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From Stool Transplants to Next-generation Microbiota Therapeutics

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Abstract

The epidemic of *Clostridium difficile* infection fueled by new virulent strains of the organism has led to increased use of fecal microbiota transplantation (FMT). The procedure is effective for even the most desperate cases, after failure of multiple courses of antibiotics. The approach recognizes microbiota to be integral to normal human physiology, and microbiota being used in FMT represents a new class of therapeutics. Imbalance in the composition and altered activity of the microbiota are associated with many diseases. Consequently, there is growing interest in applying FMT to non-*C. difficile* indications. However, this may succeed only if microbiota therapeutics are developed systematically, based on mechanistic understanding, and applying up-to-date principles of microbial ecology. We discuss two pathways in development of this new therapeutic class: whole microbial communities separated from donor stool and an assembly of specific fecal microorganisms grown *in vitro*.

Keywords

Fecal Microbiota Transplantation; Full Spectrum Microbiota; Defined Microbiota Ecosystems; Recurrent *Clostridium difficile* Infection

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Introduction

The gastrointestinal tract contains a dense population of organized and highly specialized microbial communities that regulate host immunity and metabolism. The pathogenesis of many diseases is thought to involve compositional and functional changes in commensal microbiota, caused by use of antibiotics and other environmental factors such as dietary changes¹ Consequently, there is intense interest in manipulation of the composition of commensal microbiota as a therapeutic strategy. Although the public and many physicians may not distinguish this approach from use of probiotics or dietary supplements, large-scale alteration of the intestinal microbiota requires a different set of definitions and requirements, as well as research and development of live biotherapeutics.

Regulatory agencies such as the US Food and Drug Administration (FDA) consider any agents given to patients to cure, treat, mitigate, or prevent disease to be drugs. Therefore, their approval for testing and use in patients requires demonstrations of safety and efficacy. In contrast, probiotics are defined as live microorganisms that might provide health benefits.^{2, 3} However, there is no legal definition for probiotics and these products are often marketed without scientific proof for their claims.^{4, 5}

Fecal microbiota transplantation (FMT) is highly effective in treatment of antibiotic-refractory *Clostridium difficile* infection (CDI);⁶⁻¹⁰ it is the most direct and radical way to change the composition of a patient's distal intestinal microbiota. Given the serious public health challenge posed by the *C difficile* epidemic and lack of therapeutic alternatives, the FDA, for the time being, permits FMT for CDI that does not respond to standard antibiotic therapies, even though fecal microbiota are not formally approved for this or any other indication. However, it is difficult to imagine that this situation will continue indefinitely, and it is reasonable to expect that microbiota-based therapies will be developed for other conditions. Advances in high-throughput technologies have enabled characterization of complex microbial communities in terms of taxonomy, gene and protein expression, and production of metabolites within the human host will undoubtedly move this field of research forward. We review the promises and challenges of developing microbiota-based therapeutics.

History of FMT

Coprophagy (consumption of the feces) is common in the animal kingdom, and observed in many species.¹¹⁻¹⁴ It likely contributes to accelerated development of the gastrointestinal tract, increased resistance to colonization by pathogens, and enhanced digestion of nutrients. The concept of transferring gastrointestinal contents (transfaunation) has been practiced for centuries in veterinary medicine. Indications for this procedure include ruminal acidosis in cattle and sheep and chronic diarrhea in horses; transfaunation is also used to increase resistance of newborn chicks to enteric pathogens.¹⁵⁻¹⁷

Despite an instinctive aversion to fecal material, there are records that FMT has been used in human civilization as a therapeutic agent for many centuries. Chinese medicine provided the earliest known documentation of FMT in humans, referring to various forms of fecal preparations, including fresh, fermented, dried, and infant-derived products that were used to

treat multiple gastrointestinal maladies.¹⁸ In Europe, for centuries there was widespread belief in medical applications fecal material. Franz Christian Paullini, a German physician, observed that fecal consumption was common in animals as well as humans (since manure was used as fertilizer). In 1696 he published a book *Hailsame Dreck-Apotheke* (Salutary Filth-Pharmacy) on medical uses of human and animal feces.¹⁹

In modern clinical medicine, the team of Ben Eiseman provided the earliest report of FMT, describing successful treatment of 4 patients with pseudomembranous colitis using fecal enemas.²⁰ These clinical investigators recognized that antibiotics caused this usually fatal syndrome by suppressing the native microbial communities that normally provide colonization resistance to pathogens, which in retrospect was likely *C difficile*. They anticipated that the procedure would become standardized, simplified, and tested in clinical trials.²⁰ However, vancomycin was soon identified as an effective antibiotic for treatment of pseudomembranous colitis.²¹ Nevertheless, FMT continued to be anecdotally reported worldwide—primarily for intractably recurrent CDI.⁶ Over the decades, permutations of the technique evolved, varying mainly in the route and method of administration.²² In 1989, Michael Tvede and Jorgen Rask-Madsen reported the first attempt at constructing a synthetic microbial community for treatment of recurrent CDI, using 10 bacterial strains cultured from healthy human feces.²³ Interestingly, implantation of selected human fecal microorganisms was also described in anecdotal reports in the 1970s in patients that were receiving gnotobiotic care or undergoing bone marrow transplantation and antibiotic therapy with the goal of increasing resistance to colonization by pathogens.²⁴⁻²⁷

The *C difficile* Epidemic

The most obvious reason for recent increases in the practice and reporting of FMT is the rising incidence and increasing severity of CDI in the last 2 decades.²⁸ This has been associated with emergence of more genetically diverse and virulent strains, characterized by their broad antibiotic resistance, capacity to produce toxins, and increased sporulation efficiency.²⁹⁻³² There has been also a great increase in the incidence of community-acquired CDI, even in low-risk groups such as younger adults and children.^{33, 34} CDI is the leading cause of hospital admissions and death from gastroenteritis; some estimates of yearly mortality attributed to CDI in the US exceed 100,000.^{35, 36} Frequently, the infection cannot be cured with antibiotic treatments alone. This is the case in fulminant CDI, which is associated with 50% mortality—even with the best surgical therapy.^{37, 38} Another common and challenging problem is the recurrent CDI syndrome, characterized by an indefinite cycle of relapses following multiple attempts to discontinue antibiotic therapy for CDI.⁶ This syndrome is associated with progressive patient exhaustion and can facilitate acquisition of new infections, as new pathogens emerge during continuous antibiotic administration.^{39, 40}

FMT has been reported to cure high percentages of patients with severe or recurrent CDI syndrome, although most clinical experience is with the latter. Most reports are of individual cases and single-center case series. FMT has been reported for more than 500 cases worldwide, with a cumulative efficacy of approximately 90% and few if any serious adverse events.⁶⁻¹⁰ Results from an open-label, randomized, controlled trial comparing antibiotic therapy alone or with FMT were published in 2013.⁴¹ The trial was terminated prematurely

because of the marked superiority of FMT. Consistently high rates of efficacy have been reported for FMT, irrespective of protocols for material preparation or administration, and including self-administration of fecal enemas.⁴² A group of gastroenterology and infectious disease specialists who perform FMT published a set of consensus guidelines, to provide best-practice, common sense recommendations that consider practical barriers for most clinicians.⁴³ However, practice patterns of this procedure continue to vary widely⁴⁴

Why is FMT Effective for Refractory CDI?

C difficile-associated disease is caused by enterotoxins, released by vegetative forms of toxigenic bacterial strains, that destroy the intestinal epithelium, leading to an intense inflammatory response and secretory diarrhea.³⁰ Although *C difficile* is a native of the distal gut, its growth and pathogenic activity are normally held in check by commensal microbiota. Antibiotic use is the main risk factor for CDI. Although specific classes of antibiotics pose different risks for development of CDI,⁴⁵ cumulative exposure to antibiotics over time further increases risk.⁴⁶ Notably, antibiotics are also the standard treatment for CDI, but these are broadly active against many members of intestinal microbiota. Therefore, it is likely that successive rounds of antibiotic treatments in patients with recurrent CDI weaken the resistance of the microbiota against *C difficile*, which persists in the form of antibiotic-resistant spores. Bacterial diversity is markedly decreased in fecal samples of patients with recurrent CDI; these often contain increased proportions of members of the *Enterobacteraceae* family of γ -*Proteobacteria* and reduced proportions of the *Bacteroidetes* and *Firmicutes* phyla.^{40, 41, 47} FMT promptly restores normal, donor-like composition of intestinal microbiota dominated by *Bacteroidetes* and *Firmicutes*, and containing diminished proportion of γ -*Proteobacteria*.^{40, 41, 48}

Little is known about which specific members of microbiota suppress *C difficile*, although several non-mutually exclusive mechanisms can be considered. One classic mechanism, based on principles of ecology, is competitive niche exclusion, in which organisms compete for limited amounts of nutrients. The nutritional niche for *C difficile* is not well defined. However, this mechanism might be manipulated to prevent or treat CDI, using non-toxigenic *C difficile* (NTCD). Experiments in hamsters have shown that pre-treatment with NTCD protects them from lethal challenge with toxigenic *C difficile*.⁴⁹ Early-stage clinical trials with NTCD are underway. Interestingly, highly toxigenic clinical strains of *C difficile*, characterized by high sporulation efficiency, out-compete less toxigenic strains in mouse colonies.⁵⁰ Furthermore, consortia of specific bacterial species that protect against CDI in mice and humans have little phylogenetic similarity to *C difficile*.^{50, 51} Of course, phylogenetically dissimilar bacterial species may still compete for the same limiting nutrients. Conversely, it is not known whether NTCD strains necessarily have the same nutritional requirements as TCD.

Certain gut microbes might inhibit *C difficile* growth or toxigenic activity directly, via mechanisms that involve production of phage, small molecules, or antimicrobial peptides (bacteriocins). For example, *Lactobacillus delbrueckii* ssp. *Bulgaricus* B-30892 was shown to block *C difficile* adhesion and toxicity to epithelial cells via release of unknown factors, but have no effects on vegetative growth of *C difficile* bacteria.⁵² Furthermore, bacteriocin

secreted by *Bacillus thuringiensis*, named thuricin CD, was found to have narrow-spectrum activity against *C difficile* in an in vitro model of fecal microbiota in the distal colon.⁵³ In contrast to metronidazole and vancomycin, thuricin CD spared the dominant bacterial populations.

The microbiota can also interfere with the lifecycle of *C difficile* indirectly, via the host. Colon bacteria participate in the metabolism of bile acids, which likely play an important role in the *C difficile* lifecycle. Bacteria mediate deconjugation of primary bile acids from their amino acids and 7 α -dehydroxylation to produce secondary bile acids, deoxycholate, and lithocholate. Taurocholate, a major primary bile acid, is a potent germinant of *C difficile* spores, and has long been used as a key component in *C difficile* growth media.^{54, 55} In contrast, lithocholate is an inhibitor of *C difficile* spore germination, while deoxycholate is toxic to the vegetative forms of the bacteria.⁵⁶ Therefore, an altered composition of bile, caused by antibiotic-induced changes in microbiota, might transform an environment that inhibits *C difficile* growth to one that promotes its expansion. In support of this hypothesis, mutant forms of *C difficile* that do not express a recently identified bile acid germinant receptor have decreased pathogenicity in hamsters.⁵⁷ Small intestinal and cecal extracts from mice given clindamycin contain higher proportions of primary bile salts and stimulate *C difficile* spore germination and colony formation.⁵⁸ The factor responsible for the germinant activity fits the physico-chemical characteristics of bile salts and can be neutralized by the bile salt-binding resin, cholestyramine. Fecal samples from patients with recurrent CDI have increased concentrations of primary bile salts and undetectable amounts of secondary bile acids, whereas FMT quickly restores a donor-like composition of fecal bile acid.⁵⁹ Interestingly, cholestyramine has been successful in anecdotal reports of treatment for recurrent CDI,⁶⁰⁻⁶² although it was not found to effectively treat primary CDI—possibly because it also stimulates bile acid synthesis and binds to vancomycin.⁶³⁻⁶⁶

The microbiota may also prevent colonization by *C difficile* through immune-mediated mechanisms. Toll-like receptor (TLR) signaling decreases in mice following administration of antibiotics, which could increase their susceptibility to infection.⁶⁷ Mice lacking Myd88, an adaptor protein required for signaling through most TLRs, are more susceptible to *C difficile*-induced colitis than wild-type mice following administration of antibiotics.⁶⁸ More severe disease also develops in *Tlr4*^{-/-}, but not *Trif*^{-/-}, *Tlr2*^{-/-}, or *Tlr5*^{-/-} mice.⁶⁹ In humans, polymorphisms in *TLR4* correlate with risk of hospital-acquired CDI.⁷⁰ Notably, although TLR4 is generally activated by lipopolysaccharide on Gram-negative bacteria, it also interacts with surface-layer proteins of *C difficile*.⁶⁹

In contrast, stimulation of TLR5 with flagellin derived from *Salmonella enterica* protects against colitis, slowing *C difficile* proliferation and reducing epithelial cell apoptosis.⁷¹ These findings indicate that some TLR stimulation by other members of the microbiota could promote immune regulation of *C difficile*. TLR-mediated potentiation of both innate and adaptive immune response is plausible. Paneth cells express antimicrobial peptides called α -defensins in response to TLR signaling via Myd88; these peptides can neutralize *C difficile* toxin B.^{72, 73} Furthermore, *Tlr4*^{-/-} mice do not mount an antibody response against *C difficile*, and patients with recurrent CDI have lower levels of antibodies against *C difficile* toxin than patients who are able to recover without recurrence.^{69, 74}

Challenges in Clinical Practice

At first glance, FMT appears to be a simple and inexpensive procedure that can even be performed by patients at home.⁴² However, physicians face many challenges in implementing protocols for donor material preparation in a clinic setting, and patients often have difficulty finding FMT providers, leading to the emergence of do-it-yourself protocols.

One obvious hurdle is aesthetic. Humans have a natural aversion to fecal material, and preparation of fecal slurries in a clinic can be challenging for both staff and patients. Processing human stool generally requires biosafety level 2 containment, which requires protective gear for the laboratory personnel, a biological cabinet, and dedicated working space.⁷⁵ This may seem overly cautious—after all, FMT donors are theoretically screened and tested for infections. Thus far, the FDA has approved investigational new drug protocols that involve processing of stool in patient-care areas under “universal precautions”. However, biosafety containment is ideal if blending procedures create high levels of aerosolized fecal particles, leading to significant respiratory exposure to fecal microbiota and toxic products.

Another challenge to FMT is donor recruitment, screening, and testing. Many practitioners rely on a patient's own intimate partners, family members, and friends. There is no evidence that material from related donors is better engrafted than material from unrelated donors. One argument for the use of related donors is that intimate partners are presumed to have already shared infectious exposures. However, there is extensive evidence from blood safety analyses that directed donors, i.e., donors identified by patients, are more likely to test for infectious disease markers than unrelated volunteer donors.⁷⁶ It is also prudent to consider potential risks of microbiota engraftment beyond infectious disease considerations. The intestinal microbiota regulates many aspects of energy metabolism, the immune system, carcinogenesis, and even neurologic development and behavior.⁷⁷ Therefore, it is conceivable that FMT could contribute to development of a new disease, such as type II diabetes or colon cancer.

Most FMT practitioners agree on some basic infectious agents that potential donors should be tested for, blood borne (syphilis, HIV, hepatitis B and C viruses) and enteric (*C. difficile*, *Salmonella*, *Shigella*, *Yersinia*, *Campylobacter*, *Escherichia coli* O157:H7, ova, and parasites).⁴³ However, it is also preferable that donors do not have any gastrointestinal disorders, metabolic syndrome, allergic or autoimmune diseases, or neurologic or psychiatric problems. When faced with such rigorous qualifications, most recipients are not able to identify and recruit a donor. In our own practice (Khoruts, unpublished observations), which selects donors based on these described criteria,⁴³ we have found that more than 90% of potential donors do not pass the screening evaluations. Of course, it is important to determine whether risks outweigh potential benefits. The potential risk of transmission of diabetes or another chronic disease to an elderly patient with possibly fatal refractory CDI is slight, compared to the immediate threat posed by the active infection. However, the effects of a chronic disease that requires many years to develop would be much greater in a young patient, so all potential long-term consequences should be considered.

We cannot ignore the reality that FMT receives minimal reimbursement, despite the establishment in 2013 of the Current Procedural Terminology code (44705) for physician assessment of donors and oversight of fecal microbiota preparation. Expenses not only arise through testing of donors, but also in preparation and administration of the material. For an upper route of administration, it is prudent to rule out the presence of a large hiatal hernia. Radiology fees may also be incurred with placement of a naso-duodenal tube. There are facility expenses regardless of administration route; these vary depending on procedure duration and nursing staff requirements. Gastroenterologists in the US typically perform FMT during a diagnostic colonoscopy, so patients can also be evaluated for conditions such as inflammatory bowel disease, lymphocytic colitis, and diverticulosis, which have higher prevalence among patients with recurrent CDI. This diagnostic examination is typically reimbursed by the insurance company. However, a physician-administered enema is generally not reimbursed.

Despite the challenges, the medical community, faced with the CDI epidemic and desperate patients, has increased its use of FMT. However, availability is limited in many communities. Many different protocols are used for donor testing, material preparation, and administration.⁴⁴ The out of pocket costs to patients also vary greatly, ranging from no cost to more than \$10,000 (Khoruts, patient communications). Clearly, we are in a transitional period and FMT will need to become more standardized, accessible, and affordable.

From FMT to Standardized Full-spectrum Microbiota (FSM) Therapies

The ideal therapeutic agent would be licensed by regulatory agencies, easily accessible, aesthetically unchallenged, simple to administer, and cost effective. Arguably, FMT is already cost effective for treatment of recurrent CDI, compared to the costs of antibiotics, hospitalizations, complicating infections, and loss of productivity with standard therapies that cannot clear the infection. However, FMT can become widely available only if the material becomes standardized, licensed, and ready to use.

Regulatory agencies must now wrestle with the new paradigm of FMT-based therapeutics. Although the FDA considers the fecal microbiota to be a drug, like any agent used to treat, mitigate, or prevent disease, it is certainly not like any other drug. It is incredibly complex, comprising hundreds of species of microorganisms, including Eubacteria, Archaea, Eukaryota, and viruses. The composition of the microbiota varies among individuals, within individuals at different times. The microbiota is alive, metabolically active, and highly dynamic in response to multiple environmental factors such as diet. Most of its microorganisms cannot be cultured in vitro, and currently, the therapeutic potential of microbiota can only be adequately tested in patients.

In many ways, FMT is similar to tissue or organ transplantation. In fact, the premise of FMT is that a healthy intestinal microbiota, minimally manipulated during preparation, was already optimized by nature for its stability, resilience, and mutualistic relationship with the host. At the minimum, the intestinal microbiota is a highly complex biologic that varies from lot to lot. However, this variation is an intrinsic property that allows it to rapidly adapt to daily environmental changes. Compositional complexity is common to other biologics, such as blood and cell products, and can be addressed by standardization of the

manufacturing process. Good manufacturing practice (GMP) guidelines can be adapted for production of therapeutic fecal microbiota. The GMP principles are routine in pharmaceutical manufacturing, and ensure that manufacturing processes are consistent, controlled, defined, and recorded. Under GMP, the goal of production is not to achieve precise equivalence among lots, in terms of physico-chemical composition. It is rather to ensure that the process is consistent and enforceable by regulatory agencies.

Establishing a manufacturing facility that adheres to GMP guidelines is a major investment that is certainly out of reach for individual physicians practicing FMT. However, moving forward, central manufacturing facilities (commercial and/or academic) might be established to produce therapeutic intestinal microbiota. The University of Minnesota has recently established GMP production of fecal microbiota and is currently in the feasibility stage of scaling up production (Fig. 1). The ultimate envisioned model has many parallels to blood banks, which have a network of donation locations, accredited diagnostic laboratories, storage facilities, and protocols for product release and traceability. Volunteer donors fill out initial questionnaires, their medical histories are collected, and they undergo physical examinations and laboratory tests. Considerable cost savings may be realized in the economy of scale, although that can only be achieved with dedicated volunteers who are willing to provide multiple donations. Development of this resource will require substantial and sustained efforts toward educating the public and changes to prevailing attitudes about human fecal material.

Defined Microbiota Ecosystems

Mixtures of defined microbes derived from stool are also being developed as therapeutics, based on the principle that reconstitution of the intestinal microbiota can cure recurrent CDI. With these stool-derived mixtures, the exact composition of microbes to be administered is known, and can be controlled and recreated for future treatments. Furthermore, these mixtures would not contain infectious agents and (except for their initial isolation step) do not require human donors.

In 1989, researchers in Denmark reported findings from 6 patients with recurrent CDI who received either FMT or a mixture of 10 intestinal bacterial species, isolated from human feces²³. One patient received FMT, 4 received the 10-strain mixture, and 1 received both. This latter patient did not respond to FMT but responded to the 10-strain mixture.

The mixture was comprised of facultative aerobic and anaerobic bacteria: 3 strains of *Clostridia* sp. (*Clostridium innocuum*, *Clostridium ramosum*, *Clostridium bifermentans*), 3 strains of *Bacteroides* sp. (*Bacteroides ovatus*, *Bacteroides vulgatus*, *Bacteroides thetaiotaomicron*), 2 strains of *E coli*, plus *Streptococcus faecalis* (former name for *Enterococcus faecalis*) and *Peptostreptococcus productus* (former name for *Ruminococcus productus*). Each strain was first grown in pure culture, then combined in sterile normal saline and administered to the patients by enema. As with FMT, response was rapid; patients became asymptomatic and tested negative for *C difficile* toxin within 24 hours. The choice of strains was based on the concept of replenishing the bacteria that were absent in patients with relapsing CDI (the 3 *Bacteroides* strains) and on data from in vitro assays for bacteria that inhibit *C difficile* (*R productus*, *C bifermentans*, one of the *E coli* strains). Health details

of the donors were not provided. Although the 10-strain mixture was effective, no other studies in the past 20 years have presented results of trials of synthetic microbe mixtures for patients with recurrent CDI.

In 2013, researchers described the use of stool-derived microbe mixtures to treat patients with recurrent CDI. Patients were given a mixture of 33 strains of bacteria, isolated from the stool of 1 healthy donor.⁵¹ The donor was chosen according to a standard FMT screening protocol, and was a non-smoker, with an average body-mass index, born by normal vaginal delivery and breastfed as an infant, with no exposure to antibiotics in the past 10 years. Using culture techniques developed on principles of microbial ecology, researchers used a modified continuous culture chemostat system to isolate intestinal bacteria from the donor stool, which were then characterized and banked (Figure 2A). From these isolated strains, an ecosystem of 33 non-pathogenic strains, termed “RePOOPulate”, was retained for further study. The strains were chosen based on their antibiotic susceptibility profiles, reliable culturability, and community robustness under conditions that mimic the distal intestine. Like the bacteria from the Tvede and Rask-Madsen study, the 33 strains were reconstituted in sterile normal saline (Figure 2B) and administered as a single dose, directly into the colons of two patients. In this proof-of-principle pilot study, the synthetic mixture cured 2 patients with recurrent CDI. Both patients remained well and were symptom-free at their 6-month follow-up assessment. Analysis of the microbial composition of the patient stool samples demonstrated that ribosomal RNA sequences from RePOOPulate were rare in the patient pre-treatment stool samples, but made up more than 25% of samples collected 6 months after treatment. Both patients subsequently received multiple courses of antibiotics for unrelated infections; despite these antimicrobial exposures, neither patient developed a relapse of CDI. These findings indicate that defined microbial ecosystems (microbial ecosystem therapeutics, or MET) are a viable alternative to conventional FMT for recurrent CDI.

Although there are advantages to MET, it also faces challenges not posed by FMT.⁷⁸ FMT and MET are both currently classified as biologic agents by the FDA and are therefore subject to the same stringent considerations (efficacy, safety, quality) as biologics. In addition, since MET are a defined, synthetic product, they require an in vitro culture step, creating additional technical and regulatory hurdles for their synthesis and manufacturing. For example, regulatory agencies have requested full genome sequences of the microbes in MET—a requirement that is not feasible for FMT. In addition, because MET are different from other biologics, the regulatory process will likely evolve as more is discovered about the intestinal microbiome. There will undoubtedly be special regulatory considerations unique to MET that are not yet fully defined.

Clearly, a reagent that contains 33 strains of bacteria poses formidable manufacturing challenges. Ecosystem diversity is required for the sustainability of its function and stability.⁷⁹ A reduction in the complexity of MET, or even loss of just a few strains from the mixture, could greatly reduce its efficacy. In order for MET to become an alternative to FMT, researchers must construct and manufacture a diverse microbial community that is healthy, resilient, and robust.

Future Directions

The sustained changes in the composition of fecal microbiota observed following FMT or MET for patients with recurrent CDI have raised hopes that similar approaches will be developed for other diseases associated with alterations to the microbiota. These include inflammatory bowel disease (IBD), irritable bowel syndrome (IBS), metabolic syndrome, autoimmunity, neurologic disorders, and autism.^{22, 77, 80} A compelling case can be made for plausible roles of the intestinal microbiota in all these conditions, which are currently incurable. The concept of FMT as a simple answer can be seductive to patients and clinical investigators.

However, it is important to contrast recurrent CDI with these indications. Recurrent CDI is likely a low-hanging fruit in the field of microbiota therapeutics. Patients with recurrent CDI are conditioned for optimal engraftment because they have undergone sustained exposure to antibiotics, which has suppressed their native microbiota. The compositional requirements of the replacement microbiota needed to break the cycle of CDI recurrence are probably minimal, and most healthy donors (except those who have received a recent course of antibiotics) should be adequate.

The optimal conditioning program for recipients of FMT and optimal composition of donor microbiota are not known for various non-CDI indications. Furthermore, our understanding of non-CDI conditions is incomplete. At least 163 genetic loci have been associated with IBD. Consequently, there are likely to be many more categories of IBD, beyond ulcerative colitis (UC) and Crohn's disease, and responses to microbiota therapeutics are likely to vary among them. Another potential problem is the window of response. Studies of germ-free mice and animals given antibiotics suggest that there is an early developmental time period during which the effects of the microbiota on the immune system, energy metabolism, and neurological function are established; after this point, changes in microbiota may have little effect.⁸¹⁻⁸³ Although early childhood exposure to antibiotics could contribute to later onset of allergic diseases, IBD, metabolic syndrome, or autism, changes to the intestinal microbiota after these disorders have already developed may not confer have any therapeutic effects. The duration or existence of such a window of intervention has not been demonstrated in human development, and studies to define it would not be easy.

There is intense interest in microbiota therapeutics for metabolic syndrome and obesity, which collectively present the greatest health care challenge and burden in the developed world, and a growing problem in developing nations. Analyses of how alterations to the composition and activity of the microbiota with sub-therapeutic doses of antibiotics affect farm and experimental animals, and even humans, have demonstrated the important roles of microbiota in shaping body fat and lean mass content^{82, 84-86} The microbiota and body fat content have also been linked in animals studies of FMT.⁸⁷ Notably, the effects of FMT on body mass index depend on diet—one of the main environmental factors that determines the composition and activity of the microbiota.⁸⁸ Remarkably, obese and lean phenotypes can be induced in germ-free mice by transfer of fecal microbiota from human donors.^{88, 89} These types of studies could be used to identify microbes or groups of microbes that might reduce features of metabolic syndrome, or to screen standardized full-spectrum microbiota preparations for efficacy. This type of systematic research is likely to improve on the

promising early results from studies of the effects of FMT on insulin sensitivity and fasting triglyceride levels.⁹⁰

Given the obvious challenges in treating patients with non-CDI diseases with poorly defined microbiota preparations, the data so far appear mixed. There are anecdotal reports of success – a physician reportedly cured his own steroid-refractory UC via FMT, and Borody et al. reported the efficacy of FMT for patients with UC or Crohn's disease.^{91, 92} Recently, Kunde et al. reported favorable results in a pilot trial of FMT in pediatric patients with UC.⁹³ No serious adverse events were reported, which is promising because there are concerns about systemic entry of transferred microbes in patients with compromised mucosal gut barrier. In fact, Angelberger et al. reported transient systemic toxicity, characterized by fevers and increased levels of C-reactive protein, in 5 patients with moderate to severely active UC following FMT.⁹⁴

UC became more severe in 2 patients 1 month after the FMT. Only 1 patient appears to have benefitted from the FMT—fecal samples from this patient were found to have increased presence of bacteria associated with anti-inflammatory activity. In a similar study of 6 patients with refractory UC who received FMT via colonoscopy, Kump et al. reported no clinical improvement, despite detected changes in the composition of the microbiota.⁹⁵

There are anecdotal reports of the benefits of FMT for patients with IBS, constipation, several neurologic disorders, chronic fatigue syndrome, autoimmunity, or autism. However, it is important that desperate patients are not given false hope. The success of FMT will require systematic research to determine the mechanism by which different microbiota communities contribute to various disorders. This information could lead to selection of appropriate microbial therapeutics for specific groups of patients.

Conclusion

The most promising directions in the development of next-generation microbiota therapeutics include FSM and synthetic mixtures of defined MET. These should be pursued as pharmaceutical projects with GMP principles and clinical testing. This new class of therapeutics is based on new paradigms of health and disease that have developed in the past decade, as we have increased our understanding of the importance of microbiota in human physiology.

These paradigms challenge our historical view of microorganisms grounded in infectious disease. Most medically relevant bacteria known to clinicians today are pathogens, found to cause disease through rigorous application of Koch's postulates. Antibiotics are used to treat presumed or real infections with little consideration for the long-term consequences of collateral damage to the mutualist microbiota. Probiotics are the only agents widely available to clinicians to alter the microbiota, and there is little evidence for their ability to restore indigenous microbial communities.

Microbiota therapeutics must be developed based on the scientific method; paradigms of infectious disease and classical microbiology have been supplemented by concepts from microbial ecology, in the recognition of the microbiota as an integral part of the human

super-organism. It is not adequate to view the human microbiota as a mere sum of its individual organisms. The microbiota is a highly organized community with extensive subdivisions, based on their metabolic functions and signaling networks, that interact with the host. Fecal material should be recognized as more than a waste product or source of infection, but a resource for development of therapeutics and diagnostics. FMT in its original form, which is still practiced widely today, has shown a remarkable safety record in the hands of many practitioners worldwide, over many decades. Risks should not be assessed without consideration of potential benefits. Of course, it is important to consider long-term effects of such therapeutics. Extensive follow up is required for patients who participate in clinical trials of microbiota products. However, the same considerations should apply to all currently approved drugs that act on the microbiota—including antibiotics.

The importance of this new class of therapeutics is not purely clinical; microbiota–host interaction is an exciting, growing field of science. Administration of microbes is the most direct and controlled means of altering the microbiota in patients with dysbiosis-associated diseases. Koch's postulates must be updated, to study how entire microbial communities contribute to disease pathogenesis. We have a great deal to learn about how to use FSM and synthetic microbiota to treat patients. Careful studies are needed of patient outcomes, biomarkers, and microbial signatures in patients who receive FSM. Through these types of studies, we can learn how to build defined microbial consortia that restore a mutualist microbiota and human health.

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Abbreviations

CDI	<i>Clostridium difficile</i> infection
FMT	fecal microbiota transplantation
FSM	full spectrum microbiota
MET	microbial ecosystem therapeutics
NCTG	non-toxigenic <i>Clostridium difficile</i>
RECURRENT CDI	recurrent <i>Clostridium difficile</i> infection
UC	ulcerative colitis

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Figure 1. Therapeutic unit of Full-Spectrum Microbiota

A suspension of defined number of bacteria and a cryoprotectant is frozen in a cryobag. The pigtail attached to the cryobag contains samples of the microbiota material, which will remain stored in the central manufacturing facility after the unit is released for clinical use. Each treatment unit can be traced to a specific donor and date of donation.

Figure 2. Anatomy of a Robogut

The figure shows Robogut (far right) (1) Complex media is continuously pumped in and pumped out, at a constant rate which mimics the transit time of the human gut; (2) pH is monitored continuously and controlled by computer with the addition of acid or base, as needed. Temperature is also continuously monitored and controlled by computer; (3) Anaerobic gas is gently bubbled in and the vessel is maintained in a strict anaerobic atmosphere; (4) A stirrer gently mixes the culture of bacteria.