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Opinion

Rejuvenating the human gut microbiome

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Industrial advances have caused significant loss of diversity in our gut microbiome, potentially increasing our susceptibility to many diseases. Recently, rewilding the human gut microbiome – that is, bringing it back to an ancestral or preindustrial state (e.g., by transplanting stool material from donors in nonindustrial societies) – has been hotly debated from medical, ethical, and evolutionary perspectives. Here we propose an alternative solution: rejuvenating the human gut microbiome by stool banking and autologous fecal microbiota transplantation, that is, collecting the hosts' stool samples at a younger age when they are at optimal health, and cryopreserving the samples in a stool bank for the hosts' own future use. In this article we discuss the motivation, applications, feasibility, and challenges of this solution.

Highlights

Industrial advances have been associated with large-scale changes in the human gut microbiome and a higher incidence of complex human diseases.

Rewilding the human gut microbiome by transplanting the whole gut microbial community from donors in nonindustrial societies may result in a dramatic mismatch between our industrial environment/lifestyles and the ancestral microbiome.

Emerging studies suggest that stool banking and autologous fecal microbiota transplantation (FMT), using the recipients' own stool samples collected at a younger age when they are disease-free, may be a better – or at least an alternative – solution. This leads to the idea of rejuvenating the human gut microbiome.

The conceptual similarity between stool banking for autologous FMT and cord blood banking for an autologous transplant implies the potential for rejuvenating the human gut microbiome.

Industrialized human microbiome

Trillions of microbes have coevolved with humans for millions of years. There is mounting evidence that the human gut microbiome has experienced significant changes over the past decades due to an urban/suburban lifestyle, coincident with modernization and progress in medicine, and the industrialization of food production [1,2]. Although these selective forces have improved certain aspects of our life, and have resulted in human microbiomes that are able to withstand modern conditions, these changes have resulted in the loss of microbial species and their biochemical functions. Indeed, previous studies have shown that industrial advances (e.g., antibiotics, processed foods, C-section, infant formula, and a highly sanitized environment) are associated with large-scale changes in the human gut microbiome and a higher incidence of complex human diseases, such as asthma [3], *Clostridioides difficile* infection (CDI) [4], colorectal cancer (CRC) [5], irritable bowel syndrome (IBS) [6], inflammatory bowel disease (IBD) [7], cardiovascular disease [8], and type 2 diabetes [9]. Although the **hygiene hypothesis** (see Glossary) suggests that limited exposure to microbes may lead to defects in immune system development [10], the actual links between the **industrialized microbiome** and disease risk remain unclear.

Rejuvenating rather than rewilding our gut microbiome

What would happen if we were to bring our gut microbiome back to an ancestral or preindustrialized state? This idea of **rewilding the human gut microbiome** – that is, restoring a preindustrial (ancestral) microbiome – has taken off in recent years, and it is now hotly debated from medical, ethical, and evolutionary perspectives [2,11–14]. Indeed, rewilding the human gut microbiome may result in a dramatic mismatch between our industrial environment/lifestyles and the **ancestral microbiome**. Despite the recent efforts to reconstruct ancient microbial genomes from mummies or paleofeces [15,16], the notion of an ancestral microbiome per se has not been clearly defined. Microbiome samples from some current nonindustrial hunter–gatherer societies (e.g., Hadza people in Tanzania) have been proposed to approximate the ancestral microbiome [17]. However, it is still unknown whether people in industrialized societies can gain some health benefit by restoring their microbiome to an approximate ancestral state.

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Instead of rewilding the human microbiome using approximate ancestral microbiome samples, here we argue that **rejuvenating the human microbiome** using the host's own microbiome samples collected at a younger age when they are in optimal health or free of disease may be a more appropriate or at least an alternative solution. After all, the mismatch between the hosts' current environment/lifestyles and their microbiome at a younger age should be much smaller than in the case of rewilding the microbiome. We emphasize that rewilding the human gut microbiome can be achieved through different interventions: for example, replacing lost gut microbes, engineering existing microbes to perform depleted functions, or transplanting whole gut microbial communities from donors in nonindustrial societies [11]. The first two interventions are targeted rewilding, while the last one is based on the idea of fecal microbiota transplantation (FMT) (Box 1) [18]. Rewilding the human gut microbiome by transplanting the whole gut microbial communities from donors in nonindustrial societies is heterologous FMT with very special donors, while rejuvenating the human microbiome can be considered as a special autologous FMT with host samples collected at a particular time point (long before the FMT) and stored in a stool bank.

Autologous FMT: timing of the sample collection matters

Heterologous FMT has gained popularity over the past decades due to its success in treating several human diseases such as IBD and CDI. However, the long-term safety concerns [19], the challenging donor recruitment/screening process [20], the less-than-complete success rate [21–23], as well as the FDA's struggle to regulate FMT have all limited the use of FMT[i] [24]. In particular, FMT response variability is presumed to be due to the mismatch of host factors (e.g., genetics, diet, other environmental exposures) between donor and recipient, collectively known as the donor–recipient compatibility issue. There is a clear need to control for donor–recipient compatibility issues in FMT studies. By definition, autologous FMT can naturally avoid, or at least mitigate, the donor–recipient compatibility issue, as well as many of the ethical concerns associated with heterologous FMT [25]. But the timing of the sample collection matters. Several studies have compared the clinical benefit of heterologous FMT and autologous FMT in treating diseases such as CDI [26], IBS [27], and IBD [28]. Although most of these studies showed that autologous FMT had a lower response rate than heterologous FMT, caution is needed in interpreting these results. First, in those randomized controlled clinical trials, autologous FMT was introduced as a placebo control treatment. Second, fecal samples for autologous

Box 1. Fecal microbiota transplantation

FMT involves the administration of a solution of fecal matter from a carefully screened, healthy donor into a recipient – through the lower gastrointestinal (GI) tract via colonoscope or enema, through the upper GI tract via nasogastric tube, or with a capsulized, oral, frozen inoculum – in order to directly alter the composition and function of the intestinal microbiota and confer a health benefit [18]. Depending on the source of the fecal material, FMT can be divided into two categories: *heterologous* FMT, where fecal materials are collected from prescreened healthy donors, and *autologous* FMT, where fecal materials are collected from the recipients themselves before FMT.

Although various strategies have been proposed to rebuild a healthy human microbiome, (heterologous) FMT has gained popularity over the past decade due to its success in treating several human diseases. For example, FMT is known to be a very effective treatment for rCDI, with cure rates of up to 94% in clinical trials [70]. Promising findings for FMT in rCDI has led to investigation of its application to other gut microbiome-associated diseases such as CRC [71], IBS [27], IBD [28,72], and diabetes [73]. With the development of modern techniques, a set of screening processes of potential donors has been proposed, which includes a clinical assessment (e.g., medical history, mental health condition, and known history for infectious diseases, etc.) and laboratory testing (e.g., stool and serologic screening) [74].

In spite of the clinical evidence for the effectiveness and safety of (heterologous) FMT, it still has some challenges and limitations, including the potential risk of disease transmission between the donor and recipient, mild temporary adverse effects (e.g., mild diarrhea, abdominal pain, abdominal bloating, nausea, headaches, and fatigue), long-term safety concerns (e.g., weight gain after FMT using stool from a healthy but overweight donor), the challenging donor recruitment/screening process, and patients' perceived acceptance.

Glossary

Ancestral microbiome: the human microbiome of our preindustrialized ancestors.
Cord blood banking: storage of umbilical cord blood for future use. Cord blood is an excellent source of stem cells and offers another method of definitive therapy for infants, children, and adults with certain fatal diseases (e.g., hematologic malignancies and hemoglobinopathies).
Cryopreservation: the use of very low temperatures to preserve structurally intact living cells and tissues.
Human microbiota: the total collection of microorganisms (including bacteria, archaea, viruses, protists, and fungi) that live symbiotically on and within various sites of the human body, such as the oral cavity, genital organs, respiratory tract, skin, and gastrointestinal tract. Those microorganisms and their genes are collectively known as the human microbiome.
Hygiene hypothesis: the early childhood exposure to particular microorganisms protects against allergic diseases by contributing to the development of the immune system and teaching the immune system to differentiate between harmless and harmful substances and not to overreact.
Immunocompetent: having a normal immune system which is able to produce a normal immune response following exposure to an antigen.
Immunocompromised: having a weakened immune system and hence a reduced ability to fight infections and other diseases. This may be caused by certain diseases or conditions such as acquired immune deficiency syndrome (AIDS), cancer, diabetes, malnutrition, and certain genetic disorders. It may also be caused by certain medicines or treatments, such as anticancer drugs, radiation therapy, and stem-cell or organ transplant.
Industrialized microbiome: the microbiome harbored by individuals living in an industrialized society.
Microbiota Vault: a global nonprofit initiative (www.microbiotavault.org) which sets out to preserve the biodiversity of human-associated microbiota by constructing an institution for the safe storage and preservation of microbiota samples and collections to conserve long-term health for humanity.

FMT in those studies were typically collected from the patients at the time of their treatment, or shortly before treatment when they were presumed sick, rather than from the healthy individuals. In short, results from those studies just imply that the cure rates of autologous FMT (using stool samples collected from the recipients' diseased state) are almost equal to the patients recovering on their own without the need for FMT, which is exactly what we expect for a placebo control treatment. This type of autologous FMT is certainly not what we need to rejuvenate the microbiome. Our view is that fecal samples collected well before disease onset would provide the best source for autologous FMT. Conceptually, the clinical benefit observed in current studies on autologous FMT can be further improved if in the autologous FMT we use the recipients' own stool samples collected at a younger age when they were disease-free (Figure 1). The human microbiome can be affected by many external factors, including age, lifestyle, and health status. Ideally, the microbiome sample should be collected when the participants are mature, relatively young, and healthy (e.g., preferably young adulthood 18–35 years of age). In principle, people in midlife or mature adulthood (e.g., 36–55) without chronic diseases can also store their microbiome samples for future use. Based on existing FMT studies, we anticipate that recipients will benefit from rejuvenating their microbiome in multiple microbiota-related clinical situations (Box 2).

Rejuvenating the human gut microbiome: collecting stool samples from the host at a younger age when they are in optimal health and cryopreserving the samples in a stool bank for the host's own future use by autologous FMT.

Rewilding the human gut microbiome: bringing the industrialized microbiome back to an ancestral or preindustrial state by replacing lost gut microbes, engineering existing microbes to perform depleted functions, or transplanting the whole gut microbial communities from donors in nonindustrial societies by heterologous FMT.

Stool bank: a centralized facility that screens donors, processes stool, stores FMT preparations, fulfills requests from clinicians and researchers for those preparations, and monitors the safety and efficacy of the material.

Super donors: donors whose stool results in more successful FMT outcomes compared with stool from 'normal' donors.

Rejuvenating the gut microbiome: feasibility analysis

Ecological basis

Human microbiota starts to colonize in/on the human body before [29] or immediately after birth [30]. The symbiosis of the human microbiome is gradually established from birth, and is shaped during the first few years of life [31]. Although the gut microbiome might not be expected to follow the same general trajectory of age-related physiological change, numerous studies have suggested that, in the absence of extreme perturbations (e.g., repeated antibiotic administrations, or drastic diet change), the human gut microbiome is relatively resilient and stable for adults (especially in early adulthood) [32]. This serves as the ecological basis of rejuvenating the gut microbiome by stool banking and autologous FMT. After all, an unstable microbial community is very unlikely to benefit the host, especially when they are old and **immunocompromised**. By contrast, young and healthy adults with a stable gut microbiota can store their own microbiome samples in a stool bank for future autologous FMT use.

Stool banking

The first **stool bank** (OpenBiome) was actually started at Medford (Massachusetts, USA) in 2012 [33]. Since then, many stool banks have opened worldwide – including the University Hospitals of Paris Centre (2014), AdvancingBio at Mather (California, USA, 2015), Public Health England at the Birmingham laboratory (UK, 2015), the Chinese fmtBank (Nanjing, China, 2015), and The Netherlands Donor Feces Bank (Leiden, The Netherlands, 2016) – and many more are planned [34]. To our knowledge, the main goal of these stool banks is to provide stool samples from rigorously screened healthy donors to physicians so that they can effectively treat patients with recurrent CDI (rCDI). In other words, existing stool banks are typically storing stool samples for heterologous FMT, rather than for autologous FMT. (One exception is OpenBiome's personalized microbiome banking service. But it only allowed individuals to preserve a copy of their healthy microbiome for their future treatment of CDI, not other conditions.) Those stool banks provided centralized donor screening and material preparation, which increases the quality and accessibility of FMT as a therapy. In principle, the same procedure of host screening and sample collection can be used for the purpose of rejuvenating microbiome by autologous FMT. Hence, instead of starting from scratch, the existing high-standard stool banks could be repurposed for the idea of rejuvenating the microbiome with autologous FMT. Notably, the ongoing coronavirus disease 2019 (COVID-19) pandemic has significantly affected the usage of heterologous FMT in treating rCDI patients. For example, the first stool bank, OpenBiome, has unfortunately ended its program for collecting, screening,

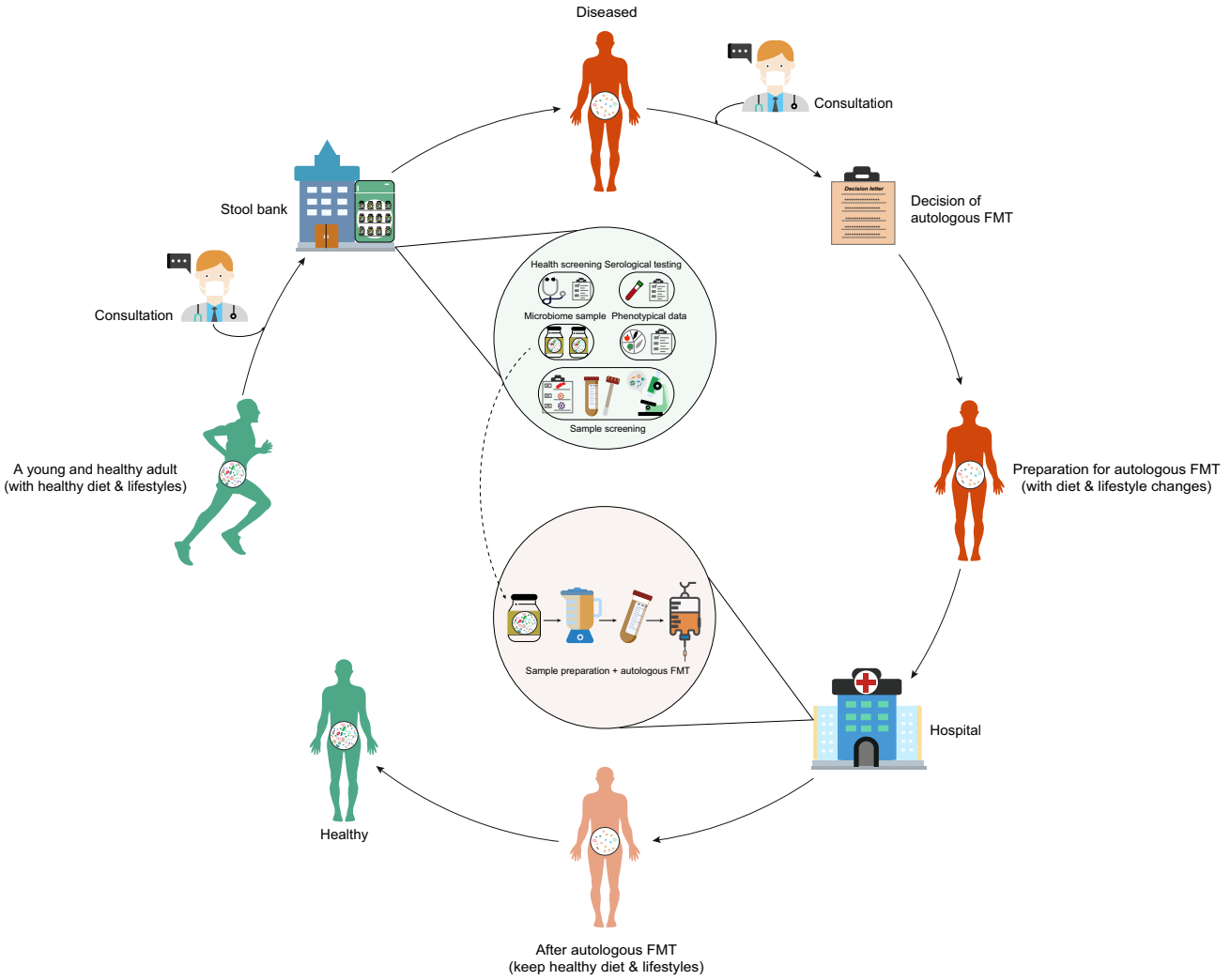

Figure 1. Hypothetic workflow of rejuvenating the human gut microbiome by stool banking and autologous fecal microbiota transplantation (FMT). Individuals who are interested in rejuvenating their gut microbiome in the future should consult with their physicians first. Once the health screening is completed, stool samples (as well as comprehensive phenotypical data such as dietary intake, medication, lifestyles, etc.) of the participants should be collected immediately by the stool bank. Part of the stool samples will be used for laboratory tests (sample screening and sequencing). The rest will be immediately cryopreserved. A set of meticulous criteria will be applied for sample screening. Only samples that pass the screening will be stored long-term at the stool bank. In the future, if participants get a disrupted gut microbiome (e.g., due to *Clostridioides difficile* infection or aging), they should consult with their physicians to decide whether they need an autologous FMT. Before the autologous FMT, participants should adjust their diet and lifestyles appropriately to match their previous healthy ones. Then cryopreserved stool samples will be resuscitated and screened again by the stool bank to ensure safety. Only samples that pass the second screening will be used for autologous FMT by gastroenterologists at hospitals. After autologous FMT, participants should keep to a healthy diet and lifestyle to enhance the efficacy of autologous FMT.

and shipping material for FMT because of COVID-19. During global pandemics such as COVID-19, if patients had already stored their fecal samples collected in a stool bank at a younger age during a disease-free period, then clinical use of FMT may not be affected at all by the global pandemic. This would certainly help us to avoid unnecessary delays in emergency cases of FMT.

Sample preparation

Based on published consensuses on the use of FMT in clinical practice [35–37], as well as on guidelines suggested by functioning stool banks [33], fresh fecal material should be suspended

Box 2. Rejuvenating the gut microbiome: potential applications

rCDI

In a previous work [75], through extensive numerical simulations using a classical community ecology model, we found that autologous FMT (using the recipient's own sample collected in the disease-free state) will always yield a higher efficacy than heterologous FMT (using an unrelated healthy donor's sample). Based on this finding, we conjecture that CDI patients can be their own '**super donors**' [76] in the FMT if the stool samples were collected from them at a younger age when they were disease-free.

IBD

Heterologous FMT for the management of patients with IBD demonstrated low clinical remission rates ranging from 24% to 50% [77]. Comparing both heterologous FMT (using samples from healthy donors) and autologous FMT (using their own fecal samples before bowel lavage) for the treatment of mild to moderate ulcerative colitis (UC, a subtype of IBD), a previous study reported that autologous stool (32%) could be as effective as heterologous (30.4%) fecal samples in inducing clinical remission [78]. A recent study suggests that autologous FMT with IBD patients' own stool samples collected at the inactive state of IBD can circumvent safety risks [79]. We anticipate that autologous FMT with the IBD patients' own stool samples collected at younger age (well before the disease onset) will not only circumvent safety risks but also have a much higher efficacy than heterologous FMT in managing IBD.

Allogeneic hematopoietic stem-cell transplantation (allo-HSCT)

Both heterologous [80] (with stool samples from healthy donors) and autologous FMT [81] (with stool samples from the patients themselves collected before chemotherapy and antibiotic administration) have been successfully applied in allo-HSCT to rebuild patients' gut microbiota and increase gut microbial diversity after chemotherapy and antibiotic administration. As gut microbiota diversity loss during allo-HSCT is associated with poorer clinical outcome, patient may benefit more if they use their own stool samples collected at a younger and disease-free age.

Obesity

A mouse study has demonstrated that autologous FMT (administration of their own feces before they developed obesity) potentiates the effects of a moderate caloric restriction on weight loss in high-fat diet-induced obese mice, by decreasing feed efficiency and increasing adipose tissue lipolysis [82]. A recent human study evaluated the efficacy and safety of diet-modulated autologous FMT for treatment of weight regain after the weight-loss phase [83]. This study found that autologous FMT (with a fecal sample collected during the weight-loss phase and administrated in the regain phase) in conjunction with a green Mediterranean diet significantly attenuated weight regain. Furthermore, the effect of autologous FMT on weight regain was associated with specific microbiome signatures and diet. We expect that autologous FMT with fecal samples collected from a prior healthy lean phase will be a powerful synergetic intervention for obesity.

Aging

A study on African turquoise killifish showed that incubating old individuals overnight with the intestinal contents of young individuals (an effective heterologous FMT) can causally induce long-lasting beneficial systemic effects that lead to life-span extension and delayed behavioral decline of the old individuals [57]. A recent mouse study reported that transplanting microbiota from young donors to aging recipients can reverse aging-associated differences in peripheral and brain immunity, hippocampal metabolome, and transcriptome of aging recipient mice, and it showed an ability to attenuate selective age-associated impairments in cognitive behavior when transplanted into an aged host [84]. Similarly, a very recent mouse study demonstrated that transplanting fecal microbiota from young into old mice can reverse several hallmarks of aging (e.g., the disrupted gut barrier integrity, systemic and tissue inflammation affecting the retina and the brain) [85]. We expect that autologous FMT (with stool samples collected from the host at a younger and healthier age) may be a more powerful therapeutic approach to promote healthy aging of the host than heterologous FMT (with stool samples collected from an unrelated young and healthy donor).

in saline using a blender or manual effort and sieved to remove fibrous material and avoid the clogging of infusion syringes and tubes in future FMT. Recently, the protocol of washed microbiota transplantation (WMT) has been developed [38], where fecal material is prepared with microfiltration based on an automatic purification system followed by repeated centrifugation plus suspension. This automatic washing procedure will drastically improve the efficiency of fecal material preparation and save the cost of **cryopreservation.**

For rejuvenating the gut microbiome, clients should have more options to use their cryopreserved samples. Depending on their particular condition/disease, the client should discuss with the

physician whether a regular FMT or a variant (e.g., fecal filtrate transfer, FFT [39–41] or fecal viral transfer, FVT [42,43]) should be administered. To ensure that the client will still have those multiple options available in the future, we suggest that the fecal material (after an appropriate 'washing' procedure) should be cryopreserved when the client is in optimal health. If we only cryopreserve a particular component of the fecal material (e.g., bacteriome, virome, mycobiome, microbial debris, metabolic products, etc.) or a particular combination of those components (e.g., sterile fecal filtrates that contain bacterial debris, proteins, antimicrobial compounds, metabolic products, and oligonucleotides/DNA), the client will have very limited options for future use. In short, we suggest that the choice of the type of procedure (FMT, FFT, or FVT) should be made in the future when the client needs it, rather than before the cryopreservation.

Cryopreservation

A key aspect in stool banking for future autologous FMT is the requirement for true long-term stool sample storage. Previous data show that the use of fecal suspensions stored (at –80°C) for up to 2 years does not undermine the clinical success of FMT for the treatment of CDI [44,45]. Indeed, OpenBiome and The Netherlands Donor Feces Bank have good experiences with –80°C storage temperature for up to 1 and 2 years, respectively [46,47]. However, for true long-term storage of microbiome samples, temperatures below the glass transition temperature of water (–137°C) should be used to protect proteins and DNA from denaturation/damage and to halt the biochemical and physiological activity of the cells [48]. This typically requires liquid nitrogen storage (–196°C). For example, an alga (e.g., *Chlorella vulgaris* CCAP 211/11B) has been shown to retain its genotypic stability for more than 40 years after serial transfer under different cultivation regimes and liquid nitrogen storage [49]. The long-term safe storage and subsequent resuscitation and cultivation of complex microbial communities (e.g., stool samples) is by itself a fundamental research question. Further research is certainly needed to systematically test longer storage times and preservation/resuscitation/cultivation procedures to inform practical guidelines for stool banking for rejuvenating the human gut microbiome. Thanks to the **Microbiota Vault** initiative[ii], research into these problems is currently accelerating. This serves as the practical basis for rejuvenating our microbiome.

Stool banking versus cord blood banking

Conceptually, rejuvenating the human gut microbiome by stool banking and autologous FMT is similar to **cord blood banking** for an autologous transplant [50]. A fundamental difference between cord blood banking and stool banking is the chance to use the cord blood and stool samples in the future. Indeed, the chance that a child would need to use his or her own cord blood is extremely low: from 1:400 to 1:200 000 over the child's lifetime [51]. However, the relationships between the gut microbiome and multiple factors – such as diet, drug use (e.g., antibiotics), lifestyle (e.g., smoking, physical activity, traveling, and sleep deprivation), age (aging), and many common disease (e.g., allergies, obesity, CDI, IBD, and cardiovascular disease) – reveals the much greater potential of stool banking compared to cord blood banking. This serves as a strong motivation to promote the idea of rejuvenating the human gut microbiome by stool banking and autologous FMT.

Regulations

Rejuvenating the human gut microbiome by stool banking and autologous FMT certainly requires careful regulation. In fact, even FMT itself requires careful regulation to ensure safety and therapy standardization [24]. Currently, FMT in many developing countries remains a 'no-man's land'. The US FDA has chosen to strictly regulate human feces as a biological product and drug[iii]. However, many gastroenterologists consider the human gut microbiota as a 'virtual organ' [52–54], and hence human feces should be regulated as 'human tissue', and safety precautions similar to

those used for transplanting human tissues (such as blood, bone, skin, and egg cells) should be taken with FMT. In the same spirit, we suggest that the procedure of rejuvenating the human gut microbiome by stool banking and autologous FMT should be carefully regulated based on regulations similar to those used for cord blood banking, including establishment registration and listing, donor screening and testing for infectious diseases, reporting and labeling requirements, and compliance with current good tissue practice regulations[iv]. In other words, the regulation policy-making process will not start from scratch but can heavily leverage existing regulations and policies on cord blood banking.

Rejuvenating the microbiome: fundamental challenges

Undoubtedly, there are some fundamental challenges with rejuvenating the human gut microbiome, as follows. Addressing those challenges warrants extensive animal and human studies.

Will a transplanted young/healthy microbiome retain its youthful/healthy characteristics for an extended period of time, or will it shortly revert to the older microbiome?

The long-term effects of FMT have not yet been extensively studied. We suspect that this will very likely depend on the specific disease or condition being treated, as well as the post-FMT host factors (e.g., diet, lifestyle, etc.). For rCDI, although previous studies have reported that heterologous FMT was a durable (maximum follow-up of 6.8 years) and safe treatment option [55,56], further investigations with larger sample sizes are needed to determine the long-term effect and changes in the microbial community after FMT. With respect to aging, the study of African turquoise killifish did find long-lasting beneficial systemic effects of heterologous FMT in older individuals using samples from younger donors [57].

For chronic diseases (e.g., type 2 diabetes, obesity) associated with a dysbiotic gut microbiome, FMT has demonstrated modest clinical efficacy with a high variability in patient response. In this case, synergetic strategies (e.g., diet intervention and lifestyle change) might have to be taken simultaneously to minimize environmental compatibility issues. Also, since the treatment effect may decline over time, repetitive FMT can be considered based on the volume of cryopreserved stool samples.

How many of us will truly be eligible for (and hence presumably benefit from) rejuvenating our microbiome?

Existing stool banks typically have a very strict donor screening process, rendering very low donor qualification rates [20,45]. For example, OpenBiome prospectively evaluated 15 317 consecutive donor candidates from February 2014 through April 2018, and found only 386 qualified donors, rendering a donor qualification rate of 2.52% [20]. We think the exclusion criteria of existing stool banks (which are currently all operating for the purpose of heterologous FMT) might be too strict for rejuvenating the microbiome (based on autologous FMT). For example, before clinical assessment, out of the 15 317 candidates, OpenBiome excluded 1876 (12.2%) of them just because those candidates did not live in the same region as the donation facility or were unable to donate on a regular basis [20]. They further excluded 3595 candidates (23.5%) because they were lost to follow-up at either clinical assessment or stool/serologic screening stage. This logistic exclusion criterion certainly should not be applied to the case of rejuvenating the microbiome. Hence, we expect the qualification rate of individuals who plan to rejuvenate their gut microbiome will be higher than the donor qualification rate reported by existing stool banks. Also, those individuals who have consistently failed their health or sample screening may consider collecting and storing stool samples from their young and healthy immediate family members (e.g., offspring and siblings), given their similar genetic backgrounds and presumably similar living environments and lifestyles [58,59].

Will the previous antibiotic exposure significantly affect the efficacy of autologous FMT in certain applications?

In our industrial society, it is difficult to find an individual who has never been exposed to antibiotics (especially during early life). Despite the controversy in societal evolution, multiple aspects of our lives (e.g., lifespan) have been improved [60]. For many of us, our gut microbiota might have already adapted well to our industrialized environment and lifestyles. Hence, we argue that it might still be meaningful to store our microbiome samples when we are younger and healthier. Also, according to our current knowledge, existing stool banks (typically serving heterologous FMT) do not completely exclude donors who have had early-life antibiotic exposure. Instead, they perform stool testing for antibiotic-resistant bacteria [20,45], such as vancomycin-resistant enterococci, methicillin-resistant *Staphylococcus aureus*, carbapenem-resistant Enterobacteriaceae, extended-spectrum β-lactamase-producing organisms, etc. We think that this is a more appropriate and smarter strategy to exclude donors with long-term and/or high-dose antibiotic exposure.

How to identify opportunistic pathogens that are benign for young adults with a strong immune system but harmful to the elderly with a weakened immune system?

Addressing this safety issue for immunocompromised individuals is actually important for both heterologous and autologous FMT. To our current knowledge, existing stool banks (usually serving heterologous FMT) do not explicitly address this question. Although existing data suggest that heterologous FMT for the treatment of rCDI in immunocompromised patients is feasible and safe, with rates of serious adverse events similar to those in **immunocompetent** patients [61,62], larger cohorts of patients are needed to establish whether heterologous FMT is safe for immunocompromised patients. A careful stool testing for well-known opportunistic pathogens (e.g., opportunistic parasites such as *Cryptosporidium* [63], *Isospora* [64], *Cyclospora* [65], *Microsporidia* [64], etc., and opportunistic bacteria such as *Bartonella* species [66], *Helicobacter pylori* [67], and *C. difficile* [68], etc.) must be preventively performed before stool banking. To further improve safety, we suggest that, before stool banking, preclinical mouse models could be used as a functional tool to determine the opportunistic infection potential of the human feces for future autologous FMT. Also, we suggest that for immunocompromised patients the decision of autologous FMT should be made very cautiously. Preclinical mouse models could again be used to test the opportunistic infection potential of the resuscitated samples.

How to ensure that a lean healthy young adult's gut microbiome will not predispose its host to develop certain diseases or phenotypes such as obesity?

This safety issue is again equally important for both heterologous and autologous FMT. There is no perfect solution to fully address this issue. For specific phenotypes such as obesity, a pioneering work has demonstrated that the microbiota from lean or obese humans induces similar phenotypes in germ-free mice [69]. This suggests that preclinical mouse models could be used as a functional tool to determine the potential of the collected human feces to predispose the host (e.g., germ-free mice) to develop a certain disease or phenotype. This may help us to minimize potential side effects of rejuvenating the microbiome based on autologous FMT.

Is the benefit–cost ratio of stool banking and autologous FMT significantly higher than that of regular heterologous FMT?

Among all the possible solutions to restoring a healthy microbiome, rejuvenating the microbiome based on stool banking and autologous FMT might be the most expensive one for patients. For certain applications (e.g., the treatment of rCDI) it is certainly not cost-effective. However, of all

Clinician's corner

Various microbiome-based therapeutic strategies have been proposed to restore a healthy human microbiome, including FMT, probiotics, prebiotics, postbiotics, diet intervention, and phage therapy. FMT has gained popularity over the past decade due to its success in treating several human diseases.

The idea of rejuvenating the human gut microbiome is based stool banking and autologous FMT. Existing stool banks serve for heterologous FMT, but they can be repurposed for autologous FMT.

Heterologous FMT is generally considered as an effective treatment for patients with rCDI and potentially a wide range of other diseases. Donor selection represents a fundamental challenge in view of the implementation of heterologous FMT programs, and this may be highly related to efficacy and safety of heterologous FMT. However, autologous FMT with the hosts' own stool samples collected at a younger age when the hosts are at optimal health can naturally avoid or at least mitigate the donor–recipient compatibility issue, as well as many ethical concerns associated with heterologous FMT. Notably, participant should be more willing to accept their own microbiome samples through autologous FMT than those of a healthy donor.

The ongoing COVID-19 pandemic has significantly affected the usage of heterologous FMT in treating rCDI patients. Rejuvenating the human gut microbiome by stool banking and autologous FMT may not be affected by the global pandemic as patients had already stored their fecal samples collected at a younger age during a disease-free period in a stool bank.

the potential applications, it might be the safest one, especially considering the advantages of autologous FMT in resolving the donor–recipient compatibility issue. Also, for all the possible applications, we think that autologous FMT should have higher patient acceptability than heterologous FMT.

How much stool should be cryopreserved for each participant?

The total volume of stool samples from a participant to be cryopreserved at the stool bank should be determined by the participant based on his or her own anticipated usage in the future. The stool bank should suggest the minimum volume (e.g., 55 g fecal material based on the standard used by OpenBiome [33]) required for a one-time autologous FMT. If the participant is interested in repeated autologous FMTs in the future, they can certainly store more samples and pay more. The detailed business model of rejuvenating the gut microbiome would be quite different from that of the current existing stool banks (which pay donors small fees as incentive to get regular donation of their stool material), but very similar to that of cord blood banks (which charge clients for the initial collection/processing fee, as well as an annual storage fee). The scale of the stool bank (as well as the related questions of facility space, energy consumption, number of mice, etc.) will be dynamically determined based on the number of clients who are willing to pay the cost of stool banking and autologous FMT. We do not anticipate that all individuals in our society would be willing to pay the cost. Developing a reasonable business model and marketing strategy would certainly require the joint forces of entrepreneurs and scientists.

Concluding remarks

It is our opinion that it would be wise to bank human stool samples at a younger age when individuals are disease-free to potentially rejuvenate the human gut microbiome using autologous FMT when the individuals age or develop diseases associated with a disrupted gut microbiota (see Clinician's corner). Of course, given the current state of the evidence, well-designed animal and human studies are warranted to further support this idea (see Outstanding questions). Also, caution is needed in promoting this idea. It is promising but certainly not a panacea. Other synergetic strategies (e.g., diet intervention and lifestyle change) might have to be taken simultaneously with autologous FMT to minimize environmental differences between the time of stool sample collection and that of autologous FMT to further enhance engraftment and improve the efficacy of autologous FMT. For chronic diseases that are associated with disrupted gut microbiota but have a strong genetic predisposition (e.g., Crohn's disease, a subtype of IBD), or autoimmune diseases with an origin in early-life gut microbiome imbalance (e.g., asthma), the efficacy of using (either heterologous or autologous) FMT to manage disease might have a very limited or no effect. In these cases, we expect that rejuvenating the gut microbiome will not help.

Basic research in cataloging, characterizing, and even engineering individual microbes (or well-defined consortia of them) and their functions (or metabolic fuels/products) is still a very promising solution to restoring a healthy gut microbiota. However, considering the daunting complexity of the human gut microbiota, both bottom-up mechanistic approaches and top-down systems approaches (based on FMT) will be needed.

Considering the massive (and possibly permanent) loss of our microbial diversity due to industrial advances, the creation of a global 'microbial Noah's ark' is warranted to protect the long-term health of humanity (Figure 2). We admire, and are grateful for, the huge efforts of the Microbiota Vault initiative[ii]. However, considering the highly personalized gut microbial compositions and the donor–recipient compatibility issue, creating a personal microbial Noah's ark using stool banks for future personal use might also be a worthwhile option.

Outstanding questions

For participants, what is the optimal age for the stool sample collection?

Should healthy people in midlife or mature adulthood store their stool sample for future use?

How should we establish a standard criterion for screening of the stool sample?

How much stool should be stored?

How long can the stool sample be stored?

Should participants consider FMT or its variant (e.g., FFT or FVT)?

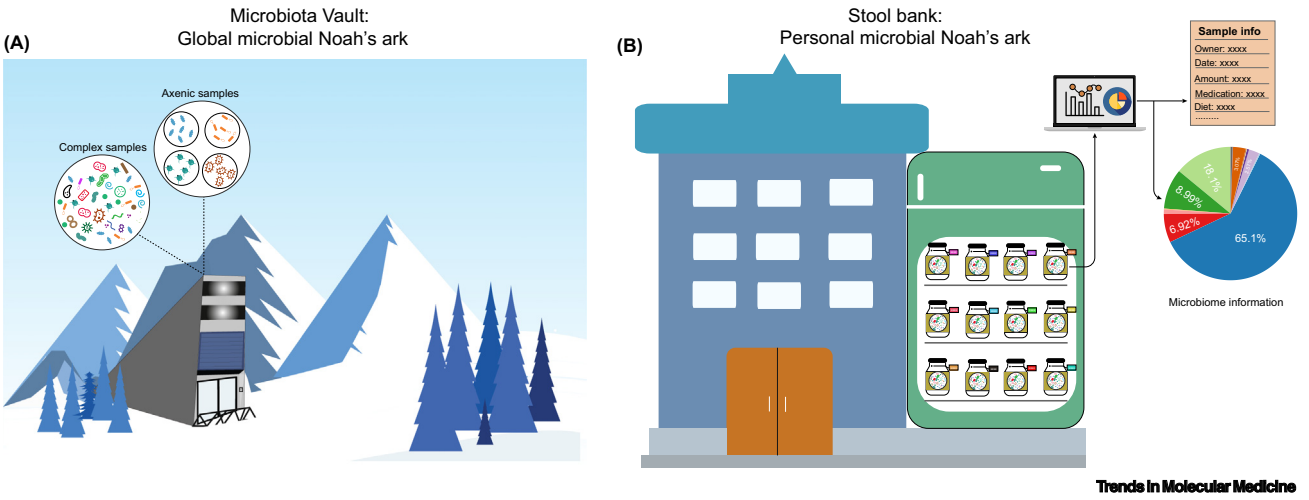

Figure 2. Microbiota Vault versus stool bank. (A) The Microbiota Vault initiative attempts to create a global 'microbial Noah's ark' to preserve the biodiversity of the human-associated microbiota by constructing an institution for the safe storage and preservation of microbiota samples and collections to conserve long-term health for humanity. (B) The existing stool banks can be repurposed to create a personal 'microbial Noah's ark' for future autologous fecal microbiota transplantation (FMT) use.

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Declaration of interests

No interests are declared by the authors.

Resources

[i]www.fda.gov/vaccines-blood-biologics/safety-availability-biologics/important-safety-alert-regarding-use-fecal-microbiota-transplantation-and-risk-serious-adverse

[ii]www.microbiotavault.org/wp-content/uploads/2021/03/Microbiota_Vault_Report_Final_20200611.pdf

[iii]www.fda.gov/regulatory-information/search-fda-guidance-documents/enforcement-policy-regarding-investigational-new-drug-requirements-use-fecal-microbiota-0

[iv]www.fda.gov/vaccines-blood-biologics/consumers-biologics/cord-blood-banking-information-consumers

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