ISSN 0003-6838, Applied Biochemistry and Microbiology, 2024, Vol. 60, No. 9, pp. 1694–1707. © Pleiades Publishing, Inc., 2024.
Russian Text © The Author(s), 2023, published in Biotekhnologiya, 2023, Vol. 39, No. 5, pp. 82–96.

MEDICAL BIOTECHNOLOGY

Microbiome and In Vitro Gut Models

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Received September 28, 2023; revised October 16, 2023; accepted November 5, 2023

Abstract—The role of the microbiota in maintaining human health and the current state of research on the human microbiome, with a focus on the development and prospects for the use of modern methodological approaches, such as the "gut-on-a-chip" technology, are described in this review. The relationship between the microbiome and metabolomics is discussed, as are the main results of international projects on the human microbiome. The limitations of both cultural and purely genetic (metagenomics) approaches for studying the microbiota bank are highlighted. The central focus is on comparison of different gut models. Arguments in favor of a microfluidic biochip approach are presented. Finally, the regulatory aspects of the practical implementation of microarrays in drug development and personalized medicine are discussed briefly.

Keywords: microbiome, cocultivation, in vitro gut models, microfluidics

DOI: 10.1134/S0003683824700261

INTRODUCTION

The intestinal microflora, or microbiota, plays a fundamental role in human health, regulating important functions such as protection against intestinal cell epithelial injury, fat deposition, and stimulation of intestinal angiogenesis. About 10^{14} (more than 1000 species) of bacteria are present in the gut, as are fungi, viruses, and protozoa. Only one-third of these microorganisms has been studied to date [1–4].

The specificity of symbiosis of microorganisms in the human gut reflects the continuous influence of ambient factors on the host organism.

In adults, the predominant types of bacteria are *Firmicutes, Bacteroidetes, Proteobacteria*, and *Actinobacteria* [5]. The microbiota is rather stable and, as has been shown in clinical studies, is able to restore its initial state after antibiotic therapy [6]. This is due to the fact that intestinal columnar cells (Goblet cells) secrete high molecular weight glycoproteins known as mucins (from the Latin *mucus*), which form a kind of physical barrier protecting intestinal cells, as well as by the presence to the so-called crypts, including the appendix, in the gut (Fig. 1). In the depths of a crypt there are only bacteria that can pass through the mucus and form stable colonies there directly interacting with epithelial cells, without being killed by an immune system attack (intestinal cells produce about 3–6 g of IgA daily) [7]. Thus, such "offshoots" are a depot of commensal bacteria in case of antibiotic therapy or other chemical impacts.

The small intestine also has special cells that secrete cationic peptides: alpha-defensins that can form pores in the membranes of attacked cells. Due to the prevalence of negative ions on bacterial membranes as opposed to more positively charged human cells, defensins perform their function without damaging host organs. The oxygen gradient in the gut lumen and near gut walls affects the distribution of anaerobic and aerobic bacteria [8, 9].

It has been shown experimentally that injury to and inflammation of the mucosal barrier are related to the high concentrations of flagellin, the major protein of bacterial flagella. Flagellin is a ligand of Toll-like receptor-5 (TLR5). Gut bacterial motility is generally limited by flagellin immunogenicity and mucus viscosity, which restricts bacterial movement [8]. Nevertheless, the chances of reaching host tissues for microorganisms such as *Salmonella* depend on flagella and chemotaxis. With a different approach, *Escherichia coli* cells have developed an alternative strategy: they secrete a serine protease that rapidly degrades mucus. Proteobacteria have an M60 peptidase for this purpose [9].

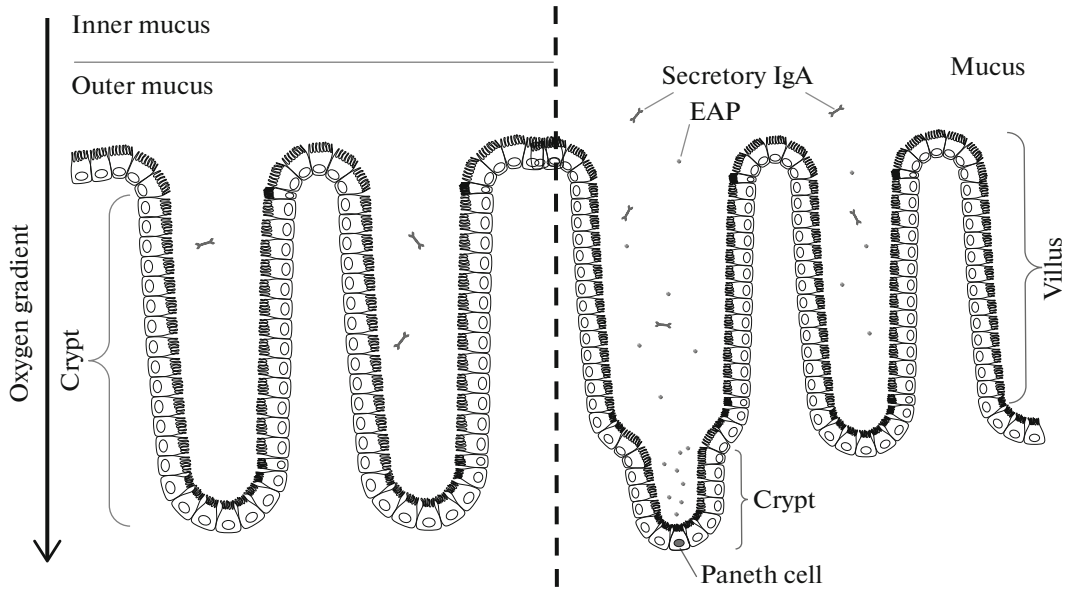

Fig. 1. Mucus layers in the large (left) and small (right) intestine. EAPs are endogenous antimicrobial peptides.

BACTERIAL ADHESION

Adhesive properties are typical both of representatives of normal microflora and pathogenic microorganisms. Due to adhesion, the resident microflora prevents colonization of the gut by pathogenic microorganisms. For the latter, adhesion is the first step on the way to formation of resistant biofilms, which makes the microbes combined in these biofilms resistant to the immune system and antibiotics. Thus, the molecular mechanisms of adhesion are versatile for both commensal and pathogenic species. As a rule, this is a selective ligand–receptor interaction between specific extramembrane proteins, capsules, lectins, adhesins, and fimbriae (pili) and the intestinal proteins and lipids.

For example, the bacterium *Helicobacter* inhabiting the stomach and the small intestine interacts with epithelial surface glycans [10, 11]. Examples of various binding mechanisms include the pathogen *Vibriocholera* (strain O395), which forms a toxin-regulated cell layer attached to the small intestinal epithelium [12]. In addition, the cholera vibrios of strains O1 and O139 are attached to mucus through an extramembrane protein that binds N-acetyl-*D*-glucosamine [13].

E. coli cells have a wide variety of lectins binding both to mucus and glycoproteins, as well as extracellular components of epithelial cells [14]. Recently, the segmented filamentous bacterium (SFB) *Candidatus savagella* has been cultured on the intestinal epithelial cell layer in vitro, although the mechanism of its attachment to the epithelium has not yet been elucidated [15].

The cells of the Gram-positive bacterium *Listeria* express a surface protein, the so-called internalin A, which first binds to epithelial cadherin and then interacts with actin, thereby inducing phagocytosis [16].

Commensal bacteria are similarly attached to mucus and the epithelium, occupying pathogen binding sites and thereby blocking their attachment. Thus, D. Savage [17] and M. Morotomi et al. [18] have shown that *Lactobacillus* bacteria forming a cell layer bound to the epithelium prevent the attachment of yeasts and staphylococci to the epithelium. F. Turroni et al. [19] have described similar mechanisms for the bacterium *Bifidobacterium bifidum*, which uses pili to bind to proteins of the extracellular matrix of the epithelium.

IMMUNOMODULATORY PROPERTIES OF MICROBIOTA

The bacteria bound to the intestinal mucosa remain there only if they do not induce an immune response from the host. The mucus contains a lot of secretory immunoglobulin A (sIgA), which is a dimer and binds to components foreign for the host, including bacteria. SigA is resistant to the proteolytic enzymes and, in contrast to IgG, does not accompany inflammation but, on the contrary, binds antigens on the surface of the mucosa and inhibits their penetration, preventing further inflammation. The binding of sIgA to commensal cells leads to the formation of a biofilm that serves as a barrier to pathogens. Thus, sIgA uses this mechanism to regulate homeostasis between the host and the microbiota, as well as between the host and the potential pathogens on the surface of mucous membranes.

An alternative view is that the immune system is not programmed explicitly to recognize symbionts;

more likely, some species have merely adapted not to induce a strong immune response. Some bacteria such as *Bacteroides fragilis* have "learned" how to send and use feedback signals from the immune system of the host [20]. A component of their capsule, polysaccharide A, sends a signal to the antigen-presenting cells by a "mediator" and stimulates the expression of interleukin-10 by T cells. The emergence of anti-inflammatory cells produced in response to this signaling allows *Bacteroides fragilis* to pass more easily through the mucous layer.

Similarly, SFBs stimulate the production of T helper cells [21]. As has been shown recently, SFBs are able to "train" the immune system: to induce a strong immune response due to intensive stimulation of T and B cell maturation. The colonies of these bacteria promote the maturation of lymphoid tissue in the intestinal mucosa and activate the mediators of innate immunity in the gut. In addition, colonization by these bacteria has an adjuvant effect on the systemic immune response [22].

It should be noted that it is not only the commensal components of the gut microbiota that perform the function of a barrier to pathogens, but opportunistic bacteria (e.g., *E. coli*, etc.) are also an integral part of healthy microflora, which also performs a stimulating function in addition to the barrier one.

THE MICROBIOME AND HUMAN DISEASES

The distribution of microbes in the gut contributes to the development and stability of the microbial community, because spatially separated niches increase the diversification of species. Partially, the microbiota can develop in the direction from proximal to distal parts, as it corresponds to the direction of feces flow. This flow, together with external factors (infection, chemicals, etc.), can physically eliminate all components of the microbiome and, consequently, cause serious disorders in the body. Apparently, in order to prevent such development, evolution resulted in the appearance of protected reservoirs, namely, crypts and the appendix, which incidentally had been considered previously as an atavism. D. Gevers et al. [23] and F. Rowan et al. [24] provide direct and indirect evidence of the positive effect of isolated reservoirs on the microbiota. In addition, it is known that the state of the microbiota influences the course of inflammatory bowel diseases, liver cirrhosis [23–25], and the development of irritable bowel syndrome (IBS) [26]. According to the results of meta-analysis of 13 clinical studies on the gut microflora, X. Zhuang et al. [27] arrived at the conclusion that patients with IBS have significantly different numbers of *Lactobacillus, Bifidobacterium*, and *Faecalibacterium prausnitzii* compared to healthy subjects. In addition, J. Beatty et al. [28] revealed the negative effect of infection by *Giardia duodenalis*: in addition to the previously known activity provoking diarrhea, giardia cause long-term dysbiosis followed by the development of IBS.

In addition to probiotics and drugs that are commonly used to normalize the intestinal microflora, there are other approaches that have not yet been widely used. For example, the possibility of transplantation of the microbiota from healthy donors to patients with impaired microflora is being studied. A number of clinical studies have shown the positive effect of transplanted microbiota on patients with IBS or with *Clostridium difficile* infection in more than 50% of cases described [29–31]. To date, there are three ways to transplant the samples from healthy donors: (i) by colonoscopy; (ii) through a nasal tube reaching the duodenum; and (iii) orally in an enteric capsule.

The clinical data obtained over the past decade confirm the crucial role of impaired gut microbiota in the course and development of many diseases [32]. Interestingly, there is a direct relationship between impaired microbiome and the risk of various neurodegenerative diseases, including Alzheimer's disease [33].

METHODOLOGY

Sample Collection and Extraction

Currently, the microbiota is studied by metagenomic analysis [3]. The latter requires a set of proven reproducible techniques to ensure the accuracy and unambiguous interpretation of data. Thus, proper DNA extraction from fecal samples or intestinal cells is the key stage in sample preparation for the analysis of the gut microflora. A number of studies have shown the effects of multiple factors on DNA extraction and subsequent analysis. It has been established that the samples for fecal occult blood tests can be preserved well for up to four days without freezing: if freezing is necessary, smears are taken, and DNA is extracted from the latter with 70% ethanol [34–36].

Metagenomics

In addition to direct studies of bacterial samples, there are various techniques such as clone libraries, terminal restriction fragment length polymorphism (T-RFLP), quantitative PCR (qPCR), transcriptome analysis, and high throughput sequencing. Note that 16S rRNA amplicon pyrosequencing and Illumina sequencing are believed to be reliable methods for studying genomic diversity and differences in expression of the genes of microbial communities of the gut [3] and mouth cavity [37]. These two technological platforms not only accelerated the metatranscriptomic analysis of the microbiome but, together with the metagenomic database, opened up new possibilities for understanding the structural and functional dependencies in microbial communities. Moreover,

these methods do not require sample cultivation and thereby allow identification of "nonculturable" microorganisms (i.e., those for which cultivation conditions have not yet been developed) within a microbiome.

Culturomics

One of the major limitations to metagenomic studies is related to the sensitivity of the method, because modern techniques are unable to detect bacteria at concentrations below 10^5 cells/g of feces. Although metagenomics makes it possible to determine DNA sequences of many "nonculturable" bacteria, further studies require viable microorganisms in a pure culture. Culture isolates will not only extend the reference base of genomic data but also ensure identification of new genes and their functions, as well as molecular targets for the development of novel therapeutic agents. For example, J. Lagier et al. [38] used 212 various cultivation conditions and matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF-MS) for the analysis of the samples obtained and identified 340 bacterial species, five fungal species, and one giant virus in three fecal samples. These most important results were a platform for restoration of the methods for cultivation and development of taxonomy for the classification of new bacteria [39].

Metabolomics

The gut microbiota is responsible for metabolic functions such as biosynthesis of amino acids, short-chain fatty acids, essential vitamins (e.g., K and B12), bio-transformation and hydrolysis of bile acids, and fermentation of nondigestible polysaccharides [40]. Metabolomics involves the large-scale study of low-molecular-weight metabolic products of cells, tissues, biological fluids, and the organism as a whole at a particular point in time. This technology is efficient for "unraveling" complex interactions between the metabolites of gut microbiota, i.e., the metabolome. Comparative studies of the metabolome of gut microbiota in healthy individuals and patients with different diseases will allow identification of unique metabolites that can be used as diagnostic or prognostic biomarkers. The two main technologies used in metabolomics are mass spectrometry (MS) and nuclear magnetic resonance (NMR). These spectral methods are characterized by a wide range of compounds to be investigated, high sensitivity, and the possibility of quantitative analysis [41]. Using this approach, G. Le Gall et al. [42] demonstrated that the metabolomic profile of fecal extracts from patients with ulcerative colitis in the presence and absence of IBS was different from that of the samples from healthy donors. It was shown that the impaired microbial metabolism in IBS leads to elevated levels of hydrogen gas, as well as indole, phenols, and other compounds [43]. Volatile organic compounds (VOCs) are released by bacteria as metabolic side products that can be determined by solid-phase microextraction followed by the gas chromatography–mass spectrometry method (SPME-GC-MS). Specific microbial VOC profiles can be considered as potential biomarkers in the diagnosis of specific diseases [44]. The modern approaches used to study the human microbiome are summarized in Table 1.

"THE HUMAN MICROBIOME PROJECT" AND FIRST RESULTS

In view of the accumulated evidence on the important role of gut microbiota, funding of The Human Microbiome Project (https://commonfund.nih.gov/hmp) was started in the United States in the early 21st century. This project is an initiative of the US National Institutes of Health (NIH) aimed at better understanding the human microflora, its significance for health, and related problems. The first phase of the project launched in 2007 was focused on identification and characterization of human microflora. The second phase known as the Integrative Human Microbiome Project was started in 2014 with the purpose to develop a resource base in order to characterize the microbiome and elucidate the role of microbes in human health. In the period of 2007–2016, this program received $170 million in financing from the NIH Common Fund. The main results of these program studies have been described in a number of papers [14, 31, 45–58]. Obvious associations between the changes in the microbiome and psoriasis, arthritis, IBS, and other pathological conditions have been found. In addition to these studies, it is worth noting the emergence of new "daughter" projects: e.g., the Alzheimer Gut Microbiome project (https://alzheimergut.org/). Despite substantial investments in this project, there is still a long way to go before defining the "reference microbiome," and many of the questions posed have not yet been answered. The main result of this project is a huge database of metadata, which formed the basis for further study of relationships between the microbiome and various diseases.

IN VITRO COCULTURE MODELS

Comparative Analysis of Models

The in vitro coculture system of gut cells and microbes has many advantages for revealing the direct involvement of bacteria in the function of this organ, provided that the system is reliable and truly mimics the digestive ecosystem. The recently developed systems for the coculture of (anaerobic) intestinal bacteria and epithelial (aerobic) cells are briefly described below.

Table 1. Modern technologies used in the study of the human microbiome

Technology	Method	Application
Metagenomics	— Clone libraries — T-RFLP — qPCR — Transcriptome analysis — High-throughput sequencing — Metagenomic analysis	(1) Discovery of new, previously not cultured species (2) Study of the structure and function of microbial communities (3) Analysis of genomic diversity and differential expression of the genes of microbial communities (4) Determination of the role of microbes in disease development
Culturomics	Different cultivation conditions for biological samples and identification of bacteria in the latter with the involvement of modern spectral methods, including MALDI-TOF-MS	(1) Isolation of microorganisms from biological samples for subsequent analysis (2) Expansion of available reference genome databases (3) Detection of new genes/functions for potential applications in the development of novel drugs
Metabolomics	Mass spectrometry, NMR	(1) Identification of metabolic products within a certain time interval (2) Study of the mechanisms of interaction between metabolites and the gut microbiome (3) Identification of unique metabolic signature for diagnostic/prognostic applications

The Transwell® coculture models (Corning Inc., United States) are an example of systems used to study cell–cell interactions. Such coculture systems seem to be particularly useful for studying the interaction between bacteria, immune mucosal cells, and intestinal epithelial cells under static, usually aerobic, conditions [59–61].

The HMI™ module (LabMET, Belgium) [62] is a custom-made coculture system consisting of two compartments: a "lumen" compartment containing intestinal bacteria and a "host" containing facultative enterocytes (e.g., Caco-2 cells). The important difference from the Transwell® co-culture system described above is that there is a continuous flow of liquid through these two compartments and that the compartments are separated by a functional double layer (a semipermeable membrane and an artificially added mucus layer).

The third system, HoxBan [63], is aimed at mimicking the host–microorganism interactions taking place at the oxic–anoxic interface of the human gut. It is a system for the coculture of *Boxteria anaerobic* and Caco-2 or DLD-1 cells (CCL-221™) cells. In contrast to the Transwell® model and the HMI™ module, the HoxBan system does not require special (including custom-made) equipment.

The fourth, relatively recently described aerobic–anaerobic coculture system is the HuMiX modular microfluidic device [64]. This "sandwich" of modular elastomeric pads between two polycarbonate covers allows the coculture of Caco-2 and *Lactobacillus rhamnosus* GG cells.

The fifth system, which has become most popular for studying host–microorganism interactions, is the "gut-on-a-chip" system. In contrast to the previously described systems, this system has not been widely used until recently for the coculture of human cells with strictly anaerobic gut bacteria [65]. This is due to the fact that it is technically difficult to maintain anaerobic conditions in the system. Nevertheless, very interesting results were obtained by J. Barrila et al. [66] when Caco-2 cells were cocultured with oxygen-tolerant intestinal bacteria. The results obtained by these authors are important for further development of true aerobic–anaerobic coculturing systems. The possibility of studying the symbiosis of bacteria and human cells for a long period of time (up to two weeks) is particularly noteworthy.

Table 2 presents detailed comparison of the models described above.

As one can see from Table 2, the "gut-on-a-chip" system has a number of significant advantages that make it a versatile and efficient model for the coculture of bacterial and intestinal cells. The main advantage is the prospect of culturing several types of organoids simultaneously (e.g., intestinal epithelial tissue, liver cells, and neuronal cells), creating near-reality models of different diseases.

"Gut-on-a-Chip": Device and Possibilities of Application

The development and implementation of "organ-on-a-chip" microfluidic models with human intestinal tissue has revolutionized research into intestinal physiology and pathophysiology. Previously it was impossible to coculture enteric bacteria with a viable epithelium for more than 24 h. Moreover, this was unachievable even for cultures of intestinal organoids. For example, in the 2D cell culture format with single-

Table 2. Characterization and comparison of in vitro models of the intestine

System components and cultivation conditions	Transwell	HMI module	HoxBan	HuMiX	Gut-on-a-chip
Type of cells	Caco-2	Caco-2	Caco-2 DLD-1	Caco-2	Caco-2
Direct contact between bacteria and host cells	Yes	No	Yes	No	Yes
Mucus layer	No	Yes (added artificially)	Yes (added artificially)	Yes (mucin layer)	Yes (expressed naturally)
Epithelial cell culture conditions	M199 + 10% FBS[a]	DMEM + 10% FBS	DMEM + 10% FBS	DMEM + 20% FBS	DMEM + 20% FBS
Bacterial cell culture conditions	Anaerobic medium M199	Mixed bacterial broth for SHIME[b]	YCFAG (anaerobic broth *Faecalibacterium prausnitzii*)	Oxygen-free medium DMEM	DMEM + 20% FBS
Possible duration of cocultivation	8 h	48 h	36 h	24 h	2 weeks
Static model or fluid flow model	Static	Flow 6.5 mL/min	Static	Flow 25 μL/min	Flow 30 μL/min
Possibility of stimulating peristalsis	No	No	No	No	Yes
Coculture with strict anaerobes	Yes	Yes	Yes	Yes	Yes
Mixed bacterial cultures	No	Yes	No	Yes	Yes
Combination with other human cells	No	No	No	Yes	Yes
Analysis of epithelial barrier function[c]	Yes (TEER, IF-staining, etc.)	Yes (diffusion of FITC-dextran)	Yes (staining)	Yes (special TEER device)	Yes (TEER)
Disease model	No	No	Yes	No	Yes

[a], FBS, fetal bovine serum; [b], SHIME®, Simulator of Human Intestinal Microbial Ecosystem; [c], TEER, transepithelial electrical resistance; IF, immunofluorescence.

layered Caco-2 cells, it is impossible to reproduce physiological conditions of the gut (such as the unique morphology of intestinal tissue with villi and the mucus produced), as well as the key differentiated functions of this part of the gastrointestinal tract, including the metabolism of cytochrome P-450-based drugs. In addition to these problems, in these coculture models, all commensal bacteria rapidly proliferate and contaminate human cell cultures. These problems have been overcome only recently due to the invention of microfluidic organ-on-a-chip models of the human intestine [67].

One of the earliest gut-on-chip devices was developed by H. Kim et al. under the supervision of D. Ingber [68]. The device was a peristaltic microfluidic two-channel gut-on-a-chip model and included a porous flexible membrane as a framework for intestinal epithelial cells. The device mimicked the dynamics (peristalsis), structure, and physiological functions (absorption and transport) of the human intestine and was suitable for culturing not only human intestinal cells but also capillary endothelial cells, immune cells, and microorganisms. Interestingly, the natural inhabitant of the human gut, *Lactobacillus rhamnosus* GG, was cocultured with intestinal cells on the surface of the intestinal lumen for two weeks. A year later, the researchers and developers of the system, D. Huh et al. [69], described in detail the microengineering underlying the manufacturing of these organs-on-chips. They described the materials and dimensions used for microdevice construction, as well as the principles of mechanical engineering that reproduce peristalsis-like movements and fluid flow mimicking the real situation in the gut. The microfluidic device was designed for assessing the permeability, pharmacokinetics, and pharmacodynamics of the tested drugs, as well as their delivery to a particular part of the intestine. Soon H. Kimura et al. [70] adapted a similar device to study the microbiome.

The possibility of studying cross-interactions between the cells of the human immune system and the microbiome and identifying new markers of chronic inflammatory diseases is an extremely important aspect of gut-on-a-chip applications [71]. It is expected that "gut-on-a-chip" will be used in the future for other disease models. Currently, the production of chips for various organoids is being developed actively. Several specialized companies offering a wide range of products and services in this field have already gained popularity. For example, the commercial gut-on-a-chip system is a perfused intestinal epithelial tubule: 3D intestinal tubules in the so-called OrganoPlate (https://mimetas.com/page/products).

Real-Time Coculture Monitoring

Biochips are positioned as modern and versatile platforms rapidly providing important information; in this case, it is most practical to use optical monitoring techniques. Symbiotic processes are studied with the involvement of some modern noninvasive optical techniques such as Raman, fluorescence, or phosphorescence spectroscopy. At the Integrated Systems Laboratory of the Swiss Federal Institute of Technology (Switzerland), fluorescence microscopy has been used successfully for monitoring the immune response induced by bacterial lipopolysaccharide in Caco-2 cells cultured on a chip together with immune cells (the NutriChip concept) [72]. In Boston (Wyss Institute for Biologically Inspired Engineering, United States), they have also used the immunofluorescence methods of monitoring under the guidance of D. Ingber [73]. It should be noted that, due to the shape of the chips themselves, similar to slides, such techniques are widely used to obtain cell culture images. In a recently published article, M. Maurer et al. [74] described the immunofluorescence methods for staining epithelial tissue markers and the applications of high-resolution scanning electron microscopy.

One of the interesting trends in fluorescence techniques is fluorescence lifetime imaging microscopy (FLIM). This method is used for noninvasive monitoring of shifts in the metabolome of Caco-2 cells: e.g., towards glycolysis or oxidative phosphorylation [75]. In addition to eukaryotic cells, FLIM has been described for fingerprinting (dactyloscopy) of bacteria, in particular, *E. coli* [76].

It should be noted that phosphorescence lifetime imaging (PLIM) has been developed as an extension of FLIM. The combination of the FLIM and PLIM techniques with time-correlated single photon counting was described in two papers back in 2017 [77, 78]. Such an approach allows obtaining highly accurate real-time data on oxygen distribution in the tissues studied. It is important that this noninvasive method of detection is suitable for studies of not only microbiota but also hypoxia of various tissues.

LIMITATIONS, PROSPECTS, AND REGULATORY STATUS OF IN VITRO MODELS

The advances in the study of the microbiota and its interactions with host cells greatly have expanded our knowledge of the impact of microflora on human health and make it possible to identify therapeutic targets and to develop new drugs on their basis. Recently, the mechanisms of contractile bionanotubes, i.e., syringe-like nanomachines for molecular puncture, have been studied. These nanotubes are produced by some bacteria: R-type bacteriocins of *Pseudomonas aeruginosa* or the R-type pyocin or R-type diffocin of *Clostridium difficile* [79]. Similar injection systems are present in contractile tail bacteriophages such as phage T4 (*Escherichia* virus T4). The mechanism of this natural antimicrobial function of the virus consists in the insertion of a hollow tube into the bacterial cell wall, the injection of viral DNA with the subsequent lysis of

the host under the action of an ion flux, and depolarization of the inner membrane [80]. This unique natural mechanism can be used to synthesize nanotubes with different specificity for target microorganisms, with substitution of the ligand recognition domains of phage receptor-binding proteins or contractile bacteriocins.

Despite the many advantages of the organ-on-chip models over other in vitro systems, they also have some limitations. Many of these models have several types of cells and improved 3D architecture, but thus far they work only on a narrow repertoire of the many cell types known today. As yet, there have been no reports on microfold cells (M cells) in the gut-on-a-chip systems. Of course, there is an urgent need for creating physically connected multi-organ "human-on-a-chip" models [69, 81]. One more limitation is the material, polydimethylsiloxane (PDMS), which is commonly used for making chips. Firstly, PDMS can absorb small hydrophobic molecules and thereby distort the results of drug screening and analysis of cellular signaling [82–84]. Secondly, there is a risk of monomer leaching into the culture, if polymerization is not fully completed, which results in cell damage [83, 84].

As mentioned above, in most modern gut models, the number of meshes is sufficient to accommodate a variety of cell types; however, some experiments may require a much larger number of such meshes, and they may not be enough, especially when it comes to extracellular matrix components [85, 86]. The modeling of infectious diseases usually involves many consecutive passages of all cells, and, therefore, several multi-well microplates are used in this work. For example, when studying colonization of a gut model, samples are collected in different periods of time. In this case, the pragmatic approach is to use several 3D model systems that will be adapted to a particular experimental problem. This is because a single model system is insufficient for consideration of all scenarios of infectious diseases.

Undoubtedly, the creation of organ-on-a-chip models and their further improvement to a specialized gut-on-a-chip model, with the possibility of coculturing with bacterial cells, should be considered as a breakthrough technology in biomedicine. The creation of a human-on-a-chip model in the near future will allow drug screening under conditions that are as close as possible to the physiological conditions. There is no doubt that such systems will become a powerful tool for personalized medicine. It should be noted that personalized medicine until recently had had only one important vector of development: oncology. However, the discovery of new data on the human microbiome has led to the emergence of a second direction: personalized gastroenterology.

This science is quite young and, from a regulatory point of view, it is too early for the replacement of clinical trials by human-on-chip models or personalized medicine to be taken seriously. At present, all medicines, including medical services/protocols, undergo mandatory clinical trials involving human volunteers in the paradigm of prehospital medicine. At the same time, at the early stages of research, the most pharmaceutical companies already resort to human organoid models and actively use them to assess both the toxicity and the efficacy of candidates under study. For more than ten years, preliminary study of the permeability of a molecule of the ground substance on Caco-2 cells has been included in the mandatory set of trials to form a registration dossier for a new oral medicinal product. This approach is not limited to the gut model. For preliminary assessment of the toxicity of a candidate drug compound, for more than 30 years the standard set of preclinical trials has included in vitro liver models based on hepatocytes cultured together with epithelial cells [87]. Microfluidic devices and 3D organoids are gaining special popularity today [88, 89]. The main purpose of using such models is to select promising compounds based on the results of the primary in vitro screening: the most active, the least toxic, and the most oxidation resistant compounds with maximum bioavailability are selected. Thus, further development of microfluidic model combinations, i.e., "3D-liver" and "gut-with-microbiota," will make it possible to replace a number of animal models in pharmacokinetics. Taking into account the volume of investments into human microbiome projects and personalized medicine in developed countries, we can expect that "organs-on-a-chip" will partially or completely (depending on nosology) replace preclinical animal studies in the next 10–15 years.

ABBREVIATIONS AND NOTATION

VOC	volatile organic compounds
PDMS	polydimethylsiloxane
IBS	irritable bowel syndrome
EAP	endogenous antimicrobial peptides
FOBT	fecal occult blood test
SFB	segmented filamentous bacteria
TEER	transepithelial electrical resistance

FUNDING

This work was supported by the Russian Science Foundation, project no. 20-15-00207.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

This work does not contain any studies involving human or animal subjects.

CONFLICT OF INTEREST

The authors of this work declare that they have no conflicts of interest.

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Translated by E. Makeeva