Effects of different treatment of faecal microbiota transplantation techniques on ulcerative colitis in rats

Fangyuan Zhu
Zhejiang Chinese Medical University
Yifan Ke
Zhejiang Chinese Medical University
Yiting Luo
Zhejiang Chinese Medical University
Jiaqian Wu
Zhejiang Chinese Medical University
Pei Wu
Zhejiang Chinese Medical University
Fangxiao Ma
Zhejiang Chinese Medical University
Yingchao Liu (jcjsylyc@163.com)
Zhejiang Chinese Medical University https://orcid.org/0000-0002-1854-8432

Research

Keywords: frozen faecal microbiota transplantation, fresh faecal microbiota transplantation, ulcerative colitis, intestinal flora

Posted Date: February 24th, 2021

DOI: https://doi.org/10.21203/rs.3.rs-227264/v1

Abstract

Background: Ulcerative colitis (UC) is a chronic non-specific inflammatory bowel disease with abdominal pain, mucus, pus, and blood in the stool as the main clinical manifestations. The pathogenesis of UC is still not completely clear, and multiple factors such as genetic susceptibility, immune response, intestinal microecological changes, and environmental factors together lead to the onset of UC. In recent years, the role of intestinal flora disturbance on the pathogenesis of UC has received widespread attention. Therefore, fecal microbiota transplantation(FMT), which changes the intestinal microecological environment of UC patients by transplantation of normal fecal bacteria, has attracted increasing attention from researchers. However, there are no guidelines at home and abroad to recommend fresh FMT or frozen FMT in the treatment of UC, and there are a few studies on this. Therefore the purpose of this experiment was to explore the effects of fresh and frozen fecal microbiota transplantation methods on the treatment of experimental ulcerative colitis models in rats.

Results: Compared with the model control group, all faecal microbiota transplantation groups achieved better efficacy, mainly manifested as weight gain by the rats, improvements in faecal characteristics and blood stools, reduced inflammatory factors, and normal bacterial flora. The efficacy of the frozen faecal microbiota transplantation group was better than that of the fresh faecal microbiota transplantation group in terms of behaviour and colon length .

Conclusions: FMT is a feasible method for treating UC. The mechanism of action may be via competitive inhibition of pathogenic microorganisms, improved immune metabolism, and reduced inflammatory response to mitigate the damage to the intestinal barrier and cause UC remission. Compared with fresh FMT, the therapeutic effect of frozen FMT may be greater.

Background

Ulcerative colitis (UC) is a chronic non-specific inflammatory bowel disease involving abdominal pain, mucus, pus, and blood in the stool as the main clinical manifestations (1, 2). The pathogenesis of UC is not completely clear, and multiple factors such as genetic susceptibility, immune response, intestinal microecological changes, and environmental factors together lead to the onset of UC (3). In recent years, the role of intestinal flora disturbance on the pathogenesis of UC has received widespread attention, as disruption of the gut bacteria can destroy the intestinal mucosa; influence T cell subgroup differentiation; cause imbalances in Thelper (Th)l, Th2, and Th17, and regulatory T cells; and lead to secretion of a large number of inflammatory mediators, such as interleukin (IL)-1β, IL-5, IL-6, IL-17, and tumour necrosis factor alpha (TNF-α) (3, 4). Additionally, changes in intestinal immune functions can occur, leading to UC and potentially to further complications. Changes in various intestinal flora genera play a key role in the incidence of UC (5, 6). Thus, maintaining the balance of the intestinal flora is essential for alleviating the symptoms of UC. Therefore, faecal microbiota transplantation (FMT), which changes the intestinal microecological environment of patients with UC via transplantation of normal faecal bacteria, has attracted increasing attention (7, 8).

At present, FMT is mainly used to treat diseases such as *Clostridium difficile* infectious diarrhoea (9, 10), inflammatory bowel disease (7, 11), irritable bowel syndrome (12, 13), and non-alcoholic fatty liver (14, 15). FMT has a clear effect on recurrent *C. difficile* infectious diarrhoea and is recommended by the European Society of Microbiology (16). The Joint Guidelines of the British Society of Gastroenterology and the Medical Infectious Society recommend that frozen FMT materials used to treat *C. difficile* infection should be considered as superior to fresh FMT preparations (level of evidence: high; recommended strength: strong) (17). However, there are no guidelines for recommending fresh FMT or frozen FMT in the treatment of UC, and these treatments have not been widely examined. Therefore, we explored whether fresh and frozen FMT methods were effective for treating experimental UC model rats.

Results

Changes in general conditions of rats in each group

To evaluate the effect of FMT in chronic intestinal inflammation conditions similar to in human patients with inflammatory bowel disease, experimental enteritis in rats was induced by administering 2,4,6-trinitrobenzenesulfonic acid (TNBS). After starting TNBS treatment, faeces from normal rats were repeatedly administered to UC model rats by gavage. Figure 1A shows the curve of the average body weight in each group. Before developing the TNBS-induced UC models, there was no significant difference between groups ($P > 0.05$). After model creation for 72 h, the weight of the rats in each model group decreased to a similar extent, with differences observed between the normal group and other groups ($P < 0.05$). After intervention, there were significant differences in body weight between the frozen FMT group, mesalazine group, and UC model group ($P < 0.05$), whereas there was no significant difference between the UC model group and fresh FMT group. There was no difference among the fresh FMT, frozen FMT, and mesalazine groups ($P > 0.05$).

After model development, each group began showing various degrees of malaise, arched back, yellow coat, diarrhoea, mucus pus, and blood in the stool, accompanied by slow weight gain or even weight loss. After intervention, the activity status, bloody stool, and diarrhoea of the rats improved. The disease activity index (DAI) score is shown in Figure 1B.

We also measured the colon length. As shown in Figure 1D, the colons of each group showed varying degrees of dilatation, oedema, and even bleeding ulcers after model creation. However, dilation and oedema of the colon of rats in the three different intervention groups were not as obvious as those in the model group, and there was no obvious ulcer bleeding. According to the colon length statistics, as shown in Figure 1C, only the normal group significantly differed from the other four groups ($P < 0.05$). The remaining four groups showed no significant differences ($P > 0.05$).

Pathological changes and scores of colonic tissues of rats in each group

The colon tissue of rats in the normal group showed a complete colonic epithelium, regular crypt structure, and small amount of inflammatory cell infiltration. The pathological changes in rats in the UC

model group were as follows: obvious erosion and ulceration was observed in the mucosal epithelium; the number and structure of epithelial crypts were changed, and their arrangement was disordered; goblet cells were significantly reduced; high inflammatory cell infiltration was observed in the lamina propria, and a large amount of inflammatory cell infiltration and oedema was observed in the submucosa. However, in pathological sections of the fresh and frozen FMT groups, inflammatory infiltration and destruction of the intestinal wall were milder than those in the UC model group.

Levels of inflammatory factors in each group of rats

According to the intervention results of each group, treatment with FMT reduced intestinal inflammation. The expression of TNF-α in the colon of the two FMT groups was significantly lower than that in the model group ($P < 0.05$) and returned to the level in normal rats. There was no significant difference between the fresh and frozen FMT groups ($P > 0.05$).

FMT treatment improves intestinal flora in UC rats

The pair end reads obtained by Miseq sequencing were first spliced according to the overlap relationship between the sequences, and the sequence quality was controlled and filtered. The samples are distinguished, after which cluster analysis and species taxonomy analysis were performed. Cluster analysis showed that various diversity index analyses could be performed and the depth of sequencing could be detected. Based on taxonomic information, community structure statistics were performed at each classification level.

Data corresponding to the richness and diversity of the gut microflora are shown in Figure 4. The Chao1 index and Shannon index showed that FMT increased alpha diversity ($P < 0.05$).

Each group of samples contained 17 known phyla from the kingdom of bacteria with an abundance of more than 0%, namely Acidobacteria, Actinobacteria, Armatimonadetes, Bacteroidetes, Chloroflexi, Cyanobacteria, Deferribacteres, Elusimicrobia, Epsilonbacteraeota, Firmicutes, Fusobacteria, Gemmatimonadetes, Nitrospirae, Patescibacteria, Proteobacteria, Tenericutes, and Verrucomicrobia.

The horizontal analysis of the phylum graph showed that Firmicutes, Bacteroidetes, Actinomycetes, and Proteobacteria were predominant (Figure 5). After TNBS-induced induction of UC, the intestinal flora of rats differed from that of normal rats at the phylum level. The difference was manifested as a decreased abundance of Bacteroidetes, significantly increased relative abundance of Firmicutes, and increased abundance of Actinomycetes and Proteobacteria. After different interventions, at the phylum level, there were significant differences between the frozen FMT group and UC model group. The specific performance was as follows: the relative abundance of Firmicutes decreased, whereas the relative abundance of Actinobacteria and Proteobacteria decreased but that of Bacteroides increased. The fresh FMT group showed a similar trend but did not significantly differ from the UC model group ($P > 0.05$).

At the genus level, the abundance comparison of a single species in each sample group is shown in Figure 6, and the Wilcoxon fit rank test was used to detect differences between groups. This study

showed that after model creation, the abundance of *Bacteroides*, *Christensenellaceae_R_7_group*, *Fusicatenibacter*, and *Allobaculum* increased, whereas *Prevotellaceae_NK3B31_group*, *Ruminococcaceae_UCG_013*, *Ruminococcaceae_UCG_014*, and *Eubacterium_coprostanoligenes_group* decreased. After FMT intervention, the abundance of *Prevotella_9* increased, and the relative abundance of *Coprococcus_2* and *Subdoligranulum* decreased.

In terms of β diversity, following intervention, the similarity of the composition of the fresh and frozen FMT group sample communities became more consistent with the normal group, as observed by principal coordinate analysis (Figure 7). In the community column chart shown in Figure 9, the composition of the colonic microflora also showed similar results: the colonic microflora composition of the frozen and fresh FMT groups gradually trended toward that in the normal group after intervention, which continued after stopping the intervention for one week.

To identify the specific types of bacteria altered by treatment, we performed linear discriminant analysis effect size (LEfSe) analysis to determine the characteristics of different groups of the gut microbiota. The clade map produced by LEfSe analysis revealed species with significant differences in abundance between groups (Figure 9). The abundance of Coprococcus 2 and Ileibacterium was significantly higher than that in the other groups, which were characteristic bacteria in the UC group. Fusobacteriaceae, c_Alphaproteobacteria, Anaerovibrio, Ruminococcaceae_UCG_008, and Prevotellaceae were characteristic bacteria in the frozen FMT group. Prevotella_9, Acidaminococcaceae, Peptostreptococcaceae, Arcobacter, Phascolarctobacterium, Eubacterium_hallii_group, Candidatus Stoquefichus, Arcobacteraceae, Faecalibaculum, and Sulfurovaceae were characteristic bacterial genera in the fresh FMT group. The abundances of Lachnospiraceae NK4A136, Ruminococcaceae_UCG_005, and Romboutsia were significantly higher than those in the other groups, which are characteristic bacterial genera in the MS group.

Discussion

The pathogenesis of UC is complex, and it is currently thought that the interaction between the host and gut microflora is a key factor. Under normal conditions, innate and acquired immunity in the host tolerates the normal microflora while preventing the invasion of harmful bacteria. When the balance of intestinal flora is disrupted, harmful bacteria in the intestinal tract are rapidly increased, and directly invade and destroy intestinal epithelial cells, leading to immune dysfunction. The release of a large amount of enterotoxin increases the permeability of the intestinal mucosa and damages the intestinal mucosal barrier (18, 19). Intestinal mucosal barrier function decreases and intestinal microbial flora shifts, further destroying the intestinal mucosal barrier, causing a vicious cycle, and exacerbating intestinal inflammation.

FMT is the process of transferring faecal bacteria from a donor to a recipient (20, 21). In recent years, FMT has made great progress in the treatment of UC. It has been reported that patients with UC treated by FMT had a higher disease remission rate than those administered traditional treatment alone (14, 22, 23).

Moreover, the diversity of intestinal flora in patients treated with FMT was significantly increased compared with that before treatment, and the level of intestinal inflammatory factors decreased (24). Faecal bacterial transplantation treatment often varies in the choice of the donor, different treatment of faecal bacteria, transplantation method, and method of the exact guide. We used fresh and frozen FMT in a rat model of UC to explore the differences between the two methods.

FMT was found to improve diarrhoea, mucous pus, and blood stool, and weight loss in rats. Histologically and pathologically, FMT effectively restored crypt injury, reduced intestinal inflammation and ulcer injury, and restored damaged villi. In addition, FMT effectively downregulated the levels of inflammatory factors. Moreover, the structure of intestinal flora was further analysed, which showed that after inoculation with exogenous faecal microflora, the colonic microflora composition of rats gradually became similar to that of the normal group. This is consistent with previous studies showing that after FMT, the intestinal flora composition of recipients and donors was consistent (25, 26).

FMT increased the number of beneficial bacteria in the gut and decreased the number of harmful bacteria. These results suggest that the mechanism of action of FMT is competitive inhibition of pathogenic microorganisms, improved immune metabolism, reduced inflammatory response, improved tight junctions between colon cells, and A reduced damage response of the intestinal barrier to alleviate the progression of UC. This is biologically reasonable, as in the early stages of UC, fluctuations in the microbiota are easier to recover (22).

However, compared with the fresh FMT group, the frozen FMT group showed a greater relief effect on UC model rats in terms of behaviour and colon length. In terms of histopathology, inflammatory factors in fresh and frozen FMT groups showed improvement in UC model rats, but the difference was not significant. A previous study showed that frozen FMT reduced the amount of gram-negative bacteria in faeces, which may explain why frozen faecal transplants are more effective than fresh faecal transplants (27).

Whether the microorganisms remain viable, particularly the beneficial bacteria, over time is unclear. Studies have shown that there is no difference in the flora of frozen and fresh faecal bacteria at 6 or 7 months. In addition, preservation of frozen bacteria requires further analysis. By studying the aerobic bacterial suspension of 10% glycerol stored at -80°C, Costello et al. found that Bifidobacteria, aerobes, total Coliforms, *Escherichia coli*, Anaerobes, and Lactobacilli survived for at least 6 months (28). Different species of bacteria have different sensitivities to freeze-thaw damage. Haines et al. found that a single freeze-thaw cycle killed 98% of *Saccharomyces cerevisiae* but only 5% of *Staphylococcus aureus* (29). Costello et al. have shown that Bifidobacterium has a better survival rate when refrigerated for 6 months compared to total coliforms and *E. coli* (28). The difference in the survival rate of different strains in cryopreservation may be clinically important for long-term preservation of FMT, which should be analysed in further studies.

In summary, we showed that frozen FMT can be used to treat UC, and the curative effect was similar to, or even better than that of fresh FMT. Previous studies on *C. difficile* infection have shown that frozen

FMT does not significantly affect the implantation and efficacy of microorganisms (30). A random clinical trial showed that the lyophilised product had a slightly lowered efficacy in *C. difficile* infection patients compared with fresh product, but resembled other treatments in microbial restoration one month after FMT (31). Another clinical randomised controlled trial with a larger sample size showed that among adults with recurrent or refractory CDI, the use of frozen compared with fresh FMT did not negatively affect the clinical resolution of diarrhoea (32). This is important for practical applications, such as supplier relationships, modes of transport, and cost-effectiveness. Compared with fresh FMT, frozen FMT reduces the frequency of donor screening (32). This approach can be applied in a wide range of healthcare settings. At present, FMT banks have been established in foreign countries by using the faecal intelligent separation system (33). China also established the Chinese Fecal Microbiota Transplantation Bank in 2015, aiming to realise the emergency treatment of faecal microbiota in different locations and the standardisation of faecal bacteria distribution, preparation, and preservation. In addition, collecting frozen stool samples through quarantine, and obtaining screening results may ease concerns that fresh FMT could transmit pathogens from donors to recipients.

There were some limitations to our study. First, the experimental period was short. Although the flora after FMT tended to be normal, it was not yet stable. The experimental period should be extended to detect the composition of colonic microflora. Second, only one model was used. Studies have shown that multiple model creation is more similar to the chronic colitis model, and multiple FMT significantly improves the treatment effect. Further studies are required to evaluate this point.

Conclusions

FMT is a feasible method for treating UC. The mechanism of action may be competitive inhibition of pathogenic microorganisms, improving immune metabolism, and reducing the inflammatory response to reduce the damage to the intestinal barrier and development of UC. Compared with fresh FMT, the therapeutic effect of frozen FMT may be greater.

Methods

Animals and groups

Forty specific pathogen-free-grade Male Sprague-Dawley rats weighing 240–250 g were purchased from Shanghai SLAC Laboratory Animal Co., Ltd. (Experimental Animal Production License Number: SCXK (Shanghai) 2017-0005). The rats were raised in the Animal Experiment Center of Zhejiang Chinese Medical University (Experimental Animal License Number: SYXK (Zhejiang) 2018–0012), which provided a barrier environment. During the feeding period, animals were given standard pellet feed and free drinking water and housed at a controlled temperature (23 ± 2°C), humidity (60%), and 12-h light cycle. Animal handling during the experiment complied with the "Guiding Opinions on the Good Treatment of Laboratory Animals" issued by the Ministry of Science and Technology Under Government of China in

2006. The study was approved by the Ethics Committee of Zhejiang Chinese Medical University (IACUC-20200506-12).

According to the random number table method, the rats were randomly divided into 2 groups: a normal (non-UC) group (n = 8) and UC model group (n = 32). After both groups were subjected to 1 week of adaptive feeding, the UC model group was created. The rats were anaesthetised with 3% pentobarbital sodium (0.15 mL/100 g). A rubber infusion tube was then inserted 8 cm into the upper anus of each rat and injected with 5% TNBS at 100 mg/kg (dissolved in 50% ethanol, total volume of 1 mL) (34, 35). To prevent fluid leakage after colonic infusion and ensure the induction of colitis, the rats were placed in the Trendlenburg position with their head down for 1 min (36). The daily weight and DAI score of both normal and UC model groups of rats were recorded. Fresh faeces were collected from each group before and after UC model creation. After the experiment, the rats were sacrificed by CO_2 inhalation, and colon tissue specimens and faeces were collected and immediately placed at -80°C.

The UC model group was randomly divided into four groups 72 h later, with eight animals in each group: UC model group (control), mesalazine group, fresh FMT group, and frozen FMT group. The rats in each group were fed separately in cages.

Main reagents and instruments

TNBS (lot: SLCD2161) was purchased from Sigma (St. Louis, MO, USA). Mesalazine (lot: 14055) was purchased from Shanghai Haoyuan Chemexpress Co., Ltd. (Shanghai, China). The rat TNF-α ELISA Kit (lot: 20200930) was purchased from Nanjing Jiancheng Bioengineering Institute (Nanjing, China). A BioTek luciferase chemiluminescence detector (Winooski, VT, USA) was also used.

Interventions

Faecal bacteria preparation

For frozen FMT preparation, seven days before transplantation, the normal group was treated as donors whose faecal samples were collected in a sterile tube, dissolved in 0.9% NaCl at a ratio of 1 g:10 mL and homogenised for 5 min, followed by adding 0.1 mL 10% glycerol, mixing, and freezing at -80°C (37). The samples were transferred to -80°C refrigerator for 1 week.

For fresh FMT preparation, on transplant day, the normal group was treated as donors whose samples were collected in a sterile tube, dissolved in 0.9% NaCl at a ratio of 1 g:10 mL and homogenised for 5 min, followed by 0.1 mL of 10% glycerol. The time from sampling to successful transplantation did not exceed 3 h (38).

Intervention pattern of each group

To create four different groups within the UC model group, the rats were administered different treatments once per day for five consecutive days. The fresh FMT group was treated with 1 mL/100 g of fresh faecal

homogenate from the normal group (non-UC) on the same day of transplantation, whereas the frozen FMT group was treated with 1 mL/100 g of frozen faecal homogenate collected from the normal group 7 days prior to transplantation. The mesalazine group was administered 0.3 g/kg of mesalazine by gavage as a positive control. The UC model group and normal group were administered 1 mL/100 g of saline by gavage as a blank control and negative control, respectively.

Specimen collection

The faeces of rats were collected at 72 h after model creation, two days after stopping drug intervention, and seven days after stopping drug intervention, and immediately placed in -80°C until analysis. After stopping the drug intervention for seven days, the rats were sacrificed by CO_2 inhalation. The rat colon tissue was separated and the most severely affected part of the rat colon tissue visually and excess fat around the tissue was cut off. The tissues were repeatedly rinsed with pre-cooled normal saline, after which filter paper was used to absorb the excess liquid, and the colon tissue was placed in 4% poly Fixed (lot:69111800, biosharp) and preserved in formaldehyde solution until haematoxylin-eosin staining. A section of severely diseased colon tissue was placed in a sterile cryopreservation tube for subsequent detection of inflammatory factors.

Detection indicator

Behavioural score

Weight was measured once every day. After model creation, the weight loss, stool traits, blood, and stool of each group were observed daily to determine the DAI score, as shown in Table 1 (39).

Histological analysis

Colon tissues fixed with pom solution were embedded in paraffin. After haematoxylin and eosin staining, the tissue structure was observed in detail under a light microscope, and the pathological score was determined according to Table 2 (39, 40).

ELISA

The collected colon samples were thawed and tested for TNF-α using an ELISA kit according to the kit instructions. The absorbance of samples in each well of the plate was measured with a multifunctional microplate reader at a wavelength of 450 nm.

Bacterial DNA extraction and 16S rRNA gene sequencing

Total genomic DNA was extracted from the samples using the CTAB/SDS method. DNA concentration and purity were monitored on 1% agarose gels. According to the concentration, DNA was diluted to 1 ng/μL using sterile water. The primers 16S V3-V4: 341F CCTAYGGGRBGCASCAG, 806R GGACTACNNGGGTATCTAAT. 16S/18S rRNA genes were tagged with a barcode and used for analysis. All

PCRs were carried out in 30 μL volume containing 15 μL Phusion®High-Fidelity PCR Master Mix (New England Biolabs, Ipswich, MA, USA), 0.2 μM of forward and reverse primers, and approximately 10 ng template DNA. Thermal cycling steps consisted of initial denaturation at 98℃ for 1 min, followed by 30 cycles of denaturation at 98℃ for 10 s, annealing at 50℃ for 30 s, and elongation at 72℃ for 60 s. The last step was performed at 72℃ for 5 min. Equal volumes of 1X loading buffer (contained SYB green) and PCR products was mixed and the samples were evaluated by 2% agarose gel electrophoresis. Samples showing bands at 400–450 base pairs were further analysed. PCR products were mixed in equivalent ratios and purified with an AxyPrepDNA Gel Extraction Kit (AXYGEN, Union City, CA, USA). Sequencing libraries were generated using an NEB Next®Ultra™DNA Library Prep Kit for Illumina (New England Biolabs) following the manufacturer's recommendations, and index codes were added. The library quality was assessed on a Qubit@ 2.0 Fluorometer (Thermo Fisher Scientific, Waltham, MA, USA) and Agilent Bioanalyzer 2100 system (Agilent Technologies, Santa Clara, CA, USA). The library was sequenced on an Illumina Miseq/HiSeq2500 platform (San Diego, CA, USA) and 250-bp/300-bp paired-end reads were generated.

Statistical analysis

The measured data were expressed as the average ± standard deviation (x ± s). SPSS software (version 20.0, SPSS, Inc., Chicago, IL, USA) was used to analyse the data. The independent sample *t*-test was used to compared data from the two groups, and one-way analysis of variance was performed to compare data between multiple groups. $P < 0.05$ indicated statistically significant results. Flora data were analysed and graphed by non-parametric test Kruskal Wallis sum rank test and Wilcoxon rank sum test with R language ggplot2, and ggpubr, and the MetagenomeSeq toolkit and Ubuntu Linux conda LEfSe software.

Declarations

Ethics approval and consent to participate

The study was approved by the Ethics Committee of Zhejiang Chinese Medical University (IACUC-20200506-12).

Consent for publication

Not applicable.

Availability of data and materials

All data generated or analysed during this study are included in this published article.

Competing interests

The authors declare that they have no competing interests.

Funding

This work was supported by Health Commission of Zhejiang Province (2020378459).

Authors' contributions

The study was conceived by KYF and LYC and they secured the funding. ZFY, LYT, KYF, WJQ, WP and MFX did experiments and performed analysis. ZFY performed the data analysis. The first draft of the manuscript was written by ZFY. All authors read and approved the final manuscript.

Acknowledgements

We thank Health Commission of Zhejiang Province (2020378459) for funding the project.

References

1. Ordás I, Eckmann L, Talamini M, Baumgart DC, Sandborn WJ. Ulcerative colitis. The Lancet. 2012;380(9853):1606-19.
2. Eisenstein M. Ulcerative colitis: towards remission. Nature. 2018;563(7730).
3. Ungaro R, Mehandru S, Allen PB, Peyrin-Biroulet L, Colombel J-F. Ulcerative colitis. The Lancet. 2017;389(10080):1756-70.
4. Danese. S, Fiocchi C. Ulcerative colitis. The New England Journal of Medicine. 2011;365(18):1713-25.
5. Kaakoush NO, Mitchell HM, Man SM. Role of emerging Campylobacter species in inflammatory bowel diseases. Inflamm Bowel Dis. 2014;20(11):2189-97.
6. Paramsothy S, Nielsen S, Kamm MA, Deshpande NP, Faith JJ, Clemente JC, et al. Specific Bacteria and Metabolites Associated With Response to Fecal Microbiota Transplantation in Patients With Ulcerative Colitis. Gastroenterology. 2019;156(5):1440-54 e2.
7. Weingarden AR, Vaughn BP. Intestinal microbiota, fecal microbiota transplantation, and inflammatory bowel disease. Gut Microbes. 2017;8(3):238-52.
8. Vindigni SM, Surawicz CM. Fecal Microbiota Transplantation. Gastroenterology Clinics of North America. 2017;46(1):171-85.
9. Kelly CR, Khoruts A, Staley C, Sadowsky MJ, Abd M, Alani M, et al. Effect of Fecal Microbiota Transplantation on Recurrence in Multiply Recurrent Clostridium difficile Infection: A Randomized Trial. Ann Intern Med. 2016;165(9):609-16.
10. van Nood E, Vrieze A, Nieuwdorp M, Fuentes S, Zoetendal EG, de Vos WM, et al. Duodenal Infusion of Donor Feces for RecurrentClostridium difficile. New England Journal of Medicine. 2013;368(5):407-15.
11. Shen Z-H, Zhu C-X, Quan Y-S, Yang Z-Y, Wu S, Luo W-W, et al. Relationship between intestinal microbiota and ulcerative colitis: Mechanisms and clinical application of probiotics and fecal microbiota transplantation. World Journal of Gastroenterology. 2018;24(1):5-14.

12. El-Salhy M, Hausken T, Hatlebakk JG. Increasing the Dose and/or Repeating Faecal Microbiota Transplantation (FMT) Increases the Response in Patients with Irritable Bowel Syndrome (IBS). Nutrients. 2019;11(6).
13. Johnsen PH, Hilpusch F, Valle PC, Goll R. The effect of fecal microbiota transplantation on IBS related quality of life and fatigue in moderate to severe non-constipated irritable bowel: Secondary endpoints of a double blind, randomized, placebo-controlled trial. EBioMedicine. 2020;51:102562.
14. Rossen NG, Fuentes S, van der Spek MJ, Tijssen JG, Hartman JH, Duflou A, et al. Findings From a Randomized Controlled Trial of Fecal Transplantation for Patients With Ulcerative Colitis. Gastroenterology. 2015;149(1):110-8 e4.
15. Craven L, Rahman A, Nair Parvathy S, Beaton M, Silverman J, Qumosani K, et al. Allogenic Fecal Microbiota Transplantation in Patients With Nonalcoholic Fatty Liver Disease Improves Abnormal Small Intestinal Permeability: A Randomized Control Trial. Am J Gastroenterol. 2020;115(7):1055-65.
16. European consensus conference on faecal microbiota transplantation in clinical practice. 2017.
17. The use of faecal microbiota transplant as treatment for recurrent or refractory Clostridium difficile infection and other potential indications joint British Society of Gastroenterology (BSG) and Healthcare Infection S. 2018.
18. Franzosa EA, Sirota-Madi A, Avila-Pacheco J, Fornelos N, Haiser HJ, Reinker S, et al. Gut microbiome structure and metabolic activity in inflammatory bowel disease. Nat Microbiol. 2019;4(2):293-305.
19. Larabi A, Barnich N, Nguyen HTT. New insights into the interplay between autophagy, gut microbiota and inflammatory responses in IBD. Autophagy. 2019;16(1):38-51.
20. Malikowski T, Khanna S, Pardi DS. Fecal microbiota transplantation for gastrointestinal disorders. Curr Opin Gastroenterol. 2017;33(1):8-13.
21. Rossen NG, MacDonald JK, Vries EMd, D'Haens GR, Vos WMd, Zoetendal EG, et al. Fecal microbiota transplantation as novel therapy in gastroenterology: A systematic review. World J Gastroenterol. 2015;21(17):5359-71.
22. Moayyedi P, Surette MG, Kim PT, Libertucci J, Wolfe M, Onischi C, et al. Fecal Microbiota Transplantation Induces Remission in Patients With Active Ulcerative Colitis in a Randomized Controlled Trial. Gastroenterology. 2015;149(1):102-9 e6.
23. Costello SP, Hughes PA, Waters O, Bryant RV, Vincent AD, Blatchford P, et al. Effect of Fecal Microbiota Transplantation on 8-Week Remission in Patients With Ulcerative Colitis: A Randomized Clinical Trial. JAMA. 2019;321(2):156-64.
24. Wei YL, Chen YQ, Gong H, Li N, Wu KQ, Hu W, et al. Fecal Microbiota Transplantation Ameliorates Experimentally Induced Colitis in Mice by Upregulating AhR. Front Microbiol. 2018;9:1921.
25. Paramsothy S, Kamm MA, Kaakoush NO, Walsh AJ, van den Bogaerde J, Samuel D, et al. Multidonor intensive faecal microbiota transplantation for active ulcerative colitis: a randomised placebo-controlled trial. The Lancet. 2017;389(10075):1218-28.
26. Lleal M, Sarrabayrouse G, Willamil J, Santiago A, Pozuelo M, Manichanh C. A single faecal microbiota transplantation modulates the microbiome and improves clinical manifestations in a rat

model of colitis. EBioMedicine. 2019;48:630-41.

27. Costello SP, Soo W, Bryant RV, Jairath V, Hart AL, Andrews JM. Systematic review with meta-analysis: faecal microbiota transplantation for the induction of remission for active ulcerative colitis. Aliment Pharmacol Ther. 2017;46(3):213-24.
28. Costello SP, Conlon MA, Vuaran MS, Roberts-Thomson IC, Andrews JM. Faecal microbiota transplant for recurrentClostridium difficileinfection using long-term frozen stool is effective: clinical efficacy and bacterial viability data. Alimentary Pharmacology & Therapeutics. 2015;42(8):1011-8.
29. The effect of freezing on bacteria. Proceedings of the Royal Society of London Series B - Biological Sciences. 1997;124(837):451-63.
30. Youngster I, Russell GH, Pindar C, Ziv-Baran T, Sauk J, Hohmann EL. Oral, capsulized, frozen fecal microbiota transplantation for relapsing Clostridium difficile infection. JAMA. 2014;312(17):1772-8.
31. Jiang ZD, Ajami NJ, Petrosino JF, Jun G, Hanis CL, Shah M, et al. Randomised clinical trial: faecal microbiota transplantation for recurrent Clostridum difficile infection - fresh, or frozen, or lyophilised microbiota from a small pool of healthy donors delivered by colonoscopy. Aliment Pharmacol Ther. 2017;45(7):899-908.
32. Lee CH, Steiner T, Petrof EO, Smieja M, Roscoe D, Nematallah A, et al. Frozen vs Fresh Fecal Microbiota Transplantation and Clinical Resolution of Diarrhea in Patients With Recurrent Clostridium difficile Infection: A Randomized Clinical Trial. JAMA. 2016;315(2):142-9.
33. Cammarota G, Ianiro G, Kelly CR, Mullish BH, Allegretti JR, Kassam Z, et al. International consensus conference on stool banking for faecal microbiota transplantation in clinical practice. Gut. 2019;68(12):2111-21.
34. Silva, Pinto, Mateus. Preclinical Study in Vivo for New Pharmacological Approaches in Inflammatory Bowel Disease: A Systematic Review of Chronic Model of TNBS-Induced Colitis. Journal of Clinical Medicine. 2019;8(10):1574.
35. Liu R, Li Y, Zhang B. The effects of konjac oligosaccharide on TNBS-induced colitis in rats. Int Immunopharmacol. 2016;40:385-91.
36. Rashidian A, Muhammadnejad A, Dehpour A-R, Mehr SE, Akhavan MM, Shirkoohi R, et al. Atorvastatin attenuates TNBS-induced rat colitis: the involvement of the TLR4/NF-kB signaling pathway. Inflammopharmacology. 2016;24(2-3):109-18.
37. Hamilton MJ, Weingarden AR, Sadowsky MJ, Khoruts A. Standardized frozen preparation for transplantation of fecal microbiota for recurrent Clostridium difficile infection. Am J Gastroenterol. 2012;107(5):761-7.
38. Smits LP, Bouter KE, de Vos WM, Borody TJ, Nieuwdorp M. Therapeutic potential of fecal microbiota transplantation. Gastroenterology. 2013;145(5):946-53.
39. Wirtz S, Popp V, Kindermann M, Gerlach K, Weigmann B, Fichtner-Feigl S, et al. Chemically induced mouse models of acute and chronic intestinal inflammation. Nat Protoc. 2017;12(7):1295-309.
40. Wang L, Tang H, Wang C, Hu Y, Wang S, Shen L. Aquaporin 4 deficiency alleviates experimental colitis in mice. The FASEB Journal. 2019;33(8):8935-44.

Tables

Table 1. Disease activity index score, DAI score as the sum of weight loss, stool consistency, and occult blood or gross blood in the stool.

Score	Weight loss	Stool consistency	Blood
0	None	Normal	None
1	1–5%	Soft but still formed	
2	5–10%	Soft	Positive hemoccult
3	10–15%	Very soft; wet	
4	>15%	Watery diarrhoea	Blood traces in stool visible

Table 2. Pathological score as the sum of the score of infiltration of inflammatory cells in the lamina propria, colonic tissue damage, and crypt damage.

Score	Infiltration of inflammatory cells in lamina propria	Colonic tissue damage	Crypt damage
0	Infrequent	None	None
1	Elevated with some neutrophils	Isolated focal epithelial damage	Loss of 1/3 of the basal layer
2	Submucosal presence of inflammatory cell clusters	Mucosal erosions and ulcerations	Loss of 2/3 of the basal layer
3	Transmural cell infiltration	Extensive damage deep into the bowel wall	Only the surface epithelium is intact
4			Loss of entire crypts and epithelium

Figures

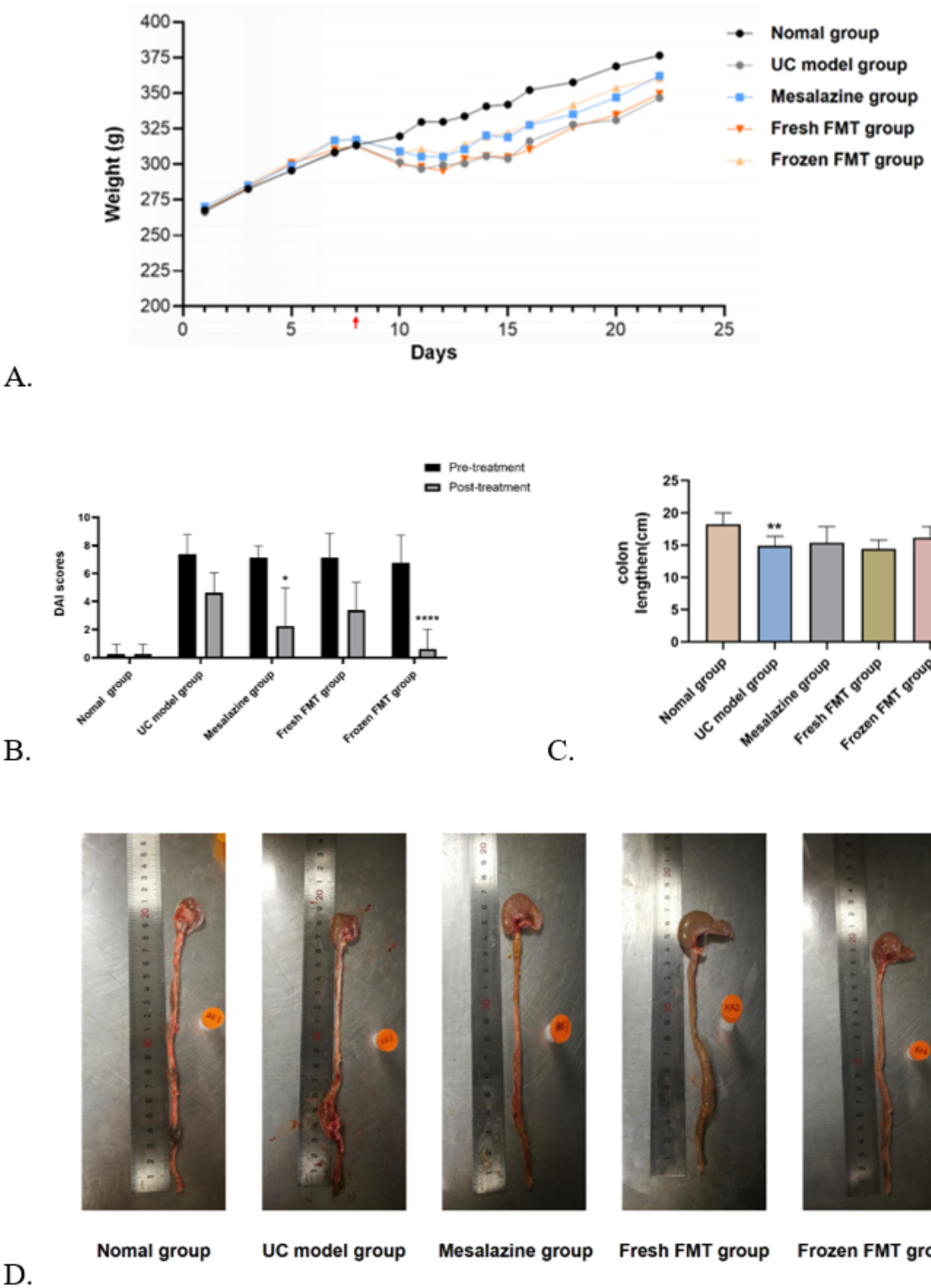
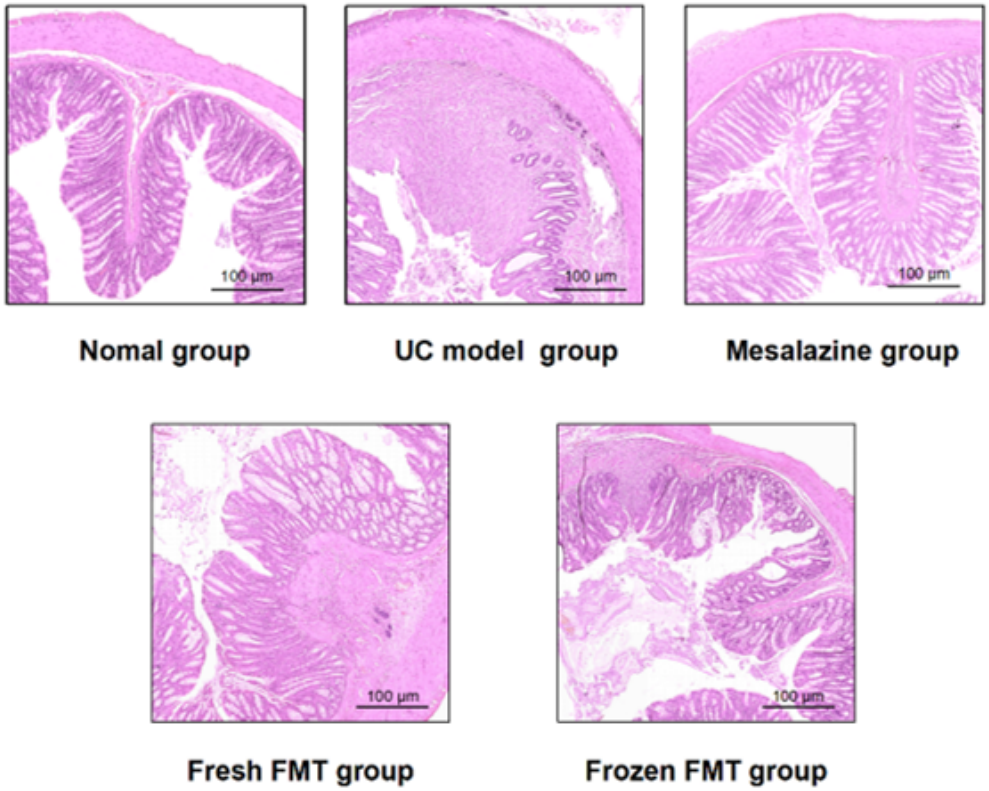
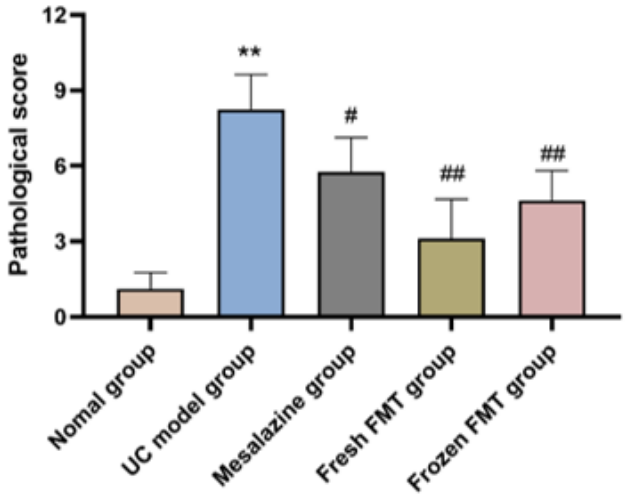


Figure 1

Changes in general conditions of rats in each group. A. Change in mean weight of each group: red arrow in the picture shows the day of model creation. Due to fasting for 24 h before the model creation, no weight was recorded on this day.; B. Disease activity index score (DAI score): as TNBS-induced colitis has a tendency to heal itself, we compared the UC group on the same day after treatment with the other groups; C. Scatter plot of colon length in each group; D. Typical colon anatomy of each group.



A.



B.

Figure 2

Pathological changes and scores of colonic tissues of rats in each group. A. Typical histological performance of each group; B. Pathological score of each group.

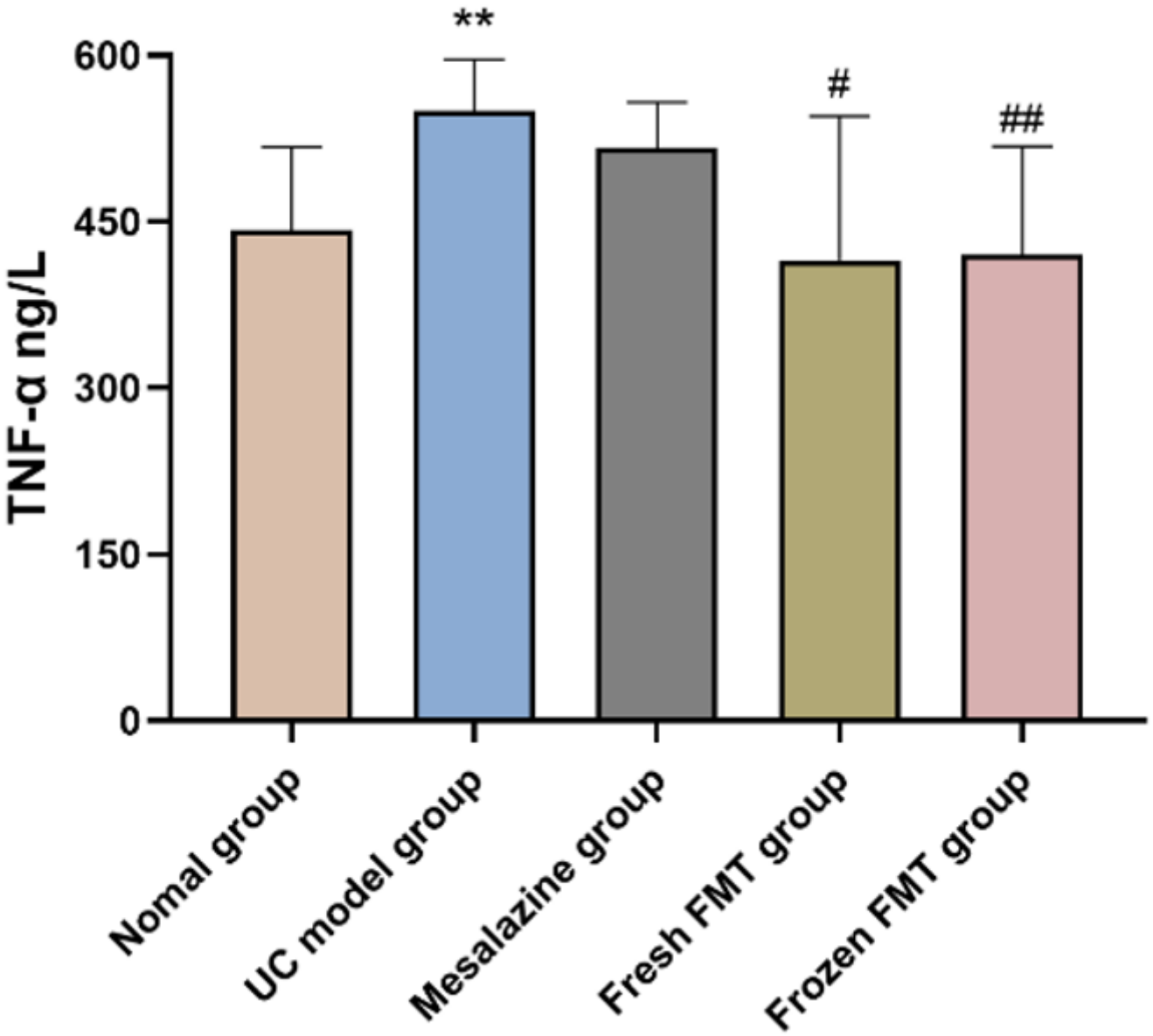


Figure 3

Colon tissue inflammation factor tumour necrosis factor alpha (TNF-α) level in each group.

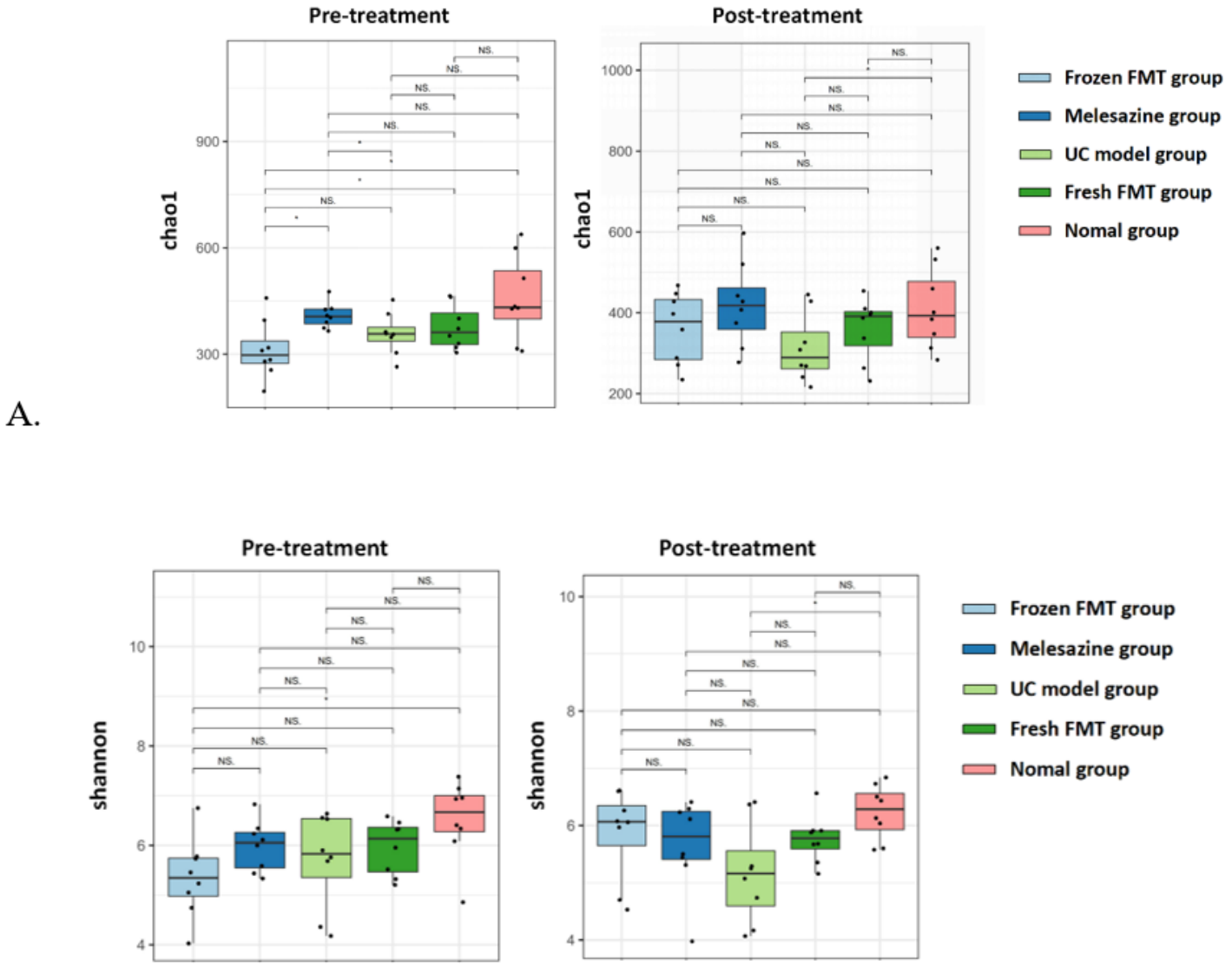


Figure 4

α diversity of bacterial groups. A. α diversity of each group of flora (Chao 1); B. α diversity of each group of flora (Shannon index).

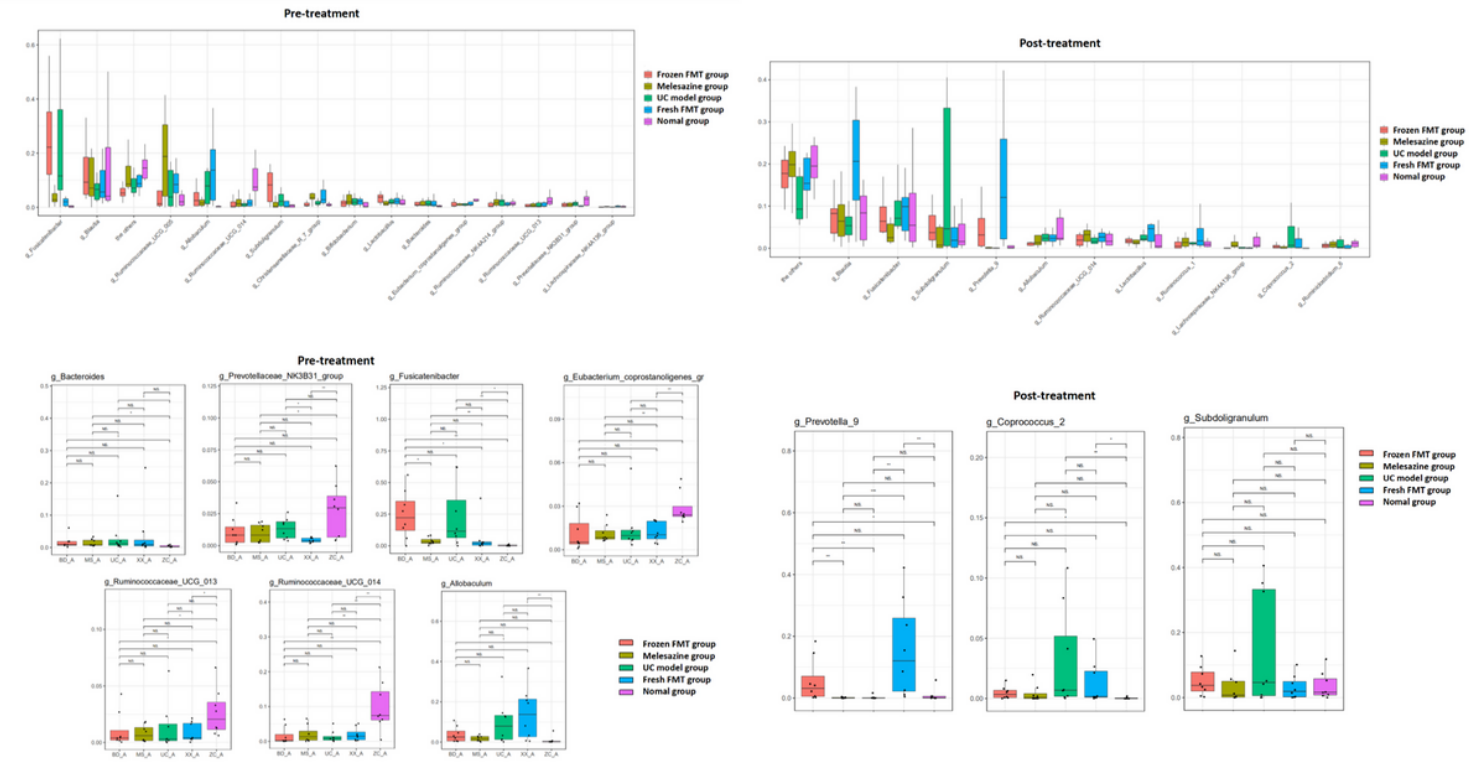


Figure 5

Comparison of species abundance at the genus level.

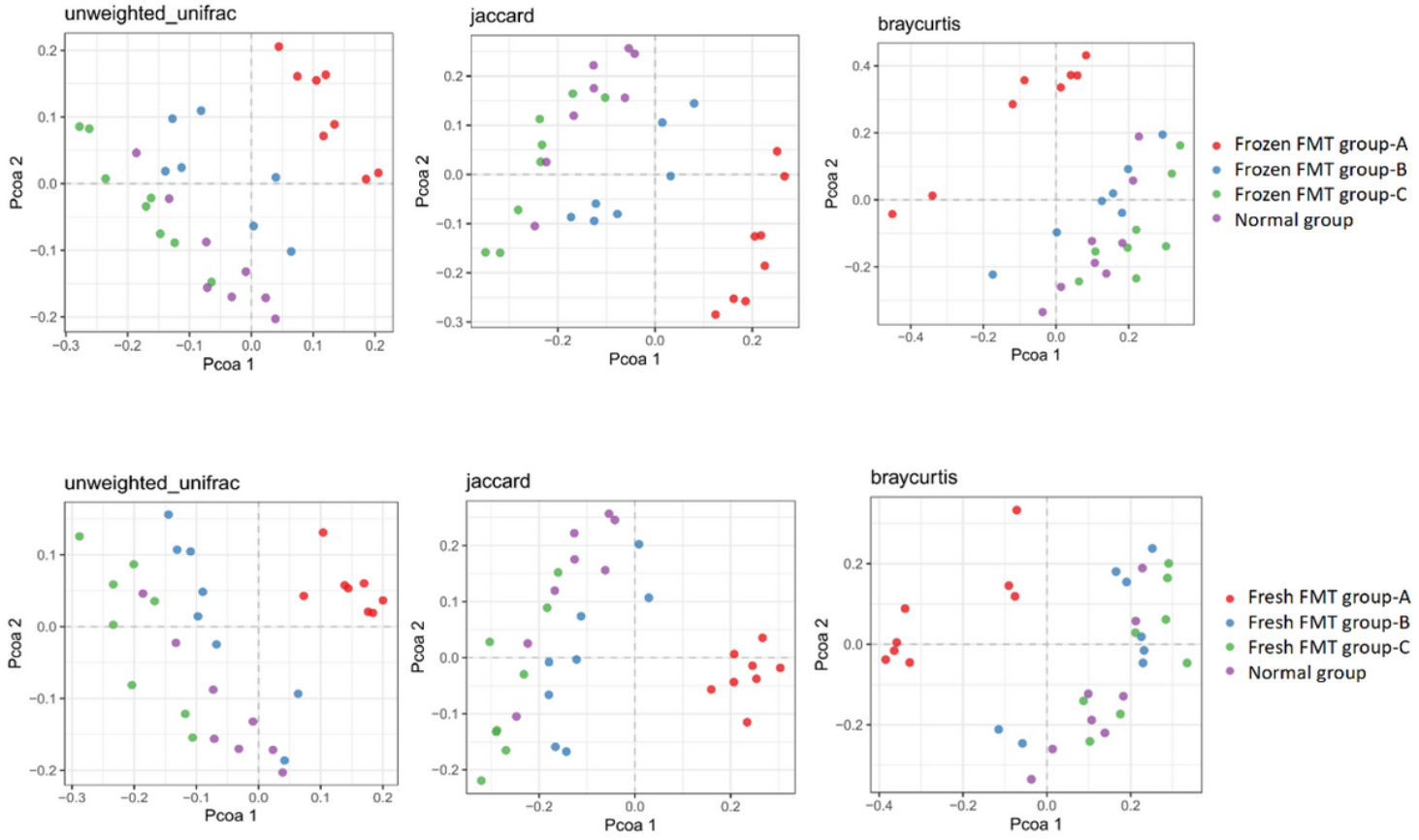


Figure 6

Principal coordinate analysis of frozen FMT group and fresh FMT group. Frozen FMT group-A = frozen FMT group before treatment; Frozen FMT group-B = Frozen FMT group 1 week after treatment; Frozen FMT group-C = Frozen FMT group 2 weeks after treatment; Fresh FMT group-A = Fresh FMT group before treatment; Fresh FMT group-B = Fresh FMT group 1 week after treatment; Fresh FMT group-C = Fresh FMT group 2 weeks after treatment.

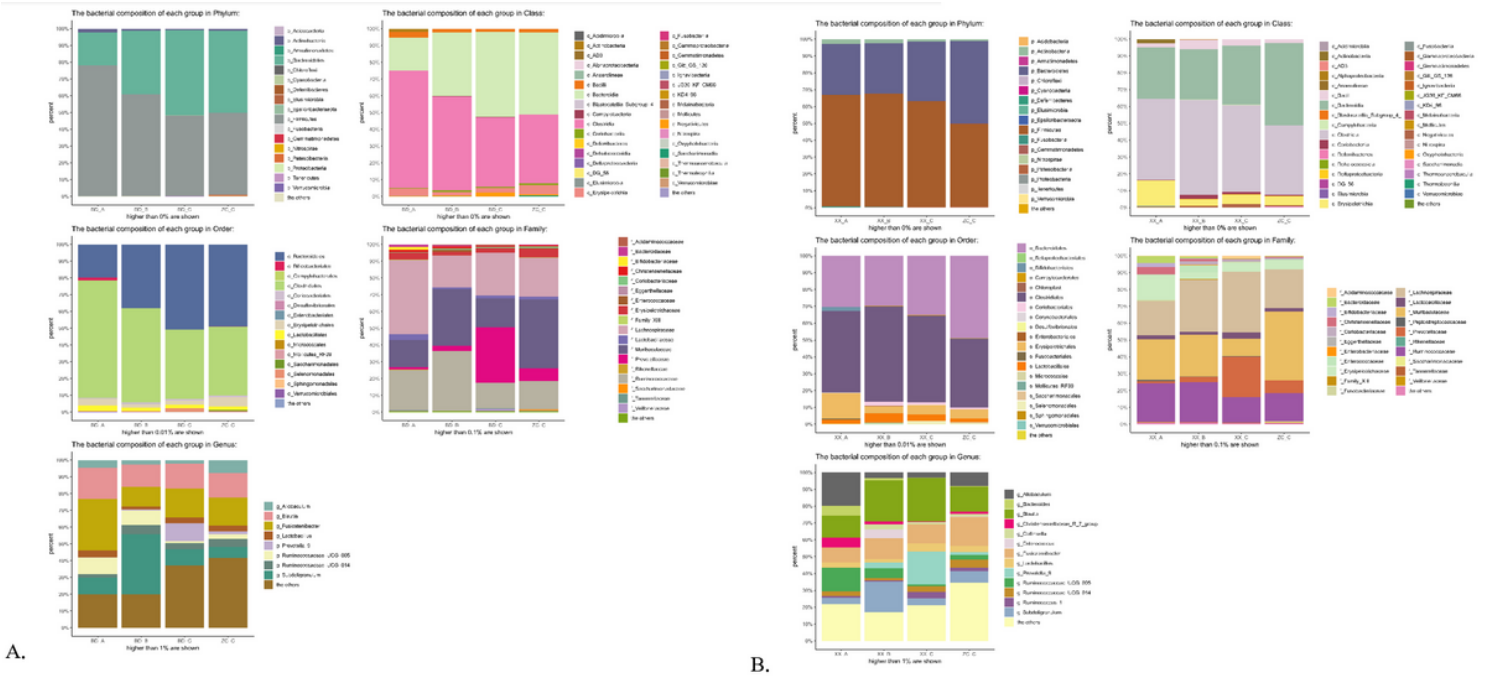


Figure 7

Bacterial community column chart of different groups. BD-A = Frozen FMT group before treatment; BD-B = Frozen FMT group 1 week after treatment; BD-C = Frozen FMT group 2 weeks after treatment; ZC-C = normal group; XX-A = Fresh FMT group before treatment; XX-B = Fresh FMT group 1 week after treatment; XX-C = Fresh FMT group 2 weeks after treatment.

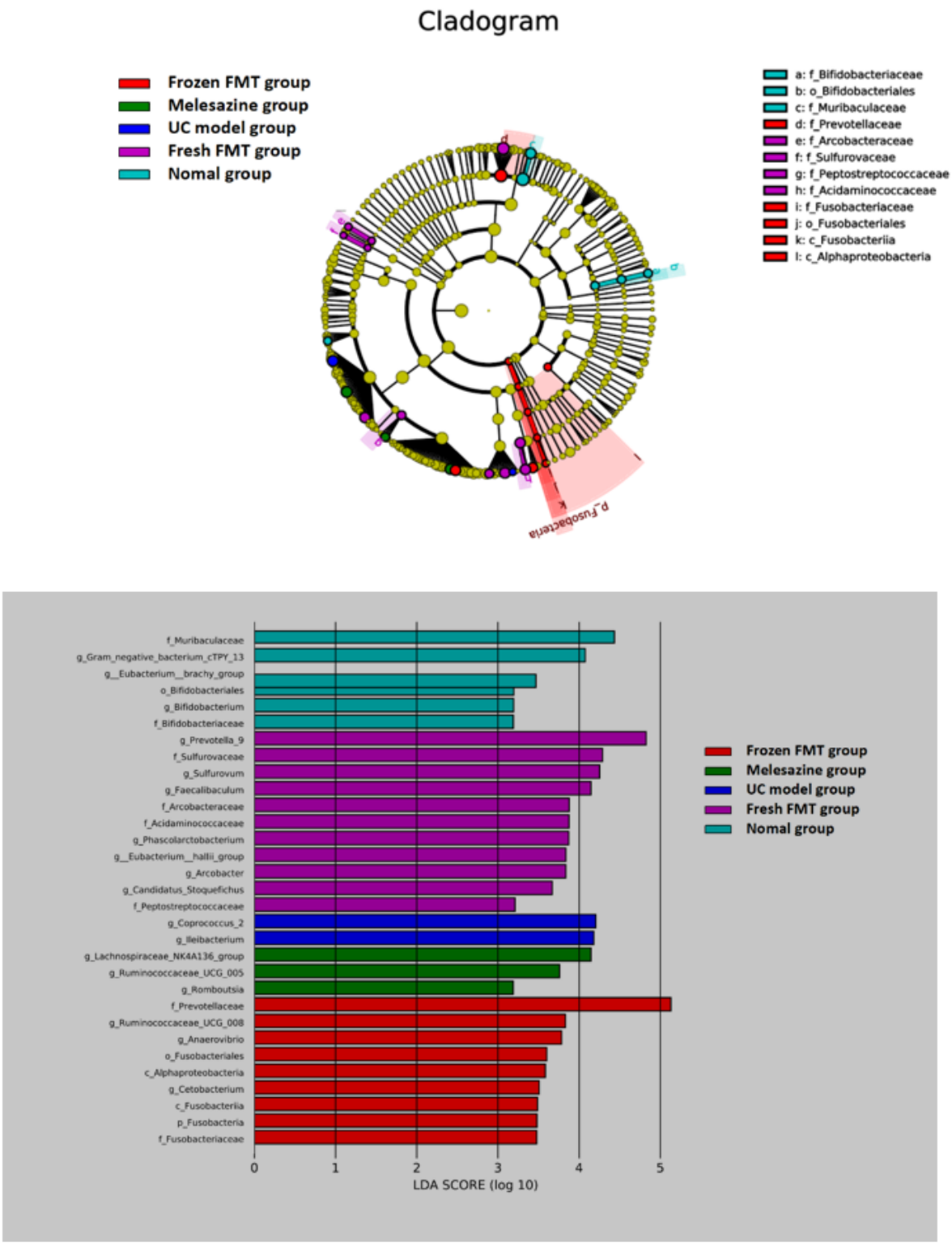


Figure 8

Characteristics of microbial community composition using LEfSe analysis.