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Emergence of fecal microbiota transplantation as an approach to repair disrupted microbial gut ecology

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Abstract

In the recent years fecal microbiota transplantation (FMT) has emerged as an effective therapeutic option for patients with refractory *Clostridium difficile* infection that is not responding to antibiotic therapy. It results in implantation of donor microbiota into recipients and restoration of normal distal gut microbial community structure. We anticipate that this form of therapy represents merely the first entry into a new class of therapeutics. There is great interest in application of FMT or defined microbial consortia to treatment of many diseases associated with dysbiosis. However, many challenges remain in development as our understanding of microbial ecology within the human body and microbiota–host interactions remain limited. Future advances in this field will be critically depending on detailed mechanistic understanding.

Keywords

Fecal microbiota transplantation; *Clostridium difficile* infection

1. Introduction

The germ theory of disease has been the foundation of some of the largest successes in medical history, which throughout recorded time has been dominated by infectious disease. While the development of antibiotic therapies allowed these diseases to be combated, such treatments focused exclusively on pathogens with little consideration of any effects on the resident microbial communities in the human host. Until recently, these commensal microbiota were largely neglected in clinical practice and mostly inaccessible to investigation. In the absence of data to suggest otherwise, host microbial communities were thought to be extremely resilient, and it was assumed that antibiotics could be used with impunity. However, there is now growing concern that changes in development and composition of host microbiota caused by pervasive use of antibiotics and dietary changes have contributed to the emergence of new diseases such as metabolic syndrome, autoimmunity, atopic conditions, and many others.

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Nowhere is the relationship between antibiotics, host microbiota, and human disease more clear than in the treatment of refractory *Clostridium difficile* infection (CDI) with fecal microbiota transplantation (FMT). Recent investigations of patients treated with FMT demonstrate that there are limits to the resilience of host microbiota to antibiotic exposure, and that entirely new microbial communities can be established in adult patients by direct implantation [1–3]. The remarkable clinical success of this procedure in extinguishing *C. difficile* bacteria and achieving resolution of associated gastrointestinal symptoms has encouraged many investigators and patients to imagine that FMT can result in similarly successful outcomes when applied to many other conditions [4]. However, while some small observational studies do support a degree of optimism, it is clear that moving forward requires deeper mechanistic understanding of how microbial communities are held together, how they interact with the host, and what activities of the microbiota are needed for therapeutic effects in specific diseases.

2. Current uses of FMT in treatment of CDI

Although fecal enemas were introduced as early as 1958 by Eiseman et al. in the treatment of pseudomembranous enterocolitis [5], a condition that is now typically associated with CDI, the practice has been relatively sparse until recently. Vancomycin, an antibiotic that suppresses vegetative forms of *C. difficile*, was introduced into clinical practice in 1959 [6], and antibiotic-refractory cases of CDI were relatively uncommon for several decades. However, new, more virulent strains of *C. difficile* emerged in the early 1990s, associated with broader antibiotic resistance, greater capacity for toxin production, and increased sporulation efficiency [7–9]. This has been accompanied by greater incidence, morbidity, and mortality associated with CDI. CDI today is the most common cause of nosocomial diarrhea in the US, and has increasingly become an important cause of community-acquired diarrhea [10,11]. Although the Centers for Disease Control conservatively attributes approximately 14,000 deaths to CDI annually, some estimates of CDI-associated mortality are >100,000 per year [12].

Antibiotics constitute the major risk factor for CDI, which is acquired by ingestion of *C. difficile* spores. Antibiotic-induced suppression of the host microbiota creates an environment favorable to *C. difficile* spore germination and growth of the vegetative form of the bacteria [13,14]. Clinical symptoms, which vary from fecal urgency and diarrhea to cessation of bowel movements in toxic megacolon, are caused by *C. difficile* enterotoxins that damage the colonic mucosa and produce inflammation. Common clinical challenges associated with CDI include recurrent infection (RCDI), severe or complicated infection refractory to antibiotic therapy, and CDI associated with underlying inflammatory bowel disease (IBD). FMT appears to be effective in all these presentations, but there are important nuances associated with each.

Antibiotic therapy alone fails to clear the infection in a significant fraction of patients (~20–30%), and each round of antibiotic treatment for CDI further increases the risk of recurrence by approximately 20% [15]. The reason for recurrence of CDI may be failure to clear the *C. difficile* spores with antibiotic treatment, which further suppresses the normal microbiota and perpetuates the underlying problem. Ultimately, a significant fraction of patients develop

recurrent *C. difficile* infection syndrome, a condition characterized by an indefinite series of antibiotic treatments and relapses. Analysis of fecal samples in such patients shows marked contraction of the normally dominant members of the Bacteroidetes and Firmicutes phyla, accompanied by dramatic expansion of γ-Proteobacteria [2,3,16,17]. Infusion of fecal material taken from a healthy donor promptly leads to establishment of donor-like composition of fecal bacteria in the recipient and normalization of gastrointestinal symptoms. Clinical efficacy of FMT, as defined by abrogation of CDI recurrence over 1–2 months following cessation of antibiotics, is approximately 90% in multiple case series of consecutive patients treated [18]. Furthermore, FMT is confirmed to be effective over vancomycin in a randomized, controlled trial [3].

Reports of FMT in severe and complicated CDI refractory to antibiotic therapy remain sparse, and the standard therapy continues to be surgical colectomy. However, surgical therapy accompanied by modern supportive care is still associated with ~50% mortality [19,20]. The prognosis is only marginally better than ~75% mortality associated with pseudomembranous enterocolitis in 1950s, when Eiseman's team first reported use of fecal enemas [5]. Isolated case reports of successful use of FMT in treatment of severe and complicated CDI suggest that this approach should be investigated further [21–25]. Commonly these patients have multiple serious comorbidities and constitute a very challenging study population. In this situation FMT is introduced during active infection when vegetative forms of *C. difficile* are still present. Nevertheless, in our own experience the response to FMT in the treatment of active, complicated CDI can be very prompt and measureable in mere hours [26]. However, we find that sequential administration of FMT is needed to achieve sustained recovery [26].

CDI is a common complication in patients with underlying IBD [27–29], and is always an important diagnostic consideration when the patient presents with a flare of IBD activity. In this context one generally does not see pseudomembranes during an endoscopic examination of the colon, and failure to make the diagnosis can lead to a delay in providing appropriate antibiotic treatment and escalation of immunosuppressive therapy that by itself may worsen the disease. FMT appears to be comparably effective in clearing the infection in RCDI patients with and without underlying IBD [30].

3. FMT and immune mediated colonization resistance in CDI

Despite its efficacy, the mechanisms of FMT are poorly understood, though the concept behind the procedure seems intuitively straightforward. Even the earliest investigators using FMT in the 1950s recognized the importance of gut microbiota in the normal function of the gastrointestinal tract, and made a connection between the use of antibiotics and the clinical syndrome caused by the infection [5]. They hypothesized that restoration of the normal microbial gut ecology by transfer of entire microbial communities from healthy donors could be curative in this disease. Recent experiments have largely validated this hypothesis, although it remains unclear how these transferred microbiota are able to combat the infection. Potential mechanisms include competition for limiting resources by other microorganisms within the same ecological niche in the intestinal tract, direct elimination of *C. difficile* or interference with its pathogenicity by microbial products, restoration of

secondary bile acid metabolism in the colon, and induction of immune-mediated colonization resistance. These various mechanisms were reviewed recently in several articles [31,32], and this review will focus primarily on immune-mediated colonization resistance.

It is well-established that the commensal microbiota provide both tonic stimulation in the mucosa, critical for epithelial tissue protection and repair, and maintenance of both innate and adaptive immune defenses. Treatment of mice with a mix of antibiotics that includes metronidazole and vancomycin, which are routinely used in the care of patients with CDI, results in downregulation of intestinal expression of RegIIIγ, a secreted C-type lectin that kills Gram-positive bacteria [33]. Human α-defensins produced by intestinal Paneth cells also neutralize *C. difficile* toxin B, which is largely responsible for CDI-associated pathology [34]. Expression of many defensive antimicrobial proteins and peptides, including RegIIIγ and α-defensins, is dependent on MyD88 stimulation [33,35]. Notably, MyD88-deficient mice suffer markedly increased mortality following CDI [36]. While it is not yet clear which MyD88-mediated protective mechanisms may be operative in CDI, one mechanism is likely to be expression of CXCL1. This chemokine recruits neutrophils, which play an important role in suppressing lethality of CDI in mice and dominate the histopathology of pseudomembranous colitis [36]. In fact, neutrophil recruitment is further potentiated by IL-1β-mediated secretion triggered by translocating commensals through the damaged intestine [37]. Therefore, decreased MyD88 stimulation following antibiotic treatment may contribute to an environment where *C. difficile* can grow and produce toxins, and makes the host vulnerable to septic complications.

Patients with RCDI are subjected to prolonged courses of antibiotics, which may affect these immune responses indirectly by altering the diversity and composition of the colonic microbiota. An average patient with RCDI in our clinical practice has had >5 antibiotic courses for the infection [30], and prolonged tapered or pulsed courses of vancomycin or similarly long sequential treatments with several antibiotics, e.g., vancomycin and rifaximin, constitute standard practice approaches in RCDI. The marked loss of microbial diversity that is observed in these patients, therefore, is not surprising. It is not uncommon for these patients to develop progressive diarrheal symptoms despite ongoing antibiotic treatment and absence of other pathology such as inflammatory bowel disease (IBD). Although explanations for this diarrhea may include alterations in bile acid composition, it is also possible that expansion of resident pathobionts or infections with viral and bacterial pathogens may contribute to some of these cases. In our experience, these patients respond well to FMT and normalize their bowel function following the procedure [17].

4. FMT beyond CDI

There is enormous interest in exploring the potential of FMT in conditions other than CDI, including inflammatory bowel disease, irritable bowel syndrome, metabolic syndrome, autoimmunity, and autism [38,39]. All of these conditions are associated with some degree of altered microbial composition and activity in the gut. However, it is not known whether these changes are causally linked to the specific diseases or result from them. Furthermore, it is possible that maladaptive host-microbial interactions have already hard-wired undesirable host physiology. For example, animal studies suggest altered immune system development

in a germ-free host leads to pathological immune responsiveness despite later conventionalization [40].

Despite potentially formidable obstacles, FMT offers the most controlled form of clinical intervention that can be used to test whether manipulation of microbiota can lead to beneficial results. However, to date the exploratory attempts to use FMT have emphasized ambitious clinical endpoints of safety and efficacy, while few attempts have been made to answer even the most basic technical questions. For example, is it necessary to condition patients with antibiotics prior to FMT for most non-CDI indications? Which antibiotics should be chosen? Are there disease-specific donor selection criteria or markers of specific microbiota for individual clinical indications? Should such FMT interventions be combined with specific diets or lifestyle changes? Similarly, most non-CDI conditions being considered for FMT are extremely heterogeneous in terms of host genetic factors and developmental periods. Therefore, it is critical to carefully characterize the recipient populations. A major distinguishing feature of CDI versus most other indications is the extensive antibiotic exposure in all CDI patients undergoing FMT. Clearly, the microbial community structure is devastated in this population, and FMT provides a quick ecological repair. In contrast, most non-CDI conditions being considered for FMT studies are associated with much more stable and resilient microbial communities, even though they may not be optimal for the host.

A carefully reasoned and systematic approach is needed to pick the next best targets and develop disease-specific protocols. Undoubtedly, repair of microbial community structure following an ecological catastrophe is likely to be an easier target. One such non-CDI example occurs in the setting of bone marrow transplantation (BMT), where patients are subjected to intensive rounds of body irradiation, chemotherapy, and broad-spectrum antibiotics during their pre-BMT conditioning period, all of which are known to disrupt gut microbiota and microbial-host homeostasis [41]. Loss of microbial diversity in fecal microbiota of these patients is comparable to that seen in patients with RCDI. In many patients groups such as the Enterococci or γ-Proteobacteria, which are more adept at gut translocation than most commensal organisms, become dominant over all other bacterial constituents of gut microbiota [41]. Such overgrowth is associated with systemic bacteremia that is commonly observed after BMT. It is reasonable to hypothesize that well-timed FMT in these patients resulting in normalization of their gut microbial communities may prevent these common infectious complications. In fact, FMT in a murine model can clear antibiotic-associated colonization by vancomycin-resistant *Enterococcus*, although potency of FMT is dependent on densities of key microbial taxa in the donor preparation [42]. It is also possible that host-microbe interactions that can enhance development of regulatory immune circuits following BMT, e.g., regulatory T cell inducing taxa of Clostridia [43], will have an important benefit of dampening pathological graft versus host responses that complicate long-term outcomes in these patients.

Interestingly, the idea of microbiota therapy, or “reconventionalization”, of antibiotic-treated immunosuppressed or immunodeficient patients dates back many decades. It was recognized then that gut anaerobes provide colonization resistance, and a handful of patients with congenital immune deficiency or undergoing bone marrow transplantation were treated with

fecal suspensions or microbiota propagated in germ-free mice [44,45]. Development did not go beyond clinical anecdotes and management of infectious complications in these challenging situations since has relied on increasingly broad-spectrum antibiotics.

5. Standardization of FMT

In addition to selecting appropriate patient populations for treatment with FMT, standardization of the procedure is critical for its continued use in RCDI and other diseases. For decades FMT was practiced rather crudely [38]. Typically, a small quantity of donor fecal material, ~50 g, but rarely actually weighed, was suspended in a saline solution, usually with an aid of a blender. The suspension was filtered to remove larger particles and administered into the patient via an enema, colonoscopy, nasoduodenal or nasogastric tube, depending on available medical expertise [4]. This process continues to be the most common clinical practice of FMT today.

This lack of standardization is one of the multiple practical barriers that prevent FMTs from becoming mainstream medicine. Other barriers include the effort required to identify willing donors, esthetic and safety concerns associated with material preparation at a clinical site, and significant time demands on providers associated with each procedure. We have described protocols for standardization and cryopreservation of fecal microbiota that overcome most of these practical difficulties [30]. It is now possible to acquire fecal microbiota material from the healthiest donors, standardize preparation and final unit dose, and store prepared material as any other human tissue in a dedicated bank. In our institution donors are selected following comprehensive screening, physical examination, and stool and blood testing. Individuals with any history or signs of metabolic syndrome, autoimmunity, atopic disease, and neurologic and psychiatric problems are excluded. The microbiota material is produced according to current good manufacturing practice protocols in a dedicated, regularly inspected facility. Such production is essential for mainstream clinical practice, and should enable conduct of clinical trials and development of next-generation microbiota products [31].

6. FMT and defined microbiota: microbiota therapeutics

Scientific advances in recent years, along with the spectacular successes of FMT in the treatment of refractory CDI, introduced the concepts of microbial ecology to clinical medicine. We now recognize that microbial communities cannot simply be understood as mere collections of individual organisms. Furthermore, we recognize that these symbiotic microbial communities are integral to normal human physiology, and therefore represent a legitimate therapeutic target.

FMT represents the first entry in an emerging new class of drugs: microbiota therapeutics. As a complex drug, FMT is appropriately regulated by the FDA Office of Vaccines, Blood, and Biologics in the United States. Unlike probiotics, which are scientifically defined as live microorganisms that benefit human health, microbiota therapeutics explicitly target disease. FMT relies on established microbial communities designed by nature over millions of years and tested for decades in individual donors, establishing a very high bar for researchers who

wish to develop new therapeutics with synthetic microbial communities grown in bioreactors. However, cultivated synthetic communities may also theoretically lower the potential FMT-associated infectious risk, improve manufacturing efficiency, and have greater potential for intellectual property that is ultimately critical for successful commercialization.

Although typically the topmost concern for providers, patients, and regulators, the infectious disease risk associated with FMT thus far appears to be extremely small. Of course, this risk will not be fully known in absence of large scale clinical trials or mandated registries and long-term follow-up of all FMT recipients. Nevertheless, as FMT is an increasingly common clinical procedure with thousands of patients being treated across the globe, the paucity of infectious complication reports that are even only possibly associated with FMT is almost surprising [46]. This is especially so given that the majority of recipients are elderly patients with multiple systemic problems and a significant fraction are patients with IBD, with disrupted mucosal gut barrier, intrinsically defective mucosal immunity, and concurrent treatment with immunosuppressive medications. Therefore, it is very likely that the commensal microbiota and vigorous mucosal immune systems of clinically healthy individuals are very successful at containing potential enteric pathogens in the donor material. An additional safety mechanism may be the limits imposed by transplant itself, which allows only one donor and a single donation to contribute to an individual dose of transplant material. This ensures that the risk posed by a single donor is always limited. For these reasons, the infectious disease safety advantages of synthetic microbiota may be minimal at best. In fact, it is worthy of note that bioreactors do not possess a natural immune system, and pathogen contamination in mass manufacturing phase can potentially present an infectious disease risk to a greater number of patients.

Thus far development of synthetic microbial communities has been guided by ability to grow organisms in culture, considerations for antibiotic sensitivity, and serendipity in terms of ability to control CDI. In fact, a relatively simple mix of bacteria has already been described in a murine model of RCDI [47]. Similarly, defined microbial communities capable of abrogating the cycle of CDI recurrence in patients were described by Tvede and colleagues in the late 1980s [48], as well as more recently [49]. These mixtures appear to engraft promptly, but become relatively minor constituents of total microbiota over time. This pattern is reminiscent of successive microbial colonization following birth, a period of gradually increasing microbial diversity until maturity at approximately 2–3 years of age. Interestingly, newborns are commonly colonized with toxigenic *C. difficile*, although they typically remain asymptomatic [50–53]. As their microbiota and the immune system mature, *C. difficile* usually disappears. It is hypothesized that newborns lack the receptor for *C. difficile* toxins, although it is also plausible that early commensal organisms are able to contain *C. difficile* pathogenicity via mechanisms that have not yet been recognized [53]. It is certainly possible that specific defined microbiota even with very limited diversity can perform some critical functions such as activation of immune-mediated colonization resistance, reconstitution of secondary bile acid metabolism, or even provision of minimal microbial community-building scaffolding that allows further diversification and maturation. One of the anticipated challenges for synthetic microbiota manufacturing will be ensuring that critical activities needed for therapeutic efficacy will not be lost during laboratory or industrial culture growth, as the organisms adapt to life outside the human host. Ultimately,

functionally relevant assays will be needed in addition to monitoring of precise composition of defined microbiota in therapeutic product development.

Progress in this new field will most certainly require systematic research. Understanding microbiota from the standpoint of microbial ecology, including identification of keystone species and deduction of community-wide metabolic networks, nutritional food webs, as well as microbiota–host interactions, will all be foundational in development of designer microbiota that will fit the needs of specific pathologic conditions. Certain attributes of microbiota may be critical for some diseases. For example, efficient production of short chain fatty acids may be important in metabolic syndrome [54,55], and may also promote induction of regulatory T cells that may be beneficial in inflammatory bowel disease [56,57]. Germ-free animal models will undoubtedly play a very useful role in this discovery process, although they do have important limitations, as human microbiota may not necessarily reproduce effects of animal host species-specific microbiota [58]. Bottom-up approaches at building microbial communities from individual organisms will likely be very important in building the required knowledge base in this emerging scientific discipline. However, existing diversity of microbial communities within the human population also represents an important discovery resource. Microbiota material can be manufactured in a standardized fashion from individuals that vary in geography, lifestyle, donor-specific physiologic characteristics, microbiota composition, and metabolic signatures of microbiota activity, and tested in interventional clinical trials. FMTs are already being done daily, and every procedure is a potential experiment that could yield new data. Eventually, it is possible that bottom-up and top-down discovery approaches will converge and complement each other. The journey is only beginning.

Abbreviations

BMT	bone marrow transplantation
CDI	*Clostridium difficile* infection
FMT	fecal microbiota transplantation
IBD	inflammatory bowel disease
RCDI	recurrent *Clostridium difficile* infection

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