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Review

Organoids – New Models for Host–Helminth Interactions

María A. Duque-Correa,^{1,*} Rick M. Maizels,² Richard K. Grencis,³ and Matthew Berriman¹

Organoids are multicellular culture systems that replicate tissue architecture and function, and are increasingly used as models of viral, bacterial, and protozoan infections. Organoids have great potential to improve our current understanding of helminth interactions with their hosts and to replace or reduce the dependence on using animal models. In this review, we discuss the applicability of this technology to helminth infection research, including strategies of co-culture of helminths or their products with organoids and the challenges, advantages, and drawbacks of the use of organoids for these studies. We also explore how complementing organoid systems with other cell types and components may allow more complex models to be generated in the future to further investigate helminth–host interactions.

Organoids to Mimic Host Interactions with Pathogens

Organoids are *in vitro* multicellular clusters containing various differentiated cell types, capable of self-renewal and organization, and exhibiting some level of functionality and architecture of the tissue of origin [1,2]. Organoids have transformed biomedical research in the past 10 years. Part of this revolution has been in their use for a better understanding of host–pathogen interactions. Organoid models have been used to investigate viral, bacterial, and protozoan parasite infections [1,3–6].

Helminth infections affect millions of people and livestock, resulting in a major social and economic burden worldwide [7,8]. Despite their impact, research into helminth infections relies heavily on the use of animals and is limited by a lack of models that more closely reproduce the interaction of the parasites with their specific hosts [9]. *In vivo* model organisms for human and livestock helminth infection have provided invaluable data on the immune response to helminths, parasite genetics, and transmission. Nevertheless, these models are expensive and complex, hindering the study of host–parasite interactions at the molecular level. Moreover, model organisms cannot fully recapitulate human helminth infections. Entirely new systems are therefore needed that allow the processes of invasion and colonization of human helminths to be studied, and that open up new possibilities for testing new drug targets and identifying vaccine candidates. Organoids from different organs infected by helminths during their life cycle (including gut, lung, bladder, and liver) have enormous potential as novel models for studying the interactions of helminths and their products with their hosts. Nevertheless, the size and complexity of helminths pose challenges on the adaptability of organoid cultures for their use in research. Here, we discuss the applicability of this technology. From our recent investigations into the interactions of whipworm larvae with caecal epithelium and evaluations of the host response to **excretory/secretory (ES) products** (see [Glossary](#)), we explore the advantages and pitfalls of using organoid systems.

The implementation of organoid models promises major new avenues for developing new therapeutics and vaccines against helminths, for understanding the anti-inflammatory effects of helminth infections, and for using helminths or their products as alternative therapies to treat inflammatory diseases.

Organoids: What, Whence, and Where to Infection Biology

Organoids can be derived from either **primary tissue stem cells** or embryonic or induced **pluripotent stem cells (PSCs)** when cultured in conditions that resemble those of the stem cell niche (SCN) for a specific organ [1,10,11]. The SCN is a specialized, dynamic and restricted microenvironment where the stem cells reside, and it is composed of physical and cellular components [12,13]. The physical niche comprises the **extracellular matrix (ECM)**, the shape and arrangement of cells and mechanical

Highlights

Organoids are multicellular *in vitro* culture systems that closely resemble the architecture and function of the tissue of origin.

Organoids are currently used to study host cell interactions with microorganisms, including viruses, bacteria, and parasites.

Helminth research is lacking models that better recapitulate the interaction of the human/livestock host and parasites.

Organoid cultures are of great potential to study helminth infections while their application in this field is in its infancy. The different components of the host–helminth *in vivo* interaction, including tissue architecture, polarization, and multicellularity, environmental cues, and biomechanical forces, should be considered when using organoids to recreate these parasitic infections.

In the future, more complex systems integrating stromal and immune cells and microengineering technologies with organoid cultures will further empower the use of this system in helminth research.

¹Wellcome Sanger Institute, Wellcome Genome Campus, Hinxton, UK

²Wellcome Centre for Integrative Parasitology, Institute of Infection, Immunity and Inflammation, College of Medical, Veterinary, and Life Sciences, University of Glasgow, Glasgow, UK

³Lydia Becker Institute of Immunology and Inflammation, Wellcome Trust Centre for Cell Matrix Research and Faculty of Biology, Medicine and Health, University of Manchester, Manchester, UK

*Correspondence: md19@sanger.ac.uk



forces, and the cellular niche refers to the resident immune and **stromal cells** embedded in the ECM that provide signaling clues to maintain stem cell division and differentiation [12,14,15].

Current organoid culture conditions combine an ECM support with exogenous growth factors and morphogens (both promoters and inhibitors) that direct the division and differentiation of daughter cells into the multiple cellular populations present in the targeted organ [1,2,10,11,16]. Organoids derived from embryonic or induced PSCs additionally require germ layer- and lineage-specific factors to direct their differentiation into several endoderm-, mesoderm-, and ectoderm-derived tissues [1,2,10,11].

Organoid systems were initially developed to recreate murine and human small intestine and colon epithelia, and involved the use of specific culture media cocktails to recreate the SCN conditions for each intestinal segment in the specific species [10,11,17]. Subsequently, organoids have been developed for a great variety of tissues and organs, including stomach, esophagus, liver, lung, pancreas, prostate, brain, kidney, mammary gland, ovary, lingual, taste bud, salivary gland, testis, endometrium, Fallopian tube, lymph nodes, blood vessels, skin, inner ear, and retina [1,2,11,18–21]. Moreover, intestinal, mammary, keratinocyte, and liver organoids from other animal models already exist, including those of bovine, porcine, ovine, chicken, feline, and canine origin [22].

Currently, organoids are used as models of different pathologies, including infectious diseases caused by viruses, bacteria, and protozoans [3–5]. Viral infection studies with organoid systems have included: norovirus, rotavirus, enteric adenovirus, and coronavirus invasion of intestinal organoids [3–6]; herpes simplex virus 1 [23] and cytomegalovirus [24] infection of cerebral organoids; Zika virus infection of cerebral [4,5] and human testicular organoids [25]; human airway organoids to model pathology and assess infectivity of emerging influenza [26], parainfluenza [27] and respiratory syncytial viruses [28]; BK virus infection in human kidney **tubuloids** [29]; and human liver organoids to study infection with hepatitis B and its related tumorigenesis [30].

Organoid models of bacterial pathogenesis include enterohaemorrhagic, enteroaggregative, and enteropathogenic *Escherichia coli*, *Vibrio cholerae*, *Salmonella*, *Clostridium difficile*, and *Shigella* infecting intestinal organoids of mouse, bovine, porcine, and human origin [3–6,31], as well as *Helicobacter pylori* colonizing gastric (stomach) organoids [4–6]. In addition, the involvement of bacteria in adenocarcinoma formation in gallbladders has been studied using murine gallbladder organoids infected with *Salmonella* [4,5]. Infection with the uropathogen *Enterococcus faecalis* has been studied in human urothelial organoids [32]. More recently, Fallopian tube organoids have served as a model to study the long-term impact of *Chlamydia trachomatis* infections in the human epithelium that may contribute to the development of ovarian cancer [18].

Organoids are also gaining in popularity to model protozoal infections. In particular, *Toxoplasma gondii* infects bovine and porcine small intestinal organoids [31]. Furthermore, the entire life cycle of *Cryptosporidium parvum* can now be modelled in murine and human small intestinal [33,34] and lung organoids [34]. Lung organoids have successfully recapitulated respiratory tract infections with *C. parvum* occurring in immune-competent and -deficient individuals [34].

Organoids as a Tool for Studying Helminth Infections

Research on helminth infections has largely focused on the use of model organisms that have helped us to understand the observations made in patient samples collected in the field (Table 1) [35]. In recent years, organoids have started to be exploited as a tool to indirectly investigate the effects on the intestinal epithelia of the immune responses triggered by intestinal helminth infections. Specifically, mouse small intestinal organoids have served as an experimental system to demonstrate the expansion of **tuft cells** as a consequence of *ex vivo* stimulation with interleukin (IL) 13. IL-13 is secreted by innate lymphoid cells in response to stimulation with IL-25, a cytokine produced by tuft cells during the early response to *in vivo* infection with the intestinal dwelling nematodes *Nippostrongylus brasiliensis*, *Heligmosomoides polygyrus*, and *Trichinella spiralis* [36–39]. These findings

Glossary

Excretory/secretory (ES) products: soluble mediators released by helminths, either actively exported through secretory pathways or diffused or leaked from the parasite soma as a consequence of physiological process such as digestion. ES products may interact with host tissues and are involved in parasite invasion and modulation of host immune responses.

Extracellular matrix (ECM): 3D network of extracellular hydrated macromolecules, such as collagen and glycoproteins, present within all tissues and organs. ECM provides essential physical scaffolding for cells, together with the biochemical and biomechanical support required for tissue morphogenesis, differentiation, and homeostasis.

Extracellular vesicles (EVs): lipid bilayer-enclosed nanoparticles released by cells and carrying complex cargoes, including proteins, lipids, and nucleic acids involved in intercellular, inter-organismal, and even interspecies communication.

Goblet cells: a mucus-producing cell population of the intestinal and bronchial tract epithelium.

Inflammatory bowel disease (IBD): chronic inflammatory disorder of the gastrointestinal tract comprising two main clinical forms: Crohn's disease and ulcerative colitis. IBD is associated with multiple pathogenic factors, including genetic variants conferring susceptibility, a dysregulated immune response, abnormal gut microbiota, and environmental changes.

Microbiota: all the microorganisms residing in a specific niche such as the human gut.

Microinjection: a method of delivery of substances into a tissue, organoid, or a single cell by injection under the microscope using a glass micropipette.

Organ-on-chip: microfabricated *in vitro* cell culture devices that model functional units of organs. They are composed of multiple, individually reachable microchambers where various cell types are grown and that are connected through microfluidics that allow the control of the culture microenvironment.

add to the evidence that helminth infections influence stem-cell proliferation and differentiation towards specific epithelial cellular populations, including the **goblet cell** and the tuft cell compartments that are key for worm expulsion [38,40]. Comparisons of organoids generated from uninfected and *H. polygyrus*-infected mice provided further evidence of the impact of helminth infections on stem cell fate within the intestinal crypts. Larval invasion of the submucosal tissue breaches the epithelial barrier, and results in a switch of stem cell phenotype from Lgr5⁺ to Sca1⁺, the latter resembling a fetal-like, proliferative, or wound-healing intestinal epithelium profile [41].

Organoids have also been utilized in experiments that aim to characterize the interactions and intestinal epithelial responses to ES products of various nematodes. In particular, stimulation of murine small intestine organoids with ES products and extracts of *T. spiralis* showed that sensing of parasitic products by tuft cell receptors results in Ca²⁺ responses [39]. Moreover, imaging experiments of murine small intestine and colon organoids, microinjected with exosome-like **extracellular vesicles (EVs)** present in the ES of *N. brasiliensis* and *Trichuris muris*, respectively, showed their internalization by host epithelial cells [42,43]. A similar approach has been taken to visualize the uptake of *Ascaris suum* EVs cocultured with canine intestinal organoids [44]. However, to date, an organoid system has not been exploited to study direct interactions (and the molecular basis of those interactions) between host epithelia and live helminths.

Various life cycle stages of helminths infect organs for which organoids of human, murine, porcine, and canine origin exist (Table 1). However, the use of organoids is currently restricted to investigations of the interactions with invasive parasitic stages or ES products from different life cycle stages, because co-cultures with established adult stages would require long-term culture and, therefore, cannot yet be recreated (discussed later).

Comparing Organoids with Other Models to Study Helminths: *In Vitro*, *Ex Vivo*, and *In Vivo*

In vitro models for helminth infections are based mainly on the use of cell lines. In particular, some host interactions with intestinal helminths have been modelled with commonly used cell lines (T84, Caco-2, and HT-29) that enable small intestine and colon epithelial function to be studied [6]. For high-throughput screening, biobanking and genetic manipulation, cell lines are excellent tools. However, they are generated by immortalization of cancerous cells, severely limiting their use as systems to understand physiological processes in normal/healthy tissues [2,6].

To date, epithelial and fibroblast cell lines of diverse origin (small intestine, colon, kidney, lung, among others) have been used to study the invasion and early colonization of *T. spiralis* [45,46]. Prolonged cultures of L1 larvae of *T. spiralis* in Caco-2 cells sustain moulting, ecdysis, development to adulthood and reproduction of the parasite [47]. *T. spiralis* also invades 2D cultures of mouse embryonic small intestinal cells [48].

Cell lines and primary small intestine and colonic epithelial cells have also been used to study the responses of epithelial cells to *H. polygyrus* [49], *T. muris* [50,51], *T. spiralis* [39], and *Ascaris* ES products and EVs [52]. While the use of cellular preparations *ex vivo* allows experiments to be performed with the different cell populations present in the tissue, the isolation procedures disrupt the architecture and polarization of the cells in the tissue, making it difficult to interpret the results. In addition, *ex vivo* cultures are short-lived, restricting their use to experiments that evaluate only very early interactions.

Organoids are an excellent alternative, bridging *in vitro*, *ex vivo*, and *in vivo* models (Table 2). Organoids derived from a single individual can be expanded and cryopreserved as individual lines, allowing several experiments to be performed using the same starting material [1,10]. Moreover, organoids can be grown in a 2D and 3D conformation, and, in contrast to traditional cell lines, they retain organ specificity and maintain genome stability, allowing their biobanking [2,6]. These features make them amenable to high-throughput screening, although it is important to consider that

Pericytes: mural cells of the vasculature that wrap around the endothelial cells that line the capillaries, arterioles and venules throughout the body.

Permeable support/transwell: cell culture device, also known as cell culture inserts, composed of a porous membrane which hangs in the centre of an outer well and provides independent access to both sides of a monolayer.

Pluripotent stem cells (PSCs): stem cells with the capacity to differentiate into any type of cell of all three germ layers: ectoderm, endoderm, and mesoderm. They include both embryonic and induced PSCs. Embryonic PSCs are derived from the inner cell mass of the blastocyst of an early-stage preimplantation embryo. Induced PSCs refer to those derived from adult somatic cells that are reprogrammed to an embryonic stem-cell-like state.

Primary tissue stem cells: stem cells present in organs from adult organisms and with the capacity to divide and differentiate into the different cell types present in the organ of origin.

Stromal cells: group of connective tissue cells of an organ that provide support, structure, and anchoring of the parenchymal cells of that organ. The most common stromal cells include fibroblasts and pericytes.

Telocyte: a particular type of stromal cell presenting extremely long and thin prolongations named telopodes.

Tubuloids: primary kidney tubular epithelial organoids representing proximal as well as distal nephron segments.

Tuft cells: chemosensory cells with blunt apical microvilli, found in the thymus and hollow organs or tubes lined by an epithelium such as the urinary tract, the digestive and respiratory systems.

Table 1. Selection of Human and Livestock Helminth Infections and Life Cycle Stages with Potential to Be Studied Using Organoids

Organ	Helminth	Host	Life cycle stage relevant to organoid models
Stomach (abomasum)	<i>Haemonchus contortus</i> (barber's pole worm)	Sheep	L3 larvae invade epithelium of abomasum and mature to adults that remain attached
Small intestine	<i>Ascaris lumbricoides</i> ; <i>A. suum</i> (roundworm)	Human; pig	L3 larvae hatch from ingested eggs, invade epithelium, L4 larvae/adults in lumen
	<i>Toxocara canis</i> , <i>T. cati</i> (roundworm)	Human, dog, cat, rodents, rabbit, birds	L2 larvae hatch from ingested eggs, invade epithelium, L3/L4/ L5 and adults in lumen
	<i>Heligmosomoides polygyrus</i>	Mouse	Ingested L3 larvae invade epithelium and submucosa; re-emerge as adults into lumen
	<i>Trichinella spiralis</i> (pork worm)	Human, pig, mouse	L1 larvae invade epithelium and mature to adults
	<i>Necator americanus</i> , <i>Ancylostoma duodenale</i> (hookworms); <i>Nippostrongylus brasiliensis</i>	Human; mouse, rat	L4 larvae/adult worms in lumen (L3 enter through skin)
	<i>Strongyloides stercoralis</i> (thread worm); <i>S. venezuelensis</i> , <i>S. ratti</i>	Human, dog; mouse, rat	L4 larvae/adult worms in lumen (L3 enter through skin)
	<i>Taenia saginata</i> , <i>T. solium</i> (tapeworms); <i>T. taeniaeformis</i> , <i>T. crassiceps</i>	Human, pig, ruminants; mouse, rat	Ingested larvae (cysticerci) attach to epithelium, grow to adults
	<i>Echinococcus granulosus</i> , <i>E. multilocularis</i> (tapeworms)	Human, dog, cat, cattle, horse, sheep, pig, rodents	Eggs ingested by the first host hatch releasing oncospheres (larvae) that penetrate the epithelium and submucosa. Cysts and protoscolices that are ingested by a second host attach to epithelium and develop into adults
	<i>Fasciola hepatica</i> , <i>F. gigantica</i> (liver fluke)	Human, sheep, cattle, mouse	Newly excised juvenile (larvae) penetrate the intestinal wall of the duodenum into peritoneal cavity
Small and large intestine	<i>Schistosoma mansoni</i> , <i>S. japonicum</i> (blood fluke)	Human, mouse	Adults in mesenteric veins produce eggs which transit intestinal wall to lumen
Large intestine	<i>Trichuris trichiura</i> ; <i>T. muris</i> ; <i>T. suis</i> (whipworm)	Human; mouse; pig	L1 larvae invade caecal/large intestinal epithelium and mature to adults
Skin	<i>N. americanus</i> , <i>A. duodenale</i> ; <i>N. brasiliensis</i>	Human; mouse, rat	Free-living L3 larvae penetrate unbroken skin
	<i>S. stercoralis</i> ; <i>S. venezuelensis</i> , <i>S. ratti</i>	Human, dog; mouse, rat	Free-living L3 larvae penetrate unbroken skin
	<i>S. mansoni</i> , <i>S. haematobium</i> , <i>S. japonicum</i>	Human, mouse	Free-swimming cercariae in freshwater penetrate unbroken skin

(Continued on next page)

Table 1. Continued

Organ	Helminth	Host	Life cycle stage relevant to organoid models
Lung	<i>T. canis</i> , <i>T. cati</i>	Human, dog, cat, cattle, horse, sheep, pig, rodents	L2/L3 larvae transit lung where may encapsulate or migrate through trachea and oesophagus to gut
	<i>N. americanus</i> , <i>A. duodenale</i> ; <i>N. brasiliensis</i> ; <i>Ascaris</i> spp	Human; mouse, rat; pig	Developing L3/L4 stages transit lung, migrate through trachea and oesophagus to gut
	<i>S. stercoralis</i> ; <i>S. venezuelensis</i> , <i>S. ratti</i>	Human, dog; mouse, rat	L3 larvae transit lung, migrate through trachea and oesophagus to gut
	<i>S. mansoni</i> , <i>S. haematobium</i> , <i>S. japonicum</i>	Human, mouse	Schistosomulae (larvae) develop prior to migration to vascular niche
	<i>E. granulosus</i>	Human, dog, cat, cattle, horse, sheep, pig, rodents	Oncospheres (larvae) circulate to the lungs where they develop into cysts and protoscolices
Liver	<i>T. canis</i> , <i>T. cati</i>	Human, dog, cat, cattle, horse, sheep, pig, rodents	L2 larvae transit liver where may encapsulate or migrate to the lung
	<i>S. mansoni</i> , <i>S. japonicum</i>	Human, mouse	Eggs frequently trapped in liver, pathogenic
	<i>E. granulosus</i> , <i>E. multilocularis</i>	Human, dog, cat, cattle, horse, sheep, pig, rodents	Oncospheres (larvae) circulate to the liver where they develop into cysts and protoscolices
	<i>F. hepatica</i> , <i>F. gigantica</i>	Human, sheep, cattle, mouse	Migrating juvenile and adults
Brain	<i>T. solium</i> , <i>Mesocostoides corti</i>	Human, mouse	Cysts form in brain
Bladder	<i>S. haematobium</i>	Human, mouse	Eggs from adults breach barrier to reach urinary tract
Lymphatics/blood vessels	<i>Brugia malayi</i> (and other lymphatic filariae)	Human	Adults live in lymphatic system, microfilariae in peripheral blood

experiments using organoids are currently subject to variability due to inherent heterogeneity in size, shape, structural organization and functional capacity, and batch variability of both the ECM and growth factors used in culture [3,16,53].

Compared with animal models, organoids are much less complex because they lack vascularization, innervation, interactions with other cells present in the organ, and **microbiota**. However, organoids do enable aspects of human physiology and disease to be studied that cannot be modelled in animals due to significant interspecies differences [2]. Most helminths are host-restricted and, in such cases, animal models are of limited use in the understanding of helminth pathogenesis. Organoids derived from the specific host could overcome this issue by providing a model with increased relevance for the specific infection; for instance, using human organoids and the human infective nematode *Trichuris trichiura*, which is currently experimentally intractable, could replace some studies in mice using *T. muris*.

The multicellular composition of organoids is critical for studying the varying roles of specific cell types in colonization, replication, transmission, host damage, and clearance of pathogens. These studies are not achievable with cell lines that often present only one or two types of cells present in the organ of interest [6]. Moreover, 'wild-type' organoids can be genome-edited using lentiviral expression systems or CRISPR/Cas9, a process that, in animal models, requires embryo mutagenesis and breeding. An additional advantage of organoids is that they can be generated from patient-derived stem cells, allowing the long-term impact of pathogen infections on histological features and physiological phenotypes to be studied.

Table 2. Advantages and Drawbacks of Organoids to Study Helminth–Host Interactions

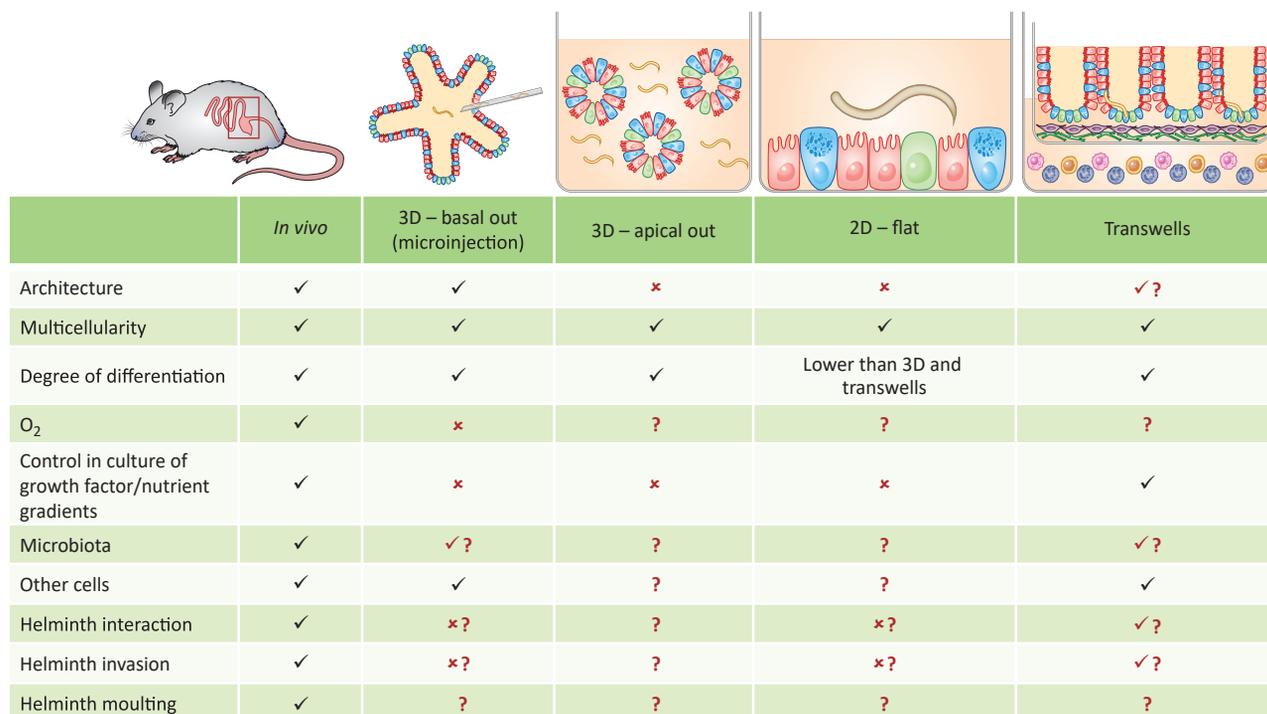
Advantages	Disadvantages
<p>Experimental</p> <ul style="list-style-type: none"> • When compared with cell lines, organoids display the different cell types present in the organ of origin and self-organize. This allows a better understanding of the molecular and cellular interactions of helminths with their host. • Multicellularity permits study of the role of different cell types in helminth invasion and colonization, host damage and responses. Moreover, up- and downregulation of cell populations and factors in tissue-specific context can be evaluated after exposure to helminths and their products. • 3D organoids reproduce tissue architecture to a certain extent. • 2D organoids allow ready apical delivery of parasites and their products and better control of the conditions of co-culture with helminths. • Both ES products and larval stages can be co-cultured with organoids. 	<ul style="list-style-type: none"> • Heterogeneity of organoids (in size, architecture, metabolism, and differentiation of cells, across samples) can impact the observations upon co-culture with helminths and their products. • <i>In vivo</i> tissue architecture is not completely recapitulated in organoids. • 2D organoids lack the cellular architecture present in 3D organoids and <i>in vivo</i> situations. • Organoids are still reductionist models lacking more complex interactions regulating infection in the native microenvironment. • Dimensional conformation and size of organoids impacts the delivery and co-culture of specific parasitic stages. • Moulting of helminth parasites in organoid culture may be restricted by the lifespan of the organoids and their passage requirements (3D organoids).
<p>Technical</p> <ul style="list-style-type: none"> • Organoid lines can be generated from small tissue biopsies from the specific host and can be shared across different laboratories. • Indefinite expansion and cryopreservation of individual organoid lines with genomic stability. • Organoids can be genetically engineered by lentiviral expression systems and CRISPR/Cas9. • 2D organoid systems are amenable to high-throughput applications. 	<ul style="list-style-type: none"> • Organoid culture is costly and there is an initial requirement to access tissue from animals or patients, which has ethical implications.

The use of organoids in experiments with live helminth parasites requires careful consideration of the different components of the *in vivo* host–parasite interaction that this system can potentially recreate (Figure 1, Key Figure) [3]. Those include specific host molecular structures sensed by helminths that are defined by tissue architecture, polarization, and multicellularity; environmental clues such as the presence of commensal microbiota, gas exchange, and nutrient gradients; and physiologically relevant biomechanical forces (e.g., fluid shear, stretch, compression) [3,6]. In addition, it is crucial to consider the life cycle stage of the parasite to be used in organoid studies as this impacts the mechanistic and physiological experimental conditions to be used. Next, we discuss our own insights on the use of intestinal organoids to model host–helminth interactions.

How 2D and 3D Conformations of Organoids Recreate *In Vivo* Conditions

Organoids can be utilized in their 3D conformation, when grown embedded in an ECM, or as 2D monolayers grown over plasticware or permeable supports/transwells coated with ECM [3–6,54,55]. The 2D models require numerous 3D organoids to be dissociated into single cells. The resulting single cells are then grown as a monolayer that requires further culturing before being differentiated [3–6,54,55]. In our experience, when the cells of intestinal organoids are cultured over plasticware they differentiate into the major cell types of the organ, but do not present any

Key Figure

Comparison of *In Vivo* Models and Organoid Systems on the Degree of Modelling of the Components of the *In Vivo* Host–Helminth Interaction

Trends in Parasitology

Figure 1. *In vivo* models allow a holistic recreation of the conditions required for parasite infection, including tissue architecture, oxygenation, multicellularity, cell differentiation and growth factor and nutrient gradients, colonization by local microbiota, and interactions with other cells that create a unique microenvironment hosting the parasite. Organoids can be grown in 2D and 3D conformations mimicking to some extent the components of the *in vivo* host–parasite interaction. '✓': fully recreated; '✗': not modelled; '?': condition modelling not known or investigated; '✓?': conditions that have been recreated to certain extent; '✗?': those that have not been successfully modelled by the authors, but are still to be tested in work with other helminths.

organization. In contrast, cultures grown on permeable supports/transwells have a more physiologically relevant appearance to those grown on conventional plasticware [3], and promote the differentiation of cells and their organization in a specific conformation that, to an extent, resembles intestinal crypts. Moreover, culturing on permeable supports/transwells allows the independent control of apical and basolateral surfaces, as cells grown on these conditions polarize [6,54,55]. This permits the creation of nutrient/growth factor gradients and an air–liquid interface that promotes cell differentiation, and the co-culture with other cell types. Nevertheless, organoids grown in a 2D fashion lack any semblance of the organ architecture and self-organization that characterize 3D organoids and *in vivo* conditions (Figure 1).

A recent publication has shown that polarity of 3D gastrointestinal organoids can be controlled [56]. When organoids are cultured embedded in ECM they present a basal-out conformation with the apical side facing the lumen of the organoid. This can be reversed to an apical-out conformation by ECM removal and culture in suspension. The barrier function and major cell types are still

maintained, and the conformation facilitates the exposure of the apical side to nutrients, drugs, and pathogens [56].

New engineering-based approaches for control over organoid composition, size, spatial organization, luminal accessibility, chemical gradients, and biomechanical forces are being developed [1,3,15,53,57,58]. These include 3D-bioprinting, chemically programmed assembly and defined ECM, microwell arrays, droplet-based microfluidics, peristaltic pumps, and bioreactors [3,15,16,58,59]. These approaches offer increasing control over physiological conditions and will enable a better understanding of how tissue architecture influences the cellular responses during helminth infection.

Strategies of Co-culture of Helminths or Their Products with Organoids

The dimensional conformation in which organoids are grown determines the accessibility and methods used for their co-culture with pathogens or their products [5]. Moreover, the size of the life stage of the helminth of interest impacts the strategy of delivery to be utilized. Sizes range from a few hundred micrometers in the eggs and infective stages (L1/L3 larvae, cercariae) up to meters in the adults of some cestodes. Thus, a combination of parasite size and organoid dimensional conformation determines the extent to which the co-culture/infection can be controlled, both in terms of the number of parasites and concentration of ES products, as well as the conditions required for potential hatching of eggs, or activation of larvae/adults and their invasion (such as oxygen levels, presence of host molecular structures, cellular polarization/architecture, and microbiota). For pathogens other than helminths, there are no reports on the impact of the dimensional conformation and architecture of organoids on the interactions with the host cells. Indeed, the life cycle of the protozoan parasite *C. parvum* can be replicated both in 3D and 2D organoids [33,34].

3D Organoids

Until recently, basal-out 3D organoids were the only approach available to perform studies on host-pathogen interactions while preserving the cellular architecture of the organoids. Basal-out 3D organoids have restricted luminal access, thus requiring delivery of parasites or their products directly into the lumen of organoids by **microinjection**. Microinjection is technically challenging, slow, and labor-intensive [3]. This technique poses problems with delivery depending on organoid size, as the number of cells per organoid and luminal volumes vary, making it difficult to perform precise quantitative experiments [3,5,6]. The volume injected also depends on the accumulation of mucus and cell debris within the enclosed organoid lumen, which restricts access to the apical surface of the epithelia [56]. In addition, microinjection can result in spillage of the cargo into the basolateral space that can lead to confounding results [56].

For microinjection, it is important to consider the diameter of the injecting needle needed to penetrate the organoid without causing major damage. Therefore, in the case of live helminth microinjection, the diameter of the needle limits the life stage of the parasite that can be injected. In our experience, microinjection is viable for ES products and larvae only (eggs and adults are too large), and is not amenable to high-throughput approaches because larvae often block the microinjecting needle. Upon microinjection of the parasites, it is important to consider the luminal conditions in the organoid, as low levels of oxygen (varying from approximately 3–10% [60]) and accumulation of dead cells can impact parasite ability to survive and invade (Figure 1).

Apical-out 3D gastrointestinal organoids offer the possibility of co-culturing with parasites or their products in suspension, potentially offering greater control over delivery, a key factor for quantitative experiments [56]. Nevertheless, when using apical-out 3D organoids the conditions required for parasite invasion need additional consideration; for instance, the nematode *T. spiralis* requires a semi-solid medium to invade host cells *in vitro* [45,46]. Furthermore, apical-out 3D organoids lack a lumen, they release mucus into the culture media, and do not seem to recreate the epithelial architecture as budding structures are missing [56]. All of these features may impact on parasite invasion and active infection.

2D Organoid Cultures

While 2D organoid cultures lack the cellular architecture that may have an important role on helminth invasion [5], they enable apical and quantitative exposure to parasites and their products to a layer of cells that, in the case of gastrointestinal organoids, is polarized and that produces and maintains a mucus layer. An additional advantage of this system is the better oxygenation of the culture, compared with 3D organoids where the low concentrations of oxygen in the lumen may be detrimental to parasite viability. Moreover, permeable supports/transwells permit access to the basolateral compartment, so that growth factor or nutrient gradients can be established, and other cells can be co-cultured, such as immune cells, to better reproduce the native microenvironment. Unfortunately, a current major limitation of this system is that live imaging of the transwells is currently not possible without disassembling the permeable support [56].

In theory, 2D organoid cultures allow interactions to be studied with many different life stages of helminth parasites and their ES products. However, it is unlikely that adult parasites will actively infect cells that, under natural conditions, would be invaded by larval stages (with subsequent moult and then development into adults in tissue). Moreover, upon successful infection of both 2D and 3D organoids by helminth larvae, it is implausible that these systems can support moulting of parasites to reach adult stage; the time interval for moulting exceeds the 48–72 h interval for splitting organoid cultures required for their culture maintenance. Moreover, in the case of 3D organoids, they grow to only a few millimeters in size due to the limitations of passive diffusion of oxygen and nutrients, and their luminal space may constrain the size of the parasite that can be contained (Figure 1).

Consequently, current organoid systems to study interactions of live helminths with their host through their life cycle may be restricted to specific parasitic stages. In the future, advances in engineering-based approaches for organoids, including vascular systems to promote extended organoid growth and function [16,53], will potentially support the development of long-term organoid cultures that could support *in vitro* life cycles of helminth parasites.

Complementing the System for Future Applications

While organoids are more physiological and complex models than cell lines, they are still reductionistic systems with consequent limitations on the interpretation of results obtained with them. In the future, complementation of organoids with the tissue components that they currently lack will be required to better mimic the host–helminth interactions regulating infection in the native microenvironment [6]. Those components include the SCN, the commensal microbiota of the specific organ, and chemical gradients and physical/mechanical forces [1,3,53].

For instance, the intestinal SCN influences stem cell behavior to govern intestinal homeostasis under physiological and pathological conditions [13] and plays a pivotal role in responses to infection [41,61]. The intestinal microbiota and their by-products shape the architecture of the intestinal epithelia [62,63], and influence the intestinal SCN renewal and differentiation, through stimulation of stromal, epithelial, and immune cells [5,63]. Moreover, during infection with intestinal pathogens, the microbiota influences their establishment in the tissue and the development of immune responses [40,64].

Hence, improving the state-of-the-art culture of intestinal organoids by complementation with stromal (including fibroblasts, myofibroblasts, **telocytes**, **pericytes**, neural, endothelial, and smooth muscle cells) and immune cells, and microbiota *in vitro*, will illuminate the intricate interactions of these components during infection with intestinal helminths (Figure 1).

Co-culture of organoids, both in 2D and 3D conformation, with other components of the intestinal SCN has been described with interesting results. Fibroblasts promote the growth of intestinal and esophageal organoids [65–67]. Similarly, neural cells induce stem cell division of gastric [68] and intestinal [69,70] organoids, while also supporting tuft cell survival [69]. Co-culture with immune cells

includes: (i) intraepithelial lymphocytes that proliferate and incorporate in the organoid epithelium [71]; (ii) macrophages that enhance barrier function and maturity of enteroid monolayers and respond to infections with *E. coli* [72]; (iii) lamina propria lymphocytes that mediate commensal protection of the epithelium [73]; and (iv) dendritic cells that alter stem cell fate by inhibiting secretory lineage and drive absorptive lineage [74]. The anaerobic nature of the majority of the members of the intestinal microbiota complicates its co-culture in 2D organoid systems that grow under normal oxygen concentrations [5,75]. However, introduction of some oxygen-tolerant microbiota members (and their components and by-products), in organoid cultures and other intestinal models, has allowed their influence on the intestinal SCN to be studied [62,73,75].

Co-cultures of induced PSC-derived organoids and other cells, such as vascular and neural cell types, to simulate embryonic organogenesis, results in their own 3D self-organization promoting higher order function [53]. However, for organoids of other origin, permeable supports/transwell systems and novel microengineered culture scaffolds have the potential to increase the complexity of the organoid models by allowing stromal cells to be added, such as fibroblasts and nerves, as separate matrix layers in a sandwich-style model [6,58,59,65,67]. These systems also permit the creation of air and nutrient gradients and the co-culture with immune cells to address their interactions with intestinal epithelial cells during infection (Figure 1).

Complementation of organoid cultures from other organs with their own SCN and tissue components will equally be crucial in the implementation of organoid systems as models to study helminth infections. Recent models include spatial separation of different cell types, microchannels, and fluidic systems that mimic perfusion, oxygen and nutrient supply, and mechanical cues simulating the tissue-specific microenvironment and further promoting organoid maturation and self-organization [16,53]. In the future, more sophisticated modelling systems such as **organ-on-chip** approaches will integrate organoids [16] and provide more complex tools to recreate and dissect the molecular mechanisms governing host–helminth interactions.

Concluding Remarks

The use of organoids in helminth research is in its infancy, but it offers an exciting approach to complement other experimental systems to better understand the interactions of helminth parasites with their specific hosts at a molecular level. The opportunity that organoids provide for working with human or livestock material, and overcoming the limitations of model organisms, is also invaluable. Moreover, the features of organoids that allow expansion and cryopreservation of organoid lines will certainly promote collaborations among laboratories studying diverse helminths, having a substantial impact in reducing the number of animals used in research and in the fight against the neglected diseases caused by helminth parasites.

While the use of organoids requires experimental fine-tuning to recreate and promote the invasion and colonization by helminth parasites, the field is quickly evolving and providing new engineering solutions to better mimic the organs that are naturally targeted (see Outstanding Questions). At present, most of the developments are focused on organoids derived from gastrointestinal tissues and will benefit investigations on helminths that reside in those organs; nevertheless, we foresee a broad application of organoids derived from other organs in the study of helminth–host interactions. Importantly, interacting molecules at the host–helminth interface will provide good candidates for novel antiparasitic interventions such as vaccine development or targets for drug discovery – and the organoid system provides an experimental set-up suitable for screening approaches. Drug discovery not only includes those to treat helminth infections, but also medicaments that target chronic noninfectious diseases resulting from a dysregulated immune system, such as **inflammatory bowel disease (IBD)**, for which alternative therapies with helminth-derived compounds are currently being developed [43,76].

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Outstanding Questions

Which other variables (e.g., mucus composition and hormone levels) affect the establishment of infection and colonization by helminths and need to be considered when recreating these processes in organoids?

Are organoids from specific genetic backgrounds required to reproduce infections with certain helminths?

Can organoids support the growth and moulting of helminths that characterize their life cycle stages? What are the constraints limiting longer term organoid culture, and how can these be rectified to accommodate the development of parasites through their life cycle?

Will the integration of organoids derived from different organs such as the lung, liver, and intestine (body-on-a-chip) sustain complex helminth life cycles, for example, hookworms and schistosomes?

To which scale can organoids sustain the screening of new drugs for antihelminth therapy and the testing of the anti-inflammatory effects of helminths on chronic non-infectious diseases?

Can we develop organoids from helminth tissue to further explore the fundamental biology of parasites?

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