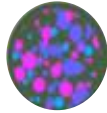
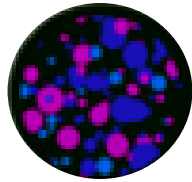


A Novel Microfloral Refinement System for Fecal Microbiota Transplantation



**Disruptive Technology Revolutionizing
Gut Microbiome-Based Therapeutics**

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1. Abstract

Clostridium difficile is an epidemic infectious intestinal pathogen that usually infects patients with a compromised gut microbiome, and is currently treated with antibiotics with minimal success. Fecal Microbiota Transplantation is an emerging alternative therapy that shows promise: essentially, stool from a healthy donor is processed and administered to a patient, in order to restore healthy bacteria to the gut ecosystem. Our objective was to create a device to efficiently isolate, extract, and package viable microflora from donated stool efficiently, for administration to a patient or for further research

2. Background and Description of the Problem

The Problem: *Clostridium difficile* (*C. diff*) is an epidemic infectious intestinal bacterium, and is the number one cause of hospital-acquired diarrhea. At its worst, *C. diff* is highly drug resistant, virulent, and deadly. The current standard of care for *C. diff* infection (CDI) is antibiotics, which are highly ineffective with recurrence rates of 35% for primary CDI, and 65% for recurrences. This has resulted in >\$3.2 billion in annual healthcare costs in the US [1] [2]. In the majority of cases, CDI is caused by the administration of antibiotics, which kill many of the important natural gut microflora that comprise the gut microbiome. Loss of this critical microbiome balance allows *C. diff* to thrive and produce toxins leading to CDI. One highly effective, yet frequently overlooked therapeutic option is Fecal Microbiota Transplantation (FMT). In contrast to the paradoxical administration of more antibiotics to treat CDI (the current standard first line treatment), FMT reconstitutes the balance of the patient's microbiome. This is accomplished by using a stool sample provided by a healthy donor (screened for transmissible pathogens). The donor's stool is liquefied and administered to the patient, usually via enema, nasogastric tube (NGT) or colonoscopy. Unlike the abysmally low efficacy of antibiotics, FMT cures 94% of even highly refractory CDI [3].

Unfortunately, FMT is not widely adopted by physicians due to its unappealing nature, lack of standardization and logistical inefficiency [4] [5]. In order to perform FMT, stool must be scooped from a toilet, transferred to a separate room with a lab hood, then transferred into and homogenized in a blender with the addition of saline. This slurry must then be filtered (typically using crude supplies such as gauze or a coffee

filter) into a receptacle such as an enema bag, where it is then either delivered directly via enema or NGT, or drawn into a syringe and delivered via colonoscope. Clear problems with the current procedure include two-way contamination risks, the aesthetics of the odor and sight of stool, numerous inefficient open-air transfers and processing steps, and lack of an easily executed and replicable standardized protocol. As a result, FMT is typically only used as a last-resort for recurrent CDI.

The Solution: To address the significant technologic gaps limiting adoption of FMT, we aim to bring FMT to the mainstream by introducing a novel collection, processing and isolation system that optimizes all sequential steps of FMT within a completely closed environment. The system thus eliminates all the aesthetic and logistical technologic barriers currently limiting FMT adoption. The device separates viable microflora from the insoluble waste, and packages its fluid product for a variety of further uses: therapeutic direct delivery (enema, colonoscopy, NGT, or even eventual enteric-coated capsules), optimized storage and distribution, or stool-based analytics.

While the upstream components of this system are in development by the company who we served as consultants, we have designed the critical downstream refinement technology enabling the system's comprehensive and versatile capabilities. At this stage, we have a set of two fully-functional 3D printed works-like/looks-like prototypes. Our product seamlessly integrates with the company's upstream device, and maintains a completely closed-system through the target endpoint of rapidly producing a highly purified pellet of microflora (with comprehensive microfloral diversity) packaged within a vial with a resealable access port. While we will ultimately demonstrate that our device produces statistically similar microfloral compositions as the state-of-the-art on a genomic level through comparative deep sequencing of the end products, we have culture data illustrating that culturable species diversity remains unchanged and is statistically the same as the state-of-the-art.

Defensibility: U.S. and international non-provisional patent applications have been filed based on professional patentability analysis and premier IP counsel suggesting that our method and system have broadly protectable claims available. Such claims protect all critical processes throughout the FMT value-chain.

Regulatory Pathway: Predicate FDA approved lab processing equipment affords a rapid initial Class I filing pathway to market. Relatively minimal clinical data is expected to be required to obtain approval for an FMT-indication through a Class II 510(k) filing. As such, we face a uniquely rapid, low-cost regulatory opportunity.

The Market: A significant market exists for our device: in 2012, there was approximately 2 million addressable cases of CDI in the U.S. alone [6] [7] [8] [9], and based on a bottom-up analysis, the annualized addressable U.S. CDI market approximates \$1 billion. Moreover, while CDI represents the most immediately addressable market, other prevalent, disabling diseases such as inflammatory bowel disease (IBD), irritable bowel syndrome (IBS), antibiotic-associated diarrhea unrelated to *C. diff*, obesity, and diabetes are thought related to disruptions in the gut microbiome, and have strong early data supporting the potential therapeutic value of FMT.

Business Opportunity: Our FMT refinement system optimizes our ability to penetrate these attractive microbiome therapeutics markets. As the most cost-effective prospect for treating CDI (and potentially other conditions), our system is appealing to payers. Initial CPT coding is already in place for FMT, affording minimal reimbursement risk for our technology. Our low COGS design facilitates an attractive margin opportunity.

3. Objective Statement

Our primary objective was to create a system that seamlessly integrates with our client's existing device to isolate, extract, and package viable microflora from donated stool efficiently, while maintaining an entirely closed-system. Secondary objectives focused on design elegance with respect to optimizing user-requirements and cost.

4. Documentation of the Final Design

The working prototype features pans made of plastic-welded polypropylene with replaceable nylon sieves housed in a 3D-printed frame that integrates with a commercially available sieve shaker base (Octagon D200 Digital Sieve Shaker). We demonstrated that novel coupling of shaking-driven porous filtration with subsequent centrifugal extraction produces the desired microfloral product, with respect to the primary specifications of our client. Specifically, the design recovered significantly higher viable microflora than both the

current upstream device to which it adapts, and the state-of-the-art method. The method extracted microflora out of solution without compromising species diversity or viability. Importantly, the design also maintained an entirely closed-system through the endpoint of producing a packaged microfloral pellet within a novel resealable vial. See Table 1 for specifications tested.

To demonstrate that we indeed accomplished these key specification objectives, we used synthetic stool inoculated with bacteria as a substitute for real human stool that could be safely tested. The synthetic stool was homogenized in a blender with saline and passed through multiple filters of varying descending pore sizes. Gravity filtration was coupled with mechanical shaking-agitation of the entire setup. This yielded a high degree of bacterial separation from fibrous waste. Subsequent centrifugation of the filtrate isolated microflora from solution with retained viability and diversity. Serial microflora recovery tests allowed us to optimize power, shaking time, and sieve volume, pore size and number.

Table 1: Optimization Specifications

Further Optimization: Specifications Tested	
Specification	Test Result
Closed-System: Fully Sealed	The design is completely watertight
Closed-System: Contamination	No contamination observed
Biological Safety	Closed system. User is not contaminated
Device Safety	EMC and LVD compliant
Isolation and Extraction	Centrifugation and novel closed-system supernatant removal system isolates and extracts microflora as a pellet packaged with in a novel resealable vial.
Microfloral Recovery	Recovery is superior to the state-of-the-art method (4.64E+08 CFU [71.51%] vs. 4.82E+06 CFU [0.74%])
Microfloral Diversity	Retains the same culturable microfloral diversity as state-of-the-art (See Figure 1)
Product Versatility	Microfloral pellet stored and analyzed easily. Reintroduction with saline produces clear, odourless solution that can be easily infused therapeutically.
Process Time	1.5 minutes
Power Consumption	Octagon uses 115V
Volume Capacity	Bottom pan holds 1L of solution
Cost	Cost allows pricing with strong product margin, where the cost to payers per CDI case is less than the cost of a standard vancomycin course per CDI case.

5. Prototype of the Final Design

Our device uses sequential gravity fed filtration and simultaneous mechanical shaking agitation of the filters to isolate microflora from unwanted fibrous matter contained within donated stool. Filtrate collected in novel vials is centrifuged to extract microflora from solution, and removal of supernatant leaves a microfloral pellet packaged in the vial. A resealable access port in the vial not only allows for closed-system supernatant removal, but also enables eventual closed-system transfer of the microflora pellet to therapeutic and analytic devices. The design is aesthetically pleasing, simple, efficient and intuitive. Please see Figure 1 for the final device design, and Figure 2 for a step-by-step illustration of how the device functions.



Figure 1: Microflora Refinement System

Please visit the following link to watch a video where we discuss the device in further detail: <http://youtu.be/ufKh52WP-pl> and

<http://youtu.be/3Z2vYUexMPM> where we have set-up a quick demonstration of our final prototypes.

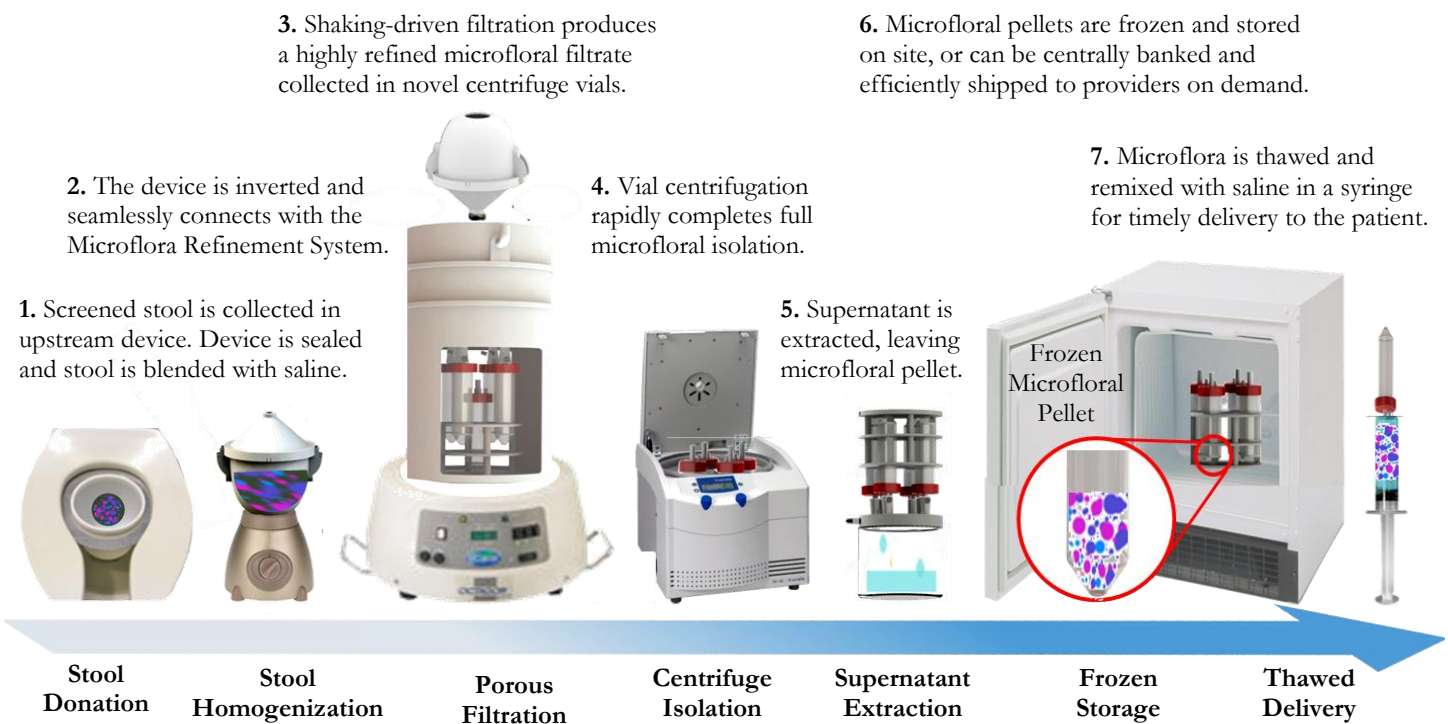


Figure 2: Step-by-Step Illustration of the Microflora Refinement System

6. Proof that the Design is Functional

To verify the functionality of our final device, we conducted a proof-of-concept test for microfloral recovery using canine fecal matter. We ran samples through both the crude state-of-the-art sieve and gauze, as well as our system, and spread diluted samples from both outputs, (in addition to an unfiltered, control sample), onto nutrient agar plates, to observe the effect of both systems on microflora growth. Our device yielded a total colony-forming unit (CFU) count approximately double that of the state-of-the-art method. Moreover, we conducted centrifugation studies to determine that the final extracted product indeed contained viable microflora as opposed to other undesired components of stool. As seen in Figure 3, the difference in the amount of bacteria between the pellet and the initial sample is small.

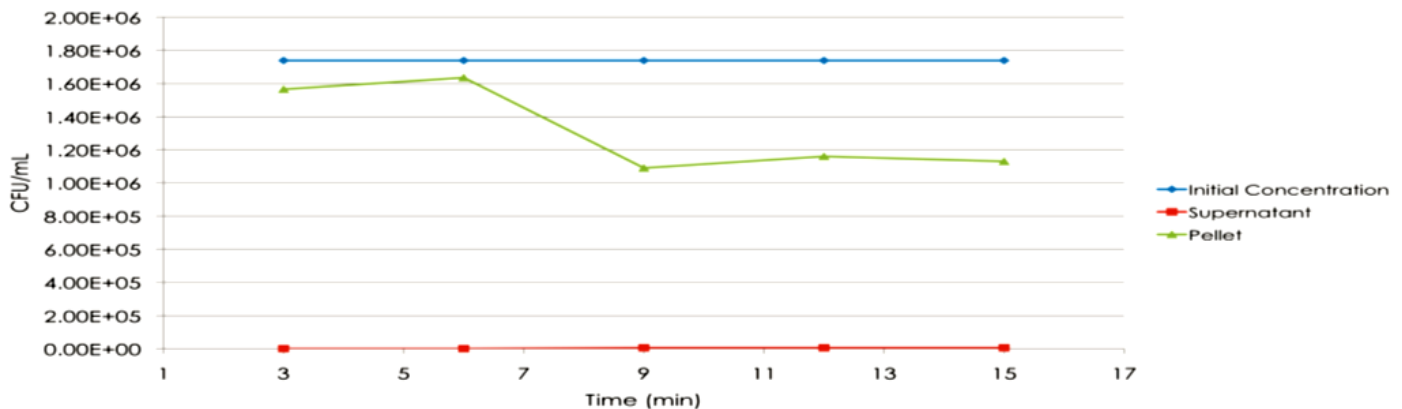


Figure 3: Centrifugation Study to Show Isolation of Microflora in Pellet (Microflora Concentration after Centrifugation at 8,000 rcf)

The question of whether our design met user-requirements was based both on highly positive feedback from our client who has studied such requirements extensively, and feedback received from target users who demoed our device at Dartmouth-Hitchcock Medical Center (DHMC). Our prototype was considered “intuitive” and “easy to use” (See Appendix D for a user guide for our device). Both our client and the DHMC doctors believed that the device made the MFT process simple, efficient, and standardized, and removed the aesthetic and contamination concerns. Taken together, it was felt that our device represented a critically important, enabling innovation offering significant practical and versatile value to providers.

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