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Gut Microbiota and Dietary Modulation in Osteonecrosis: Mechanistic Insights and Food-Based Therapeutic Strategies

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ABSTRACT: Osteonecrosis is a progressive and debilitating bone disease characterized by compromised vascular supply and impaired bone remodeling, currently lacks effective therapeutic strategies. Emerging evidence implicates the gut microbiota (GM) as a pivotal regulator of bone health via the gut-bone axis. Whereas GM dysbiosis, characterized by reduced beneficial taxa (e.g., *Lactobacillus*, *Bifidobacterium*) and increased pathobionts, undermines skeletal integrity through diminished short-chain fatty acids (SCFAs) production, compromised nutrient absorption, and heightened systemic inflammation. This review synthesizes current evidence on GM-derived mechanisms in osteonecrosis, focusing on dietary and microbial metabolites such as SCFAs, polyamines, and hydrogen sulfide (H_2S) as well as immune-nutritional crosstalk. We critically examine food-based and microbiota-targeted interventions, including probiotics and prebiotics that restore microbial balance and enhance SCFA synthesis; functional foods such as polyphenols and fiber-rich diets with osteoprotective and anti-inflammatory properties; postbiotics (e.g., microbial-derived exopolysaccharides) as direct immunomodulators; and emerging therapies such as fecal microbiota transplantation (FMT) and glucagon-like peptide-1 receptor agonists (GLP-1RAs) that offer metabolic and vascular bone benefits. Additionally, the potential of GM signatures as biomarkers for early diagnosis and the development of personalized nutrition strategies are discussed. Despite promising insights, challenges remain regarding interindividual variability, lack of standardized protocols, and limited clinical translation. By integrating advances in food science, microbial ecology, and translational medicine, this review underscores the potential of GM and diet-based therapeutics as novel, noninvasive strategies to mitigate osteonecrosis progression and improve bone health outcomes.

Keywords: Osteonecrosis, Gut microbiota, Gut-bone axis, Dysbiosis, Immune-nutritional crosstalk, Functional foods

1. Introduction

Osteonecrosis, also known as avascular necrosis, is a pathological condition characterized by the death of bone tissue due to disrupted blood supply, leading to structural collapse and functional impairment. It most commonly affects weight-bearing joints such as the femoral head but can also affect the jaw (osteonecrosis of

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the jaw, ONJ), humerus, or knee (Chan & Mok, 2012; Lohiya et al., 2023). The etiology is multifactorial, involving traumatic events such as fractures or dislocations that disrupt blood flow, as well as nontraumatic risk factors such as prolonged high-dose corticosteroid use, excessive alcohol consumption, and medical conditions such as sickle cell anemia, systemic lupus erythematosus, and radiation therapy (Wells & Dunn, 2022). The pathophysiology involves ischemia-induced death of osteocytes and bone marrow cells, followed by mechanical weakening of the bone, which may fragment and collapse, precipitating secondary osteoarthritis (Vincent et al., 2022). While vascular comprise is a well-established driver of osteonecrosis, emerging evidence suggests that extra-skeletal factors, particularly those originating from GM, may influence bone metabolism and disease progression through systemic inflammatory, immune, and metabolic pathways (Lyu et al., 2023). This has opened new perspectives on the role of the gut-bone axis in the pathogenesis of osteonecrosis.

The GM, which is composed of trillions of microorganisms residing in the gastrointestinal tract, plays a critical role in regulating various aspects of host physiology, including immune responses, metabolism, and endocrine function (Khan et al., 2024). Its effects on skeletal health are mediated through the gut-bone axis, a bidirectional communication network involving immune, endocrine, and metabolic pathways (Han et al., 2024; Wei et al., 2025). Through this axis, the GM enhances calcium and magnesium absorption, which is critical for bone mineralization, and synthesizes vitamins such as vitamin K2, which support bone matrix proteins (Akbari & Rasouli-Ghahroudi, 2018). Conversely, when balanced GM is disturbed, a condition known as dysbiosis, it can adversely affect bone health. Alterations in GM composition may impair nutrient absorption, disturb immune regulation, and alter microbial metabolite production, which can contribute to bone-related disorders including osteoporosis, osteoarthritis, and osteonecrosis (Cheng & Mirzaei, 2024).

The development of GM-related bone disorders has no standardized diagnostic criteria and further large-scale clinical trials are required to confirm the efficacy of microbiota-targeted strategies (Scassellati et al., 2021). Moreover, microbiomes of individual are extremely heterogeneous, posing an additional challenge, as responses to probiotics, prebiotics and diet manipulation may vary depending on a microbial composition of each patient (Chandra et al., 2024). Diet is one of the strongest modulators of GM composition and function, with different nutrient profiles favoring distinct microbial communities and metabolic activities that influence bone health (Barrea et al., 2020). Certain dietary patterns can promote beneficial metabolite production, such as SCFAs, which support bone formation, whereas others may induce dysbiosis and contribute to bone degeneration (Serrano et al., 2021).

Given the limited exploration of GM's role in osteonecrosis, this review aims to synthesize current evidence on the interplay between GM dysbiosis and osteonecrosis pathogenesis. It also shows the prospect of therapeutic usage of targeting the gut-bone axis to improve clinical outcomes. In addition, it has also indicated the established mechanism as SCFAs and emerging mediators as polyamines and H_2S to provide comprehensive framework for understanding microbiota-based approach in prevention and treatment of osteonecrosis.

2. Gut-Derived Metabolites and Immune Pathways in Bone Health and Remodeling

2.1 SCFAs as Microbial Mediators of Bone Health

GM has emerged as a key factor of bone health due to its influence in calcium uptake and overall bone balance because the GM regulates the absorption of micronutrients and the synthesis of vital vitamins that support bone health and maintain their density like calcium, vitamin D, and magnesium (Ferrante et al., 2025; Upadhaya & Kim, 2020). It also enhances absorption of the calcium molecule either through active transcellular pathway or passive paracellular diffusion (Ciosek et al., 2021) via generation of particular metabolites, including SCFAs, and calcium is among the main nutrients that are involved in the regulation of the bone (Kokate et al., 2023).

SCFAs (e.g., acetate, propionate, and butyrate) are byproducts of gut bacteria such as *Bifidobacterium* which facilitates mineral absorption and supports gut barrier integrity (Patra et al., 2018). SCFA abundance has been correlated to increased bone mass and the prevention of bone loss *in vivo*. Moreover, they enhance the translocation of paracellular calcium across the intestinal epithelium (Patra et al., 2018; Portincasa et al., 2022; Wawrzyniak & Sulibursk a, 2021). However, they also increase the translocation of paracellular calcium across the intestinal epithelium (Thammayon et al., 2024) and are also generated by the fermentation of amino acids (Ferreira et al., 2014). In the meantime, butyrate stimulates the signaling cascade GPR109A/HCA2 in immune cells and propionate regulates gene expression by inhibiting histone deacetylases (HDAC3 and HDAC4) and thus promotes regulatory T cell (Treg) activity and intestinal homeostasis (Sanford et al., 2016). Additionally, Yan et al. (2016) demonstrated that microbiota-derived SCFAs enhance bone formation by stimulating insulin-like growth factor 1 (IGF-1), thus it demonstrated a direct mechanism through which the microbiome regulates bone metabolism.

SCFAs, particularly propionate and butyrate, have been implicated in the regulation of bone density and osteoclast activity *in vivo*. Mechanistically, propionate and butyrate alter osteoclast metabolism, thereby suppressing bone resorption (Lucas et al., 2018). Beyond modulating bone cells, they exert immunoregulatory effects, notably decreasing pro-inflammatory cytokines like TNF-α and IL-6, often found at high concentrations in osteonecrosis. By expanding regulatory T cell populations and inhibiting inflammatory pathways, SCFAs help maintain immune homeostasis in the bone marrow microenvironment, particularly under ischemic stress (e.g., osteonecrosis).

The diverse biological effects of SCFAs position them as key microbiota-derived mediators connecting gut microbial balance with skeletal health. By influencing nutrient absorption, immune regulation, and cellular signaling pathways, SCFAs show strong potential for integration into innovative therapeutic approaches for bone-related disorders. Future clinical strategies may focus on targeting SCFA-mediated mechanisms to prevent and treat osteonecrosis in high-risk populations

2.2 Polyamines as Powerhouses in Bone Formation and Resorption Control

Polyamines, including putrescine, spermidine, and spermine, are organic polycations synthesized from three major sources such as host cell biosynthesis, dietary intake, and synthesis by GM (Amin et al., 2021; Bui

et al., 2023). The genera of bacteria, including *Bifidobacterium*, *Lactobacillus*, and *Clostridium*, play an important role to intestinal polyamine pool, converting the amino acids, such as arginine and ornithine, to bioactive polyamines (C. Zhang et al., 2025). Polyamines play a pivotal role in cell proliferation and differentiation as they are regarded as important processes during bone health (C. Zhang et al., 2025; Zhang et al., 2023). They can stimulate differentiation of osteoblasts and promote bone formation stimulating osteogenic markers such as Runx2 and alkaline phosphatase required in bone mineralization (Zhang et al., 2023). The effects are mediated through AMP-activated protein kinase (AMPK) and mechanistic target of rapamycin (mTOR), which critically regulate osteoblast function and differentiation (Galasso et al., 2023). For instance, spermidine stimulates the AMPK pathway, which results in activation of osteoblast differentiation and bone formation *in vivo* (Yadav et al., 2024). Also, it stimulates macrophage differentiation and production of anti-inflammatory cytokines, such as IL-10 and transforming growth factor-beta (TGF-β) and thereby favors osteoblast functions and bone formation (Karadima et al., 2025). The exogenous administration of polyamines (spermidine, spermine) has been identified to prevent bone resorption in the animal model with TRAP-staining showing an increase in bone volume and trabecular thickness in OVX rats (Makletsova et al., 2022). Spermidine and spermine prevent osteoclast maturation and activity in the osteoclastogenesis process including inhibiting the resorptive potential by inhibiting the primary osteoclastogenic pathways and especially the RANKL/NF-kB pathway. These polyamines suppress the expression of key osteoclastogenesis-related genes (RANK, NFATc1 and TRAF6) and inhibit actin ring formation, which is essential for osteoclast-mediated bone resorption (S. Li et al., 2025; Yamamoto et al., 2012; Zhang et al., 2023). Also, reduce the expression of osteoclastogenic genes, such as RANK, NFATc1, and TRAF6, and inhibit the formation of actin rings, which are essential for osteoclast-mediated bone resorption, Moreover; polyamines suppress the activity of resorptive enzymes like cathepsin K, further inhibiting osteoclast function (S. Li et al., 2025; Zhang et al., 2023). In conditions like osteomyelitis, polyamines have also shown potential in reducing the severity of bone infection, as demonstrated by studies on obese/type 2 diabetic mice. Supplementation with prebiotics such as oligofructose led to increased polyamine levels and a reduction in osteomyelitis severity, suggesting polyamines mediate some bone-protective effects of dietary fiber (Zikou et al., 2024). **Collectively, polyamines exert dual regulatory effects by promoting bone formation and inhibiting resorption, positioning them as promising therapeutic agents for skeletal disorders such as osteoporosis, osteomyelitis, and potentially osteonecrosis.**

2.3 Hydrogen Sulfide as a Silent Regulator of Bone Remodeling and Health

Hydrogen sulfide (H_2S), a gaseous signaling molecule, plays a multifaceted role in bone remodeling, modulating both osteoblast and osteoclast activity. H_2S synthesized endogenously by enzymes such as cystathionine γ-lyase (CSE) and cystathionine β-synthase, which are involved in sulfur amino acid metabolism (Liu et al., 2021). The GM such as *Desulfovibrio*, *Bilophila wadsworthia*, and *Fusobacterium nucleatum* play role in regulating its systemic levels by metabolizing these amino acids (Awarun et al., 2024;

Braccia et al., 2021; Wang et al., 2023). It can also affect bone metabolism by a variety of biological mechanisms, such as by modulating the reactive oxygen species levels (ROS), which have known to interfere with the osteoblast activity and stimulating bone resorption in order to balance the bone formation and resorption processes (Xie et al., 2024), by triggering the nuclear factor erythroid 2-related factor 2 (Nrf2) signaling pathway which up regulates the level of antioxidant enzymes like heme oxygenase-1 and glutathione peroxidase (Gáll et al., 2020). Moreover, it contributes to increasing the functioning of osteoblasts by increasing their mitochondrial ATP production, which is crucial to osteoblast differentiation and mineralization (Hao et al., 2021). This is achieved by S-sulfhydration of mitochondrial protein involved in the electron transport chain including mitofusin 2, dynamin-related protein 1 which enhances the energy metabolism of cells and promotes the functions of osteoblasts (J. Liu et al., 2024).

H_2S is also essential in osteoclastogenesis because it alters the RANKL/NF- kB pathway. It inhibits the expression of receptor activator of nuclear factor kappa-B ligand (RANKL) through the inhibition of NF-kB activation, thereby preventing the differentiation of osteoclast and bone resorption (Song et al., 2024). Furthermore, it induces a post-translational modification of the RUNX2 transcription factor by sulfhydration and stimulates its osteogenic and promotes the synthesis of the bone matrix (Behera et al., 2018; Nasi et al., 2021). Also, it has been reported to modulate extracellular vesicles (EVs) released by mesenchymal stem cells (MSCs), increasing their capacity to facilitate osteogenic differentiation, and it could be used to promote bone regenerative therapies (Cheng et al., 2023). Another mechanism through which it may alleviate osteonecrosis is by inhibiting apoptosis of the osteoblasts which occurs by activating the anti-apoptotic proteins like Bcl-2 and inhibituting the expression of caspases. In addition, it enhances angiogenesis through upregulation of an essential vascular endothelial growth factor (VEGF) through hypoxia-inducible factor-1 alpha (HIF-1 alpha), which plays a key role in the revascularization of necrotic bones (Y.-Z. Wang et al., 2021). Similarly, H_2S donors (e.g. NaHS) returned bone mass, biomechanical bone health, and balanced bone turnover markers when systemically administered (Hao et al., 2021).

Together, polyamines, H_2S, and SCFAs act synergistically to support bone remodeling dynamics, positioning them as key components of the gut-bone axis. In osteonecrosis, restoring these microbial metabolites may re-establish bone regeneration. Understanding their interrelated roles offers promising insights for developing combinatorial therapeutic approaches targeting the gut–bone axis to treat osteonecrosis and other bone-related disorders.

2.4 Complex interactions among the GM, immunity, and bone health

The interplay between GM, the immune system, and bone metabolism is dynamic and context-dependent. In the field of osteoimmunology, immune cells, and their signaling molecules regulate osteoclastogenesis, osteoblast differentiation, and bone resorption (Xu et al., 2017).

A study by Sjögren et al. (2012) established a direct connection between GM and bone physiology, reporting that germ-free (GF) mice exhibited significantly higher bone mass compared to conventionally raised (CONV-R) mice. The observed differences are likely mediated by immunological mechanisms;

absence of microbial colonization results in reduced secretion of pro-inflammatory cytokines, lower counts of $CD4^+$ T cells, and fewer osteoclast precursors in the bone marrow compartment. Restoration of a balanced microbiota in GF or dysbiotic hosts can reverse these immune alterations, thereby supporting skeletal health. Evidence indicates that probiotic strains such as *Lactobacillus acidophilus* and *Bacillus clausii* enhance regulatory T cell populations, attenuate Th17-associated inflammatory responses, and limit osteoclast formation (Dar, Pal, et al., 2018).

The increased bone mass observed in GF mice and the bone-protective effects of microbiota restoration can be reconciled by considering the context-dependent nature of host-microbe interaction. In GF mice, the increased bone mass is a consequence of an underdeveloped immune system, lacking antigenic stimulation. The resulting suppression of immune signaling in this state constrains osteoclast development and reduces the rate of bone resorption. However, this immunological pattern reflects neither a fully competent nor an adaptable immune-bone axis and therefore may not accurately represent physiological bone health (D'Amelio & Sassi, 2017). In contrast, microbiota restoration in dysbiotic or disease contexts introduces beneficial microbial taxa, such as *Lactobacillus*, *Bifidobacterium*, and certain *Clostridium* species, which modulate immune responses towards a bone-protective phenotype (Foley et al., 2020; Ticinesi et al., 2025). These microbes upregulate anti-inflammatory responses and suppress pro-inflammatory cytokines which are typically elevated in dysbiosis and lead to osteoclastogenesis. Thus, the goal of GM restoration is not merely to return to a "conventional" microbiome, but to establish a health-associated microbial profile that actively suppresses pathological inflammation, promotes regulatory immune responses, and supports bone remodeling and health (Bhardwaj et al., 2021; Jensen et al., 2022).

Among immune cell populations regulated by the GM, Th17 cells are key mediators of bone metabolism. These $CD4^+$ T cell subsets secrete IL-17 and IL-22, cytokines central to innate immunity and inflammation. In the gut, segmented filamentous bacteria (SFB) promote Th17 differentiation, thereby enhancing IL-17–driven signaling (Goto et al., 2014). IL-17 is a primary osteoclastogenic cytokine that promotes bone resorption through upregulation of RANKL in osteoblasts and stromal cells (Li et al., 2019). Moreover, intestinal filamentous bacteria stimulate the production of IFN-γ and IL-17, cytokines that promote bone formation and protect against osteoporosis, which also observed in OVX mice, suggesting that microbiota modulation may represent a potential therapeutic strategy (Duque et al., 2011).

Unlike the Th17 cells, Tregs have a bone protective effect and are important to the regulation of systemic immune balance and bone health. Notably, the CD4+FOXP3+ Treg subsets are highly reliant on the type of GM, and the microbial-immune cross-talks are of remarkable importance in bone control (Atarashi et al., 2011). The undifferentiated progenitor cells of Treg populations have subsequently been demonstrated to be increased by certain species of *Clostridium*, lowering systemic immune responses, and protecting the skeleton against inflammation (Goto et al., 2014). Tregs mediate their bone-protective function by inhibiting osteoclast differentiation and activity, largely through CTLA-4-mediated downregulation of RANKL expression. The same effect of protection is observed with probiotic intervention; the use of *Bacillus clausii* improves the

production of Treg and bone formation and suppresses bone resorption in OVX mouse models, suggesting the promising therapeutic effect of this probiotic (Dar, Pal, et al., 2018). Also, *Lactobacillus acidophilus* enhances the maintenance of Th17/Treg balance, which inhibits osteoclastogenesis and bone resorption and reinforces the indispensability of Tregs in terms of their relevance in bone metabolism and immune homeostasis (Dar, Shukla, et al., 2018). Besides the control of T-cells, the GM also has an impact on B-cells, which are also involved in the homeostasis through the control of bone resorption, by the release of osteoprotegerin (OPG), which is an analog of receptor (RANKL), thus suppressing differentiation of osteoclast and bone resorption (Zhang et al., 2018). The GM hence controls the innate and adaptive immune cell functions that control the re-modeling of the skeleton.

Certain bacteria in the gut, including *Fusobacterium nucleatum* and *Bacteroides fragilis*, can activate the Wnt/β-catenin pathway (Rubinstein et al., 2013). Since a lack of β-catenin was linked to impaired bone formation, this indicates that the GM composition provides a direct effect on bone development and bone mass regulation (Gregson & Duncan, 2020). In addition to that, *Lactobacillus rhamnosus* GG (LGG) has been demonstrated to enhance bone anabolism through CD8+T cell Wnt10b control by Treg, further confirming the relationship between GM, immune activation, and bone homoeostasis (Tyagi et al., 2018).

3. The role of gut dysbiosis in osteonecrosis pathogenesis

3.1 Intestinal barrier dysfunction and systemic inflammation

The intestinal epithelial barrier is a fundamental component that preserves the gut integrity. This barrier contains a monolayer of epithelial cells connected by tight junction proteins that provide a selective permeability of the barrier and do not allow pathogens and toxins into the circulation and supporting the intestinal absorption of nutrients (Gieryńska et al., 2022). Dysbiosis characterized by reduced diversity and the reduction of beneficial bacteria, disrupts this barrier, leading to increased gut permeability, commonly referred to as "leaky gut" (Chae et al., 2024). It is related to a reduced production of SCFAs and other metabolites vital to the tight junction maintenance because of a reduction in beneficial bacteria including *Bifidobacterium* as well as *Lactobacillus* (N. Wang et al., 2021). Dysbiosis-induced *Bifidobacterium* depletion impairs nutrient absorption and elevates systemic inflammation, promoting bone resorption and reducing bone mineral density. As shown in **Figure 1A**, when the barrier is compromised, harmful substances such as Lipopolyscarride (LPS), which are components of the outer membrane of gram-negative bacteria, can translocate from the gut lumen into the systemic circulation (Ulluwishewa et al., 2011). Osteonecrosis can be exacerbated by systemic inflammation resulting from intestinal barrier disruption. After LPS has entered the blood, it also becomes a potent endotoxin that stimulates the immune system by toll-like receptors (TLRs) on immune cells and triggers the release of proinflammatory cytokines, including interleukin-6 (IL-6), tumor necrosis factor-alpha (TNF- α), and interleukin-1 (IL-1) (Hou et al., 2013). They are important in bone metabolism, IL-6 enhances osteoclastogenesis, bone resorption and reduction of bone density (Di Benedetto et al., 2013); TNF-α increases osteoclast differentiation, and reduces bone density (Xu et al., 2023); and IL-1 increases bone resorption and osteoclast activity by stimulating the expression of RANKL that promotes the

differentiation of osteoclasts (Souza & Lerner, 2013). Conversely, the overgrowth of harmful bacteria such as *Bacteroides* and *Dialister* is associated with increased levels of LPS and systemic inflammation. This disruption can exacerbate intestinal inflammation and impair nutrient absorption, leading to deficiencies in calcium and vitamin D, which contribute to reduced bone mineral density (He et al., 2020; Wei et al., 2021; Xu et al., 2020). Since osteonecrosis is closely associated with bone resorption caused by inflammation, immune activation caused systemically by the translocation of LPS could be pivotal in disease progression. Chronic LPS exposure can disrupt bone remodeling by shifting the balance toward osteoclast activation, ultimately damaging bone integrity and increasing susceptibility to osteonecrosis.

3.2 Oxidative stress

Dysbiosis can significantly increase oxidative stress by promoting the generation of ROS such as superoxide anion radicals ($O_2\bullet^-$), hydrogen peroxide (H_2O_2), and hydroxyl radicals (•OH), are highly reactive molecules that can damage cellular components such as lipids, proteins, and DNA (Singh et al., 2022). This imbalance increases ROS production while simultaneously compromising cellular antioxidant defenses, which results in detrimental effects on bone health (**Figure 1B)**. Elevated ROS levels induce cellular dysfunction across osteoblasts, osteocytes, and osteoclasts, leading to the creation of a pathological bone microenvironment (Tao et al., 2020). The oxidative environment particularly impairs osteoblast function, diminishing their capacity for bone formation and matrix synthesis. Concurrently, it abnormally activates osteoclast-mediated bone resorption. This dual disturbance of the bone remodeling process leads to progressive bone loss and reduced mineral density (Zhu et al., 2022). These pathological effects are particularly severe in osteonecrosis, where oxidative damage synergizes with vascular impairment to accelerate bone deterioration. GM dysbiosis can promote excessive ROS production (H. Li et al., 2024). Notably, certain pathogenic bacteria generate ROS as metabolic byproducts, contributing to both intestinal and systemic oxidative stress (Marciano & Vajro, 2017). The consequences of ROS overproduction extend beyond the gut microenvironment, creating systemic conditions that predispose to osteonecrosis. Dysbiosis may simultaneously compromise the microbiota's capacity to generate antioxidant compounds, disrupting redox homeostasis. This dual dysfunction; involving both the depletion of beneficial antioxidant producing bacteria and the proliferation of pro-oxidant pathogens begins a reinforcing cycle of oxidative stress. Such redox imbalance contributes to osteonecrosis pathogenesis through multiple mechanisms as impairing osteocyte viability, disrupting bone remodeling processes, and promoting tissue necrosis (Kubo et al., 2021).

ROS can damage osteoblast, the cells involved in bone synthesis, which results in compromised bone formation and regeneration (Domazetovic et al., 2017). Further, oxidative stress has the ability to promote the activity of the osteoclasts leading to faster resorption and loss of bone. These processes can aggregate to result in the degeneration of the bones and amplify the occurrence of the bones-related problems, such as osteoporosis and osteonecrosis (Galliera et al., 2021). Specifically, in osteonecrosis, oxidative stress inducing damage of osteocytes and related vascular dysfunction also leads to further development of the condition. Research has indicated that those therapies that target the reduction of oxidative stress, including antioxidants

and anti-inflammatory forms of treatment, are capable of minimizing bone loss and enhancing bone health (Marcucci et al., 2023). Thus, managing oxidative stress through dietary interventions, lifestyle changes, or therapeutic interventions may play a central role to prevention and treatment of diseases in the modality of osteonecrosis, by way of bone degeneration, which is induced by increased dysbiosis and oxidative damage.

3.3 Altered Bone Marrow Microenvironment

The GM is important in shaping the bone marrow microenvironment. Such a dynamic system includes several cell components, the extracellular matrix, cytokines, and growth factors that control stem cell fate, bone formation, and an immune response (X. Liu et al., 2024). This delicate microenvironment can be disrupted through a dysbiosis leading to the development of osteonecrosis and other adverse effects on bone health. As a result of the dysbiotic microbiome, there is low-grade chronic inflammation, an increase in circulating levels of enzymes and cytokines of the proinflammatory nature. These cytokines invade into the bone marrow niche resulting in the instability of the balance between osteogenic and osteoclastic activity (**Figure 1C**) (Amato et al., 2025). Particularly, chronic inflammation hinders the MSC differentiation into cells that produce bone, including osteoblasts, but promotes the differentiation of hematopoietic stem cells (HSCs) into cells that destroy the bone, including osteoclasts (Bartold et al., 2019), thereby contributing to bone loss and impaired bone quality which is key factors of osteonecrosis.

Along with stem cell differentiation interference, dysbiosis could also influence the expression of growth factors and adhesion molecules in bone marrow microenvironment that play a crucial role in proper functioning of stem cells (Sevcikova et al., 2024). Remarkably, processes such as angiogenesis and preservation of bone marrow vasculature are found in VEGF and stromal cell-derived factor-1 (SDF-1) to make sufficient oxygen and nutrients available to bone-forming cells (**Figure 1D**) (Ramchandani & Weber, 2015). Research has also demonstrated that VEGF are capable of activating the expression of SDF-1 and vice versa thus, exhibiting a synergism enhancement of angiogenesis and tissue repair. SDF-1 is commonly higher in inflamed tissues which may enhance the reaching of endothelial progenitor cells and aid in vascular remodeling. Thus, VEGF and SDF-1 expression could increase under the inflammatory condition of gut dysbiosis, affect vascular functionality, and bone marrow oxygenation (Jin et al., 2013; H. Yang et al., 2022). This compromised blood supply leads to local hypoxia which is a significant cause of apoptosis of osteocytes, failure of bone healing and osteonecrosis development. Serving as an added beneficial effect, OGR1 signaling upregulation in the endothelial progenitor cell (EPCs) inhibits angiogenesis and enhances apoptosis through activation of PTEN and inhibition of mTOR, facilitates EPC recruitment through inhibition of the CXCR7/CXCL12 pathway (Ding et al., 2019; Yang et al., 2020). These disturbances reduce the vascular supply that is vital to the health of the bone to result in ischemic conditions and apoptosis that forms the pathogenesis of osteonecrosis.

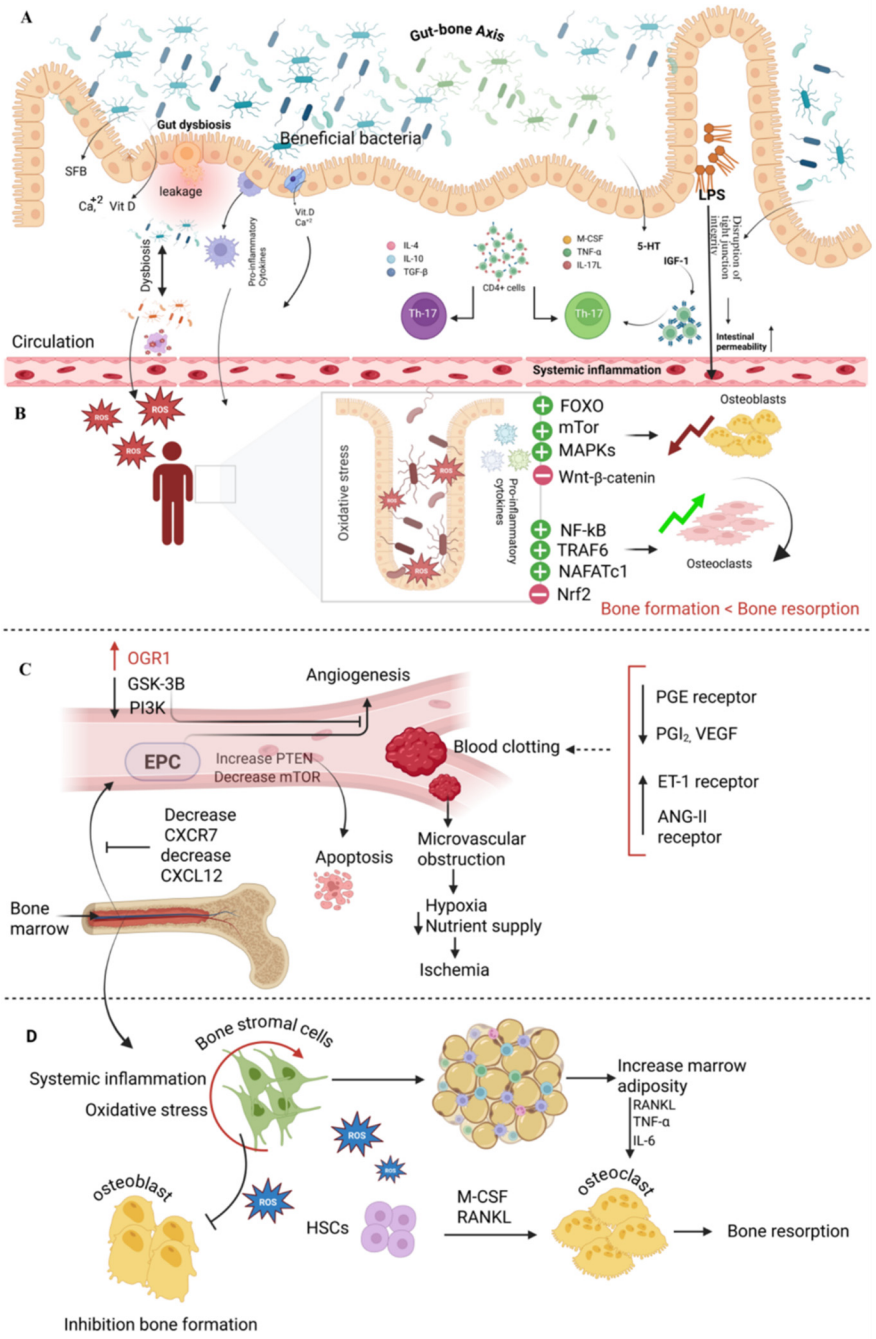

Figure 1. This Sketch illustrates mechanistic insights into gut dysbiosis-induced Osteonecrosis. Gut dysbiosis and intestinal hyperpermeability contribute to osteonecrosis pathogenesis through synergistic inflammatory, oxidative, and vascular pathways. (A) Gut-derived bacterial products (e.g., LPS) enter systemic circulation, triggering Th17-mediated inflammation (elevated TNF-α, IL-17) and suppressing Treg activity. This cytokine storm promotes osteoclast activation and impairs osteoblast function, disrupting the bone remodeling equilibrium critical for osteonecrosis occurrence. (B) Resultant oxidative stress exacerbates bone ischemia by inhibiting osteogenic pathways (Wnt/β-catenin) while stimulating osteoclastogenesis (via NF-κB), accelerating trabecular bone loss and necrotic lesion formation. (C) Vascular compromise occurs through endothelial dysfunction, where oxidative damage and impaired angiogenic signaling (OGR1/GSK-3β/PI3K axis, PTEN/mTOR dysregulation) reduce bone perfusion, a hallmark of osteonecrosis. (D) In the bone marrow microenvironment, these perturbations induce adipocyte accumulation, RANKL-driven osteoclast hyperactivity, and impaired hematopoietic support, culminating in the avascular necrosis characteristic of advanced osteonecrosis. Together, these mechanisms delineate how gut dysbiosis propagates osteonecrosis through intertwined inflammatory, metabolic, and microvascular disruptions. The figure is generated with Biorender.com. TGF-β (Transforming growth factor beta); MC-SF (Macrophage colony-stimulating factor); 5-HT (5-hydroxytryptamine, serotonin); IGF-1 (Insulin-like growth factor 1); MAPKs (Mitogen-activated protein kinases); NF-kB (Nuclear factor kappa-light-chain-enhancer of activated B cells); TRAF6 (Tumor necrosis factor receptor-associated factor 6); NAFATc1 (Nuclear factor of activated T-cells, cytoplasmic 1); EPC (Endothelial progenitor cells); CXCL12 (C-X-C motif chemokine ligand 12); PGE (Prostaglandin E); ET-1 (Endothelin 1); ANG-II (Angiotensin II); M-CSF (Macrophage colony-stimulating factor).

3.4 Impact of microbial metabolite imbalance on bone remodeling

Microbial metabolites play a critical role in the regulation of bone health, and during dysbiosis, the production and composition of these metabolites can be significantly altered, potentially contributing to the development of osteonecrosis. Certain microbial metabolites, such as SCFAs and secondary bile acids, have both direct and indirect effects on bone metabolism and overall bone integrity (Han et al., 2024).

Nevertheless, during dysbiosis, the synthesis of SCFAs may decrease dramatically, which causes the abnormality of osteoblast development and reduction of bone formation (Lucas et al., 2018). This can cause imbalance in bone formation and resorption with more emphasis on bone loss since the SCFA will have reduced and this contributes to conditions like osteonecrosis whereby the bone tissues become weak and easily damaged and may cause proinflammatory state and resorption of bones leading to osteonecrosis (Zheng et al., 2022). Deficiencies in minerals as a result of dysbiosis may worsen the bone quality by reducing SCFA levels further creating an improvement of the risk of osteonecrosis. Gut dysbiosis reduces SCFAs synthesis, an ability that diminishes the osteogenic potential of the MSC and ability to renew the bone marrow. Besides immune regulation, another important effect of dysbiosis is that it changes the expression of important growth factors and adhesion molecules in the bone marrow microenvironment that directly modulates stem cells activity (X. Liu et al., 2024).

Dysbiosis-driven overproduction of secondary bile acids, notably deoxycholic acid (DCA) and lithocholic acid (LCA), can profoundly disrupt bone metabolic regulation. While bile acids play essential roles in lipid digestion and signaling, excessive levels of these microbial metabolites under dysbiotic conditions lead to oxidative stress and chronic inflammation within bone tissue (**Figure 2**) (X.-J. Li et al., 2024). These bile acids can increase the production of ROS in bone cells, leading to oxidative damage that impairs the function of osteoblasts and promotes osteoclast-mediated bone resorption (Yadav et al., 2024). This oxidative imbalance is closely associated with overactivation of the RANKL signaling cascade (**pathway II**), resulting in increased osteoclastogenesis and subsequent bone degradation. Furthermore, secondary bile acids can disrupt gut barrier integrity, facilitating LPS translocation into the systemic circulation. Circulating LPS further amplifies NF-κB–mediated immune responses (**pathway III**), promoting a proinflammatory milieu that exacerbates bone resorption and impairs osteoblast survival (Calzadilla et al., 2022; Domazetovic et al., 2017). Taken together, these processes enhance the likelihood of osteonecrosis, which is typified through microfracture, ischemia, and apoptosis of osteoblasts as well as osteocytes.

Polyamines and H_2S could be at an abnormal level and disrupt skeletal balance due to dysbiosis. An elevated polyamine leads to higher oxidative stress and apoptosis in osteocytes, lower H_2S may result in decrease in osteoblast survival and vascularization. Together, these imbalances may contribute to impaired bone remodeling and elevate the risk of osteonecrosis, especially when combined with SCFA and bile acid dysregulation. Moreover, it was demonstrated that DCA and LCA can disrupt Wnt/β -catenin signaling pathway that is the major regulator of osteoblast differentiation and bone formation (Cho et al., 2013). This pathway is repressed by dysbiosis-induced changes in the bile acid composition to decrease osteoblastic

activity and weaken bone matrix integrity. All these bile acid-mediated perturbations play out to impaired bone remodeling, fragility and susceptibility to osteonecrosis as shown pictorially in **Pathway IV**. Understanding the complex interactions among microbial metabolites, immune pathways, and bone remodeling mechanisms offers promising targets for preventive and therapeutic strategies aimed at preserving bone health in individuals with dysbiosis.

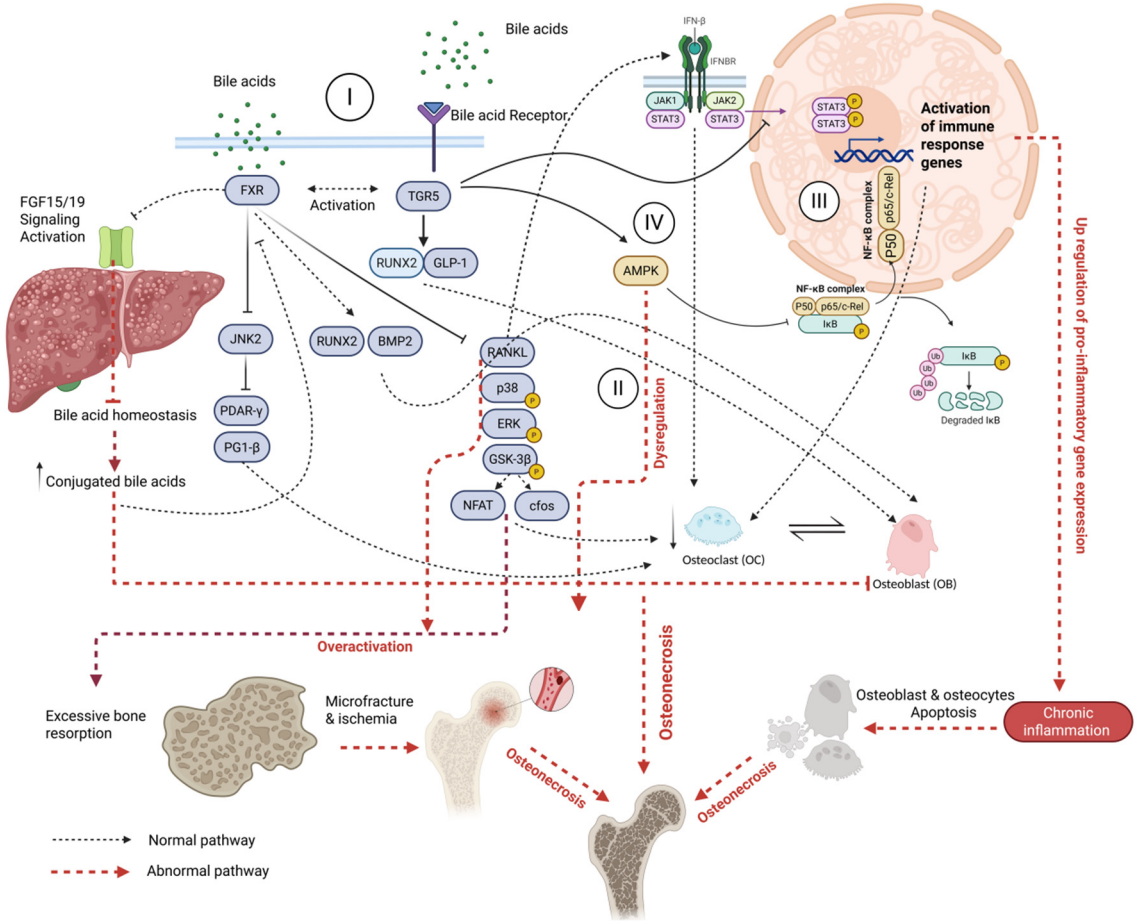

Figure 2. Bile Acid mediated Gut-Bone Axis Dysregulation in Osteonecrosis Pathogenesis. The pathological mechanisms linking bile acid signaling, gut immune responses, and bone remodeling in osteonecrosis. Bile acids activate FXR and TGR5 receptors, modulating FGF15/19 and GLP-1 pathways to influence osteoblast differentiation (via RUNX2/BMP2) while disrupting bile acid homeostasis. Dysregulated RANKL signaling (via p38, ERK, NFAT, c-Fos) promotes excessive osteoclast activity, leading to bone resorption and microarchitectural damage. Concurrently, JAK/STAT and NF-κB activation (STAT1/3, p50/p65) drives systemic inflammation, exacerbating osteocyte apoptosis and vascular injury. Impaired AMPK signaling further disrupts metabolic balance, aggravating osteoblast dysfunction and chronic inflammation. Collectively, these pathways lead to induce bone ischemia, necrosis, and impaired repair, perpetuating osteonecrosis progression. This figure created via BioRender.com. FXR (Farnesoid X receptor); TGR5 (Takeda G protein-coupled receptor 5); RUNX2 (Runt-related transcription factor 2); BMP2 (Bone morphogenetic protein 2); FGF15/19 (Fibroblast growth factor 15/19); ERK (Extracellular signal-regulated kinase); GSK-3β (Glycogen synthase kinase 3 beta); NFAT (Nuclear factor of activated T-cells); cfos (Cellular proto-oncogene fos); JNK2 (c-Jun N-terminal kinase 2); PPAR-γ (Peroxisome proliferator-activated receptor gamma); PG1-β (Prostaglandin I2 synthase-beta); IFN-β (Interferon beta); IFNBR (Interferon beta receptor); JAK1/2 (Janus kinase 1/2); STAT3 (Signal transducer and activator of transcription 3); p65, p50, c-Rel (NF-κB subunits); IκB (Inhibitor of kappa B).

3.5 Endocrine Pathways Linking Dysbiosis to Osteonecrosis

The endocrine system plays a critical role in balancing bone formation and resorption, and in maintaining calcium and phosphate homeostasis. The key hormones involved in this process include parathyroid hormone, PTH-related peptide, calcitonin, vitamins A and D, sex hormones, and growth hormone (Al-Suhaimi, 2022).

According to the recent evidence, the GM improves calcium absorption and bone mineralization, as well as affects the bone health via the effect on the endocrine system (Behera et al., 2020). The GM can change the endocrine system and its impacts on skeletal health via gut-endocrine axis, including through modulation of hormone secretion (Ibrahim et al., 2022), which may contribute to osteonecrosis. The presence of glucocorticoids such as cortisol (steroid hormone) is relevant in the metabolism of the bones especially when being controlled by the hypothalamic pituitary adrenal (HPA) axis that regulates the functions of the physiological systems (Janssen, 2022). Disrupted HPA axis due to dysbiosis may elevate glucocorticoids, inhibiting osteoblasts and enhancing osteoclastogenesis (Misiak et al., 2020; Shah et al., 2015), thus impairing bone structure and promoting osteonecrosis.

Sex hormones help the integrity of bones in terms of density and strength. Specifically, estrogen is known to protect the bone tissue through decreasing resorption and stimulating the formation of bones (C.-H. Cheng et al., 2022). Gut Dysbiosis may disrupt sex hormone regulation, promoting inflammation and increasing pro-inflammatory cytokines, osteoclastogenic, which include TNF-α, RANKL and IL-17 (Li et al., 2016). In GF mice, sex steroid deficiency never causes the production of osteoclastogenic cytokines. These findings suggest that the GM plays a critical role in modulating bone metabolism in response to sex hormone levels (Bellavia et al., 2016).

Vitamin D is important in bone health and immune regulation as well as absorption of calcium. Dysbiosis of the gut, however, is capable of interfering with the metabolism of vitamin D, resulting in the bone health being negatively impacted. Among the major effects of the dysbiosis is the hyper-population of Proteobacteria, including *Helicobacteraceae*, that enhance the inflammatory state and decline bone remodeling (Di Stefano et al., 2023). In addition, dysbiosis also leads to a loss of Tregs essential in regulating inflammation and preserving bone tissue (Campbell & Rudensky, 2020). Moreover, the shortage in vitamin D promotes dysbiosis, suppressing the epithelial surfaces and immune cells expression of E-cadherin, stimulating host inflammation and reduced ability to recover bone to lead to the risk of osteonecrosis (Y. Chen et al., 2022). Such multifactorial impairment, including hormonal imbalance and gut barrier dysfunction, undermines bone strength and blood flow, creating a pathological environment in which the onset, continuation and development of osteonecrosis succeeds. Therefore, the gut-endocrine-bone axis has been shown to become one of the crucial ways through which dysbiosis is able to promote the pathogenesis of osteonecrosis indirectly.

3.6 From Dysregulation to Degenerating Bone: Translational Insights into Osteonecrosis

Recent advances in microbiome research have elucidated the mechanistic connections between gut dysbiosis, other mechanistic contributions and osteonecrosis. Critical findings from clinical and preclinical studies are shown in **Table 1**, highlighting microbiota alterations, molecular pathways, and therapeutic implications across diverse osteonecrosis etiologies.

Table1. Comparative Analysis of Preclinical and Clinical Studies investigating Osteonecrosis

Study	Model	Osteonecrosis Type and induced method	Microbiota Alterations	Mechanisms Involved	Key Findings	Biomarker	Methodological Limitations
(Williams et al., 2020)	six-week-old female C57BL/6J mice	Medication-related osteonecrosis of the jaw (MRONJ) then treated with 125 μg/kg ZOL, and ligature placement for LIP induction.	1. Shift from *Methylobacterium komagatae* and Enterobacteriaceae to Bacteroidales family S24-7. 2. Clostridiales and S24-7, and decreases dominant taxa like *Methylobacterium* and Enterobacteriaceae, allowing increased colonization by rare taxa such as *Ruminococcus gnavus* and *Roseburia.*	Indigenous microbiota influences the inflammatory response and subsequent bone resorption processes that are critical in the pathogenesis of MRONJ. The disruption of this microbiota through antibiotic treatment can lead to increased susceptibility to osteonecrosis due to altered immune responses and inflammatory cytokine levels.	1. Normal oral flora protects against inflammation-induced osteonecrosis 2. Antibiotic-induced dysbiosis leads to increased empty lacunae and necrotic bone in certain conditions. 3. Histological assessments indicated that reduced oral bacterial load correlates with changes in osteoclast activity and bone mass	Not specifically mentioned	1. Model Limitations: Used a single mouse strain (C57BL/6J), which may not capture the genetic diversity relevant to human osteonecrosis. 2. Microbiota Analysis: Antibiotic-induced dysbiosis was employed, which may not accurately reflect natural microbiota alterations in osteonecrosis. 3. Histological Assessment: While histological changes were observed, the study did not quantify the extent of bone resorption or correlate it with microbiota changes.
(Williams et al., 2014)	Mouse model	Intravenous administration of mouse anti-RANKL Ab or the bisphosphonate zoledronate	-	Impaired bone resorption due to suppressed osteoclast function. The pathogenesis is consistent with a defect in jawbone physiologic remodeling or wound healing	Unresorbed bone is associated with ONJ development	undetectable tartrate-resistant acid phosphatase level in the serum	1. Model Limitations: The use of bisphosphonates and anti-RANKL antibodies may not accurately mimic the human condition of MRONJ. 2. Biomarker Analysis: The study did not identify specific biomarkers associated with the observed histological changes. 3. Mechanistic Insights: The study linked impaired bone resorption to ONJ development but did not explore underlying molecular mechanisms.
(Okazaki et al., 2013)	Male Wistar rats	Rats were fed a Lieber–DeCarli liquid diet containing 5% ethanol to induce ONFH.	-	Chronic alcohol intake led to hepatic steatosis (fatty liver), hepatic dysfunction, and hyperlipidemia. These liver-related changes were associated with the development of ONFH. However, the study did not find a significant relationship between the pro-inflammatory response via Toll-like receptor 4 (TLR4) signaling and the development of alcohol-induced ONFH.	1. ONFH was observed within seven days of initiating the 5% ethanol-containing liquid diet. 2. Histopathological analysis revealed empty lacunae within necrotic bone trabeculae and adipocytic necrosis in the bone marrow of the femoral head, resembling findings in human ONFH cases. 3. The study suggests that liver damage, such as hepatic	Not specified	1. Model Limitations: Chronic alcohol intake in Wistar rats may not fully replicate human alcohol-induced osteonecrosis due to species differences. 2. Inflammatory Pathways: The study did not establish a direct link between TLR4 signaling and osteonecrosis development. 3. Metabolic Analysis: While liver-related changes were observed, the study did not assess

					steatosis and dysfunction, may contribute to the pathogenesis of nontraumatic ONFH.		the impact of these changes on bone metabolism.
(Park et al., 2021)	Male Sprague–Dawley rats (60 rats)	Osteonecrosis was induced using MPS, a glucocorticoid, to model glucocorticoid-induced ONFH.	-	1. Suppression of the TLR4/NF-κB Pathway: Calycosin significantly inhibits the activation of TLR4 and nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) signaling pathway, which plays a pivotal role in inflammation associated with glucocorticoid-induced ONFH. 2. Promotion of Osteogenic Differentiation: *In vitro* studies have shown that calycosin enhances the osteogenic differentiation of human bone mesenchymal stem cells (hBMSCs). This is evidenced by increased Alizarin red staining, elevated alkaline phosphatase activity, and upregulation of osteogenic-related genes.	1. Reduction in ONFH Incidence: *In vivo*, calycosin administration decreased the incidence of ONFH and alleviated pathological manifestations within the femoral head. 2. Preservation of Osseous Blood Supply: Calycosin helped maintain the blood supply to bone tissue, which is crucial for preventing osteonecrosis. 3. Enhancement of Bone Formation: Dynamic bone formation was promoted in calycosin-treated rats, indicating improved bone health and regeneration	Not specified	1. Model Limitations: The study focused on a single compound (calycosin), which may not represent the broader therapeutic landscape for osteonecrosis. 2. Mechanistic Insights: While TLR4/NF-κB pathway suppression and osteogenic differentiation were observed, the study did not explore the long-term effects of calycosin treatment. 3. Biomarker Analysis: The study did not identify specific biomarkers associated with the therapeutic effects of calycosin.
(Yue et al., 2021)	Rat model	Administration of steroids to induce ONFH	-	1. miR-335 Regulation: Icariin administration was found to regulate the expression of miRNA-335, which is implicated in the pathogenesis of ONFH. 2. Protection of Bone Microvascular Endothelial Cells: Icariin demonstrated protective effects on bone microvascular endothelial cells, which are essential for bone health and integrity.	Icariin effectively prevented the development of ONFH in the rat model by modulating miRNA-335 expression and protecting endothelial cell function.	Changes in miR-335 expression levels were used as a biomarker	1. Model Limitations: The study employed a steroid-induced ONFH rat model, which may not fully replicate human osteonecrosis due to species-specific differences. 2. Molecular Analysis: The study focused on miRNA-335 regulation but did not assess the functional consequences of this regulation on bone metabolism. 3. Biomarker Analysis: Changes in miR-335 expression were used as a biomarker but were not validated in human samples.
(Cai et al., 2025)	Genetic data from the MiBioGen Consortium and the UK Biobank to explore associations between GM and ON in a human population.	-	1. Positively Associated with Increased ON Risk: Order *Erysipelotrichales* Family Erysipelotrichaceae Class *Erysipelotrichia* 2. Negatively Associated with ON Risk (Protective Effect): Christensenellaceae	1. Lipid Metabolism: Alterations in GM may influence lipid metabolism, contributing to ON pathogenesis. 2. Immune Modulation: GM can modulate systemic inflammation and immune responses, potentially affecting bone health and susceptibility to ON. 3. Osteogenic Differentiation: Certain microbial taxa might impact the differentiation and activity of	1. Identification of a causal relationship between specific GM taxa and ON risk. 2. Positive associations of *Erysipelotrichales*, Erysipelotrichaceae, and *Erysipelotrichia* with increased ON risk. 3. Negative associations of Christensenellaceae R and Family XIII with ON risk,	1. Risk Indicators: Increased abundance of *Erysipelotrichales*, Erysipelotrichaceae, and *Erysipelotrichia* may serve as indicators	1. Model Limitations: The study utilized genetic data from large cohorts, which may not fully capture the complexity of osteonecrosis pathogenesis. 2. Microbiota Analysis: The study identified associations between specific microbial taxa and osteonecrosis risk but did not establish causal relationships. 3. Genetic Insights: While 20 host

				osteoblasts and osteoclasts, thereby influencing bone remodeling processes relevant to ON.	suggesting a protective effect. 4. Annotation of 20 host genes potentially linked to ON pathogenesis, providing insights into genetic factors that may mediate the relationship between GM and ON.	of heightened ON risk. 2. Protective Indicators: Higher levels of Christensenellaceae R andFamily XIII could indicate a reduced risk of developing ON.	genes were annotated, the study did not assess their functional roles in osteonecrosis development.
(Q.-Y. Zheng et al., 2024b)	Human subjects diagnosed with NONFH	The study focuses on NONFH resulting from two primary etiologies: steroid use and alcohol consumption	1. In patients with alcohol-induced ONFH, there was an increased abundance of *Lactobacillus* and *Roseburia*. 2. In patients with steroid-induced ONFH, higher levels of Megasphaera and Akkermansia were observed.	The study suggests that both steroids and alcohol can induce changes in the GM, leading to alterations in fecal metabolites. These changes, driven by different pathways, may contribute to the progression of NONFH.	1. Metabolomic analysis revealed that alcohol-induced ONFH patients had elevated levels of L-Lysine and Oxoglutaric acid. 2. Steroid-induced ONFH patients exhibited increased levels of Gluconic acid and Phosphoric acid. 3. Ten metabolic pathways showed significant differences between the two patient groups, indicating distinct metabolic disruptions associated with each etiology.	1. Elevated levels of L-Lysine and Oxoglutaric acid were observed in patients with alcohol-induced NONFH. 2. Higher levels of Gluconic acid and Phosphoric acid were noted in patients with steroid-induced NONFH.	1. Model Limitations: The study focused on human subjects diagnosed with NONFH, which may not fully represent the broader spectrum of osteonecrosis cases. 2. Microbiota Analysis: The study identified microbial alterations in alcohol- and steroid-induced ONFH but did not assess the functional implications of these changes. 3. Metabolic Analysis: While metabolomic differences were observed, the study did not explore the impact of these differences on bone metabolism.
(Yue et al., 2024b)	Humans	AONFH	Altered abundance of specific bacterial species (e.g., *Basidiobolus*, *Mortierella*).	Changes in GM metabolites and altered abundance of specific microbial species, such as *Basidiobolus*, *Mortierella*, *Phanerochaete* and *Ceratobasidium*.	Interplay between gut microbiome and metabolome in AONFH pathogenesis; specific bacterial alterations associated with disease	Not specified	1. Model Limitations: The study focused on alcohol-induced ONFH in humans, which may not fully capture the complexity of osteonecrosis pathogenesis. 2. Microbiota Analysis: The study identified specific bacterial species alterations but did not assess the functional consequences of these changes on bone metabolism. 3. Biomarker Analysis: The study did not identify specific biomarkers associated with the observed microbiota changes.
(B. Li et	Humans	Drug-associated	Alterations in various	GM composition changes linked to	Certain bacterial families and	Not specified	1. Model Limitations: The study

al., 2024)		osteonecrosis	gut bacterial taxa	drug-induced osteonecrosis	genera have protective or negative causal effects on osteonecrosis due to drugs		focused on drug-associated osteonecrosis in humans, which may not fully represent the broader spectrum of osteonecrosis cases. 2. Microbiota Analysis: The study identified alterations in various gut bacterial taxa but did not assess the functional implications of these changes on bone metabolism. 3. Mechanistic Insights: The study did not explore the molecular pathways linking microbiota alterations to osteonecrosis development.
(Gómez-Barrena et al., 2021)	Human clinical trial involving patients diagnosed with ONFH	The study focuses on patients with ONFH of varying etiologies, including corticosteroid use, alcohol consumption, and idiopathic causes.	-	1. Cell Therapy: Autologous MSCs are expanded ex vivo and injected into the necrotic lesion of the femoral head. 2. Bone Regeneration: MSCs are hypothesized to promote bone repair and regeneration through their differentiation potential and paracrine effects.	1. Safety: The procedure was found to be safe, with no significant adverse events reported over a minimum follow-up period of 5 years. 2. Efficacy: Clinical outcomes indicated improvement in hip function and pain relief, suggesting the potential of MSC therapy in halting or reversing the progression of ONFH.	-	1. Model Limitations: The clinical trial involved patients with ONFH of varying etiologies, which may introduce heterogeneity into the findings. 2. Therapeutic Approach: The study focused on autologous MSC therapy but did not compare this approach with other potential treatments. 3. Long-Term Outcomes: While safety and efficacy were assessed over a minimum follow-up period of 5 years, the study did not evaluate long-term outcomes beyond this period.
(Fang et al., 2024)	*In vivo*: Rat model of steroid-induced ONFH, using methylprednisolone (MP) plus LPS. *In vitro*: Rat bone marrow mesenchymal stem cells (BMSCs) treated with MP.	Steroid-induced ONFH, induced via combined administration of MP and LPS in rats.	-	1. Glucocorticoids down-regulate serum α-2-macroglobulin (α2M), reducing antioxidative protection2. Increased oxidative stress upregulates SIRT2 in BMSCs. 3. SIRT2 deacetylates the BMP2 promoter, suppressing BMP2 expression. 4. Resulting oxidative stress and BMP2 repression promote BMSC apoptosis and ONFH development.	- GC-induced oxidative stress reduces α2M, elevating SIRT2 levels. - SIRT2-mediated deacetylation suppresses BMP2, promoting apoptosis in BMSCs. - Knockdown of SIRT2 restores BMP2 expression and mitigates ONFH in both cell and animal models.	(α2M): α2M downregulation correlates with oxidative stress and ONFH progression	- No gut microbiota or metabolite analysis performed. - Findings limited to rat models and *in vitro* cells. - Long-term outcomes, translational relevance, and therapeutic interventions were not tested.

Note: SONFH: Steroid-induced osteonecrosis of the femoral head; COL-I: Collagen type I; MPS: Methylprednisolone; TRAP5b: Tartrate-resistant acid phosphatase 5b; LIP: Ligature-induced periodontitis; TLR4: Toll-like receptor 4; hBMSCs: Human bone marrow mesenchymal stem cells; ONFH: Osteonecrosis of the femoral head; miRNA: MicroRNA; GAON: Glucocorticoid-associated osteonecrosis; OCN: Osteocalcin; ON: Osteonecrosis; GC, glucocorticoid; AONFH: Alcohol-induced osteonecrosis of the femoral head; MSCs: Mesenchymal stem cells; NONFH: Non-traumatic osteonecrosis of the femoral head; UK Biobank: United Kingdom Biobank; MiBioGen: Microbiome Genome Consortium.

4. The GM and Drug Metabolism as Hidden Factors in Osteonecrosis Risk

Emerging research underscores the significant role of the GM in influencing drug metabolism through its vast collection of enzymes (Rao et al., 2024). Direct effects of this microbial activity may modify the chemical structure of the administered drugs with regard to efficacy and toxicity (Pant et al., 2023). As an example, the gut bacteria could inactivate digoxin by altering its structure and thus causing the therapeutic failure. On the other hand, there is the risk of the gut microbial imbalance caused by some antibiotics that will result in drug non-metabolization and a change in the drug efficacy (C.-Y. Chen et al., 2022a).

In the context of ostonecrosis, the interplay between drug metabolism and GM is particularly significant. Glucocorticoid therapy has been found to cause a shift in the composition of the gut microbiome and specifically a loss of beneficial bacteria extracellular vesicles that has been implicated in the pathogenesis of ONFH (Schepper et al., 2019). This indicated that a healthy GM might play a pivotal role in preventing glucocorticoid-induced bone loss. Moreover, such composition-restoring interventions demonstrated the prevention of ONFH development during animal studies, emphasizing the possible preventive approach of microbiota therapeutic strategies against glucocorticoid-related osteonecrosis (C.-Y. Chen et al., 2022a).

Another example involves the administration of a bisphosphonate known as zoledronate (ZOL) applied in treating osteoporosis but has been cited in observing MRONJ. It has been found that the indigenous microbiota prevents MRONJ, which is provided by the occurrence of the microbiota. In rodent models, GM depletion by the administration of broad-spectrum antibiotics caused mice to be more susceptible to MRONJ when administered ZOL and teeth extracted. This observation indicates that a balanced gut microbiome possesses a protective effect on a negative impact of some specific medications on bones (Aguirre et al., 2021; Bassan Marinho Maciel et al., 2024).

Moreover, studies using Mendelian randomization (MR) may differentiate certain gut microbial taxa that may have a protective or harmful effect against drug-induced osteonecrosis. Protective associations were found with bacterial groups such as *Lentisphaerae*, and *Bifidobacterium*, and a higher risk was found with *Methanobacteria* and *Bacillales*. Such results show that there is a strong and perhaps contradictory connection between the composition of GM and the side effects of some drugs, so individual differences in stabilizing microbes can affect the likelihood of developing osteonecrosis at the stage of drug treatment (Chai et al., 2024).

These case studies support the complicate connect between GM and the drug induced osteonecrosis. Such results emphasize the significance of taking into account individual differences in the GM composition in the evaluation of osteonecrosis associated risk provided by certain medicines. Understanding the role of GM in drug metabolism offers promising avenues for personalized medicine (Ford, 2025). Through profiling healthcare provider and individual's microbiota can anticipate reactions to the drug and develop a prescriptive plan to reduce such side effects as osteonecrosis. This method undermines the significance of observation of microbial effects on pharmacotherapy to settle approaches to alter the microbiota and enhance the safety and effectiveness of drugs.

5. Microbiota-driven therapeutic approaches for osteonecrosis

5.1 Probiotics, Prebiotics and Their Synbiotic Role

Probiotics (live beneficial microorganisms) and prebiotics (indigestible fibers that stimulate microbial growth) offer a dual approach to GM modulation, with potential applications in bone health optimization (de Sire et al., 2022; S. Li et al., 2024); Such modulation of gut health by microbiota-targeted therapies offers a new direction in the treatment of osteonecrosis and may influence bone metabolism (Lyu et al., 2023).

Nondigestible prebiotics are complex carbohydrates, including galactooligosaccharides and inulin, which have been demonstrated to enhance proliferation and activity of probiotics; they have a great impact in changing the microbiome of the gut, and therefore they might influence the bone health of patients with osteonecrosis (Schepper et al., 2019). The interaction of prebiotics and the GM results in formation of SCFAs, may exert various positive impacts on bone metabolism (Zaiss, 2018). Such therapeutic possibilities also can be promoted by prebiotics enhancing the creation of SCFAs as the results of gut fermentation (C.-Y. Chen et al., 2022a; Fernandes et al., 2024; Zhang et al., 2022). Besides the enhancement of intestinal homeostasis and immune response modulation, it is possible to mitigate the consequences of medication associated with osteonecrosis (Zhang et al., 2022).

Synbiotics, which are combinations of probiotics and prebiotics, synergistically restore the balance in the microbiota of the gut and optimize bone-protective action. Synbiotics stimulate the production of SCFAs, as mentioned previously, thereby minimizing bone resorption (Papa et al., 2024). They also aid in the recovery of microbial diversity, which is impaired in corticosteroid-induced osteonecrosis. Some synbiotic combinations include *L. acidophilus* with inulin and *Bifidobacterium breve* with resistant starch, both of which have been found to be promising for improving bone metabolism (Kim et al., 2017). On the basis of these attributes, synbiotics could be promising adjunct therapies for the prevention and treatment of osteonecrosis. Overall, the approach of microbiota-targeted therapies holds great promise for preventing drug-induced osteonecrosis. The reconstitution of the gut microbiome and improvement of its positive influence on bone metabolism could provide an intervention that is complementary to standard pharmacological treatments. Further investigations are needed regarding their clinical efficacy.

5.1.1 Probiotic Interventions Alleviate Multifactorial Osteonecrosis

The therapeutic targeting of GM represents a promising strategy for managing osteonecrosis across alcohol-induced, trauma-induced, drug-induced, and sickle cell disease-induced forms, leveraging the gut-bone axis through distinct mechanisms. Alcohol-induced osteonecrosis, probiotic interventions like *Lactobacillus animalis* restore microbial balance, enhance SCFA production (e.g., butyrate), and reduce inflammation, thereby mitigating bone loss (Q.-Y. Zheng et al., 2024a). Also, glucocorticoid-induced osteonecrosis, probiotic treatment ameliorated bone loss in murine models by promoting VEG, suppressing apoptotic pathways (e.g., caspase-3), and restoring bone microarchitecture. Co-housing glucocorticoid-exposed mice with healthy controls transferred a protective microbiome that reduced osteonecrosis severity by 60% (Chen et al., 2020). Furthermore, in sickle cell disease, where vaso-occlusion

compromises bone blood flow, engineered melatonin-producing probiotics (e.g., *Lactobacillus* strains) or butyrate supplementation inhibit RANKL-induced osteoclastogenesis, enhance osteocalcin expression, and improving bone density (Zemanova et al., 2022).

While preclinical studies have demonstrated therapeutic potential, clinical validation in humans remains limited (Kiousi et al., 2022; Zou et al., 2024), as summarized in **Table 3,** which overviews preclinical and translational studies on GM-targeted interventions in osteonecrosis. Selected strains, such as *Lactobacillus reuteri*, can regulate osteoclastogenesis by suppressing pro-inflammatory cytokines and RANKL signaling, potentially preventing bone loss in conditions like osteoporosis and osteopenia, particularly in postmenopausal women or type 2 dabetics (T2D) with gut dysbiosis associated with bone density loss (Chu et al., 2025).

Advanced delivery systems, e.g., engineered microencapsulation or biomaterial carriers (e.g., sterosomes), enhance gastric survival and site-specific release of probiotics, allowing localized therapies in preclinical models of infection-associated bone deficits, wherein strains such as *Lactobacillus* used in conjunction with osteoinductive agents promote healing through simultaneous suppression of pathogens such as methicillin-resistant *Staphylococcus aureus* (MRSA) and augmentation of osteoblast function (Asgari et al., 2020). However, significant limitations constrain broad clinical adoption, including strain-specific variability in efficacy, where benefits observed in animal models or niche populations (e.g., preterm infants) fail to consistently translate to human trials, as seen in T2D patients where prebiotics like inulin-type fructans showed no significant improvement in serum bone turnover markers (P1NP, CTX-1) or mineral levels despite theoretical microbiota-bone cross-talk (Andrade et al., 2020).

Existing studies suffer from methodological frailties, including short-period, small-number human trials (e.g., 6-week trials) with diverse cohorts, resulting in 'very low quality' evidence according to GRADE systems that are incapable of establishing definitive dosing and strain protocols (Jafari et al., 2025), as delivery and stability issues remain due to probiotics' low gastric survivability absent sophisticated engineering that requires nanoencapsulation to tolerate acidic breakdown and ensure colonic survival (A. Chen et al., 2025; Sun et al., 2025).

The interindividual response to probiotics in bone disorders is due to several host-specific factors such as preexisting GM composition, genetic predisposition, hormonal status (e.g., estrogen deficiency in postmenopausal osteoporosis), disease phase, and comorbidities (e.g., T2D or RA). For instance, T2D patients exhibited no statistically significant changes in bone turnover markers (P1NP, CTX-1) following prebiotic supplementation in spite of theoretical gut-bone axis advantages, illustrating metabolic disease-induced non-response (Kverka & Stepan, 2024). Similarly, rheumatoid arthritis patients exhibit stage-specific microbial dysbiosis, where probiotics may only ameliorate bone erosion in early inflammation phases but fail in advanced stages with established joint damage (M. Cheng et al., 2022).

Several barriers hinder the clinical translation of probiotics for osteonecrosis therapy. One major challenge is their poor gastric survivability, necessitating advanced delivery platforms such as engineered

delivery systems (e.g., microencapsulated *Lactobacillus* proteins), show promise for enhancing gastric survival and targeted osteoimmunomodulation, but still facing a stringent approval processes due to undefined biocompatibility and ecological risks like horizontal gene transfer (D'Amico et al., 2025). Additionally, safety concerns cover risks of bacteremia in immunocompromised individuals and a seeming immune activation (e.g., heightened IL-6 in chronic inflammation), as well as governing concerns over long-term effects and environmental risks like horizontal gene transfer in CRISPR-engineered strains (Thomas et al., 2017). Moreover, commercial probiotic formulations lack quality control, with inconsistent viability and unverified strain claims, while region-specific microbiome variations (e.g., Megamonas-BMD correlations in Chinese cohorts) challenge global standardization (Nadeem-Tariq et al., 2025). Thus, probiotics represent a promising adjunct for osteonecrosis management via immunomodulation and GM restoration. However, successful clinical translation depends on addressing interindividual variability, delivery limitations, safety concerns, and regulatory barriers through standardized trials and patient-specific approaches.

5.2 Postbiotics

According to the International Scientific Association for Probiotics and Prebiotics (ISAPP), postbiotics is a manufacturing state of inactive microorganisms and/or constituents that has a health advantage in the host (Salminen et al., 2021). These preparations consist of nonviable microbial cells, structural parts (cell walls), and microbial metabolites that can be involved in the health outcome attained upon the death or inactivation or inanimation of microorganisms (Vinderola et al., 2022). Noteworthy, postbiotics obtained from well-characterized microorganisms with identified genomic sequences and manufactured through standardized procedures (Boyte et al., 2023).

The postbiotics have the potential of treating osteonecrosis due to their immunomodulatory and anti-inflammatory effects. The evidence suggests that inanimate microorganisms and/or parts may cause health benefits provided they are administered in adequate quantities to the host. These agents have been shown to regulate the immune system pathways, in particular, by modulating the host cells and GM, cytokine production, and decreased production of inflammatory cytokines (Liu et al., 2022). Research has demonstrated that specific postbiotics, including heat-inactivated *Lactobacillus* and *Bifidobacterium* strains, downregulate proinflammatory cytokines and upregulate the production of anti-inflammatory mediators (Martorell et al., 2021). Based on the fact that postbiotics can perform their action in host immune cells and the microbiota of the gastrointestinal tract, they can be used to prevent the inflammatory processes leading to osteonecrosis development. Through acting on host immune cells and inhibiting the GM, postbiotics can therefore limit the sustained inflammation that underlies osteonecrosis and microbial products like SFCAs are directly beneficial to the bones (Yadav et al., 2024). Given that postbiotics are not live bacteria, it is less likely to cause any risk and is likely to be more stable than probiotics especially in immunocompromised patients, or patients with gastrointestinal sensitivities (Yeşilyurt et al., 2021). This safety attribute is particularly important to high risk patient populations including patients undergoing glucocorticoid therapy that have been shown to suppress a patient’s immune system and alter the GM composition. Long-term glucocorticoid treatment is

among the major risk factors for osteonecrosis since glucocorticoids, owing to their ability to manage inflammation, oxidative stress, and microbial metabolites without introducing live microbes it may serve as potential therapies for glucocorticoid-induced osteonecrosis without impairing gut and immune homeostasis (Bourebaba et al., 2022).

To use postbiotics in the treatment of osteonecrosis, they can be incorporated into functional foods, dietary supplements, or pharmaceuticals. Food supplements represent a promising category for the development of new postbiotic products because loss of viability may increase the stability of postbiotic products compared with that of probiotic food supplements. Future work must focus on the identification of certain postbiotic strains or metabolites that possess the capacity to directly affect bone metabolism and inflammatory pathways involved in osteonecrosis. Overall, they constitute a promising and novel treatment approach for osteonecrosis that acts on microbiota-driven inflammation and bone health by means of safe, stable, and bioactive microbial components.

5.3 Dietary interventions

Dietary interventions play crucial roles in the treatment, management, and prevention of osteonecrosis by influencing bone metabolism, the GM, and systemic inflammation. Excess phosphorus intake has been shown to affect bone metabolism, and studies indicate that cheese can decrease serum parathyroid hormone (PTH) and bone resorption, which is relevant to osteonecrosis, as excessive bone resorption contributes to disease progression (Karp et al., 2007). By modulating phosphorus intake, dietary interventions may help stabilize bone turnover and prevent excessive bone degradation in patients with osteonecrosis. Additionally, anthocyanins from blackcurrants have been found to modulate GM and suppress osteoclastogenic cytokines such as IL-1β, IL-2, and RANKL, which are directly involved in excessive osteoclast activity that leads to bone necrosis; thus, anthocyanin-rich foods may mitigate osteonecrosis progression by reducing inflammation-induced bone resorption (Nosal et al., 2024). Fermentable fibers, polyphenols, anthocyanins, and selenium-containing substances improve intestinal permeability and reduce oxidative stress, which is particularly relevant since osteonecrosis is linked to systemic oxidative stress and inflammation that can be exacerbated by intestinal barrier dysfunction (Hair et al., 2021; Hidalgo-Liberona et al., 2020; Hung & Suzuki, 2018; Qiao et al., 2022). Dietary strategies that promote GM diversity, such as high-fiber and polyphenol-rich diets, could support osteonecrosis management by enhancing nutrient absorption and reducing inflammatory responses. Moreover, some dietary patterns, e.g., consumption of high-fat, high-glucose, or high-protein diets, are found to hamper the work of the gut and GM composition, thus causing chronic inflammation and the decrease in SCFA production, both of which are prime cause-factors osteonecrosis, especially when corticosteroid usage or occurrence of metabolic disorders is involved (Bass et al., 2013; Muñoz-Garach et al., 2020; Wong et al., 2018). Conversely, anti-inflammatory dietary patterns including the Mediterranean diet, energy-restricted diet and ketogenic diet, have also occurred dual impact on bone health, specifically, regulating the composition of the GM, mediating the production of SCFA, and exerting effects on systemic inflammation, each of which may affect the progression and cure of osteonecrosis (Zhang et al., 2024).

Personalized diets that incorporate the intake of adequate amounts of calcium and vitamin D supplements and dietary changes are necessary, and these are important since they play an essential role in the structure of repair and model bone in individuals with osteonecrosis. Furthermore, nutritional education and dietary intercession programs should be given a chance to improve the osteonecrosis treatment by establishing commitment to bone-supporting diets and long-haul inspection of eating regimens and their effects singularly on bone health where only nutritional intercessions will do little to change osteonecrosis, yet will have significant advantages of slowing its advancement and improvement of recovery (Park et al., 2017). In summary, the treatment of osteonecrosis with dietary interventions is possible, as it regulates bone remodeling, balances the microbiota and formation of SCFA in the gut, decreases systemic inflammation, and prevents additional damage to bone structures by limiting high-fat and high-sugar consumption, accompanied by personalized nutrition schedules, which may enhance the treatment. Dietary intervention may be the most significant element of management of osteonecrosis since incorporation of high-fiber, polyphenol-rich and even anti-inflammatory diets by avoiding processed foods can not only enhance the gut health and decrease the inflammation but also promotes bone regeneration.

5.4 Fecal microbiota transplantation: Clinical Applicability, Risks, and Regulatory landscape

FMT entails the transfer of a well-characterized, functionally active gut microbial ecosystem from a thoroughly screened healthy donor to a recipient, aiming to restore gut microbial homeostasis and manage both gastrointestinal and systemic diseases associated with dysbiosis (Zheng et al., 2025). FMT is emerging as a novel treatment for osteonecrosis, particularly due to its potential to restore the balance of the GM and improve bone health (Biazzo & Deidda, 2022). A summary of therapeutic strategies involving FMT for osteonecrosis through GM restoration is provided in **Table 2**.

5.4.1 Clinical applicability

According to established interconnection between GM and bone metabolism, FMT has a clinical potential as a future therapy option that may help patients with the risk of drug-induced osteonecrosis (Bailey & Fraser, 2023), as in **Table 3**. The restoration of an intact microbial community in the gut by FMT may mitigate the adverse side effects of drugs, such as bisphosphonates or corticosteroids, which are known to disrupt GM balance and impede normal bone health (Y. Yang et al., 2022; Zemanova et al., 2022). Furthermore, FMT has been found to enhance immune function and decrease chronic inflammation leading to reduced levels of inflammatory markers (Burrello et al., 2018; Sahle et al., 2024). These effects could prevent the pathological processes leading to osteonecrosis and help maintain bone integrity. Beyond *C. difficile* infection (CDI), FMT has been investigated for other disorders, including inflammatory bowel disease (IBD), irritable bowel syndrome (IBS), metabolic syndrome, and even cancer (Bailey & Fraser, 2023; Jamal et al., 2023).

Table 2. A Summary of therapeutic Approaches for Osteonecrosis through GM Restoration via FMT.

Category	Sub-Category	Details	Approved Products	Dose & Administration	Mechanism of Action	Clinical Evidence	Safety & Risks	Regulatory Status	References
Donor Criteria	Health Status	- No chronic/metabolic diseases (diabetes, IBD) - BMI 18.5-25 kg/m²	N/A	N/A	N/A	N/A	N/A	N/A	(Adolph et al., 2024)
	Microbiome Profile	- High alpha diversity - Bacteroidetes/Firmicutes ratio >1.2	N/A	N/A	N/A	N/A	N/A	N/A	(Pinart et al., 2021)
	Screening	- Stool PCR for pathogens - Blood tests (HIV, hepatitis)	N/A	N/A	N/A	N/A	N/A	N/A	(Yong, 2024)
Recipient Profile	Indications	- Early-stage osteonecrosis (ARCO I-II) - Steroid-induced cases	N/A	N/A	N/A	Pilot studies show 40% improvement at 6 months	N/A	Off-label use	(Larson et al., 2018)
	Contraindications	- Immunocompromised - Active GI infection	N/A	N/A	N/A	N/A	Risk of sepsis	N/A	(Kovacs et al., 2014)
FMT Products	FDA-Approved	- Rebyota® (lyophilized) - Vowst™ (oral capsules)	30-50 g equivalent per dose	Colonoscopy or oral capsules	Microbiota restoration	90% efficacy for *C. difficile*	Mild GI symptoms	Approved for *C. difficile*	(Kovacs et al., 2014)
	Investigational	- FIN-524 (Seres) - MTP-101 (Mikrobiomik)	50-100 g fresh equivalent	Enema or nasoduodenal tube	Immune modulation	Phase II for IBD	Theoretical risk of bacteremia	Clinical trials	(Hou et al., 2025)
Treatment Protocol	Acute Protocol	- Single 100 g dose via colonoscopy	Off-label use of approved products	100 g fresh stool in 500 mL saline	Rapid microbiome alteration	Case reports show pain reduction	Transient fever possible	IRB-approved	(Porcari et al., 2023)
	Chronic Protocol	A single 150 mL dose. Administered rectally.	Rebyota® (multiple doses)	A prepackaged, single-dose fecal microbiota suspension in a 250 mL bag. An administration tube set is provided separately	While the exact mechanism is not fully elucidated, REBYOTA™ is believed to restore GM balance. By introducing a diverse consortium of spore-forming and non-spore-forming bacteria, it aims to reestablish a healthy microbial community, thereby preventing CDI recurrence.	The safety profile of REBYOTA™ was evaluated across five prospective clinical trials, including three Phase II trials (PUNCH CD, PUNCH CD2, PUNCH Open-Label) and two Phase III trials (PUNCH CD3, PUNCH CD3-OLS). Participants were adults with recurrent CDI who had completed standard antibiotic therapy. Depending on the trial design, they received one or two rectal doses of REBYOTA™ or a placebo. Ongoing trials	Tolerability: REBYOTA™ was generally well-tolerated among study participants. Common Adverse Events: Reported side effects included abdominal pain, diarrhea, abdominal distension, flatulence, and nausea. Serious Adverse Events: The incidence of serious adverse events was low and comparable between REBYOTA™ and	The U.S. Food and Drug Administration (FDA) approved REBYOTA™ on November 30, 2022, for preventing CDI recurrence in adults following antibiotic treatment for recurrent CDI.	(Feuerstadt et al., 2024; Lee et al., 2023)

						for osteonecrosis	placebo groups.		
Mechanistic Effects	Short-term (1-7 days)	-GM engraftment -SCFA production ↑	All products	Dose-dependent	Microbial colonization	Metagenomic sequencing data	Dysbiosis if dose too high	N/A	(Yue et al., 2024a)
	Long-term (1-12 months)	- TNF-α/IL-6 reduction - Bone perfusion improvement	FIN-524 showing promise	Requires maintenance doses	Immune-bone axis modulation	MRI improvements in 60% cases	Autoimmunity risk (theoretical)	N/A	(Y. Li et al., 2025)
Monitoring	Microbiome	- 16S rRNA sequencing at 1, 3, 6 months	N/A	N/A	N/A	Confirms donor engraftment	N/A	N/A	(Smillie et al., 2018)
	Clinical	- MRI at 6 months - Pain scales monthly	N/A	N/A	N/A	30%-50% show radiological improvement	N/A	N/A	(Baal et al., 2021)

Table 3. Summary of preclinical and translational studies on GM-targeted interventions in osteonecrosis.

Intervention	Study Type & Model	Endpoints	Key Outcomes	Limitations	Clinical Applicability	Regulatory Status	Reference
Probiotic supplementation with *Lactobacillus reuteri* PTA ATCC 6475 (3.3×10^8 CFU/mL in drinking water)	Preclinical, murine model: male C57BL/6J mice aged 16 weeks received a slow-release subcutaneous glucocorticoid pellet (2.5 mg/kg/day) for 8 weeks with or without L. reuteri supplementation	- Femoral head vascularization (vWF staining) - Apoptosis of osteoblasts/osteocytes (TUNEL assay) - Marrow adiposity - Bone structure (via μCT) -Gene expression of TNF-α and Bax	- GC treatment caused ~50% reduction in blood vessels—completely prevented by L. reuteri - L. reuteri blocked GC-induced apoptosis and marrow fat accumulation - μCT showed full preservation of bone microstructure -Prevention of GC-induced rise in TNF-α and Bax levels	- Single species and strain used - Exclusively male mice; no females - Probiotic delivered via drinking water—not easily translatable to human dosing - No long-term or post-intervention follow-up data - Mechanistic pathways (e.g., gut-bone axis) inferred,not directly proven	Promising for prophylactic use in patients on chronic glucocorticoids to prevent osteonecrosis. However, translation to humans requires: - Dose optimization - Verification in larger animal models - Human clinical trials to determine safety, efficacy, and optimal delivery	Not yet approved for this indication. *L. reuteri is* commercially available as a probiotic, but nothing is registered or approved specifically for preventing glucocorticoid-induced osteonecrosis.	(Kang et al., 2020)
Sodium butyrate supplementation in GAON rat model	Translational human-clinical + preclinical. Bidirectional MR using microbiome GWAS data Cross-sectional clinical study: GC-treated patients who developed GAON (n=17), GC-treated non-GAON (n=15), untreated controls (n=15). Rat GAON model with sodium butyrate (NaB) supplementation (n=10), GAON (n=10) , and blank group (n=10)	Lachnospiraceae % in GM - Levels of fecal and serum SCFAs, especially butyrate- Serum IL-17A, IL-33, TNF-α- MR causal associations- Rat femoral head necrosis severity via μCT and histology in control, GAON, and GAON + NaB groups	MR analysis correlated lower Lachnospiraceae with GAON (IVW P = 0.011)	- Cross-sectional design in humans: association but not causation - Small sample size (17 GAON cases) - MR analysis: potential residual confounding - Rat model may not fully replicate human GAON - Focused only on select cytokines (IL-17A, IL-33, TNF-α); broader inflammatory milieu not assessed. No long-term or dose-response data on butyrate supplementation	Biomarkers: Lachnospiraceae abundance, butyrate levels, and inflammatory cytokines may help early GAON risk stratification. - Intervention potential: Butyrate supplementation shows promise as a preventive strategy in GC-treated patients -needs validation in prospective clinical trials. - Feasibility depends on optimizing oral butyrate dosing, delivery	Butyrate is an unregulated dietary supplement, not approved for GAON prevention and not registered clinical indications.	(Guo et al., 2024b)

					methods, safety, and determining efficacy in humans.		
Antibiotic-induced GM depletion prior to GA-ONFH modeling in rats- FMT to restore dysbiosis- Butyric acid supplementation in vitro (bone marrow BMSCs) and *in vivo* in GA-ONFH rats	Preclinical study using Sprague–Dawley rat model of glucocorticoid-induced ONFH. Includes multi-omics: 16S rDNA GM profiling, targeted metabolomics, in vitro BMSC assays, and *in vivo* histology and μCT.	- GM composition (16S sequencing) - Fecal and serum butyric acid levels - BMSC proliferation, migration, osteogenic differentiation (ALP, scratch wound) - Gene expression by RNA-seq - Inflammatory cytokines in BMSCs: TNF-α, IL-2, IL-4 (western blot, immunofluorescence) - Rat femoral head pathology (μCT, HE staining, immunohistochemistry)	- Antibiotic-induced dysbiosis worsened GA-ONFH - FMT restored butyrate levels and attenuated disease - Butyric acid boosted BMSC proliferation, migration, osteogenic differentiation *in vitro* - RNA-seq: butyrate modulated T cell–related inflammatory cytokine genes in BMSCs - Western blot/IF: butyrate reduced TNF-α and IL-2, and raised IL-4 in BMSCs - *In vivo*: butyrate supplementation improved bone microstructure, decreased necrosis, reduced TNF-α and IL-2 expression in femoral head	- Animal-only model: no human data - Multi-omics brings correlation but lacks mechanistic in-depth intervention analysis - Dose–response and long-term effects of butyrate not fully explored - Focus limited to select cytokines (TNF-α, IL-2, IL-4); other pathways remain unexamined	- Highlights the protective effect of microbiota-derived butyric acid on bone via modulating BMSC inflammation - Suggests FMT or butyrate supplementation as preventive/therapeutic strategies for GA-ONFH - Translation to humans will require clinical trials to optimize dosing, delivery method, and confirm safety/efficacy	- Butyric acid (as sodium butyrate) is an unregulated supplement, not officially approved for osteonecrosis prevention - FMT is clinically used for recurrent *C. difficile*, but off-label for bone-related conditions and not GA-ONFH	(He et al., 2025)
Cohousing GC-treated mice with healthy mice or colonization via normal mouse GM- Oral supplementation with *Lactobacillus animalis*- Administration of *L. animalis*–derived extracellular vesicles (EVs)	Preclinical murine study using a glucocorticoid (methylprednisolone)-induced ONFH model in male mice	Bone structure: μCT metrics (trabecular BV/TV, thickness, number, spacing) and histology (H&E)- Vascularization: μCT-angiography and CD31 immunostaining- Osteogenesis (osteocalcin) and apoptosis (TUNEL assay)- 16S sequencing of GM, tracking L. animalis abundance- Tracing labeled EVs into femoral tissue	Cohousing or GM transfer rescued bone microarchitecture, improved vascular metrics, boosted osteoblast number, and reduced apoptosis. - GC treatment decreased abundance of L. animalis; supplementation restored it. Oral L. animalis mitigated ONFH, improving bone and vascular health, and reducing apoptosis. L. animalis–derived EVs entered the femoral head and produced pro-angiogenic, pro-osteogenic, anti-apoptotic effects, mirroring whole-cell effects	- Mouse-only model; may not fully translate to humans - Focused on *L. animalis*; contribution of other bacteria not explored - Long-term outcomes and optimal dosing of EVs not assessed - Mechanistic depth limited; underlying molecular pathways of EV action not fully elucidated - EV purification and stability for potential clinical use remain undeveloped	- EVs could serve as novel therapeutics for GC-induced ONFH; may bypass issues with live probiotics - Tracking and supplementing *L. animalis* could be part of preventive strategies in GC-treated patients - Clinical translation requires studies on safety, dosing, delivery, EV stability, and human efficacy	- L. animalis supplements and bacterial EVs are not approved for bone disease therapy - EV-based treatments are investigational, requiring extensive preclinical development and regulatory approval	(C.-Y. Chen et al., 2022b)

SHD administered orally to rats at two dosages: low (4.86 g/kg/day) and high (9.72 g/kg/day) for 8 weeks	Preclinical study using male Sprague–Dawley rats (n=40). SONFH induced via combined LPS + methylprednisolone, then treated with SHD	GM : 16S rRNA sequencing, focus on Verrucomicrobia, *Allobaculum*, Burkholderiales, *Jeotgalicoccus*, *Clostridium*, *Corynebacterium*, rc4-4 Network pharmacology: Analysis identified 7 key molecular targets (CYP19A1, CYP1A2, CYP1B1, CYP2C9, CYP3A4, MIF, HSD11B1) and 2 major pathways: tyrosine metabolism and steroid hormone biosynthesis	SHD improved bone morphology, increasing BV/TV, trabecular thickness/number and reducing separation, as seen on Micro CT and histology Upregulation of osteogenic markers (RUNX2, OCN, COL I). Metabolite restoration: 21 hydroxypregnenolone and tyramine normalized post SHD Microbiome modulation: SHD increased beneficial taxa, including Verrucomicrobia, *Allobaculum*, and Burkholderiales Integrated mechanism: A network linking herbs → metabolites → enzyme targets → microbial taxa, pointing to multi-level regulation via GM, host metabolism, and bone regeneration	Animal model only: No human clinical data. Complex intervention: Herbal mixture with multiple components complicates attribution of specific effects. Causality unclear: Associations from omics approaches; direct mechanistic validation is lacking. Duration and safety: Long-term effects, dose optimization, and toxicity not addressed.	SHD shows promise as a multi-modal therapy targeting the gut-metabolism–bone axis for SONFH. Before clinical use, required steps include: 1. Isolation of active components 2.Dose standardization and safety profiling 3. Early-phase human trials to confirm efficacy and optimal microbiome/metabolome modulation	Traditional Chinese medicine decoctions like SHD are not regulated as pharmaceuticals in most countries. Translational progress requires active ingredient characterization and clinical validation for regulatory approval	(Zhu et al., 2025)

Note: GA-ONFH, glucocorticoid-associated osteonecrosis of the femoral head; μCT, micro-computed tomography; Bax, Bcl-2-associated X protein; vWF, von Willebrand factor; TUNEL, terminal deoxynucleotidyl transferase dUTP nick end labeling; BV/TV, bone volume/total volume; CYP, cytochrome P450 (e.g., CYP19A1, CYP3A4); MIF, macrophage migration inhibitory factor; HSD11B1, 11β-hydroxysteroid dehydrogenase type 1.

Despite its therapeutic potential, current limitations underscore the need for further studies to elucidate the specific mechanisms and establish the clinical efficacy of FMT in preventing or treating osteonecrosis. The efficacy of FMT is influenced by complex interactions between the GM of the donor and recipient, and the degree of donor microbiota engraftment is not necessarily positively correlated with the success rate of FMT (Danne et al., 2021). The efficiency of FMT can be greatly influenced by such factors as bowel preparation, co-administered drug use, diet, lifestyle and severity of underlying illness of the patient (Liu et al., 2025). Preservation of bacteria or bacterial viability may be affected by practical issues of freezing and oxidizing during FMT preparations, thus interfering with the overall quality and efficacy of the transplant. The major problem is that it has not been possible yet to define the exact mechanism of FMT efficacy, and the difference in outcomes might be attributed to uncharacterized components (Arantes et al., 2025; Shaheen et al., 2025). FMT's poorly defined biological nature, originating from human waste, complicates product characterization and leads to lack of batch-to-batch consistency. These challenges hinder the application of traditional Good Manufacturing Practice (GMP) standards and create difficulties in defining potency, quality control, and reproducibility. Cold chain logistics and storage also present challenges for temperature-sensitive FMT products (Waheed et al., 2024).

5.4.2 Interindividual Variability

Human donor-derived FMT products entail an inbuilt microbial diversity that cannot be removed even with restrict screening procedures. As manufacturers attempt to make the donated stool standardized, it is not an easy task to achieve the full reproducibility of products (Wilson et al., 2019). The health condition, lifestyle, body weight, gender, age, and genetic relatedness of donors along with the specific microbial composition of their gut may all impact how effective the transplant (Danne et al., 2021; Liu et al., 2025). Donor-recipient compatibility, conceptually belonging to the non-random categories, is becoming a predominant parameter in the context of FMT, whereas some light must be shed on possible consequences of sex discordant and age discrepant FMT. This inherent donor variability is a fundamental challenge for FMT, moving it away from a standardized "drug" model towards a more personalized, "tissue-like" therapeutic (Ianiro et al., 2022). That means success in the future depends on either creating stronger donor-recipient matching standards or shifting to specific microbial consortia to achieve reproducible clinical outcomes.

5.4.3 The long-term concern

Current research generally indicates that FMT is safe in the long term for recurrent CDI, with a low risk of serious or lasting adverse effects when proper screening and preparation protocols are followed (Sandhu & Chopra, 2021). However, the risk of pathogen transmission remains a critical safety concern. Rare cases of transmission of multidrug-resistant organisms (e.g., ESBL-producing *E. coli*) have occurred due to inadequate donor screening, prompting the implementation of stricter regulatory guidelines (Ng et al., 2024). Emerging or unknown pathogens not routinely screened for may still pose risks, underscoring the importance of ongoing updates to donor screening protocols (Ng et al., 2023). Immunocompromised patients, who are often at higher risk for osteonecrosis, also carry a higher theoretical risk of infection from FMT (Williams et al., 2023). The

risk of pathogen transmission, though rare with rigorous screening, represents a critical safety hurdle, especially for a therapy derived from human waste. This necessitates continuous vigilance, adaptation of screening protocols for emerging pathogens, and reinforces the drive towards "cleaner," defined microbial products.

5.4.4 Lack of Regulatory Consensus

FMT presents a unique challenge to existing regulatory frameworks, often described as a "square peg" not easily fitting into traditional "round holes" due to its poorly defined biological origin and complex, unprocessed nature (Hoffmann et al., 2025). In the United States, the Food and Drug Administration (FDA) classifies FMT as a biological drug product. However, for rCDI unresponsive to standard therapies, the FDA has exercised "enforcement discretion" to permit clinical use while formal approval pathways are being established (Hoffmann et al., 2025; Scheeler, 2019). Recent FDA approvals of Live Biotherapeutic Products (LBPs) in 2022 and 2023 for recurrent CDI mark a step toward standardized, microbiota-derived therapeutics (Berry & Khanna, 2025). In Europe, regulation is fragmented. Some countries treat FMT as a medicinal product requiring stringent premarket approval (e.g., France, Germany, Spain), while others regulate it as a Substance of Human Origin (SoHO), similar to tissue and cell therapies (e.g., Italy, Belgium) (Paaske et al., 2025). Meanwhile, countries like Austria and Denmark apply less stringent oversight. The European Medicines Agency (EMA) recognizes the complexity of regulating FMT, citing challenges such as the undefined active component, poor product characterization, and lack of batch-to-batch consistency (Gisbert et al., 2025). These issues hinder the application of standard GMP controls (Zain et al., 2022). This global regulatory fragmentation, whether FMT is classified as a drug, tissue, or medical procedure, creates barriers to clinical trial harmonization, commercialization, and equitable patient access (El Boukhari et al., 2025). To overcome these limitations, emerging strategies such as the development of defined microbial consortia and synthetic microbiota-based products are being actively explored. These approaches aim to enhance safety, reproducibility, and regulatory compliance by offering standardized formulations with well-characterized microbial compositions (Waheed et al., 2024).

While FMT shows considerable therapeutic promise, these challenges underscore the necessity for rigorous patient selection, standardized donor screening, and long-term safety monitoring to mitigate potential risks. Furthermore, ongoing development of defined microbial consortia and synthetic microbiota-based therapies may offer safer and more controlled alternatives, addressing current limitations and paving the way for broader clinical application. Such measures are critical to ensuring that FMT evolves into a reliable and ethically responsible treatment modality.

5.5 GLP-1RAs as a Novel Gut-Bone Axis Modulator in Osteonecrosis Therapy

GLP-1 receptors (GLP-1Rs) were detected in the human osteosarcoma cell line including MG63 and immature osteoblastic TE-85 cells but not the mature Saos2 cells, thus indicating cell-specific activities (Zhao et al., 2024). GLP-1RAs have been involved in increasing the differentiation of osteoblasts and inhibiting the production of osteoclasts at the same time promoting bone formation and inhibiting bone destruction. The

process of action of GLP-1Rs on osteoblastogenesis through molecular signaling pathways, including MAPK, PKC, PKA, PI3K/AKT, and GSK3b, therefore exaggerates the process of bone formation. In addition, these agents counteract osteoclastogenesis by blocking the activation of NF-kB and MAPK, thereby inhibiting bone resorption (Li et al., 2020). It is suggested that this osteoanabolic effect occurs through the reduction of Wnt signaling, i.e. through the stabilization of β-catenin and the activation of LRP5/6 (H. Liu et al., 2024).

GLP-1RAs are widely used for glycemic control in type 2 diabetes mellitus (T2DM), with emerging evidence supporting their extra-glycemic benefits in osteonecrosis. These include modulation of bone metabolism, promotion of compensatory angiogenesis, and suppression of inflammatory pathways (Wikarek et al., 2024). They are gaining importance in the context of bone homeostasis, and their role is thought to have an effect on osteoblastogenesis, osteoclast, and vascular processes (Bendotti et al., 2022). Although these are encouraging results, the underlying mechanisms are not yet clarify. Also, GLP-1RAs can inhibit proinflammatory cytokines that are known to promote the development of osteonecrosis (Alharbi, 2024). Since a common complication of osteonecrosis is vascular insufficiency, then the proangiogenic activity of GLP-1RAs may be of specific benefit. These agents are revealed to stimulate endothelial nitric oxide synthesis, which improves vasodilation and angiogenesis (Mabilleau et al., 2018) leads to increase blood flow to the ischemic areas, may prevent ischemic damage and promote bone healing. Moreover, they have been found to decrease the adiposity of bone marrow by modulating the action of peroxisome proliferator-activated receptor gamma (PPAR-γ) activity, which is in close connection with development of osteonecrosis. (S. Zhang et al., 2025).

Emerging evidence suggests a bidirectional relationship between GLP-1RAs and GM, which plays a significant role in bone health. GLP-1RAs have been shown to modulate the composition of GM, increasing beneficial bacterial populations that produce SCFAs, which are known to influence bone metabolism (Singh et al., 2024). Additionally, GM can regulate GLP-1 secretion, suggesting a bidirectional interaction that may impact the efficacy of GLP-1RAs in osteonecrosis management. The ability of these agents to improve gut barrier integrity and reduce systemic inflammation further supports their role in maintaining bone health.

Although they exhibit promising mechanisms relevant to bone health, their therapeutic role in osteonecrosis remains speculative, with no clinical trials currently available to validate their efficacy. However, there is cases related osteoporosis associated with T2D, is due to their multifaceted mechanism of action. The drugs promote bone formation by activating GLP-1 receptors on BMSCs, promoting osteogenic differentiation via Wnt/β-catenin and AMPK/mTOR/SREBP2 pathways, reducing cholesterol accumulation in osteoblasts, and inhibiting adipogenesis (Mabilleau et al., 2018). Meanwhile, osteoclast activity is inhibited through regulation of the OPG/RANKL/RANK system, and bone marrow-derived macrophages are polarized toward anti-inflammatory M2 phenotypes. As a result, pro-inflammatory mediators like IL-1, IL-6, and TNF-α, which contribute to bone loss, are reduced (Wan et al., 2025). Despite these advantages, there are drawbacks, such as the lack of direct GLP-1 receptors on osteoblasts and osteoclasts. As a result, their effects are limited to intermediary cells like macrophages and neurons, which may reduce efficacy (Z. Zheng et al.,

2024). Gastrointestinal side effects (e.g., nausea, vomiting) and the blood-brain barrier's restriction of central nervous system (CNS) access further complicate dosing and tolerability, necessitating higher doses for CNS-mediated anti-inflammatory effects (Liu, 2024). Interindividual variability in response to GLP-1RA is due to genetic polymorphisms (e.g., GLP-1R, TCF7L2, or SORCS1 genes) that affect receptor affinity and downstream signaling, leading to varying effects on bone turnover markers across populations (Yu et al., 2019). Regulatory challenges arise from the absence of osteoporosis indications for GLP-1RAs, which are currently approved only for T2DM and obesity. Repurposing would require large-scale trials showing reduced fracture risk, an especially difficult endpoint due to slow bone turnover and the need for extended follow-ups (M. Chen et al., 2025). While GLP-1RAs demonstrate considerable therapeutic potential for osteonecrosis, additional investigations are needed to definitively establish their clinical efficacy, determine optimal dosing regimens, and evaluate long-term safety profiles. Future research should prioritize randomized controlled trials examining GLP-1RAs' modulation of the gut-bone axis as a mechanistic pathway for osteonecrosis management. These studies may reveal novel pharmacotherapeutic approaches to promote bone regeneration and optimize clinical outcomes in affected patients.

5.6 Personalized microbiota-targeted therapies on the basis of individual microbial profiles

On the basis of individual microbial profiling, these therapies offer a promising avenue for mitigating the risk of osteonecrosis, particularly in patients receiving treatments that include medications known to potentially contribute to this condition (Behera et al., 2020). With studies further demonstrating the close connection between GM and bone health, individual microbiome composition may help to enhance efficacy and safety of interventions (Behera et al., 2020). Such analysis of personal microbial profile could allow clinicians to determine the most efficient probiotic consortia or prebiotic combinations to use to restore bone health and prevent drug-induced osteonecrosis (McCabe et al., 2015).

In addition, personalized treatments can entail FMT, and it has been related to increased susceptibility to osteonecrosis. In different contexts, FMT has been promising and has promoted a wide range of the microbiome that is contributive to good health and can improve bone density and strength (Du et al., 2022). Also, the relationships between GM and drug therapies can be applied to create the individual approaches that would reduce the adverse effects of the medications like bisphosphonates or corticosteroids. For instance, specific measures may be imposed to prevent the occurrence of potential inflammation or bone metabolism alteration in the case of predisposition identification of a patient attributed to the microbial profile even prior to drug treatment (Ma et al., 2021).

Clinical trials testing the application of probiotics on specific patients with osteonecrosis are scarce, but the protective nature of commensal bacteria would promote a beneficial outcome using probiotic treatments (Seely et al., 2021). Probiotics could aid in the restoration of microbial balance and both reduce and alleviate inflammation, which is relevant in the context of the conditions like MRONJ (Damhorst et al., 2021). The applicability of FMT in osteonecrosis has not been extensively studied yet but has been shown to be useful in a number of other gastrointestinal disorders and may therefore prove as a new form of intervention in patients

especially at risk of osteonecrosis as a result of antibiotic induced dysbiosis (Sedghizadeh et al., 2012). The microbiome should be considered as a potential factor in osteonecrosis because modulation with probiotics, prebiotics, or more selective antibiotics, it is maybe a way to reduce the associated risks in patients. These studies prove the hypothesis that targeting of the microbiota with theraputics can offer an innovative means of osteonecrosis cure.

5.7 Therapeutic Microbiota Modulation and Targeted Antibiotic Stewardship as a Novel Approach

Modulation of microbiota is an emerging novel treatment that takes advantage of the protective effects of indigenous gut and oral microbiota to avoid and cure osteonecrosis, especially glucocorticoid- and bisphosphonate-related pathologies (Jelin-Uhlig et al., 2024). Alteration of beneficial bacteria caused by glucocorticoid therapy leads to oncreasing risk of ONFH, so the revision of the useful guide bacteria with the help of probiotic supplements may become a highly effective treatment (Luo et al., 2023; Lyu et al., 2023). The study results conclude that the gastrointestinal microbiome and its products can be used to develop new treatment options that have the potential to improve the healing capabilities of bone repairs and alter the extent of osteonecrosis (Han et al., 2024). Besides GM, another component of oral microbiome also plays a role in the prevention of MRONJ. Experimental studies have proven that antibiotic-induced dysbiosis enhances bone necrosis, suggesting that maintenance of a healthy oral microbiota can minimize MRONJ risk (Williams et al., 2020). It is suggested by research that female C57/BL6 mice treated with broad-spectrum antibiotics accumulate more osteoclasts after the administration of Zoledronic acid and develop intensive osteonecrosis (Jiang et al., 2023; Wang et al., 2024). There is also clinical evidence that an improvement in the level of oral hygiene before receiving a bisphosphonate leads to reduced cases of MRONJ in patients (Du et al., 2022). Such outcomes mean that the oral microbiome maintenance or restoration as specific probiotics and selected antibiotics can be part of osteonecrosis prevention protocols. Combining the microbiota modulation procedure with the principles of antibiotics stewardship may enhance the management of bone infections, as inappropriate or excessive antibiotic use can disrupt beneficial microbial communities (Yan & Charles, 2017). The management of antibiotics, in turn, which implies the serious and sensible consumption of antimicrobial drugs, contributes to the balance between infection control and microbiota preservation (Williams et al., 2020). To address osteonecrosis or minimize it, it is necessary to optimize the use of antibiotics, that is, to prevent exposing the microbiome to broad-spectrum antibiotics, unnecessary prolonged antibiotic treatment, and complications, such as *Clostridioides difficile* infection which has been associated with the loss of bone tissue and systemic inflammation (Vidal et al., 2022). A study on osteonecrosis-related infection suggested that omission of antibiotics against gram-negative bacteria in case of positive culture after five days could avoid inappropriate disruption of the microbiome without losing efficacy in managing the infection (Vidal et al., 2022).

The combination of antibiotic stewardship with microbiota-based therapy may enhance the outcomes of treatment of osteonecrosis in terms of treating the microbiota with the balance of antimicrobial therapy (Tan et al., 2020). This integration can be made easier with the implementation of several strategies, one of which is

the prescription of precision antibiotics, meaning that broad-spectrum empirical treatments are replaced with narrow-spectrum antibiotics based on the results of cultures, which should avoid disturbing the microbiota (Vidal et al., 2022). It has also been reported that coadministration with probiotics increases the bone mineral content and established that *Lactobacillus* and *Bifidobacterium* strain can restore the microbiota balance in case they are combined with antibiotics (Parvaneh et al., 2014). Prebiotic diets, calcium-based supplements are also dietary interventions that maintain diversity of gut microbes and enhance the performance of probiotics (de Sire et al., 2022). To improve the practical application of the proposed intervention, future studies need to consider the optimization of probiotic formulas, expedite antibiotic stewardship practice, and discover long-term advantages of microbiota manipulation as a method of managing osteonecrosis. The above strategies underscore the centrality of the GM in maintaining osteoimmunological pathways, systemic inflammation, and metabolic bone remodelling that are essential in the pathogenesis of osteonecrosis. These multifactorial interventions have the potential to restore microbial health, enhance bone integrity, and impede disease progression, positioning GM modulation as a viable therapeutic paradigm in osteonecrosis management (**Figure 3)**.

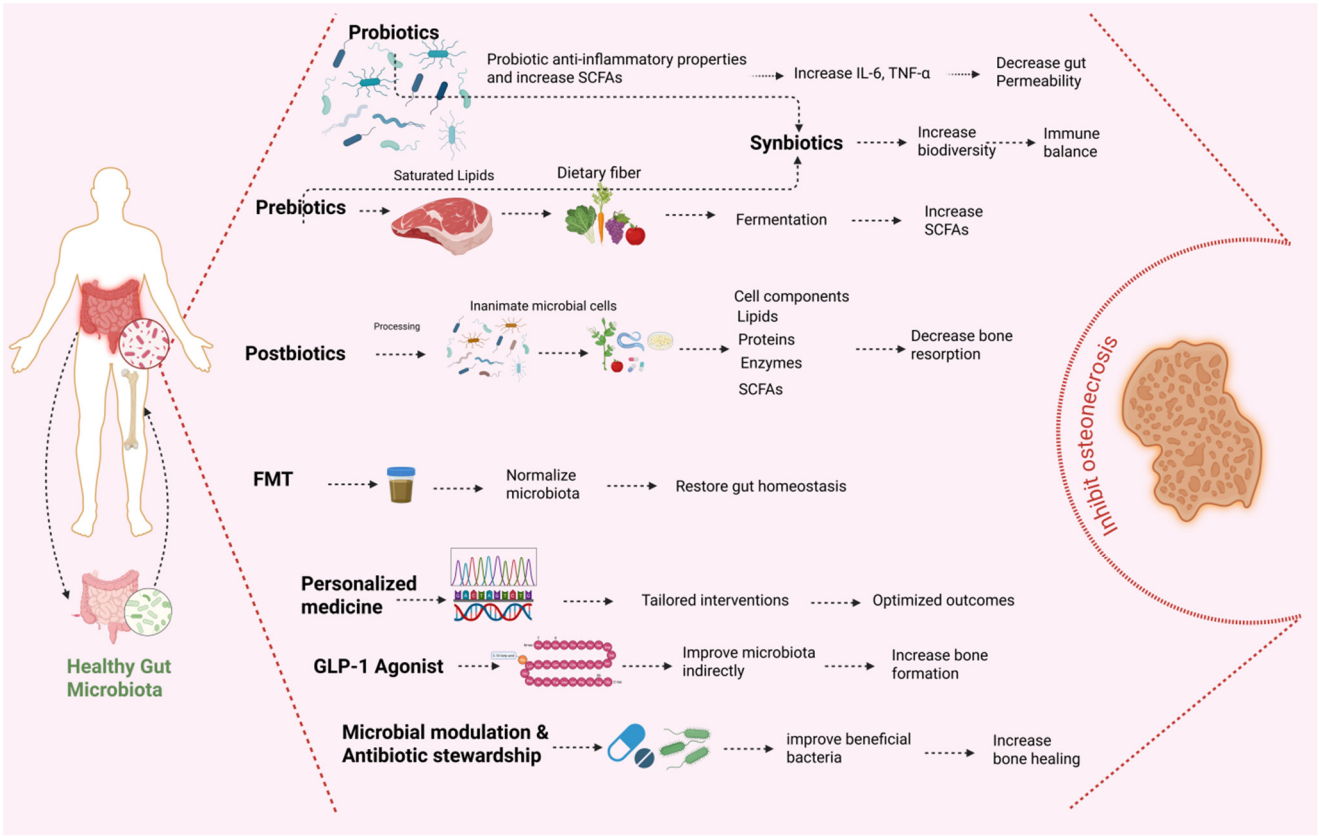

Figure 3. Overview of microbiota-based therapeutic approaches for mitigating osteonecrosis. This figure illustrates how various interventions as probiotics, prebiotics, synbiotics, postbiotics, FMT, GLP-1 receptor agonists, personalized microbiome-based therapies and microbial modulation act to restore GM balance, enhance intestinal barrier integrity, and regulate immune responses. These strategies promote the production of SCFAs, reduce pro-inflammatory cytokines such as IL-6 and TNF-α, and support bone remodeling processes, collectively contributing to osteonecrosis prevention. Dashed arrows indicate directionality of effects or relationships. This Figure created via BioRender.com.

6. Integrating GM into Osteonecrosis Management

The emerging recognition of the gut-bone axis requires a transition of the current osteonecrosis management toward an approach that encompasses microbial considerations within its diagnostic and

therapeutic models (Z. Li et al., 2024). It comprises the use of the latest technologies in microbial profiling, developing of robust biomarkers, and precision medicine strategies (Rello et al., 2018).

6.1 Microbial Signatures with Clinical Utility, Risk Stratification, and Validation Framework

Recent research highlights that gut microbial signatures hold strong potential as diagnostic and prognostic biomarkers for osteonecrosis, particularly in glucocorticoid- and alcohol- associated cases. Microbiota analyses revealed that a reduction in Firmicutes, increased Bacteroidetes, and lower levels of beneficial genera such as *Lactobacillus* and *Bifidobacterium* in osteonecrosis cases (Kim et al., 2024). In preclinical study, ONFH has shown that glucocorticoid treatment leads to a marked reduction in *Lactobacillus animalis.* Restoration of this strain, either via cohousing with healthy mice or oral supplementation, significantly protects against ONFH by promoting angiogenesis and osteogenesis and reducing apoptosis through extracellular vesicle signaling (C.-Y. Chen et al., 2022a). Likewise, in a rat model of GAON, the bacterial ecosystem depletion of Lachnospiraceae led to the decreased serum and fecal levels of butyrate, increased systemic inflammation (e.g., IL-17A, TNF-α) and ONHF incidence; these changes were restored by supplementation with sodium butyrate, allowing to minimize the disease progression (Guo et al., 2024a). Clinical studies of alcohol-induced ONFH have also revealed gut microbial imbalances, including increased *Klebsiella*, *Pseudomonas*, and *Holdemanella*, alongside disruptions in vitamin B6, retinol, and glycerophospholipid metabolic pathways (Yue et al., 2024a). These findings suggest that reduced abundance of protective taxa (e.g., *L. animalis*, Lachnospiraceae) and diminished SCFA production, particularly butyrate, may serve as early microbial indicators of ONFH susceptibility. In anthor study, High-throughput 16S rDNA sequencing has revealed distinct microbial shifts; alcohol-associated osteonecrosis is characterized by elevated level of *Roseburia*, whereas glucocorticoid exposure increases *Megasphaera* and *Akkermansia* (Q.-Y. Zheng et al., 2024a). Such signatures could facilitate patient stratification prior to high-dose corticosteroid exposure, enabling preventive measures like probiotic or SCFA supplementation. Microbial profiling may also support personalized treatment strategies by identifying specific taxonomic deficiencies, informing probiotic strain selection, adjusting dosing regimens, and quantifying dysbiosis severity (Bubnov & Spivak, 2023).

To clinically validate these microbial biomarkers, we would propose a multi-tiered framework beginning with prospective cohort studies that track serial stool samples using 16S rRNA or metagenomic sequencing combined with SCFA profiling, and that evaluate outcomes such as MRI-confirmed osteonecrosis, bone turnover markers (e.g., CTX, PINP), and inflammatory cytokines. This should be followed by randomized controlled trials testing microbiota-based interventions (e.g., *L. animalis* or butyrate supplementation), with primary endpoints including changes in BMD, osteonecrosis, cytokine profiles, and imaging-based bone assessments. Methodological standardization is essential across the research pipeline, from fecal sample stabilization and −80 ℃ storage, to consistent sequencing depth (≥20,000 reads/sample), validated bioinformatics platforms (e.g., QIIME 2, DADA2), and SCFA quantification via GC-MS. Furthermore, the establishment of well-defined clinical thresholds for α-diversity (e.g., Shannon index), and

taxon-specific cut-offs will be crucial to improve cross-study reproducibility and clinical interpretability. Despite the promise of microbiome-based diagnostics, several practical barriers remain, including high assay costs, inter-individual gut variability, regulatory complexity, and the growing demand for clinician proficiency in translating microbiome data into clinical decision-making. Overcoming these challenges; will be essential for the successful integration of microbiota-informed risk prediction and personalized intervention into routine osteonecrosis care.

6.2 Multiomics Approaches in Osteonecrosis Research

Building on this foundation, advanced multiomics technologies and machine learning approaches are now being used to deepen our understanding and enhance the precision of microbiota-based osteonecrosis management. Additionally, new multiomics methods, including metagenomics, metabolomics, and proteomics, provide us with significant understanding of host–microbiome relationships and their role in developing osteonecrosis pathophysiology (Seely et al., 2021). Metagenomic sequencing thus permits the detection of microbial communities without the process of culturing, which enables the study of microbiome diversity and that of functional gene expression (Neelakanta & Sultana, 2013). The research on biophosphate-related osteonecrosis of the jaw (BRONJ) has found distinctive microbial communities paired with high abundance of *Actinomyces* suggesting once more the role of dysbiosis in the development of osteonecrosis (Seely et al., 2021). Moreover, metabolomics demonstrated that the participants with osteonecrosis declare a shift in the SCFAs, tryptophan metabolites, and bile acids which regulate bone remodeling and immune response (Xu et al., 2022). The proteomics approach has shown the modifications of bone-related proteins, including RUNX2, important during osteoblast differentiation, and PTGS2 , the protein that plays a role in the inflammatory response, which further demonstrates the impairment of bone remodeling related to the microbiota (Cheng et al., 2021; Kawane et al., 2018).

6.3 Machine Learning & Precision Medicine Approaches

Combining high-throughput sequencing (HTS) and machine learning models will allow discovering the microbiome-generated biomarkers, which will transform the osteonecrosis diagnostic and treatment process (Cheung & Lin, 2023). Using the predictive modeling of the GM composition it is possible to detect the possibility of developing osteonecrosis early in the course of treatment with glucocorticoids thereby permitting the introduction of customised microbiota-based interventions, i.e. supplementation with probiotics working on a patients individual gut profile. Also, microbiome-derived biomarkers can be used to monitor progression of the disease in real-time and deliver information that can be used to treat disease and know how it responds to specific treatment. Moreover, machine learning-based microbiome profiling strategies are under development with integrative multiomics, particularly enabling researchers to correlate proliferation of microbial genes, production of metabolites, and inflammatory responses in the host, with severity of the disease, enabling new and more personalised forms of treatment (Bansal et al., 2023; Wu et al., 2024).

6.4 Biomarker Discovery and Clinical Translation

SFCA and tryptophan metabolites (e.g., indole) have been studied to serve as metabolic biomarkers that affect the functioning of osteoblasts and osteoclasts (Lu et al., 2021). Notebly, H_2S and polyamines also considered as emerging key players in osteonecrosis pathogenesis. Towards translating them to clinics, recent studies have tried to validate microbiome-based biomarkers on large-scale clinical trials, create a diagnostic panels designed according to an individual that incorporate microbiota sequencing, metabolomics, and host inflammatory markers as well as between-study harmonization and regulatory considerations of microbiome-based diagnostics in clinical trials (Scherz et al., 2021; Swann et al., 2020).

By integration of multiomics, microbiome profiling, and modeling approaches, the future of osteonecrosis management might shift towards the implementation of precision medicine strategies that allow addressing common issues early, engaging in personal therapies, and leading to better patient outcomes. The combination of these developments represents a paradigm shift in osteonecrosis research away towards the traditional symptom-focused diagnostics to a systemic research focus, based on a systems biology perspective, of the gut microbiome, host metabolism, and immune-related processes. Artificial intelligence models and multiomics technologies are enhancing the capability of solving complicated host-microbe relations, providing real-time and individualized predictive and management tools in diseases.

7. Ethical and Regulatory Challenges

The integration of microbiota-based treatments in the management of osteonecrosis also raises a number of ethical issues and concerns that should be resolved in effective way regarding the security, effectiveness and accessibility of care to the patients with the further development of microbiota-based therapies, it is important to make sure that patients completely comprehend the essence of the treatment (Chaudhary, 2024; Ogunjobi et al., 2024). Patients are required to know microbiota modulation uncertainties, the possible risks, and benefit. As the gut and the oral microbiome are complicated, the communication should be made evident to enable informed decision-making (Rajasekaran et al., 2024). Regarding patient autonomy, respect should be given by giving patients the option to undergo microbiota-based treatments (Jordens & Montgomery, 2018). This incorporates the authority to turn down treatment without any pressure or boundless impact by the health officials. Clinicians should create an atmosphere in which patients feel that they decide about how to be treated (Szalados, 2021).

The therapies involving microbiota remain quite experimental and require intense clinical studies to determine the safety and effectiveness of this method (Manrique et al., 2024). Ethical issues also concern the provision of therapeutical services that lack appropriate basis of well-designed studies. The regulatory bodies have to see to it that treatment is adequately tested before it can be administered to patients. The modulation of microbiota may have unintended side effects in form of dysbiosis or adverse effects (Hrncir, 2022). For instance, antibiotics are used to modify GM, but instead of improving the situation, might worsen the disease conditions, including osteonecrosis (de Sire et al., 2022).

Ethically it is necessary that microbiota-based therapies be available to all patients equally as the field becomes more widespread. Healthcare inequalities may make some people have unequal opportunities of

getting healthcare, which may be dependent on socio economic status or geographical location (Braveman et al., 2010). Parties involved in making policies and healthcare systems should strive to avail such therapies to different populations. Microbiota-based therapies may have large repercussions on finances (Hoffmann, 2019). High costs associated with probiotics, prebiotics, or advanced diagnostic tools may limit access for some patients. Ethical frameworks should address how to balance innovation with affordability in healthcare (Ilse, 2024). The rapid development of microbiota-based therapies poses challenges for regulatory agencies tasked with ensuring safety and efficacy (Khoruts et al., 2019). There is a need for clear guidelines governing the use of such therapies in clinical practice (Weisz et al., 2007).

8. Critical Evaluation and Emerging Framework

8.1 A Novel Microbiota-Immune-Bone Axis: Toward a Systems-Level Understanding of Osteonecrosis

Microbial dysbiosis, particularly the loss of beneficial taxa such as *Lactobacillus*, *Bifidobacterium*, *Roseburia*, and Lachnospiraceae, is associated with diminished production of key microbial metabolites (e.g., SCFAs), reduced calcium and vitamin D bioavailability, and increase intestinal permeability. These disruptions enable systemic translocation of microbial components such as LPS, triggering inflammatory cascades that impair bone vascularization, promote osteoclastogenesis, and suppress osteoblast activity. Whereas at the immune level, this dysbiotic state leads to dysregulation of Treg and Th17 cell populations within the bone marrow microenvironment, mediated in part by reduced SCFA levels and other microbial or dietary signals. This immune-nutritional crosstalk further disrupts bone remodeling and contributes to the vascular damage and apoptosis characteristic of osteonecrotic lesions.

The distinguish aspect of this framework is its integration of microbial, immune, and nutritional dynamics, and drawing attention to a broad spectrum of gut-derived mediators, ranging from SCFAs and microbial exopolysaccharides (EPS) to polyamines, H_2S, and dietary polyphenols that collectively influence bone health. This approach also provides a scaffold to evaluate microbiota-targeted interventions such as probiotics, prebiotics, polyphenol-rich functional foods, postbiotics, and FMT, as well as emerging metabolic modulators like GLP-1RAs.

Ultimately, the microbiota-immune-bone axis underscores the potential for noninvasive diet and microbiota based strategies to prevent or mitigate osteonecrosis. It also highlights the value of GM-derived biomarkers for early diagnosis and personalized nutrition approaches tailored to individual microbial profiles. Key of this effort is identifying microbial signatures with clinical utility to enable risk stratification and develop robust validation frameworks for diagnostic and therapeutic use. Future research should focus on integrating multi-omics tools, dietary intervention trials, and mechanistic models to translate this method into clinical solutions.

8.2 Critical Assessment of Current Literature: Limitations, Methodological Nuances, and Conflicting Findings

The increasing interest in the field of GM and osteonecrosis research, whereas it is characterized that this research direction has several inherent limitations, methodological complexities, and seemingly contradictory findings that warrant careful consideration. A thorough understanding of these aspects is crucial for advancing the field towards robust clinical translation.

8.2.1 General Limitations Hindering Translational Progress

One of the major challenges in diagnostic criteria and methodological protocols, which make it challenging to identify, categorize, and compare patient populations across studies is heterogeneity. It also limits the ability to generalize and makes it difficult to determine the clear causal links between individual microbial profiles and osteonecrosis or confirm the faithful microbiota-specific therapies. As a result, it becomes a laborious task to compile quality evidence on different studies, in a bid that could lead to universality in the results on the same that could be used in formulating clinical guidelines. Adding to this, large scale clinical trials to prove the effectiveness of microbiota-targeted interventions on human populations is required, and this is missing critical evaluation. Although several preclinical studies and small human trials have provided encouraging results about the nature of relationships in the gut-bone axis, it is not possible to reliably deploy them in routine clinical practice unless high powered and large-scale human trials are drawn upon. Although preclinical models are invaluable in describing mechanistic pathways they can often fail to establish the complex physiological and pathologic heterogeneity that occurs in humans. Likewise, the small sample size trials, although they initially demonstrate some potential, often lack statistical power to identify some weak therapeutic or rare undesirable effects, which are essential for the application of the specified clinical practice and its authorization. This dependency on pre-existing results is one step of the disparity between encouraging research and adoption in the clinical world.

Moreover, the heterogeneity of individual microbiomes is an obstacle in itself to the formulation of generalized treatment. Probiotics, prebiotics and nutritional interventions have variable therapeutic potential in different patients and are specific to the individual microbiome. This deep interindividual variation implies that the specific modulation of microbiota cannot be discussed as a one-shot solution, which is going to be effective with osteonecrosis across the board. It requires the basic transition to the strategies of personalized medicine, in which the enhanced microbial profiling methods and complex predictive modeling should be applied to modify an individual's specific microbial landscape. Finally, most existing literature focuses broadly on skeletal disorders, with limited specific exploration of microbiota’s role in osteonecrosis. As a result, current evidence remains fragmented, often extrapolated from related conditions such as osteoporosis or arthritis.

8.2.2 Methodological Nuances and Their Impact on Interpretation

Methods and data partitioning can affect the realism of results in computational linguistics, variations in experimental design within gut-bone axis studies can introduce considerable variability. For instance, the choice of animal model (e.g., GF vs. conventionally raised mice, different osteonecrosis induction models), the methods of sample collection, the sequencing technologies employed (e.g., 16S rRNA gene sequencing

versus whole-genome metagenomics), and the specific bioinformatics pipelines used for data analysis can all profoundly impact the observed microbial shifts or the strength of mechanistic links reported. This means that contradictory results in the literature might sometimes stem from these underlying methodological differences rather than fundamental biological discrepancies.

Statistical methods, such as alpha and beta diversity metrics, are commonly used to evaluate microbial richness and composition, though biases due to sequencing depth must be addressed (Finotello et al., 2016). Functional profiling tools like PICRUSt and HUMAnN predict microbial activity, but their results depend heavily on taxonomic classification accuracy (Ijoma et al., 2021). Confounding factors, such as diet and medication, must be accounted for in the analysis, and multiple testing corrections are crucial to control false discovery rates. The integration of these methodological considerations ensures that GM results are reliable and reproducible, although challenges in data interpretation persist (Behera et al., 2020).

Additionally, human studies are often underpowered, with weak causal inference in Mendelian randomization analyses and insufficient adjustment for confounders like age, diet, or metabolic conditions, while translational gaps persist due to overreliance on preclinical models that poorly mimic human physiology and a paucity of mechanistic data on immune-endocrine pathways (e.g., NF-κB/PI3K signaling) in human contexts (Taschler et al., 2022; Yao et al., 2024).

8.2.3 Conflicting Findings and the Nuance of Microbial Influence

Inconsistencies in reported effects of specific microbial taxa further complicate the field. For instance, some studies highlight *Lactobacillus* and *Bifidobacterium* as beneficial, while others show reduced abundance or no clear impact. Such discrepancies likely stem from differences in host species, disease models, microbiota profiling depth, or ecological interactions within the gut. At the immunological level, the gut-bone axis is mediated by complex interactions involving **Th17 cells, Tregs, dendritic cells, and inflammatory cytokines** (e.g., IL-17A, TNF-α, IL-6). However, studies rarely dissect these pathways in detail. While Th17 cells are typically pro-osteoclastogenic, Tregs exert anti-resorptive effects, yet the net immune response in osteonecrosis appears to depend on subtle shifts in microbial-derived metabolites (e.g., SCFAs, LPS) and host immune system. These **bidirectional and dynamic immune responses** are often oversimplified in current studies, leading to ambiguities in interpreting whether microbiota-induced immune modulation ultimately promotes or protects against bone loss. Likewise, future investigations must adopt multi-parametric approaches, combining immune phenotyping, metabolomics, and microbiome sequencing to fully capture the dynamics of this axis in osteonecrosis.

This axis and critical evaluation of the literature underscore both the *promise and complexity* of the gut-bone relationship in osteonecrosis. Addressing the limitations discussed above while leveraging systems-level approaches may pave the way for more precise diagnostics and personalized interventions in future clinical applications.

9. Conclusion

The literature review proposed a comprehensive microbiota-immune-bone axis framework, integrating microbiome, immunometabolic, and nutritional evidence to explain osteonecrosis pathology. GM profiling can also serve as a source of diagnostic biomarkers that may enable early diagnosis of osteonecrosis and the selection of an individualized strategy for management. Gut-derived metabolites (SCFAs, polyamines, H_2S) are crucial mediators of bone health, regulating bone remodeling by influencing nutrient absorption, osteoblast/osteoclast activity, immune responses, and cellular signaling pathways. Gut dysbiosis disrupts this gut-bone axis, leading to intestinal barrier dysfunction, systemic inflammation, oxidative stress, and altered bone marrow environments that promote bone resorption, impair formation, and contribute to osteonecrosis pathogenesis. Targeting the microbiome and its metabolites offers significant therapeutic potential for restoring bone homeostasis and developing treatments for bone disorders like osteonecrosis. The gut microbiome significantly influences osteonecrosis risk by modulating drug metabolism (e.g., glucocorticoids, bisphosphonates), where dysbiosis can exacerbate drug toxicity and bone damage, while a healthy microbiota offers protection. Microbiota-targeted therapies; including probiotics, prebiotics, synbiotics, postbiotics, dietary interventions, FMT, and GLP-1RAS; show promise for preventing and treating osteonecrosis by restoring gut balance, enhancing beneficial metabolites (SCFAs), reducing inflammation, and improving bone remodeling. Significant challenges remain for clinical translation, including strain-specific efficacy, interindividual variability, delivery/stability issues (especially for probiotics), safety concerns (FMT pathogen risk), regulatory hurdles (FMT classification), and the need for larger, standardized human trials.

10. Future Research Direction

While emerging evidence supports the therapeutic potential of GM modulation in osteonecrosis, more studies must be performed to translate these findings to clinical applications.

Large-scale human trials must be undertaken to determine the efficacy and safety of probiotics, prebiotics, FMT, and GLP-1RAS modulate the microbiota to prevent and treat osteonecrosis in corticosteroid-exposed individuals with metabolic disorders or dysbiosis-related inflammation.

Precision medicine strategies employing GM profiling to tailor dietary, probiotic, or pharmaceutical interventions to a patient's microbial community and bone health state may optimize therapeutic benefits.

Genetically engineered probiotics and other advances in microbiota engineering hold promise for customized bone-protective functions such as augmented production of SCFAs, improved calcium uptake, and targeted immune modulation to reduce inflammation-induced bone loss.

Future research must also study host-microbiome interactions in bone remodeling, investigate long-term outcomes of microbiota-directed therapies, and determine standard biomarkers of gut-bone axis dysregulation to permit early diagnosis and intervention.

Integrating microbiome research with regenerative medicine and precision therapeutics may revolutionize osteonecrosis management and create opportunities for microbiome-based treatment of bone diseases.

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Declaration of competing interest

No potential conflicts of interest were reported by the author(s).

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