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Evolution of fecal microbiota transplantation in methodology and ethical issues

Faming Zhang[1,2], Ting Zhang[1,2], Heming Zhu[3] and Thomas J Borody[4]

Fecal microbiota transplantation (FMT), the core therapy for remodeling the gut microbiota with a long medical history, has gained great attention worldwide in recent years. Increasing studies have explored its indications, methodology, efficacy, safety, and ethics. Purified forms of FMT, using an automated method for the purification of fecal microbiota from stool, has become a reality. Colonic transendoscopic enteral tubing makes frequent FMT delivery into the whole colon feasible. This review focuses on the recent progress in laboratory preparation, updated clinical strategies, novel delivery methods, and ethical issues surrounding FMT in clinical studies.

Addresses
[1] Medical Center for Digestive Diseases, The Second Affiliated Hospital of Nanjing Medical University, Nanjing 210011, China
[2] Key Lab of Holistic Integrative Enterology, Nanjing Medical University, Nanjing 210011, China
[3] Department of Acupuncture and Oriental Medicine, Maryland University of Integrative Health, Laurel, MD, United States
[4] Centre for Digestive Diseases, Five Dock, NSW 2046, Australia

Corresponding author: Zhang, Faming (fzhang@njmu.edu.cn)

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Introduction

Humans have always been inseparable from the use of healthy human stool to treat diseases for thousands of years [1]. The core of this ancient therapy for using stool, named fecal microbiota transplantation (FMT), has experienced a renaissance in recent years with great attention worldwide. FMT has been classified as a treatment for recurrent *Clostridium difficile* infection (CDI) [2••]. Recently, series of pilot studies on ulcerative colitis (UC) [3–7,8••], Crohn's diseases (CD) [9–11], irritable bowel syndrome [12••], constipation [13], liver diseases [14–18], blood diseases [19,20•], autism [21], epilepsy [22], and recurrent urinary tract infections [23], have showed potential effectiveness of FMT in these diseases beyond CDI. However, the crude methods, inhuman clinical work-flow, and underdetermined long-term safety have limited the perceived clinical benefit of FMT [1]. This review focuses on the recent progress in the laboratory preparation, novel delivering methods, updated clinical strategies, and the ethical recognition of FMT in practice and pilot studies. Published literature was mainly based on recent randomized clinical trials, pilot series studies, research group statements, consensus statements, and guidelines.

Multidimensional exclusive methods for donor screening

Donors for FMT can be divided into two types, allogeneic donors and autologous donors, according to the source of stool. The allogeneic source is most commonly adopted because it is easy to meet the requirement for one-to-many treatments, and it has shown improved efficacy compared with an autologous source [12••,24,25,26••]. The autologous source is currently used in very few controlled studies in patients who had diseases. The strategy to screen healthy stool donors for FMT is to use the concept of exclusive methods [26••,27••,28••,29••]. The current exclusive methods for screening best allogeneic donors were recently updated to Eight Dimensions of Screening [27••,29••]: age, physiology, pathology, psychology, veracity, time, living environment, and recipients.

There are no well-defined age restriction on donors. Individuals aged 6–24 are preferred as donors for FMT treating all potential conditions [1] by the China FMT-standardized Study Group, but the latest joint British FMT guideline in 2018 recommended an age range of 18–60 years old for CDI [26••]. Body weight index beyond the normal range is one of the exclusive physiological criteria. All existing current known microbiota-related diseases in potential donors should be excluded. The veracity, donating time, living environment of donors, and status of recipients are required to be considered [1]. Honesty is important for ensuring correct information from the donors in center bank, and persons with questionable integrity should be excluded. If donors travelled to places with low hygiene or high infection risk for endemic diarrhea, they need to be retested for absence of possible bacteria or viral infection before they join the donation again [30,31]. Potential individuals living in extreme environments such as regions of high altitude, high temperature, alpine cold, high humidity, severe pollution, and saline alkali, are also excluded [29••].

The status of donors is not the only factor affecting clinical outcome. The assessment on the status of recipients including their age, immune function, and nutritional status should be integrated to predict the clinical outcomes of FMT [32].

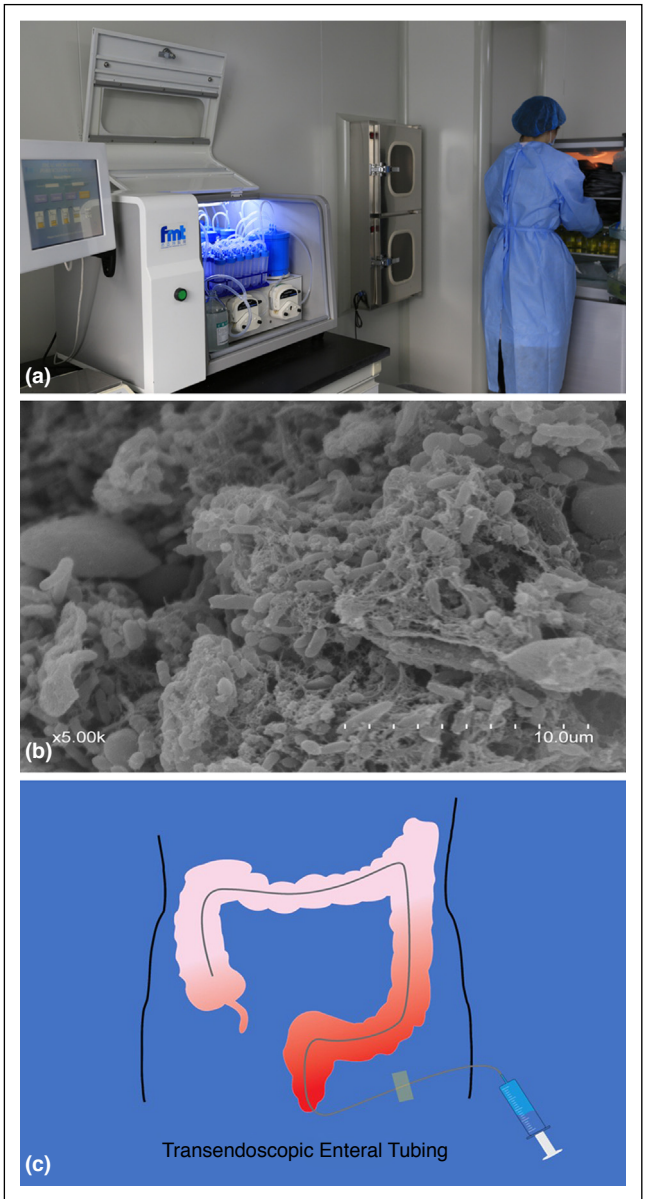
Laboratory preparation is moving away from crude FMT

The methods of laboratory preparation for FMT reported in the literature are classified into 'Rough Filtration', 'Filtration plus Centrifugation (FPC)', and 'Microfiltration plus Centrifugation (MPC)' [1,11]. For example, the method of fecal microbiota manipulation in Hamilton's [33] report was defined as FPC, another method based on the automatic microbiota purification system (GenFMTer FMT medical, Nanjing, China) was defined as MPC [7,10]. The latest British FMT working group in 2018 considered only studies that used the administration of manipulated whole stool for the purposes in their guideline [26••]. However, it is timely to consider the popular aesthetic standard, discomfort of the technician, and quality control of the process.

The latest automated method developed for purification of the microbiota suspension aims to minimize the processing time for preservation of living bacteria [1,5]. According to the composition analysis between the enriched fecal bacteria by MPC and the original feces, there was no significant difference in bacteria diversity [10]. The process of purification significantly reduced the adverse events in CD, but did not change the efficacy of FMT [27••]. This protocol achieved support from the experimental evidence [5,34]. After the intestinal microbiota is removed from the body and exposed to oxygen for an extended period, it would have a significant effect on the survival of the bacterial communities. For example, *Faecalibacterium prausnitzii* cannot survive beyond two minutes if exposed to an oxygen-rich environment [5]. Chu's recent experimental study [35] demonstrated that oxygen exposure degraded fecal bacterial communities. The stool was permitted to be used within 6 hours according to the latest recommendations from Europe [26••,30] and 5D framework in USA for CDI [36••]. A method called a 'one-hour FMT protocol', defined the process time from the donor's defecation to the enriched bacterial material be infused into the patient's intestines within one hour based on a new automatic purification system GenFMTer [7,10] (Figure 1). Using the 'one-hour FMT protocol', the high level of clinical response rates was reported in FMT treated inflammatory bowel disease (IBD) [7,10,20•,27••,29••,37,38]. Beyond the time control for preparation, another advancement in the laboratory is using anaerobically prepared stool in UC [8••,38].

The laboratory has been suggested to match a Good Manufacture Practice (GMP) facility corresponding to the level required by pharmaceutical companies for the manufacture of oral medicine [1,5] (Figure 1). The laboratory for fecal bacteria preparation must have a dedicated room [1,5], where unrelated individuals, animals, or biological samples are not allowed to enter. Additionally, to achieve better traceability, donors' fecal samples should be stored in deep cryopreservation for at least two years [5] according to the FMT-standardized study group [1]. This concept of the work-flow on FMT has been used in

Figure 1



The evolution of methodology for FMT. **(a)** Working GenFMTer in GMP lab, **(b)** Electro-scan for microbiota from the manipulation process for stool by GenFMTer and the following centrifugation and washing. **(c)** Frequent FMTs to whole colon can be delivered through colonic transendoscopic enteral tubing.

Hvas's trial for CDI in Denmark [32], though they did not introduce the time for the retention of the materials.

Colonic transendoscopic enteral tubing (TET) makes frequent FMTs possible

The delivery methods for FMT include three routes through the upper-gut, mid-gut and lower-gut [39]. Oral intake of fecal matters in ancient practice [40] and in a recent trial on autism children [21], as well as microbiota capsules [41,42] are direct delivery methods through the upper-gut.

The microbiota suspension can be infused into the small intestine beyond the second duodenal segment, which was defined as the mid-gut, through endoscopy, nasojejunal tube, mid-gut transendoscopic enteral tubing (TET), small intestine stoma or percutaneous endoscopic gastro-jejunostomy (PEG-J) [1,39,43•]. FMT can be also delivered to the lower-gut through colonoscopy, enema, distal ileum stoma, stoma after colostomy and colonic TET [1,39].

The latest progress on FMT delivering is colonic TET (Figure 1). The methods of colonic endoscopic procedure for TET is generally suggested as: the marketing available TET tube (tube dimeter 2.7 mm, FMT medical, Nanjing, China) is inserted into the cecum through the endoscopic channel, the tube is then kept within the colon and the endoscope is taken out of the colon. The endoscope is then reinserted into the cecum, where the loop of the tube is fixed onto the wall by clips. It has been a safe and convenient procedure for multiple FMTs and colonic medication administration [29••,39] in populations aged over seven years old. The FMT through colonic TET for children under seven years old [39] indicated the clinical significance of alleviating pain from children and family.

There is no single best universal delivery method that matches all patients, but the most suitable choice should be made for each individual. When considering the delivery route of FMT, disease condition, aesthetic factors, psychology, and privacy should be considered carefully during the entire work-flow. All efforts to reduce the incidence of nausea and vomiting mentioned in different reports [7,10,44–47] have been highlighted as an important detail of work-flow in practice [1].

These delivery routes have been widely used in various types of studies. Paramsothy *et al.* [49] reported that FMT enemas induced 27% of active UC patients to achieve steroid-free clinical remission with endoscopic remission or response (Mayo score $\leq$2, all subscores $\leq$1, and $\geq$1 point reduction in endoscopy subscore) at week 8. In accordance with the largest case series in CD up to now, Wang *et al.* [27••] reported that FMT via mid-gut for mild to severe CD induced 55.6% of patients to achieve clinical remission. Ding *et al.* [29••] reported that a lower rate of FMT-related adverse events (AEs) was associated with using an automatic system to prepare the fecal microbiota and using colonic TET as the delivery method.

The above studies only illustrate the effectiveness of a single approach of FMT. A recent study based on 134 UC patients [29••] proved that no difference in efficacy was observed between patients who received FMT from mid-gut and those from colonic TET. The combined FMT delivery within one treatment course has recently been used in two randomized clinical trials with endoscopic colonic delivery followed by repeat enema [8••,48].

Using FMT for refractory or serious diseases needs clinical care

The clinical outcomes of FMT varied widely in different studies [4,7,10,25,37,49]. The effectiveness from a single FMT might be limited for severe and refractory microbiota-related conditions. Recently Fischer *et al.* [50] reported that severe and complicated indication, inpatient status during FMT, and the number of previous CDI-related hospitalizations were strongly associated with early failure of a single FMT. Using FMT for microbiota related disease beyond CDI should be more dependent on the strategy of treatment than CDI. A protocol called the step-up FMT strategy was designed for the treatment of refractory microbiota related conditions, consisting of three steps: step 1 means single FMT; step 2 means multiple FMTs ($\geq$2); step 3 means the combination of one or more FMTs with the followed typical treatments (e.g. steroids, cyclosporine, anti-TNF antibody). The clinical improvement could be observed during the following 12–36 hours post-FMT, and FMT daily can be tolerated. Generally, patients underwent 2–3 FMTs within several days in step 2. Each FMT used the same or similar dose in all steps [29••]. It has been reported to treat steroid-dependent UC with demonstrated efficacy [7,29••] following case series and prospective studies. Shimizu *et al.* [3] reported a child with steroid and anti-TNF refractory UC successfully achieved clinical remission and low-dose steroid control by repeated FMTs. Seth *et al.* [51] reported the first case of UC in India who underwent this strategy and finally maintained clinical and endoscopic remission for more than eight months. Another study from India reported that a multisession FMT, which corresponds to the step-up FMT strategy, successfully induced clinical remission for steroid-dependent UC in a real world intention-to-treat analysis [52•]. Additionally, this FMT strategy was applied in the treatment of steroid refractory intestinal acute graft-versus-host disease. Eight patients who received FMT achieved a higher progression-free survival as compared to those did not receive FMT [20•]. In summary, refractory microbiota-related diseases, including steroid-resistant/dependent acute graft-versus-host disease [19,20•], steroid ineligible severe alcoholic hepatitis [14], and cancer [53•],

had achieved promising results from pilot studies based on FMT and the followed typical medication treatment.

If patients achieved clinical improvement after FMT, they would be recommended to receive the second course of FMT at three months later for maintaining the clinical benefits from the first course. This strategy has been reported in recent studies on CD and UC from Zhang' group [24,29••].

Non-CDI antibiotic use was common after successful FMT and significantly increased the risk of a new episode of CDI. In a study with 404 CDI cases, Allegretti *et al.* found that the prophylactic use of anti-CDI antibiotics or probiotics was not protective [54]. An interesting study on FMT treating CDI demonstrated that hemoglobin was the strongest and only statistically significant covariate associated with failure of FMT. The presence of anemia, that is, a hemoglobin below the gender-specific reference interval, was associated with a 6.3 times increased risk of failure (95% CI 1.3–30.9) [32].

Ethical issues of FMT will continue to progress

FMT mainly presents the ethical and social issues in five areas [55]: (1) informed consent and the vulnerability of patients; (2) determining what a 'suitable healthy donor' is; (3) safety and risk; (4) commercialization and potential exploitation of vulnerable patients; and (5) public health implications. Authorities must regulate FMT to safeguard patients and donors, promote further research into safety and efficacy, and avoid abuse of the treatment. North America has strict regulation on FMT since 2013. The regulations from governments in Europe, Austria and China are ongoing to shape.

The patients treated with FMT for diseases beyond CDI in China are much more than those in other countries. It is based on the basic theories of Nature-Human Unity and Yin-Yang Harmony in traditional Chinese medicine [1], which believes that everything in nature live together as an entity, for example, bacteria and human body co-exist in one body to maintain body's health and wellbeing [56,57]. At the same time everything in nature contains two opposing and interdependent parts, Yin and Yang. In short, the microbiota is the 'Yin' and the host is the 'Yang'. Either part's problems may cause disharmony in the body. To rethink the ancient medical history of orally FMT designed by nature may be useful to avoiding the thinking of 'Chicken Little' [55].

However, oral administration of the fecal solution and primitive methods of preparation should not be widely acceptable in current practice, though it has been used in a recent trial in older children aged 7–17 years old [21]. Additionally, the cost-effective analysis demonstrated FMT as the best strategy for the treatment of recurrent CDI [58]. A recent study demonstrated that FMT showed its cost-effectiveness, particularly in improving quality of life and decreasing the medical and societal cost, for the moderate to severe IBD in a Chinese cohort [59]. However, FMT faces potential robust competition [55]. This is the main reason why FMT was strictly restricted in 2013 by the FDA [60], though the over-restriction of FMT actually hindered the professional care at hospitals and even led to patient self-administration of FMT at home [61]. In a word, the ethics of FMT should progress, but not from one extreme to another.

Conclusion

The best way is to move standardized FMT forward given that human beings have been inseparable from the use of healthy human stool to treat diseases for thousands of years. The latest progress in methodology of FMT will make more physicians willing to accept and adopt FMT, and, therefore, bring more benefits to patients. In conclusion, emerging evidence of FMT is opening a new era of therapeutics revolution, though it is facing a lot of challenges.

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Conflict of interest statement

Faming Zhang invented the concept of GenFMTer and transendoscopic enteral tubing and devices related to it. Ting Zhang, Heming Zhu and Thomas J Borody declare no conflict interest.

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