassart *et al* showed that recapitulating the gut forces during epithelial barrier establishment and during infection is critical for *Shigella flexneri*²¹. In this study a human colonic cell line was matured in a 2-channels PDMS-made OoC mechanically stimulated with both FSS and peristaltic motion²¹. This intestine-on-chip recapitulates the crypt-like structures as well as mechanical forces found *in vivo*, and these parameters fueled *S. flexneri* infectivity²¹. Furthermore, the peristalsis significantly aided *Shigella* invasion, perhaps modifying the membrane's rigidity and the intercellular connections, increasing the epithelium's vulnerability to invasion^{21, 22}.

1-2-2 Protozoan pathogens

The parasite *Entamoeba histolytica*, like *Shigella*, takes advantage of peristalsis to destroy the colonic barrier and to cause amebiasis. *E. histolytica* has a strong adhesion mechanism to human cells and is known to begin by adhering to the epithelium's apex²³. The same intestine-on-chip used for *Shigella*²¹, showed that peristalsis sped up and deepened parasite penetration, while weakening the connections under increased stress, amplifying the consequences of the lysing activity²². Further research needs to be done to determine how the mechanical stress, the dynamic reactions of the parasites and the host cells synergize to increase the virulence of *E. histolytica* during peristalsis. Additionally, this study proposes a methodology to examine the dynamic and mechanical stress in deformable OoC, useful for mechanobiology studies²². The protozoan *Cryptosporidium* is another parasite affecting the human gut, but its pathophysiology is still unclear, partly due to the lack of a long-term culture method due to its intricate and long life cycle²⁴. Intestinal organoids are revealed to be a better model than 2D cultures to investigate this parasite because they allow a longer culture, the direct interaction between the protozoa and the epithelial cells, and the formation of oocysts^{25, 26}. Unfortunately, those oocysts are smaller than what is found in an infected host and the infection is decreasing over time. However, a recent OoC using a scaffold microfluidic device seeded with organoids and recreating villi and crypt structures of the human intestine, has been successful in recreating the complete and long-term *Cryptosporidium Parvum* intestinal infection²⁷, demonstrating the high potential of OoC combined to organoids to mimic host-pathogen interaction.

1-2-3 Viruses

Viruses are also present in the human gut and they can be studied using intestinal organoids and OoC devices. One example is SARS-CoV-2, a virus that can infect lung cells with the interaction of

its spike protein with angiotensin-converting enzyme 2 (ACE2) receptor. ACE2 is also highly expressed in the human intestine, explaining why some COVID-19 patients exhibit gastrointestinal disorders^{28, 29}. SARS-CoV-2 can infect human intestinal organoids, particularly enterocytes, where the virus alters gene expression²⁹. This supports the use of intestinal organoids to study the SARS-CoV-2 gut invasion³⁰. Another work uses a human colonic cell line and primary endothelial cells matured in a 2-channels OoC stimulated with FSS, to show that SARS-CoV-2 can infect the intestine³¹. This study highlights that intestinal coronavirus infection causes villi destruction, damages the barrier integrity and mucosal secretion, eventually inducing a significant increase in cytokine- and chemokine-related genes^{28, 31}, as it was shown in infected human intestinal organoids³².

1-2-4-Microbiota interactions

The intestine is in a symbiotic relationship with the gut microbiota, which is fundamental for human health. Dysbiosis has been linked to several intestinal diseases, including celiac disease, inflammatory bowel disease (IBD), and gastrointestinal cancer. For these reasons, researchers have tried to develop *in vitro* models that allow the combination of intestinal cells and microbiota. A recent study describes a multi-layered hydrogel to sustain the complex gut microbiota environment and taxonomic diversity *in vitro*. The aim of this study was to establish a gradient in the hydrogel to test several anti-tumoral drugs and examine how the microbiota can impact drug metabolism³³. Another OoC model also succeeded in the co-culture of the commensal bacterium *Lactobacillus rhamnosus* GG within the gut-on-chip seeded with human colonic cell line¹⁰. This model of intestine-on-chip was improved using eight different probiotic bacterial strains, showing a gene expression similar to the human ileum. Peristalsis was necessary to maintain tissue homeostasis and avoid bacterial overgrowth, but also stimulation by LPS and co-culture with immune cells demonstrates how this model can be used to study the immune response³⁴. However, to establish physiological conditions for the gut microbiota, it is necessary to recreate an oxygen gradient. *Jalili-Firoozinezhad et al* added a microscale oxygen sensor in the intestine-on-chip to monitor the amount of oxygen *in situ*. Microvascular endothelium and intestinal epithelium were developed in separate chambers that were connected by an ECM-coated porous membrane. The whole system was put inside an anaerobic chamber to create an oxygen gradient between the upper epithelial and lower endothelial layers. This configuration enables stable co-culture of anaerobic and aerobic commensal bacterial communities and intestinal epithelium in the same channel³⁵. Another alternative is the HuMiX microfluidic device that consists of three microfluidic chambers that are divided by semipermeable membranes and is used to study human-microbial interaction. The lower chamber serves as a perfusion chamber, the middle one serves as a cell culture chamber, and the upper one serves as a microbiological culture chamber. Under oxygen and biomolecule gradients, the device enables the co-culture with either

obligate anaerobe bacterial strain (*Bacteroides caccae*) or facultative anaerobe bacterial strain (*Lactobacillus rhamnosus*)³⁶.

2- Liver

Various metabolic, digestive, immune, and detoxifying functions occur in the liver. Additionally, the liver exhibits remarkable tissue-regenerative properties when challenged biologically, chemically, and physically. The most abundant liver pathogens are hepatitis viruses and *Plasmodium* parasites. These liver pathogens exhibit a narrow range of hosts, where the hepatitis virus infects mostly (or only) humans, the outcome of which is heterogeneous among individuals. As the architecture of the human liver is complex and highly organized, containing multiple cell subtypes including parenchymal and non-parenchymal cells, having a good liver model is critical to study the above-mentioned features of liver pathophysiology and host-microbiome interaction.

2-1-Studying hepatic infections using organoid-based models

3D Liver organoids (LOs) and cholangiocyte organoids can be derived from primary hepatocytes/cholangiocytes isolated from fetal/mature tissues, and from differentiation of iPSCs. These LOs are emerging as valuable tools to study hepatic virus interaction. Functional human LOs, generated by co-culturing of human iPSC (hiPSC)-derived endoderm cells (hiPSC-endoderm), human bone marrow mesenchymal stem cells (BM-MSCs), and human umbilical endothelial cells (HUVECs), supported hepatitis B virus (HBV) infection and long-term HBV propagation³⁷. HBV infection in these LOs induced dose-dependent hepatic dysfunction and liver failure revealed by down-regulation of hepatic gene expression profile, increased aminotransferase (ALT)/lactate dehydrogenase (LDH) expression, and altered hepatic ultrastructure³⁸. Additionally, a study of *ex vivo* HBV infection in the LOs derived from healthy human liver tissue showed the potential of LOs as a material to screen antiviral drugs and to address drug toxicity³⁸. Furthermore, genetic characterization of LOs derived from non-tumor HBV-infected patient tissues displayed early hepatocellular carcinoma (HCC)-like signature³⁸, indicating the possible discovery of novel biomarkers for HCC development in HBV-infected patients. The proof-of-concept for LOs as a drug screening platform against the hepatitis virus was demonstrated in the hepatitis E virus (HEV) infection model³⁹. The adult/fetal liver stem cell-derived LOs supported the full life cycle of HEV infection, leading to robust type I interferon response, implicating the choice of proper therapeutic reagents based on their mechanism of action. By screening safe-in-human broad-spectrum antiviral agents in LOs, the authors identified two novel compounds, brequinar & homoharringtonine, that inhibited viral replication at very low concentrations³⁹.

Another liver pathogen *Plasmodium falcifarum* (*Pf*) parasites cause malaria by reaching the liver and invading hepatocytes where they massively replicate, differentiate, and eventually rupture the hepatocytes and enter the bloodstream. Given that a small number of *Pf* can lead to severe malaria, understanding the initial hepatic phase of *Pf* infection is critical for disease control and vaccine development. A main bottleneck in the *Pf* research had been the absence of proper liver models. However, recently LOs were used to address the initial *Pf* pathophysiology⁴⁰. LOs support complete *Pf* development from sporozoites to schizonts expressing typical merozoite surface protein. scRNA-seq of infected LOs revealed that upon *Pf* infection, the host metabolism changes to accommodate energy consumption by upregulating lipid metabolism and glycolysis. Interestingly, the authors also detected upregulation of SR-B1, known to be a host receptor of *Pf* invasion, the function of which remains to be determined⁴⁰.

2-2-Hepatic infection using OoC approaches

While Liver-on-chips can be generated with human primary hepatocytes with other non-parenchymal cells^{41, 42}, a more advanced Liver-on-chip method was reported using LOs and matched blood samples to address adaptive immune responses against the hepatitis C virus (HCV). Natarajan *et al.* used microfluidic chips to co-culture ACS/TSPC-LOs pulsed with HCV-specific peptides and HLA-matched primary human CD8⁺ T cells specific for the HCV peptides. HCV peptide-pulsed LOs are embedded in the central channel that is sandwiched with top and bottom media channels, one of which being loaded with specific CD8⁺ T cells. Applying a lateral pressure gradient to allow T-cells to translocate from the media channel to the central channel where LOs are located, the authors observed T-cell-mediated, specific killing of infected LOs⁴³. This microfluidic co-culture system can be applied to identify HCV-specific epitopes and to understand the following adaptive immune responses to design HCV vaccines. The same principle can contribute to investigate (personalized) hepatic (or other) cancer immunotherapy.

3-Respiratory system

The cellular composition of the different epithelia along the respiratory tract varies both in nature (differ cell types) and in proportion. Advances in stem cell engineering of lung tissue directly impacted the study of respiratory diseases. Differentiation pathways from cell lines, PSCs, and ASCs towards mature airway epithelium have yet to be fully understood. Some level of differentiation has been achieved using 2D cultures from ESCs^{44 45}.

3-1- Lung infections using organoid-based models

The advent of 3D-culture systems and especially organotypic cell culture systems have provided more mature lung epithelium. Different parts of lung tissue have been modeled. The early branching structure could be recapitulated using both PSCs⁴⁶ and fetal tissue extracts⁴⁷. Organoids resembling bronchiolar⁴⁸ and self-renewing bronchiolar organoids have been derived from adult lung extracts⁴⁹. Alveolar epithelial spheroids have been derived from hPSCs⁵⁰ and from bud tip organoids as well⁴⁷. Translating organoid engineering into infection assays needs optimization as most pathogens interact with the apical side of the epithelium. For instance, a study described the use of opened human bronchiolar organoids to enable the analysis of SARS-CoV-2 infections⁴⁹. Another study used microinjection into the lumen of the organoids to demonstrate the ability of *Mycobacterium* bacteria to infect the epithelial cell⁵¹. While very useful for studying infection mechanisms, this method is not suited for large screening assays. Another approach consisting of inverting the polarity of the organoids was used to provide a platform for both respiratory virus infection and antiviral screening on bronchiolar organoids⁵². Interestingly, human neutrophils were attracted and interacted with Respiratory Syncytial Virus (RSV) infected organoids⁴⁹. This shows organoid culture could at least probe the initial stages of immune response.

3-2-Lung infections using OoC-based models

Other 3D-cell culture techniques can be used to circumvent some of the drawbacks linked to organotypic cell culture. As lung tissue naturally exhibits an air-liquid interface, the use of membranes and microfluidic devices provides interesting spatial compartmentalization. For instance, static porous membranes can be used to grow cells with an air-liquid interface (ALI). In static conditions, it was demonstrated that SARS-CoV-2 impaired mucociliary clearance⁵³. Moreover, multiple systems were developed to apply mechanical stimuli to lung epithelium. Briefly, lung epithelial cells were seeded on a membrane or structure that could be stretched as it was embedded in a microfluidic device^{54,55,56} to mimic alveolar compartments. Adding a mechanical actuation improves the system's biomimicry. It was shown that physiological stretching of the epithelium led to an increase in nanoparticles crossing the epithelial barrier⁵⁵. These configurations also enable ALI conditions thus providing even more physiologically relevant systems. Finally, the compartmentalization naturally enables co-cultures of endothelial cells in the vicinity of the epithelium. Recreating the alveolar interface to such an extent enabled Thacker et al⁵⁷ to model *Mycobacterium tuberculosis* infection in a murine model. The accessibility to the epithelial side proved crucial in demonstrating surfactants play a key role in containing bacterial growth. In a human system, Bai et al⁵⁸ show that mechanical stimuli activate the immune response in both epithelium and endothelium to suppress Influenza and SARS-CoV-2 infections. The use of such OoC devices was not restricted to alveolar systems. It was shown that airway

epithelium within OoC devices could be used to screen antiviral therapies with some alternative configurations enabling high throughput screening⁵⁹.

Most of the OoC platforms have demonstrated their capabilities using either cell lines or primary cells. However, several studies are now taking advantage of both organotypic cultures and porous membrane technologies (OoC, transwell). For instance, Lamers et al. ⁶⁰ expanded the fetal bud tip organoids prior to seeding these cells on transwell membranes. Moreover, patient-derived cells could be expanded into organoids and then seeded on microfluidic chips⁶¹. The latter study exemplifies how organoids and OoC pave the way for personalized screening. Overall, the ability to model infections and probe therapies relies on the ability to produce the complexity of the lung environment. While great advances have shown the ability to recreate the lung epithelium, it has not been demonstrated that the endothelial part in OoC systems provides a physiological model.

4-Urinary system

The urinary system, encompassing the intricate interplay between the kidneys and bladder, plays a critical role in maintaining homeostasis and filtration within the human body. The kidneys have a complex architecture, densely vascularized, and organized in nephrons and glomerulus units composed of multiple cell populations, including glomerular podocytes, proximal tubular epithelial cells, distal tubules, and collecting duct cells. These specialized cells collectively orchestrate the fine-tuned filtration and reabsorption processes, effectively maintaining fluid and electrolyte balance within the body. The bladder, a hollow muscular organ situated in the pelvic cavity, serves as the reservoir for urine storage. Structurally, it is a stratified organ composed of smooth muscle layers, transitional epithelium, and connective tissue, enabling its distension during urine accumulation. The arrangement of the detrusor muscle and the presence of urothelial cells lining the inner surface of the bladder play a crucial role in maintaining urinary continence and preventing urine backflow into the kidneys⁶².

The urinary system is subject to several mechanical forces such as strong fluid shear stress (FSS), stretching, and compressive forces. More specifically, high blood flow is involved in its filtration through the glomeruli, by hydrostatic pressure forcing water and solutes out of the blood. Peristaltic contraction (stretching and compression forces) of smooth muscle layers in the ureter propels urine through the ureters and into the bladder. This mechanical force ensures unidirectional flow and prevents urine reflux from the bladder back into the kidneys. Also, in the bladder, the detrusor muscle is responsible for emptying the organ during micturition (urination). When the bladder reaches a certain level of distension due to the accumulation of urine, stretch receptors signal the detrusor muscle to contract. This contraction generates mechanical force, increasing the intravesical pressure

and expelling urine through the urethra. All these mechanical forces are essential for the function of the urinary system and can strongly impact infections^{63 52}.

The urinary tract infections (UTI) are very common and may be fatal. The majority are caused by uropathogenic *Escherichia coli* (UPEC) bacteria invading the bladder⁶³. In some cases, UPEC or other Gram-negative bacteria ascend into the kidney causing pyelonephritis leading to Acute Kidney Injury (AKI), urosepsis, or even death. In addition to bacteria several viruses have been reported to infect the kidney including HIV, Hepatitis, Dengue, Monkeypox, and even SARS-CoV-2 viruses.

4-1-Kidney and bladder infections using organoids

While pathogens can easily be detected in the urine, investigating infection in the kidney or bladder is challenging. This led researchers to develop several *in vitro* models that mimic the urinary system. Many reports described kidney organoids generation from h(i)PSC⁶⁴, but while their transcriptional profiles are close to mature kidneys, their cell organization and polarity are not complete, especially showing a lack in apically located transporters, essential for the function of the nephron. However, a recent work described an enhanced protocol for proximal tubule (PT) organoids by extending the mesodermal patterning step⁶⁵. These enhanced PT organoids have an increased expression of apical transporters and other markers like ACE2 and enable efficient uptake tests, and drug screening as well as SARS-CoV-2 invasion⁶⁵. In addition, using another protocol to generate human kidney organoids with tubular and glomerular structures as well as stromal and endothelial cells, another group was able to successfully observe the complete infectious cycle of the Monkeypox virus, showing for the first time the direct invasion of this virus into the kidney⁶⁶. Bladder organoids have been developed and successfully used to analyze in real-time solitary UPEC invading the deeper layer of the epithelium⁶⁷.

4-2- Kidney and bladder infections using OoC systems

Outside of kidney organoids, the most promising kidney surrogate to investigate host-microorganism is kidney-on-chip models. The kidney-on-chip approach aims to closely mimic the dynamic and flowing environment of the kidney using a 2D/3D cellular model within a microfluidic device. The system consists of a 3D architecture made up of renal cells grown on an ECM interface or next to perfusable microchannels, allowing media, blood, or urine to flow across the cell surface. These models have demonstrated that fluid shear stress (FSS) significantly influences the phenotype of renal tubular cells, making them valuable for studying nephrotoxicity and renal function⁶⁸. Different types of kidney-on-chip models have been developed to mimic various segments of the renal unit like Glomerulus-on-chip or (proximal/distal) Tubule-on-Chip, that have been already well described⁶⁹. So far only one study describes the use of a very simple microfluidic chip to investigate host-pathogen interactions in the kidney⁷⁰. In this work, MDCK cells were used to recapitulate tubule-on-chip with physiological FSS to

analyze pseudorabies virus infection and its impact on kidney function⁷⁰. The authors observed for the first time the efficient invasion and replication of the virus and the profound effect of this infection on the barrier integrity and on basic functions of renal tubules like the Na⁺ reabsorption capacity⁷⁰.

Sharma et al. used a commercially available bladder-on-chip to investigate UPEC infection⁷¹. This chip is composed of two channels separating bladder (umbrella) epithelial cells from microvascular endothelial cells, under a flow of urine fluid and nutrient media respectively, in order to recapitulate the bladder filling and micturition cycles⁷¹. Using this model, the authors monitored an intracellular bacterial community (IBC) in real-time after several cycles of antibiotic treatment and observed that a minor proportion of IBC was resistant to antibiotics and that this was sufficient to allow these bacteria to proliferate between two courses of antibiotics, explaining the difficulty of treating UPEC in patients^{63,71}. In this study, recapitulating the physical forces of the bladder (emptying/filling), was key to identifying the role of IBC in the persistence of UTIs⁷¹. Urinary system organoids-on-chip are currently under intensive development and several models are already available with proven physiological relevance; they should be used in future infectious diseases studies.

5-Brain

The *in vitro* study of infectious diseases with a neurological tropism hinges on the availability of pertinent assays that can disentangle the pathogen ingress processes (Blood Brain Barrier (BBB) models) and on advanced cell culture systems that can recapitulate the pathogenesis (Brain organoids). In the last decade both fields of research have made considerable progress and today several investigations targeting brain pathogens have benefited from the latest advancements in BBB-on-chip and in brain organoid derivation.

5-1- Brain microfluidic devices to investigate neural infections

Recapitulating the tightness and selectivity of the human BBB is very challenging as both architecture and cell composition contribute to the BBB function. Relying on cell self-organization in flat Transwell™ culture systems was partially successful in achieving physiological tightness⁷². The use of topographically structured microfluidic chips based on hydrophobic pinning⁷³ or viscous fingering⁷⁴ allowed the production of microdevices where different cells can be compartmentalized and perfused with different medium compositions. This compartmentalization allowed the coculture of different tissues on chips. Typically, endothelial lining and neural tissue could be juxtaposed, and diffusive exchanges could be established and quantified between the two tissues on the same chip. These chips, when seeded with human primary cells including endothelial, pericytes, and other astrocytes, microglia, or neurons demonstrated good levels of self-organization into functioning microtissues with short-term permeability comparable to *in vivo* data⁷⁵⁻⁷⁷ with typical astrocytic feet and pericytes

engulfing vasculature. Typical assays consisted of measuring Dextran diffusion or transendothelial electric resistance (TEER) across the engineered endothelial/neural interface as a proxy for physiological BBB permeability. Currently, these BBB-on-chip systems are still undergoing further developments in order to implement adaptive immune responses, active transport, and long-term tightness (multiple days). BBB and other neurovascular units on-chips were essentially studied to investigate transport/permeability⁷⁷ and neurodegenerative disorders⁷⁸. The first report on the use of a BBB-on-chip model to investigate infectious diseases looked at a fungal infection⁷⁹. The study by Kim et al. allowed to dissect the potential mechanisms of action of *Cryptococcus neoformans* and could show that the fungus achieved barrier crossing without destabilizing the tight junctions of the endothelial lining pointing to the involvement of transcytosis mechanisms.

5-2- Brain organoids to investigate neural infections

On the other hand, the seminal work of Lancaster et al. showed that human pluripotent stem cells can be steered to self-organize *in vitro* into human cerebral organoids (hCO) that emulate neural tissue architecture and function⁸⁰. Further investigations showed that recapitulating specific regions of the human brain including the forebrain, midbrain, and hypothalamic region⁸¹ was possible. Finally, the production of brain assembloids that merge two or more brain organoid types was also demonstrated⁸². Very interestingly efforts towards vascularizing these microtissues are also ongoing⁸³ with the objective to engineer a BBB in these models hCOs were very quickly utilized for infection studies. The concomitance of the Zika outbreak in 2015 with the active development of brain organoids helped with the use of these models to investigate viral infections first^{81,84}. These papers showed that infected brain organoids can recapitulate the microcephaly phenotype associated with Zika virus infection through the abrogation of neurogenesis during development. Dang et al.⁸⁵ further reported the involvement of TLR3-triggered apoptosis in brain organoids infected with Zika virus. SARS-CoV-2 infection was also associated with neurological symptoms. Reports using brain organoids confirmed the neurotropism of this new virus appeared as early as 2020. SARS-CoV-2 was shown to specifically infect neurons and to trigger cell death through the aberrant phosphorylation of the Tau protein⁸⁶. In contrast in a choroid plexus (ChP) model, the infection of neurons was not confirmed but the destruction of the epithelium and the loss of tightness was reported upon SARS-CoV-2 infection⁸⁷. Human cerebral organoids were also utilized to investigate other virus-mediated diseases including CMV or HSV-induced microcephaly (^{88,89}respectively) and La Crosse mediated encephalitis⁹⁰. It is also worth noting that the majority of the brain organoid studies (excluding the ChP models) are not able to address the pathogen barrier crossing step. There, the produced results should be understood in the context of a fully compromised barrier function and outside of any competition for receptors located on the epithelial/endothelial side of the BBB. OoC devices can, in fact, facilitate a more

comprehensive investigation by considering the tissue's physiology, dynamics, and interactions between multiple cellular populations coexisting in the same microenvironment in addition to the cellular point of view.

Conclusions and perspectives

Overall, organoid and OoC technologies are evolving rapidly and have shown great potential for mimicking human tissues function. Hence these systems are particularly interesting to investigate infections. Key tissues, including the gut and lungs epithelia, can be accurately replicated by organoids but the use of OoC devices has led to a better understanding of the complexity of host-pathogen interactions. This method allows taking in consideration biophysical cues that might affect the pathogen's invasion. It is worth noting that, complex, non-epithelial tissues such as the brain still cannot be successfully emulated by OoC technology. Full brain-on-chip systems will still require intensive development efforts. On the other hand, organoids, can bridge this gap. Cerebral organoid strategy appeared relevant to study viral infections that trigger massive cell death or that impair normal brain development leading to microcephaly.

Ultimately the combination of organoids and OoCs was shown to be a very strong asset for the reconstruction of highly physiological tissue models. Organoids-on-chip models are gaining traction as they combine best of both worlds: accurate cell composition and faithful architectures or geometries. These tissue surrogates are very powerful when dealing with questions where no suitable animal or *in vitro* models exist, such as human restricted pathogens, live imaging of deeply buried cellular processes, or to study the impact of tissue mechanics, among others. In the future, establishing comprehensive disease models will certainly entail the coupling of the OoC and organoid technologies. This may be a useful way to obtain a more physiological model, even *in vitro*. The scientific community must continue to develop these technologies, as several organs, such as the brain, are still far from being fully recapitulated. We hope that this review will encourage the scientific community to adopt these technologies to study host-microorganism interactions, which until now have been marginally used. Hopefully the better availability of source material and the development of practical on-chip solutions that can be efficiently deployed by infection biology specialist will make these approaches more popular.

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Figure Legend

Figure 1: Organoids and OOC can recreate a specific organ and allow the study of host-microorganism interaction. Human or animal induced Pluripotent Stem Cells (iPSCs), Embryonic Stem Cells (ESCs) or Adult Stem Cells (ASCs), enable to generate organoids, closed structures with 3D topology, liquid-liquid interface, and a high cellular diversity. Organoids can then be matured in organ-on-chip, which allows to have not only the 3D topology and the cellular diversity, but also different kind of interfaces and the mechanical stimulation. Both models can be used to study host-pathogen interaction, injecting microorganisms by microinjection in organoids' closed lumen or directly in the microfluidic system for organ-on-chips. Human cell lines cannot be used to generate organoids, but they can be matured with mechanical stimulation in organ-on-chips, recreating a 3D topology and a cellular diversity. The figure was created using BioRender software.

