



# In vitro models and ex vivo systems used in inflammatory bowel disease

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## Abstract

Inflammatory bowel disease (IBD) is a chronic, relapsing gastrointestinal condition. Ulcerative colitis and Crohn's disease are types of inflammatory bowel disease. Over many decades, the disease has been a topic of study, with experts still trying to figure out its cause and pathology. Researchers have established many in vivo animal models, in vitro cell lines, and ex vivo systems to understand its cause ultimately and adequately identify a therapy. However, in vivo animal models cannot be regarded as good models for studying IBD since they cannot completely simulate the disease. Furthermore, because species differences are a crucial subject of concern, in vitro cell lines and ex vivo systems can be employed to recreate the condition properly. In vitro models serve as the starting point for biological and medical research. Ex vivo and in vitro models for replicating gut physiology have been developed. This review aims to present a clear understanding of several in vitro and ex vivo models of IBD and provide insights into their benefits and limits and their value in understanding intestinal physiology.

**Keywords** Inflammatory bowel disease · In vitro cell lines · Ex vivo systems · Organoids · Caco-2 cell lines · Gut-on-chip

## Introduction

Inflammatory bowel disease (IBD) is a type of chronic gastrointestinal inflammatory condition that includes Crohn's disease (CD) and ulcerative colitis (UC). IBD is marked by periods of stomach discomfort, bloody diarrhea, loss of weight, and infiltration of neutrophils and macrophages that release cytokines, proteolytic enzymes, and reactive species that cause inflammation and ulceration in the intestinal mucosa [1]. The colon and rectum are usually affected by ulcerative colitis. Crohn's disease affects any region of the gut, although the terminal ileum is the most commonly affected [2, 3]. The incidence of IBD differs widely both within and between geographic areas. Crohn's disease ranges from 8.9 to 17.45 in Europe, whereas ulcerative colitis has an incidence ranging from 4.1 to 21.47 [4]. The specific cause of IBD is unknown, but growing evidence shows that in genetically predisposed individuals, the development of

IBD may be caused by a disruption in the finely controlled equilibrium between host response and gut commensal bacteria in the intestinal epithelium. Environmental variables like antibiotics, dietary components, tobacco, and nicotine may have a role in initiating and recurrence of ulcerative colitis or Crohn's disease, or both, by modulating host immunological responses [5–9]. Since the specific etiology of IBD is unknown, several in vivo models or animal models have been developed throughout the years to investigate the disease's pathophysiology. Chemically induced models include dextran sodium sulfate (DSS)-induced, trinitrobenzene sulphonic acid (TNBS)-induced, oxazolone-induced, acetic acid-induced, and carrageenan-induced models of IBD. Bacterial-induced models such as adherent invasive *E. coli* and salmonella models are also developed. Other in vivo models include genetically engineered, transgenic mice, mutation knock-in, and adoptive transfer models [10].

In vivo animal models are commonly used to simulate IBD. Still, they are too complicated to adjust the multiple IBD factors separately. Moreover, because of the vast interspecies variability between animal models and people, the success of IBD therapy is difficult to predict [11]. As a result of the constraints of using in vivo animal models to understand the pathophysiology of IBD and identify new medicines for treating it, researchers have attempted to establish

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numerous in vitro and ex vivo models of IBD. In both normal and diseased situations, these in vitro and ex vivo models investigate gastrointestinal physiology, intestinal epithelial function, host-microbiome interaction, absorption of the drug, and cell-cell co-culture.

The current review focuses on several in vitro and ex vivo models of inflammatory bowel disease and their benefits and drawbacks. The review also focuses on the most recent application of each model in research investigations to better understand inflammatory bowel disease.

## In vitro models

### Caco-2 cell line

Caco-2 cells, also known as cancer-coli-2 cells, are immortalized human colorectal adenocarcinoma cells [12]. Fogh created these cells in 1977 at the Sloan-Kettering Institute for Cancer Research [13]. Caco-2 cells develop independently in culture into a diverse collection of intestinal epithelial cells [12]. Hidalgo and his colleagues discovered the use of Caco-2 cells in research in the late 1980s [14]. While colon cancer produces these cells, they resemble enterocytes that line the intestines [15].

The differentiated Caco-2 cells show features identical to small bowel enterocytes, such as brush-border microvilli, and the production of characteristic digestive enzymes such as peptidases, sucrase-isomaltase, dipeptidyl peptidase IV, lactase, and aminopeptidase N [16, 17]. Caco-2 cells also possess the ability to produce various cytokines, which include interleukin 6, 8, and 15 (IL-6,8,15), tumor necrotic factor- $\alpha$  (TNF- $\alpha$ ), and thymic stromal lymphopoietin (TSLP) [18–20]. Thus, the production of these cytokines enables the researchers to utilize the Caco-2 cells in those research involving the study of inflammation such as IBD. Several investigations correlated absorption data to Caco-2 permeability coefficients and found a significant connection, especially for substances carried passively [21–24]. The Dulbecco's Modified Eagle Medium [25] is routinely used to preserve Caco-2 cell lines.

Caco-2 cell lines, as previously stated, are extremely useful for studying intestinal diseases owing to their close similarity to intestinal epithelial cells; yet, there are numerous restrictions or downsides to utilizing these cell lines. Not only do enterocytes exist in the normal epithelium, but there are many other types of cells as well. When Caco-2 cell lines are being used, mucus and an unstirred layer of water are not present in the solution. A variety of non-cellular factors influence a compound's absorption in cells. Thus, phospholipids and bile acids have a significant impact on lipophilic component transport, and compound solubility in the mucus

layer and the unstirred water layer near the epithelium has a considerable influence on in vivo absorption [12].

Many experts in colitis research have utilized and continue to use these cell lines extensively. Maria-Ferreira et al. conducted in vivo experiments on rhamnogalacturonan and in vitro tests on Caco-2 cells. The findings demonstrated that rhamnogalacturonan enhanced wound healing, reduced epithelial barrier dysfunction, and suppressed IL-1-induced IL-8 production in Caco-2 cells [26]. Chen et al. published research on astragalus polysaccharides revealed that astragalus inhibited ferroptosis in a mouse model of colitis and human Caco-2 cells by disrupting the NRF2/HO-1 cascade [27]. Another study conducted by Song et al. showed Epac-2 expression aided in repairing the intestinal barrier in the LPS-induced cell co-culture environment by increasing TJ protein expression and decreasing Caco-2 cell death [28]. Li et al. investigated the anti-inflammatory impact of allicin on ulcerative colitis induced by trinitrobenzene sulphonic acid. Allicin reduces the phosphorylation of P38 and JNK and the expression of NF- $\kappa$ B p65 in Caco-2 cells, which were raised after IL-1 $\beta$  treatment [29]. Effect of corn protein hydrolysate digests on TNF- $\alpha$ -induced iNOS, COX-2 protein expression, and IL-8 production in Caco-2 cells was studied by Liang et al. [30].

### HT29 cell line

HT29, like Caco-2, is a human colon cancer-derived cell line. In 1964, Fogh and Trempe derived it from a 44-year-old Caucasian woman's primary tumor [31]. Since several human colon cancer cell lines have been developed, these cell lines were initially employed to investigate elements of human cancer biology. These cells drew attention due to their ability to express mature intestinal cell features like enterocytes or mucus generating cells [32]. There are several similarities between Caco-2 and HT29 cells. Similar to Caco-2 cells, HT29 cells differentiate. HT29 cells also release digestive enzymes comparable to Caco-2 cell lines [33].

Previous research revealed that, like Caco-2 cell lines, HT29 cell lines release cytokines comparable to Caco-2 cells [34]. Another significant distinction between Caco-2 and HT29 cells is that HT29 cells could generate mucin at a reasonably high level [35]. The high rate of glucose consumption is the peculiar characteristic of HT29 cells. Hence, a high concentration of glucose is required in the medium. These HT29 cells did not exhibit any characteristics resembling intestinal epithelial cells when exposed to high glucose concentrations. They developed as a multilayer of undifferentiated, unpolarized cells [36]. As a result, the discovery of substituting glucose with galactose was made. Pinto et al. [37] discovered that replacing the media in which HT29 was put with galactose triggered enterocytic differentiation.

Many professionals in colitis studies have and continue to make substantial use of these HT29 cell lines. In a rat

model of DSS-induced colitis, Rajendiran et al. used HT29 cells to study the efficacy of *Alpinia officinarum* extract on cell sustainability and expression of NF- $\kappa$ B and COX-2. The results revealed that increasing the concentration of extract increased cell mortality and lowered the viability of HT29 cells [38]. In another study performed by Lei et al., baicalin reduced LPS-induced HT29 cell death by inhibiting the PI3K/AKT signaling pathway and considerably lowered TNF- $\alpha$ , IL-6, and IL-1 $\beta$  release while increasing the production of IL-10. Studies have also shown that HT29 cells are popularly used in those studies involving adhesion and survival of microorganisms. Wang et al. have performed a similar analysis using the *Lactobacillus* strain. In that study, four different species and three *Lactobacillus* strains were utilized to study their adhesion to HT29 cells [39]. Barbosa et al. employed HT29 cells to study goniiothalamine's proapoptotic effects on these cells. The researchers discovered that goniiothalamine induced apoptosis in HT29 cells, involving the generation of reactive oxygen species (ROS), the activation of MAPK, and caspases [40]. In a recent study on ulcerative colitis, Xiaotian et al. established the in vitro effectiveness of p-hydroxy benzaldehyde derived from a cyanobacterium, *Nostoc commune*. p-Hydroxy benzaldehyde increased mRNA expression in lipopolysaccharide-stimulated HT29 cells [41]. Another comparable recent work done by Balaji et al. on thymoquinone utilizing HT29 found that thymoquinone lowered pro-inflammatory chemokines such as CXCL-1 and COX-2 production in HT29 cells. The PPAR- $\gamma$  activity was also investigated using HT29 cells, and it was observed that thymoquinone enhanced their expression [42]. In vitro models of IBD are increasingly being used in research on the involvement of TLR (toll-like receptor) in the IBD. Several researchers have used HT29 cell lines to conduct recent studies on TLR receptors [43–47].

## Organoids

Organoids are a type of cell produced from pluripotent or adult tissue stem cells. They are three-dimensional and can change into different kinds of cells to perform the functions of the relevant organs [48–50]. Scientists have been attempting to maintain tissue explants in vitro since 1992 [51, 52]. However, in 2008 and 2009, Eiraku et al. and Sato et al. established the first practical way of producing stem cell-derived structures, which are now known as “organoids” [53, 54]. Sato et al. developed them using Lgr5+ adult stem cells (ASCs), initially isolated from rodents and then human intestinal crypts [54]. Other anatomical regions, such as colonic, esophageal, and gastric tissues, have yielded good organoid cultures [55–57]. Colonoids are organoids that are formed from colonic tissue. Enteroids or small bowel organoids are organoids generated from small intestinal tissue [58]. They may be grown from a small volume of tissue,

such as biopsies, and developed into mature enterocytes, tuft cells, goblet cells, and paneth cells [48, 59]. Many of the source tissue processes, including filtration, endocrine, and paracrine secretion molecular transport, contraction, and absorption, are likewise performed by gut organoids [48].

Even though they are inexpensive and may be created quickly, these organoids cannot recreate the complex environment of an organism [60]. Only one sort of cell may be manufactured. Cell types that are uncommon, such as tuft cells, are difficult to cultivate. Additionally, organoids cannot replicate the mucosal barrier or mucus production, and standardization protocol is not globally accepted [49]. Furthermore, because organoids are formed from malignant cells, they exhibit inherently distinct qualities from those of non-malignant cells, notably adhesion, cell polarity, and epithelial integrity, among other characteristics [61].

Because organoids may imitate the physiology of intestinal epithelial cells, they are great models for demonstrating IBD, as one of the critical features of IBD is abnormalities in epithelial cell activities. It is also feasible to co-culture gut organoids with non-epithelial cell lines such as macrophages and lymphocytes to better understand the intestinal mucosa [62, 63]. In addition to epithelial alterations, host-microbial interactions play an essential role in the pathogenesis of IBD. These organoids may imitate the gut's microbial ecology, which can help researchers better understand the host-microbial connections that occur in IBD [64–66]. Organoids generated from an adult, and fetal mouse tissues have also been used to investigate the developmental expression profiles of parts of the innate immune system, such as NOD2, TLR4, and TLR5, in the laboratory [67]. Patients with active inflammatory bowel illness can be used to culture intestinal organoids [68–70]. When organoids were stimulated with particular inflammatory cytokines, specific biomarkers of inflammation were upregulated. In research done by Michael et al., interferon-gamma was employed to activate the human-derived organoid. The results revealed that E-cadherin was upregulated [71]. The NF- $\kappa$ B signaling pathway was activated in mouse colonic organoids subjected to chronic inflammatory stimuli, as demonstrated by Hibiya et al. [72]. Another recent study conducted by Verstockt et al. explored the influence of intestinal inflammation on viral intestine invasion mechanisms (SARS-CoV-2), especially ACE2, in IBD. The researchers found that intestinal inflammation changes SARS-CoV-2 coreceptors in the colon and ileum. In the ileum, HNF4A appears to be a critical upstream modulator of ACE2. At the same time, interferon transmission may be dominant in the colon [73]. Some researchers have also made efforts to use organoids produced from pediatric IBD patients to illustrate and understand the pathophysiology of IBD [74, 75].

Consequently, primary organoid cultures from damaged or inflamed tissue appear to be a suitable model for studying the epithelial and mucosal abnormalities in IBD.

### Co-culture of Caco-2/HT29-MTX

The HT29 cell line was discussed in the earlier part of this review. According to preliminary findings, researchers have attempted to cultivate HT29 cells in the presence of methotrexate (MTX), according to preliminary results [76]. These were done to investigate if methotrexate affected the ability of HT29 cells to secrete mucus. The scientists discovered that exposing fast-growing HT29 cells to higher doses of MTX caused them to develop into mucus-secreting differentiated cells [76]. Since then, co-cultures of Caco-2 and HT29-MTX have been employed extensively in various research, including microbial adhesion, intestinal absorption, colonic permeability, and intestinal barrier integrity [77–80].

The stability and repeatability of the Caco-2 and HT29-MTX co-culture models are pretty close to that of the monocultures. During the 21-day polarization stage, the cells are cultured together. Between days 21 and 25, the co-cultures must be employed for experimentation [81]. Caco-2 cell lines are derived from absorptive cells in the intestine, whereas HT29-MTX cells are derived from goblet cells [82]. As previously noted, the Caco-2 cell line is of colonic origin. Still, when cultivated in culture, it exhibits traits of small intestine epithelium, such as the presence of a brush border, typical intestinal enzymes, and columnar absorptive cells. However, one significant drawback of the Caco-2 cell line is its inability to generate a mucus layer. Because HT29-MTX cells are derived from goblet cells, they can develop gel-forming MUC5 and membrane-bound MUC1. The co-cultivation of these two cell lines, Caco-2 and HT29-MTX, results in a model consisting of normal epithelium, goblet cells, and enterocytes when these two cell lines are grown together [82]. Walter et al. identified a strong relationship between the two factors in a study to measure the permeability of Caco-2/HT29 co-culture [83].

Caco-2 and HT29-MTX cells are cultured independently. Before seeding, the two cell cultures are combined. The most physiologically appropriate ratios (Caco-2/HT29-MTX) are from 90:10 and 75:25 [84]. This proportion provides the optimal compromise between model responsiveness and the occurrence of a mucus layer. The co-culture is maintained at 37 °C in a 5% concentration of carbon dioxide in Dulbecco's Modified Eagle Medium (DMEM) containing 10% of heat-denatured juvenile bovine serum, 2 mM concentration of L-glutamine, 1% concentration of non-essential amino acids, and 100 U/ml concentration of penicillin along with 100 g/ml concentration of streptomycin. During 21 days, the culture media is replaced every 2–3 days.

Caco-2/HT29-MTX co-cultures are widely employed in research on IBD, colon cancer, and other disorders affecting intestinal barrier integrity or mucus layer adhesion abnormalities. Rasha et al. used Caco-2/HT29-MTX co-culture in recent work to investigate the pathophysiology of IBD. The study's rationale was to look into using L-pNIPAM hydrogel to develop a 3D co-culture model under circumstances similar to inflammation [85]. Another recent work has shown that Caco-2/HT29-MTX co-culture may be used to explore the harmful effects of phycotoxins on the intestinal barrier. Reale et al. studied the toxicity of four phycotoxins on Caco-2/HT29-MTX co-cultured cells [86]. Other studies explore the use of Caco-2/HT29-MTX co-culture in colon cancer [87, 88]. The potential of different plants to stabilize barriers has also been investigated using co-cultures of Caco-2 and HT29-MTX cells [20]. Song et al. recently investigated the transcellular permeability of epigallocatechin-3-gallate using Caco-2 and HT29-MTX co-cultures [89].

Cell lines are a good choice since they are widely accessible and quickly produced in a lab setting, assuring excellent model system reproducibility. The system is simple to implement and successful in screening findings. These two cell lines do not generate some of the transporters or carriers found in average healthy epithelia; hence, the production of the necessary molecules should be established in the case of transport investigations. To create a homogenous mucin layer, the ratio of the two cell types is also critical. Even though cell lines are a viable alternative, they have stability and survival challenges.

### T84 cell line

The T84 cell line is another often utilized cell line in the study of intestinal research. Murakami et al. developed it in 1980 to study the hormonal regulation of human colon cancer [90]. T84 cells contain microvilli on their surfaces, and when activated by transforming growth factors, they can develop into crypt-like cells [91].

T84 cells, like Caco-2 cells, can develop quickly into an absorptive stratum of epithelial cells. When T84 cells are stimulated by human transforming growth factors and/or mesenchymal factors, they can develop into crypt-like cells [92]. T84 cell lines are typically acceptable and effectively mimic the colon milieu, making them useful for host-microbe research in general [93].

T84 cell lines have been used to investigate mechanisms involving electrolyte transport [94], intestinal permeability [95, 96], and, most importantly, signaling pathways involved in the inflammatory response of the intestines [97, 98], making it one of the most widely used models in the study of inflammatory bowel disease [99–102]. Researchers have been employing these cells to better understand the

complicated pathophysiology of inflammatory bowel disease since their discovery. Li et al. proposed that aberrant phosphorylation of colonic claudins might be linked to alterations in intestinal barrier function in a study that examined the influence of LPS on claudin phosphorylation in T84 colonic cells [103]. Manzini et al. used T84 cells in their research. They found that administering the energy drink to the cells lowered the release of the pro-inflammatory cytokines interleukin-6 and tumor necrosis factor- $\alpha$  [104]. Yueying et al. recently used T84 cells in a novel study. Their goal was to use cDNA microarray probe genomics to investigate the function of long coding RNAs in UC [105]. T84 cells can also be employed in studies involving a primary IBD pathophysiology [106–109].

T84 cell lines are one of the models used in research involving the colon. Still, they can also be utilized in general studies regarding inflammatory bowel disease. However, the T84 cell line, like Caco-2 and HT29 cell lines, has limitations. Because of its malignant origin and lack of epithelium-specific activity exhibited in vivo, the T84 cell line has drawbacks similar to Caco-2 and HT29 cell lines [92].

## Intestinal-immunological cultures

Researchers created 2D co-cultures as gut inflammation models to improve simple cellular models. The interaction between intestinal and immune cells was used to create these models. Primary cells such as human peripheral blood mononuclear cells (PBMCs) [110] and macrophage cell lines from either human (THP-1) [111] or murine origin (RAW 246.7) are the principal immune cells employed to generate the various co-culture systems with intestinal cell lines.

### PBMC

Human PBMCs are obtained from healthy donors' or buffy coats' peripheral blood and utilized to study the effects of dietary bioactive on immune cells. Dendritic cells, lymphocytes, and monocytes make up PBMC. These cells are stimulated by polyclonal activators, which cause them to release cytokines [112]. As a result, these cells are acceptable for IBD research involving immune responses [113–116]. Though PBMC is a convenient source of human immune cells, they have a fundamental drawback: phenotypic variations exist between immune cells and cells of the intestinal mucosa [112].

### THP-1

THP-1 cell lines are human leukemia cell lines initially obtained from a patient with acute monocytic leukemia. Tsuchiya et al. were the first to create it in 1980 [117]. THP-1 cells are similar to primary monocytes and macrophages in

terms of differentiation. They transform into macrophages after being exposed to PMA (phorbol-12-myristate-13-acetate) [118]. These cells may be maintained in vitro for approximately 3 months (passage 25) without loss of sensitivity or activity. This cell line has been used to research ROS generation and inflammation. It has been described as a model for immunological regulation [119, 120]. THP-1 cells have high reproducibility in studies and low variability of cellular phenotypes due to their homogeneous genetic background. THP-1 is more suited and efficient than PBMC cell lines due to this property [91]. Another benefit of THP-1 cells over other primary monocytes is that siRNA allows for easier down-regulation of the expression of particular proteins [121]. The most often utilized media for storing THP-1 cell lines is RPMI 160 medium [121]. THP-1 cell lines are now being used in inflammatory bowel disease research due to their high vitality, extended storage period, and ability to accurately simulate inflammation [122–126].

### RAW 246.7

The RAW 246.7 cell line was obtained from a mouse tumor using the Abelson leukemia virus [127]. RAW 246.7 cell lines are frequently co-cultured with Caco-2 cells [128, 129]. In terms of pattern recognition receptors and reactivity to microbial ligands, including TLR, these cells have been demonstrated to imitate murine bone marrow-derived macrophages [130]. RAW 246.7 cells are often utilized in studies of intestinal inflammation [131–133]. Recent studies have also revealed that these cells are widely employed in inflammatory bowel disease research due to their capacity to accurately simulate the gut environment and their ability to successfully mimic TLRs, which play a key role in the pathogenesis of IBD [134–138]. One of the key pathologies implicated in IBD is the host-microbiome interactions. The RAW 246.7 cell lines have simulated host-microbiome interactions in studies [139–142]. Despite the widespread use of these cells in numerous fields of study, the authors concluded that when utilizing RAW 246.7 cells, caution should be exercised when interpreting the data due to alterations during continuous culture. Various cross-reactions should also be considered when merging human and mouse cells (e.g., co-culture of Caco-2/RAW 246.7) [143].

## Ex vivo models

### Ussing chamber

Hans Ussing, a Danish zoologist, invented the Ussing chamber in 1950. It was created to understand better the mechanics driving active transport in ex vivo intestinal tissue [144]. The usage of a Ussing chamber is

commonly employed to evaluate ion transport in tissues such as the gut mucosa [145]. The discovery of the sodium-potassium ATPase pump was aided by the usage of this chamber [146].

Typically, the Ussing chamber is divided into two compartments, separated by monolayers of cells between them. This chamber contains electrodes, and the voltages and short circuit currents measured by these electrodes may be used to determine the absorption and movement of ions through the epithelium (Figure 1) [147].

Clinical studies on rectal biopsies of patients with cystic fibrosis have demonstrated that ion transport function may be effectively assessed using the Ussing chamber [148]. Gut integrity and intestinal permeability investigations on mouse and human colon biopsies have also been published [149]. The use of the Ussing chamber also assisted the researchers in determining that in the instance of a human colon, the left colon had more permeability than the right colon [145]. The Ussing chamber's usage is restricted to investigating intestinal permeability or intestinal barrier integrity. Still, it is also frequently used in the study of host-microbiome interaction, which is an essential factor in the etiology of IBD [150–153]. The first demonstration of enterotoxin from *Shigella* in rabbits occurred in 1975 when the Ussing chamber was used [154]. Bifico, a commercially available probiotic, was found to increase the epithelial barrier function of the colon in mice that had been deprived of the interleukin-10 (IL-10) cytokine [155]. Another research on the effects of *Enterococcus faecalis* was conducted in a Ussing chamber, and the results were positive. After doing their study concluded that bacterial translocation in the colon mucosa was caused by an aggregation product produced by *Enterococcus faecalis* [156]. Using the Ussing chamber, several modern

studies have attempted to investigate the physiology of many receptors [157–160].

Although the Ussing chamber has numerous advantages, it also has a few limitations or drawbacks. Long-term experiments are impossible because the ex vivo tissue explants employed in the chamber are only viable for 5 h. Another downside of the chamber is its poor throughput and handling complexity [92].

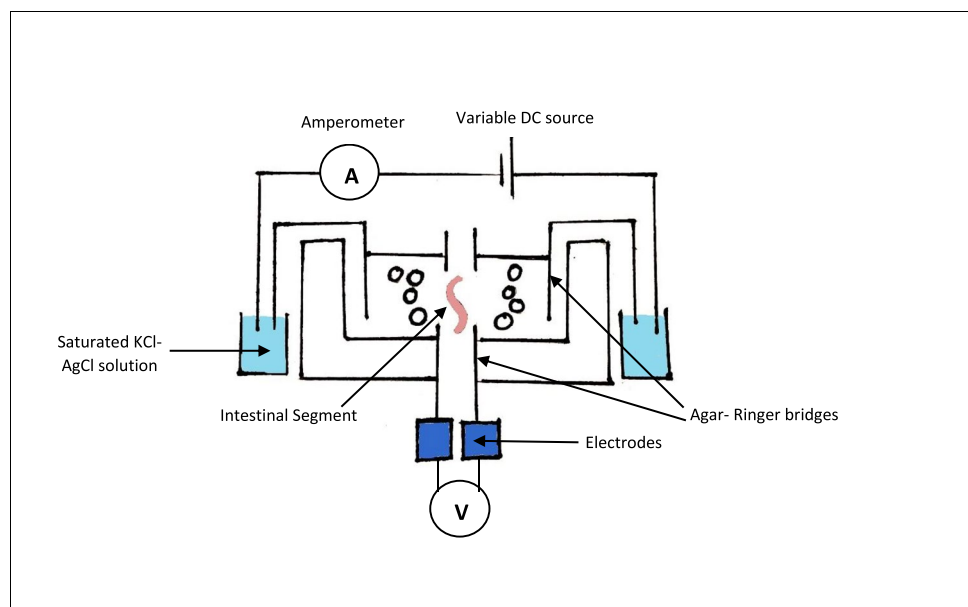
### Everted gut sac

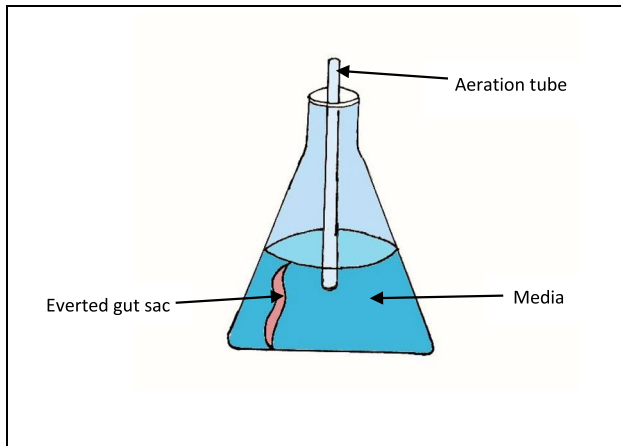
Wilson and Wiseman initially established the everted gut sac model in 1950 to thoroughly understand the kinetics and mechanism of drug absorption [161]. Generally, the everted gut sac model is used to investigate gastrointestinal drug interactions, multidrug resistance, and drug pharmacokinetics and pharmacodynamics [162].

Figure 2 represents the everted gut sac system. The initial step in preparing the gut sac is to segment the chosen region of the intestine (duodenum, jejunum, ileum, or colon) and then evert the segments. The everted parts are then washed with a physiological solution over a glass rod. After that, the sac is filled with Kreb's solution and transferred to a flask at 37 °Celsius [163].

Few early research employed the everted gut sac model to investigate p-glycoprotein function in rat intestines [164]. It was also utilized to investigate paracellular transport across the small intestine [165]. Since its development, the everted gut sac has been widely used to examine the intestinal permeability of various medications, and in the case of colitis research [166, 167]. Videla et al. conducted a similar trial with polyethylene glycol (PEG) on TNBS-induced colitis. They employed everted colonic sacs to investigate PEG's

**Fig. 1** Ussing chamber





**Fig. 2** Representation of the everted gut sac system

intestinal permeability. Pretreatment with PEG resulted in a long-term decrease in epithelial permeability in everted colonic sacs [168]. Recent research on the targeted dual drug administration of 5-aminosalicylic acid and curcumin to inflamed colon has also used the everted gut sac [169].

Although the model closely reflects gastrointestinal tract environments, one of the model’s primary limitations is tissue survivability. Under typical settings, the tissue is only viable for 2 h. Another shortcoming of the system is its sensitivity; the system depends on various conditions. Furthermore, this model is not ideal for using the human

intestine; instead, the rat intestine is employed, eliminating the intricacy of the human gut [162, 164].

**Microfluidic gut-on-chip**

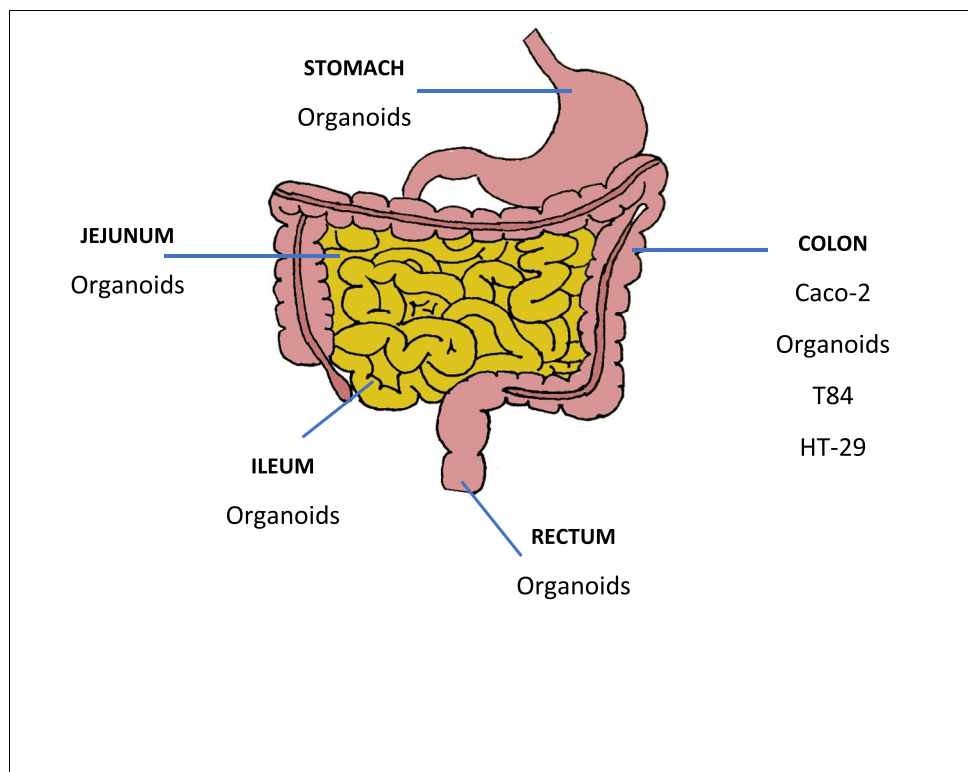
Different in vitro and ex vivo models have previously been detailed in earlier sections of the paper. Still, their usage is limited owing to their drawbacks. For example, the ex vivo models (everted gut sac and Ussing chamber) have a restricted throughput. The in vitro models cannot replicate the intestine’s blood vessels and immune cells.

As a result, a few scientists created the microfluidic gut-on-chip in 2008 and 2009 to overcome the aforementioned restrictions and take in vitro research to the next level. The initial designs of microfluidic gut-on-chip devices were created using a silicon-based polymer, polydimethylsiloxane (PDMS), and had two chambers [170, 171]. In 2009, researchers attempted to change the original model significantly. Sung et al. and Kim et al. created a 3D hydrogel villi microfluidic device using calcium alginate and collagen. Their research indicated that growing Caco-2 cells on this device increased CYP3A4 enzymatic activity [172, 173].

Different organs-on-chip have also been designed based on where the organ is derived. A gut chip based on Caco-2 cells, a duodenum-intestinal chip, a colon-intestinal chip, and a jejunum-intestinal chip have been produced [174–176].

Since its development, the usage of microfluidic gut-on-chip technology in IBD research has been steadily rising

**Fig. 3** Region of origin of various in vitro and ex vivo models



**Table 1** Advantages and disadvantages of in vitro and ex vivo models of inflammatory bowel disease.

Sr. no.	Models/systems	Advantages	Disadvantages
1.	Caco-2 cell line	Features identical to small bowel enterocytes (presence of brush-border microvilli) [16]. Produces specific digestive enzymes (e.g., peptidase and lactase) [16]. Ability to produce various cytokines [18–20]. Absorption-related studies are easy to perform [21–24].	Presence of other cells other than enterocytes in the epithelium [12]. Mucus and unstirred water are unable to be considered [12]. Various non-cellular factors hinder the study [12].
2.	HT29 cell line	Cytokine release pattern similar to Caco-2 cells [34]. Higher mucin production [35]. Cell differentiation pattern similar to Caco-2 cells [33]. Produces typical digestive enzymes (e.g., peptidase and lactase) [33].	A longer time of differentiation [33]. High consumption of glucose [36]. Undifferentiated cells even when high glucose concentration is provided [36].
3.	Organoids	Imitate physiology of intestinal cells [62, 63]. Co-culturing of gut organoids with non-epithelial cells is also possible. [62, 63] Imitate gut microbial ecology [64–66]. Investigation of innate immune systems possible [67]. Patient-derived organoids can be used [68–70].	Lacks specific cells/tissues [49]. Unable to recreate the complex environment of an organism [60]. Uncommon cell types (e.g., tuft cells) are difficult to cultivate [49]. Unable to replicate mucosal barrier [49].
4.	Caco-2/HT29-MTX co-culture	Excellent for research involving microbial adhesion, colonic permeability, etc. [81]. Able to generate mucus membranes [82]. Stability and reproducibility similar to monocultures [82]. Increased mucin production [82].	Unable to generate some transporters [82]. Perfect ratio between Caco-2 and HT29 cells required for a homogenous mucin layer [82]. Cell viability is challenging [82].
5.	T84 cell lines	Development into crypt-like cells [92].	Similar to Caco-2 and HT29 cells [92].
6.	Intestinal-immunological cultures	Appropriate for use of studying immune-related mechanisms.	Variations between immune cells and intestinal cells.
7.	Ussing chamber	Presence of two compartments [147]. Presence of electrodes to measure transport of ion [147]. Used to study intestinal permeability [149]. Used to study host-microbiome interactions [151]. Use of both animal and human tissue possible [149]. Physiology of receptors can easily be studied [157–160].	Cell viability up to 5 h only [92]. Since viability of cell is less, long-term studies not possible [92]. Poor throughput [92]. Complex handling [92].
8.	Everted gut sac	Intestinal tissue is intact [92]. Mucus layer is present [92]. Presence of large surface area [92].	Not much relevant to human as it is ideal for rat tissue. System sensitivity depends on a number of factors. Tissue viability is 2 h only [164].
9.	Microfluidic gut-on-chip	Location-based organ-on-chip can be developed [174–177]. Co-culturing of cells and macrophages possible [177]. Pluripotent stem cells can also be used [180].	Complex system [92]. PDMS used can itself adsorb compounds, hindering the research [185, 186]. High labor intensive [92].

over time. Beurivage et al. used human intestinal cells and monocyte-derived macrophages on a gut-on-chip device to replicate the human intestine. This environment was shown to closely mimic the expression profile of the human colon

in vivo. When the model was activated, IEC secreted polarized IL-8, CXCL10, and CCL-20 [177]. Another recent study found that direct interaction between DSS-sensitized mucosa and immune cells increases oxidative stress, with



intestinal microbial stimulation eliciting inflammatory cytokines and immune cell recruitment [178]. One research found that the microfluidic technique of the gut-on-chip device may also be used to evaluate the efficacy of colon targeting of dietary fiber, according to the findings of a recent study [179]. Biopsy-derived organoids or induced pluripotent stem cells (iPSCs), which maintain their own diseased morphology *ex vivo*, are also employed to replicate the IBD phenotype on a microfluidic device [180]. One of the most complicated models of IBD on the chip was developed by Trapecar and colleagues. They built an IBD model that included primary liver and gut cells and circulating immune cells. They evaluated the influence of short-chain fatty acids (SCFAs) on IBD-related inflammation [181]. The microfluidic gut-on-chip is also employed in research to understand better the host-microbiome interaction, which is one of the factors implicated in the pathogenesis of IBD. Jalili-Firoozinezhad et al. have recently attempted to cultivate commensal anaerobic and aerobic bacteria on an intestine-on-chip to understand better the interactions between the host microbiome and the commensal bacteria [182]. Similar experiments employing a colon-on-a-chip technology to explore host-microbiota interactions have recently been described [183, 184].

Thus, microfluidic intestine-on-a-chip devices, which can more precisely imitate *in vivo* intestinal architecture and environment than standard *in vitro* culture methods, provide a fresh and more accurate way to study intestinal function and disease. Although the intestine-on-a-chip approach has its pros and cons, there are certain common obstacles. Because cell and organoid-based intestine-on-a-chip technologies lack varied cell types and associated tissue structures, they accurately depict the intestinal wall. Although some researchers have demonstrated that different cell types may be added to a system, their systems are still complex and labor-intensive. Another disadvantage is the chip substance, which can significantly influence drug response bioassays when intestine-on-a-chip models are employed. PDMS is capable of adsorbing a broad spectrum of compounds [185, 186].

Figure 3 depicts the region of origin of several *in vitro* and *ex vivo* models.

## Conclusion

Over the last few decades, numerous *in vitro*, *in vivo* animal models, and *ex vivo* models have been designed to understand IBD's pathophysiology and etiology better. *In vivo* animal models cannot perfectly imitate the condition. Reliability is a challenge due to species differences between humans and animals. As a result, most contemporary research is conducted in cell culture or *ex vivo* settings. Cell cultures are

highly desirable since they allow easy, immediate access to and assessment of tissues. Several models can imitate IBD, but each has its advantages and disadvantages. The review presents a clear understanding of the use of *in vitro* and *ex vivo* models of IBD along with their own set of advantages and limitations (Table 1); many of the systems are promising but do not perfectly replicate intestinal function. However, advancements in **microfluidic gut-on-chip** systems and organoids may provide solutions to those unanswered problems. Each model is distinctive; for example, PBMC, THP-1, or RAW 246.7 cells can be used for immune-related investigations. In crypt-related research, the T84 cell line would be chosen. Because each has its own set of drawbacks, no model is an ideal model for inflammatory bowel disease. As a result, a battery of models is advised to be employed for IBD research. Furthermore, an increased understanding of diverse *in vitro* and *ex vivo* models is required to answer outstanding IBD research problems.

**Author contribution** The idea for the article, performance of the literature search, drafting, and revision of the work was done by AJ. Final approval for submission of the manuscript as given by AS and SA.

## Declarations

**Conflict of interest** The authors declare no competing interests.

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