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Short Communication

In vitro human colon microbiota culture model for drug research

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ABSTRACT

The colonic microbiota, comprising 500 species and 40 trillion bacteria, is influenced by various factors, such as diet, habits, and constitution, which impact human health and disease. This paper discusses the significance of colonic microbiota in human health and explores various *in vitro* colonic microbiota culture models to evaluate the effects of functional ingredients on gut microbiota. Traditional evaluation methods involve animal experiments and human intervention studies. However, ethical and practical challenges remain. This study introduces the Kobe University Human Intestinal Microbiota Model (KUHIMM) as an innovative *in vitro* culture system. This study details the operational methods and distinctive features of the KUHIMM, highlighting its capacity to accurately reproduce the diversity of the colonic microbiota and the metabolites in individual human donors. Various applications of the KUHIMM have been presented, ranging from the assessment of dietary fibers and probiotics to drugs and herbal medicines. The ability of the model to predict health effects and its sensitivity in evaluating different drugs make it a valuable tool for research and development. This study acknowledges its limitations, including the absence of an absorption system for metabolites, but anticipates the increasing importance of *in vitro* gut microbiota culture systems in advancing the understanding of human health and expediting the development of effective interventions.

1. Introduction

Various types of bacteria live in the human digestive tract, and these bacterial communities are collectively referred to as "colonic microbiota." In the upper digestive tract, spanning from the oral cavity to the small intestine, as well as in the lower digestive tract, extending from the large intestine to the anus, the intestinal microbiota adapts to the unique conditions of each environment. The colonic microbiota, specifically, comprises a diverse array of 500 species, totaling an impressive 40 trillion bacteria. The types and numbers of bacteria constituting the colonic microbiota are largely influenced by the environment, habits, diet, and constitution of the human host and vary among individuals and races [1]. The colonic microbiota has been reported to affect not only the homeostasis of the human digestive tract but also organs located far from the intestines [2]. Research and development of pharmaceuticals and supplements aimed at improving overall health by influencing intestinal bacteria are in progress. Until recently, the effects of these products on the intestinal microbiota were evaluated mainly through animal experiments and human intervention tests. However, there is a growing emphasis on reducing animal experimentation from the standpoint of animal welfare, as highlighted in the international guidelines known as ARRIVE (Animal Research: Reporting of In Vivo Experiments) [3]. In addition, the results of animal studies cannot be extrapolated to humans because the gastrointestinal tract structure and intestinal microbiota of animals are very different from those of humans. However, in human intervention studies, there are ethical barriers to testing ingredients with unknown functions or for which there is no dietary experience. In addition, there is a remarkable burden in terms of time and cost to evaluate a large number of candidate ingredients simultaneously, which is a major factor slowing down research and development. Under such circumstances, the Food and Drug Administration (FDA) Modernization Act 2.0 was officially passed by the U.S. House of Representatives on December 23, 2022, and signed into law by President Joe Biden, allowing preclinical testing as an alternative to animal testing [4]. Therefore, it is expected that alternative methods for animal testing to evaluate drugs and dietary supplements will be

Abbreviations: FDA, Food and Drug Administration; KUHIMM, Kobe University Human Intestinal Microbiota Model; SCFA, short chain fatty acid; SHIME, Simulator of the Human Intestinal Microbial Ecosystem; TIM, TNO Intestinal Model.

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explored, and research on *in vitro* and *in silico* systems for formulating testing guidelines will accelerate considerably in the future. In this paper, we review *in vitro* gut microbiota culture models for evaluating the effects of drugs and functional ingredients on the gut microbiota, focusing on models developed by our group and used by other groups.

2. Intestinal behavior of foods and drugs on human health

Most food components orally ingested by humans are absorbed in the small intestine and utilized for vital activities through digestion by mastication and digestive enzymes in the oral cavity, as well as by gastric acid, enzymes, and intestinal bacteria in the small intestine. In contrast, components that escape digestion and absorption (e.g., dietary fiber, unabsorbed phytochemicals, and useful bacteria) reach the large intestine. Among the food components that reach the large intestine, useful substances are called prebiotics, and useful bacteria are called probiotics [5]. These prebiotics and probiotics activate the growth of colonic microbiota in the large intestine, resulting in the production of metabolites, such as short-chain fatty acids (SCFAs), in the colon [6]. These metabolites are absorbed by intestinal epithelial cells and affect receptors and various organs via the portal vein. These metabolites play critical roles in the maintenance of the intestinal barrier and homeostasis. Furthermore, many immune cells in the human body are concentrated around the intestinal tract, and intestinal bacteria are in close proximity to these immune cells and are actively engaged in metabolic activities [7]. Therefore, the types and concentrations of metabolites are considered crucial factors in human health. Recently, it has been reported that the diversity of intestinal microbiota and their metabolites are related not only to colorectal cancer and inflammatory bowel disease, but also to coronary artery, heart, and neurodegenerative diseases [8] (Fig. 1). Therefore, *in vitro* culture models evaluating the effects of drugs, supplements, prebiotics, and probiotics may require a high degree of reproducibility with respect to the intestinal bacterial species, bacterial abundance, and metabolites.

3. Objectives and methods of *in vitro* colonic microbiota culture

An *in vitro* intestinal microbiota culture model is a general term for a system that reproduces the human intestinal environment *in vitro* by culturing human feces as the inoculum source. A non-additive system—where test ingredients such as food components, dietary supplements, and drugs are not added to the culture—is used as a control. A comparative analysis is performed between the bacterial species, their abundance, and metabolites of the additive and non-additive cultures to predict and evaluate the functionality of the additives [9]. In general, the hurdles to start an *in vitro* experiment are fewer than those for human intervention studies because approval can be more easily obtained from a clinical research ethics committee for a noninvasive observational study.

Various *in vitro* gut microbiota culture models with different characteristics have been developed and can be divided into two main types according to their concepts. The first type, called the "human digestive tract composite culture model," reproduces the intestinal environment using an *in vitro* culture system that simulates the entire digestive tract, including the stomach and small intestine, by connecting multiple reaction chambers and culture vessels. Representative examples include the Simulator of the Human Intestinal Microbial Ecosystem (SHIME) developed by Ghent University in Belgium [10] and the TNO Intestinal Model (TIM) developed by the Netherlands Organisation for Applied Scientific Research (TNO) in the Netherlands [11]. These models can comprehensively evaluate the degradation or modification of ingested ingredients in the digestive tract and their effects on intestinal bacteria, making them highly useful, particularly for understanding the intestinal dynamics of food ingredients and drugs. However, because this is a culture method in which substrates are added to a complex system consisting of several connected vessels, it takes time for culture, and differences from the actual human intestinal environment can easily occur owing to dilution of the culture medium and reduction in anaerobic activity. SHIME was later improved, and an *in vitro* culture system for intestinal bacteria (referred to as M−SHIME), including the mucosal layer (mucin layer) on intestinal epithelial cells in the colon, was developed [12]; however, the reproducibility of the inoculum source for the microbiome after culture still needed further investigation.

The second type focuses on the reproduction of the intestinal microbiota and involves performing batch culture in a single culture chamber (Fig. 2). The *in vitro* human colon microbiota culture model (KUHIMM; Kobe University Human Intestinal Microbiota Model) developed in Japan [13] and the MiPro (96-deep well plate–based culturing model) reported by a research group at the University of Ottawa in Canada [14] fall into this category. Compared to the aforementioned methods, the reproducibility of the colonic bacterial population is high, and the simplicity of the culture system facilitates small-scale, high-throughput, and diverse analyses. This model is particularly useful for evaluating the efficacy of components intended to affect bacteria in the colon. In the following sections, we explain the operational method, features, and application examples of the KUIHMM developed by our group.

4. Operational methods and features of the KUHIMM

The KUHIMM is an *in vitro* culture model specialized for colonic microbiota, as described above, and is operated as a 100-mL anaerobic

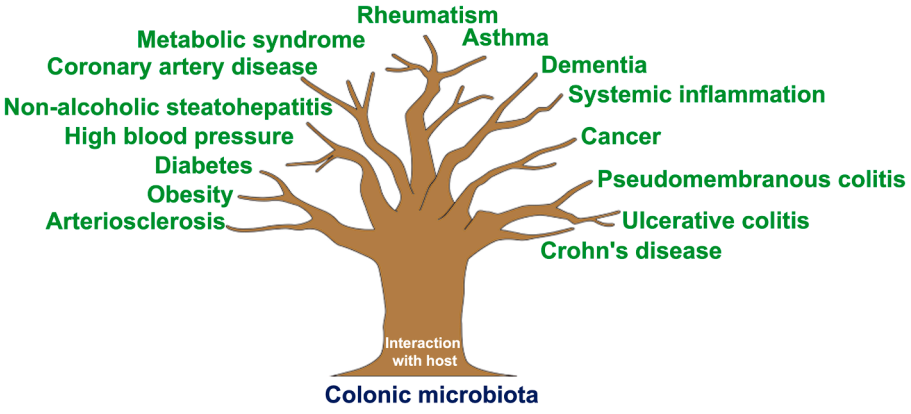

Fig. 1. Tree diagram from colonic microbiota to health and disease. The tree diagram shows that the diversity and metabolites of the colonic microbiota are associated with many diseases, including cancer, inflammatory bowel disease, coronary artery disease, heart disease, and neurodegenerative diseases. This figure was heavily modified from the figure on Front Med (Lausanne). 2014; 1: 15. [48].

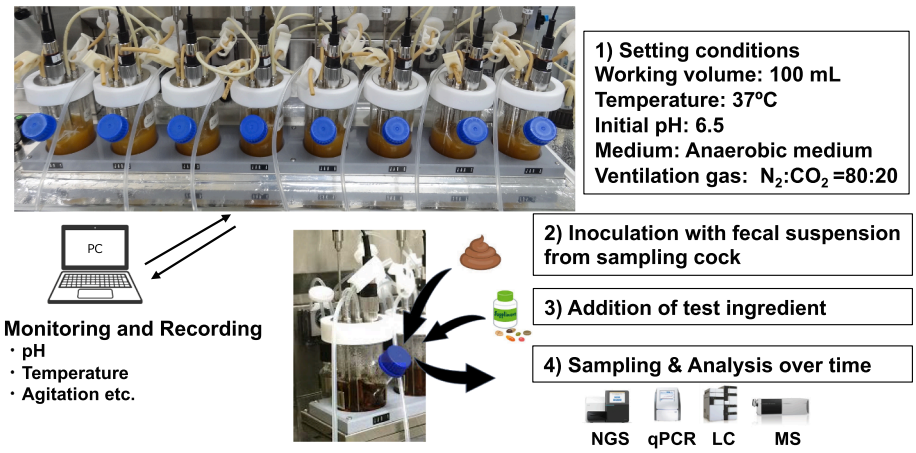

Fig. 2. The KUHIMM and its experimental scheme. The KUHIMM is operated as a 100-mL anaerobic batch monovessel culture using human feces as the inoculum source (100–200 μL of fecal suspension in phosphate buffer is usually used). The operating conditions are 37 °C with an initial pH of 6.5, using anaerobic nutrient medium (Gifu anaerobic medium is usually used) with ventilation of anaerobic gas (N_2:CO_2=80:20). pH, temperature, and agitation speed are monitored and recorded. After fecal inoculation and addition of the test ingredient, the culture is incubated for 24–72 hours, during which time the culture medium is periodically sampled through the sampling cock. The culture medium over time is analyzed for the total number of bacteria, the quantity of beneficial bacterium and the composition of the colonic microbiota with Next-generation sequencing (NGS) or/and quantitative PCR (qPCR), whereas the culture supernatant is used to analyze the metabolites with Liquid chromatography (LC) or/and Mass Spectrometry (MS).

batch monovessel culture (one person's feces is inoculated into one culture vessel) using human feces as the inoculum source (Fig. 2). After fecal inoculation, the culture is incubated for 24–72 hours, during which time the culture medium is sampled periodically. The culture medium is separated into bacteria and culture supernatant by centrifugation, following which nucleic acids are extracted from the bacteria to analyze the total number of bacteria and the population and balance of the colonic microbiota, and the culture supernatant is used to analyze the major metabolites of intestinal bacteria, such as SCFAs. By comparing the results of these analyses over time with and without the addition of the component under evaluation, it is possible to evaluate the effect of the component on the colonic microbiota and its metabolites.

The most important feature of the KUHIMM is that it can be implemented while maintaining the diversity of the colonic microbiota of an individual and can maintain and reproduce its bacterial community structure to a high degree without bias after culturing (Fig. 2). Additionally, it can reproduce the composition ratio of the major metabolites of intestinal bacteria, represented by SCFAs (compared to the report by Commings et al. [15]), and can be used to quickly and easily evaluate the safety of various types of drugs and food ingredients as well as their effects on colonic microbiota. These advantages could possibly be attributed to the consumption of carbohydrate sources and the production of metabolites in the colon by culturing the colonic microbiota only in the medium at the start of culture; crossfeeding (nutrient symbiosis) [16] between microorganisms occurs in the subsequent culture time, allowing selective growth of the microbiota in the colon under highly anaerobic conditions. The pH transition of the culture medium over time is similar to that of the actual colon (ref. Cummings et al. 1987 [15]). In addition, most animal experiments reported in the field of colonic microbiota research involve feeding experiments, in which the doses administered to animals are considerably overestimated when converted to human doses, which may overestimate the functionality of foods, supplements, and drugs for intestinal bacteria. However, when using the KUHIMM, the quantity of ingredients evaluated is determined considering the daily dietary intake and colonic contents of humans. The studies are conducted in such a way that the extrapolation of the results of the evaluation to humans is highly plausible. Even when compared with human intervention studies, the KUHIMM is not directly affected by daily dietary conditions, and the optimal dose is easy to set, allowing for a more sensitive detection of the response to the test drug, which is difficult to detect in clinical trials.

5. Extrapolation and applicability of the KUHIMM

Here, we discuss the extrapolation and applicability of the KUHIMM in an experiment using dietary fibers. Resistant maltodextrin (DEX), α-cyclodextrin (αCD), and dextran (DXR) were selected as dietary fibers, and a realistic daily oral intake for humans was set at 6 g/day, based on commercially available prebiotic intake. Overall, eight healthy volunteers participated in the study, and fresh feces samples collected and cultured separately were used as a non-additive control to estimate the changes in the microbiota and to measure the increase or decrease in metabolites during incubation with each dietary fiber added separately. Note that 0.2 % (0.2 g/100 mL) dietary fiber was added to each culture chamber, considering the water intake of humans (3 L/day) and the amount of dietary fiber consumed per day, as mentioned above. For more details on colon microbiota culture, please refer to the study by Sasaki et al. [17].

Analysis of SCFAs in the KUHIMM post-culture samples revealed considerable increases in the concentrations of acetate and propionate with the addition of each dietary fiber to the culture. However, there was no change in butyrate concentrations. Correlating with the metabolite results, the results of the microbiota analysis demonstrated an increasing trend in the density of *Bacteroides* spp. (acetate and propionate–producing bacteria responsible for the degradation of polysaccharides with glycosidic linkages in the colon), which has also been reported to increase with dietary fiber intake in *in vivo* studies, whereas the results of the principal coordinates analysis showed no significant changes in the microbiota upon the addition of dietary fibers. In other words, no major changes in the microbiota were observed when adequate amounts of dietary fiber were ingested. However, it was predicted that the activation of *Bacteroides* spp. would increase the production of these SCFAs in the colon. In addition, an intervention study was conducted with the same volunteers. They received 6 g/day of DEX or αCD daily for 7 days. This was followed by a 14-day washout period, and the fecal microbiota on the last day of each period was then compared with that before the study commenced. As in the KUHIMM test, an increase in *Bacteroides* spp. was detected; however, no significant change in the microbiota was detected before or after dietary fiber intake. The KUHIMM not only captured changes in the microbiota with the same resolution as done by the human intervention test but also showed that changes in SCFAs, which are difficult to measure in real environments in the human intervention test, can be used to predict

health effects in humans.

6. Evaluation of various agents that affect the colonic environment

Because of the features described in the previous section, the KUHIMM has been used to evaluate the functionality and safety of various agents, including prebiotics and probiotics, functional ingredients, drugs, herbal medicines, and antibodies, as well as ingredients of functional foods for specific health applications and foods with functional claims. Below are almost all the currently published examples of functional components evaluation using the KUHIMM. The prebiotic examples were fructan-containing barley grass extract, yeast mannan, guar gums, and indigestible maltodextrins. Probiotics included the lactiate-producing bacteria *Wezmznnia coagulans*, the metabolite of *Bacillus subtilis*, and the algae *Euglena gracilis*. Furthermore, examples of medicines were the traditional Japanese herbal medicine Daikenchuto, D-β-hydroxybutyrate, and recombinant immunoglobulins. Lastly, we featured various glycosaminoglycans and glucosamine as dietary supplements.

6.1. Prebiotics

The effects of various prebiotics were investigated using the KUHIMM. When 1.5 % fructan-containing barley grass extract was added to the KUHIMM, an increase in *Bifidobacteria* and butyrate-producing bacteria was observed [18]. The increase in butyrate was consistent with the increase in butyrate-producing bacteria, such as *Faecalibacterium* and *Roseburia* [19]. Butyrate is an important energy source for colonic epithelial cells that maintain intestinal homeostasis [20]. Yeast mannan, a water-soluble polysaccharide, selectively increases *Bacteroides thetaiotaomicron* [21]. Yeast mannan treatment increased the production of acetate and propionate, which are major metabolites of the Bacteroidetes phylum [22].

Furthermore, the effects of two types of partially hydrolyzed guar gums with different molecular weights, high and low molecular weights, on the human colonic microbiota and the production of SCFAs were evaluated using the KUHIMM [23]. The propionate production capacity of the *Bacteroides* species, especially *Bacteroides uniformis*, which has a high propionate production capacity, was also evaluated. This increase coincided with an increase in propionate production in the culture medium, especially in the low molecular–weight types. The relative abundances of the genera *Faecalibacterium*, Unclassified Lachnospiraceae, and *Bifidobacterium* also increased.

It should be noted that the KUHIMM can more accurately predict the functionality of test ingredients with different chemical structures by simultaneously evaluating them [24]. For example, indigestible dextrin is a polymer of glucose derived from starch; in addition to the original product, many generic products with slightly different molecular structures have been developed. A slight difference in the molecular structure of these digestion-resistant dextrins can be detected using the KUHIMM with high sensitivity based on the difference in fermentability, and an accurate prediction can be made regarding the difference in functionality.

The KUHIMM can accurately detect a slight difference in dietary fibers to investigate potential functional expression at the human individual level. Overall, six types of dietary fibers were selected and tested. These fibers have different proportions of glycosidic bonds [α- or β-(1 → 4), (1 → 6), (1 → 2), and (1 → 3) bonds] and molecular weights (Molecular weight: 1000–5000). The fibers include: 1) polydextrose (PDX), which is reported to be persistent with a high proportion of (1 → 2) and (1 → 3) bonds; 2) resistant glucan (RGN); 3–5) three types of resistant maltodextrin DEX (Nutriose (DEX-1), Promitor (DEX-2) and Fibersol-2 (DEX-3)) with different proportions of (1 → 4) and (1 → 6) bonds; 6) isomaltodextrin (IMD), which specifically contains more easily degradable (1 → 6) bonds. Analysis of the respective degradation rates based on the quantification of dietary fiber using high-performance liquid chromatography detected marked differences in the binding modes and molecular weights of the samples after KUHIMM incubation. Both PDX and RGN, as well as DEX-1 and DEX-2, showed similar degradation rates, suggesting that only DEX-3 and IMD were relatively easily degraded in the real environment. The ratio of (1 → 2)+(1 → 3) bonds and the degradation rate were found to have a high negative correlation. Among acetate and propionate, which are reported to be produced by intestinal bacteria upon fiber intake, the concentration and degradation rate of propionate increased considerably when DEX-3 and IMD were added; its concentration was found to be highly positively correlated with the degradation rate.

6.2. Probiotics

There are several examples of the effects of probiotic bacteria on the KUHIMM. For example, when *W. coagulans* (formely *Bacillus coagulans*) was added to the KUHIMM, it inhibited the growth of Enterobacteriaceae, including *Escherichia coli*, promoted the growth of Lachnospiraceae, including butyrate-producing bacteria, and promoted the production of butyrate [25]. Moreover, another study showed the addition of 0.1 % peptide produced by *B. subtilis* promoted the growth of *Bifidobacterium* spp. [26]. These results were consistent with those of a clinical study [27] that reported an increase in *Bifidobacterium* spp. after 24 weeks of intake of 3.4 x 10^9 CFU/day of *B. subtilis*.

The algae *Euglena gracilis* (*E. gracilis*) has attracted attention as a health food in recent years; however, its effects on the human colon microbiota remain unknown; therefore, a KUHIMM study was conducted [28]. The KUHIMM (n = 11 healthy subjects), which simulates human colon microbiota, revealed that the addition of *E. gracilis* stimulated the growth of the symbiotic *Faecalibacterium*. Furthermore, the addition of *E. gracilis* enhanced butyrate production by *Faecalibacterium prausnitzii*; paramylon (β-1,3-glucan polysaccharide), an insoluble dietary fiber that accumulates in and is a major component of *E. gracilis*, did not stimulate *Faecalibacterium* growth in the KUHIMM. In this study, a human intervention experiment was conducted to demonstrate the performance of the KUHIMM. Overall, 28 human participants received 2 g of *E. gracilis* daily for 30 days. Changes in daily bowel movements before and after daily consumption (2 g/day) of *E. gracilis* were determined using the Bristol Stool Form Scale. An increase in *Faecalibacterium* abundance in the KUHIMM has also been confirmed in human intervention studies.

6.3. Drugs

Pharmaceutical ingredients were also evaluated using the KUHIMM. Daikenchuto (DKT) is a traditional Japanese herbal medicine (Chinese herbal medicine) containing ginseng, processed ginger, and Sansho or flower pepper. We established the KUHIMM using feces collected from nine healthy volunteers supplemented with 0.5 % DKT, which concentration was set by considering the volume of the colon (400 mL) and the amount of one dose of the medicine [29]. DKT treatment markedly increased the relative abundance of bacteria related to the genus *Bifidobacterium*. In pure cultures of several monomicrobial species, DKT extensively promoted the growth of *Bifidobacterium adolescentis*. In a study using pure cultures of *B. adolescentis* and in the study using the KUHIMM, it was found that *B. adolescentis* converts the active ingredient contained in DKT and uses it for proliferation.

D-β-hydroxybutyrate (DBHB) is a type of ketone body that has been investigated for its potential use in drugs or supplements. In the KUHIMM inoculated with feces from subjects who were speculated to respond well to DBHB, DBHB treatment increased the relative abundance of *Coprococcus* spp. and correlated with increased butyrate production [30]. Based on a predicted functional genes analysis, the amount of β-hydroxybutyrate dehydrogenase in KUHIMM from DBHB responders following DBHB addition was higher than that of the KUHIMM

from non-responders. DBHB responders and non-responders were identified in this study, and no increase in butyrate production was observed in DBHB non-responders. Moreover, the presence or absence of effects of taurine, a bioactive amino acid, on colonic microbiome under anaerobic conditions have been investigated for other drug-related components using KUHIMM [31].

Furthermore, the KUHIMM has been used to study the physiological effects of recombinant immunoglobulins because of fewer ethical restrictions for KUHIMM. Immunoglobulin IgA W27 is known to inhibit the growth of pathogenic *E. coli*, and its effect on human colonic microbiota has been studied using the KUHIMM [32]. When IgA W27 (final concentration, 0.5 μg/mL) was added to the KUHIMM, the relative abundance of *Escherichia* spp. decreased. This study revealed the suppressive effect of IgA W27 on *Escherichia* and the usefulness of KUHIMM to evaluate the effect of a medicine candidate or a gut microbiota regulator on human colonic microbiota.

6.4. Dietary supplements

Glycosaminoglycans are linear polysaccharides with a repeating structure of disaccharides, including amino sugars, and are the major components of the extracellular matrix secreted by animal cells. Chondroitin is a representative glycosaminoglycan used as a dietary supplement or over-the-counter drug to relieve joint pain. Although glycosaminoglycans have been reported to be degraded and utilized by some intestinal bacteria, their structures vary widely, and it is not fully understood which glycosaminoglycans specifically affect the human colon environment. We separately added 0.3 % (w/v) of each of seven different glycosaminoglycans to the KUHIMM using human feces as the inoculum source and cultured them to examine their effects on the production of SCFAs and the structure of the colon microbiota [33]. The results showed that the addition of chondroitin, chondroitin sulfate, and heparosan increased the production of acetate and propionate. The addition of chondroitin sulfate to a specific structure markedly increased the percentage of *Bacteroides* involved in these processes. Furthermore, comprehensive relative quantification of metabolites in the supernatant of the culture medium by metabolome analysis revealed that the addition of chondroitin, chondroitin sulfate, and heparosan considerably increased the production of some amino acids, such as lysine and ornithine.

Glucosamine, which is often taken up simultaneously with chondroitin [46], has also been investigated with KUHIMM. Glucosamine is used as a supplement worldwide to improve the overall health/well-being of joints. Recently, a few large-scale epidemiological studies reported that glucosamine intake, regardless of its effect on the joints, contributes to reduced mortality in humans [47]. On the other hand, it has been reported that approximately half of orally ingested glucosamine is absorbed in the upper gastrointestinal tract, while the remaining half escapes absorption and reaches the large intestine [34]. However, there are few reports on the effects of glucosamine without chondroitin on the colonic microbiota. Therefore, we analyzed the effect of glucosamine addition on the human colonic microbiota using the KUHIMM with feces collected from thirteen volunteers (unpublished results). The results showed that the addition of 0.2 % (w/v) glucosamine increased butyrate production. Next, we analyzed the diversity of the microbiome and found that the addition of glucosamine had no obvious effect on the composition of the microbiota; however, statistical analysis confirmed that the addition of glucosamine selectively increased certain butyrate-producing *Anaerostipes* bacteria to produce butyrate with beneficial effects on the host.

7. Construction of disease model

Dysbiosis is a condition in which the structure and diversity of colonic microbiota and their metabolism are disrupted, causing various diseases. Our group is also working on the development of a pathological model using the KUHIMM. For instance, ulcerative colitis (UC) is not a single-factor disease but a multifactorial disease, and it has been strongly suggested that the colonic microbiota is involved in the pathogenesis of UC as one of several environmental factors that may trigger the development of UC in individuals with single or multiple genetic mutations [35]. One of the characteristics of dysbiosis is a decrease in butyrate-producing bacteria, such as those of the genus *Roseburia* and family Lachnospiraceae [36]. Butyrate is known as a molecule that induces the differentiation of regulatory T-cells, a process known to play a pivotal role in the suppression of intestinal inflammation [37], and it has been shown that the concentration of SCFAs, including butyrate, is decreased in patients with UC [38]. This suggests that colonic microbiota and their metabolites in the intestinal environment are involved in UC pathogenesis. Our group has successfully developed a "UC pathological model KUHIMM," which reproduces the characteristic dysbiosis described prior in the culture model, by using fecal specimens from patients with UC as an inoculum source [38] (Fig. 3). This model could potentially allow us to identify an additive ingredient that improves the bacterial population in this pathological condition [25,39]. It is noteworthy that this pathological model was able to detect an unbalanced SCFAs profile compared with healthy subjects. This may reflect the function of colonic microbiota in the colon of patients with UC. In addition to this UC model, we have succeeded in reproducing the dysbiosis characteristics of patients with coronary artery disease [40,41]. We reported the beneficial effect of resistant starch, a prebiotic, on the colonic microbiota and its metabolites in KUHIMM using feces from patients with coronary artery disease [42]. More recently, we have examined the application of this method to pathological models of diabetes and patients with cancer.

8. Conclusion

As noted in the introduction, several human gut models have been developed to date. When comparing KUHIMM with SHIME and TIM, it is important to note that KUHIMM is a single-batch fermenter, which results in a large morphological difference from that of human organs. On the other hand, it is evident that KUHIMM has a significant advantage in terms of the high reproducibility of colonic microbiota composition and metabolites [39]. It is impossible to directly measure changes in microbiota and metabolite concentrations in the gastrointestinal tract when humans ingest test ingredients; however, an *in vitro* culture system such as the KUHIMM that fully reconstructs the human colon microbiota can simulate such changes without actual human ingestion. Additionally, we have been more recently developing a multiplexed model of the conventional KUHIMM with higher reproducibility of the colonic microbiota. A large-scale cohort study have revealed that drugs and supplements are correlated with the structure and diversity of the gut microbiota [43]. Thus, the value of the *in vitro* colon microbiota culture model described in this study is expected to become more pronounced in the future because of its ability to provide quick and easy evaluations. However, it must be recognized that the lack of an absorption system for metabolites produced, their accumulation, and the inability to analyze interactions with intestinal epithelial cells and immune cells are issues that need to be addressed in all existing *in vitro* colon microbiota culture models, including the KUHIMM. Currently, as a method to solve these problems, the intestinal bacteria-organoid-on-a-chip, which combines intestinal bacteria culture with a two-layer culture system of cultured cells or an *in vitro* organoid culture system, is being developed [44,45]. In addition, the construction of a composite culture system that can evaluate the interaction between the human colon and other organs is currently in progress. We hope that *in vitro* gut microbiota culture systems, whose extrapolation to the human gastrointestinal tract has been further advanced through these efforts, will be utilized for the rapid screening of candidate drugs to maintain human health, improve pathological conditions, and accelerate the development of effective drugs and dietary supplements.

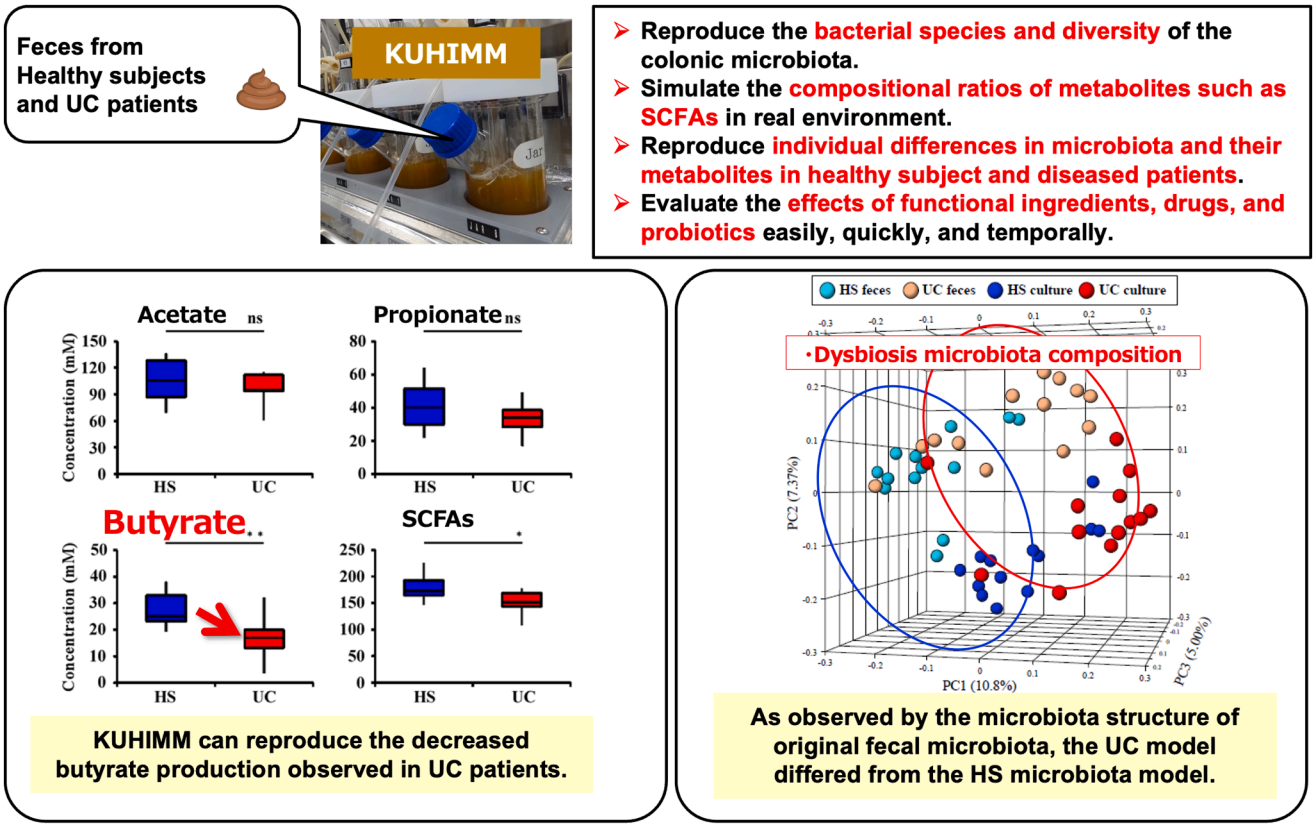

Fig. 3. Construction of the UC-KUHIMM. Inoculation with the feces of patients with ulcerative colitis (UC) could maintain patient microbiomes and pass on patient features. The microbial diversity of cultures with feces samples from patients with UC was very close to that of feces samples from patients with UC, unlike that of HS (healthy subjects). This model successfully reproduced the altered metabolites (e.g., decrease in butyrate) and dysbiosis (e.g., microbial diversity). Note that this figure was heavily modified from that in Biotechnol J. 14, 1,800,555 (2019) [38].

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CRediT authorship contribution statement

Tomoya Shintani: Writing – original draft, Funding acquisition, Data curation. **Daisuke Sasaki:** Writing – original draft, Visualization, Data curation, Conceptualization. **Yasushi Matsuki:** Project administration, Funding acquisition, Conceptualization. **Akihiko Kondo:** Writing – review & editing, Validation, Supervision, Resources, Project administration, Funding acquisition, Conceptualization.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: The authors declare no conflicts of interest. Tomoya Shintani was an employee of the Matsutani Chemical Industry Co., Ltd. (Itami, Japan) until December 2022. Daisuke Sasaki has been an employee of Bacchus Bio innovation Co. Ltd. (Kobe, Japan) since April 2023.

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References

[1] Hollister EB, Gao C, Versalovic J. Compositional and functional features of the gastrointestinal microbiome and their effects on human health. Gastroenterology 2014;146(6):1449–58. https://doi.org/10.1053/j.gastro.2014.01.052. PMID 24486050.

[2] Sommer F, Bäckhed F. The gut microbiota–masters of host development and physiology. Nat Rev Microbiol 2013;11(4):227–38. https://doi.org/10.1038/nrmicro2974. PMID 23435359.

[3] Percie du Sert N, Hurst V, Ahluwalia A, Alam S, Avey MT, Baker M, et al. The ARRIVE guidelines 2.0: updated guidelines for reporting animal research. PLOS Biol.2020;18(7):e3000410. doi: 10.1371/journal.pbio.3000410, PMID 32663219.

[4] Wadman M. FDA no longer has to require animal testing for new drugs. Science 2023;379(6628):127–8. https://doi.org/10.1126/science.adg6276. PMID 36634170.

[5] Pandey KR, Naik SR, Vakil BV. Probiotics, prebiotics and synbiotics- a review. J Food Sci Technol 2015;52(12):7577–87. https://doi.org/10.1007/s13197-015-1921-1. PMID 26604335.

[6] Vinolo MAR, Rodrigues HG, Nachbar RT, Curi R. Regulation of inflammation by short chain fatty acids. Nutrients 2011;3(10):858–76. https://doi.org/10.3390/nu3100858. PMID 22254083.

[7] Peterson LW, Artis D. Intestinal epithelial cells: regulators of barrier function and immune homeostasis. Nat Rev Immunol 2014;14(3):141–53. https://doi.org/10.1038/nri3608. PMID 24566914.

[8] Hou K, Wu ZX, Chen XY, Wang JQ, Zhang D, Xiao C, et al. Microbiota in health and diseases. Signal Transduct Target Ther 2022;7(1):135. https://doi.org/10.1038/s41392-022-00974-4. PMID 35461318.

[9] Payne AN, Zihler A, Chassard C, Lacroix C. Advances and perspectives in in vitro human gut fermentation modeling. Trends Biotechnol 2012;30(1):17–25. https://doi.org/10.1016/j.tibtech.2011.06.011. PMID 21764163.

[10] Alander M, De Smet I, Nollet L, Verstraete W, von Wright A, Mattila-Sandholm T. The effect of probiotic strains on the microbiota of the simulator of the human intestinal microbial ecosystem (SHIME). Int J Food Microbiol 1999;46(1):71–9. https://doi.org/10.1016/s0168-1605(98)00182-2. PMID 10050686.

[11] Blanquet S, Zeijdner E, Beyssac E, Meunier JP, Denis S, Havenaar R, et al. A dynamic artificial gastrointestinal system for studying the behavior of orally administered drug dosage forms under various physiological conditions. Pharm Res 2004;21(4):585–91. https://doi.org/10.1023/b:pham.0000022404.70478.4b. PMID 15139514.

[12] Van den Abbeele P, Belzer C, Goossens M, Kleerebezem M, De Vos WM, Thas O, et al. Butyrate-producing clostridium cluster XIVa species specifically colonize mucins in an in vitro gut model. ISME J 2013;7(5):949–61. https://doi.org/10.1038/ismej.2012.158. PMID 23235287.

[13] Takagi R, Sasaki K, Sasaki D, Fukuda I, Tanaka K, Yoshida K, et al. A single-batch fermentation system to simulate human colonic microbiota for high-throughput evaluation of prebiotics. PMID 27483470 PLoS One 2016;11(8):e0160533.

[14] Li L, Abou-Samra E, Ning Z, Zhang X, Mayne J, Wang J, et al. An in vitro model maintaining taxon-specific functional activities of the gut microbiome. Nat Commun 2019;10(1):4146. https://doi.org/10.1038/s41467-019-12087-8. PMID 31515476.

[15] Cummings JH, Pomare EW, Branch WJ, Naylor CP, Macfarlane GT. Short chain fatty acids in human large intestine, portal, hepatic and venous blood. Gut 1987;28(10):1221–7. https://doi.org/10.1136/gut.28.10.1221. PMID 3678950.

[16] Engel P, Moran NA. The gut microbiota of insects – diversity in structure and function. FEMS Microbiol Rev 2013;37(5):699–735. https://doi.org/10.1111/1574-6976.12025. PMID 23692388.

[17] Sasaki D, Sasaki K, Ikuta N, Yasuda T, Fukuda I, Kondo A, et al. Low amounts of dietary fibre increase in vitro production of short-chain fatty acids without changing human colonic microbiota structure. Sci Rep 2018;8(1):435. https://doi.org/10.1038/s41598-017-18877-8. PMID 29323180.

[18] Sasaki D, Sasaki K, Kadowaki Y, Aotsuka Y, Kondo A. Bifidogenic and butyrogenic effects of young barely leaf extract in an in vitro human colonic microbiota model. AMB Express 2019;9(1):182. https://doi.org/10.1186/s13568-019-0911-5. PMID 31721000.

[19] Rivière A, Selak M, Lantin D, Leroy F, De Vuyst L. Bifidobacteria and butyrate-producing colon bacteria: importance and strategies for their stimulation in the human gut. Front Microbiol 2016;7:979. https://doi.org/10.3389/fmicb.2016.00979. PMID 27446020.

[20] Litvak Y, Byndloss MX, Bäumler AJ. Colonocyte metabolism shapes the gut microbiota. Science. 2018;362(6418):eaat9076. doi: 10.1126/science.aat9076, PMID 30498100.

[21] Oba S, Sunagawa T, Tanihiro R, Awashima K, Sugiyama H, Odani T, et al. Prebiotic effects of yeast mannan, which selectively promotes bacteroides thetaiotaomicron and Bacteroides ovatus in a human colonic microbiota model. Sci Rep 2020;10(1):17351. https://doi.org/10.1038/s41598-020-74379-0. PMID 33060635.

[22] den Besten G, van Eunen K, Groen AK, Venema K, Reijngoud DJ, Bakker BM. The role of short-chain fatty acids in the interplay between diet, gut microbiota, and host energy metabolism. J Lipid Res 2013;54(9):2325–40. https://doi.org/10.1194/jlr.R036012. PMID 23821742.

[23] Sasaki D, Sasaki K, Abe A, Ozeki M, Kondo A. Effects of partially hydrolyzed guar gums of different molecular weights on a human intestinal in vitro fermentation model. J Biosci Bioeng 2023;136(1):67–73. https://doi.org/10.1016/j.jbiosc.2023.04.002, PMID 37105857.

[24] Sasaki D, Sasaki K, Kondo A. Glycosidic linkage structures influence dietary fiber fermentability and propionate production by human colonic microbiota in vitro. Biotechnol J 2020;15(10):e1900523.

[25] Sasaki K, Sasaki D, Inoue J, Hoshi N, Maeda T, Yamada R, et al. Bacillus coagulans SANK 70258 suppresses enterobacteriaceae in the microbiota of ulcerative colitis in vitro and enhances butyrogenesis in healthy microbiota. Appl Microbiol Biotechnol 2020;104(9):3859–67. https://doi.org/10.1007/s00253-020-10506-1. PMID 32146494.

[26] Hatanaka M, Morita H, Aoyagi Y, Sasaki K, Sasaki D, Kondo A, et al. Effective bifidogenic growth factors cyclo-val–leu and cyclo-val–ile produced by Bacillus subtilis C-3102 in the human colonic microbiota model. Sci Rep 2020;10(1):7591. https://doi.org/10.1038/s41598-020-64374-w. PMID 32372037.

[27] Takimoto T, Hatanaka M, Hoshino T, Takara T, Tanaka K, Shimizu A, et al. Effect of Bacillus subtilis C-3102 on bone mineral density in healthy postmenopausal Japanese women: a randomized, placebo-controlled, double-blind clinical trial. Biosci Microbiota Food Health 2018;37(4):87–96. https://doi.org/10.12938/bmfh.18-006. PMID 30370192.

[28] Nakashima A, Sasaki K, Sasaki D, Yasuda K, Suzuki K, Kondo A. The alga Euglena gracilis stimulates faecalibacterium in the gut and contributes to increased defecation. Sci Rep 2021;11(1):1074. https://doi.org/10.1038/s41598-020-80306-0. PMID 33441865.

[29] Sasaki K, Sasaki D, Sasaki K, Nishidono Y, Yamamori A, Tanaka K, et al. Growth stimulation of Bifidobacterium from human colon using daikenchuto in an in vitro model of human intestinal microbiota. Sci Rep 2021;11(1):4580. https://doi.org/10.1038/s41598-021-84167-z. PMID 33633259.

[30] Sasaki K, Sasaki D, Hannya A, Tsubota J, Kondo A. In vitro human colonic microbiota utilises D-β-hydroxybutyrate to increase butyrogenesis. Sci Rep 2020;10(1):8516. https://doi.org/10.1038/s41598-020-65561-5. PMID 32444846.

[31] Sasaki K, Sasaki D, Okai N, Tanaka K, Nomoto R, Fukuda I, et al. Taurine does not affect the composition, diversity, or metabolism of human colonic microbiota simulated in a single-batch fermentation system. PMID 28700670 PLoS One 2017;12(7):e0180991.

[32] Sasaki K, Mori T, Hoshi N, Sasaki D, Inoue J, Shinkura R, et al. W27 IgA suppresses growth of Escherichia in an in vitro model of the human intestinal microbiota. Sci Rep 2021;11(1):14627. https://doi.org/10.1038/s41598-021-94210-8. PMID 34272464.

[33] Inokuma K, Sasaki D, Kurata K, Ichikawa M, Otsuka Y, Kondo A. Sulfated and non-sulfated chondroitin affect the composition and metabolism of human colonic microbiota simulated in an in vitro fermentation system. Sci Rep 2023;13(1):12313. https://doi.org/10.1038/s41598-023-38849-5. PMID 37516730.

[34] Shintani T, Shintani H, Sato M, Ashida H. Calorie restriction mimetic drugs could favorably influence gut microbiota leading to lifespan extension. GeroScience 2023;45(6):3475–90. https://doi.org/10.1007/s11357-023-00851-0. PMID 37389698.

[35] Khor B, Gardet A, Xavier RJ. Genetics and pathogenesis of inflammatory bowel disease. Nature 2011;474(7351):307–17. https://doi.org/10.1038/nature10209. PMID 21677747.

[36] Machiels K, Joossens M, Sabino J, De Preter V, Arijs I, Eeckhaut V, et al. A decrease of the butyrate-producing species Roseburia hominis and Faecalibacterium prausnitzii defines dysbiosis in patients with ulcerative colitis. Gut 2014;63(8):1275–83. https://doi.org/10.1136/gutjnl-2013-304833. PMID 24021287.

[37] Rubtsov YP, Rasmussen JP, Chi EY, Fontenot J, Castelli L, Ye X, et al. Regulatory T cell-derived interleukin-10 limits inflammation at environmental interfaces. Immunity 2008;28(4):546–58. https://doi.org/10.1016/j.immuni.2008.02.017. PMID 18387831.

[38] Sasaki K, Inoue J, Sasaki D, Hoshi N, Shirai T, Fukuda I, et al. Construction of a model culture system of human colonic microbiota to detect decreased Lachnospiraceae abundance and butyrogenesis in the feces of ulcerative colitis patients. PMID 30791234 Biotechnol J 2019;14(5):e1800555.

[39] Hoshi N, Inoue J, Sasaki D, Sasaki K. The Kobe University human intestinal microbiota model for gut intervention studies. Appl Microbiol Biotechnol 2021;105(7):2625–32. https://doi.org/10.1007/s00253-021-11217-x. PMID 33718974.

[40] Yoshida N, Yamashita T, Osone T, Hosooka T, Shinohara M, Kitahama S et al. Bacteroides spp. promotes branched-chain amino acid catabolism in brown fat and inhibits obesity. iScience. 2021;24(11):103342. doi: 10.1016/j.isci.2021.103342, PMID 34805797.

[41] Yoshida N, Yamashita T, Kishino S, Watanabe H, Sasaki K, Sasaki D, et al. A possible beneficial effect of Bacteroides on faecal lipopolysaccharide activity and cardiovascular diseases. Sci Rep 2020;10(1):13009. https://doi.org/10.1038/s41598-020-69983-z. PMID 32747669.

[42] Yoshida N, Sasaki K, Sasaki D, Yamashita T, Fukuda H, Hayashi T, et al. Effect of resistant starch on the gut microbiota and its metabolites in patients with coronary artery disease. J Atheroscler Thromb 2019;26(8):705–19. https://doi.org/10.5551/jat.47415. PMID 30587666.

[43] Nagata N, Nishijima S, Miyoshi-Akiyama T, Kojima Y, Kimura M, Aoki R, et al. Population-level metagenomics uncovers distinct effects of multiple medications on the human gut microbiome. Gastroenterology 2022;163(4):1038–52. https://doi.org/10.1053/j.gastro.2022.06.070. PMID 35788347.

[44] Moossavi S, Arrieta MC, Sanati-Nezhad A, Bishehsari F. Gut-on-chip for ecological and causal human gut microbiome research. Trends Microbiol 2022;30(8):710–21. https://doi.org/10.1016/j.tim.2022.01.014. PMID 35190251.

[45] Cameron O, Neves JF, Gentleman E. Listen to your gut: key concepts for bioengineering advanced models of the intestine. Advanced Science (Weinh) 2024;11(5):e2302165. PMID: 38009508.

[46] Navarro SL, Levy L, Curtis KR, Lampe JW, Hulluar MA. Modulation of gut microbiota by glucosamine and chondroitin in a randomized, double-blind pilot trial in humans. Microorganisms 2019;7:610.

[47] Shintani T, Shintani H, Ashida H. Shift the focus of d-glucosamine from a dietary supplement for knee osteoarthritis to a potential anti-aging drug. Hum Nutr Metab 2021;26:200134.

[48] Nagpal R, Yadav H, Marotta F. Gut microbiota: the next-gen frontier in preventive and therapeutic medicine? Front Med 2014;1:15.