Fecal Microbiota Transplantation for Ulcerative Colitis: An Evolving Therapy

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Background: Fecal microbiota transplantation (FMT) is currently an approved treatment for recurrent and refractory *Clostridioides difficile* infection. However, its use in ulcerative colitis is at an early stage and significant gaps remain in our understanding of the mechanisms and logistics of its practical application.

Methods and results: This article aims to look into specific issues which remain unsettled for use of FMT in ulcerative colitis including donor and recipient selection, route of administration, and duration of therapy. We also discuss optimal ways to assess response to FMT and the current state of FMT regulations. In addition, we postulate the impact of diet on the microbiome profile of the donor and recipient. We also suggest a change in the nomenclature from FMT to fecal microbiome transfer.

Conclusion: FMT is an evolving therapy. There are several considerations for its use in UC but its use and role should be directed by further clinical trials.

Lay Summary

This article explores fecal microbiota transplantation (FMT) in the treatment of ulcerative colitis in regards to donor and recipient selection, administration route, therapy duration, and donor diet. We discuss assessment of FMT response and the current state of FMT regulation.

Key Words: fecal microbiome transfer, fecal microbial transplantation, ulcerative colitis, randomized controlled trial

INTRODUCTION

Fecal microbiota transplantation (FMT) is defined as the administration of *healthy donor whole stool* (that consists of microbial communities and their functional ecologies) into the gastrointestinal (GI) tract of an individual with the aim of correcting dysbiosis. It is currently the approved treatment for recurrent/refractory *Clostridioides difficile* infection (rCDI).[1,2] However, the use of FMT in ulcerative colitis (UC) is still evolving with many issued remaining unsettled.

FMT was used for UC in 1989 by Bennet and Borody.[3,4] However, interest in FMT for inflammatory bowel disease (IBD) was heightened after 2 randomized trials evaluating FMT for induction of remission in UC were published with conflicting results in the year 2015.[5,6] Since then there have been 2 more randomized controlled trials (RCTs, both from Australia) demonstrating efficacy of FMT in inducing remission in UC.[7,8] Benefit with multisession colonoscopic FMT in inducing remission in patients with steroid-dependent UC has also been reported.[9] Though the literature suggests that

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FMT increases the proportion of participants achieving clinical remission in UC, no firm conclusions can be drawn at the moment.[10] However, it has been consistently shown that responders to FMT experience microbial enrichment (increase in microbiota diversity) and a shift in composition that resembles the profile of their donor, similar to what has been found in rCDI patients.

A comprehensive literature search was carried out on MEDLINE, MedIndia, EMBASE, and Cochrane Central Register of Controlled Trials for relevant literature published on use of FMT in UC. All the original articles, systematic reviews, meta-analyses, conference abstracts, and review articles were included. Medical Subject Headings (MeSH) terms used to coin search strategies were "faecal microbiota transplant" "fecal microbiome transplant" "fecal microflora transplant" "fecal bacteriotherapy" "stool transplant" "fecal transfusion" "donor feces infusion," "fecal transplant," "fecal transplantation," "fecal microbiota transplantation," "intestinal microbiota transfer," "ulcerative colitis," "colitis gravis," "inflammatory bowel disease," "IBD," and "bowel diseases, inflammatory." The methodologies in published trials on FMT in UC are heterogeneous. The available literature lacks uniformity on practical application of FMT especially with regard to patient and donor selection, dose, route, and frequency, and long-term follow-up policy. This review discusses the information and gaps in our understanding of the mechanisms of FMT pertaining to its application in UC.

HOW DOES FMT WORK IN UC

Gut microbiome within an individual is variable and dynamic and therefore defining a "normal" healthy gut microbiome is not possible at the moment. The gut-microbial health is characterized by 3 parameters: (1) resistance to disturbances in the intestinal ecologies, (2) resilience to revert back to original composition even if disturbed, and (3) functional redundancy if there is delay in regaining the predisturbance composition so that compositional shifts do not affect the functions of ecological niches. The gut-microbial health therefore hangs in a delicate balance with the potential to be influenced by a large number of environmental factors. If the effects induced by external factors exceed the ability of microbial community to resist the change, resulting dysbiosis can affect the functionality of the intestinal microenvironment culminating in unfavorable consequences.[11,12]

Whether the immune-mediated damage in IBD is due to recognition of particular bacterial epitopes or due to molecular mimicry-mediated autoimmune reactions is still obscure. Further, whether dysbiosis is a *cause* (that sets the inflammatory process in motion) or an *effect* (of altered immune and metabolic environment of the inflamed mucosa) is unclear and it complicates the feasibility of an in-depth analysis of changes brought by FMT in patients with IBD. Dysbiosis in IBD is characterized by reduction in *Bacteroidetes*, reduced diversity within *Firmicutes* and an increased proportion of *Proteobacteria*.[13] FMT is expected to correct this dysbiosis and thereby reduce gut inflammation in UC. However, to date, no specific group of microbes have been established in relation to response to FMT in UC. Fuentes et al reported that an increase in *Anaerostipes caccae*, *Coprococcus eutactus*, or *Eubacterium rectale* and decrease in *Enterococcus* species were associated with good clinical response while persistence of *Proteobacteria* and *Bacteroidetes* and low levels of *Clostridium* clusters IV and XIVa were associated with treatment failure.[14] Butyrate producing *Eubacterium hallii*, *Roseburia inulinivorans*, *Eggerthella* species, and *Ruminococcus bromii* also correlated with response to FMT whereas *Fusobacteria*, *Escherichia*, *Sutterella*, *Streptococcus*, and *Prevotella* were associated with lack of remission in another RCT.[15] However, Costello et al identified a different set of microbes (*Anaerofilum pentosovorans*, *Bacteroides coprophilus*, *Clostridium methylpentosum*, *Acidaminococcus intestine*, and *Senegalimassilia anaerobia*) in association with response to FMT. Interestingly, fecal short chain fatty acid concentrations, including butyrate, did not correlate with any observed treatment effect with FMT.[7]

The microorganisms within the intestinal tract not only synergistically cooperate in nutrient digestion and metabolism but also intensely compete with each other for nutrients and space. The exclusion of competitive niche by FMT-induced restoration of microbial communities (and thereby preventing opportunistic pathogens to persist and proliferate) is one of the plausible mechanisms for its therapeutic effect.[16–18]

The mucosal immune system is characterized by its exclusivity. Commensal microorganisms play an important role in development as well as maturation of the mucosal immunity. Several immune mechanisms that work in tandem with the microbiota to establish and maintain gut homeostasis are dysregulated in patients with IBD.[19–23] FMT attempts to restore the equilibrium by correcting microbial dysbiosis. Animal models have shown reduced colonic inflammation following FMT due to reestablishment of colonic CD4+ and Treg cells, increase in interleukin-10 (IL-10) production, reduced ability of antigen presenting cells to present bacterial antigens to the colonic T cells, and restoration of intestinal memory/effector T cell populations.[24–26] The only clinical trial assessing the immunological changes with FMT failed to demonstrate any significant changes in mucosal T cell subsets.[7] The current understanding of immunoregulatory changes with FMT is limited and well-designed focused studies with adequate sample sizes are needed.

UNSETTLED ISSUES REGARDING FMT FOR UC

Patient Selection

The majority of the participants in the randomized trials published so far have been middle-aged with mild-to-moderately active UC who were on stable doses of

concomitant medications.[5–8] As these RCTs are heterogeneous with regard to the route and dose of administration, number of treatment sessions, choice of placebo, and the exact indication for FMT, the results cannot be compared. The lower response rates observed with severe disease in these trials suggest that severe disease is less likely to respond.[5,6] While no significant interaction between age, sex, disease duration and distribution, and concomitant medications was observed in the post hoc analysis of RCTs,[5,7] the authors in India found younger age, disease extent E2 and endoscopic Mayo subscore 2 to be associated with achievement of clinical remission (unpublished work).

As is evident from RCTs and cohort studies, none of the studies used FMT as the only therapeutic agent or as initial therapy. Patients not responding to standard pharmacotherapy including corticosteroids, 5-aminosalicylates (5-ASA), thiopurines, and/or biologics were subjected to FMT. In the first focused open label uncontrolled study evaluating role of FMT in a selective group of steroid-dependent patients with moderately severe UC, high rates of clinical response (75%) and steroid-free clinical remission (46%) were documented.[9] With limited use of antibodies to tumor necrosis factor (anti-TNF) biologics in developing countries due to high cost and prevalence of tuberculosis, FMT potentially presents itself as a salvage therapy in such patients. This observation however needs further corroboration in large randomized trials.

Donor Selection

The objective of donor selection through stringent screening is to prevent any adverse event related to the infused fecal material. Donor screening is usually conducted in accordance with local regulatory authorities or governing bodies. The first step in selecting the potential donor is a detailed medical history and risk behavior assessment by use of a dedicated questionnaire. Once a potential donor has been found suitable based on the donor questionnaire, he/she is subjected to a structured physical and laboratory evaluation.[27–29] Interactions between the donor screening staff and donors need to be scheduled on a regular basis (preferably every 1–2 months) to identify problems related to donation.

Apart from screening for infectious agents and multidrug-resistant organisms, profiling microbial diversity and functionality holds the key to optimal donor selection. The genetic background and dietary intake of both recipients and donors (which affects the gut microbiome) is not routinely analyzed currently but may become part of the standard workup in the future.

Moayyedi et al observed varied response to different stool donors. Taxonomic profiles of the donors highlighted distinct microbial differences between the 2 donors. Of the 9 patients who entered remission, 7 had received FMT from the same donor, the so-called "super donor." Microbial diversity of the donor stool was the most important predictor of FMT outcome.[30] If this hypothesis was true, multidonor FMT infusions to ensure greater microbial diversity than from individual donors should result in a higher response rate.[8] Despite using multidonor intensive FMT, Paramsothy et al did not significantly increase the response rate (27% vs 24% in Moayyedi et al).[5,8] Therefore, "one stool fits all" approach may not hold true in the context of treating microbial dysbiosis-associated chronic diseases. Detailed characterization of donors and recipients with a multiomics approach (metagenomics, metatranscriptomics, and metabolomics) in an attempt to correct functional deficiencies through appropriate matching may potentially improve efficacy.[31]

Preparation of the Fecal Slurry

The process of preparation of the fecal slurry has evolved from using a blender to a more refined centrifugation plus filtration (using automated blenders) and centrifugation plus microfiltration (using automated purification system based on GenFMTer).[32] The refined techniques minimize manual handling of the fecal sample, prevent contamination, and result in uniform homogenization of the slurry. The experience with laminar flow cabinet or tissue culture hood with UV-C germicidal lamp during preparation of slurry is limited.

There is evidence that the proportion of viable bacteria reduces when donor stool sample is processed in ambient air compared to anaerobic processing.[33] However, trials comparing aerobic and anaerobic preparations are lacking at this moment in time. The impact of storing and freezing donor stool on the microbial viability and therapeutic efficacy remains unknown. Costello et al reported that the microbiome remained largely unchanged after 6 months of storage.[34] On the contrary there are reports of declining microbial viability when stool is stored for more than 8 hours.[35] The ideal situation would be to deliver the fecal slurry into the recipient bowel as early as possible in its *purest* form, free of preservatives. By incorporating this time control for preparation, higher rates of clinical response can be expected.[32] However, this may be challenging because of logistic difficulties and restrictions.

Route of Administration

There is no consensus on the most appropriate route of administration of FMT. The RCTs with a colonic or rectal instillation of fecal slurry[5,7,8] have shown better response rates compared to the upper GI route of administration.[6] In a meta-analysis based on cohort studies, the pooled proportion of clinical remission rates with upper and lower GI tract administration of fecal slurry were 8% and 31%, respectively.[36] Gastric acid can impairing the growth and survival of *Bacteroides* and *Firmicutes* may be responsible for lower response rates with upper GI delivery.[37] However, this may be negated by using proton pump inhibitors or delivering the fecal slurry into

duodenum or jejunum. The response rates when fecal slurry is delivered by enema varies from 24% to 32% in RCTs.[5,7,8] In comparison when multisession colonoscopic approach was used, steroid-free clinical remission was achieved in 46.3% of patients.[9] A small open label pilot study has demonstrated the efficacy of capsule-based long-term multidonor FMT. However, the number of participants was small and 20% developed serious adverse effects and dropped out.[38] Further studies with head to head comparisons of the oral and colonoscopic route are needed to determine the optimal approach.

Need for Maintenance Therapy

Because of complex and persistent pathologic mechanisms in UC, therapeutic microbial manipulation with a single session of FMT is unlikely to have sustained benefits.[39] In one study the median time for maintaining clinical response with FMT in Crohn disease patients was about 4 months.[40] In another study in patients with UC, all 9 patients who achieved remission with FMT relapsed on follow-up.[5] Maintenance therapy with FMT is, therefore, needed. A randomized pilot study from India, evaluated 8 weekly colonoscopic infusion of FMT for maintaining remission in UC. Patients who achieved clinical remission with FMT were randomized to receive either FMT or placebo in addition to stable doses of 5-ASA plus azathioprine. Among participants allocated to FMT plus pharmacotherapy 27 of 31 (87.1%) were able to maintain steroid-free clinical remission at week 48 vs 66.7% (20/30) patients assigned to the pharmacotherapy alone ($P = 0.111$). Endoscopic [FMT: 18/31 (58.1%) vs placebo: 8/30 (26.7%), $P = 0.026$] and histological [FMT: 14/31 (45.2%) vs placebo: 5/30 (16.7%), $P = 0.033$] remissions were maintained in a significantly higher number of patients receiving FMT in addition to pharmacotherapy.[41]

The optimal interval between 2 sessions of FMT remains to be determined. Microbial engraftment in patients with rCDI has been demonstrated to increase from days 2 to 6 after FMT and it plateaus by days 28 to 45. This engraftment is typically sustained for months.[42] However, in a persistent state of dysbiosis of UC, repeated FMT sessions are likely to be required for sustained efficacy. Although there is no consensus, intervals ranging from 1 to 12 weeks have been described.[5–8,43] In the Indian studies, a colonoscopic route for infusion of FMT was used and the sessions were scheduled at weeks 0, 2, 6, 10, 14, 18, and 22 for induction of remission and subsequently every 8 weeks for maintenance of remission.[9,41] The authors acknowledge that multisession colonoscopic FMTs may not be an economically viable option in developed countries. But for developing countries where colonoscopies are not expensive (eg, US$50 per session in India) and use of biologics have constraints, FMT is an economically less demanding endeavor. The annual cost of FMT is comparable to the costs incurred on 5-ASAs. However, it is an invasive and labor intensive procedure. This strategy of using frequent FMT sessions is not likely to be feasible in North America and Europe. The development of purified capsular forms of select intestinal microbiome components may provide a more efficient and less expensive method of maintenance.

Impact of Diet on Response to FMT

Diet is one of the most important determinants of gut-microbial composition.[44–46] It has been proposed that the habitual diet consumed over the long term decides the fundamental microbial health.[12] Complex interactions between dietary nutrients and microbiota shape the immune responses that trigger/sustain the disease. Maintaining a healthy diet is hence important for both the donor and the recipient of FMT. A donor who is screened and selected today may not be acceptable tomorrow if there are significant deviations in dietary patterns or lifestyle. Regular dietary follow-up of selected donors may nearly be as important as screening for infections. Similarly, tracking the dietary habits and patterns of the recipient are crucial. FMT attempts to restore the microbial dysbiosis in UC. Once the dysbiotic microbiome is "corrected" by FMT, it has to be sustained over time for a prolonged benefit. The authors believe that, apart from maintenance FMTs, a healthy diet that regulates gut homeostasis and maintains a diversified microbiome, is vital for long-term response to FMT. Consumption of a "pro-inflammatory diet" (comprising of animal proteins, refined carbohydrates, n-6 polyunsaturated fatty acids, food additives and emulsifiers, etc.) by a FMT recipient is expected to increase the relative abundances of unfavorable microbes like *Proteobacteria*, *Actinobacteria*, *Bacillus* species, *Alistipes* species, *Bilophila* species, *Clostridium leptum*, *Escherichia coli*, *Mycobacterial* species, *Staphylococcus*, *Streptococcus*, *Klebsiella*, *Salmonella*, and *Pseudomonas* species laying the foundation for persistence of an inflammatory milieu in the intestinal lumen, ultimately leading to therapeutic inefficacy. The dietary patterns and practices of the donor and the recipient may thus influence the long-term effects of FMT. Further studies addressing the optimal diets for recipients responding to FMT are needed.

Assessing Response to FMT

Response to FMT depends on successful bacterial engraftment of the donor microbiota. However, many factors like age, severity and behavior of underlying disease, genetic makeup of the patients, dietary patterns, indication for FMT, comorbidities, and concomitant medications can influence the outcomes with FMT.

Newer microbiota-targeted approaches including bacterial genome reconstruction, studying functional capacity (including metabolomics, metaproteomics, and metatranscriptomics) and strain variation, and analyzing viruses and eukaryotes ("kingdomagnostic" metagenomics) have facilitated the assessment of the viability and engraftment of

transplanted gut microbiota.[47] Successful engraftment is also dependent on host immune responses and dietary patterns of both the donor and the recipient as they shape the microstructure of gut microbiome.[48] Li et al demonstrated that new microbial strains from the donor had a higher likelihood of engrafting if the recipient already possessed that species.[49] Further research focusing on these aspects will undoubtedly reveal significant information and enrich the existent knowledge.

FMT in the COVID-19 Era

As the world reels under the coronavirus disease (COVID-19) pandemic, concerns regarding the screening of donors of cellular or tissue-based products have been raised. The severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) RNA has been isolated from both intestinal tissue and fecal specimen. Interestingly, the viral RNA is detected in fecal samples even after the respiratory samples test negative for COVID-19.[50] The viral excretion in feces opens up the possibility for transmission of SARS-CoV-2 via feces, though the evidence is lacking at the moment. Nevertheless, the international expert panel on FMT and stool banking recognizes the risk and suggests screening of the donors for presence of typical COVID-19 symptoms (including fever, fatigue, dry cough, myalgia, dyspnea, and headache) and inquiring about history of travel/close contact with individuals with proven or suspected infection, within the previous 30 days.[51] The US Food and Drug Administration (FDA) recommends that stool donated before December 1, 2019, can be used until proper testing and screening protocols are available.[52] Development and standardization of stool tests for SARS-CoV-2 have been a hurdle, though a protocol for stool SARS-CoV-2 viral quantification has been proposed.[53] Since viral RNA can persist in stool even in the absence of respiratory symptoms, it may be prudent to test donors at multiple timepoints. Appropriately equipped biosafety level 2 laboratories with trained staff and expertise in specimen handling would be required.

FMT Regulations

FMT for patients with UC is still in an experimental stage and is currently not recommended as the standard of care. Its use beyond clinical trials is therefore not recommended. Even for rCDI the FDA exercises its discretion and places it under the ambit of an investigational new drug. The experimental nature of FMT, as well as its potential long-term consequences and the likelihood of achieving therapeutic goals should be discussed with the patient in detail. FMT can be administered through various routes, none of which is standardized at present. The recipient should have sufficient opportunity to discuss and have all of his/her questions answered to his/her satisfaction upon which the informed decision about undergoing the procedure should be made.[54]

Stool is a complex mixture. The exact composition of FMT is not known, and will vary even if obtained from the same donor on different days. This poses a significant challenge for the regulatory agencies since stool is unlike any other therapeutics approved for clinical use. Reports of transmission of drug-resistant *E. coli* by FMT, though in immune-compromised recipients, have further complicated the issue.[55] As of now, there is no consensus on how FMT should be classified or regulated. Currently FMT is regulated as a drug in Canada and in the United States and as a biologic in Australia; yet it remains unregulated in many countries. In North America, a treating physician can offer FMT to patients suffering from rCDI without the need to apply for an Investigational New Drug (USA) or Clinical Trial Application (Canada), which are required for other indications. In the United Kingdom, a hospital can prepare FMT and treat its own patients under pharmacy exemption. If FMT is to be sent to another hospital, a special license would be required, and for use in a clinical trial, additional license (IMP license) is necessary. For many countries, an investigator simply needs to submit an application to the institutional ethics boards before conducting FMT trials. An entirely new framework is needed for the purpose of regulation. On the one hand, we do need regulation of FMT for patient safety. At the same time, we do not want to create barriers in the process to hinder patient access and scientific progress.

NOMENCLATURE

The intestinal microbiome (comprising both structural and functional ecosystems of the microbial community) has an important role in maintaining health and any imbalance in the composition and diversity can cause several diseases.[56] The idea that normalization of an altered microbiome and the restoration of balance alleviates disease is the backbone of the science of transplantation (FMT). However, the term FMT seems to be a misnomer. The *phrase* fecal microbiota, in strict terms, refers only to luminal microbial community and does not represent the functional/metabolomic components. Substituting the word "microbiota" with "microbiome" is likely to portray the true picture.[57] Secondly, the term transplantation denotes the process of taking an organ or living tissue and implanting it permanently in another part of the body or in another body. FMT is not precisely "transplantation" as the composition of transferred microbiome is not constant and organism engraftment is affected by various host and environmental factors. Therefore, replacing the word "transplantation" with "transfer" appears scientifically logical. Although, the term FMT has been widely reported in the literature to date, and others have suggested an alternative,[58] the authors believe that the term *fecal microbiota transplantation* should be replaced by *fecal microbiome transfer*.

THE WAY FORWARD

FMT has caught the imagination of researchers around the globe. The future holds much promise for the potential applications of this approach in management of UC. Although our

understanding of the microbiome and mucosal immune system is moving forward rapidly, the science of the gut microbiome is still in its infancy and the clinical use beyond clinical trials is not recommended at the moment. Despite the encouraging results from clinical trials, many issues need to be settled. Selecting appropriate patients and advancements in fecal slurry preparation and mode of delivery are necessary. Many unanswered questions like whether shifting to selective microbiota transplantation tailored according to a particular disease can substitute the whole stool with its biological and chemical ecosystems; or should the donors and recipients be matched for genotype, diet, or environment; and to what extent can diet modulate the intestinal microbiota to influence disease development, need to be resolved. A greater understanding of these factors is expected to identify the place of FMT in the therapeutic armamentarium of UC and optimize its use in clinical practice.

DATA AVAILABILITY

Data sharing is not applicable to this article as no new datasets were generated or analyzed during the current report.

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