Review

Microbiota Transplantation in an Antibiotic-Induced Bacterial Depletion Mouse Model: Reproducible Establishment, Analysis, and Application

Lijun Shang [1,2]**, Jiayu Tu** [1,2]**, Ziqi Dai** [1,2]**, Xiangfang Zeng** [1,2] **and Shiyan Qiao** [1,2,*]

[1] State Key Laboratory of Animal Nutrition, Ministry of Agriculture Feed Industry Centre, China Agricultural University, Beijing 100193, China; shanglj1996@163.com (L.S.); s20213040636@cau.edu.cn (J.T.); daiziqi@cau.edu.cn (Z.D.); ziyangzxf@163.com (X.Z.)

[2] Beijing Bio-Feed Additives Key Laboratory, Beijing 100193, China

[*] Correspondence: qiaoshiyan@cau.edu.cn; Tel.: +86-10-62733588; Fax: +86-10-62733688

Abstract: The fecal bacteria transplantation (FMT) technique is indispensable when exploring the pathogenesis and potential treatments for microbiota-related diseases. For FMT clinical treatments, there are already systematic guidelines for donor selection, fecal bacterial separation, FMT frequency, and infusion methods. However, only a few studies have demonstrated the use of standardized FMT procedures for animal models used in theoretical research, creating difficulties for many new researchers in this field. In the present paper, we provide a brief overview of FMT and discuss its contribution to the current understanding of disease mechanisms that relate to microbiota. This protocol can be used to generate a commonly used FMT mouse model and provides a literature reference of customizable steps.

Keywords: antibiotic; application; FMT; microbiota; protocol

1. Introduction

The animal body is inherently metagenomic, not only in relation to the eukaryotic genome that makes up the body, but also the genomes of the microbiomes colonizing the surface of the body, which includes bacteria, archaea, fungi, protozoa, and viruses [1]. A growing body of research has shown that commensal microbial communities interact with almost all physiological aspects of the host in health and disease [2–7]. The microbiome within the gut is the most widely studied because its microbial biomass exceeds that of other bodily habitats by a large order of magnitude, and it is separated from the host only by a single layer of epithelial cells [5]. Numerous studies have shown that gut microbiota dysbiosis contributes to the onset of many diseases, from gastrointestinal and metabolic disorders to immune and neuropsychiatric diseases [8–11].

In this context, fecal microbiota transplantation (FMT), an important means of regulating the composition and functions of the gut microbiota [12], is often used in studies of the gut microbiota. Clinically, FMT, also known as fecal transplantation, is a procedure in which stool from a healthy donor is placed into another patient's intestine [13]. However, in experimental studies, its definition is broader. Common research modes include transplanting wild-type (WT) mice or healthy human microbiota into disease-model recipients, [14–16] transplanting disease model microbiota into recipients [17–19], or even transplanting a customized combination of microbiota (selective microbiota transplantation, SMT) to achieve specific experimental purposes [20]. Some studies have also applied a combination of several modes, for example, the inclusion of a model group that acts as both the donor and recipient to control for handling and allows for the analysis of confounding factors that may affect the experimental groups [21].

In clinical situations, there are already systematic FMT treatment guidelines in place that are constantly being updated and improved [22–24]. There have been many reports

on donor selection, the separation of fecal bacteria, the frequency of FMT, and infusion procedures [13,22]. Nevertheless, only a few studies have been designed to explore the methodology of FMT, and even fewer have provided standardized FMT procedures for use in animal models used in research [25], creating difficulties for many beginners in microbiota research. Therefore, the aim of the present review is to provide a simple and repeatable FMT protocol, as well as a summary of the literature references for each adaptable step to aid in customizing microbiota. Moreover, necessary analyses related to FMT, as well as common patterns among studies that have developed this technique to investigate the disease mechanisms related to the intestinal ecosystem, are discussed.

2. The Development and Overview of the Procedure

The history of using stool from healthy people to treat human diseases dates back to the fourth century AD [26]. Hong Ge, a Chinese doctor during the Dong Jin Dynasty (AD 300–400), recorded the treatment of "Wen Bing" (febrile disease) and "Shang Han" (typhoid fever) by drinking the fecal suspension or fermented feces [27,28]. Later, in the Compendium of Materia Medica, which is the most comprehensive record of resolving diseases in traditional medicine, Shizhen Li described more than 20 indications that can be effectively treated with fecal suspension or fermented feces [29]. In 1958, Eiseman et al. successfully treated patients with severe pseudomembranous enteritis using a fecal suspension, which was the first recorded instance of such a treatment in the English literature [30]. In 2011, the method was officially termed fecal microbiota transplantation (FMT) [31], and, in 2013, it was included in the medical guidelines for the treatment of refractory *Clostridiodies difficile* infection (CDI), which represented a milestone in the history of FMT application [32]. The most successful application of FMT, to date, has been in the treatment of refractory CDI [22,33], and there is growing evidence that FMT also has great potential for interventions in other enteric-related diseases and in neurological conditions [14,20,34–36].

FMT, as a strategy to modulate gut microbes, is not only a breakthrough medical technique, but also a breakthrough in technological and theoretical research. In theoretical studies, interest in FMT has not been limited to its use as a therapeutic method (in which the fecal microflora from healthy donors is transmitted to patients to restore a healthy microbial composition to the gut), because it has also involved the transfer of bacteria from one individual to another to induce a desired physiologic effect. Potential microbial material from donors is not limited to feces, and may also comprise intestinal contents or specially modified microbiota (such as SMT) [19,20]. Researchers have often applied FMT to studies of mouse models, which, because of their genetic proximity to humans, our ability to genetically manipulate their genomes, and the availability of many tools, mutants, and inbred strains, have become the mammalian model of choice [37,38]. Therefore, recipients are not limited to germ-free mice and may also be genetically engineered mouse models [15,39].

Of all the available research methods, the most basic is the transplantation of the microbiota of target mouse donors into antibiotic-induced bacterial-depletion mouse recipients, and other research models can be modified on this basis. In Sections 3 and 4 of this review, we provide a brief overview of the selected models of FMT (mouse donors; antibiotic-induced bacterial-depletion mouse recipients), along with the literature references and considerations for each step. In the following sections, we provide some of the necessary analyses related to FMT. Finally, we discuss the contribution that FMT has made to the current understanding of disease mechanisms related to gut microbiota.

3. Protocol

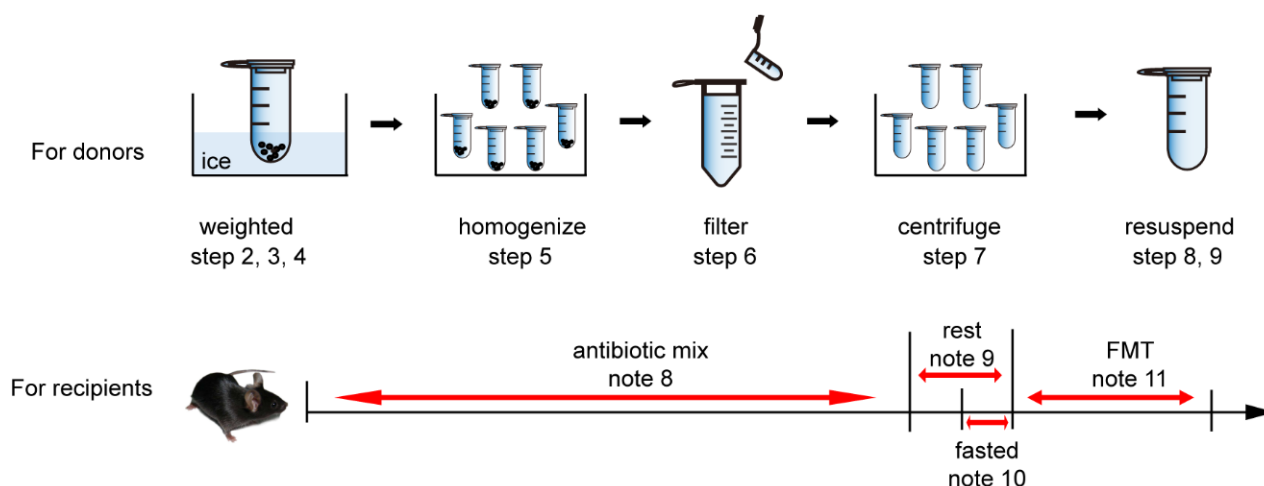A schematic of all the steps can also be observed in Figure 1.

Figure 1. Schematic overview of the models, for which setup is described in the Procedure Section.

3.1. Materials

(1)　Phosphate-buffered saline (PBS), sterile.
(2)　Sample storage buffer: Glycerin in PBS = 1:1 (*v/v*), sterilization (see Note 1).
(3)　Placebo: Glycerin in PBS = 1:4 (*v/v*), sterilization (see Note 2).
(4)　Broad-spectrum antibiotic mix: 500 mg/L of vancomycin, 1 g/L of ampicillin, and 1 g/L of neomycin (see Note 3).
(5)　A 40 µm cell strainer.
(6)　Lavage needle, size 9 (Table 1) (see Note 4).

Table 1. Common type and application range of the intragastric needle [40].

Type	Length	Needle Diameter	Apply
6	5 cm	0.6 mm	Nude mice, about 5 weeks old
7	5 cm	0.7 mm	≤25 g mice
8	4.5 cm	0.8 mm	≤30 g mice
9	6 cm	0.9 mm	≤30 g mice
12	4 cm	1.2 mm	~50 g mice
12	5 cm	1.2 mm	~100 g mice
12	6 cm	1.2 mm	~150 g mice
16	8 cm	1.6 mm	150~250 g rat
16	9 cm	1.6 mm	~250 g rats
16	11 cm	1.6 mm	~350 g rats
18	10 cm	1.8 mm	≥350 g rats
20	10 cm	2.0 mm	Rats (≥350 g), dogs, rabbits, and birds

(7)　A total of 2 mL of cryotubes, round bottom (see Note 5).
(8)　A 1 mL syringe (see Note 6).
(9)　Cage, tweezers, cryotubes, and all other appliances and reagents need to be sterilized before use.

3.2. Procedure

3.2.1. Donors

(1)　All the donors are raised in separate cages. Place all the regents and buffers on ice.
(2)　Weigh the dry, empty 2 mL cryotubes.
(3)　Collect fresh feces in cryotubes and weigh (see **Note 7**).
(4)　Add an appropriate amount of pre-cooled sterile PBS (V_1) to produce a feces concentration of 50–100 mg/mL. Reach as close to the upper limit as possible.
(5)　Homogenize the solution at 4 °C.
(6)　Filter the solution through a 40 µm filter and collect the supernatant in a new tube.

(7) Centrifuge at $8000 \times g$ for 5 min at 4 °C.

(8) Discard the supernatant without disturbing the sediment.

(9) Transplantation solution: Resuspend the pellet obtained from the steps above in cold PBS (the original volume, V_1), and add an equal volume of sample storage buffer (see **Note 1**).

(10) If not used immediately, freeze in liquid nitrogen and store at −80 °C.

3.2.2. Recipients

(1) Administer to the recipients the broad-spectrum antibiotic mix instead of drinking water for at least 14 days (see **Note 8**), and allow to "rest" for 1–2 days (see **Note 9**).

(2) Before intragastric administration, fast all recipients but allow them to drink freely for 1 day (see **Note 10**).

(3) Intragastrically administer each recipient with 200–300 μL (see **Note 6**) of transplantation solution once a day for 5 consecutive days (Table 2) (see **Note 11**).

Table 2. Maximum intragastric intake of commonly used laboratory animals at one time [41].

Animal Species	Weight (g)	Gastric Volume (mL)
Mice	20–24	0.8
	25–30	0.9
	≥30	1.0
Rats	100–199	3.0
	200–249	4–5
	250–300	6.0
	≥300	8.0
Guinea pigs	250–300	4–5
	≥300	6.0
Rabbits	2000–2400	100
	2500–3500	150
	≥3500	200
Cats	2500–3000	50–80
	≥3000	100–150
Dogs	10,000–15,000	200–500
Pigs	-	500

4. Notes

(1) When the concentration of glycerol in the sample storage buffer is 50%, add the sample storage buffer to the resuspended solution at a ratio of 1:1 to prepare the transplantation solution and obtain a final glycerol concentration of 25%. The final concentration glycerol can be adjusted within the range of 10–30% according to different experimental purposes and conditions.

(2) Use a placebo as a control for the transplantation solution. The final glycerol concentration in the placebo should be equal to that of the transplantation solution.

(3) Metronidazole can be added to the broad-spectrum antibiotic mix, but it must be used with caution. This can achieve better bacterial depletion results, but may cause weight loss in mice [42,43]. You can try to gradually introduce metronidazole to the solution [42].

(4) The procedure is suitable for commonly used 6-to-8-week-old mice (25–30 g). If using other target animals, refer to Tables 1 and 2.

(5) Compared to a tapered-bottom tube, using a round-bottom tube can achieve better homogenization effects.

(6) A 1 mL syringe is suitable for commonly used 6-to-8-week-old mice (25–30 g). If using other target animals, refer to Table 2. The volume received by each recipient (200–300 μL) is calculated for approximately 10–20 mg feces/mouse. Generally, the

ideal effect can be obtained from a feces concentration of 50–100 mg/mL in 200 µL of transplantation solution. To determine the optimum conditions for a particular model, a pilot experiment is required.

(7)   Generally, each mouse can provide 50–100 mg of fresh feces (6-to-8-week-old mice, 25–30 g). However, if the donors are enteritis-mouse models, there will be less feces. Therefore, the stool from mice of matched weight and sex can be mixed depending on the experimental design. The number of animals used can be customized to the experiment. Fresh feces should be used for transplantation within 6 h [22,36,44], as oxygen exposure degrades the fecal bacterial communities [45]. If frozen feces are required for subsequent use, aim to complete the freezing operation within 15 min as much as possible [46].

(8)   The duration of the broad-spectrum antibiotic mix treatment of mice can be customized, but, generally, it lasts for at least 14 days.

(9)   Before FMT, a "rest" period of 12–48 h is required [22,36,42,44].

(10)   Fasting should be started for at least 4–8 h before gavage to avoid the gastric contents hindering gavage injection and affecting the drug absorption rate.

(11)   The transplanting of recipients once a day for 5 consecutive days is suitable for commonly used 6-to-8-week-old mice (25–30 g). To determine the optimum conditions for a particular model, a pilot experiment is required. For the solutions to other common problems, see Table 3.

Table 3. Troubleshooting table.

Problem	Possible Reason	Solution
High incidence of animal death	Unskilled intragastric operation, resulting in excessive stress	Gavage should be painless. If the animal persistently struggles, has difficulty breathing, or resists needle insertion, immediately stop needle insertion and pull the needle out. Try again after the animal has become calm. After the mice have been injected and released, and observations have been conducted for any respiratory abnormalities, the success of the gavage insertion should be confirmed
Low colonization efficiency	(1) Low volume of feces collected; (2) short duration of intragastric administration of bacterial liquid; (3) operation from solution preparation to intragastric administration more than 6 h; (4) and inappropriate glycerin concentration	(1) To increase the amount of feces collected, feces from several mice can be mixed on the basis of experimental needs; (2) intragastric administration should last at least 2 weeks; (3) operation should be fast to reduce the exposure time under oxygen; and (4) the glycerin concentration should be appropriate
High degree of variability within experimental groups	(1) Excessive differences in donor flora; (2) Failed intragastric administration of some recipients	(1) Donors should be rigorously selected, and detection of donor flora is necessary; (2) gavage operations should be skillfully conducted to ensure that mice do not spit out the bacterial fluid

5. Microbiological Analyses

5.1. The Detection of Donors

The detection of the donor microbiota is a necessary stage. First, the homogeneity of the donor microbiota can be determined to avoid a great degree of variability within recipient groups after FMT. Second, the composition of the donor microbiota is used to verify the successful separation of fecal bacteria.

5.2. The Detection of the Transplantation Solution

First, the similarity between the microbiota of transplantation solution and that of the donor is detected, because a low similarity indicates that the separation of the flora has failed. Second, the microbial composition of the transplantation solution is clarified, which provides a data reference for subsequent analysis and experiments.

Most studies suggest that the microbiota in recipients after FMT tends towards the donor composition [35,47–49]. Therefore, this test can also evaluate the success of FMT.

5.3. The Detection of the Recipients

5.3.1. Baseline

Before fecal transplantation, baseline microbial composition testing is critical. Studies have shown that the recipient's microbial diversity at baseline predicts their responses to FMT [50]. Generally, broad-spectrum antibiotic mix treatment depletes mice of their intestinal microbiota by hundreds of times to a level similar to that observed in germ-free mice [42,43].

5.3.2. After FMT

After FMT, 16S rRNA gene sequencing is recommended for both the FMT and placebo group to identify the microbiota. In general, a statistically significant change in the microbiota composition in the FMT group, compared to the placebo group, was found after treatment [34].

Most studies compare the changes in the flora of fecal samples to determine the success of FMT [44]. Ishikawa et al. stated that the feces, luminal contents, or mucosa of the target intestinal segment can be analyzed on the basis of the needs of the experiment, and even the sites of the sampling can be chosen as microbial donor sites. Different detection sites illustrate different mechanisms. Stool is almost identical to the contents of the rectum, and the contents of a particular intestinal site are more reflective of the physiological conditions at that particular location in the intestine than feces. Their mucosa provides the best reflection of the colonization of microorganisms and, due to their proximity, may have an advantage in reflecting microbial interactions within the enteric nervous system or mucosal immune system.

6. Application

Research into FMT, combined with sequencing, bioinformatics techniques, and an up-to-date holistic understanding of the microbiome, provides new intuitive evidence for the treatment and mechanisms of microbiota-related diseases.

6.1. Healthy Individuals as Donors

In general, this method of applying FMT is used to transplant the microbiota of healthy individuals to regulate the microbiota of diseased recipients, thereby verifying the therapeutic effect of the microbiota on the disease. For example, Ishikawa et al. found that FMT, following antibiotic pretreatment with Amoxicillin, Fosfomycin and Metronidazole, may be useful for the treatment of ulcerative colitis [44]. Claudia et al. transplanted microbiota from normal donors into a dextran sodium sulphate-induced colitis mouse model and found that restoring a normobiotic core ecology contributed to the resolution of inflammation [14].

Researchers often combine FMT with other techniques to further elucidate the underlying mechanisms of FMT treatment of diseases. Using the sorting and sequencing of immunoglobulin (Ig) A-coated microbiota (called IgA-seq) techniques, Lima et al. identified immune-reactive microbiota during FMT [49].

In recent years, the active communication between the gut microbiota and the nervous system was discovered [9,10,51]. Studies have shown that FMT treatment can improve abnormal gut microbiota and cognitive deficits and, therefore, its potential as a therapeutic strategy for cognitive dysfunction and Alzheimer's disease (AD) [15,16].

6.2. Disease Models as Donors

As the donor, the bacterial community of the disease model is generally significantly different from that of the healthy control, and is often used to study the disease's characteristic microbial impact on various aspects of the body's physiology. Studies have shown that donor mice display disease-related phenotypic alterations that can be transferred from donors to recipients by FMT [19,52,53]. Furthermore, other techniques, such as using genetically engineered mice or META analysis, can be combined to further understand the mechanisms of the microbial influence on disease development, such as core flora [19] or immune regulation [49,52]. Furthermore, human donors can be used to transplant microbiota to mice. After the colonization of germ-free mice with hypertensive patient-derived strains, elevated blood pressure was observed and, although needing to be transferred through the microbiota, illustrating a novel causal role for abnormal gut microbiota in the pathogenesis of hypertension [54].

6.3. Customized Microbiota as Donors

Once a substance or gene is known to have a positive effect on a disease, then the substance-modified microbiota from donors can be used in FMT as a transplantation solution. For example, a ketogenic diet (KD) is known to be useful in the treatment of refractory epilepsy, but the mechanisms underlying its neuroprotective effects remain unclear. By transplanting the most abundant microbiota from the KD-diet mice into antibiotic-treated mice, Olson et al. revealed a potential mechanism by which the gut microbiota modulates the host's metabolism and susceptibility to seizures [20]. Similarly, phlorizin (PHZ), a phytonutrient in apples, can promote good body health. Zhang et al. performed FMT by transplanting the feces of PHZ-fed mice to the high-fat-diet (HFD)-fed mice, confirming that feeding HFD mice the gut contents of the PHZ-modulated mice attenuates HFD-induced metabolic disorders [55]. Similar studies also used genetically engineered mice or known disease-tolerant races as bacteria donors [56,57]. Another method is the application of a mixture of several bacteria from a donor. These can be core microbiota found through meta-analysis in previous studies, and their function can be re-validated by FMT [2,58].

6.4. A Combination of the above Donors

It is more common to use combinations of multiple models than the above two models alone. Compared with healthy individuals, using disease models as donors can reproduce a disease phenotype in recipient mice [39,59–61]. Sharon et al. conducted further studies, including in vivo metabolome and validation tests, proposing that the gut microbiota regulates behaviors in mice via the production of neuroactive metabolites [61]. Similarly, Kundu et al. used metagenome analysis to select sodium butyrate as a candidate metabolite and, on in vivo re-validation, reproduced the phenotype of FMT [62]. Britton et al. combined the results of 16S rRNA gene sequencing with the detection of homeostatic intestinal T-cell responses to interpret a general mechanism for the microbial contribution to inflammatory bowel disease [63].

7. Conclusions and Perspectives

Strategically FMT is the most direct method used to change the composition of gut microbiota. In the present study, we provided a brief overview of the FMT protocol and summarized the research progress of FMT. However, this review has some limitations. One limitation is that we only included oral administration, the most common route. The protocol provided in a previous section may not be generalizable to other routes, such as rectal FMT. Additionally, we only provide the primary means of FMT and solutions to common problems, and some other difficulties and innovations are not included. These limitations may mean that the instructions are only informative for beginners. However, with the rapid progress of gut microbiology, it is hoped that more studies will be conducted in the

future to further clarify the application prospects of FMT and seek more comprehensive and optimized FMT strategies.

Abbreviations

Alzheimer's disease: AD; *Clostridiodies difficile* infection: CDI; Fecal microbiota transplantation: FMT; high-fat diet: HFD; immunoglobulin: Ig; ketogenic diet: KD; Phosphate-buffered saline: PBS; phlorizin: PHZ; selective microbiota transplantation: SMT; wild type: WT.

References

1. Ley, R.E.; Hamady, M.; Lozupone, C.; Turnbaugh, P.J.; Ramey, R.R.; Bircher, J.S.; Schlegel, M.L.; Tucker, T.A.; Schrenzel, M.D.; Knight, R.; et al. Evolution of mammals and their gut microbes. *Science* **2008**, *320*, 1647–1651. [CrossRef] [PubMed]
2. Pedersen, H.K.; Gudmundsdottir, V.; Nielsen, H.B.; Hyotylainen, T.; Nielsen, T.; Jensen, B.A.; Forslund, K.; Hildebrand, F.; Prifti, E.; Falony, G.; et al. Human gut microbes impact host serum metabolome and insulin sensitivity. *Nature* **2016**, *535*, 376–381. [CrossRef] [PubMed]
3. Le Chatelier, E.; Nielsen, T.; Qin, J.; Prifti, E.; Hildebrand, F.; Falony, G.; Almeida, M.; Arumugam, M.; Batto, J.M.; Kennedy, S.; et al. Richness of human gut microbiome correlates with metabolic markers. *Nature* **2013**, *500*, 541–546. [CrossRef] [PubMed]
4. Karlsson, F.; Tremaroli, V.; Nielsen, J.; Bäckhed, F. Assessing the human gut microbiota in metabolic diseases. *Diabetes* **2013**, *62*, 3341–3349. [CrossRef] [PubMed]
5. Sender, R.; Fuchs, S.; Milo, R. Revised Estimates for the Number of Human and Bacteria Cells in the Body. *PLoS Biol.* **2016**, *14*, e1002533. [CrossRef]
6. Turnbaugh, P.J.; Hamady, M.; Yatsunenko, T.; Cantarel, B.L.; Duncan, A.; Ley, R.E.; Sogin, M.L.; Jones, W.J.; Roe, B.A.; Affourtit, J.P.; et al. A core gut microbiome in obese and lean twins. *Nature* **2009**, *457*, 480–484. [CrossRef] [PubMed]
7. Dominguez-Bello, M.G.; Godoy-Vitorino, F.; Knight, R.; Blaser, M.J. Role of the microbiome in human development. *Gut* **2019**, *68*, 1108–1114. [CrossRef]
8. Leshem, A.; Liwinski, T.; Elinav, E. Immune-Microbiota Interplay and Colonization Resistance in Infection. *Mol. Cell* **2020**, *78*, 597–613. [CrossRef]
9. Kabouridis, P.S.; Lasrado, R.; McCallum, S.; Chng, S.H.; Snippert, H.J.; Clevers, H.; Pettersson, S.; Pachnis, V. The gut microbiota keeps enteric glial cells on the move; prospective roles of the gut epithelium and immune system. *Gut Microbes* **2016**, *6*, 398–403. [CrossRef]
10. Agirman, G.; Hsiao, E.Y. SnapShot: The microbiota-gut-brain axis. *Cell* **2021**, *184*, 2524. [CrossRef]
11. Meroni, E.; Stakenborg, N.; Viola, M.F.; Boeckxstaens, G.E. Intestinal macrophages and their interaction with the enteric nervous system in health and inflammatory bowel disease. *Acta Physiol.* **2018**, *225*, e13163. [CrossRef]
12. Wargo, J.A. Modulating gut microbes. *Science* **2020**, *369*, 1302–1303. [CrossRef]
13. Gupta, A.; Khanna, S. Fecal Microbiota Transplantation. *JAMA* **2017**, *318*, 102. [CrossRef] [PubMed]
14. Burrello, C.; Giuffrè, M.R.; Macandog, A.D.; Diaz-Basabe, A.; Cribiù, F.M.; Lopez, G.; Borgo, F.; Nezi, L.; Caprioli, F.; Vecchi, M.; et al. Fecal Microbiota Transplantation Controls Murine Chronic Intestinal Inflammation by Modulating Immune Cell Functions and Gut Microbiota Composition. *Cells* **2019**, *8*, 517. [CrossRef]
15. Sun, J.; Xu, J.; Ling, Y.; Wang, F.; Liu, J. Fecal microbiota transplantation alleviated Alzheimer's disease-like pathogenesis in APP/PS1 transgenic mice. *Transl. Psychiatry* **2019**, *9*, 189. [CrossRef] [PubMed]
16. Zhan, G.; Yang, N.; Li, S.; Huang, N.; Luo, A. Abnormal gut microbiota composition contributes to cognitive dysfunction in SAMP8 mice. *Aging* **2018**, *10*, 1257–1267. [CrossRef]

17. Fujii, Y.; Nguyen, T.; Fujimura, Y.; Kameya, N.; Morita, H. Fecal metabolite of a gnotobiotic mouse transplanted with gut microbiota from a patient with Alzheimer's disease. *Biosci. Biotechnol. Biochem.* **2019**, *83*, 1–9. [CrossRef]
18. Sampson, T.; De Belius, J.; Thron, T.; Janssen, S.; Shastri, G.; Ilhan, Z.; Challis, C.; Schretter, C.; Rocha, S.; Gradinaru, V. Gut Microbiota Regulate Motor Deficits and Neuroinflammation in a Model of Parkinson's Disease. *Cell* **2016**, *167*, 1469–1480. [CrossRef] [PubMed]
19. Shang, L.; Liu, H.; Yu, H.; Chen, M.; Yang, T.; Zeng, X.; Qiao, S. Core Altered Microorganisms in Colitis Mouse Model: A Comprehensive Time-Point and Fecal Microbiota Transplantation Analysis. *Antibiotics* **2021**, *10*, 643. [CrossRef]
20. Olson, C.A.; Vuong, H.E.; Yano, J.M.; Liang, Q.Y.; Nusbaum, D.J.; Hsiao, E.Y. The Gut Microbiota Mediates the Anti-Seizure Effects of the Ketogenic Diet. *Cell* **2018**, *173*, 1728–1741. [CrossRef] [PubMed]
21. Boehme, M.; Guzzetta, K.E.; Bastiaanssen, T.F.S.; van de Wouw, M.; Moloney, G.M.; Gual-Grau, A.; Spichak, S.; Olavarría-Ramírez, L.; Fitzgerald, P.; Morillas, E.; et al. Microbiota from young mice counteracts selective age-associated behavioral deficits. *Nat. Aging* **2021**, *1*, 666–676. [CrossRef]
22. Cammarota, G.; Ianiro, G.; Tilg, H.; Rajilić-Stojanović, M.; Kump, P.; Satokari, R.; Sokol, H.; Arkkila, P.; Pintus, C.; Hart, A.; et al. European consensus conference on faecal microbiota transplantation in clinical practice. *Gut* **2017**, *66*, 569–580. [CrossRef] [PubMed]
23. Sokol, H.; Galperine, T.; Kapel, N.; Bourlioux, P.; Seksik, P.; Barbut, F.; Scanzi, J.; Chast, F.; Batista, R.; Joly, F.; et al. Faecal microbiota transplantation in recurrent Clostridium difficile infection: Recommendations from the French Group of Faecal microbiota Transplantation. *Dig. Liver Dis.* **2016**, *48*, 242–247. [CrossRef]
24. Mullish, B.H.; Quraishi, M.N.; Segal, J.P.; McCune, V.L.; Baxter, M.; Marsden, G.L.; Moore, D.J.; Colville, A.; Bhala, N.; Iqbal, T.H.; et al. 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 Society (HIS) guidelines. *J. Hosp. Infect.* **2018**, *67*, 1920–1941.
25. Zhang, F.; Cui, B.; He, X.; Nie, Y.; Wu, K.; Fan, D. Microbiota transplantation: Concept, methodology and strategy for its modernization. *Protein Cell* **2018**, *9*, 462–473. [CrossRef] [PubMed]
26. Zhang, F.; Luo, W.; Shi, Y.; Fan, Z.; Ji, G. Should we standardize the 1700-year-old fecal microbiota transplantation? *Am. J. Gastroenterol.* **2012**, *107*, 1755. [CrossRef] [PubMed]
27. Hong, G. *Zhou Hou Bei Ji Fang*; Tianjin Science & Technology Press: Tianjin, China, 2000.
28. Mei, Q.X.; Hui-Fei, W.U. The Book Zhou Hou Bei Ji Fang Make a Great Contribution to the Progress of Medicine. *Study J. Tradit. Chin. Med.* **2005**. Available online: https://xueshu.baidu.com/usercenter/paper/show?paperid=1343fc06ebec0f7b5b467a92fbfbad4b&site=xueshu_se&hitarticle=1 (accessed on 27 February 2022).
29. Li, S. *Ben Cao Gang Mu*; Huaxia Press: Beijing, China, 2011.
30. Eiseman, B.; Silen, W.; Bascom, G.S.; Kauvar, A.J. Fecal enema as an adjunct in the treatment of pseudomembranous enterocolitis. *Surgery* **1958**, *44*, 854–859.
31. Borody, T.J.; Campbell, J. Fecal microbiota transplantation: Current status and future directions. *Expert Rev. Gastroenterol. Hepatol.* **2011**, *5*, 653–655. [CrossRef]
32. Surawicz, C.M.; Brandt, L.J.; Binion, D.G.; Ananthakrishnan, A.N.; Curry, S.R.; Gilligan, P.H.; McFarland, L.V.; Mellow, M.; Zuckerbraun, B.S. Guidelines for diagnosis, treatment, and prevention of Clostridium difficile infections. *Am. J. Gastroenterol.* **2013**, *108*, 478–498, 499. [CrossRef]
33. Allegretti, J.R.; Mullish, B.H.; Kelly, C.; Fischer, M. The evolution of the use of faecal microbiota transplantation and emerging therapeutic indications. *Lancet* **2019**, *394*, 420–431. [CrossRef]
34. Moayyedi, P.; Surette, M.G.; Kim, P.T.; Libertucci, J.; Wolfe, M.; Onischi, C.; Armstrong, D.; Marshall, J.K.; Kassam, Z.; Reinisch, W.; et al. Fecal Microbiota Transplantation Induces Remission in Patients with Active Ulcerative Colitis in a Randomized Controlled Trial. *Gastroenterology* **2015**, *149*, 102–109. [CrossRef] [PubMed]
35. Suskind, D.L.; Brittnacher, M.J.; Wahbeh, G.; Shaffer, M.L.; Hayden, H.S.; Qin, X.; Singh, N.; Damman, C.J.; Hager, K.R.; Nielson, H.; et al. Fecal microbial transplant effect on clinical outcomes and fecal microbiome in active Crohn's disease. *Inflamm. Bowel Dis.* **2015**, *21*, 556–563. [CrossRef] [PubMed]
36. Ishikawa, D.; Sasaki, T.; Takahashi, M.; Kuwahara-Arai, K.; Haga, K.; Ito, S.; Okahara, K.; Nakajima, A.; Shibuya, T.; Osada, T.; et al. The Microbial Composition of Bacteroidetes Species in Ulcerative Colitis Is Effectively Improved by Combination Therapy with Fecal Microbiota Transplantation and Antibiotics. *Inflamm. Bowel Dis.* **2018**, *24*, 2590–2598. [CrossRef] [PubMed]
37. Cheon, D.J.; Orsulic, S. Mouse models of cancer. *Annu. Rev. Pathol. Mech. Dis.* **2011**, *6*, 95–119. [CrossRef]
38. Vanhooren, V.; Libert, C. The mouse as a model organism in aging research: Usefulness, pitfalls and possibilities. *Ageing Res. Rev.* **2013**, *12*, 8–21. [CrossRef]
39. Seishima, J.; Iida, N.; Kitamura, K.; Yutani, M.; Wang, Z.; Seki, A.; Yamashita, T.; Sakai, Y.; Honda, M.; Yamashita, T.; et al. Gut-derived Enterococcus faecium from ulcerative colitis patients promotes colitis in a genetically susceptible mouse host. *Genome Biol.* **2019**, *20*, 252. [CrossRef]
40. Administrator. Guidelines for Routine Administration of Drugs in Animals. Available online: https://lac.nankai.edu.cn/2020/0529/c19628a275201/page.htm (accessed on 29 May 2020).
41. Huo, Y. *Experimental Zoology of Cardiovascular Diseases*; People's Medical Publishing House: Beijing, China, 2011.

42. Vicentini, F.A.; Keenan, C.M.; Wallace, L.E.; Woods, C.; Cavin, J.; Flockton, A.R.; Macklin, W.B.; Belkind-Gerson, J.; Hirota, S.A.; Sharkey, K.A. Intestinal microbiota shapes gut physiology and regulates enteric neurons and glia. *Microbiome* **2021**, *9*, 210. [CrossRef] [PubMed]

43. Reikvam, D.H.; Erofeev, A.; Sandvik, A.; Grcic, V.; Jahnsen, F.L.; Gaustad, P.; McCoy, K.D.; Macpherson, A.J.; Meza-Zepeda, L.A.; Johansen, F.E. Depletion of murine intestinal microbiota: Effects on gut mucosa and epithelial gene expression. *PLoS ONE* **2011**, *6*, e17996. [CrossRef] [PubMed]

44. Ishikawa, D.; Sasaki, T.; Osada, T.; Kuwahara-Arai, K.; Haga, K.; Shibuya, T.; Hiramatsu, K.; Watanabe, S. Changes in Intestinal Microbiota Following Combination Therapy with Fecal Microbial Transplantation and Antibiotics for Ulcerative Colitis. *Inflamm. Bowel Dis.* **2016**, *23*, 116–125. [CrossRef] [PubMed]

45. Chu, N.D.; Smith, M.B.; Perrotta, A.R.; Kassam, Z.; Alm, E.J. Profiling Living Bacteria Informs Preparation of Fecal Microbiota Transplantations. *PLoS ONE* **2017**, *12*, e170922. [CrossRef]

46. Gorzelak, M.A.; Gill, S.K.; Tasnim, N.; Ahmadi-Vand, Z.; Jay, M.; Gibson, D.L. Methods for Improving Human Gut Microbiome Data by Reducing Variability through Sample Processing and Storage of Stool. *PLoS ONE* **2015**, *10*, e134802. [CrossRef] [PubMed]

47. Cui, B.; Li, P.; Xu, L.; Zhao, Y.; Wang, H.; Peng, Z.; Xu, H.; Xiang, J.; He, Z.; Zhang, T.; et al. Step-up fecal microbiota transplantation strategy: A pilot study for steroid-dependent ulcerative colitis. *J. Transl. Med.* **2015**, *13*, 298. [CrossRef] [PubMed]

48. Fang, H.; Fu, L.; Wang, J. Protocol for Fecal Microbiota Transplantation in Inflammatory Bowel Disease: A Systematic Review and Meta-Analysis. *BioMed Res. Int.* **2018**, *2018*, 8941340. [CrossRef]

49. Lima, S.F.; Gogokhia, L.; Viladomiu, M.; Chou, L.; Putzel, G.; Jin, W.; Pires, S.; Guo, C.; Gerardin, Y.; Crawford, C.V.; et al. Transferable Immunoglobulin A–Coated Odoribacter splanchnicus in Responders to Fecal Microbiota Transplantation for Ulcerative Colitis Limits Colonic Inflammation. *Gastroenterology* **2022**, *162*, 166–178. [CrossRef] [PubMed]

50. Paramsothy, S.; Kamm, M.A.; Kaakoush, N.O.; Walsh, A.J.; van den Bogaerde, J.; Samuel, D.; Leong, R.; Connor, S.; Ng, W.; Paramsothy, R.; et al. Multidonor intensive faecal microbiota transplantation for active ulcerative colitis: A randomised placebo-controlled trial. *Lancet* **2017**, *389*, 1218–1228. [CrossRef]

51. Carabotti, M.; Scirocco, A.; Maselli, M.A.; Severi, C. The gut-brain axis: Interactions between enteric microbiota, central and enteric nervous systems. *Ann. Gastroenterol.* **2015**, *28*, 203–209.

52. Kim, N.; Jeon, S.H.; Ju, I.G.; Gee, M.S.; Do, J.; Oh, M.S.; Lee, J.K. Transplantation of gut microbiota derived from Alzheimer's disease mouse model impairs memory function and neurogenesis in C57BL/6 mice. *Brain Behav. Immun.* **2021**, *98*, 357–365. [CrossRef]

53. Chevalier, G.; Siopi, E.; Guenin-Macé, L.; Pascal, M.; Laval, T.; Rifflet, A.; Boneca, I.G.; Demangel, C.; Colsch, B.; Pruvost, A.; et al. Effect of gut microbiota on depressive-like behaviors in mice is mediated by the endocannabinoid system. *Nat. Commun.* **2020**, *11*, 6363. [CrossRef]

54. Li, J.; Zhao, F.; Wang, Y.; Chen, J.; Tao, J.; Tian, G.; Wu, S.; Liu, W.; Cui, Q.; Geng, B.; et al. Gut microbiota dysbiosis contributes to the development of hypertension. *Microbiome* **2017**, *5*, 14. [CrossRef]

55. Zhang, X.Y.; Chen, J.; Yi, K.; Peng, L.; Xie, J.; Gou, X.; Peng, T.; Tang, L. Phlorizin ameliorates obesity-associated endotoxemia and insulin resistance in high-fat diet-fed mice by targeting the gut microbiota and intestinal barrier integrity. *Gut Microbes* **2020**, *12*, 1–18. [CrossRef]

56. Zhang, P.P.; Li, L.L.; Han, X.; Li, Q.W.; Zhang, X.H.; Liu, J.J.; Wang, Y. Fecal microbiota transplantation improves metabolism and gut microbiome composition in db/db mice. *Acta Pharmacol. Sin.* **2020**, *41*, 678–685. [CrossRef] [PubMed]

57. Zhang, Y.; Huang, R.; Cheng, M.; Wang, L.; Chao, J.; Li, J.; Zheng, P.; Xie, P.; Zhang, Z.; Yao, H. Gut microbiota from NLRP3-deficient mice ameliorates depressive-like behaviors by regulating astrocyte dysfunction via circHIPK2. *Microbiome* **2019**, *7*, 116. [CrossRef] [PubMed]

58. Bárcena, C.; Valdés-Mas, R.; Mayoral, P.; Garabaya, C.; Durand, S.; Rodríguez, F.; Fernández-García, M.T.; Salazar, N.; Nogacka, A.M.; Garatachea, N.; et al. Healthspan and lifespan extension by fecal microbiota transplantation into progeroid mice. *Nat. Med.* **2019**, *25*, 1234–1242. [CrossRef] [PubMed]

59. Yu, F.; Han, W.; Zhan, G.; Li, S.; Jiang, X.; Wang, L.; Xiang, S.; Zhu, B.; Yang, L.; Luo, A.; et al. Abnormal gut microbiota composition contributes to the development of type 2 diabetes mellitus in db/db mice. *Aging* **2019**, *11*, 10454–10467. [CrossRef]

60. Trikha, S.; Lee, D.M.; Ecton, K.E.; Wrigley, S.D.; Vazquez, A.R.; Litwin, N.S.; Thomas, K.N.; Wei, Y.; Battson, M.L.; Johnson, S.A.; et al. Transplantation of an obesity-associated human gut microbiota to mice induces vascular dysfunction and glucose intolerance. *Gut Microbes* **2021**, *13*, 1940791. [CrossRef]

61. Sharon, G.; Cruz, N.J.; Kang, D.W.; Gandal, M.J.; Wang, B.; Kim, Y.M.; Zink, E.M.; Casey, C.P.; Taylor, B.C.; Lane, C.J.; et al. Human Gut Microbiota from Autism Spectrum Disorder Promote Behavioral Symptoms in Mice. *Cell* **2019**, *177*, 1600–1618. [CrossRef]

62. Kundu, P.; Lee, H.U.; Garcia-Perez, I.; Tay, E.; Kim, H.; Faylon, L.E.; Martin, K.A.; Purbojati, R.; Drautz-Moses, D.I.; Ghosh, S.; et al. Neurogenesis and prolongevity signaling in young germ-free mice transplanted with the gut microbiota of old mice. *Sci. Transl. Med.* **2019**, *11*, eaau4760. [CrossRef]

63. Britton, G.J.; Contijoch, E.J.; Mogno, I.; Vennaro, O.H.; Llewellyn, S.R.; Ng, R.; Li, Z.; Mortha, A.; Merad, M.; Das, A.; et al. Microbiotas from Humans with Inflammatory Bowel Disease Alter the Balance of Gut Th17 and RORγt+ Regulatory T Cells and Exacerbate Colitis in Mice. *Immunity* **2019**, *50*, 212–224. [CrossRef]