FECAL MICROBIOTA TRANSPLANTATION AND EMERGING APPLICATIONS

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Abstract:

Fecal Microbiota Transplantation (FMT) has been used sporadically across Europe, North America and Australia for over 50 years. The recent epidemic of *Clostridium difficile* infection (CDI) in North America and Europe has resulted in the increased and more frequent use of FMT given its high efficacy in eradicating CDI and resolving clinical symptoms. As more patients request treatment and more clinics develop facilities to incorporate FMT into their treatment repertoire, we are beginning to see reports of applications outside of CDI, paving the way for the employment of FMT in a number of idiopathic conditions. Interest in this therapy has in part been driven by recent research into the composition of gut microbiota, which can now be appreciated as a microbial human organ with important roles in energy metabolism as well as development and function of the immune system. This new paradigm allows the possibility that many common diseases result, at least partially, from microbiota-related dysfunction. This understanding invites investigation of FMT into areas of inflammatory bowel disease, irritable bowel syndrome, metabolic syndrome, autoimmune and allergic diseases, neurodevelopmental disorders, among others. The field of microbiota-related disorders is currently in its infancy. It certainly is an exciting time in the burgeoning science of fecal microbiota transplantation and we expect to see new and previously unexpected applications for FMT in the near future. It is now time to further define these microbiota-related conditions with the use of well designed and well executed randomized controlled trials.

Introduction:

Microbial communities populate all surfaces of the human body, but are present at their greatest density in the distal gut where they exceed the total number of human cells by an order of magnitude.[1] In fact, it is fair to consider the distal gut microbiota as a distinct human organ responsible for multiple physiologic functions, including various aspects of energy metabolism and the development and modulation of our immune system.
Like any organ in the body, the gut microbiota is made up of specialized cells that work symbiotically with each other and the host.[2] However, not all gut microbial species are dependent on host health, and relationships with these microbes can become problematic[3].
In the past six decades, our gut microbes have been under assault from antibiotics in the form of medical therapy and farming practices. The concerns over potential unanticipated health consequences are only now beginning to be realized, with multiple diseases associated with the current Western lifestyle hypothesized as being causally linked to alterations in the gut microbiota.[3-5] Examples include constipation, irritable bowel syndrome, inflammatory bowel disease, neurological diseases, cardiovascular diseases, obesity, metabolic syndrome, autoimmunity, asthma, and allergic diseases, many of which have reached epidemic proportions in recent years.

Technological limitations have until recently hampered our attempts to enumerate the various microbial populations comprising the gastrointestinal microbiota, with the vast majority of dominant anaerobic species largely unculturable by traditional microbiological techniques. However, the introduction of high-throughput DNA sequencing technologies, rising computational capabilities, and new analytical techniques have revolutionized this area of science and provided the opportunity to speculate about the existence of a 'phylogenetic core', a core microbiota persistent and abundant among large fractions of the population. Major efforts are now underway, such as the Human Microbiome Project in the U.S and the MetaHIT project in Europe, aimed at characterizing the microbial communities of the human body to determine their role in human health and disease.[6]

The notion of the gut microbiota as a regulator of both health and disease dates back to Elie Metchnikoffs work more than a century ago,[7] where he hypothesized that toxins produced by putrefactive microbes in the colon could accelerate senescence, and that useful microbes could be used to replace harmful ones. Metchnikoff noted the large

consumption of fermented milk in certain Eastern European rural populations famed for their purported longevity, and introduced sour milk into his own diet, noticing a subsequent improvement in his own health. This formed the foundation for the development of live microbial food supplements, termed probiotics.[8]

A problem facing probiotic development today is a quantitative one. Oral probiotic doses are typically three to four orders of magnitude lower than the 100 trillion native microorganisms contained within the colon. This number is likely reduced further following their passage through the harsh environments of the stomach and small bowel. Furthermore, although most species used in probiotic formulations have originated in the gut, they have likely lost some of their adaptation to this environment during ex-vivo cultivation. However all of these may not be insurmountable problems. Even small numbers of certain microorganisms can exert profound effects on large microbial communities. They can promote biofilm formation by facilitating microbial co-aggregation and production of biosurfactants; produce bacteriocins, which can selectively kill microorganisms and are important in maintaining stability of microbiota; enhance gut barrier function through their effects on the epithelia, and can signal the host immune system and elicit immunomodulatory effects[6]. One mechanism that isn't likely to play much of a role in how probiotics may work is competitive exclusion, a mechanism envisioned by Metchnikoff over 100 years ago and still one most commonly understood and articulated by patients. They generally believe that probiotics are the good bacteria intended to replace the bad. It is, of course, naïve to consider gut microbiota as a mere assembly of individual microbial species. They comprise an interdependent microbial community, which as a whole is much more than a sum of individual parts.

Disruption of gut microbiota in *Clostridium difficile* infection

Arguably, one of the best examples of a disease resulting from major disruption of the gut microbiota by antibiotics is *C. difficile* infection. Generally acquired following antibiotic treatment and ingestion of environmental spores, CDI has become a growing public health problem in the last two decades. In the United States alone, the National Hospital Discharge Survey revealed a two-fold increase in CDI between 1996 and 2003 to approximately 0.6/1,000.[9] A more recent survey of 12.5% of all U.S. acute care facilities showed a CDI prevalence rate among inpatients of 13.1/1,000.[10] This rise has been

accompanied by increasing rates of colectomy and death, with approximately 100,000 people dying annually in the U.S. with CDI,[10, 11] in part driven by the emergence of more virulent *C. difficile* strains, such as PCR ribotype 027/North American Pulsed Field type 1 (NAP1), which is characterized by resistance to fluoroquinolones, increased toxin production due to tcdC gene mutation, as well as binary toxin production.[12, 13]

Standard treatment of CDI is based on antibiotics, such metronidazole and vancomycin, which have broad activity against the dominant phyla of colonic microbiota, but can themselves perpetuate recurrence of the CDI following their discontinuation. The risk of relapse following initial treatment of CDI is approximately 20-25%.[14, 15] This is increased further by the use of additional interim antibiotics for treatment of other infections.[16] Thus, a fraction of patients can develop chronic, recurrent form of CDI that can last indefinitely. Chang and colleagues, analyzing the fecal microbiota of seven patients with CDI using 16S rDNA sequencing, found a progressive reduction in species diversity in patients with initial CDI compared to normal controls, and patients with recurrent CDI compared to those with initial infection.[17] In fact, in the three patients with recurrent CDI disruption of distal gut microbiota was evident at the phylum level with marked reduction in *Bacteriodetes* species and relative increases in *Proteobacteria* and *Verrucomicrobia* species, both usually only minor constituents of the fecal microbiota. This finding is consistent with the 1989 report by Tvede *et al.,* which noted the absence of *Bacteroides species* in similar patients and reversal of deficiencies with successful implantation.[18] Interestingly, the new macrocyclic antibiotic fidaxomicin, which spares *Bacteriodes* species, reduced the initial relapse rate of CDI in half compared to Vancomycin, but did not differ from it in recurrence rate for the virulent PCR 027/NPA1 strain.[15] While it is hoped that the new emerging narrow-spectrum antibiotics will permit restoration of gut microbiota in patients with chronic relapsing form of CDI, they are yet to be tested in this patient population. Similarly, it is not known whether newer antibiotics will lower the unacceptably high current rates of mortality and colectomy associated with severe and fulminant forms of CDI

Fecal Microbiota Transplantation

Pseudomembranous colitis, one of the more severe clinical manifestations of CDI, was recognized as a complication of antibiotic therapy shortly after their inception into

clinical practice. It was also quickly realized that restoration of the normal gut microbiota could solve this problem. The most frequently quoted earliest report is that by Eiseman and colleagues, a team of surgeons from Colorado, who successfully treated four patients with fecal enemas in the late 1950s.[19] Three of the four patients had fulminant pseudomembranous colitis, which at the time carried a 75% mortality rate. The patients were treated with antibiotics, hydration, vasopressors, hydrocortisone, and *Lactobacillus acidophilus* without success. In desperation, the physicians resorted to fecal retention enemas, which resulted in the prompt recovery of their critically ill patients, all able to leave the hospital within days of treatment. The authors further expressed hope that a "more complete evaluation of this simple therapeutic measure can be given further clinical trial by others".

Fecal microbiota transplantation (FMT), previously also known as 'fecal bacteriotherapy', has been offered for decades in select centers across the world, primarily as a last-ditch therapeutic option for recalcitrant CDI. This form of CDI is characterized by rapid infection recurrence upon discontinuation of antibiotics. Infection cycles ultimately become predictable with near certainty and are frequently accompanied by considerable morbidity and mortality. In these most difficult cases the reported cumulative success rate of FMT in clearing the infection is approximately 90%.[20] At the time of this writing the published FMT experience is approximately 264 patients, consisting of small case series and individual case reports, virtually all on patients with relapsing disease. Early procedures were initially performed using fecal enemas, with nasoduodenal tube[21] and colonoscopy administration[22, 23] introduced later. No adverse events were ever reported. Two extensive reviews have since summarized our current knowledge on FMT for recurrent CDI. Evan Nood *et al.* covered some of the history, screening of donors, pretreatment processes and routes of fecal infusion.[24] The review by Johan S. Bakken covers similar ground but also focuses on patient preparation and methodology for instillation of the donor stool slurry.[20] Borody *et al.* in 2010 also deals with this subject comprehensively in UpToDate and suggests some of the practical pointers in methodology for carrying out FMT in recalcitrant CDI.[25] Some important aspects of donor selection will be clarified below. Selection of the route of administration is likely dependent on the

clinical situation, although transcolonoscopic infusion is likely to be favored for the vast majority.[26]

Severely ill patients may require several transcolonoscopic infusions given that the disease may impair deep instrumentation of the colon, and burden of *C. difficile* organisms may be higher. Even in these extreme situations, enema or transcolonoscopic infusion into the distal colon is likely to result in clinical success.[27] Naso-duodenal or naso-gastric infusions in such cases may not succeed due to ileus. Data on the success of FMT in fulminant disease is unavailable at this stage, except for one published case.[28] Although CDI occurs in acute, relapsing and fulminant categories, most data currently stems from the relapsing category. Much more work is needed in order to reverse the high mortality in fulminant CDI - in excess of 50%[29] - and the high rate of colectomy. With the growing success of FMT we hope that there will be a fundamental and systematic re-evaluation of standard antibiotic regiments in treatment of CDI, and therapeutic approaches will be aimed at minimizing further gut microbiota disruption and optimizing their restoration. The prompt reconstitution of normal microbiota, even with a single infusion, is so complete and durable[30-32] that it is reasonable to consider early incorporation of FMT into standard treatment algorithms. The challenge now is to develop methods, such as stored transplant material, which can be rapidly accessed and deployed for patients with severe CDI and early signs of fulminant disease.

How does FMT work?

Unlike the concept of probiotics, which at best aim to somehow alter the metabolic or immunologic activity of the native gut microbiota, the idea behind FMT has always been to introduce a complete, stable community of gut microorganisms to repair or replace the disrupted native microbiota. This was in fact documented in one case report of FMT for recalcitrant CDI, with the fecal microbiota composition of the patient closely resembling that of the donor two weeks and one month post-FMT.[31] Engraftment of donor microbiota was accompanied by normalization of the patient's bowel function. The exact mechanism that achieved this normalization remains to be elucidated.

Why isn't the use of FMT widespread?

Today FMT still remains at the relative fringes of medicine for reasons that have little to do with the efficacy of the procedure, although any remaining doubts should be

satisfied by ongoing randomized placebo-controlled studies in the near future.[24] It is more likely that various other factors have played a role in preventing the procedure from becoming a standard therapeutic option. First is the issue of simple aesthetics. Although the "yuck" factor associated with fecal material is virtually non-existent in patients with recalcitrant CDI, it can be a challenge in the medical office or endoscopy suite. Material preparation isn't easily performed without a well-ventilated, dedicated microbiological safety cabinet removed from proximity to other patients and health care staff. Currently insurance companies do not cover FMT in the U.S., and expenses associated with donor screening and material preparation are considerable and have typically been assumed by the patient. Finding a suitable healthy donor is often a challenge to be solved by the patient, which can be a formidable task for someone already ill. Even donation itself is not necessarily trivial, with some donors unable to produce the material on demand.

The challenge of the CDI epidemic has forced the reevaluation of FMT as a procedure, one which cries out for further development. Unfortunately, as the donor material is both widely available and complex in composition, little interest has been expressed by the pharmaceutical industry involving technological development of FMT-based therapeutics. Therefore, development has largely been driven by individual clinicians who are facing increasing numbers of patients requiring FMT as an optimal, and potentially life-saving treatment.

We recognize FMT to be a form of organ transplantation. The idea of a human microbial organ is a novel paradigm, but one well supported by modern science. This organ presents a unique set of challenges and considerations, but these should not ultimately deny patients of a potentially lifesaving therapy. In some aspects FMT is simpler to perform than other organ transplants, without the need for immunologic matching of donor and recipient or the need for immunosuppression following the procedure. However, there are many unknowns. At this time, no formal standard practice guidelines exist for performing FMT. Different institutions have devised individual protocols regarding donor and recipient selection, material preparation, and route of administration. Recently, a group of infectious disease and gastroenterology specialists with experience in this procedure have proposed current best practice recommendations.[33] We believe that for the procedure to become

widely available, the process requires standardization, and rigorously tested donor material should be made readily available for therapeutic application to practitioners.

The first step in moving toward this aim is careful donor selection. Although each individual's gut microbiota composition is unique, there is no convincing evidence at this time to suggest that FMT has to aim for restoration of the precise original microbial composition. Instead, a reasonable goal is engraftment of a functional microbial community that will be stable in the new recipient and able to benefit his or her health. This issue is one deserving of further detailed study, but experience thus far suggests that the success rate of FMT is comparable between related and unrelated donors. There is, however, a great deal of compelling evidence that the composition of the gut microbiota can be a factor in the pathogenesis of many diseases particularly common in Western societies. Unfortunately, as we cannot at this time predict the behavior of the gut microbiota by any kind of compositional assay, the primary focus in donor selection should be overall donor health. We suggest that a number of criteria need to be satisfied in universal donor selection (Table 1). Obviously, the risk of infection transmission is an immediate concern, but one that is minimized with a careful history, physical examination, and screening tests. Another consideration is the possible transmission of traditionally non-infectious gastrointestinal or systemic diseases. The intestinal microbiota are involved in the pathogenesis of inflammatory bowel disease, irritable bowel syndrome, and colon cancer in ways not currently understood. It is reasonable to exclude donors with any gastrointestinal disease or potential concern about such possibility. A great deal of evidence from both animal and human studies has emerged recently to implicate a role for altered gut microbiota in the obesity epidemic and pathogenesis of metabolic syndrome.[34-36] One can similarly build a compelling case that the rise of allergic and autoimmune diseases in the industrialized world at least in part has been driven by wide use of antibiotics and altered diet, factors that likely impacted the composition of gut microbiota.[3, 37, 38] Therefore, it is reasonable to exclude donors with history of any of these disorders. In addition, it is important that the donor microbiota are intact and stable, with recent antibiotic exposure or history of major intestinal surgery constituting absolute exclusion criteria.

Table 1.

Donor Exclusion Criteria Evaluation

1. Infectious Risk	
a) HIV and Hepatitis	Risk factors and laboratory testing
b) Syphilis	Laboratory testing
c) Enteric infections	History and laboratory testing
2. Gastrointestinal Disorders	
a) Inflammatory bowel disease	History
b) Irritable bowel Syndrome	History
c) Chronic constipation or diarrhea	History
d) Abdominal surgery or GI neoplasms	History and physical examination
3. Systemic medical conditions	
a) Metabolic Syndrome	Physical and laboratory evaluation
b) Systemic Autoimmunity	History and physical examination
c) Atopic diseases (e.g., asthma, eczema)	History and physical examination
d) Food and respiratory allergies	History
e) Any chronic pain syndromes	History
f) Neurologic disorders	History and physical examination
g) Neurodevelopmental disorders	History and physical examination
e) Use of prescription medications	History
4. Antibiotics for any indication within 6 months	

Clearly, if donor selection is to be as rigorous as we suggest, it would be unreasonable to burden patients who are often quite ill with sourcing potential donors. A possible solution to this problem is the establishment of donor programs, where volunteers are recruited and screened. This, for example, is what we have done both at the University of Minnesota and Centre for Digestive Diseases in Sydney, where the vast majority of FMT is performed using volunteer donor material. This protocol has greatly simplified procedural coordination and markedly decreased the costs of laboratory donor screening.

The second step in standardizing FMT is the actual material preparation. Different protocols currently exist between various institutions, and while most appear quite successful, they are not universally practical for any clinical setting. The duration of

viability of diverse gut microbiota constituents outside the human body whilst exposed to oxygen is unknown. As in any organ transplantation, it seems fitting to ensure minimal manipulation of donor material before transplanting it into the new recipient. Therefore, as FMT development moves forward, we envision the development of protocols that will ensure prompt material processing. Given the current state of knowledge it would be futile to conduct detailed taxonomic characterization of donor fecal microbiota. However, it would be reasonable to ensure that the number of microorganisms present in each preparation be measured and confirmed to be in a therapeutically effective range. At first glance, all of these steps during material preparation may appear cumbersome and reduce the practicality and accessibility of FMT. However, if these steps are standardized and performed by dedicated facilities, the result would be far more practical than is currently the case in most institutions. In the foreseeable future, the task would be best conducted by a few centralized facilities, which will process the donor material and ship it to individual providers in frozen, lyophilized, or encapsulated forms.

Emerging FMT applications- Beyond CDI

Whilst recalcitrant and severe CDI constitutes the most immediate indication for FMT that urgently warrants further development for wider dissemination in clinical practice, it is reasonable to consider other potential indications where this procedure may be effective. As already noted, alterations in the intestinal microbiome have been implicated in the emergence of many common diseases of the Western world. Recent publicity concerning scientific advances in describing and understanding the gut microbiota has already convinced some patients that FMT can be curative for their individual conditions. Both authors of this review frequently field inquiries about the possibility of FMT for a variety of clinical problems, including inflammatory bowel disease, irritable bowel syndrome, obesity, anorexia nervosa, systemic autoimmunity, food allergies, eosinophilic disorders of the gastrointestinal tract, as well as neurodegenerative and neurodevelopmental disorders. However, more basic science and preliminary clinical work needs to be performed to develop optimal protocols that could be implemented in systematic clinical trials to test the therapeutic potential of FMT in these indications. Unlike recalcitrant CDI where the native microbiota have been severely damaged by multiple courses of antibiotics, microbial communities in these diseases may be quite resilient to

change. Is there a need for an antibiotic conditioning regiment to suppress or eliminate the native microbiota prior to FMT? What antibiotics should be used and how long? Is one FMT infusion sufficient, or should multiple scheduled infusions be administered?

The scientific rationale for FMT development for indications beyond CDI is in fact quite compelling, although few clinical reports are currently available. Hence, we will consider only several potential indications here. As originally with CDI, treatment with FMT can lead to clinically gratifying outcomes in other suspected microbiota infections such as 'irritable bowel syndrome' and constipation,[39, 40] which lack uniformly measurable markers such as toxin detection in CDI. Such clinical observations urgently need to be followed with well designed randomized trials. It is likely that the therapeutic action of FMT in the IBS area is similar to that operating in the treatment of CDI.

The central role of the gut microbiota in the pathogenesis of inflammatory bowel disease is well established.[41, 42] However, the current paradigm places the dominant focus on host factors such as the immune system and the gut barrier, whilst the microbiota is regarded more generically as sources of microbe-associated molecules that can stimulate inflammatory responses. Yet, recent work has clearly demonstrated non-equivalence of different gut microorganisms with respect to their interaction with the host immune system. Thus, some species were found uniquely capable of inducing Th17 cells,[43] while others augmented responses of regulatory CD4 T cells.[44, 45] Some patients with Crohn's disease have a reduction in *Faecalibacterium prausnitzii* in mucosa-associated microbiota.[46] This microorganism, a member of the dominant phylum *Firmicutes*, secretes metabolites that can lower production of pro-inflammatory cytokines such as IL-12 and interferon-γ, increase production of IL-10, and inhibit development of colitis in a murine model. Similarly, Qin *et al.* have also reported reduced diversity of the faecal microbiota in patients with IBD, finding that on average, IBD patients harbor 25% fewer microbial genes than healthy controls.[47] If these alterations are somehow involved in the pathogenesis of IBD, it is probable that replacement with a more favorable composition can be therapeutic. Prolonged remissions of ulcerative colitis following FMT have been reported in the literature[48, 49], demanding systematic clinical trials. It should be noted, however, that unlike CDI where a single infusion of FMT is curative in most patients, recurrent infusions are

typically required to induce profound remission in UC patients. Clearly, the FMT mechanism of action in UC is quite different to that of CDI.

Energy metabolism is a well-recognized function of gut microbiota. Therefore, problems associated with this function are a reasonable target for FMT. The epidemic of the metabolic syndrome, associated with obesity and numerous other health problems, is arguably the greatest single health care challenge in the industrialized world, one now rapidly spreading to encompass less developed nations. The potential role of the gut microbiota and their impact on body size has long been acknowledged in the usage of low dose antibiotics in farming practices.[50, 51] In fact, similar effects of low dose antibiotics have been shown in humans in the 1950s in the absence of any effects on rates of clinically significant infection.[52] Interestingly, comparisons in the distal gut microbiota of obese and lean individuals, as well as genetically obese and lean mice have revealed differences in the composition of the distal gut microbiota and their metabolites.[53-55] Furthermore, the gut microbiota were shown to be involved in multiple elements of energy metabolism, including energy harvest, metabolic rate, and energy storage.[56-59] Germ-free mice, which have naturally low body weight, gain more body fat following colonization with gut microbiota from obese compared to lean mouse donors without increases in food consumption or obvious energy expenditure.[54] More recently, Vrieze *et al*. reported a significant improvement in insulin sensitivity in 18 obese males with metabolic syndrome treated with FMT from lean donor individuals.[60]

Whilst using FMT to treat UC or constipation-predominant 'irritable bowel syndrome' in the Sydney clinic, we have also observed serendipitous improvements in extra-intestinal conditions not previously considered to be microbiota-related. These include the virtually complete and prolonged (>15 years) normalization of severe multiple sclerosis in three patients whose constipation was the target of FMT,[61] and progressive normalization of platelet counts in a patient with idiopathic thrombocytopenic purpura whose UC was successfully treated with FMT.[62] Although such observations are exciting and provocative starting points, they should prompt the systematic study of microbiota composition pre- and post-FMT in sufficiently powered randomized trials.

In summary, the FMT of today for its use in CDI may be focused on repairing the most obvious severe damage induced by antibiotic medications, but it may be only the first chapter in a much larger task- that of gut microbiota restoration on a society-wide scale and determination of the potential microbiota-associated illnesses where it may benefit.

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