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Organ-on-Chip: The Future of Nutrition Research in a One Health World

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ABSTRACT

The One Health approach emphasizes the interconnectedness of human, animal, and environmental health, recognizing that the health of each is interdependent and influenced by shared ecosystems. Nutrition research plays a critical role in improving health outcomes across these domains, with implications for sustainability and food security. Organ-on-chip (OoC) technologies have emerged as innovative tools replicating key organ functions, supporting disease modeling, drug discovery, and personalized medicine. They also hold promise as alternatives to traditional animal models. This systematic review examines the potential of OoC technologies within the One Health framework and nutrition research, focusing on (1) their ability to replicate human and animal organ functions, (2) applications in food safety and ecotoxicology, and (3) their use in studying food components' health effects. Challenges and future directions for adoption are also discussed. Although fully replicating the complexity of in vivo physiology remains a challenge, OoCs offer a promising platform to simulate organ functions and interactions. These systems hold significant potential for advancing food safety assessments, studying food impacts on health, and addressing sustainability in food systems. Challenges such as standardization, scalability, accessibility, and biases toward traditional models remain. Despite these hurdles, current advancements underscore the versatility and promise of OoCs, positioning them as valuable tools for driving innovation in nutrition research, food and feed safety, and ecotoxicology. With continued progress, OoCs are poised to make significant contributions to the goals of the One Health framework.

1 | Background

An organ-on-chip (OoC) system, often referred to as a "microphysiological system" (MPS), is a sophisticated microfluidic cell culture platform designed to replicate the structure and function of human organs and tissues in vitro. Unlike the traditional concept of organs, OoC systems do not aim to replicate entire organs but rather focus on key functional units, such as liver cell aggregations, cardiac muscle segments, or epithelial–endothelial interfaces. These systems capture essential physiological characteristics, including enzyme secretion, contraction force, and barrier function, offering insights into organ-specific responses.

OoC platforms are characterized by four distinct features: (1) the incorporation of multiple cell types to reflect the diversity of tissues, such as vascular, stromal, parenchymal, and immune cells; (2) microfabricated structures and fluidic channels that simulate the native tissue environment under biophysical stimuli like shear stress; (3) interfaces, including membranes or pillar structures, that mimic nutrient, drug, and oxygen transport, as well as cellular trafficking; and (4) microstructured compartments that allow tissue constructs to replicate core organ functions.

Advanced iterations, known as multi-OoC systems, connect individual OoC devices representing distinct organs through microfluidic channels, enabling researchers to study inter-organ communication and systemic responses. These integrated platforms can support a variety of cell cultures, from monolayers to organotypic models like organoids and spheroids, with systems modeling up to 13 interconnected organs, earning the designation "body-on-chip" (Miller and Shuler 2016).

The applications of OoCs are rapidly expanding, encompassing human pathophysiology, pharmacological testing, environmental toxicology, and personalized medicine (Ingber 2022). For instance, models of the stomach (Ferreira et al. 2023; Lee et al. 2018), intestine (Kulthong et al. 2018; Lee et al. 2023; Seiler et al. 2020), liver (Banaeiyan et al. 2017; Ewart et al. 2022), pancreas (Tian et al. 2023), and brain (Liu et al. 2019; Zhao et al. 2021) demonstrate the versatility and potential of this technology to mimic diverse organ functions.

Traditional methods, such as static cell cultures or animal models, face significant limitations in replicating the complexity of human physiology. Cell cultures fail to mimic three-dimensional (3D) structures and tissue interactions, whereas animal models present translational challenges due to species-specific differences. OoCs address these gaps by providing human-relevant models that closely emulate physiological functions, enhancing the validity of research findings while contributing to the reduction of animal testing.

"One Health" is an interdisciplinary framework that recognizes the intrinsic connections among human, animal, and environmental health. It emphasizes collaborative, multi-sectoral, and transdisciplinary approaches to address global health challenges, such as (foodborne) zoonotic diseases, antimicrobial resistance, food safety, the impact of environmental (food chain) contaminants on health, and climate change. By integrating knowledge and expertise from diverse fields, One Health aims to develop sustainable strategies that promote health and well-being across all three domains.

Nutrition research plays a critical role in advancing human, animal, and environmental health, with implications for sustainability and food security. The One Health framework aligns well with the potential of OoC technology within the context of nutrition research. By replicating key physiological processes in vitro, OoCs offer a platform to explore interactions among environmental contaminants, nutrition, and health. These systems can advance food safety by enabling the study of environmental toxicants like pesticides, heavy metals, and persistent organic pollutants (POPs), which pose significant risks to human and animal health. OoCs also hold promise for evaluating sustainable food systems, contributing to safer and healthier diets.

This systematic review explores the current and potential applications of OoC technology in nutrition research within the One Health framework, highlighting their versatility in addressing human, animal, and environmental health challenges. Additionally, the review highlights the limitations and challenges that need to be addressed to fully realize the potential of this emerging technology, while proposing potential solutions to overcome these obstacles.

2 | Methods

2.1 | Search Strategy and Study Selection

A systematic search of the literature was conducted to identify relevant studies for inclusion in this review. The electronic database PubMed was searched for articles published from January 2015 to December 2024. The search terms included combinations of keywords related to OoC technology and (1) relevant organs or systems (e.g., intestine, liver, and multi-organ systems), (2) food safety, (3) environmental contaminants, and (4) human and animal health. The list of keywords with MeSH terms and Boolean operators utilized is detailed in Supporting Information Appendix A.

2.2 | Study Selection

The titles and abstracts of retrieved articles were independently screened by two reviewers to identify potentially relevant studies. Full-text articles were then evaluated for eligibility based on predetermined inclusion and exclusion criteria. Studies meeting the following criteria were included: (1) focusing on or related to the use of OoC technology and (2) addressing human or animal health outcomes in the context of nutrition research, food safety, ecotoxicology, and environmental toxicology. Both primary research articles and gray literature were considered for inclusion. Additionally, both the reference lists of selected full-text articles and the list of similar articles retrieved from PubMed underwent scrutiny to identify any potentially overlooked scientific articles or related documents, which were included if they met the predefined inclusion/exclusion criteria. Exclusion criteria comprised studies unrelated to the topic, reviews, studies published before 2015, and duplicate publications or contents. However, review papers were included in the qualitative analysis and discussion

sections to provide broader context, identify gaps in the literature, and highlight potential future directions. This approach allowed for a comprehensive and nuanced discussion while maintaining a focus on primary research for the quantitative results. Similarly, studies published before 2015 were considered in the discussion to contextualize findings, reinforce scientific rigor, strengthen the evidence base, and provide historical perspectives where relevant.

2.3 | Data Extraction

The following information was extracted: study characteristics (author, year of publication), study design, OoC platform used, cell types and source, nutritional/chemical/pathogen/drug exposures, outcomes measured, and main findings.

2.4 | Quality Assessment and Data Synthesis

The quality of included studies was assessed independently by two reviewers using appropriate tools depending on the study design. Any discrepancies in quality assessment were resolved through discussion or consultation with a third reviewer. Data synthesis was conducted to summarize the relevant findings or statements of the included research studies. Descriptive statistics were used to summarize the characteristics of primary research studies where applicable.

3 | Results

The search yielded 4930 results, out of which 97 were considered eligible on the basis of their title or abstract. Following full-text screening, 90 articles were included. The publication dates span from 2015 to 2024. A PRISMA flow diagram depicting the selection procedure has been created; please refer to Figure 1.

To enhance readability, we have divided the results into thematic sections exploring the potential of OoC platforms within the One Health framework. First, we provided an overview of the main OoC models of potential relevance to nutrition research, exploring their capacity to replicate key aspects of in vivo physiology. In the second section, we investigate the current and potential applications of OoCs in the assessment of food safety. We examine how OoC technology can be leveraged to evaluate the safety of food and feed products by assessing possible hazards, including environmental contaminants in the food chain, toxins, and foodborne-zoonotic pathogens. Additionally, we address broader ecotoxicological impacts. Lastly, we identify the applications of OoCs in the study of the potential influences of specific foods or food-derived compounds as well as food additives on human health.

3.1 | Advancements in Gastro-Intestinal OoC Technologies: Replicating Key Aspects of In Vivo Organ Physiology and Interactions

A wide range of human and animal tissues and organs have been modeled through OoC technology. In regard to the digestive system and its associated organs, the stomach, small and large intestines with associated microbiota, and liver have been successfully modeled. These models are increasingly being integrated into multi-organ systems to study various inter-organ axes, such as the gut–liver and gut–brain axes, providing valuable insights into their complex interactions and systemic effects. It is important to note that OoCs do not replicate the function of an entire organ or organs system; rather, they represent living 3D cross-sections of key functional subunits of organs such as the intestinal villi, the microbiota–intestine interface, liver lobule, or stomach epithelium.

3.1.1 | Stomach-on-chip

The stomach plays a crucial role in the digestive process, serving as the primary site for food storage, mixing, and initial digestion. Its intricate structure and function make it a pivotal focus in nutrition research. Lee et al. (2018) pioneered a bioengineered framework aimed at introducing luminal flow-through human pluripotent stem cell-derived gastric organoids (hGOs) to enhance the representation of in vivo gastric functions. They described an inventive microfluidic imaging setup housing hGOs with controlled peristaltic luminal flow in a laboratory setting. This human stomach-on-chip technology enables robust, sustained 3D growth of hGOs while facilitating luminal delivery through a peristaltic pump. Additionally, this system enables researchers to rhythmically apply stretch and contraction to the organoid, simulating gastric motility. This platform holds promise for long-term replication of nutrient delivery into the stomach in vitro, offering a valuable tool for investigating human gastric physiopathology (Lee et al. 2018). Ferreira et al. (2023) developed a stomach-on-chip device that faithfully reproduces both the structural and functional features of the native organ, encompassing the gastric epithelial barrier function and its selective permeability. Their model facilitated a sustained 3D co-culture of epithelial and mesenchymal cells over an extended period, preserving cell homeostasis and viability without adverse effects. Notably, they demonstrated its ability to evoke organ-level responses, as the cells exhibited phenotypic, molecular, and functional traits characteristic of normal gastric mucosa, traits typically unobservable in conventional two-dimensional (2D) cultures. This design affords convenient access to both the apical and basal sides of the gastric epithelium, presenting a versatile model suitable for investigating or simulating luminal and systemic stimuli on the gastric mucosa. Another advantage of this system is its utilization of a biomimetic supporting extracellular matrix, serving as an analog to the lamina propria (Ferreira et al. 2023).

Various stomach diseases arise from the dysregulation of the gastric mucosal barrier by pathogens. A recent study introduced a human stomach MPS (hsMPS) combining organoid and microfluidic technology. The hsMPS facilitated functional maturation of gastric epithelial cells through enhanced fluid flow, leading to the recreation of a mesh-like mucus layer rich in protective peptides and well-developed epithelial junctions. Additionally, the hsMPS demonstrated successful in vivo-like gastroprotection mechanisms against pathogens (Jeong et al. 2023).

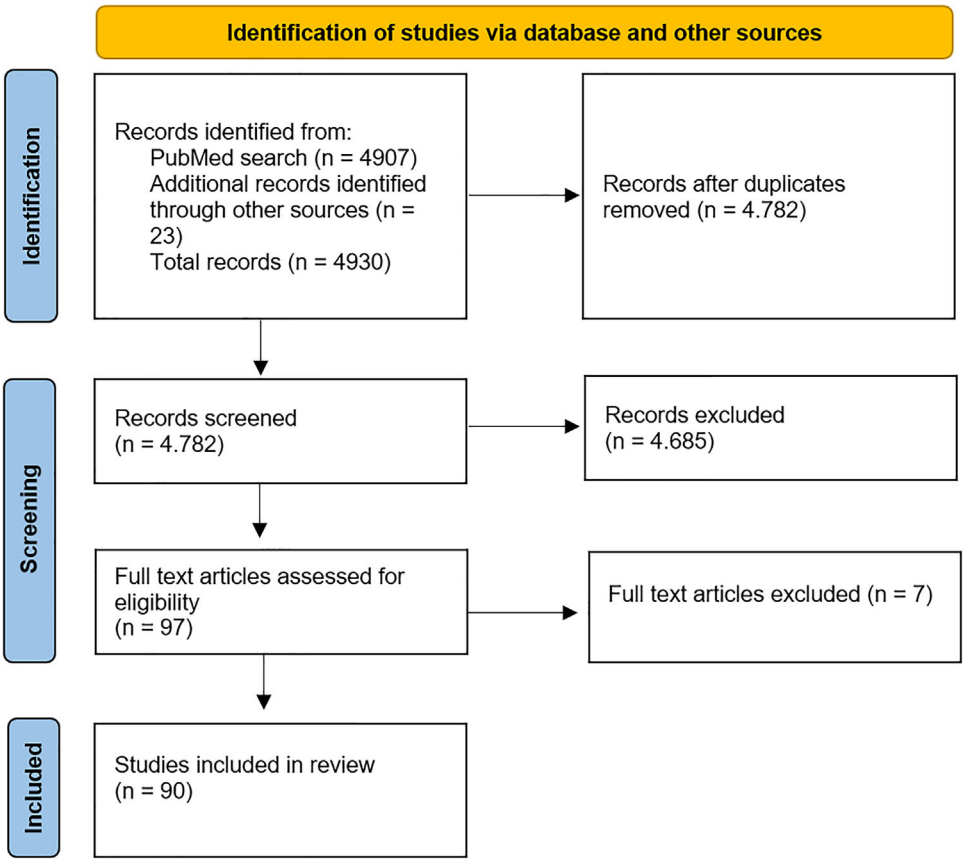

FIGURE 1 | A PRISMA flow diagram of the included literature.

3.1.2 | Intestine-on-Chip and Gut-Microbiota-on-Chip

The intestinal mucosa represents a complex physical and biochemical barrier that serves a multitude of essential functions. It facilitates the transportation, absorption, and metabolism of nutrients and xenobiotics, while also fostering a symbiotic relationship with the microbiota and preventing the invasion of microorganisms. The functional interplay among various cell types and their surrounding physical and biochemical environment is crucial for the establishment and maintenance of intestinal tissue homeostasis. Flow, achieved through various methods like syringes (Kulthong et al. 2018; Zhao et al. 2022), pumps (Jing et al. 2020; Tovaglieri et al. 2019), or gravity (Seiler et al. 2020; Shim et al. 2017), is integral to microfluidic gut-on-chip models, enhancing physiological relevance by replicating in vivo fluid flow and exposing cells to corresponding shear stresses. Dynamic fluidic culture improves cell differentiation (Workman et al. 2018; Yin et al. 2021), morphogenesis (Maurer et al. 2019), barrier integrity (Jeon et al. 2022; Maurer et al. 2019), enzyme activity (Shim et al. 2017), and transcriptome profiles (Kasendra et al. 2020; Kulthong et al. 2021) compared to static conditions. Flow also aids in prolonging epithelial integrity and enhancing nutrient access (Nikolaev et al. 2020; Verhulsel et al. 2021).

Mechanical forces, such as the rhythmic contractions in the intestine, are vital for gut propulsion and impact intestinal homeostasis and development. Altered gut motility is implicated in several diseases (Bassotti et al. 2020). Research utilizing mechanical gut-on-chip models has shown that cyclic strain can affect intestinal epithelial cells permeability and enzyme activity, as well as enhance pathogen virulence and gene expression (Apostolou et al. 2021; Boquet-Pujadas et al. 2022; Grassart et al. 2019). Computational studies highlight peristalsis as a critical factor affecting gut-on-chip systems, potentially influencing cell differentiation (Borwornpiyawat et al. 2022). In addition, fluidic flow and peristalsis have been found to be a critical factor in modulating active host–microbiota interplay. Kulkarni et al. (2022) outlined a comprehensive protocol for creating intestine-on-chip models mimicking both the human duodenum (duodenum-on-chip) and colon (colon-on-chip), followed by their cultivation under sustained flow conditions and peristalsis-like movements. They illustrated techniques for evaluating drug metabolism and CYP3A4 induction in the duodenum-on-chip using standard inducers and substrates (Kulkarni et al. 2022).

Over the past 6 years, the development of novel models mimicking physiological hypoxia has facilitated the co-cultivation of strict anaerobes alongside human cells. Advances in oxygen sensor technology, coupled with its miniaturization, have led to the creation of new platforms featuring integrated oxygen sensors and increased throughput (Azizgolshani et al. 2021; Khalid et al. 2022). Microfluidic models have emerged as key tools in refining the simulation of intestinal oxygen gradients, thereby replicating physiological conditions for co-culturing both aerobic and anaerobic microorganisms, ultimately enhancing the mimicry of host–microbial interactions (Jalili-Firoozinezhad

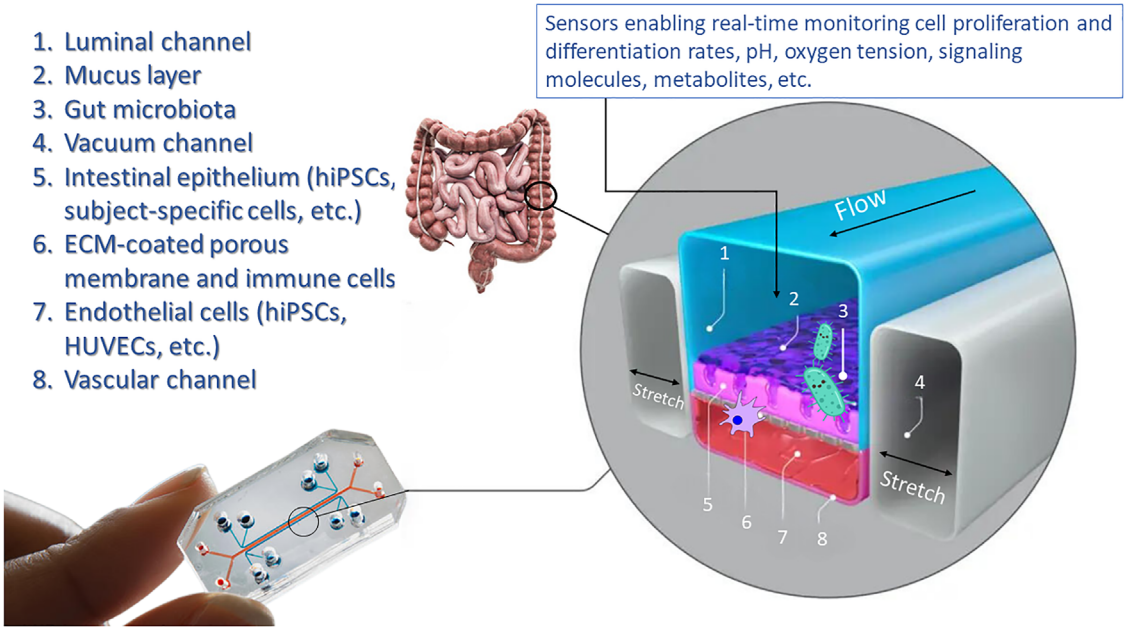

FIGURE 2 | A magnified schematic depicts the typical architecture of a sophisticated, mechanically dynamic human intestine-on-chip system. Subject-specific intestinal epithelium, complete with resident microflora, immune cells, and vascular endothelium, are arranged on opposing surfaces of an ECM-coated flexible porous membrane. This setup is subjected to fluid flows and peristalsis-like strains. ECM, extracellular matrix; hiPSCs, human-induced pluripotent stem cells; HUVECs, human umbilical vein endothelial cells. *Source:* Adapted from https://wyss.harvard.edu/technology/human-organs-on-chips/ and from https://elifesciences.org/articles/50135.

et al. 2019). Gut-on-chip devices can be integrated with transepithelial electrical resistance (TEER) sensors and electrochemical sensors for dynamically simulating the formation of the physical intestinal barrier and monitoring the transport and absorption of nutrients and xenobiotics (Lucchetti, Werr et al. 2024; Ogulur et al. 2023; Pöschl et al. 2023; Wang et al. 2023). TEER is a method used to evaluate the integrity of the epithelial barrier formed by the intestinal cells in the OoC device. It measures the electrical resistance across this cell layer, providing insights into how tightly the cells are joined together. Higher TEER values indicate a more intact and functional barrier, reflecting the ability of the intestinal cells to prevent the passage of molecules and pathogens from the luminal side to the bloodstream.

The intestinal mucus forms a barrier between the epithelium and the lumen. The loss of mucus homeostasis is an important factor in the pathogenicity and severity of several diseases, including foodborne-zoonotic diseases. Despite its significance, the incorporation of a mucus component in OoC models remains limited. Hagiwara et al. (2022) applied a mucus layer on the surface of Caco-2 cells to shield them from bile acids and mimic intestinal fluid properties. Meanwhile, Sontheimer-Phelps et al. (2020) utilized patient-derived colon organoids to construct a model featuring mucus-secreting goblet cells, forming a bilayered mucus structure akin to in vivo observations.

Several gut-on-chip models have incorporated a vascular element to emulate the interface between intestinal and endothelial cells (Jalili-Firoozinezhad et al. 2019; Jeon et al. 2022; Jing et al. 2020; Maurer et al. 2019; Seiler et al. 2020), immune compartment (Ramadan et al. 2022), and fibroblasts (Seiler et al. 2020). Figure 2 describes the typical architecture of a sophisticated, mechanically dynamic human intestine-on-chip system.

Gut-on-chip models hold significant potential for mimicking the gastrointestinal environments of various species, contributing to a holistic understanding of health within the One Health framework. In this context, Drieschner et al. (2019) developed the first fish-gut-on-chip model, which reconstructs the intestinal barrier of rainbow trout by culturing epithelial and fibroblastic cell lines in a controlled microenvironment. The system incorporates ultrathin, porous silicon nitride membranes as basement membrane analogues, a microwell plate-based microfluidic bioreactor for parallelized experiments and fluid flow simulation, and integrated electrodes for non-invasive impedance sensing of cell health. This model enables the investigation of epithelial cell responses to in vivo-like shear stress and the interplay between epithelial and fibroblast cells under optimized flow conditions. The fish-gut-on-chip provides a novel platform for advancing fish physiology studies, optimizing aquaculture feed, and assessing chemical uptake and bioaccumulation for environmental risk analysis. The system's design principles hold potential for broader applications in other barrier-on-chip models (Drieschner et al. 2019) (Figure 3).

Although many intestine-on-chip models primarily utilize immortalized cell lines or primary cells, an increasing number of systems are now employing induced pluripotent stem cells (iPSCs) (Naumovska et al. 2020; Ogulur et al. 2023; Moerkens et al. 2024; Sedrani et al. 2023; Workman et al. 2018).

3.1.3 | Liver-on-chip

The liver, a key organ in nutrient and xenobiotic metabolism, is highly organized to fulfill its functions. Comprising about 70% of the liver's content, hepatocytes are accompanied by

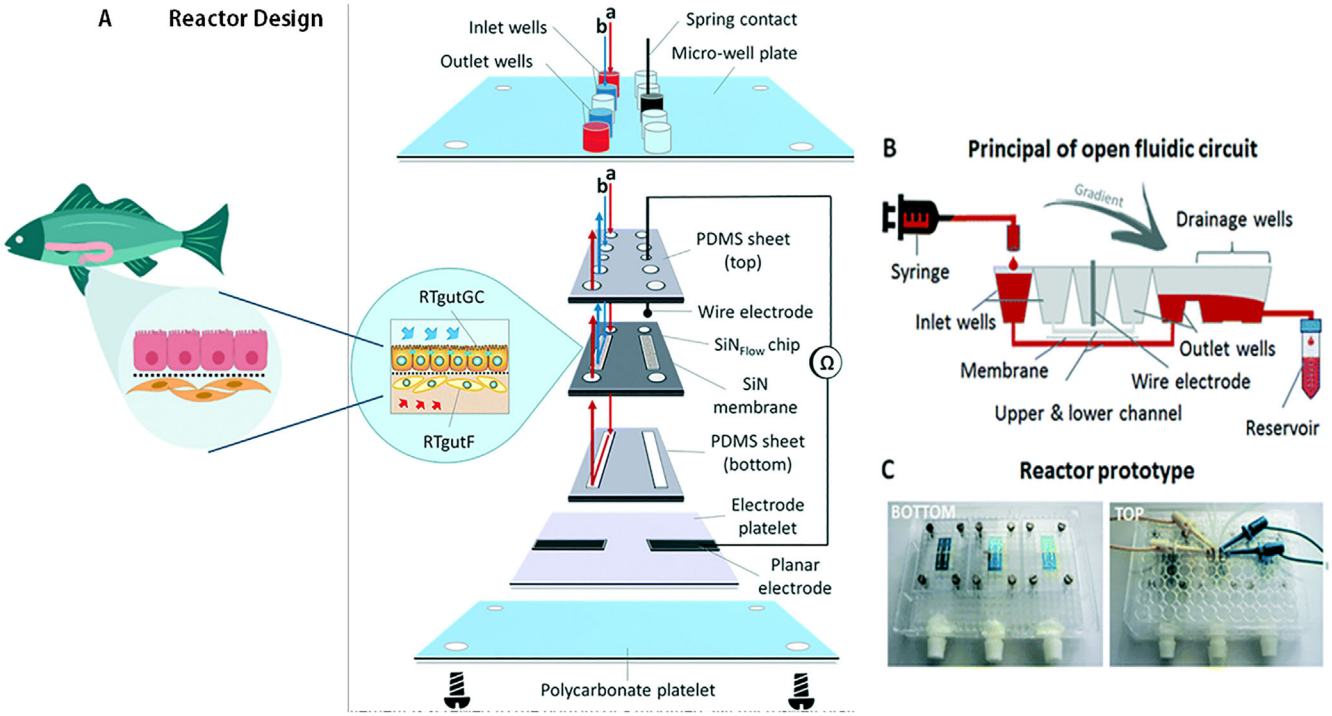

FIGURE 3 | **The fish-gut-on-chip model**. (A) The device features a modular stacked assembly comprising the SiNFlow chip positioned between two PDMS sheets and an electrode plate at the base. This structure is secured to the bottom of a modified 384 microwell plate using a polycarbonate plate. The microwell plate format enables simplified perfusion of the upper and lower microfluidic channels through the top microwells and facilitates connection to reactor-integrated electrodes via spring contacts. A magnified view of the membrane illustrates the conceptual cell culture, showing gut epithelial RTgutGC and fibroblastic RTgutF rainbow trout cell lines cultured on opposite sides of the membrane. (B) The working principle of the open microfluidic circuit is illustrated for the lower microfluidic channel, shown in a partial cross-section of the microwell plate. (C) Photographs of the reactor are presented, showing the bottom and top views. The bottom view displays three SiNFlow chips assembled on the well plate, whereas the top view shows the middle chip connected to tubing for perfusion and cables for impedance spectroscopy. PDMS, polydimethylsiloxane; RTgutF, rainbow trout gut fibroblastic cells; RTgutGC, rainbow trout gut gill cells; SiNFlow, silicon nitride flow chip.

non-parenchymal cells like cholangiocytes, liver sinusoidal endothelial cells, hepatic stellate cells, and immune cells. Hepatocytes are structured into hexagonal lobules, delineated into periportal, pericentral, and midlobular zones. Nutrient and oxygen-rich blood from the gut flows through the portal vein, traverses liver sinusoids, and exits through the central vein. Hepatocytes play roles in nutrient uptake, secretion, and hormonal sensing, creating distinct gradients across liver zones. Additionally, hepatocytes are polarized, forming a barrier between sinusoidal blood flow and bile flow. Banaeiyan et al. (2017) developed a liver-lobule-on-chip device, featuring a network of hexagonal tissue-culture chambers resembling liver lobules. This device allowed for flow circulation between layers, simulating the function of the central vein in a liver lobule. Functionality tests, including albumin excretion and urea synthesis, along with cell morphology, were conducted and maintained. Additionally, the study observed the formation of 3D tissue-like structures and bile-canaliculi features within the device (Banaeiyan et al. 2017). In another investigation, researchers established a microfluidic platform featuring a liver-on-chip structure resembling a tree shape to emulate rat and human liver zonation observed in vivo (Kang et al. 2018). Vernetti et al. (2016) developed a model to assess drug safety in liver disease, comprising a 3D microfluidic system with sequentially layered cell types, including primary human hepatocytes, along with human endothelial, immune, and stellate cells. Additionally, the platform incorporated protein biosensors for mechanistic readouts and a system database to analyze the acquired data. The exposure to a diverse array of drugs elicited anticipated liver effects, such as acute or delayed toxicity, validating the model's relevance for human in vivo scenarios (Vernetti et al. 2016). Jang et al. (2019) utilized microengineered OoC technology to develop liver-on-chip models for rats, dogs, and humans. These models incorporated species-specific primary hepatocytes interfaced with liver sinusoidal endothelial cells, with or without Kupffer cells and hepatic stellate cells, and were cultured under physiological fluid flow conditions. Species-specific toxicities were observed when the models were exposed to specific tool compounds (Jang et al. 2019).

The liver-chip has been characterized as a reproducible and functional model of human liver tissue (Ewart et al. 2022; Jang et al. 2019; Shi et al. 2022), with prior studies demonstrating its capacity to detect drug-induced toxicities, including cholestasis, steatosis, and fibrosis. Notably, the liver-chip exhibited robust transcriptional, morphological, and biomarker profiles that align with industry guidelines for liver MPS used in drug-induced liver injury (DILI) prediction (Baudy et al. 2020).

Järvinen et al. (2021) implemented a fish-liver-on-chip model by optimizing protocols for microwell fabrication (including well shape and size) and surface functionalization to support the culture of fish hepatocytes as scaffold-free 3D spheroids under

microfluidic flow. The authors observed that fish hepatocytes adhered more strongly to the chip surface compared with human hepatocytes, necessitating careful optimization of the pegylation process to promote forced floating and aggregation within the microwells. Under these optimized conditions, the hepatocytes formed compact 3D spheroids, enabling more accurate toxicity testing. For instance, the threshold toxic concentration of ketoconazole (a bioaccumulative antifungal) was significantly lower under microfluidic flow compared with static 2D or 3D cultures, demonstrating the system's potential for studying bioaccumulation and time-dependent toxicity in fish (Järvinen et al. 2021).

The exploration of liver-on-chip models employing immortalized cell lines (Bircsak et al. 2021; Solan et al. 2023), or primary hepatocytes (Docci et al. 2022; Ewart et al. 2022; Jellali et al. 2021), considered the current gold standard, has been extensively researched. Nevertheless, due to the restricted availability of primary hepatocytes, there has been increasing interest in utilizing iPSCs (Bircsak et al. 2021; Fanizza et al. 2023; Schepers et al. 2016).

3.1.4 | Multi-OoC Systems

Although each OoC system provides a limited representation of the individual organ it models, it can be integrated with other OoC platforms. Similar to the interdependence of all organs in the human digestive system, it is feasible to construct a comprehensive network of microfluidic systems that replicate the entire path of a specific substance from one organ to another. With such integrated tools, it becomes possible to assess the effects of dietary bioactive compounds, nutraceuticals, or xenobiotics following ingestion in vitro. Miller and Shuler (2016) introduced a human "body-on-chip" comprising 13 chambers representing various organs. The linking of diverse OoCs within multi-OoC platforms, which replicate the interface and communication among barriers, parenchymal tissues, and systemic circulation, has provided novel opportunities to investigate the in vitro absorption, distribution, metabolism, and bioactivity of nutritional compounds or xenobiotics with unprecedented physiological accuracy.

de Haan et al. (2021) introduced a novel, integrated, in vitro gastrointestinal system combining three compartments into one hyphenated, flow-through setup. In the first compartment, a compound undergoes dynamic exposure to enzymatic digestion in three consecutive microreactors, simulating processes in the mouth, stomach, and intestine. The resulting solution (chyme) then flows to the second compartment, a flow-through barrier model of the intestinal epithelium allowing absorption of the compound and its metabolites. Effluents from the barrier model can be analyzed offline by electrospray-ionization-mass spectrometry (ESI-MS) or online in the final compartment using chip-based ESI-MS. Two model drugs, omeprazole and verapamil, were used to evaluate the integrated model. Omeprazole underwent breakdown upon exposure to gastric acid but reached the cell barrier intact when introduced to the system mimicking an enteric-coated formulation. In contrast, verapamil remained unaffected by digestion. Additionally, reduced uptake of verapamil was observed when dissolved in apple juice, a simple food matrix (de Haan et al. 2021).

Lucchetti, Aina et al. (2024) introduced a multi-OoC platform combining the human gut-microbiota-on-chip with liver-on-chip, replicating the bidirectional interplay between the gut and liver known as the gut–liver axis. Kim et al. (2024) explored the effects of microbiota-derived metabolites and exosomes on neurodevelopment and neurodegenerative disorders employing neurons derived from human iPSCs within a gut–brain axis chip.

Vernetti et al. (2017) assessed five human OoC models to examine functional coupling, which involves studying organ interactions through a process mimicking in vivo sequential transfer of media between organs. The multi-OoC system (body-on-chip) under evaluation represented key organs involved in absorption, metabolism, and clearance, including the jejunum, liver, and kidney, as well as skeletal muscle and neurovascular models (Vernetti et al. 2017).

3.2 | OoC and Food Safety Assessment

Food safety assessment refers to the systematic evaluation of food products to ensure that they are safe for consumption. This process involves identifying potential hazards, such as chemical, microbiological, or physical contaminants, and assessing the risks they pose to human and animal health. It encompasses all steps involved in the production, handling, storage, and preparation of food to prevent contamination and minimize the risk of foodborne illness or injury to consumers.

3.2.1 | OoCs and Environmental Contaminants in the Food Chain

Pesticides, Persistent Environmental Pollutants, and Heavy Metals. A recent study developed and validated an advanced human placenta OoC model to investigate the effects of endocrine-disrupting compounds (EDCs) on placental function. EDCs, including bisphenol A (BPA), bisphenol S, and polybrominated diphenyl ethers, were gradually introduced to the chip and evaluated over 72 h. Results showed that EDCs induced oxidative stress, cell-specific endocrine effects, and limited cell death in certain placental cells, as well as localized inflammation. However, the placenta-on-chip compensated for the exposure to EDCs, suggesting resilience to these compounds even at doses associated with adverse pregnancy outcomes (Vidal et al. 2024).

Polyfluoroalkyl substances (PFAS), known for their persistence in the food chain and chronic toxicity, are posing an emerging ecological and environmental crisis. A microfluidic-based assay was recently developed to replicate the intestine–vessel–liver interface in 3D, enabling high-resolution, real-time imaging and precise quantification of interactions during PFAS biotransformation. Unlike traditional 2D plates, this 3D platform formed a dense honeycomb network of vascular endothelium with longer tubular structures. Epithelial cells cultured in this platform exhibited a thicker, more closely arranged cell layer compared to planar cultures. By combining the chip with a solid-phase extraction-MS system, researchers dynamically monitored metabolic crosstalk in the intestinal-vascular endothelium-liver interaction under fluorotelomer alcohols (PFAS precursors) exposure. Their findings revealed that endothelial cells were involved in fluorotelomer

alcohol metabolism, producing toxic metabolites that impacted angiogenesis. This system holds promise as an enhanced surrogate model for studying food contaminants exposure and for biomedical research (Xu et al. 2023). Another study by Solan et al. (2023) employed a liver-on-a-chip model to examine the effects of five short-chain PFAS at low (1 nM) and high (1 μM) concentrations on gene expression profiles relevant to toxicity. Using a gene expression assay, they observed marked upregulation (up to 4-fold) of a gene encoding the breast cancer resistance protein for most short-chain PFAS tested. In addition, some PFAS reduced the expression of a liver-specific transporter gene, whereas others increased the expression of genes encoding specific cytochrome P450 enzymes (CYP450s). This imbalance might affect the removal of natural and foreign substances. This study offered insights into how short-chain PFAS affect liver function and potential health risks. The liver-on-chip model combined with gene expression assays shows promise for future rapid toxicology screening (Solan et al. 2023).

BPA, an organic substance present in materials that enter in contact with foods, including beverage containers and baby bottle, is an endocrine-disrupting chemical that may lead to many diseases; for these reasons, in the last years new technologies have been developed to detect its presence and quantity (Han et al. 2023). Lee et al. (2016) developed a novel approach to assess the toxicity of BPA using 3D cultures of human hepatoma cells encapsulated in alginate. The cells were cultured on a micropillar chip coupled with a panel of metabolic enzymes on a microwell chip. By incorporating various metabolic enzymes, including CYP450s, UDP-glycosyltransferases (UGTs), sulfotransferases (SULTs), alcohol dehydrogenase (ADH), and aldehyde dehydrogenase 2 (ALDH2), they were able to mimic human metabolic pathways. Results showed that BPA toxicity was influenced by the presence of different enzymes, with higher toxicity observed in samples containing CYP2E1 compared to controls. Conversely, the toxicity was alleviated in the presence of ADH, ALDH2, and SULT1E1 enzymes. The authors also confirmed cytochrome CYP2E1-mediated cytotoxicity by quantifying unmetabolized BPA using high-performance liquid chromatography with fluorescence detection. Overall, these findings suggest that this micropillar/microwell chip platform could serve as a promising tool for evaluating BPA toxicity in human metabolic systems, offering higher throughput and requiring smaller sample volumes compared to conventional methods (Lee et al. 2016).

Micro- and nanoplastics (MNP) are pervasive in the environment and food chain, exposing humans and animals through inhalation and ingestion (Iqbal et al. 2023). Donkers et al. (2022) utilized physiologically relevant human-based in vitro models for the lung and gut, as well as an intestinal explant barrier-on-chip, to assess MNP membrane passage and their cytotoxic, barrier disruptive, and proinflammatory effects. Various MNP materials, shapes, and sizes were examined, including some fluorescently labeled particles. Nylon fibers and high-density polyethylene (HDPE) disrupted the lung epithelial barrier without affecting cell viability. Polystyrene particles and pristine HDPE fragments reduced colon tissue functionality, whereas all polystyrene particles affected tissue viability in porcine jejunum, ileum, and colon. Nylon fibers induced proinflammatory cell activation in the lung compartment and human colon tissue. MNP permeability across lung and intestinal tissues varied, with larger polystyrene spheres demonstrating the highest permeability. Confocal microscopy confirmed MNP translocation across epithelial barriers. These findings highlight the ability of MNPs to cross epithelial barriers and induce adverse effects in advanced in vitro models, necessitating further research into the impact of MNP characteristics on global health (Donkers et al. 2022).

Paraquat, a highly toxic and long-lasting herbicide used in wheat crops in various countries, presents significant health risks. Xia et al. (2022) investigated how paraquat poisoning leads to lung fibrosis, a complex condition with no specific treatment. They created a lung-on-chip model test the effects of paraquat on lung epithelial cells and fibroblasts and found that higher paraquat concentrations decreased the viability of cells. They also observed changes in specific proteins associated with lung fibrosis. When lung epithelial cells and fibroblasts were grown together in the chip, they showed more severe signs of fibrosis compared to when they were grown separately. This model could help researchers better understand pesticide-induced lung fibrosis (Xia et al. 2022).

Several studies have linked pesticide exposure to metabolic disorders, particularly concerning dichlorodiphenyltrichloroethane (DDT) and permethrin (PMT), common pesticides associated with liver lipid and glucose metabolism dysregulation, and nonalcoholic fatty liver disease (NAFLD). However, the combined effects of DDT and PMT mixtures, as well as the underlying mechanisms, remain unclear. Jellali et al. (2021) utilized a rat liver-on-chip model to investigate the effects of DDT, PMT, and their mixture on liver damage. Hepatocytes were exposed to two concentrations (15 and 150 μM) for 24 h under perfusion, and multi-omic analysis was employed to assess the outcomes. Transcriptome and metabolome analyses showed that low pesticide doses had profiles similar to the control, whereas high doses led to changes in gene and metabolite expression, with each pesticide and their mixture showing unique patterns. Transcriptome analysis revealed changes associated with liver inflammation, steatosis, necrosis, peroxisome proliferator-activated receptor (PPAR) signaling, and fatty acid metabolism. Metabolome analysis detected consistent patterns among all treatments, such as altered lipid and carbohydrate production and decreased levels of amino acids and Krebs cycle intermediates (Jellali et al. 2021). This study highlights the effectiveness of integrating OoC technology with multi-omic approaches for toxicological assessments, providing valuable resources for chemical risk evaluation, particularly when using hepatocytes derived from the relevant species of interest.

Zhao et al. (2021) designed a brain-on-chip with a 3D structure to mimic the natural growth environment of brain tissues in vivo. The chip included a porous filter and 3D brain cell particles, fitting into a standard 96-well plate. Using computer modeling and 3D printing, they fabricated molds for the filter and particles. Mouse embryonic brain cells suspended in sodium alginate were poured into the particle mold, forming hydrogel pieces after solidification. The chip was then used to assess pesticide neurotoxicity. After exposure to varying concentrations of chlorpyrifos (CPF) or imidacloprid, cell proliferation, acetylcholinesterase activity, and lactate dehydrogenase (LDH) release were measured for toxicity evaluation. The embryonic brain cells grew and proliferated normally within the hydrogel particles in the filter.

Both pesticides inhibited cell growth and proliferation in a dose-dependent manner, as well as reducing acetylcholinesterase activity and increasing LDH release. However, imidacloprid demonstrated less pronounced effects compared to CPF. It is important to note that using rat-derived cells may yield results that are not necessarily applicable to humans or other species (Estévez-Priego et al. 2023). Utilizing cells derived from humans or specific species could offer a promising platform for effectively evaluating the neurotoxicity of chemicals, including pesticides, and for studying pharmacodynamics and disease mechanisms within a species-specific context.

Liu et al. (2019) explored the effectiveness of a 3D tetraculture brain MPS for screening neurotoxic chemical agents. This platform comprises neuronal tissue with embedded neuroblastoma cells in extracellular matrix, along with microglia, astrocytes, and vascular tissue featuring dynamic flow and endothelial layer culture without membranes. The scientists examined the model's suitability for testing organophosphate pesticides, as well as substances affecting GABA and/or opioid receptors. Through various assays, including measuring barrier integrity, acetylcholinesterase inhibition, viability, and residual organophosphate concentration, they validated the MPS platform. Results indicated that organophosphates infiltrated the blood–brain barrier (BBB) and inhibited acetylcholinesterase activity. Overall, this study underscores the potential of the membrane-free tetraculture MPS for assessing neurotoxicity (Liu et al. 2019). A variant of this model consisting of a 3D brain-on-chip platform with human iPSC-derived neurons and astrocytes was developed to evaluate organophosphate pesticides toxicity and therapeutic compounds for treatment after acute exposure (Liu et al. 2020). Miller et al. (2021) developed a new method to study how exposure to a common pesticide, CPF, affects the BBB. Using this model, they tested different doses of CPF. At lower doses, they did not find CPF or its byproducts crossing the BBB, but at higher doses, they detected one byproduct, trichloropyridinol, in the brain. When they kept the CPF dose constant, they still did not find CPF crossing the BBB, but they did find an increase in acetylcholine levels, a sign of nerve disruption. These findings show how CPF can affect brain function and confirm the usefulness of this model for studying pesticide effects on the brain (Miller et al. 2021). Permethrin, an insecticide, is approved for application on various food and feed crops, as well as livestock, livestock housing, transportation, and structures, including food handling establishments. Kühnl et al. (2021) employed a dynamic skin and liver multi-organ-chip model to evaluate the impact of various exposure scenarios on PMT's pharmacokinetics and pharmacodynamics. Their study incorporated reconstructed human epidermis models (EpiDerm) and HepaRG-stellate spheroids. Remarkably, the model accurately replicated the excretion process observed in human in vivo studies for PMT, demonstrating significant comparability. These findings underscore the utility of the skin and liver multi-organ-chip model in assessing the disposition of parent and metabolite compounds, thereby contributing to the risk assessment of insecticides and other chemicals at the topical level (Kühnl et al. 2021).

Dioxins are toxic chemicals that persist in the environment and accumulate in the food chain. In their study, Kulthong et al. (2018) developed a gut-on-chip model to investigate the transport of food contaminants. They cultured intestinal epithelial cells (Caco-2) on a porous membrane within a microfluidic chip setup and exposed these cells to a mixture of dioxin compounds for 24 h, using advanced analytical techniques to assess dioxin transport. They found that the amount of transported dioxin was similar in both the gut-on-chip and traditional transwell systems. Moreover, the transport pattern of individual dioxin congeners corresponded to their chemical properties. These findings demonstrate the utility of the gut-on-chip model for studying the transport of lipophilic compounds like dioxins, offering a promising alternative to traditional static methods (Kulthong et al. 2018). Li et al. (2017) introduced a kidney-on-chip model with three compartmentalized culture chambers to study cadmium-induced nephrotoxicity at various concentrations in primary rat glomerular endothelial cells. Cadmium exposure led to significant cytotoxicity, disrupted expression of the tight junction protein zonuline-1, and increased permeability of the endothelial layer to large molecules such as immunoglobulin G and albumin (Li et al. 2017). Importantly, this study explored cadmium-induced nephrotoxicity in rat-derived cells. Utilizing this model with primary or iPSC-derived glomerular endothelial cells from humans or different animal species could facilitate the investigation of species-specific cadmium-induced kidney disfunction and glomerular disease across various organisms. Koning et al. (2021) developed a multi-OoC approach to investigate how oral exposure to nickel can cause systemic toxicity leading to immune activation in skin. Reconstructed human gingiva and human skin, incorporating an immune compartment, were integrated and cultured within a dynamic microfluidic system. They found that gingival nickel exposure resulted in an increased activation of immune cells in the dermal compartment, describing for the first time systemic toxicity and immune cell activation in a multi-organ setting (Koning et al. 2021). In a study of 2023, researchers developed a novel bioelectronic organoid called Taste Organoids-on-a-Chip (TOS), which mimics the human sense of taste ex vivo. By coupling taste organoids with an extracellular potential sensor array, the TOS system demonstrated high stability and repeatability. The taste organoids maintained key taste receptor expression and high cell viability during on-chip culture. Importantly, the TOS accurately distinguished among sour, sweet, bitter, and salt stimuli, as well as varying concentrations of these stimuli. This analytical method relied on signal feature extraction and principal component analysis. The bioelectronic tongue holds promise for applications in food quality control (Wu et al. 2023).

OoCs and Ecotoxicology. Ecotoxicological studies play a crucial role in understanding how environmental contaminants, such as pesticides, heavy metals, and microplastics, accumulate in ecosystems and subsequently enter the food chain. These substances can bioaccumulate in plants and animals, posing significant risks to global health. Investigating these pathways is essential for improving food safety and implementing strategies to mitigate risks associated with environmental contamination.

OoC platforms offer the potential to evaluate the ecological risks associated with various potentially harmful substances, including those utilized in agricultural practices or livestock farming. OoCs technology could potentially provide a versatile platform for replicating organs and biological systems from fish and other ecologically significant or sensitive species. As an example, fish intestinal epithelia play a crucial role as gatekeepers

and mediators between fish and their surrounding environment, regulating interactions with water, food, microorganisms, and xenobiotics. Given that fish serve as early indicators of aquatic ecosystem health, understanding compromised intestinal barrier function is of great importance in environmental toxicology and the aquaculture industry. Our current understanding of the fish intestine largely stems from in vivo experiments or ex vivo gut sac preparations. However, the growing preference for ethically justifiable, cost-effective, and simplified in vitro systems highlight the need for the development of fish cell line-based models of the intestinal barrier (Drieschner et al. 2019). A recent study introduced the first fish-gut-on-chip model, which reconstructs the intestinal barrier using two rainbow trout intestinal cell lines in a microfluidic microenvironment. Initial experiments investigated epithelial fish cell responses to in vivo-like shear stress rates, while examining epithelial-fibroblast cell interplay under optimal flow conditions. This model could enhance predictions of chemical uptake and bioaccumulation in fish for environmental risk assessment (Drieschner et al. 2019).

Järvinen et al. (2021) developed a fish-liver-on-chip system featuring scaffold-free 3D cultures of rainbow trout hepatocytes (RTH-149) within a microfluidic platform. The system mimics chronic drug exposure in aquatic environments, enabling the study of time-dependent toxicity and bioaccumulation of environmental pharmaceuticals in vitro. By integrating microfluidic flow and optimized microwell arrays, the model demonstrated the enhanced sensitivity of hepatocytes to accumulative toxicants like ketoconazole compared to static cultures, highlighting its potential for improving environmental risk assessments of pharmaceutical residues in aquatic ecosystems (Järvinen et al. 2021).

Naturally Occurring Toxins in Food and Feed. Santbergen et al. (2020) employed a gut-on-chip model coupled with an ultra-performance liquid chromatography quadrupole time-of-flight mass spectrometer for alternate analytical assessments of the apical and basolateral concentrations of ergotamine epimers, which are natural-occurring toxins found in food. This pioneering study provided the first evidence of epimer-specific ergotamine transport across the gut epithelium (Santbergen et al. 2020).

Deoxynivalenol (DON) is a prevalent mycotoxin in human food (Shen et al. 2022; You et al. 2023). Pöschl et al. (2023) investigated the effects of DON, on intestinal barrier function using a gut-on-chip model, which incorporates intestinal flow. Caco-2 cells were cultured in a 3-lane microfluidic system and exposed to various concentrations of DON through either the apical or basolateral channel. Permeability was assessed through continuous TEER measurements and barrier integrity assays. Levels of zonulin-1, LDH activity as an indicator of toxicity, and proinflammatory status (interleukin [IL]-8) were analyzed. Results showed that DON exposure led to a dose-dependent decrease in barrier integrity, particularly with basal application, although lower concentrations had TEER-enhancing effects. Apical DON induced IL-8 secretion, whereas the effects of DON on barrier integrity were more pronounced with basolateral exposure. The study suggests that the gut-on-a-chip model provides a promising and advanced tool for investigating the bidirectional effects of DON under flow conditions (Pöschl et al. 2023). Imaoka et al. (2020) investigated how the human kidney responds to different doses of ochratoxin A (OTA), a mycotoxin contaminant in food stuffs that can cause nephrotoxic, carcinogenic, teratogenic, and immunotoxic effects. Using a 3D human kidney proximal tubule MPS, they found that OTA can cause kidney damage at concentrations similar to those seen in urine of affected individuals. Surprisingly, the researchers did not observe increased levels of kidney injury markers after OTA exposure, but rather a decrease, suggesting a complex response. Further experiments showed that certain enzymes, called glutathione *S*-transferases and P450, play a role in either detoxifying or activating OTA in the kidney. RNA analysis revealed changes in genes involved in detoxification, shedding light on the mechanism of OTA toxicity. Additionally, this study suggests that specific transport proteins in the kidney may be involved in OTA movement. These findings improve our understanding of OTA-related kidney damage and may influence safety regulations and food risk assessments related to OTA exposure (Imaoka et al. 2020).

Understanding the harmful effects of enterotoxins on the intestines is crucial for food safety and nutrition research. With concerns about enterotoxin contamination in food and increasing interest in their clinical applications, new platforms are needed. In a recent study, Morelli et al. (2024) recently demonstrated the utility of a microfluidic platform with Caco-2 tubules for studying enterotoxin effects on the human intestinal epithelium, reflecting their distinct pathogenic mechanisms. They found dose-dependent reductions in barrier permeability measured by TEER after exposure to various enterotoxins, including OTA and patulin, with higher sensitivity than previous models. Combining different cell assays allowed comprehensive evaluation of toxin cytotoxicity, particularly evident with OTA exposure. Overall, this study highlights the potential of the Caco-2 tubular model as a multi-parametric and high-throughput tool (Morelli et al. 2024).

3.2.2 | OoCs and Foodborne-Zoonotic Pathogens

Mortensen et al. (2016) developed a microfluidic device cultured with a human intestinal cell-line (Caco-2 cells) and used metabolomics to analyze the response to the bacterial pathogen *Campylobacter jejuni*. The study used both microfluidic devices and transwells to compare the effects of *C. jejuni* infection. Metabolomics analysis distinguished infected and non-infected media collected from microfluidic devices, revealing impacts on branched-chain amino acid metabolism, glycolysis, and gluconeogenesis. In contrast, the transwell media did not show any distinction between infected and non-infected samples. Microfluidic conditions provided a more metabolically homogeneous cell population and facilitated the study of host–pathogen interactions over extended periods (Mortensen et al. 2016).

Lee et al. (2023) developed a gut–kidney axis (GKA)-on-chip to co-culture gut and kidney cells and observe the effects of Shiga toxin-producing *Escherichia coli* (STEC) infection and Shiga toxin intoxication. The study showed that, without antibiotic treatment, STEC O157:H7 killed both gut and kidney cells on the chip. Treatment with ciprofloxacin reduced O157 infection in the gut cells but increased Shiga toxin-induced damage in the

kidney cells. In contrast, gentamicin treatment reduced both O157 infection in the gut cells and Shiga toxin-induced damage in the kidney cells. This study highlights the utility of the GKA-on-chip for studying the effects of antibiotics on the risk of hemolytic–uremic syndrome associated with STEC infection (Lee et al. 2023).

Humans are highly susceptible to enterohemorrhagic *E. coli* (EHEC) infection, unlike mice. Using OoC technology, researchers found that metabolites derived from the human gut microbiome induced greater epithelial injury than those from mouse microbiomes. Four specific human microbiome metabolites were identified as sufficient to enhance EHEC pathogenicity by promoting flagellin expression. This research highlights how these metabolites may contribute to human susceptibility to EHEC infection and suggests potential therapeutic strategies for modulating microbe products to prevent and treat foodborne-zoonotic bacterial infections in humans (Tovaglieri et al. 2019). Thaker et al. (2020) created a murine lung-on-chip infection model and utilized time-lapse imaging to expose the intricacies of host-Mycobacterium tuberculosis interactions at an air–liquid interface, achieving a spatiotemporal resolution not achievable with traditional animal models. This approach also allowed them to investigate the direct involvement of pulmonary surfactant in early infection (Thacker et al. 2020). A variation of this model could be adapted to study *Mycobacterium bovis* and *Mycobacterium caprae*, which are key pathogens in zoonotic tuberculosis affecting bovines, small ruminants, and humans.

Grassart et al. (2019) effectively simulated the influence of flow and peristalsis on the foodborne pathogen *Shigella* within a 3D colonic epithelium employing human gut-on-chip technology. The study found that *Shigella* invasion closely mirrored clinical observations. Additionally, *Shigella* was found to exploit the intestinal microenvironment by capitalizing on microarchitecture and mechanical forces to efficiently invade the intestine (Grassart et al. 2019).

Table 1 summarizes the contemporary applications of various OoC platforms in exploring food and feed contaminants of global health concern.

3.3 | OoCs to Study the Effects of Food, Specific Food Components, Food-Supplements or Additives on Health and Disease

OoC systems are increasingly recognized as valuable tools for modeling diseases in a species-specific and clinically relevant manner, as well as for evaluating the effectiveness of diverse treatments. In the realm of nutritional research, their application is emerging with notably promising outcomes.

A recently developed intestine-on-chip model coupled with an immune compartment has been successfully utilized for the activation and quantification of inflammatory cytokine secretion in human immune cells, with the objective of evaluating dietary supplements for their anti-inflammatory properties. This chip comprised three distinct fluidic layers designed for perfusion, immune cell culture, and cytokine capture and quantification. To mimic the intestinal epithelial layer, a biomimetic membrane separated the perfusing media from the cell culture. Immune-responsive cells were modeled using a human peripheral blood monocytic cell line and its induced macrophages. These cells were sequentially stimulated with lipopolysaccharides and two well-known dietary supplements that modulate inflammation, namely, curcumin and docosahexaenoic acid (DHA). Both curcumin and DHA demonstrated anti-inflammatory effects by reducing the secretion of tumor necrosis factor (TNF)-α, IL-6, IL-1β, and IL-10. This study highlights the potential of the developed system for screening the inflammatory or anti-inflammatory properties of dietary supplements or compounds (Ramadan et al. 2022). Vernetti et al. (2017) employed a multi-OoC system integrating models of the jejunum, liver, kidney, skeletal muscle, and neurovascular system to assess the organ-specific processing of trimethylamine (TMA) as a potential microbiome-derived toxin, and vitamin D3. Their research revealed that the metabolism of these compounds by individual organs aligned with clinical findings. Furthermore, they observed that trimethylamine-*N*-oxide (TMAO), a metabolite of the gut-microbiota originating from choline, phosphatidylcholine, betaine, and l-carnitine—nutrients plentiful in seafood, dairy, egg yolks, muscle, and organ meats, and linked to various human diseases—had the capacity to cross the BBB (Vernetti et al. 2017).

In 2019, researchers utilized a modular, microfluidics-based gut-on-chip model to investigate the impact of short-chain fatty acids (SCFAs) released by probiotic *Lactobacillus rhamnosus* on colorectal cancer (Greenhalgh et al. 2019). Specifically, they observed alterations in SCFAs and lactate production under conditions simulating a high-fiber diet, compared to a control medium containing only simple sugars. Notably, the simulated high-fiber diet upregulated the expression of oncogenes and proinflammatory signaling in the absence of *L. rhamnosus* supplementation. However, in the presence of probiotics, both gene clusters exhibited significant downregulation, which correlated with a reduced cell proliferation rate of primary colorectal cancer cells. This investigation underscores the capacity of gut-on-chip systems to meticulously dissect various facets of the microbiota–host–diet interaction at the metabolic level. Liu et al. (2023) recently utilized a gut-on-chip model to investigate the impact of *Bifidobacterium bifidum* supplementation on inflammatory bowel disease (IBD). This particular probiotic has demonstrated efficacy in preventing, alleviating, and treating IBD in humans. Additionally, it has been confirmed to enhance the integrity of the intestinal epithelial barrier by averting disruption and fostering the repair of damaged intestinal epithelial cell layers (Liu et al. 2023).

Kostrzewski et al. (2020) developed a liver-on-chip platform providing long-term (> 1 month) in vitro cultures of primary human hepatocytes, Kupffer, and stellate cells in 3D constructs that capture key NAFLD and non-alcoholic steatohepatitis hallmarks such as intracellular fat accumulation, inflammation, and fibrosis. This model could enable researchers to delineate the specific mechanistic effects of compounds (including dietary-derived compounds) and to manipulate the model to suit specific research needs (Kostrzewski et al. 2020).

A recent study investigated the impact of commonly used food emulsifiers, polysorbate 20 and polysorbate 80, on an

TABLE 1 | An overview of the present-day applications of various organ-on-chip platforms in the investigation of food and feed contaminants spans a wide range, from environmental pollutants like pesticides to foodborne pathogens.

Organ/system-on-chip used	Food contaminants/xenobiotics studied				
	Pesticides, persistent environmental pollutants	**Heavy metals**	**Naturally occurring toxins**	**Pathogens**	**Other xenobiotics**
Intestine	Dioxins (Kulthong et al. 2018)		Ergotamine epimers (Santbergen et al. 2020), DON (Pöschl et al. 2023), Patulin (Morelli et al. 2024), OTA (Morelli et al. 2024)	*Campylobacter jejuni* (Mortensen et al. 2016), EHEC (Tovaglieri et al. 2019), *Shigella* (Grassart et al. 2019)	Triton-X as a proof-of-principal xenobiotic (fish-gut-on-chip) (Drieschner et al. 2019)
Liver	PFAS (Solan et al. 2023), BPA (Lee et al. 2016), DDT (Jellali et al. 2021), PMT (Jellali et al. 2021)				Ketoconazole (fish-liver-on-chip) (Järvinen et al. 2021)
Kidney		Cadmium (Li et al. 2017)	OTA (Imaoka et al. 2020)		
Brain/BBB	Chlorpyrifos (Estévez-Priego et al. 2023; Miller et al. 2021), Imidacloprid (Estévez-Priego et al. 2023), OPP (Liu et al. 2020), Substances affecting GABA and/or opioid receptors (Liu et al. 2020)				
Lung	Paraquat (Xia et al. 2022)			Mycobacteria (Thacker et al. 2020)	
Placenta	BPA, BPS, and polybrominated diphenyl ethers (PDE) (Vidal et al. 2024)				
Multi-organ-chip systems (body-on-chip)	PFAS (intestine–vessel–liver-on-chip) (Xu et al. 2023); MNP (lung–gut-on-chip) (Donkers et al. 2022); PMT (skin–liver-on-chip) (Kühnl et al. 2021)	Nickel (gengiva–skin-on-chip) (Koning et al. 2021)		STEC (gut–kidney-axis-on-chip) (Lee et al. 2023)	

Abbreviations: BPA, bisphenol A; BPS, bisphenol S; DDT, dichlorodiphenyltrichloroethane; DON, deoxynivalenol; EHEC, enterohemorrhagic *Escherichia coli*; MNP, micro- and nanoplastics; OPP, organophosphate pesticides; OTA, ochratoxin A; PMT, permethrin; PFAS, polyfluoroalkyl substances; STEC, Shiga toxin-producing *Escherichia coli*.

iPSC-derived colon organoid-on-chip model, revealing cytotoxicity and barrier disruption at concentrations as low as 0.1%. Even concentrations as low as 0.05% induced transcriptome alterations indicative of a proinflammatory response, highlighting potential adverse effects on gastrointestinal health (Ogulur et al. 2023).

Tian et al. recently constructed a new type of endocrine pancreas-on-chip to mimic pancreatic secretion stimulated by glucose or sugar substitutes. The impact of five sweeteners (glucose, erythritol, xylitol, sodium cyclamate, and sucralose) on pancreatic islet cell viability and insulin and glucagon secretion was examined. The developed endocrine pancreas-on-a-chip has shown promise for assessing the safety of sugar substitutes in food additives, thus broadening the utility of OoCs in food safety research and offering a novel platform for evaluating diverse food additives (Tian et al. 2023).

4 | Discussion

This review explores how OoC technology could revolutionize our understanding of nutrition and its impact on human, animal, and environmental health within the One Health framework. Despite limited applications in nutrition, food safety, and ecotoxicology, OoCs show immense potential as physiologically relevant, species-specific, and ethical research platforms. These systems can

- Replicate key aspects of cellular, organ, and multi-organ architecture, including cell differentiation, mechanical forces, and fluid flow.
- Provide species-specific insights into food-derived compounds, xenobiotics, and microbiota interactions, potentially surpassing traditional models in relevance.
- Facilitate the study of zoonotic pathogens and host–pathogen interactions.
- Reduce reliance on animal testing by offering human-relevant alternatives.

The potential ecological implications of OoC technology within the One Health framework are profound, especially in studying environmental pollutants in the food chain and their effects on human, animal, and ecosystem health. OoCs can evaluate the impact of industrial and agricultural chemicals, including pesticides and heavy metals, providing critical insights into their pathways and interactions. This knowledge can inform the development of less toxic and more sustainable chemicals, encouraging environmentally friendly production practices.

OoCs also offer powerful tools to model how contaminants interact with biological systems in a species-specific context. For instance, the gill-on-chip model developed by Glawdel et al. (2009) replicates the physiological environment of fish gills, enabling detailed ecotoxicological studies and supporting portable water quality testing. Fish-liver-on-chip models may facilitate the in vitro assessment of pollutant bioaccumulation and toxicity in fish, providing a more ethical alternative to traditional in vivo methods (Järvinen et al. 2021). Additionally, fish-gut-on-chip systems (Drieschner et al. 2019) could evaluate contaminant absorption, including PFAS, and their effects on aquatic organisms, offering valuable data to mitigate bioaccumulation in ecosystems.

PFAS, hazardous chemicals with extensive persistence and bioaccumulation potential, pose serious risks to ecosystems, biodiversity, and overall health. Despite their widespread presence, as highlighted by EFSA assessments in 2020 (EFSA Panel on Contaminants in the Food Chain (EFSA CONTAM Panel et al. 2020), their toxicity remains incompletely understood. OoC platforms present an advanced alternative to traditional animal models for investigating PFAS exposure and toxicity, supporting the establishment of maximum residual limits for animal-derived food products while aligning with sustainable and ethical research practices.

Novel foods offer significant opportunities for promoting healthier diets and supporting sustainable food systems. Examples include alternative vitamin sources, plant-derived extracts, cell-based foods, and agricultural products from diverse regions (Mazac et al. 2023). As their commercial production grows, ensuring their safety becomes increasingly important. To address safety concerns, particularly for cell-based foods such as cultured meat, poultry, seafood, dairy, and eggs, the FAO and WHO have released a comprehensive report identifying 53 potential hazards, including metals, microplastics, and allergens (FAO-WHO 2023). OoCs provide a potential valuable platform to assess these risks, facilitating rigorous safety evaluations. The rising popularity of edible insects in Western markets has raised concerns about allergenicity. Risk assessments require a deeper understanding of insect allergens, sensitization pathways, cross-reactivity, and the effects of food processing (De Marchi et al. 2021). Although current gut and skin OoC models simulate some immune interactions, they lack specific models to study food protein allergenicity via the gut–immune–skin axis. Developing such models, incorporating both innate and adaptive immune cells, would allow for systematic evaluation of allergen sensitization and mechanisms (Janssen et al. 2024). These advancements could significantly enhance the safety assessment of novel foods and their integration into sustainable diets.

OoC technologies leverage subject-specific cells for personalized nutrition research by capturing individual genetic and epigenetic traits, as well as gene–environment interactions. Studies have highlighted the role of genetic variability in responses to dietary components, toxins, food additives, and environmental contaminants (McGlynn et al. 1995; Minatel et al. 2018), as well as in species-specific and genetic predispositions to zoonotic foodborne illnesses (Gilchrist et al. 2015; Tovaglieri et al. 2019). These findings underscore the importance of considering genetic diversity in understanding health outcomes across humans and animals.

In veterinary medicine, OoC models derived from farm animal cells could offer valuable insights into host–pathogen interactions, especially for pathogens that cause clinical or subclinical infections in farm animals, leading to long-term environmental contamination and zoonotic transmission. Using cells from asymptomatic carrier species, these models could also explore protective mechanisms of the host (Ambrosini and Tachibana 2022). While still in its infancy, the application of OoC technology

in veterinary medicine holds great promise. Advances such as the generation of animal iPSCs (Dutton et al. 2019; Pillai et al. 2021; Tsukamoto et al. 2024) and animal-derived organoids (Ambrosini and Tachibana 2022; Kawasaki et al. 2022) are expanding the field. The establishment of a dedicated OoC facility for veterinary species by the Royal Veterinary College further highlights its transformative potential (Royal Veterinary College [RVC] 2023). By replicating animal tissue structures and predicting responses to stimuli like pathogens, vaccines, and environmental factors, OoCs aims to address critical knowledge gaps. Additionally, they can reduce reliance on in vivo testing, promoting more ethical and humane practices in veterinary research.

4.1 | OoCs as Ethical and Human-Relevant Alternatives to Animal Testing

OoC systems hold significant potential to reduce reliance on animal models by providing human-relevant alternatives, addressing ethical concerns, and fostering more humane research practices. Recent advancements in OoC technologies offer more physiologically and clinically reliable in vitro preclinical models for assessing physiopathology and pharmacological responses compared to many animal studies (Ingber 2020).

Several examples demonstrate the ability of OoCs to faithfully mimic human responses, outperforming traditional methods, including animal models. For instance, a proximal tubular OoC identified the nephrotoxicity of SPC-5001, an experimental oligonucleotide. This toxicity, undetected in preclinical testing on mice and non-human primates, was observed in Phase 1 clinical trials, showcasing the OoC model's predictive accuracy (Nieskens et al. 2021). Another kidney-on-chip model replicated cisplatin-induced toxicities via a human-specific efflux transporter absent in animals (Jang et al. 2013). Ewart et al. (2022) evaluated the emulate liver-chip for predicting DILI using criteria from the Innovation and Quality (IQ) Consortium for MPS. The study demonstrated 87% sensitivity in detecting hepatotoxic drugs, including those missed by traditional models, and identified compounds responsible for 242 deaths due to DILI. These findings highlight the liver-chip's potential to improve safety and reduce the progression of hepatotoxic drugs to clinical trials (Ewart et al. 2022). Similarly, a blood vessel-on-chip successfully replicated thrombotic toxicities caused by a therapeutic monoclonal antibody, which were undetected in preclinical animal studies but led to fatalities in human trials (Barrile et al. 2018). Mimicking the human immune system remains a challenge for animal models; however, recent studies suggest that a human OoC can support the self-assembly of B and T lymphocytes into germinal center-like lymphoid follicles (Goyal et al. 2018).

Although these examples do not directly address nutrition and food safety, they underscore OoCs' potential as ethical, physiologically relevant alternatives to animal models. In the One Health context, OoCs provide opportunities for human-relevant research, promote ethical advancements by replacing animal testing, and reduce the emotional burden on researchers associated with animal experimentation (Grimm 2023). Further studies are needed to fully demonstrate the superiority of OoCs.

Figure 4 provides a schematic overview of the current applications and future potential of OoC technologies within the One Health framework.

4.2 | Limitations, Challenges, and Future Perspectives

OoCs face technical and systemic challenges that must be addressed for their widespread adoption and effective use. Key technical issues include material limitations, architectural complexity, and challenges in replicating in vivo conditions. For instance, polydimethylsiloxane, commonly used in microfluidic chips, can adsorb molecules, affecting the precision of toxicity tests (Ewart et al. 2022). Complex tissue models may hinder imaging techniques, and many gut-on-chip models lack the full four-layered intestinal wall structure, limiting their ability to simulate complex interactions (Hu et al. 2024). Future advancements will require improved designs to enhance physiological relevance.

Scalability remains a major barrier, as transitioning from laboratory-scale models to standardized, high-throughput systems involves developing cost-effective manufacturing processes, standardizing designs and protocols, and ensuring a reliable supply of human-specific cells, such as iPSCs, and chemically defined media (Weber et al. 2024). Overcoming technical hurdles in multi-organ systems is also crucial to maintaining physiological relevance and functional interactions at scale. These challenges underscore the need for innovation and harmonized standards to facilitate widespread adoption.

The adoption of new approach methodologies (NAMs), including OoCs, has been slow, partly due to the lack of global standards and concerns about performance reliability (Ewart and Roth 2021). Harmonization across cell types, media, biomaterials, and readouts is critical for reproducibility. A recent roundtable highlighted the importance of global collaboration and data sharing to address standardization challenges (Parvatam et al. 2024). Voluntary data sharing from MPS studies was identified as a key strategy to enhance collective expertise and support regulatory integration.

Validation studies are essential for building confidence in OoCs. For example, a qualification study using 870 liver chips to assess DILI followed IQ MPS Consortium guidelines, employing a double-blind design to ensure reliability (Baudy et al. 2020; Ewart et al. 2022). Such studies are resource–intensive but critical for broader adoption.

Investment in MPS technologies is often hindered by perceived high risks despite their high potential rewards. Establishing a regulatory framework that links risk capital to viable products is vital to attracting private investors. Increased funding for validation studies is also crucial for achieving regulatory acceptance and unlocking the full potential of OoC technology (Moruzzi et al. 2023).

Another significant barrier to the widespread adoption of OoCs is the bias favoring traditional animal models. Ingber (2020) observed that researchers submitting grant proposals or manuscripts based on in vitro findings often face reviewers

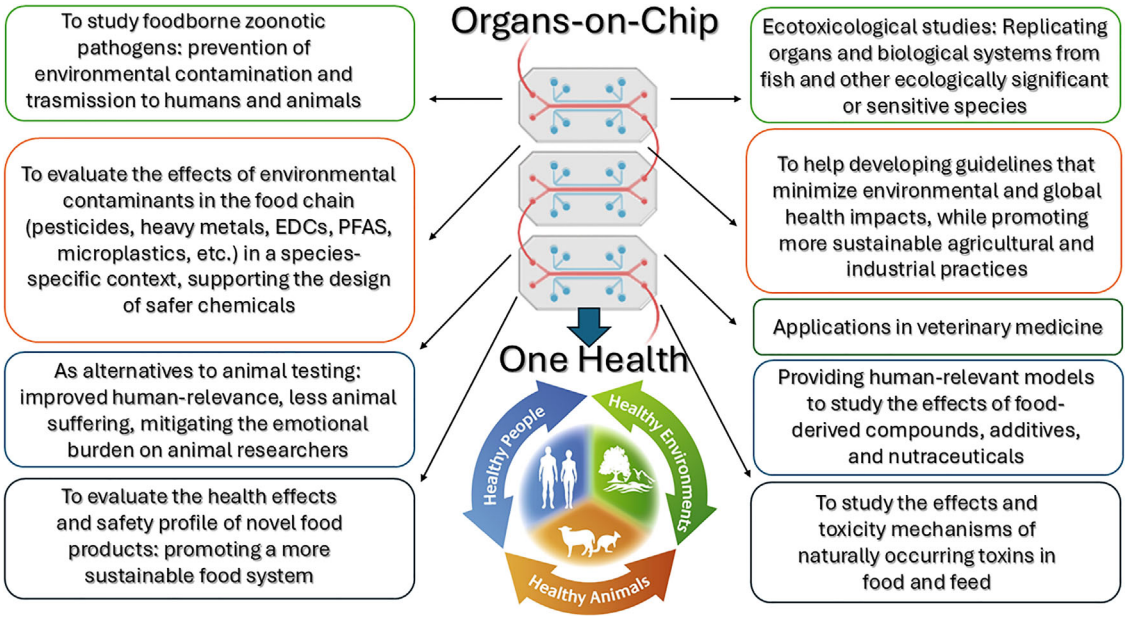

FIGURE 4 | **A schematic overview of the current and potential applications of OoC technologies within the One Health framework.** OoC technologies provide innovative platforms to study foodborne-zoonotic pathogens, environmental contaminants, and ecotoxicological impacts in species-specific contexts. They support sustainable agricultural and industrial practices, serve as alternatives to animal testing, and facilitate the evaluation of novel food safety and toxicity mechanisms. Additionally, OoCs hold promise in veterinary medicine and in advancing human-relevant models for assessing food-derived compounds, additives, and toxins, promoting interconnected health for humans, animals, and the environment. EDCs, endocrine-disrupting chemicals; PFAS, Per- and Polyfluoroalkyl Substances.

demanding additional animal experiments to validate results before approval (Ingber 2020). This "animal-methods bias" reflects a preference for animal-based methods, even when human-relevant alternatives are more suitable, potentially delaying manuscript acceptance or funding decisions (Krebs et al. 2023). Ingber emphasizes that many animal models lack physiological relevance for complex human contexts, rendering their use counterproductive when requested without clear scientific justification (Ingber 2020). Addressing this bias requires targeted education and awareness efforts involving researchers, students, journal editors, funding agencies, and policymakers. Training programs and campaigns can highlight the advantages of human-relevant technologies like OoCs. Additionally, integrating OoC technologies into academic curricula could enhance their acceptance and widespread integration.

Table 2 summarizes key limitations and challenges associated with OoC technology, alongside proposed solutions and future directions to promote broader adoption.

The successful integration of innovative alternative methods, as demonstrated by the cosmetics industry's adoption of validated human reconstructed skin models for regulatory testing under OECD guidelines, highlights the power of collaboration among agencies, industry, and researchers (Silva and Tamburic 2022). Similarly, the EMA's *Guideline on the Principles of Regulatory Acceptance of 3Rs (Replacement, Reduction, Refinement) Testing Approaches* acknowledges the potential of liver-on-chip and heart-on-chip technologies for assessing DILI and cardiotoxicity (European Medicines Agency 2023). These advancements exemplify the readiness of advanced OoC models for regulatory applications, inspiring their adoption in nutrition research. By fostering interdisciplinary collaboration and leveraging these innovations, OoCs can emerge as pivotal tools in advancing a One Health approach that bridges human, animal, and environmental health.

5 | Conclusion

OoC technology represents a transformative advancement in nutrition research and food safety. By accurately replicating key aspects of human and animal physiology, these platforms offer unprecedented opportunities to study the effects of food components, additives, and xenobiotics, contributing to the development of more sustainable and health-conscious food systems. Furthermore, they hold significant potential for reducing or replacing the use of animals in research, addressing both ethical concerns and limitations associated with traditional models.

Despite these advancements, challenges such as standardization, validation, and scalability remain, along with the need to address biases favoring traditional methods. To fully realize the potential of OoCs within the One Health framework, greater cross-sector collaboration is essential, involving researchers, policymakers, industry stakeholders, and regulatory bodies. Increased investment in research and development, as well as funding dedicated to non-animal methodologies, will also play a crucial role in overcoming existing obstacles.

By addressing these challenges through multidisciplinary cooperation and sustained support, OoC technology is well positioned to revolutionize nutrition research, enhance food safety, and

TABLE 2 | Summary of limitations, challenges, potential solutions, and future directions for organ-on-chip technology.

Limitations and challenges	Potential solutions	Future directions
Microfluidic chip materials like polydimethylsiloxane (PDMS) adsorb molecules, compromising test accuracy	Develop alternative microfluidic materials that minimize adsorption and maintain substance integrity	Design next-generation OoCs with improved materials optimized for reproducibility and accuracy
Architectural complexity of OoCs hinders deep-tissue imaging and functional replication of organ features	Enhance OoC architecture to better replicate organ-specific structures, such as the Disse space in liver models. Implement more robust multi-organ-on-chip systems to study complex interactions	Expand collaborations between engineers and biologists to optimize model design and functionality
Gut-on-chip models lack a complete four-layer intestinal wall, limiting simulation of complex interactions	Augment gut-on-chip designs to include all intestinal layers for comprehensive interaction simulations	Integrate advanced computational tools with OoCs to better simulate and analyze multi-layered interactions
Absence of globally harmonized standards, scalability, and reproducibility concerns hinder adoption	Establish globally harmonized standards for OoC design, operation, and evaluation	Facilitate data sharing across institutions to support standardization and validation efforts and to build confidence in OoCs
High costs and resource–intensive nature of validation studies	Increase dedicated funding for validation studies and establish cost-sharing collaborations	Attract private investment by demonstrating clear pathways for OoCs to transition from research to application
Biases favoring traditional methods	Concerted effort to educate stakeholders at all levels	Training programs and awareness campaigns targeting students, early career researchers, editors, project evaluators, reviewers, and so on

Note: This table highlights key limitations and challenges faced by OoC technology, alongside proposed solutions and future directions to overcome these obstacles and promote broader adoption, reliability, and functionality.

contribute meaningfully to the interconnected health of humans, animals, and the environment.

Conflicts of Interest

The authors declare no conflicts of interest.

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Supporting Information

Additional supporting information can be found online in the Supporting Information section.