



# Fecal microbiota transplantation as a promising treatment option for osteoporosis

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## Abstract

Osteoporosis is a systemic metabolic bone disease characterized by the descending bone mass and destruction of bone microstructure, which tends to result in the increased bone fragility and associated fractures, as well as high disability rate and mortality. The relation between gut microbiota and bone metabolism has gradually become a research hotspot, and it has been verified that gut microbiota is closely associated with reduction of bone mass and incidence of osteoporosis recently. As a novel “organ transplantation” technique, fecal microbiota transplantation (FMT) mainly refers to the transplantation of gut microbiota from healthy donors to recipients with gut microbiota imbalance, so that the gut microbiota in recipients can be reshaped and play a normal function, and further prevent or treat the diseases related to gut microbiota disorder. Herein, based on the gut–bone axis and proven regulatory effects of gut microbiota on osteoporosis, this review expounds relevant basic researches and clinical practice of FMT on osteoporosis, thus demonstrating the potentials of FMT as a therapeutic option for osteoporosis and further providing certain reference for the future researches.

**Keywords** Fecal microbiota transplantation · Gut microbiota · Osteoporosis · The elderly

## Introduction

Osteoporosis is a systemic metabolic bone disease characterized by the descending bone mass and destruction of bone microstructure, which tends to result in the increased bone fragility and fractures [1, 2]. Therein, the brittle fractures caused by osteoporosis mainly occur in stress concentration parts, including the vertebral bodies, hip and distal radius, which significantly enhances the disability rate and the mortality of the middle-aged and elderly individuals [3–5]. Moreover, in accordance with previous studies, more than 150 million individuals worldwide suffer from osteoporosis every year, and one-third of women and one-fifth of men around the world might suffer from osteoporotic fractures at least once during their whole life [6, 7], which is a major challenge for the patients themselves, their families and social medical security system [8, 9].

Currently, the prevention and treatment of the osteoporosis mainly depend on the interventions of various kinds of drugs and the adjustment of lifestyles of middle-aged and elderly people [10]. The drugs for osteoporosis mainly include bisphosphonates, selective estrogen receptor modulators, hormone replacement agents, calcitonin, and so on,

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which have already obtained the effective clinical efficacy and safety [11]. However, the potential complications and corresponding side effects of partial drugs are need to be concerned. For example, the bisphosphonates may result in the severe bone, joint or muscle discomfort in partial patients [12]. Long-term use of alendronate is associated with the occurrence of femoral intertrochanteric fracture and femoral shaft fracture [13]. As a kind of selective estrogen receptor modulator, raloxifene may enhance risk of venous thromboembolism and stroke [14]. In hormone replacement therapy, estrogen might increase the risk of endometrial hyperplasia and cancers in the elderly women, and enhances the incidence rates of gallstone disease and venous thromboembolism by two to three times [15]. Hence, the existing side effects of drugs limit its long-term use in the osteoporosis, and it is urgent to explore alternative approaches with fewer side effects to help get out of this dilemma.

Regarding this, numerous studies in recent years have revealed that there is a close relationship between the gut microbiota and bone metabolism [16, 17]. As the largest microbial repository in human body, the dynamic balance of intestinal micro-ecosystem is closely associated with the health of body. Meanwhile, the changes in internal and external environment of body can also disrupt the balance between the gut microbiota and body, triggering kinds of inflammatory and metabolic diseases, such as obesity, inflammatory bowel disease, diabetes, rheumatoid arthritis, osteoporosis, and so on [18–22]. Moreover, in previous critical reviews, based on concept of “brain-gut-bone” axis, we have also summarized the modulatory effect and implication of the gut microbiota to osteoporosis [23], and regulative effect and repercussion of probiotics and prebiotics on osteoporosis [24]. Hence, in view of the close association between the gut microbiota and bone metabolism, it is suggested that gut microbiota may be regarded as a potential target for preventing and treating the osteoporosis to a certain extent.

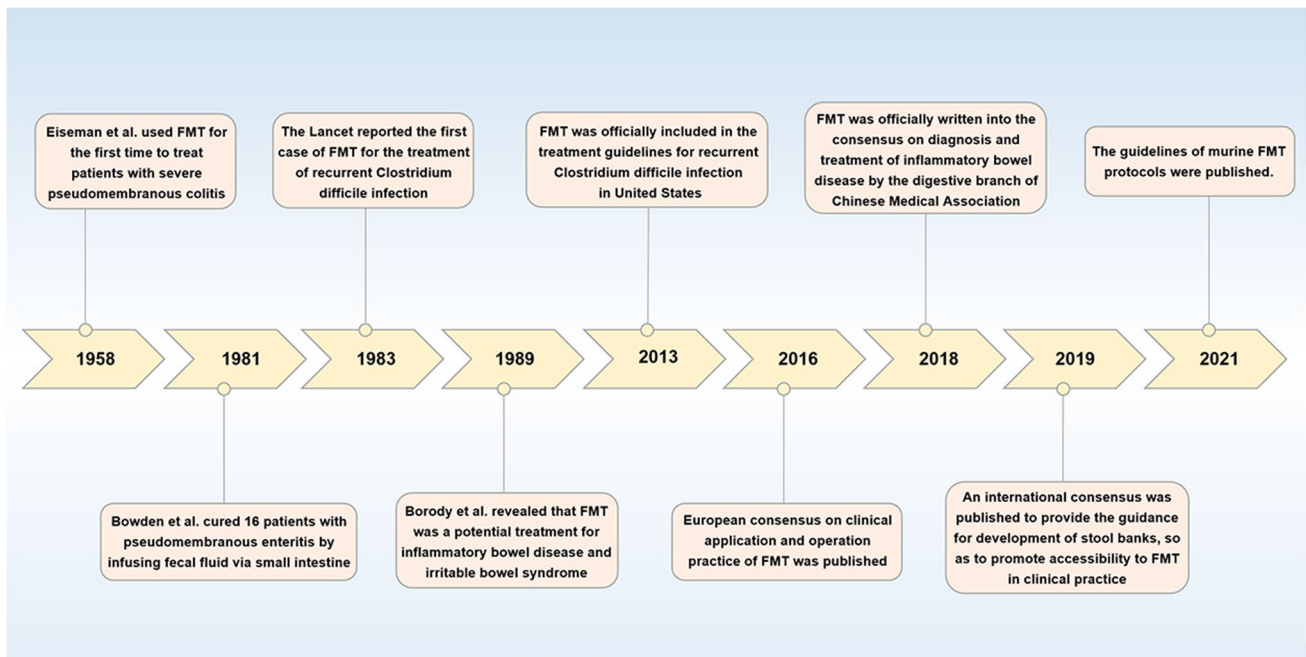
In addition, the current interventional strategies for gut microbiota in clinical and scientific research stages mainly include the antibiotic application, dietary adjustment, supplementation of probiotics and prebiotics, fecal microbiota transplantation (FMT), and so on [25–28]. Therein, the FMT, as a novel “organ transplantation” technique, has gradually attracted the close attention of researchers. Specifically, the core meaning of FMT is to extract the beneficial microbiota from the feces of healthy people or animals after the screening, centrifugation, filtration and other steps, and transplant it into the gastrointestinal tract of corresponding recipients, so that the microbiota in the recipients can be reshaped and play a normal function, and further prevent or treat the diseases related to gut microbiota disorder [29]. Meanwhile, FMT can also reshape the intestinal microecology of recipients, improve the inflammatory, immune and metabolic status of the recipient's intestine, and provide

a new treatment concept and approach for various intestinal and non-intestinal diseases [30]. In a single FMT, the time for the receptors' microbiota to maintain the normalization is generally 3–6 months [31]. Since each human body has its own internal environment, the healthy microbiota system can be successfully colonized soon after FMT. However, due to the effects of its own internal environment and external interventions, partial bacterial components might slowly disappear after 3 to 6 months, and need to be transplanted again [32, 33].

To date, FMT has been gradually verified to be effective for a variety of intestinal diseases, such as irritable bowel syndrome (IBD), *Clostridium difficile* infection, colon cancer, and so on [23, 34, 35]. FMT has exhibited a cure rate of more than 90% in clinical trials for the treatment of recurrent *Clostridium difficile* infection and accompanied with less side effects. More importantly, FMT is regarded as potential life-saving “last chance” for the treatment of recurrent *Clostridium difficile* infection, which has been written into the diagnosis and treatment guidelines [23, 34]. The development history and significant time nodes of FMT since the modern times are exhibited in Fig. 1 [36–44]. Moreover, FMT has been widely explored to many metabolic and immune diseases, such as the obesity, diabetes, fatty liver, arthritis, psoriasis and osteoporosis [45–49]. Herein, combined with relevant studies and based on the relevant basic studies and clinical practice, this current review is aimed to discuss the potential mechanisms, application, therapeutic feasibility, and existing challenges of FMT on osteoporosis and propose the corresponding prospect, thus demonstrating the potentials of FMT as a therapeutic option for the osteoporosis and further provide certain reference for future researches.

## The relationship between gut microbiota and osteoporosis

Gut microbiota is a collection of the microorganisms colonized in the host intestine, including about 10 trillion bacteria, and its total gene number is 150 times that of host cells. The gut microbiota is mainly composed of dominant bacteria (obligate anaerobic bacteria) and the secondary bacteria (aerobic bacteria or facultative anaerobic bacteria), including beneficial bacteria, harmful bacteria and neutral bacteria [50–52]. Currently, the relationship between gut microbiota and bone metabolism has gradually become a research hotspot, especially in recent years, it has been verified that gut microbiota is closely associated with the reduction of bone mass and incidence of osteoporosis [53–55]. Gut microbiota might alter the relative activity of the osteoclasts and osteoblasts by affecting its intestinal metabolites, host metabolism, inflammatory and immune system, thus influencing



**Fig. 1** The development history and significant time nodes of FMT since modern times

the bone metabolism. Moreover, in a previous review, based on concept of “brain–gut–bone” axis, we have summarized the effects of gut microbiota on osteoporosis through the brain–gut bidirectional regulation, immune regulation and endocrine regulation [23].

Regarding this, in terms of animal studies, Tu et al. [56] revealed that by mimicking postmenopausal osteoporosis, estrogen deficiency may cause the various host changes, such as the impaired intestinal mucosal barrier function, imbalance of gut microbiota composition and abundance, and enhanced immune response. During this process, the metabolites of intestinal pathogenic bacteria are able to enter into the body circulation through the damaged intestinal mucosal barrier, further induce the immune response mediated by CD4 + T cells, generate various kinds of pro-osteoclastogenic cytokines (such as interleukin-1 $\beta$  (IL-1 $\beta$ ), IL-6, tumor necrosis factor- $\alpha$  (TNF- $\alpha$ )), and ultimately mediate the activation of osteoclasts and enhancement of bone resorption [57, 58]. Yan et al. [59] verified that the beneficial bacteria can ferment the dietary fiber in food to produce short-chain fatty acids (SCFAs), and SCFAs can decrease the local pH value of intestine and reduce the formation of complexes between calcium ions and phosphorus in intestine, thus promoting the calcium absorption and regulating the bone metabolism. Sjögren et al. [60] indicated that the expression of TNF- $\alpha$  and IL-6 in the germ-free (GF) mice was reduced, the number of CD4 + T cells was less than that of normal mice, and the number of osteoclasts in the bones was reduced and the bone quality was better

than that of the normal mice, suggesting that gut microbiota may be involved in the osteoclastogenesis by regulating the immune system and local inflammatory responses. Huo et al. [61] also suggested that the GF mice were able to exhibit the strong activity of hypothalamic pituitary adrenal (HPA) axis under mild stress, and the function of HPA axis could return to normal after supplementing an appropriate number of probiotics and prebiotics. Meanwhile, gut microbiota could also directly or indirectly regulate a variety of hormones or chemicals (such as 5-hydroxytryptamine (5-HT), epinephrine (E), vasoactive intestinal polypeptide (VIP),  $\gamma$ -aminobutyrate (GABA), norepinephrine (NE), and so on), and these substances will influence distal organs and body functions after being released into blood, thus speculating that gut microbiota may regulate bone metabolism through neuroendocrine metabolic pathways [62, 63].

In terms of the clinical researches, Fuhrman et al. [64] conducted a study on 60 randomly selected healthy postmenopausal women, and results indicated that the fecal microbial diversity and relative abundance of *Clostridium* and *Bacillus* were positively correlated with urinary estrogen metabolites, and the diversity of gut microbiota increased with proportion of hydroxylated estrogen metabolites in the urine. Several studies have indicated that prebiotics, such as inulin, *Fructooligosaccharides* and *Galactooligosaccharides*, can contribute to the increase of the number of probiotics (*Bifidobacteria*, *Lactobacillus* and *Clostridium butyricum*), promote the secretion of SCFAs, reduce the intestinal PH value, improve the solubility of calcium in

intestinal cavity, increase the calcium absorption by body, and ultimately enhance the contents and density of bone minerals in individuals [65, 66]. Moreover, Abrams et al. [67] revealed in a population-based study that supplementing *Frucooligosaccharides* for a duration of 9 days to 1 year could enhance the intestinal calcium absorption, and the continued supplementation of it for more than 1 year might enable to prevent bone loss. Similarly, van den Heuvel et al. [68] revealed in a previous study that supplementation of *Transgalactooligosaccharides* could increase the intestinal calcium absorption in postmenopausal women, so as to further enhance the bone mass. In a double-blind, placebo-controlled, randomized, and multi-center study, Jones et al. [69] revealed that supplementing *Lactobacillus reuteri* was associated with enhancement of the contents of serum 25 hydroxyvitamin D in the body, thereby increasing the synthesis of vitamin D and further preventing osteoporosis.

Collectively, more and more studies have gradually verified that gut microbiota can regulate the metabolism, immunity and inflammation of the body. However, with the enhancement of age, alteration of lifestyle, and alteration of physiological and immune characteristics, the intestinal mucosal barrier damage induced by aging and estrogen deficiency may lead to a surge of inflammatory reaction in the intestine and circulation, which could further contribute to the ecological imbalance of gut microbiota. On the contrary, the imbalance of gut microbiota may also increase the intestinal permeability, thereby enhancing the leakage of gut microbiota and its metabolites, and such positive feedback process might enhance with aging. Meanwhile, it can also act as a significant regulator of bone metabolism, affect the generation of osteoclasts, and play a critical role in regulation of bone. The emerging studies on the regulation of bone metabolism by probiotics and prebiotics have also suggested that the increase of beneficial bacteria can promote the enhancement of bone mass and reverse the bone loss, which can be regarded as a target for the prevention and treatment of osteoporosis. However, the researches on the relationship between gut microbiota and osteoporosis are still in the initial stage, and more studies are needed to further clarify its deep-level mechanisms in future.

### The potential regulation of FMT on bone metabolism

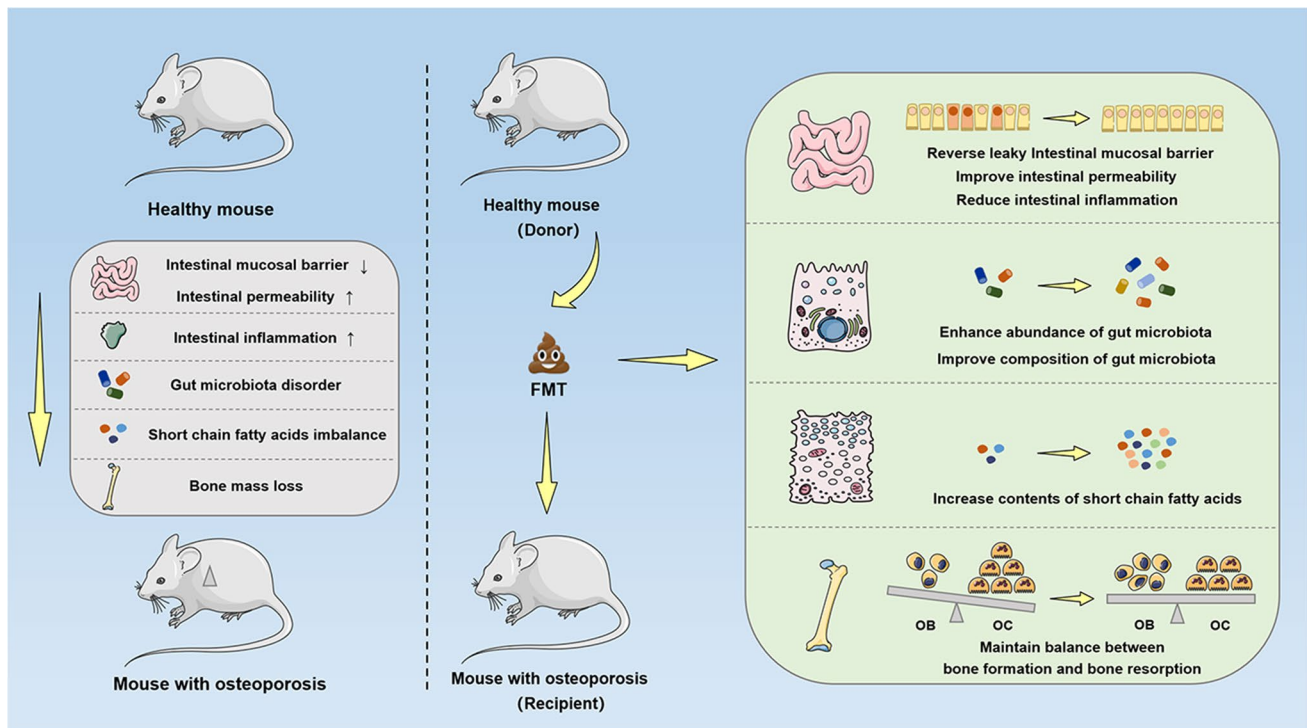
On the one hand, bone remodeling is a dynamic and balanced process, which is mainly composed of the osteoblast-mediated bone formation and the osteoclast-mediated bone resorption [70]. On the other hand, the underlying mechanisms of FMT mainly lie in reconstruction of normal intestinal micro-ecology, which participates in the regulation of bone metabolism by correcting gut microbiota disorder,

reversing intestinal metabolites (SCFAs, indole derivatives, vitamins, cholic acids, polyamines) imbalance, repairing the intestinal mucosal barrier, regulating the immune response, and improving the intestinal permeability. Meanwhile, different from the application of probiotics, prebiotics and other single microbial targets, FMT maintains the integrity of whole gut microbiota and metabolites during the overall process, which could retain the original function of gut microbiota to the greatest extent, significantly improve gut microbiota-related disorder, and restore the homeostasis of intestinal microenvironment more quickly and efficiently. Moreover, given that the diseases-induced physiological disorders may alter the composition and abundance of gut microbiota, and conversely, gut microbiota disorder can also trigger or exacerbate diseases. Hence, the experiment of transferring host phenotype by the means of FMT is essential in researches of disease pathogenesis. Herein, the potential regulatory mechanisms involving the FMT on bone metabolism are represented as follows, and the Fig. 2 exhibits the potential mechanism diagrams.

### The involvement of intestinal metabolites

Accumulating research evidence indicates that a variety of intestinal metabolites, including SCFAs, vitamins, 5-HT, cholic acids, polyamines and indole derivatives, are involved in the regulation of bone metabolism. Therein, SCFAs are mainly produced by the bacterial fermentation of amino acids or indigestible carbohydrates in the colon, and its specific components include acetic acid, propionic acid, butyric acid, and so on, which is one of the most vital metabolites of intestinal beneficial bacteria in the process of FMT [71–73]. Furthermore, after performing FMT, the SCFAs may influence the process of bone metabolism via various mechanisms, including inhibiting inflammatory response, improving intestinal calcium absorption, promoting osteoblast differentiation via regulatory T cells (Tregs), directly inhibiting osteoclast differentiation, and so on [20, 74–76]. Yan et al. [59] also highlighted in a previous study that SCFAs indirectly participate in the protection of bone mass by affecting the level of serum insulin-like growth factor-1 (IGF-1). Hence, it is recognized that the implementation of FMT may enhance the contents of SCFAs, so as to further participate in the regulation of bone metabolism through various different mechanisms, maintain the balance between bone formation and bone resorption, and effectively obtain the purposes of protecting bone mass and preventing osteoporosis.

Next, in addition to the SCFAs, other intestinal metabolites produced after FMT may also be involved in the regulation of bone metabolism. For example, 5-HT is able to be produced by the culture of *Corynebacterium*, *Streptococcus*, and *Escherichia coli* [77–79]. As a neurotransmitter



**Fig. 2** The potential mechanism diagrams of regulatory mechanisms involving FMT on bone metabolism. Based on the gut–bone axis, the underlying mechanisms of FMT mainly lie in reconstruction of normal intestinal micro-ecology, which could participate in the regulation of bone metabolism by repairing intestinal mucosal barrier,

regulating immune response, improving intestinal permeability, correcting gut microbiota disorder, and reversing intestinal metabolites imbalance. *FMT* fecal microbiota transplantation, *OB* osteoblast, *OC* osteoclast

produced by intestinal chromaffin cells, more than 90% of 5-HT is synthesized by the intestine, and it can be targeted by osteoblasts and participates in regulation of osteoblast proliferation via the signal pathways, such as Htr1b, Htr2a, Htr2b, and so on [80]. The vitamins (including vitamin C, vitamin D, vitamin K) are also significant for maintaining the balance between bone formation and bone resorption [81, 82]. As a common intestinal mucosal growth factor, polyamine is also accompanied with the process of FMT and verified to be conducive to protecting bone mass [83]. Thus, it is recognized that the FMT might regard the intestinal metabolites as one of the bridges to play a role in balancing bone formation and bone resorption, so as to further protect the bone mass and prevent osteoporosis.

### The involvement of intestinal mucosal barrier

Intestinal mucosal barrier is a physical barrier and the first line of defense against intestinal pathogens. It can not only selectively absorb the water and nutrients, but also restrict the invasion of exogenous harmful antigens into the host [84]. Meanwhile, the intestinal mucosal barrier is also an interface between the host and gut microbiota, which is a significant link in the occurrence and development of

osteoporosis mediated by gut microbiota [85, 86]. With regard to this, Schepper et al. [87] suggested that the intervention of antibiotics on normal mice may cause gut microbiota disorder, damaged intestinal mucosal barrier and enhancement of intestinal permeability, which further caused the bone loss and induced osteoporosis. Chen et al. [88] also indicated that the supplementation of lactulose could reverse ovariectomy (OVX)-induced bone loss in mice by improving intestinal mucosal barrier function, reducing inflammatory response, and optimizing composition and abundance of gut microbiota and SCFAs. Li et al. [89] also revealed that puerarin modulated the gut microbiota disorder to elicit the anti-osteoporosis effects in OVX-induced rats through improving bone microenvironment through repairing the intestinal mucosal integrity and regulating the SCFAs levels. Li et al. [90] demonstrated that tuna bone powder could ameliorate the glucocorticoid-induced osteoporosis in mice by blocking pro-inflammatory cytokines, coregulating signaling pathways, repairing intestinal mucosal barrier and modulating gut microbiota. Wang et al. [91] indicated that the supplementation of *Prevotella histicola* could prevent OVX-induced bone loss in mice through improving intestinal mucosal barrier function, reducing intestinal inflammatory response, and optimizing composition and abundance

of gut microbiota. More importantly, Ma et al. [46] also reported that using young healthy rats as donors and providing the feces as materials to conduct the FMT might alleviate the senile osteoporosis in the aged rats through enhancing the composition and abundance of gut microbiota and improving intestinal mucosal barrier function. Wang et al. [92] suggested that the dysbiosis of gut microbiota by transferring the feces from senile osteoporotic rats to young rats can induce osteoporosis, and the changed gut microbiota and impaired intestinal mucosal barrier contributed to the pathogenesis of osteoporosis. Zhang et al. [93] also showed that FMT plays an active role in remodeling the gut microbiota and improving the bone loss in OVX-induced mice with osteoporosis. Specifically, by correcting imbalance of gut microbiota, improving the level of SCFAs, optimizing intestinal permeability and inhibiting release of pro-inflammatory cytokines, FMT inhibited the excessive generation of osteoclasts and obtained the balance between bone formation and bone absorption, thus ameliorating the bone loss in OVX-induced mice with osteoporosis. Notably, these studies provide the direct and strong evidence that FMT is able to improve the bone mass and prevent the osteoporosis.

### The involvement of immune regulation

A variety of immune factors and immune cells in the intestine can participate in the regulation of bone metabolism by maintaining the balance between the osteoblasts and osteoclasts [94]. Meanwhile, the gut microbiota also affects the production of cytokines and differentiation of lymphocytes in the lamina propria of intestinal mucosa, especially the differentiation of CD4+T cells to T helper cells 17 (Th17 cells) and Treg cells [95]. In a previous study, Atarashi et al. [96] showed that the transplantation of *Clostridium IV* and *XIVa* isolated from the normal mice into GF mice could enhance the number of systemic and lamina propria Treg cells in vivo. Moreover, SCFAs were also involved in inducing the differentiation of Treg cells, and Treg cells can further influence osteoclastogenesis by secreting the IL-4, IL-10 and transforming growth factor- $\beta$  (TGF- $\beta$ ). Moreover, the estrogen could also activate the Treg cells and enhance the production of TGF- $\beta$ , or indirectly inhibit the formation of osteoclasts and prevent postmenopausal osteoporosis by inhibiting the differentiation of Th17 cells and decreasing production of TNF- $\alpha$  and receptor activator of nuclear factor- $\kappa$  B ligand (RANKL) through the estrogen receptor [97]. In addition, Goto et al. [98] revealed that the transplantation of segmental filamentous bacteria (SFB) into GF mice increased the number of Th17 cells, resulting in enhanced levels of IL-1 $\beta$ , IL-17 and TNF- $\alpha$ , inducing the expression of RANKL, and thereby promoting the formation of osteoclasts and inducing the bone resorption. As a kind of intestinal colonization bacterium, SFB could also induce the

serum amyloid A protein (SAA) in the terminal ileum, which can further promote the differentiation of Th17 cells [99].

### The involvement of endocrine regulation

As a virtual endocrine organ, the gut microbiota can directly produce or regulate a variety of hormonal chemicals, which are released into blood to regulate the functions of distant organs and systems [100]. A variety of hormones produced by gut microbiota are also the neurotransmitters of the central nervous system (CNS). For example, the GABA produced by *Lactobacillus* is one of the most significant inhibitory transmitters in the brain, while the monoamines (such as NE and dopamine) produced by the *Bacillus phlei* are excitatory transmitters, which play an indirect role in complex endocrine network, especially on HPA axis, resulting in close relationship between the secretion of estrogen, glucocorticoid and gut microbiota [101, 102]. In a FMT experiment, Schepper et al. [103] transplanted the gut microbiota of the glucocorticoid-induced osteoporosis mice to the mice treated with antibiotics for one week, and then observed that the bone mass of recipient mice decreased, indicating that effects of glucocorticoid on bone mass can be transferred via FMT. Thus, the potential therapeutic feasibility and effects of FMT on osteoporosis and its corresponding mechanisms have broad research prospects and application space. In future, more and more in-depth explorations are still needed in terms of the basic researches and clinical trials.

### The current operative approaches and security of FMT

The normal gut microbiota tends to be in a harmonious and stable state. However, when the number of certain bacteria increases, decreases or lacks, the body will exhibit corresponding pathological state [104]. FMT is usually applied to relieve or treat the gut microbiota imbalance-related diseases, mainly by centrifuging and purifying feces collected from the healthy patients to obtain the functional bacteria, and then transferred to recipient's intestinal tract via upper gastrointestinal pathway, middle gastrointestinal pathway and lower gastrointestinal pathway, thus reconstructing the stable intestinal micro-ecological environment, relieving the relevant symptoms and treating the diseases [105–107]. With regard to this, whether FMT can obtain the expected efficacy depends on several aspects, mainly including the extraction method and obtained components of fecal bacteria, the filtration steps and preservation methods of bacterial liquids, the dose, frequency and location of fecal bacteria infusion, the operative approaches, and so on [108, 109]. Therein, the aspect of operative approaches of FMT plays a significant role in the efficacy of FMT [110]. Herein, the

current operative approaches and the security of FMT are shown as follow, and Fig. 3 exhibits the corresponding illustration. Moreover, the main mode of operation, advantages and drawbacks of each operative approach are also summarized in Table 1.

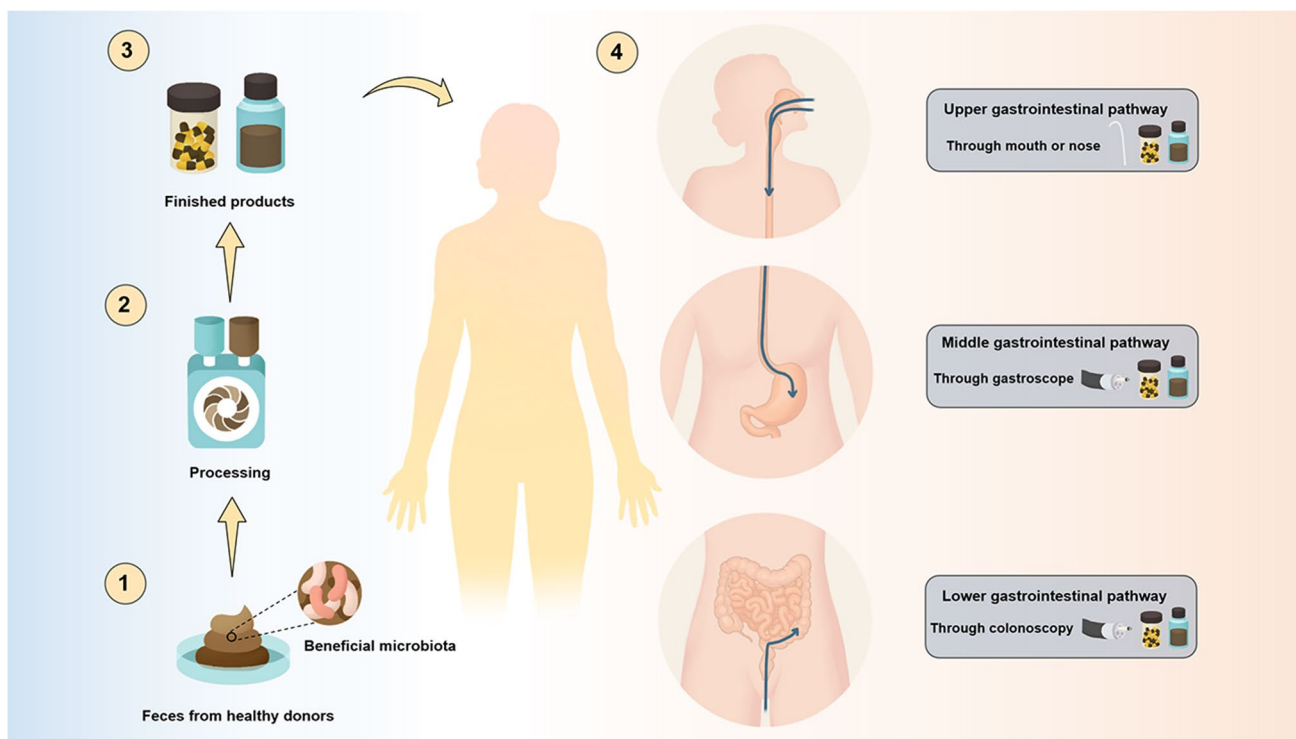
### Upper gastrointestinal pathway

Among the routes of the upper gastrointestinal pathway, the oral administration and nasogastric tubes are widely used, which are easy to be performed and have relatively low overall risk. However, during this process, the asphyxia may be caused by bacterial fluid reflux or suction, and if the body itself has obstruction, the transplanted bacterial fluid may not reach the intestinal tract accurately. Therein, the oral approaches mainly refer to encapsulated fecal bacteria materials, which is prepared by mixing the extracted fecal bacteria with a cryoprotectant (mainly glycerol), followed by multiple packaging to protect the FMT materials from gastrointestinal environment [111, 112]. Meanwhile, the operators could further select the material of capsule shell according to the expected release position. Currently, the commercially available capsule shells are often targeted at the release of stomach or colon, and are formulated to ensure the survival rate and the colonization of bacteria along with the gastrointestinal tract [113]. Collectively, the FMT through the upper gastrointestinal pathway has the

advantages of no need for expensive instruments, less damage to patients after repeated use, and is well tolerated by patients. This pathway is suitable for the patients who are not able to tolerate the use of naso-intestinal tube and gastroscopic transplantation, and the patients who require the oral customized bacteria. Moreover, during the process of making capsules, fungi, parasites, viruses and partial inflammatory mediators that may exist in asymptomatic donors can also be removed to reduce potential probability of diseases transmission [114, 115].

### Middle gastrointestinal pathway

Middle gastrointestinal pathway mainly refers to the operations of naso-intestinal transplantation and trans-endoscopic enteral tubing (TET), and TET is able to realize the whole intestinal administration [116]. Therein, the naso-intestinal tubes are stretched and straightened through the guide wire, which can pass through the pylorus automatically under the condition of normal gastrointestinal motility and further reduce the bacterial translocation when used for FMT. With regard to this, in a randomized controlled trial, van Nood et al. [117] reported that the FMT through the naso-intestinal tubes was superior to the use of vancomycin alone in the treatment of *Clostridium difficile* infection, while the patients in FMT group also experienced the adverse events, such as diarrhea, cramp and constipation. Moreover, TET



**Fig. 3** The preparation process and current main operative approaches of FMT

**Table 1** The main mode of operation, advantages and drawbacks of operative approaches for FMT

Approaches	Main mode of operation	Advantages	Drawbacks
Upper gastrointestinal pathway	<ol style="list-style-type: none"> <li>1. Oral administration</li> <li>2. Nasogastric tubes</li> </ol>	<ol style="list-style-type: none"> <li>1. Easy to perform and have relatively low overall risk</li> <li>2. Protect the FMT materials from the gastrointestinal environment</li> <li>3. No need for expensive instruments</li> <li>4. Less damage to patients after repeated use</li> <li>5. Well tolerated by patients, especially for the elderly patients</li> <li>6. Reduce the potential probability of diseases transmission</li> </ol>	<ol style="list-style-type: none"> <li>1. Asphyxia may be caused by the bacterial fluid reflux or suction</li> <li>2. If the human body itself has an obstruction, the transplanted bacterial fluid may not reach targeted intestinal tract accurately</li> </ol>
Middle gastrointestinal pathway	<ol style="list-style-type: none"> <li>1. Naso-intestinal tubes</li> <li>2. Mid-gastrointestinal pathway-mediated TET</li> </ol>	<ol style="list-style-type: none"> <li>1. Reduce the bacterial translocation</li> <li>2. Improve the efficacy of FMT</li> <li>3. The mid-gastrointestinal pathway-mediated TET is convenient and easy to maintain</li> <li>4. The mid-gastrointestinal pathway-mediated TET does not require further confirmation of the intestinal position by other medical instruments</li> </ol>	<ol style="list-style-type: none"> <li>1. The incidence of adverse events was relatively high, including diarrhea, cramp and constipation, and so on</li> <li>2. It is expensive for the patients who require repeated FMT</li> <li>3. It is accompanied with high risk of operation</li> </ol>
Lower gastrointestinal pathway	<ol style="list-style-type: none"> <li>1. Enema</li> <li>2. Colonoscopy</li> <li>3. Colonic pathway-mediated TET</li> </ol>	<ol style="list-style-type: none"> <li>1. Enema is a less invasive and relatively simple operation</li> <li>2. It can completely display the situation of colon and biopsy suspicious tissues, which is conducive for the diseases staging</li> <li>3. It allows operators to directly assess the intestinal inflammation and infuse a sufficient amount of donor fecal bacteria at the appropriate site</li> <li>4. The gut microbiota can be accurately transplanted into the influenced intestinal segment, and the bacteria can further be retained in the targeted intestinal segment</li> <li>5. Sufficient amount of donor fecal bacteria can be accurately infused, which can improve the treatment efficiency</li> </ol>	<ol style="list-style-type: none"> <li>1. Enema is accompanied with the risk of the bacteria of FMT is not able to reach the colon and splenic flexure</li> <li>2. The incidence of adverse events was relatively high, including aggravating intestinal reactions and causing intestinal perforation</li> <li>3. The patients with severe conditions may not able to tolerate colonoscopy transplantation or relevant anesthesia procedures</li> <li>4. It is expensive for the patients who require repeated FMT</li> </ol>

FMT fecal microbiota transplantation, TET trans-endoscopic enteral tubing



specifically refers to the endoscopic-assisted implantation of a fixed tube and the fixation in the deep part of intestinal tract, while the external end communicates with the outside along the intestinal tract. TET mainly includes the types of colonic pathway-mediated TET and mid-gastrointestinal pathway-mediated TET. Colonic pathway-mediated TET requires the application of colonoscopy, and it is expensive to use this route for repeated FMT. In contrast, mid-gastrointestinal pathway-mediated TET is more convenient and easier to maintain [118]. Since mid-gastrointestinal pathway-mediated TET does not require further confirmation of intestinal position by X-rays or other medical instruments after endoscopic surgery, for the patients who are not able to undergo bowel preparation for colonoscopy, or who require both the repeated FMT and enteral nutrition, the mid-gastrointestinal pathway-mediated TET is also the primary option [119].

### Lower gastrointestinal pathway

Lower gastrointestinal pathway mainly refers to operations of enema, colonoscopy, and colonic pathway-mediated TET. Therein, the enema is a less invasive and relatively simple operation. Enema is well tolerated by patients and does not require the expensive instruments, which effectively decreases the risk of operation. However, this approach requires the retention of infused fecal suspension for a relatively long time, the patient remains supine to reduce the fecal excretion, while the repeated enemas are easy to be accepted by patients [120]. In addition, the proposition that whether the bacteria of FMT can be retained in intestinal segment by enema is still unclear, and this approach is also accompanied with the risk of not reaching the colon and splenic flexure, which requires multiple perfusions to obtain the curative effect and longer operation time to make up for the defect of the low gut microbiota retention rate. Meanwhile, due to the retention of bacterial fluid, this approach may not be suitable for the patients with anal sphincter relaxation or urinary incontinence [121]. Moreover, in terms of the colonoscopy and colonic pathway-mediated TET, it is equipped with a variety of advantages, mainly including: (1) It is able to completely display the situation of colon and biopsy suspicious tissues, which is conducive for the diseases identification and staging; (2) It allows operators to directly assess the intestinal inflammation and infuse a sufficient amount of donor fecal bacteria at appropriate sites; (3) Gut microbiota can be accurately transplanted into the influenced intestinal segment, and bacteria can further be retained in targeted intestinal segment; (4) Sufficient amount of donor fecal bacteria could be accurately infused, which can improve the treatment efficiency to a certain extent. However, these operations are also accompanied by partial adverse events, including aggravating intestinal reactions,

causing intestinal perforation, and patients with severe conditions may not be able to tolerate colonoscopy transplantation or relevant anesthesia procedures [122, 123].

### The clinical application prospect and existing challenges of FMT on osteoporosis

To date, more and more patients around the world have benefited from FMT, and the medical community of numerous countries is also actively working to promote the wider clinical application of FMT [124]. Based on the proven regulatory effects of gut microbiota on the osteoporosis, the gut microbiota disorder might enhance the risk of occurrence and progression of osteoporosis, and further normalizing the composition and abundance of gut microbiota could reconstruct the gut–bone axis and maintain the balance between bone formation and bone resorption, so as to prevent and treat the osteoporosis. Hence, FMT is regarded as a novel approach of reconstructing the gut microbiota and a novel target for the prevention and treatment of osteoporosis at the present and future stages.

In addition, although FMT has become increasingly mature as a treatment method for the common gastrointestinal and metabolic diseases, there are still certain problems to be solved in the clinical prevention and treatment of osteoporosis. For one thing, the discomfort associated with current FMT approaches through traditional methods, such as nasogastric tube and naso-intestinal tube, may reduce the compliance of patients, as well as concomitant adverse events. In addition to this, the administration frequency of FMT is also inconclusive, mainly including single administration, and intermittent or continuous administration. The repeated FMT can improve the success rate of treatment to a certain extent, and the success of FMT may also depend on amount of suspension of transplanted [125, 126]. For another thing, the choice of FMT approaches might be influenced by the clinical experience of specific operators. Currently, the most suitable FMT approach has not been verified in clinical practice [127]. With regard to this, Paramsothy et al. [128] collected and sorted out the knowledge and experience of 52 gastroenterologists on the FMT in past. Therein, 37% of them regarded that the most appropriate FMT approach was colonoscopy-assisted FMT, 17% thought it was naso-intestinal route, 13% thought it was enema, and 8% thought it was oral capsules. Hence, it is concluded that each FMT approach has its advantages and drawbacks, and the specific FMT approach should be comprehensively selected in accordance with the type of diseases, position to obtain the effects, professional judgment of operators, and psychological acceptance of the patients. Additionally, the utilization of human-derived gut microbiota and

complications associated with severe infection are indeed a safety issue worthy of attention. Especially in the era of multi-infectious disease pandemics represented by the coronavirus disease 2019 (COVID-19), this issue is particularly noteworthy [129–131]. Several studies have showed that during the process of COVID-19 infection, the expression of intestinal angiotensin-converting enzyme 2 (ACE2) is down-regulated, resulting in the decreased secretion of antimicrobial peptides (AMPs) and improved survival of pathogens [132, 133]. With the global spread of COVID-19 pandemic, it is urgent to take preventive measures to screen FMT donors for COVID-19 to prevent the potential risk of COVID-19 transmission. Indeed, COVID-19 might be transmitted from asymptomatic donors to recipients via FMT, especially those who are negative for COVID-19 through respiratory samples but positive for stool samples [134, 135]. To address this vital issue, FMT donor screening should be rigorous and follow the guidelines from different jurisdictions around the world.

Nevertheless, although the current application of FMT in prevention and treatment of osteoporosis is still immature, to further realize the wide application of FMT, the specific solutions include: (1) Deepen the basic researches of FMT in the fields of osteoporosis and bone metabolism, thus providing a solid theoretical basis for its further clinical trials. Meanwhile, during the process of further clinical trials, gradually expand the research samples of FMT in prevention and treatment of osteoporosis, and deeply understand the relevant mechanisms on the gut–bone axis; (2) Further researches require appropriate phenotypic analysis of patients with osteoporosis and rational application of techniques, including functional imaging and deep microbial pyrosequencing. Using high-throughput sequencing techniques, comprehensively analyze the composition and abundance of gut microbiota in the patients with osteoporosis, and deeply analyze the microbiota structure and functional metabolism characteristics in specific environment, so as to provide a theoretical basis for FMT to improve osteoporosis and then conduct individualized targeted FMT therapy [136]. Hence, it is recognized that the application of precision medicine to conduct the personalized FMT for different individuals is a novel research direction on this field in future; (3) On the basis of current FMT-related researches, further explore the technique of synthetic microbiota transplantation (SMT) [137, 138]. The SMT mainly refers to the extraction of bacteria from the feces of healthy individuals, cultures it *in vitro*, and then uses conventional FMT approaches, such as oral administration and nasointestinal tube, to treat patients with osteoporosis; (4) On the basis of FMT, Zhang et al. [139] proposed the concept of washing microbiota transplantation (WMT) for the first time through intelligent separation system and strict quality control system. WMT belongs to the category of FMT, while

the fecal bacteria have been washed more strictly, which could also further promote the development of FMT to a certain extent. Moreover, various previous clinical evidence and animal studies have verified that WMT has significant clinical therapeutic value for the patients with inflammatory bowel diseases, and also significantly reduces the incidence rate of adverse events without reducing the efficacy of FMT [140]. Currently, there is still no related research on application of WMT in the prevention and treatment of osteoporosis, while its future prospect is still worthy of our expectation and belief; (5) As another indelible aspect related to the efficacy of FMT, it is also essential to further optimize the extraction method of fecal bacteria, develop the filtration steps and preservation methods of bacterial liquids, and explore more proper guidelines of doses, frequency and location of infusion; (6) Based on existing studies, it is necessary to further explore how the gut microbiota plays a critical role in human health and the related mechanisms by which FMT works. More high-quality researches still need to be promoted to screen out which patients are suitable for the FMT and whether there are definite age, gender, race, physical quality, genetic correlation, and other requirements for potential fecal bacteria donors [38]. Meanwhile, it is essential to identify the safe, effective and stable methods to implement FMT for different individuals, and design scientific randomized controlled trials and long-term follow-up planning to obtain a reliable analysis of the efficacy and safety of FMT on osteoporosis in future.

Ultimately, during the process of FMT, the therapeutic stools are collected from the healthy donors, and the success or failure of the treatment is likely to be determined by the composition of the donors' fecal microbiota. Thus, the screening and evaluation of donors are crucial. Regarding this, in combination with previous studies and relevant guidelines, the selection criteria for recruiting donors could be summarized as follows: (1) Individuals aged 12 to 30, and living and working in the same area as the recipients; (2) Preference is given to the healthy adolescents aged 18 to 25 with normal body mass index (BMI) and normal bowel habits; (3) Preference is given to those with more than two long-lived people (over 80 years old) in their family; (4) The donors should have a basic regular life schedule, proper exercise, a balanced and diversified diet, good mental health, and a regular bowel movement [141]. Moreover, the exclusion criteria mainly include: (1) Individuals who have used antibiotics in recent 3 months; (2) Individuals with a history of traumatic infections, allergies and immune disorders in the past years; (3) Individuals with the abnormal defecation habits; (4) Individuals with a suspected or definite pathogen infection; (5) Individuals with a history of cancer or living in special environments [142]. In addition, especially for the prevention and treatment of patients with osteoporosis via FMT, the selection of donors pays more attention to the screening of bone, family history of osteoporosis

and bone-related genetic history. Specifically, the donor's bone mineral density (BMD) should be within the normal range (T value > -1), have adequate dietary intake of calcium, vitamins and other bone-related nutrients, have adequate and regular sleep, regularly participate in various forms of exercise, have no history of hormone use, and so on.

## Conclusion and perspectives

With the increasing understanding of microbe–host interactions in recent years, the involvement of gut microbiota has become a novel, ingenious and non-negligible way to regulate the host health. On the basis of gut–bone axis and proven regulatory effects of gut microbiota on osteoporosis, more and more attention has been paid to the role of FMT in regulating bone metabolism and maintaining balance between bone formation and bone absorption. Indeed, the application of FMT in the prevention and treatment of osteoporosis is a novel proposition full of unknowns and challenges. It still needs to be recognized that although the osteoporosis can be divided into multiple subtypes, which subtypes of patients are eligible for the FMT treatment, and whether there are specific subtypes of patients who can obtain greater benefits in the process of FMT treatment, these questions are still worthy of further exploration and consideration in future.

Currently, although FMT has been widely applied in treatment of gastrointestinal diseases, the FMT as a potential treatment method for the osteoporosis has rarely been reported, and further large-scale animal experiments and potential human prospective studies are still needed, as well as the randomized controlled trials for exploring efficacy and incidence of adverse events of FMT, and the changes in the structure and function of gut microbiota after the FMT treatment. Notably, FMT is expected to become an effective supplement or even an alternative to the traditional drugs for the prevention and treatment of osteoporosis, and has broad research and application prospects. In the further researches, with in-depth exploration of donors screening, patients' preparation, transplantation routes, fresh storage of fecal bacterial fluid, times of transplantation, combined medication and other issues, we can fully expect that the standard, efficient and safe FMT will bring novel hope for the prevention and treatment of patients with osteoporosis in future.

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## Declarations

**Conflict of interest** All the authors declare that they have no potential conflict of interests, nor biomedical financial interests.

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