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Improvement of the inflammation-damaged intestinal barrier and modulation of the gut microbiota in ulcerative colitis after FMT in the SHIME® model

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Abstract

Background Fecal microbiota transplantation (FMT) seems to be a promising approach in ulcerative colitis (UC) management with the aim of repopulating a patient's dysbiotic microbiota with beneficial bacteria and restore its metabolic activity to its healthy characteristics. Metabolites present after FMT may improve the function and integrity of the intestinal barrier, reduce inflammation, and thus induce remission in an UC patient. In this study we evaluated whether the Simulator of the Human Intestinal Microbial Ecosystem (SHIME®) model may be a suitable non-invasive alternative for studying and modifying the dysbiotic microbiota in UC by FMT application.

Methods SHIME® model was used to investigate microbial and metabolic changes in the gut microbiota of UC patient induced by FMT application. FMT-modified metabolites from SHIME® were applied to an in vitro model of the intestinal barrier (differentiated Caco-2 and HT-29-MTX-E12 cell lines) compromised by pro-inflammatory cytokines to study the effect of FMT on the intestinal barrier.

Results Qualitative and quantitative microbial analyses showed that FMT increased the diversity and variability of the microbiota in UC patient associated with a significant increase in total bacteria, *Bacteroidota* and *Lactobacillus*, as well as an increase in butyrate levels. In addition, an increase in the relative abundance of some important species such as *Faecalibacterium prausnitzii* and *Bifidobacterium longum* was observed, and there was also an enrichment of the microbiota with new species such as *Blautia obeum, Roseburia faecis, Bifidobacterium adolescentis, Fusicatenibacter saccharivorans* and *Eubacterium rectale*. Furthermore, microbial metabolites modulated by FMT from the SHIME® model prevented intestinal barrier damage and inhibited interleukin 8 (IL-8) and monocyte chemoattractant protein 1 (MCP-1) secretion when cell barriers were pretreated with FMT medium for 24 h. In summary, this study confirmed that a single dose of FMT beneficially modulated the composition and metabolic activity of the UC microbiota in the SHIME® model.

Conclusions FMT favorably modulates the gut microbiota of UC patient cultured in the SHIME® model. FMT-modulated SHIME-derived microbial metabolites improve intact and inflamed intestinal barrier properties in vitro. Repeated applications are necessary to maintain the beneficial effect of FMT in SHIME® model.

Keywords Ulcerative colitis, Fecal microbiota transplantation, Dysbiosis, Intestinal barrier, Inflammation

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Introduction

Ulcerative colitis (UC) is a chronic idiopathic inflammatory bowel disease (IBD) that mostly affects adults in their 20 s–30 s. This condition is characterized by relapsing and remitting mucosal inflammation that starts distally in the rectum and extends proximally to the entire large intestine. The primary symptoms of active disease are abdominal pain, bloody diarrhea, fatigue, fecal incontinence and additionally, extra-intestinal manifestations of other organs may occur, thus strongly affecting the quality of life [1]. Aminosalicylates (5-aminosalicylic acid, 5-ASA) are the main treatment of choice for patients with mild-to-moderate UC. Glucocorticoids can be used to treat patients with UC flares or failure of response to 5-ASA treatment, while immunosuppressants and biological agents are used in moderate and severe states [2–5].

In UC, exaggerated gut mucosa reactions are triggered by the presence of microorganisms and their antigens, followed by intestinal barrier disruption and decreased levels of tight junction proteins and junctional adhesion molecules, thus promoting systemic inflammation and tissue damage with increased permeability, bacterial translocations, and undesirable exposure to luminal content. Although it is still unclear whether the disrupted intestinal barrier is a cause or consequence of microbial dysbiosis, modulation of the gut microbiota–intestinal barrier interactions is one of the targets in UC therapy [6, 7]. A dysbiotic microbiota is associated with an increased prevalence of potential pathogens, whose presence stimulates epithelial cells to produce chemokines such as interleukin- 8 (IL-8) and monocyte chemoattractant protein- 1 (MCP-1), which in turn attract neutrophils and monocytes to the mucosa and leads to acute inflammation [8]. Furthermore, permanent and abnormal stimulation of the immune cells residing in the intestinal mucosa by microbial antigens leads to the overproduction of pro-inflammatory cytokines such as interleukin- 1 (IL-1), tumor necrosis factor alpha (TNF-α), and interleukin- 6 (IL-6), which disrupts the epithelial cell layers, resulting in leaky gut syndrome in UC [9, 10]. Therefore, strategies aimed at restoring and treating inflamed and disrupted intestinal mucosa are desirable.

Over the past few years, a highly effective therapeutic alternative, fecal microbiota transplantation (FMT), has gained popularity, mainly because of its success in severe cases of several gastrointestinal diseases [11]. The most effective method in *Clostridioides difficile* infection treatment is considered to be FMT, with an efficiency of approximately 90% [12]. The aim of FMT is to repopulate the patient's dysbiotic microbiota with beneficial bacteria, which seems to be a promising approach in UC management, as it helps to restore the composition of the microbial ecosystem, and its metabolic activity to its healthy characteristics by the administration of prescreened fecal microbiota from a healthy donor (taking into account inter-individual compositional differences) to a patient with dysbiosis. Furthermore, FMT may improve the function and integrity of the intestinal barrier, reduce inflammation, and thus induce remission in patients with UC. Since FMT is associated with the risk of transfer of infectious agents, careful selection and screening of healthy donors are required to minimize the health risks that can be inflicted on the recipient [13–15]. A possible future for the application of a well-defined pathogen-free FMT, as well as repeated application of identical gut microbiota with associated metabolites to the patient, is the use of human gut microbiota cultured anaerobically in vitro. Such a comprehensive in vitro system is the Simulator of the Human Intestinal Microbial Ecosystem (SHIME®), a dynamic, highly flexible, computer-controlled model that mimics the conditions of the large intestine, enabling the cultivation of a complex, well-defined, and stable microbial community in bioreactors without loss of its metabolic activity. Furthermore, SHIME® enables non-invasive testing of novel probiotics, prebiotics, drugs, or new plant extracts [16, 17]. However, the use of this in vitro model has several limitations in comparison with clinically administered FMT; most notably, it lacks a physiological host environment, such as gut epithelium, stress factors, presence of antibodies or antimicrobial agents, and variation in diet [18, 19]. As well as the official protocol of FMT supported by studies of Mocanu et al. [20] and Ishikawa et al. [21] points out that antibiotic pre-treatment in patients with IBD is associated with improved microbial engraftment and overall response as well as induction and maintenance of remission following FMT. Despite these limitations, the overall microbial colonization profile still matches the in vivo colonization process, enabling the investigation of interactions between complex microbial ecosystems, and this fact was important for our study [17, 22]. To overcome the limitations of SHIME® at least partially, an intestinal barrier cell model composed of differentiated Caco-2 and HT-29-MTX cell lines was used to assess the impact of gut microbiota metabolites on intestinal barrier parameters. The Caco-2 cell line growing on semi-permeable membrane inserts for 14–21 days functionally differentiated into enterocytes combined with HT-29-MTX mucin-producing cells represents a reliable model of the intestinal barrier and is widely used in research on IBD, colon cancer, and other diseases associated with compromised intestinal barrier integrity and function [23, 24]. According to literature, the most physiologically appropriate ratios of Caco-2/HT-29-MTX are between 90:10 and 75:25. These ratios of differentiated cells are

characterized by a similar mucus pore size, microviscosity, and elastic modulus to those in the human intestine [25, 26].

The aim of this study was to evaluate whether the SHIME® model may be a suitable non-invasive alternative for studying and modifying the dysbiotic microbiota in UC by FMT application. The application of FMT to the SHIME® model has only been done once so far by Liu et al. [27], to monitor the efficacy of FMT to restore the vancomycin-disturbed microbiota. Furthermore, this study verifies if metabolites of UC patient's modulated microbiota after FMT treatment in the SHIME® model can support the function of a compromised intestinal barrier in vitro.

Material and methods

Human fecal samples

Biological material: fresh stool samples from a patient with severe UC (mayo score 11, female, age 27) and from clinically healthy volunteer (man, age 35) as FMT donor were collected. Stool from UC patient was processed and used as main inoculation sample of the SHIME® model. Inclusion criteria for FMT donor, ages 20 to 35 years old, with no use of antibiotics in the last 6 months, and prebiotics or probiotics, medication for gastrointestinal or metabolic disease, and dietary supplements in the last 3 months. The study and collection of biological materials were approved by the Ethics Committee of Louis Pasteur University Hospital in Košice (2018/EK/1001).

Simulator of the Human Intestinal Microbial Ecosystem (SHIME®)

The dynamic in vitro gut model for the luminal microbiota SHIME® used in this experiment consisted of five glass bioreactors with a double bottom, allowing water to circulate and ensure incubation at a defined temperature. The SHIME® model was assembled in a standard arrangement – stomach, small intestine, ascending colon (AC; pH 5.6–5.9, volume 500 mL), transverse colon (TC; pH 6.15–6.4, volume 800 mL), and descending colon (DC; pH 6.6–6.9, volume 600 mL) connected through peristaltic pumps. At the beginning of the experiment, the assembled model was supplemented with an appropriate volume of sterilized growth medium PD-NM001B (ProDigest, Belgium). Composition of the SHIME® growth medium was: 1.2 g/L arabinogalactan, 2.0 g/L pectin, 0.5 g/L xylan, 4.0 g/L starch, 0.4 g/L glucose, 3.0 g/L yeast extract, 1.0 g/L special peptone, 3.0 g/L mucin, and 0.5 g/L L-cysteine-HCl. Pancreatic juice composition was: 0.9 g/L pancreatin, 6 g/L Oxgall (ProDigest, Belgium) and 12.5 g/L $NaHCO_3$ (Sigma-Aldrich, Germany). Growth medium (140 mL) and pancreatic juice (60 mL) were automatically supplied to the system three times a day until the end of the experiment. The respective conditions in the bioreactors were controlled by software, automatically adjusting the pH with HCl or NaOH addition, and the anaerobic environment was maintained by flushing with nitrogen periodically. The inoculum was prepared from the fresh stool of a patient with UC in total volume of 40 mL in sterilized phosphate-buffered saline solution (PBS) in a 1:5 ratio and applied to bioreactors of the lower digestive tract (colon compartments only). During the first 12 days of the experiment, a stabilization phase occurred, and the inoculated gut microbiota adapted to the conditions of each bioreactor. The fecal transplant was prepared from the fresh stool of a healthy donor, mixed in sterilized PBS in a 1:5 ratio prior to the transplantation process. A volume equivalent to 5% of each of the AC, TC and DC bioreactors was injected twice on the 13th and 15th experimental days. Samples of bacterial suspensions were collected one day before the first transplantation (sample no.1 or AC1/TC1/DC1), after the first transplantation (sample no.2 or AC2/TC2/DC2), after the second transplantation (sample no.3 or AC3/TC3/DC3) and seven days after FMT treatment (sample no.4 or AC4/TC4/DC4) (Fig. 1). We collected a 35 mL sample from each bioreactor (AC/TC/DC) – 5 mL for microbial molecular analyses, 10 mL to SCFA and biochemical analyses, and 20 mL for cell culture experiments. After collection and centrifugation of the bacterial suspensions, pellets were obtained and stored at − 20 °C for microbial analyses. Supernatants were filtered through a 0.22 µm syringe filter and stored at − 80 °C. For cell culture experiments, the supernatants were diluted 1:5 in Dulbecco's modified Eagle's medium (DMEM; Sigma, Germany), according to Daguet et al. [28]. For SCFA analysis, samples were frozen and stored at − 20 °C.

Analysis of microbial communities

Bacterial DNA was extracted from the collected samples using the GRS Genomic DNA Kit—Bacteria (GRiSP, Portugal), following the manufacturer's instructions. The concentrations of extracted DNA were repeatedly recorded in triplicate using a Qubit® 3.0 fluorometer with a Qubit dsDNA HS Assay kit (Thermo Fisher Scientific, USA).

Changes in microbial community structure and composition were analyzed using molecular methods, such as denaturing gradient gel electrophoresis (DGGE), real-time quantitative polymerase chain reaction (qPCR), and next-generation sequencing (NGS).

DGGE analysis

The V3 variable region of the 16S rRNA genes was amplified using universal primers (forward 338 F with GC clamp and 518R) and Q5® Hot Start High-Fidelity DNA

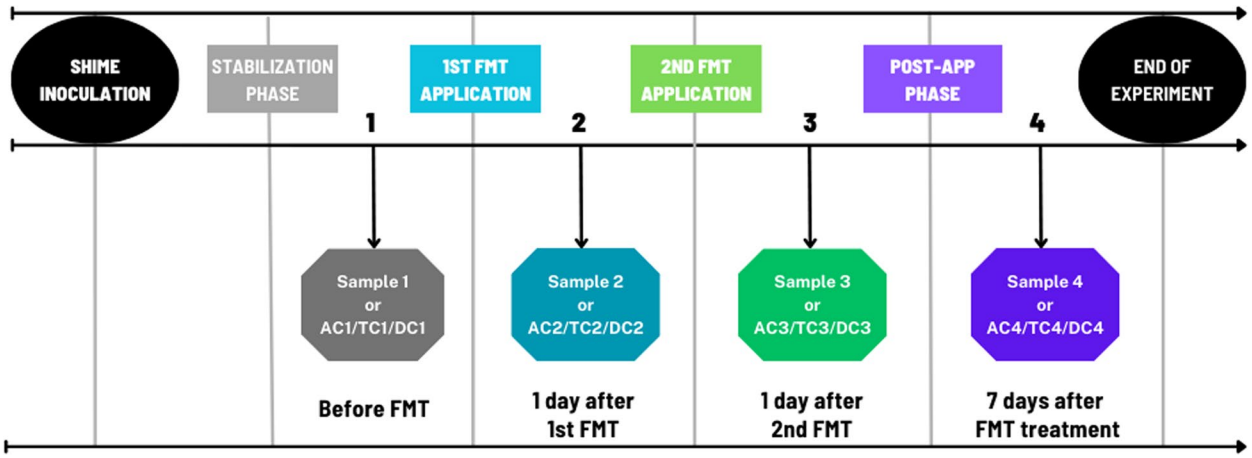

Fig. 1 Timeline of the experiment in SHIME® in vitro model. AC – colon ascendens; TC – colon transversum; DC – colon descendens; FMT – fecal microbiota transplantation

Polymerase (New England Biolabs, USA). PCR amplification was verified by standard DNA electrophoresis on agarose gel in TAE buffer solution. One-step PCR with universal primers 338 F-GC and 518R (Table S1) [29–36] was performed for total bacteria (domain *Eubacteria).* For *Bacteroidota, Lachnospiraceae, Lactobacillu*s and *Bifidobacterium* groups were used nested PCRs—first with group-specific primers shown in Table S1 and then second PCR with universal primers 338 F-GC and 518R. The PCR product from the first reaction was diluted 100-fold and then used as a template for the secondary PCR.

The amplification reactions were carried out in a total volume of 50 µL, and the reaction mixture consisted of 1× Q5 Reaction buffer, 200 µM each dNTP, 0.5 µM each primer, 0.02 U/µL Q5® Hot Start High-Fidelity DNA Polymerase, nuclease-free water and template DNA (1 µL). Amplification program included an initial denaturation at 98 °C for 2 min followed by 40 cycles of denaturation at 98 °C for 20 s, 20 s annealing step at annealing temperature (Ta) according to the primer pair (Table S1), and extension at 72 °C for 20 s and final extension step at 72 °C for 40 s. PCR was performed with the T-Personal 48 Thermocycler (Biometra, Analytic Jena, Germany). Samples were analyzed by DGGE with denaturing gradient 45–65% for *Eubacteria* and *Bacteroidota*, gradient 45–60% for *Lactobacillus* and *Bifidobacterium* and 45–55% for *Lachnospiraceae* group (100% concentration corresponds to 7 M urea and 40% (v/v) formamide). Electrophoretic separation was performed at 3 V/cm and room temperature for 16 h. The gel was then stained with a solution of 1× GelStar™ Nucleic Acid Gel Stain (Lonza, Switzerland).

Real-time qPCR analysis

Real-time qPCR amplification of 16S rRNA genes of total bacteria (*Eubacteria)*, *Bacteroidota* and *Lactobacillus* group was performed using the LightCycler® 480 SYBR Green I Master kit on a LightCycler® 480 device (Roche, Switzerland). Each 20 µL reaction mixture contained 10 µL LightCycler® 480 SYBR Green I Master 2× concentrated, 1 µL (0.5 µM) of each primer (Table S1), 3 µL PCR grade water and 5 µL template DNA (2 ng/µL). Optimized conditions for qPCR were: first step incubation for 5 min at 95 °C followed by three step PCR for 10 s at 95 °C; 10 s at primer's Ta and 8 s at 72 °C repeated 40 times and a final step „melting curve analysis " – temperature decreased from 95 °C to 65 °C and subsequently increased from 65 °C to 95 °C by 0.5 °C every 5 s. Absolute quantification was performed using a standard curve. Standard curves were generated using plasmid DNA standards carrying the entire 16S rRNA gene of *Bacteroides fragilis* (serving as a standard for *Bacteroidota* and total *Eubacteria*) or *Lactobacillus plantarum* (standard for the *Lactobacillus* group). The target gene was cloned into the pET- 21(d) +vector via a single NotI restriction site, yielding a final construct of 6924 bp. The concentration of plasmid DNA was determined using a Qubit 3.0 Fluorometer, and ten-fold serial dilutions were prepared, ranging from 1×10^1 to 1×10^8 gene copies in a 5 µL reaction volume. The standard curves were used to quantify bacterial 16S rRNA gene copy numbers in experimental samples. Data analysis was performed using LCS480 Software version 1.5.1.62.

NGS analysis

Quantitative 16S rRNA gene-targeted NGS on the Illumina platform according to Props et al. [37] was used for the sample analysis. This method is based on PCR, in which microbial sequences are amplified until the bases of the reaction are exhausted, thereby obtaining the proportional abundance (%) of different taxa at different phylogenetic levels (phylum, family, operational taxonomic unit (OTU) [38].

The 16S rRNA gene-targeted sequencing analysis included amplification of the 16S rRNA gene. The V3–V4 hypervariable regions were amplified by PCR using the standard Illumina 16S Amplicon PCR Primers (Table S1). Genomic DNA extracted from the collected samples of TC bioreactors in a volume of 40 µL was sent to the Institute of Applied Biotechnologies, a.s. (Czech Republic) for library preparation and sequencing on an Illumina MiSeq platform with v3 chemistry. Sequencing data analysis was performed using the BaseSpace Sequence Hub – Illumina tools and Microsoft Excel. DNA-seq data have been deposited in the Sequence Read Archive (SRA) and are available under accession PRJNA1127476.

SCFA analysis

The levels of SCFA (acetate, propionate and butyrate) in individual SHIME® reactors AC, TC, and DC were analyzed by gas chromatography (Agilent 7890 A; Agilent) using a capillary column DB-FFAP column (Agilent J&W GC columns Agilent, 30 m 0.53 mm 0.25 mm), with diethyl ether extraction (using 2-methyl hexanoic acid as internal standard) [39]. Helium was used as the carrier gas at a flow rate of 20 mL/min. The column temperature was set to 100 °C, and the temperatures of the injector and detector were set to 180 °C.

Model of intact and inflamed intestinal barrier

The intestinal barrier was created by co-cultivation of the human colorectal cell lines Caco-2 (ATCC, USA) and HT-29-MTX-E12 (ECACC, UK). Both cell lines were cultivated in DMEM (Sigma, Germany) supplemented with 2 mM L-glutamine (Gibco, Carlsbad, USA), 100 U/mL penicillin + 100 µg/mL streptomycin solution (Hyclone, USA), and 10% fetal bovine serum (Sigma, Germany) at 37 °C in an atmosphere of 5% CO_2. When cells reached 80% confluence, they were exposed to trypsin, counted, and seeded onto Transwell® inserts (1.12 cm^2, 0.4 µm pore size; Corning, USA) at concentration 10^6 cells/mL and seeding ratio 80:20 Caco-2:HT-29-MTX. Cells were grown on a semi-permeable membrane of inserts for 21 days to allow the differentiation and establishment of tight epithelial monolayers. Fresh culture medium was changed every three days. A cytokine cocktail (cytomix) consisting of interleukin 1 beta (IL-1β) (25 ng/mL; Abcam, UK), TNF-α (50 ng/mL; Abcam, UK), and interferon gamma (IFN-γ) (50 ng/mL; Abcam, UK) was added to the basolateral side of the well to induce inflammation and barrier dysfunction [40].

To study the effects of the FMT-modulated medium from a DC bioreactor on the intestinal barrier, three different conditions were evaluated:

(1) The FMT medium was added to the apical part of the chamber for 24 h (effect on healthy intestinal barrier).
(2) The FMT medium was added to the apical part of the chamber, and after 24 h, the medium was changed and cytomix was added to the basolateral part of the chamber for a further 24 h (preventive effect).
(3) Cytomix was added to the basolateral part of the chamber for 6 h and then replaced with fresh DMEM, and the apical part of the chamber was filled with FMT medium for a further 24 h (therapeutic effect).

Determination of Transepithelial Electrical Resistance (TEER) and permeability

The TEER was measured using an epithelial voltohmmeter EVOM2 (World Precision Instruments, USA) connected to an STX4 electrode. Only confluent monolayers of differentiated Caco- 2/HT- 29 MTX E12 cells with TEER $\leq 300\ \Omega/cm^2$ were used in the experiments. Intestinal cell barriers were treated with FMT-conditioned medium, and TEER changes were monitored at different time points according to the experimental settings. The individual TEER values were calculated by subtracting the value of the empty insert and multiplying it by the surface area of the membrane (1.12 cm^2). Then, the TEER values at 8 and 24 h were normalized to the TEER value of the insert at 0 h (to account for the differences in TEER of the different inserts). All measurements were performed in triplicate.

The permeability of Caco-2/HT-29-MTX monolayers was analyzed after 24 h of treatment by measuring the flux of the fluorescent dye lucifer yellow (LY; Sigma, Germany) from the apical to the basal compartment of the well. Cell monolayers were first washed with Hanks' balanced salt solution (HBSS; Sigma, Germany), and then 500 µL of LY solution in HBSS (40 µg/mL) was added to the apical side and 1300 µL of HBSS was added to the basal side of the well, followed by incubation on a shaker in the dark. After 1 h, the solution was collected from the basal side of the well and the fluorescence intensity was measured on a Synergy H4 hybrid plate reader (Bio-Tek Instruments, USA) using 485 nm excitation and 530 nm emission filters.

Detection of chemokines

After 24 h of FMT treatment, cell supernatants from the lower chamber were collected, centrifuged, and stored at − 80 °C for chemokine analysis. Commercially available enzyme-linked immunosorbent assay (ELISA) kits were used to detect IL-8 (Abcam, Cambridge, UK) and MCP-1

(Invitrogen, Carlsbad, CA, USA) in the cell supernatant, according to the manufacturer's instructions. The plates were read at 450 nm wavelength using an Eon™ Microplate Spectrophotometer (Bio-Tek Instruments, USA).

Immunofluorescent detection of tight junction proteins

Caco-2 and HT-29-MTX-E12 cells were seeded at a concentration of 10^5 cells/well in 4-well cultivation chambers (SPL Life Sciences Co., Korea) and grown for 2 weeks. The differentiated cell monolayers were treated with cytomix for 6 h and subsequently with FMT-conditioned medium for the next 24 h. Afterwards, media were removed, and cells were fixed with 4% formaldehyde, washed, and incubated for 1 h with blocking and permeabilization buffer (PBS containing 5% goat serum (Sigma, Germany), 0.03% Triton X (Sigma, Germany). Subsequently, the slides were incubated overnight at 4 °C with primary rabbit antibodies against zonulin- 1 (ZO-1; 1:200; Cell Signaling Technology, USA) and occludin (1:400; Cell Signaling Technology, USA), followed by incubation with Alexa Fluor® 555-conjugated secondary goat anti-rabbit antibody (1:2000, Cell Signaling Technology, USA) for 1 h at room temperature in the dark. Cells were counterstained, mounted with ProLong® Gold Antifade Reagent with 4′,6-diamidino- 2-phenylindole (DAPI; Cell Signaling Technology, USA), and observed under an Olympus BX43 fluorescent microscope.

Statistical analysis

Real-time qPCR data and differences between treatments in cell line experiments were compared using one-way ANOVA with Dunnett's or Tukey's multiple comparison test, as appropriate. Statistical significance was set at $p < 0.05$. Cell experiments were repeated at least three times in triplicate. Statistical analyses were performed using GraphPad Prism 8 software.

Results

FMT induces changes in microbial communities

PCR-DGGE was used to characterize the composition of luminal communities in the SHIME® model after FMT application. Microbial qualitative PCR-DGGE analyses at different taxonomic levels demonstrated changes in the gut microbiota composition and enrichment of new species caused by FMT, supported by the emergence of new signals (Fig. 2A). We observed change of microbial profiles and enrichment of new bands/species in total bacteria in samples 2, 3, and 4 compared to sample 1. Detailed changes are more apparent in the smaller taxonomic groups- *Bacteroidota, Lachnospiraceae, Lactobacillus* and *Bifidobacterium*. The most pronounced changes in microbial profiles are observed in the *Bifidobacterium* and *Lachnospiraceae* groups, where the number of new bands was visibly increased in samples 2/3/4 after FMT (Fig. 2A).

Quantitative analysis of samples after FMT (2: 1 day after first FMT treatment, 3: 1 day after second FMT treatment and 4: 7 days after FMT treatments) also showed a significant increase (** $= p <$ 0.01, *** $= p <$ 0.001) in total bacteria (*Eubacteria*) and bacteria of the phylum *Bacteroidota* and *Lactobacillus* group in comparison with sample 1 representing UC microbiota in SHIME® after the stabilization phase (Fig. 2B).

To confirm the qualitative changes in the gut microbiota composition of UC patient in the ideal environment, samples from TC bioreactor were analyzed by NGS 16S rRNA sequencing (Fig. 3). Mainly, *Bacillota* (*Firmicutes*), *Bacteroidota* (*Bacteroidetes*) and *Pseudomonadota* (*Proteobacteria*) phyla were present in sample 1 at levels corresponding to the UC microbial profile (Fig. 3A). The bacterial composition was substantially altered by the application of the first fresh FMT from a healthy donor, rich in bacteria from the phylum *Bacteroidota*. A decrease in *Bacillota* (49.6% → 36.7%) and a significant increase in *Actinomycetota* (*Actinobacteria*) (3.5% → 16.1%) were observed throughout the experiment. However, a slight decrease of *Bacteroidota* (38.9% → 27.5%) and *Pseudomonadota* (5.4% → 4.0%) phyla in both post-FMT samples (2 and 3), with their subsequent increase (33.7% and 10.5%, respectively) towards microbial profile of the donor in the post-application phase, was demonstrated. Negligible changes in *Verrucomicrobiota* and other phyla were also observed throughout the experiment.

Figure 3B shows that changes in the abundance and composition of dominant bacterial families after the application of fresh FMT were relevant. NGS analysis revealed an increase in the number of bacteria from *Lachnospiraceae* and *Bifidobacteriaceae* families. An increase in the relative abundance of *Oscillospiraceae* (*Ruminococcaceae*) and *Coriobacteriaceae* families after both FMT applications (samples 2 and 3) was also observed, but changes seemed to have only short-term effects according to sample 4. In contrast, there was an opposite trend in the families *Veillonellaceae* and *Bacteroidaceae* observed throughout the experiment. Furthermore, depletion of *Porphyromonadaceae* and *Enterobacteriaceae* was observed.

Figure 3C shows changes in the abundance and composition of dominant bacterial genera and illustrates the 35 main genera. The most pronounced changes are observed in the increase of the genera *Bifidobacterium, Collinsella* (both *Actinomycetota*), *Faecalibacterium* and *Clostridium* cluster IV (*Bacillota*) in samples 2, 3, and 4 in comparison with 1. On the other hand, a decrease in

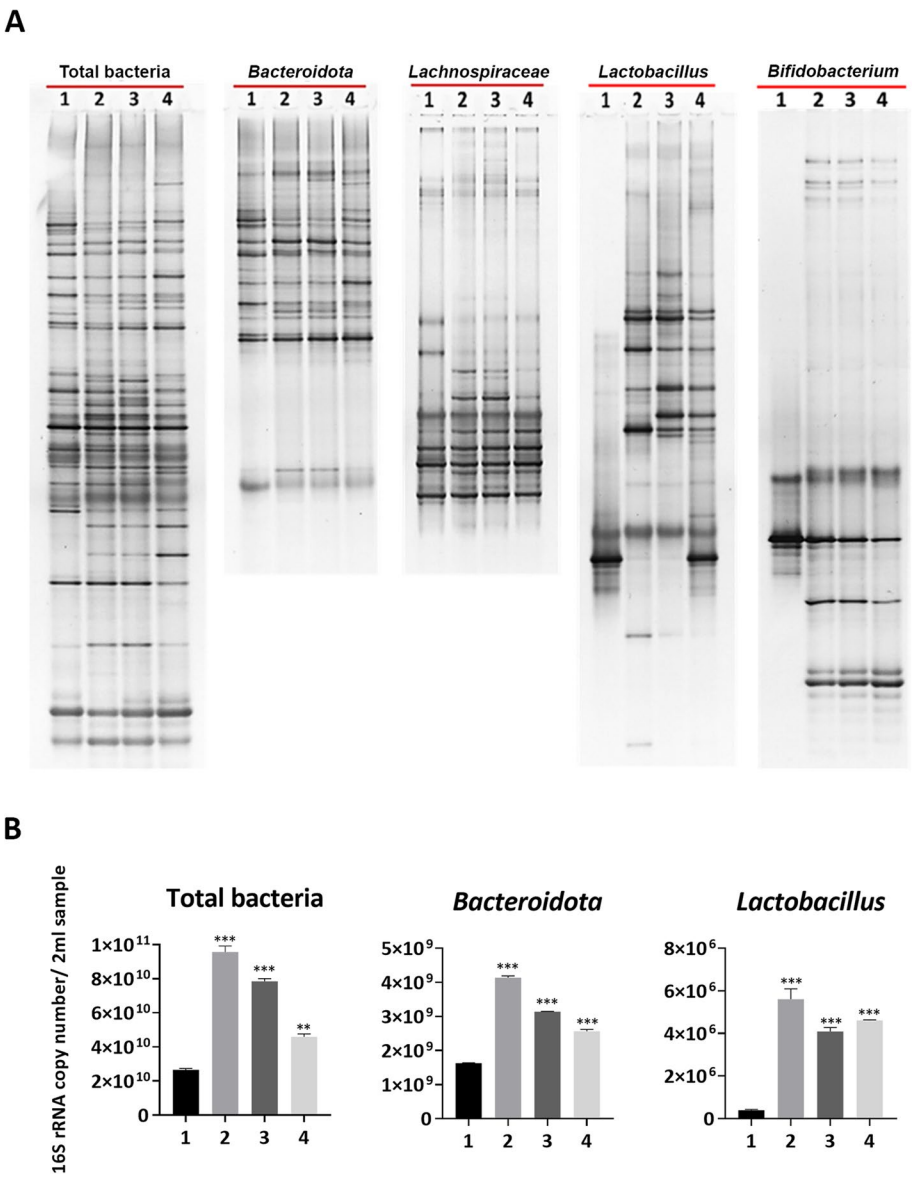

Fig. 2 Microbial profile of total bacteria and selected bacterial groups cultivated in SHIME®. **A** DGGE profiles of microbial communities of samples obtained from colon transversum reactor of SHIME® model before and after FMT applications. **B** Quantitative changes of bacterial 16S rRNA genes after FMT. One-way ANOVA with Dunnet's multiple comparison test was performed to calculate the statistical difference in comparison to sample 1 (*$p < 0.05$; **$p < 0.01$; ***$p < 0.001$) 1: UC sample before FMT treatment; 2: 1 day after first FMT treatment; 3: 1 day after second FMT treatment; 4: 7 days after FMT treatments

the abundance of the genera *Anaeroglobus, Parabacteroides, Veillonella* and *Parasutterella,* was observed.

The enrichment of new genera was observed after FMT application, from 27 genera in the sample 1 to 41 in the sample 2 (Table 1). The newly acquired genera belong to the phylum *Bacillota (Roseburia, Clostridium* cluster XVIII a XIVb, *Anaerostipes, Fusicatenibacter, Coprococcus, Lachnospiracea* incertea sedis), P*seudomonadota (Azospirillum, Desulfococcus, Sutterella*), and *Bacteroidota (Odoribacter, Barnesiella*). More detailed changes are listed in Supplementary Data Table S2.

The data in Tables 1 and 2 confirm the changes in bacterial composition, increased variability, and microbial enrichment of the analyzed samples after FMT. Table 3 shows a list of new species (OTU) in the UC microbiota obtained from a healthy donor through FMT. The identified species are especially SCFA- and

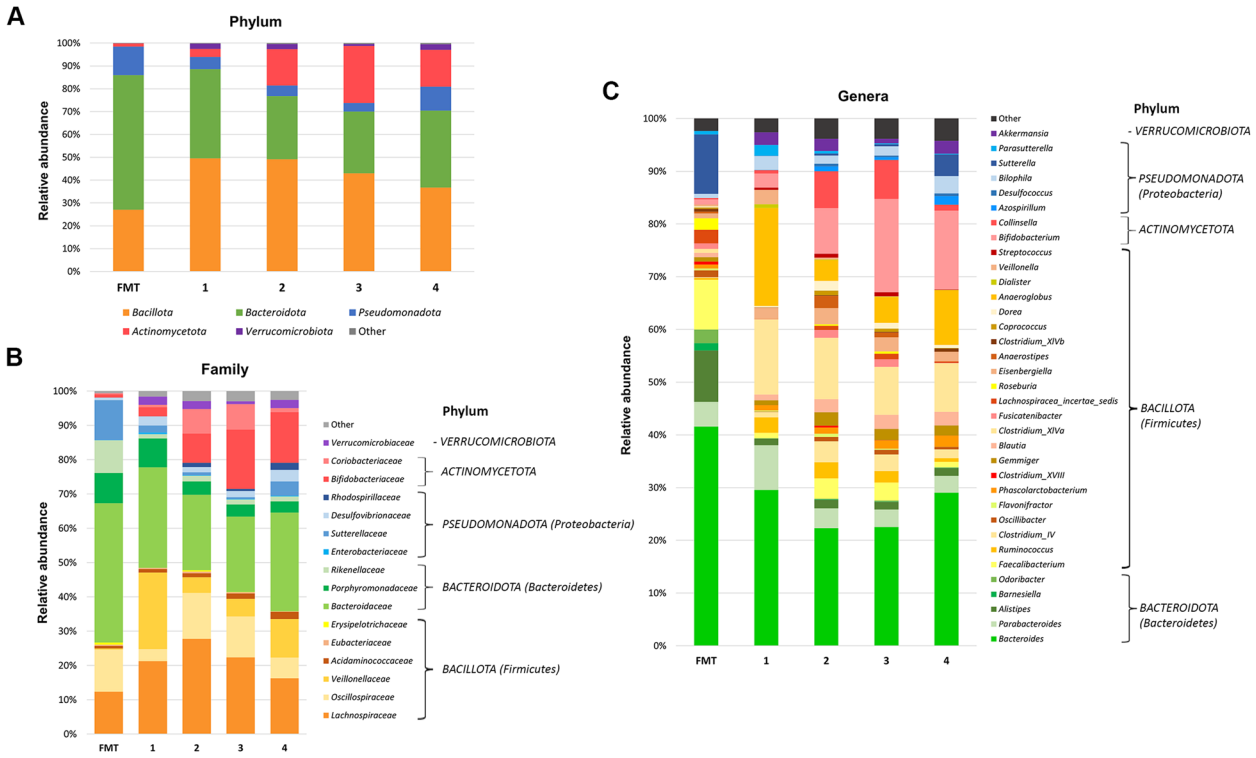

Fig. 3 16S rRNA sequencing and metagenomic analysis of gut microbiota of SHIME® samples from colon transversum. Changes in relative abundance and composition of dominant bacterial phyla (**A**), families (**B**) and genera (**C**) after FMT application. FMT – fresh fecal microbial transplant from healthy donor; 1: UC sample before FMT treatment; 2: 1 day after first FMT treatment; 3: 1 day after second FMT treatment; 4: 7 days after FMT treatments

butyrate-producing bacteria, which could provide health benefits to the recipient.

FMT induces changes in SCFA levels

Changes in SCFA levels reflect the activity of the microbial community. FMT treatments caused a gradual decrease in both acetate and propionate levels in all reactors. On the other hand, after the first dose of FMT butyrate level in the DC reactor increased significantly ($p < 0.01$), and its concentration continually increased till the end of the experiment. A significant increase ($p < 0.001$) of butyrate levels in the other two AC and TC reactors were detected after the second dose of FMT. Both FMT applications caused the levels of total short-chain fatty acids to decrease in all reactors compared with the pre-FMT sample (Fig. 4).

FMT medium improves functionality of intact and inflamed intestinal barrier

The effect of media containing microbial metabolites obtained after the first or second dose of FMT and five days after the second dose of FMT on the intact intestinal barrier was analyzed (Fig. 5A, B). All media samples after

Table 1 Changes in the number of bacterial taxa at genus and species level (major abundance > 1% and > 0.1%) in the samples after FMT

	GENUS		SPECIES	
Samples	**> 1%**	**> 0.1%**	**> 1%**	**> 0.1%**
1	15	27	21	46
2	19	41	27	71
3	18	37	27	61
4	17	36	24	59

FMT treatment (2,3,4) induced a significant ($p < 0.001$) decrease in TEER after 8 h. After 24 h, the TEER level of cell monolayers treated with FMT metabolites increased and was comparable to the negative control (DMEM treatment only). In addition, sample 2 significantly ($p < 0.01$) increased TEER compared to both the negative control and sample before FMT treatment, which was confirmed by permeability measurements. Since TEER is a nonspecific marker of barrier function, we also evaluated the effect of FMT medium on paracellular permeability by measuring the LY flux from the apical to the

Table 2 Increase of main bacterial species (OTU) abundance after FMT 1: sample before FMT treatment; 2: 1 day after first FMT treatment; 3: 1 day after second FMT treatment; 4: 7 days after FMT treatments

OTU	Family	1		2	3	4
Bacteroides vulgatus	*Bacteroidaceae*	3.7%	→	5.2%	3.0%	3.6%
Bacteroides massiliensis	*Bacteroidaceae*	3.0%	→	3.0%	3.6%	9.0%
Bifidobacterium longum	*Bifidobacteriaceae*	2.4%	→	3.1%	3.0%	0.7%
Bacteroides uniformis	*Bacteroidaceae*	1.2%	→	3.1%	4.4%	5.7%
Faecalibacterium prausnitzii	*Oscillospiraceae*	1.2%	→	4.5%	4.1%	1.2%
Gemmiger formicilis	*Eubacteriales incertae sedis*	1.0%	→	2.9%	2.6%	2.3%
Clostridium leptum	*Oscillospiraceae*	0.9%	→	4.2%	2.8%	1.8%
Collinsella aerofaciens	*Coriobacteriaceae*	0.7%	→	7.5%	8.1%	1.2%
Blautia luti	*Lachnospiraceae*	0.6%	→	1.2%	1.1%	0.7%
Eisenbergiella massiliensis	*Lachnospiraceae*	0.5%	→	2.1%	2.0%	1.4%
Bacteroides dorei	*Bacteroidaceae*	0.4%	→	1.6%	1.7%	1.6%
Bacteroides cellulosilyticus	*Bacteroidaceae*	0.1%	→	0.5%	1.5%	0.8%
Dorea longicatena	*Lachnospiraceae*	0.2%	→	2.0%	1.2%	0.6%

Table 3 List of main newly acquired taxa (OTU) after FMT treatment

OTU	FAMILY	PHYLUM
Bacteroides eggerthii	*Bacteroidaceae*	*Bacteroidota*
Bacteroides coprocola	*Bacteroidaceae*	*Bacteroidota*
Fusicatenibacter saccharivorans	*Lachnospiracea*	*Bacillota*
Bifidobacterium adolescentis	*Bifidobacteriaceae*	*Actinomycetota*
Bifidobacterium bifidum	*Bifidobacteriaceae*	*Actinomycetota*
Phascolarctobacterium succinatutens	*Acidaminococcaceae*	*Bacillota*
Blautia obeum	*Lachnospiracea*	*Bacillota*
Flintibacter butyricus	*Eubacteriales incertae sedis*	*Bacillota*
Clostridium ramosum	*Erysipelotrichaceae*	*Bacillota*
Roseburia faecis	*Lachnospiracea*	*Bacillota*
Oscillibacter sp.	*Oscillospiraceae*	*Bacillota*
Sutterella wadsworthensis	*Sutterellaceae*	*Pseudomonadota*
[Eubacterium] rectale	*Lachnospiracea*	*Bacillota*
[Eubacterium] eligens	*Lachnospiracea*	*Bacillota*
[Eubacterium] ramulus	*Lachnospiracea*	*Bacillota*
Streptococcus salivarius	*Streptococcaceae*	*Bacillota*
Ruminococcus bromii	*Oscillospiraceae*	*Bacillota*
Ruminococcus champanellensis	*Oscillospiraceae*	*Bacillota*

basal compartment of the well after 24 h of FMT treatment. Although FMT did not induce significant changes in permeability, no deleterious effects were observed after 24 h. Consistent with the TEER results, sample 1 induced an increase in permeability, whereas sample 2 (after the first dose of FMT) induced significant ($p < 0.05$) decrease in LY flux in comparison with sample before FMT (1) (Fig. 5B).

Subsequently, the preventive effect of FMT media on the inhibition of inflammation-induced intestinal barrier damage was investigated (Fig. 5C). The addition of inflammatory cytomix to all samples induced a significant ($p < 0.01$) decrease in TEER at 8 and 24 h. Pre-treatment with FMT sample 2 had a protective effect after 24 h, although TEER did not reach the TEER value of the negative control. The sample 2 also had a significant protective effect on TEER reduction compared with sample 1 (before FMT application), after both 8 and 24 h of concurrent application with cytomix. Cytomix induced a significant increase in LY flux, whereas 24 h treatment with medium after the first dose of FMT significantly ($p < 0.01$) reduced the amount of leaked LY dye (Fig. 5D).

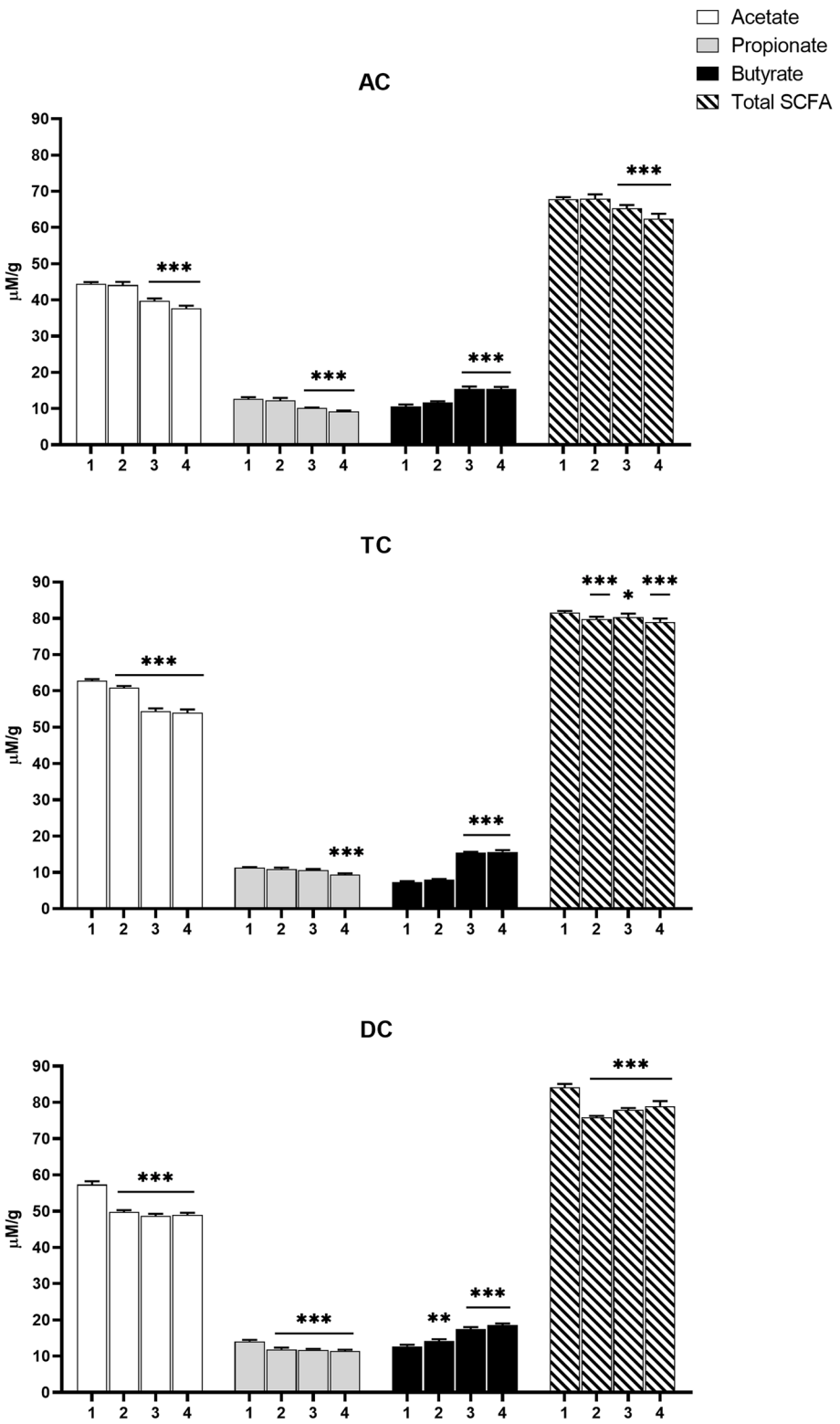

Fig. 4 Changes in concentration of SCFA (acetate, propionate, butyrate) after FMT modulation of UC microbiota in individual reactors of SHIME® model. AC – colon ascendens; TC – colon transversum; DC – colon descendens; 1: UC sample before FMT treatment; 2: 1 day after first FMT treatment; 3: 1 day after second FMT treatment; 4: 7 days after FMT treatments. One-way ANOVA with Dunnet's multiple comparison test was performed to calculate the statistical difference in comparison to sample 1 (*$p < 0.05$; **$p < 0.01$; ***$p < 0.001$)

The therapeutic effect of FMT metabolites on the intestinal barrier was analyzed after initial exposure of the barrier to cytomix for 6 h. Inflammatory cytomix reduced TEER by 20% from the initial value 6 h after the inflammatory stimulus (80.45 ± 5.25% vs 99.77 ± 4.01%), and such compromised cells were treated with FMT media (Fig. 5E). After 24 h, none of the FMT-treated monolayers showed a significant improvement in TEER. The same results were confirmed by the paracellular permeability analysis. Cytomix induced a significant ($p < 0.001$) increase in LY permeability compared with the untreated cell monolayer (C-), and only sample 2 showed a non-significant decrease in LY flux compared with cytomix (Fig. 5F).

The secretion of chemokines IL-8 and MCP-1 into the culture medium was measured to determine the effect of FMT media on the inflammatory response of intestinal cells to cytomix (Fig. 6). IL-8 and MCP-1 were not detected in untreated cells (negative control), but increased chemokine levels were detected in cytomix-treated monolayers. Media after the first and second dose of FMT (sample 2 and 3) significantly inhibited the secretion of both IL-8 ($p < 0.001$) and MCP-1($p < 0.01$) in comparison to the cytomix-treated cell monolayers when the barrier was pretreated with media for 24 h. Interestingly, treatment of the intestinal barrier with medium before FMT application (sample 1) did not induce any changes in comparison with cytomix treatment alone.

Effect of FMT-modulated microbial metabolites on tight junction proteins

The effect of FMT-modulated microbial metabolites from SHIME® on the tight junction proteins ZO-1 and occludin was studied as a possible mechanism involved in protection from inflammatory damage. The results in Fig. 7 show that in the untreated monolayer of Caco-2/HT-29-MTX cells, the immunofluorescence signals of ZO-1 and occludin were localized at the apical membrane junctions and appeared as continuous structures surrounding the cell. Treatment with pro-inflammatory cytomix for 6 h induced disruption of these structures. After the treatment of Caco-2/HT-29-MTX cell monolayers with the first dose of FMT (sample 2), a slight upregulation of occludin expression was observed compared to cytomix-treated cells, whereas ZO-1 expression was not affected by FMT treatment.

Discussion

The therapeutic manipulation of gut microbiota, known as FMT, is of great interest in UC therapy. Supported by ongoing research, FMT seems to be a reliable and relatively safe treatment concept. However, multiple factors affect the success of FMT, such as route of administration, precise donor selection, as well as repeated repopulation of the gut microbiota of UC patients is required in order to induce long-term effects. FMT changes the composition of the gut microbiota of patients, thus rapidly re-establishing the dominance of favorable bacteria, which can alleviate the symptoms of the disease and support its treatment. While FMT has been shown to achieve prolonged and accelerated clinical outcomes compared to conventional treatments, the available trial data are not entirely consistent, reflecting the influence of the aforementioned factors [41–44].

The aim of this study was to evaluate whether the SHIME® model may be a suitable non-invasive alternative for studying and modifying the dysbiotic microbiota in UC by FMT application. Until recently, the SHIME® model was mainly used to monitor the effect of dietary supplements, prebiotics or probiotics and drugs on changes in the gut microbiome and metabolome [16, 28, 45]. The application of FMT to the SHIME® model has only been done once so far, to monitor the efficacy of FMT to restore the vancomycin-disturbed microbiota [27]. Thus, in our study, the efficacy of FMT to alter the microbiota of the UC patient in SHIME® model was analyzed. Furthermore, the supportive effect of FMT modulated metabolome was tested on the intact and inflammation-damaged intestinal barrier in vitro.

Results provide evidence that SHIME® model is a non-invasive option in the study of UC management. After the application of FMT, the composition of gut bacteria changed, and their diversity and abundance increased. The gut microbiota changes observed were immediate

(See figure on next page.)

Fig. 5 The effect of FMT-modulated microbial metabolites from SHIME® model on changes in TEER and permeability of the intact and inflamed intestinal barrier. Changes in TEER (**A**) and paracellular permeability (**B**) of intact intestinal barrier treated with FMT-modulated microbial metabolites. The preventive effect of FMT-modulated microbial metabolites on the changes in TEER (**C**) and paracellular permeability (**D**) of intestinal barrier exposed to pro-inflammatory cytokine cocktail (cytomix) for 24 h. E) Therapeutic effect of microbial metabolites on the changes in TEER (**E**) and paracellular permeability (**F**) of intestinal barrier exposed to pro-inflammatory cytokine cocktail for 6 h and then treated with FMT medium for 24 h. Barrs represent mean ± SD of three independent experiments. One-way ANOVA with Tukey's multiple comparison test was performed to calculate the statistical difference (*$p < 0.05$; **$p < 0.01$; ***$p < 0.001$). *—comparison to control cells; # comparison to cytomix treated cells; + comparison to sample 1. C- control, untreated cells; Cytomix – cell treated with cocktail of cytokines IL-1β, TNF-α, IFN-γ; samples: 1: before FMT treatment; 2: 1 day after first FMT treatment; 3: 1 day after second FMT treatment; 4: 7 days after FMT treatments

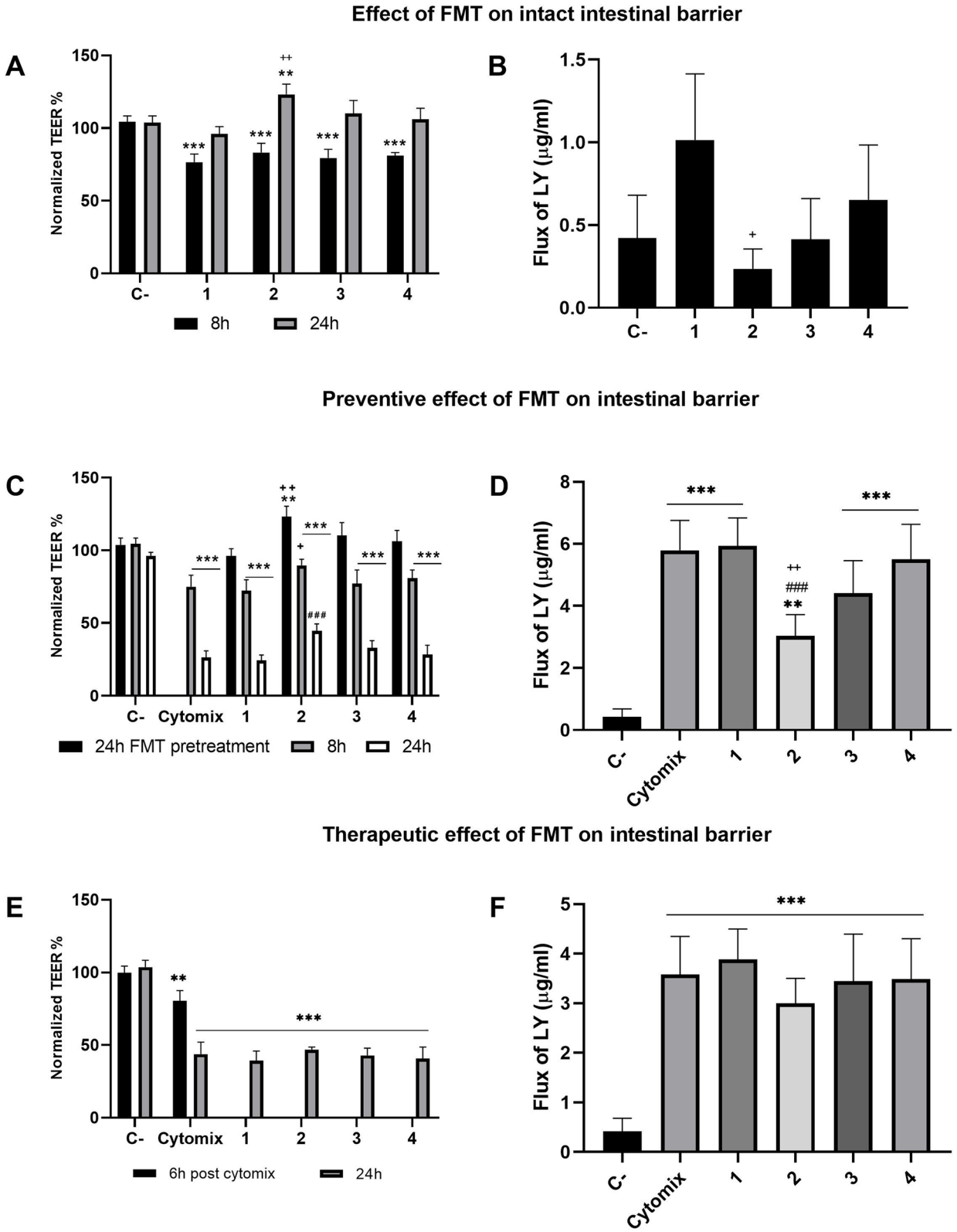

Fig. 5 (See legend on previous page.)

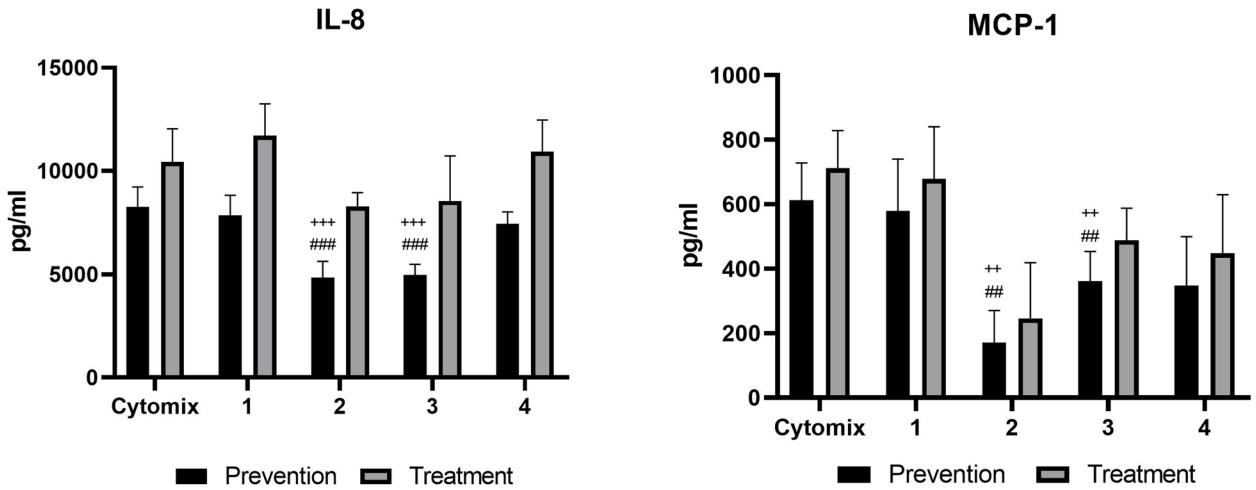

Fig. 6 The preventive and therapeutic effect of the FMT-modulated microbial metabolites from SHIME® model on the secretion of chemokines IL-8 and MCP-1. Barrs represent mean ± SD of three independent experiments. One-way ANOVA with Tukey's multiple comparison test was performed to calculate the statistical difference (*$p < 0.05$; **$p < 0.01$; ***$p < 0.001$). # comparison to cytomix treated cells; + comparison to sample 1; Cytomix – cell treated with cocktail of cytokines IL-1β, TNF-α, IFN-γ; samples: 1: before FMT treatment; 2: 1 day after first FMT treatment; 3: 1 day after second FMT treatment; 4: 7 days after FMT treatments

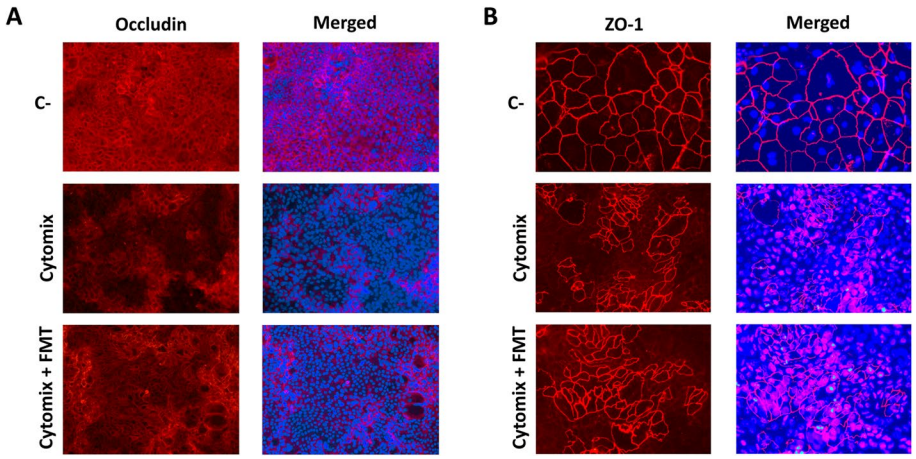

Fig. 7 Immunofluorescence localization of tight junction proteins occludin (**A**) and zonulin 1 (ZO-1) (**B**) after 24 h treatment with FMT-modulated microbial metabolites on differentiated co-culture of Caco-2/HT-29-MTX pretreated with cytomix for 6 h. Representative images of immunofluorescently labelled tight junction proteins occludin (**A**) and ZO-1 (**B**) (red). Nuclei of cells were stained with DAPI (blue); magnification 400x

but short-term in nature. These findings are supported by many studies that have shown that the sustainability of changes is not permanent unless repeated FMT administration is applied [43, 46–48]. Patients with UC can be distinguished from healthy individuals by the dysbiotic state present with a lower total bacterial count and reduction in bacterial diversity, which is attributable to decreased *Bacillota* (specifically *Lachnospiraceae*) and *Bacteroidota* compared to healthy controls [49, 50]. Real-time qPCR analyses showed a significant increase in the number of total bacteria, as well as *Bacteroidota* and *Lactobacillus* spp. (*Lactobacillaceae*) in the sample after the first FMT application, with slight reductions throughout the experiment. Ishikawa et al. [21] and Blanchaert et al. [51] also showed post-FMT increase and recovery of *Bacteroidota* and *Clostridium* clusters IV and XIVa. After the first FMT application increased microbial diversity and variability, particularly *Lachnospiraceae* family as a part of *Clostridium* cluster XIVa was enriched by approximately 30%, and new OTUs, such as *Fusicatenibacter saccharivorans*, *Blautia obeum*, *Roseburia faecis*, *Eubacterium rectale* and *Eubacterium eligens* were identified in the samples by

16S rRNA metagenomic analysis. Members of the *Lachnospiraceae* family are known mainly for butyrate and other SCFA production, thus playing an important role in the maintenance of intestinal homeostasis. SCFA are the major metabolites produced during fermentation of indigestible dietary fibers in the cecum and colon by beneficial microbes and they are also important for host health, maintenance of the intestinal epithelial barrier, anti-inflammatory activity in the mucosa, and serve as energy source for colonocytes. Thus, microbial changes observed in patients with UC are also associated with altered SCFA content, especially butyrate and propionate [52–54]. Takeshita et al. [55] and Vermeiren et al. [56] observed a significant reduction in the abundance of *F. saccharivorans* and *E. rectale* in active UC, supported by lower SCFA concentrations in the feces of patients with UC than in healthy individuals. It has been confirmed that *F. saccharivorans* suppresses intestinal inflammation, thus preventing UC through SCFA production in humans and furthermore, heat-killed *F. saccharivorans* was able to suppress colitis in colitis mouse models. Modulation of the gut microbiota towards an increase of SCFA producers represents a desirable strategy in the treatment of UC. Furthermore, administration of butyrate alone to patients with UC appears to be effective adjuvant therapy, even in patients resistant to standard therapy [57–59]. Although our data showed that FMT applications helped to increase the number of butyrate-producing bacteria in the UC environment, thus positively shifting the gut microbiota composition towards a healthy one, it is probably necessary to incorporate the simulation of the mucus layer in order to maintain the observed changes, for example, by using mucosal SHIME (M-SHIME®) which supports the growth of bacteria living in mucus. However, the overall colonization profile of several dynamic models that work with luminal content still matches the in vivo colonization process [17]. Similarly, propionate exhibits anti-inflammatory and antimicrobial properties and stimulates antimicrobial compounds such as mucosal regenerating islet-derived lectins- 3, making it a valuable compound in the treatment of UC [60]. Propionate is synthesized through three different biochemical pathways. Its elevated concentration is associated with the presence of *Clostridia* species, as well as *Akkermansia muciniphila* and several bacteria from *Bacteroidota* [61], which were also increased in our study after FMT. However, we observed a slight reduction in propionate in all reactors after FMT application. This observation is supported by the fact that SCFA production requires at least 15 days, even approaching 20 days, to adapt to the prevailing environmental conditions in the SHIME® model [17].

Other post-FMT identified SCFA- producers were *Roseburia* spp., *E. eligens* and *Blautia* spp., which belong to microbial species whose altered levels help to predict health status and disease activity in IBD. They are often associated with a healthy state and are potential functional microorganisms with probiotic properties [62–65]. On the other hand, the identification of increased relative abundance of *Sutterella wadsworthensis* in UC patients, which also reappeared in our post-FMT samples, may play an important role in the response to FMT treatment, as a predictor of ineffective donor doses that are unable to induce stable remission [66]. The most frequently used bacteria for probiotic purposes are lactic acid bacteria belonging to lactobacilli and bifidobacteria, and the *Bifidobacterium* genus was significantly increased after FMT treatment in our study. Many studies have suggested that *Bifidobacterium* spp. supplementation has a strain-specific health-promoting effect on a host and significantly reduced levels of *Bifidobacterium adolescentis* and *Bifidobacterium bifidum* were found in UC patients in comparison with healthy controls [67–69]. Animal studies using colitis models have shown that administration of both *B. adolescentis and B. bifidum* alleviate colitis symptoms, reduce diarrhea scores and spleen weight, increase colon length associated with a rearrangement of the species diversity of the gut microbiota (reduced *Pseudomonadota*, elevated *Clostridia*) [70], ameliorate intestinal inflammation [68, 71] and improve intestinal barrier functions [72]. We also identified post-FMT reappearance and abundance of *Bacteroides coprocola*, which is prominently reduced in UC patients. Cuffaro et al. [73] showed that strain *B. coprocola* AS101 exhibits characteristics that are able to reduce inflammation and improve intestinal barrier function.

Disruption of the gut microbiota ecosystem has many consequences, including disruption of the intestinal barrier and imbalance in the host immune and metabolic systems. Since microbial changes alone may not be sufficient to induce remission of UC, we investigated whether FMT-modulated microbial metabolites could also improve intestinal barrier parameters. Therefore, in this study, following FMT modulation of the gut microbiota of a UC patient, we applied SHIME-derived microbial metabolites to a model of an intact and inflamed intestinal barrier to observe simulated interactions with the host epithelium. In the sample after the first dose of FMT, there was an increase in the number of the families *Lachnospiraceae* and *Bifidobacteriaceae* involving butyrate producers mentioned above, and this modulated microbial medium was able to improve the properties of the intact intestinal barrier, such as TEER and permeability. When the medium obtained after FMT was applied prophylactically, it had a protective effect against the negative action of the pro-inflammatory cytokines IL-1β, TNF-α, and IFN-γ, which was reflected by a higher

TEER, reduced permeability, and inhibition of the secretion of the chemokines IL-8 and MCP-1. However, this effect was attenuated after the second dose of FMT. The reduced efficacy of the FMT media over time could be partly due to decreased concentration of total SCFA including propionate. Although butyrate levels increased over the course of the experiment, the protective activity of the FMT-modulated medium decreased. The reason for the reduced beneficial effect of the medium may also be due to the absence of immune cells in the intestinal barrier model, which are directly modulated by butyrate toward the differentiation of immunoregulatory T cells [74]. In addition, our results suggest that increasing butyrate levels alone only partially mitigates inflammation-induced intestinal barrier damage, and other microbial metabolites or interactions with the microbiota itself may be necessary for this effect [75]. Nevertheless, the first dose of FMT induced sufficient changes to alter the microbiota and its metabolism, which favorably protected the intestinal barrier from damage, having a preventive effect. This is in accordance with other studies confirming the protective effects of FMT on inflamed mucosa [76–79]. Li et al. [80] showed that transplantation of a bacterial consortium derived from healthy mice into DSS-treated mice led to a decrease in intestinal permeability and improvement in gut dysbiosis, particularly due to an increase of the genera *Bacillus* and *Bifidobacterium* abundance. The alleviation of disease symptoms was associated with an increase in the number of γδT cells in the intestinal lamina propria, which is a major source of gut-protective IL-17, important for the restoration of disrupted intestinal epithelium associated with the regulation of subcellular localization of occludin [81]. Huang et al. [82] suggested that FMT and associated enrichment of *Faecalibacterium prausnitzii* might explain the improvement in the imbalance of the Th17/Treg axis and alleviated intestinal inflammation in the responders'group. In our study, the improvement of the inflamed intestinal barrier could be associated with the species *F. prausnitzii, Bifidobacterium longum, Bacteroides vulgatus, Clostridium leptum* (cluster IV), *Blautia luti, Eisenbergiella massiliensis, Dorea longicatena, Roseburia faecis, Eubacterium rectale, Eubacterium eligens, or Ruminococcus bromii,* which were increased after first dose of FMT. Microorganisms identified in our study, potentially responsible for the improvement of inflammation-compromised intestinal barrier in UC patients, may also lead and represent a foundation for the following identification and development of potential next-generation probiotics targeting this group of patients. As well as, administration of "cocktail" comprising the right combinations and levels of specific microorganisms and metabolites, pre-analyzed in suitable in vitro model or combination of models simulating the human gastrointestinal tract, instead of whole microbiota transplantation could be safer option.

While the SHIME® model provides valuable insights into the microbial dynamics within the human gastrointestinal tract, it is important to acknowledge certain limitations associated with its use. The main significant limitation is that the SHIME® model lacks several physiological features present in a living human system, such as the intestinal epithelium and immune system. These components play a critical role in gut immunity and epithelial barrier function, which are important in the pathophysiology of UC. The absence of these physiological factors could potentially influence the interactions between the gut microbiota and the host immune system, limiting the model's ability to fully replicate the complexity of UC in vivo. To overcome these limitations, a cellular model of the gut barrier was used in this study, simulating the interplay between gut microbiota and epithelium under conditions of inflammation and partially simulating the pathogenesis of UC. Furthermore, different kind of stressors (acute, chronic, hormonal, etc.) can affect gut microbiota composition and their metabolites. The gut microbiota produces and releases various metabolites, which are then processed and converted into final products in organs throughout the body. This process involves a complex interplay between the gut microbiota, the host's immune system, and various organs such as liver or pancreas. Thus, absence of these organs and whole immune system in SHIME® model limits us to study only direct products of gut microbiota's metabolism. Additionally, the SHIME® model does not account for factors such as dietary variations and host-specific genetic factors, all of which can significantly affect the gut microbiota in UC patients. Therefore, while the SHIME® model offers a controlled and reproducible environment to study microbial behavior, absence of these important physiological elements should be considered when interpreting the results. Despite these limitations, the SHIME® model remains a useful tool for investigating microbial interactions and offers valuable insights into gut microbial dynamics in UC patients. Furthermore, SHIME® model could potentially be used as a non-invasive method to test the donor-recipient match based on colonization effectiveness and changes in metabolite levels and profiles, because one of the important parameters that should be evaluated is gut microbiota compatibility check, because incompatibility may cause graft ineffectiveness or the need for repeated application of FMT numerous times, thus leading to poor transplant success rates.

Conclusion

In summary, our pilot study showed that a single dose of FMT favorably modulated the composition and metabolic activity of the UC microbiota in the in vitro SHIME® model. In addition, FMT-modulated metabolites also improved important properties of the intact and cytomix-impaired intestinal barrier, with concomitant attenuation of pro-inflammatory chemokine secretion. However, repeated administration of FMT is required to maintain the beneficial changes achieved in the SHIME® model.

Abbreviations

5-ASA	5-Aminosalicylic acid
AC	Ascending colon
C-	Untreated cell monolayer
DAPI	4′,6-Diamidino-2-phenylindole
DC	Descending colon
DGGE	Denaturing gradient gel electrophoresis
DMEM	Dulbecco's modified Eagle's medium
ELISA	Enzyme-linked immunosorbent assay
FMT	Fecal microbiota transplantation
HBSS	Hanks' balanced salt solution
IBD	Inflammatory bowel disease
IFN-γ	Interferon gamma
IL-1	Interleukin-1
IL-1β	Interleukin 1 beta
IL-6	Interleukin-6
IL-8	Interleukin-8
LY	Lucifer yellow
MCP-1	Monocyte chemoattractant protein-1
NGS	Next generation sequencing
OTU	Operational taxonomic unit
PBS	Phosphate-buffered saline solution
qPCR	Quantitative polymerase chain reaction
SCFA	Short-chain fatty acids
SHIME®	Simulator of the Human Intestinal Microbial Ecosystem
SRA	Sequence Read Archive
TC	Transverse colon
TEER	Transepithelial Electrical Resistance
TNF-α	Tumor necrosis factor alpha
UC	Ulcerative colitis
ZO-1	Zonulin-1

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12906-025-04889-9.

Supplementary Material 1.

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Authors' contributions

Conceptualization - AK, MK, JŠ, Methodology – AK, LA, RL, IB, ZH, EH, JŠ; Data interpretation – AK, LA, MK, ZH, MJ, JŠ; Clinical samples collection – MJ; Writing – original draft preparation – AK, LA, MK, RL, JŠ; Writing – review and editing – AK, MK, ZH, JŠ; Project administration – IB, JŠ; Visualization – MK. All authors reviewed the manuscript.

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Data availability

DNA-seq data have been deposited in the Sequence Read Archive (SRA) and are available under accession PRJNA1127476.

Declarations

Ethics approval and consent to participate

All subjects gave their informed consent for inclusion before they participated in the study. The study was conducted in accordance with the Declaration of Helsinki 1964 and its later amendments or comparable ethical standards. The study protocol was approved by the Ethics Committee of Louis Pasteur University Hospital in Košice (2018/EK/1001). Clinical trial number: not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare no competing interests.

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