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Modern experimental systems for studying various functions of the gastrointestinal tract

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Abstract. The integrity and functioning of intestine are crucial for the digestion and absorption of nutrients, immunological homeostasis and the prevention of the ingestion of pathogens. Currently, the improvement and development of new experimental systems for studying various functions of the intestine is a pressing task in biology and medicine. The aim of this review was to describe modern experimental systems for studying various functions of the intestine, focusing on their application, advantages and limitations. To achieve this goal, publications from 2019-2025 selected from PubMed and Google Scholar were analysed and summarised. Based on the analysis of literature data and author's experimental studies, it was established that methods such as "everted intestine" and "Ussing chamber" are effective for studying the mechanisms of kinetics and absorption of drugs in the small and large intestines. The "InTESTine™" system is a tool for assessing intestinal permeability and predicting intestinal absorption of food and drugs. Systems such as "intestinal organoids", "enteroid systems" and "gut-on-a-chip" used to study dynamic processes, secretion, and absorption in the intestine have been analysed. It has been shown that transformed and cultured "epithelial cell lines" (Caco-2, HT-29 and T84) have certain disadvantages in use and cancerous origin of cells. An "epithelial cell suspension" was investigated, which is a simple and promising *ex vivo* system for studying the direct and immediate effects of chemicals on the intestinal epithelium. The practical value of the work lies in comparing experimental methods for studying the intestine, which can contribute to the effectiveness of preclinical research, the improvement of medical care diagnostics, and the development of personalised medicine

Keywords: intestine; *in vitro* model; *ex vivo* mode; enterocytes; intestinal epithelial cell suspension

INTRODUCTION

Research into experimental systems for studying gastrointestinal (GI) functions is an important area in modern physiology, gastroenterology and biomedicine. Improvements in methods allow for a deeper understanding of the mechanisms of digestion, nutrient absorption, microbiota regulation, and the pathogenesis of various gastrointestinal diseases (in particular, inflammatory bowel disease, irritable bowel syndrome and cancer). One of the main reasons for the relevance of this research is the high prevalence of gastrointestinal diseases. Using experimental methods (*in vitro*, *in vivo*, *ex vivo* and *in silico*), researchers model pathological conditions, test drugs, and analyse the impact of various factors on intestinal function. Thus, the development and improvement of experimental methods for studying intestinal functions remains a relevant task that contributes to the progress of both fundamental science and clinical practice.

Models for studying the human and animal digestive systems have undergone significant improvements with the aim of enhancing their predictive ability regarding the bioavailability of various compounds, studying absorption mechanisms, and investigating the immune response of the intestine. In their work, R. Moerkens *et al*. [1] developed a "small intestine-on-a-chip" model, which is characterised by its ability to self-organise into a villous structure and includes mesenchymal and nerve cells. This platform provided apical/basolateral access and demonstrated mature barrier and metabolic gene programmes. This approach promotes the development of personalised *in vitro* modelling of intestinal physiology and drug response.

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In a study by S.M. Parigi *et al.* [2], spatial transcriptomics was developed for the entire mouse colon. They revealed previously underappreciated molecular regionalisation and identified spatially organised healing programmes (including p53-associated zones), providing a rich reference atlas for intestinal regeneration studies. Comparing colon tissues at rest and after mucosal healing (in treated mice), spatial transcriptional processes of tissue repair, immune cell activation/recruitment, pro-regenerative pathways, and tissue remodelling were identified.

In a Ukrainian study by S. Soloviov *et al.* [3], the authors created an *in vitro* model to assess how metabolites derived from *Lactobacillus* modulate the course of enterovirus infection in intestinal cells. This practical experimental approach made it possible to analyse the interactions between the host, the microbe and the virus, which is important for studying the barrier function of the intestine. In a research study conducted by M.N. Zhayvoronok & V.M. Zalesky [4], *ex vivo* samples of human intestinal tissue were used to investigate fibrogenesis in inflammatory bowel disease. The authors demonstrated that the accuracy of the obtained results strongly depended on the quality of resected specimens, highlighting the potential of such models for translational research. In a paper by E.M. Holloway *et al.* [5], a vascular component was formed in intestinal organoids, including endothelial cells. This significantly increased their physiological plausibility and potential for modelling tissue invasion or inflammation. In the work of J. Kassis & Z. Wan [6] presented the integration of organoids and microfluidic intestine-on-a-chip platforms for testing drug permeability – in particular, a Caco-2 chip directly connected to mass spectrometry, which allows for high-throughput parallel testing of drug efficacy. M.R. Kuhn *et al.* [7] used hydrogels with a specific natural protein composition (collagen, fibrin, hyaluron) that proved suitable for culturing human intestinal organoids. These matrices promoted long-term growth, were capable of differentiation, and maintained cellular heterogeneity.

Generally, the studies presented demonstrated a wide range of approaches, from microfluidic platforms to organoid models with vascular and immune components. At the same time, a number of problems remained: lack of standardisation of methods, limited reproducibility of experiments, and the complexity of modelling the full interaction between the microbiota, immune and nervous systems. Therefore, the aim of this work was to describe, analyse and systematise current experimental models used to study the functions of the small and large intestines.

To achieve these goals, a detailed literature review was conducted using the PubMed, Google Scholar and Scopus databases only sources published within the last six years were considered. Initially, about 120 sources (2019-2025) were selected, of which 45 articles were retained for detailed consideration after critical analysis. The selection criteria included the originality of the data, the availability of clinical or experimental results, and the novelty and relevance of the research area. Experimental publications describing methods for studying the gastrointestinal tract, namely the processes of absorption, transport and absorption of nutrients and drugs, as well as review articles, were analysed. A systematic search strategy was applied using relevant keywords and search terms. The information was systematised by model type (*ex vivo*, *in vivo*, *in vitro*, etc.), which made it possible to identify the main trends and determine the existing gaps in methods for studying intestinal functions.

TYPES OF EXPERIMENTAL APPROACHES TO INTESTINAL RESEARCH

There are many models of the GIT that allow the study of transport mechanisms and predict the pharmacokinetics of drugs. Establishing the *in vitro* – *in vivo* relationship is extremely important for patient-centered drug development from a product quality perspective. This review summarised and structured information about modern experimental systems for studying various intestinal functions, focusing on their application, advantages and limitations (Table 1).

Table 1. Modern experimental models for intestinal research

Model	Description	Examples of use
In vivo	Animal systems preserving systemic circulation and neural pathways.	Mice, rats, pigs, dogs, monkeys.
Ex vivo	Intestinal tissue sections isolated from animals, incubated under controlled conditions.	Everted gut sac, Ussing chamber, InTESTine™, intestinal suspension.
In vitro	Intestinal epithelial cell lines cultivated on flat surfaces.	Caco-2, HT-29, T84.
3D cell culture	Scaffold-based cultures mimicking the intestinal microenvironment and cell architecture.	Hydrogel-based systems, ECM scaffolds.
Organoids	Mini-gut structures derived from stem cells.	Human intestinal organoids, enteroids.
Microfluidic devices	«Gut-on-chip» platforms simulating intestinal mechanics, flow, and epithelial interactions.	Intestine-on-a-chip, enteroid chip.
In silico	Computational simulations of absorption, metabolism, and barrier function based on biological data.	Physiologically based pharmacokinetic modelling, AI-driven GI-simulations.

Source: compiled by the author based on A.P. Lytvynenko [8] and F. Abbas *et al.* [9]

Modern 3D bioprinting models of the intestine allow the creation of complex three-dimensional structures with crypt villi and laminated layers of epithelium and stroma. In 2024, a tubular model of the intestine with bioinclusions has been developed that independently generates epithelial micromorphogenesis, which allows for *in virto* modelling of drug cytotoxicity [10]. Bioprinting technology made it possible to recreate a model of the human small intestine. The process involved forming tubular structures consisting of inner and outer layers formed by epithelial cells. This ensured the integration of vascular endothelial cells and smooth muscle cells necessary to reproduce the

characteristic structure of the intestinal wall. The proposed model is significantly closer to the natural architecture of the small intestine and exceeds the level of structural similarity achieved in previous *in vitro* systems. Thus, this method of coaxial bioprinting to construct 3D tubular structures somewhat overcomes the limitations associated with structural and functional similarity to native tissue. *In silico* models provide mechanistic simulation of drug absorption, effectively predicting pharmacokinetic parameters within 2-fold accuracy, which facilitates early bioavailability assessment [11]. Nonclinical models of absorption to study transport mechanisms, determine intestinal permeability, and predict plasma pharmacokinetic profiles are extremely important.

Thus, modern microfluidic systems facilitate drug testing by simulating intestinal absorption and barrier function, providing greater relevance for pharmacokinetic and toxicological studies. The development of new platforms allows for the co-cultivation of intestinal epithelium with immune cells, vascular endothelium, and microbial communities to model host-microbiome interactions and immunological interactions *in vitro*. The prospect of developing microfluidic devices requires their verification, testing, and comparison with living native systems.

MODERN *EX VIVO* INTESTINAL EXAMINATION METHODS

Ex vivo methods involve the use of functional tissues extracted from organisms and maintained under controlled laboratory conditions; however, the absence of blood circulation and neural regulation in such setups may influence the reliability of certain results. One of the earliest and still widely used *ex vivo* models is the everted intestinal sac, introduced by Wilson and Wiseman in the 1950s to investigate the kinetics and mechanisms of drug absorption across various segments of the gastrointestinal tract. This model remains relevant, especially for evaluating the absorption rates of bioactive compounds in experimental studies on rats [12]. The intestinal sac evisceration method involves isolating a specific segment of the intestine (duodenum, ileum, or colon) and then thoroughly washing it with saline solution. The intestine is then everted onto a glass plate and filled with Krebs buffer solution in an oxygenated incubation system at 37°C. The result depends on age, sex, species, segment origin, and experimental factors (pH and temperature). Data obtained by K. Miyazaki *et al*. [13] indicated that modifications such as removal of the serous and muscular layers can improve permeability assessment but lead to inaccuracy and biological variability.

Using an inverted intestinal sac model, it was found that lanthanum interacts with iron during absorption. This indicated pharmacokinetic barriers to the simultaneous administration of these elements [14]. *Ex vivo* methods, including isolated intestinal segments and tissue explants, allow for accurate assessment of the effect of nanoparticles on intestinal physiology without systemic *in vivo* exposure. For example, using *ex vivo* perfused rat intestinal explants, M. Qi *et al.* [15] assessed the effect of Ag nanoparticles on neurotransmitter release (NO, serotonin) and local motility in real time. It was noted that even short-term exposure to metal nanoparticles can alter intestinal neuroregulation, which is critical for understanding potential toxicological risks. Based on this, S. Guo *et al.* [16] conducted a detailed analysis of the absorption stages of model nanoparticles of different sizes, surface charges, and levels of hydrophobicity or hydrophilicity. It has been proven that chemical composition and physicochemical parameters influence the interaction of nanoparticles with the intestinal barrier. Subsequently, research by Y. Zheng *et al.* [17] confirmed that particle size and surface modifications play a decisive role in trans-epithelial transport. The results obtained point to potential strategies for improving the oral administration of nanoparticle-based drugs by adapting the surface properties of the particles. Additional evidence was also obtained in studies using the inverted intestinal sac method [18], which demonstrated that surface characteristics may be a key factor determining absorption efficiency. These findings support the view that *ex vivo* intestinal models, despite their limitations, remain highly relevant for the mechanistic assessment of nanomaterial safety. Nevertheless, the model remains an accessible, reproducible, and effective method for studying the kinetics of transport and absorption of drugs, including nanoparticles, in both the small and large intestines *ex vivo* [19].

The Ussinger chamber was developed in the 1950s by Hans Ussinger to measure GI-permeability using paracellular flow and electrical measurements and has been adapted for both mouse and human tissues [20]. The chamber consists of two compartments separated by monolayers of tissue or cells, isolating the apical and basolateral sides of the epithelium. The Ussinger chamber is used to measure permeability and ion transport through epithelial tissues and cells. This led to the discovery of the Na+/K+ATPase pump. Thus, it has been established that a decrease in mucosal integrity is closely associated with the pathogenesis of ulcerative colitis, Crohn's disease, and colorectal cancer [21, 22]. These disorders are accompanied by impaired barrier function, which can subsequently cause chronic inflammation. In this context, S.C. Pearce *et al.* [23] emphasised the value of the Ussing chamber as a versatile experimental tool. Unlike many *in vitro* cell models, this system allows researchers to measure permeability, tissue integrity, and transport processes in intact intestinal samples under controlled conditions. However, *ex vivo* tissue explants used in Ussinger chambers are viable for five hours. Thus, the Ussinger chamber remains a valuable tool for measuring intestinal integrity (for measuring the permeability of *ex vivo* colon tissue samples from mice and humans). This method preserves tissue physiology, allowing for accurate assessment of absorption, barrier function, and transporter activity. Low throughput, laborious setup, and the need for fresh tissue remain drawbacks of this system.

The InTESTine™ platform was developed to study the passage and absorption of biological, nutritional, and pharmacological substances through the intestinal barrier. This system also uses different segments of the intestine, such as the duodenum, jejunum, ileum, and colon. The segments are arranged in such a way as to maintain distinct apical and basal-lateral divisions [23]. However, unlike a traditional chamber, the InTESTine™ system is compatible with standard 6- and 24-well plates, providing higher throughput and simple horizontal positioning [24]. Due to its practicality and convenience, this platform is well suited for *ex vivo* analysis of various gastrointestinal functions.

Thus, viable intestinal tissue mounted in the InTESTine™ system can be used as a reliable tool for evaluating intestinal permeability. This is suitable for predicting intestinal absorption in humans of substances such as digested foods and drugs and for studying complex intestinal processes due to the presence of many cell types in the tissue *ex vivo*.

In study A. Fedi *et al*. [25] a suspension of small intestinal epithelial cells was obtained from the upper parts of villi. It has been shown that such enterocytes can be used as an effective alternative for comparative analysis of the characteristics of nutrient transport in the epithelium. Light and immunofluorescence microscopy confirmed that the isolated cells retain a differentiated phenotype, form intercellular contacts and restore polarity, with a clear division into apical and basolateral domains. All experiments were performed after 7-9 hours of incubation, when the cells remained viable. Y. Kimura *et al*. [26] and A.P. Lytvynenko *et al*. [27] described a method for obtaining functional epithelium using differentiated enterocytes collected from the upper part of the villi of the small intestine of adult mice. Three main types of differentiated intestinal epithelial cells can be identified in the area of differentiated villi: enterocytes, goblet cells and enteroendocrine cells. The method allows the isolation of morphologically identifiable intestinal epithelial cells: elongated columnar and spherical cells. Columnar cells usually originate from the tip of the villi, and spherical cells from the middle and lower parts of the villi. Thus, the effect of dextran-polyacrylamide polymers, charged and uncharged, as well as their carriers of gold and silver nanoparticles, on the regulated cell death of ileal enterocytes in mice was evaluated. Preparation of epithelial monolayers from freshly isolated mouse enterocytes allowed quantitative assessment of fluid movement in the apical part of villi and paracellular filtration. These processes depend on gap junctions and their integrity. Thus, there is a direct mechanical link between intestinal motility and intestinal fluid regulation. The isolation of enterocyte suspension is a simple method, although it has certain limitations, such as possible mechanical damage to the cells. Therefore, to avoid significant deviations, the enterocytes are stained with trypan blue and morphological assessment of cell viability is performed. This method is authorised for use in experimental research, testing and improvement.

2D and 3D cultures of GI-organoids are actively developing, so immunohistochemistry on *ex vivo* tissues is a relevant area for improving these systems. C.M. Wang *et al*. [28] have attempted to recreate the tissue environment, matrix, and receptors that influence cell signalling, changing basic cell phenotypes. S. Deyaert *et al*. [29] developed a dynamic, long-term model of the ileal microbiota using SHIME® technology. This system mimics a synthetic bacterial consortium of 12 species. This provides representative modelling of the ileal bacterial community, facilitating the study of microbiota dynamics and activity. This approach allows the interaction between the food matrix, the microbiome and the intestine to be modelled. However, the role of this specific microbial community in influencing the absorption of nutrients and drugs remains unclear. Table 2 summarised the described methods for *ex vivo* intestinal research.

Table 2. *Ex vivo* intestinal examination methods

Method	Advantages	Disadvantages
Ex vivo models using resected tissues	high biological relevance; preserved tissue architecture.	limited tissue viability; donor variability.
Ussing chambers	classic method for epithelial ion transport; barrier function study.	technically demanding; requires fresh samples.
Intestinal epithelial cell suspension	simple method isolating enterocytes.	some mechanical damage to the cells.
Immunohistochemistry on *ex vivo* tissues	precise localisation of proteins and cytokines; preserved microenvironment.	static snapshot, lacks dynamic context.

Source: compiled by the author

Thus, *ex vivo* systems remain effective for studying the mechanisms of kinetics and absorption of drugs in the small and large intestines. *Ex vivo* models provide high biological relevance, but their use is complicated by the short viability of tissues and the variability of donor material. Ussing chambers remain the gold standard for analysing barrier function and ion transport, but require high technical skills and access to fresh samples. The use of enterocyte suspensions is a simple approach, but is accompanied by mechanical damage to the cells. Immunohistochemistry on *ex vivo* samples allows for clear determination of the localisation of proteins and cytokines, although its static nature limits the ability to study dynamic processes.

EPITHELIAL CELL LINES: CACO-2, HT-29 AND T84

Immortalised cell lines derived from the human intestine, such as Caco-2, HT-29 and T84, remain indispensable in permeability and transport studies. Caco-2, first described in 1977, consistently differentiated into polarised enterocyte-like monolayers containing microvilli and tight junctions. It continues to serve as the gold standard for assessing intestinal drug absorption, despite limitations such as overly tight barrier, low hydrophilic permeability, limited metabolism [30]. N. Panse & P.M. Gerk [31] have improved its physiological characteristics by co-culturing with mucin-secreting cells (HT29-MTX). Thus, although Caco-2 retains its status as the “gold standard”, its limitations point to the need for combined systems. Table 3 provided specific examples of co-cultivation. Combination with other cell lines gradually improves the system, reduces the artificiality of the model, and brings it closer to *in vivo* conditions. T84, obtained from human colorectal carcinoma in the 1980s, is characterised by the formation of dense cell layers with a large number of intercellular contacts. This cell line is actively used to study electrolyte transport and barrier properties of the epithelium [32]. T84 does not reproduce the biochemical phenotype of mature small intestine

enterocytes characteristic of Caco-2. Similar to Caco-2, T84 cells can form absorptive monolayers of epithelial cells and are capable of differentiating into crypt-like structures [33]. The T84 line is less suitable for modelling nutrient absorption, but it is valuable for studying electrolyte transport and the functioning of tight junctions. At the same time, its ability to form crypt-like structures opens up prospects for researching regenerative processes in the intestine.

Table 3. Co-culture of Caco-2 epithelial cells

	Caco-2	Caco-2 + HT29-MTX	Caco-2 + HT29 + T84
Application	Drug transport model.	Mucus barrier model for nutrient uptake and bacterial adhesion.	Complex intestinal physiology (barrier + mucus + Cl^- secretion).
Advantages	High reproducibility; widely accepted for permeability screening.	Closer to *in vivo* mucus interaction; modulates permeability via 90:10 cell ratio.	Simulates inflammation, leaky gut; includes secretory and immune-like activity.
Disadvantages	No mucus layer; limited immune function modelling.	Incomplete MUC2 expression; variable transporter levels.	Complex culture setup; low throughput; interpretation requires caution.

Source: compiled by the author based on M. Anjum *et al.* [34], S. Peddibhotla *et al.* [35], M. Belaid *et al.* [36], D. Marescotti *et al.* [37]

HT-29*,* a colorectal adenocarcinoma cell line established in 1964, can differentiate into heterogeneous epithelial populations, including goblet cells that secrete mucus. M.J. Haddad *et al.* [30] have focused on the effects of specific stimuli, such as methotrexate or sodium butyrate. Co-cultures HT-29 with Caco-2 contribute to the creation of more physiologically relevant epithelial models by combining the barrier properties of mucus with absorptive functions. Caco-2 and HT-29 cell lines have similar properties of enterocytes during differentiation, but they also have some differences. While Caco-2 cells differentiate to a monolayer of absorptive phenotype without mucus granules, HT-29 cells show high heterogeneity in the formation of absorptive and goblet cells and, therefore, are capable of producing mucus [31]. The unique ability of HT-29 cells to form goblet-like cells makes them an important tool for modelling the intestinal mucosa. Their use in co-cultures with Caco-2 cells allows for the reproduction of more complex barrier and secretory properties of the epithelium.

Transformed and cultured "epithelial cell lines" (Caco-2, HT-29 and T84) have certain disadvantages: cancer origin, lack of specific epithelial function, different expression, in particular, of glucose transporter protein, the absence of a mucosal layer, etc. Whereas, the 'epithelial cell suspension' of enterocytes collected from the upper part of the small intestinal villi provides an alternative approach for comparative studies and responses to various conditions and stimuli in the GIT. However, these cell systems continue to evolve through new approaches of advanced engineering and co-culture strategies. These systems are very valuable in predicting drug permeability, mimicking epithelial function and modelling intestinal barrier physiology.

3D CELL CULTURE, INTESTINAL ORGANOID AND MICROFLUIDIC DEVICES

In 2023, microfluidic technologies have been intensively developed to create organ-on-a-chip devices designed to reproduce the three-dimensional topography of organs. A new coaxial bioprinting of small intestine technique has enabled the fabrication of multicellular 3D constructs that show improved structure and gene expression compared to traditional 2D monolayers [38]. New models with an open apical surface of organoids facilitate the study of pathogen invasion and nutrient uptake. Advances in the field of scaffolds for biomaterials have enabled the transition from Matrigel to synthetic hydrogels based on polyethylene glycol and collagen. This improves reproducibility and allows for the influence on the morphology and differentiation of organoids [39, 40]. Such developments highlighted the rapid advancement of 3D intestinal culture technologies. These models combine microengineering, advanced biomaterials and automation to more accurately mimic the *in vivo* gut environment. In 2021, the development of three-dimensional culture systems known as organoids has enabled the cultivation of cryptic villi that mimic many aspects of intestinal physiology. Organoids are cellular aggregates cultured in three dimensions and have the same characteristics as the tissue of origin. Due to their ability to self-renew and proliferate, organoids can be maintained in culture for a long time. Organoids have become a key platform for studying the intestinal barrier, tight junction regulation, and epithelial renewal *in virto*. These systems are suitable for studying permeability and inflammation because they respond to external stimuli such as cytokines, bacterial components, etc. [21, 41].

Organoid culturing has also been used I.A. Parente *et al.* [42] to study GI-diseases, gut-microbe interactions, and colorectal cancer. Organoids have proven to be valuable in the reproduction of inflammatory and neoplastic intestinal disorders. It has been shown that the destruction of tight junction proteins, such as claudin-2, claudin-7 and claudin-15, in mouse organoids allows us to study the mechanisms and causes of barrier disruption associated with chronic intestinal inflammation. Organoids provide a platform for researching infectious diseases, hereditary pathologies and cancer using genetic engineering and stem cell techniques. Moreover, transplantation of organoids into animal models allows tissues to be reconstituted and their reactions to be monitored *in vivo* [43]. These living biobanks offer insight into inter-patient variability and facilitate the identification of personalised therapeutic options. Thus, significant progress has been made in the development of intestinal epithelial cell cultivation technologies. The intestinal organoid is a widely used model system for studying the dynamic processes occurring at

the host-microbe interface and the mutual interactions between intestinal epithelial cells and cells of local immune homeostasis. Despite the obvious advantages of using multicellular "intestinal organoids" compared to single-cell lines, organoids still lack certain parts of intestinal physiology, such as the stroma, vasculature, immune system and microbiome, which limits the representation of the *in vivo* situation.

Intestine-on-a-chip allows for dynamic analysis of epithelial barrier function, providing simultaneous access to the apical and basolateral sides. This platform maintains continuous fluid perfusion, which promotes the development of a mucus layer and the creation of an oxygen gradient, allowing the cultivation of intestinal cells simultaneously with commensal or pathogenic microorganisms [43]. Compared to 3D organoid cultures, the gut-on-a-chip model more accurately reflects the duodenal transcriptome *in vivo*. In addition, this system allows for real-time sampling of light flux, which allows for monitoring mucus production, nutrient absorption, and barrier integrity. Intestine-on-a-chip allows for the formation of a protective mucus layer and an oxygen gradient, which supports the co-cultivation of cells and microbes for a longer time [44]. This model is technically complex and expensive, but it allows for the integration of different cell types (epithelial, stromal and microbial components), making it ideal for multivariate studies of intestinal function and disease mechanisms. Enteroid or mini-gut is derived from $LGR5^+$ intestinal stem cells isolated from the small or large intestine. It is a physiological model for the study of intestinal transport and host-pathogen interactions, for the study of viral, bacterial and protozoan parasitic infections [45]. This model allows the study of secretion and absorption processes in specific areas of the intestine under the influence of various stimuli.

Organoids derived from the intestine serve as valuable systems for studying dynamic interactions, including host-microbe relationships and communication between epithelial and immune cells. However, these systems do not fully reproduce the physiology of the intestine, as they lack components such as stromal tissue, blood vessels, immune cells, and resident microbiota, which limits their ability to completely mimic *in vivo* conditions. The enteroid model of the intestine, in turn, allows the study of segmental secretory and absorptive functions. Whereas the "gut chip" system is a new approach to studying gastrointestinal functions by incorporating several cell types and/or the intestinal microbiome into the system. Table 4 summarised the advantages and disadvantages of the systems described.

Table 4. Advantages and disadvantages of 3D organoids, Enteroid, Intestine-on-a-chip

Model	3D Organoids	Enteroid	Intestine-on-a-chip
Key features	Three-dimensional cellular aggregates capable of self-renewal and proliferation; cultured from LGR5+ stem cells.	Models derived from small or large intestine segments; LGR5+ cells.	Microfluidic systems with continuous fluid flow, mucus layer formation, and oxygen gradient.
Advantages	Closely resemble the original tissue, long-term culture possible, suitable for genetic studies.	Segment-specific functional studies, early molecular events can be observed.	Dynamic modeling of intestinal physiology, integration of multiple cell types, realistic transcriptome.
Limitations	Lack stroma, vasculature, immune system, and microbiome.	Limited modelling of multicellular interactions.	High technical complexity and cost.

Source: compiled by the author

Thus, modern technologies allow reproducing three-dimensional intestinal architecture with varying levels of complexity. 3D organoids are a powerful tool for studying cellular processes, barrier function, and pathogenic influences, while enteroids provide segment-specific functional research. Innovative microfluidic platforms such as intestine-on-a-chip allow the integration of multiple cell types and microbiota, providing a more accurate modelling of intestinal physiology *in vitro*. At the same time, none of the models fully reproduces all aspects of *in vivo*, so their selection depends on the specific research goal, and the results require accurate interpretation and comparison with living systems.

CONCLUSIONS

It has been found that modern models of intestinal research allow for detailed study of intestinal physiology, substance transport, and its barrier function. The analysis showed that *ex vivo* tissues allow for accurate assessment of absorption kinetics and ion transport. Suspensions of enterocytes collected from the villi tip are a simple and effective tool for studying the absorption of nutrients, chemical reagents, and nanoparticles. Co-culture of epithelial cells, such as Caco-2 with HT29-MTX or T84, has been shown to improve physiological fidelity by combining absorption, secretory, and barrier functions. Studies have shown that 3D organoids and enteroids reproduce the architecture of the intestine and segment-specific functionality, allowing the study of interactions with the microbiota and early molecular events during infections. Microfluidic intestine-on-a-chip platforms further enhance modelling accuracy by integrating multiple cell types, forming oxygen gradients, and co-culturing with microbes over extended periods. It has been proven that no single model can fully reproduce the physiology of the intestine *in vivo*, which emphasises the need for combined approaches. In addition, the problem of standardising methods and reproducibility of results between laboratories remains relevant. Promising areas for improving gastrointestinal research methods include the use of enterocyte suspensions in preclinical studies of intestinal effects, personalised organoid models, and the use of multi-omics approaches. This will deepen

fundamental knowledge and expand the clinical application of experimental systems in the diagnosis and treatment of gastrointestinal diseases.

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Сучасні експериментальні системи для вивчення різних функцій шлунково-кишкового тракту

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Анотація. Цілісність та функціонування кишечника мають вирішальне значення для перетравлення та поглинання поживних речовин, імунологічного гомеостазу та запобіганню потрапляння шкідливих антигенів. Вдосконалення та розробка нових експериментальних систем для вивчення різноманітних функцій кишечника є актуальним завданням біології та медицини. Метою даної статті був опис сучасних експериментальних систем для вивчення різних функцій кишечника, зосереджуючись на їх застосуванні, перевагах та обмеженнях. Для досягнення мети проведено аналіз та узагальненнння публікацій 2019-2025 рр., відібраних у PubMed та Google Scholar. На основі аналізу даних літератури та авторських експериментальних досліджень встановлено, що такі методи як «вивернута кишка» та «камера Усенга» є ефективними для вивчення, механізмів кінетики та всмоктування ліків у тонкій і в товстій кишці. Системи «InTESTine™» є інструментом для оцінки кишкової проникності й для прогнозування кишкового всмоктування їжі та ліків. Проаналізовано такі системи як «кишкові органоїди», «система ентероїду» та «кишечник на чіпі», що використовуються для дослідження динамічних процесів, секреції та всмоктування в кишечнику. Показано, що трансформовані та культивовані «лінії епітеліальних клітин» (Caco-2, HT-29 і T84) мають певні недоліки у використанні та ракове походження клітин. Було досліджено «суспензію епітеліальних клітин», яка є простою перспективною системою *ex vivo* для вивчення прямого та негайного впливу хімічних речовин на кишковий епітелій. Практична цінність роботи полягає в порівнянні експериментальних методів вивчення кишечника, що може спияти ефективності доклінічних досліджень, удосконалення діагностики медичної допомоги та розвитку персоналізованої медицини

Ключові слова: кишечник; моделі *in virto*; моделі *ex vivo*; ентероцити; суспензія епітеліальних клітин кишечника