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Review article

Microbiota *in vivo* imaging approaches to study host-microbe interactions in preclinical and clinical setting

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HIGHLIGHTS

- *In* vivo imaging can enhance knowledge of the host-microbiome interactions.
- Microbiota animal models enable investigating host-microbiome interactions.
- Bacteria can be isolated, labelled, and re-administered.
- Preclinical knowledge can be translated into the molecular imaging field.
- Molecular nuclear imaging is increasingly becoming a promising clinical approach.

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ABSTRACT

In vivo imaging in preclinical and clinical settings can enhance knowledge of the host-microbiome interactions. Imaging techniques are a crucial node between findings at the molecular level and clinical implementation in diagnostics and therapeutics. The purpose of this study was to review existing knowledge on the microbiota in the field of *in vivo* imaging and provide guidance for future research, emphasizing the critical role that molecular imaging plays in increasing understanding of the host-microbe interaction.

Preclinical microbiota animal models lay the foundation for the clinical translatability of novel microbiota-based therapeutics. Adopting animal models in which factors such as host genetic landscape, microbiota profile, and diet can be controlled enables investigating how the microbiota contributes to immunological dysregulation and inflammatory disorders. Current preclinical imaging of gut microbiota relies on models where the bacteria can be isolated, labelled, and re-administered. *In vivo*, optical imaging, ultrasound and magnetic resonance imaging define the bacteria's biodistribution in preclinical models, whereas nuclear imaging investigates bacterial metabolic activity.

For the clinical investigation of microbe-host interactions, molecular nuclear imaging is increasingly becoming a promising approach. Future microbiota research should develop selective imaging probes to investigate *in vivo* microbiota profiles and individual strains of specific microbes. Preclinical knowledge can be translated into the molecular imaging field with great opportunities for studying the microbiome.

1. Introduction

Complex multifactorial diseases caused by interactions between genetic and environmental factors often manifest themselves through epigenetic changes that primarily affect the immune system [1]. In recent years, changes in individual microbiomes and host-microbe interaction have been linked with a plethora of diseases, including cardiovascular diseases [2, 3], central nervous system disorders [4], metabolic syndrome [5, 6], and cancer [7]. Thanks to these exciting advances that have changed our perception of the microbiome, researchers need to understand how resident microbes contribute to immune system development and host defence through their host-interacting genes, proteins, and metabolites [8, 9]. Several studies revealed some of the fundamental mechanisms underpinning collective behaviours in bacterial populations. An emerging theme is that bacterial-host cross-talk is not univocal but involves the complex interactions of various microbial cell surface–derived molecules with various host receptors in different cell types, affecting host metabolism and immunity. Surface immunomodulatory

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macromolecules emerged as critical factors in inflammatory bowel disease as they serve an essential function in maintaining healthy commensal-host interactions [10].

In an attempt to elucidate bacterial-host cross-talk mechanisms, the development of imaging procedures has gained considerable interest as a fundamental approach to addressing various microbiology aspects. Advanced labelling methods have opened up new ways to studying bacteria with different imaging techniques. Reporter gene-based techniques have been successfully applied to bacteria: acoustic reporter genes to be detected with ultrasound imaging [11], luciferase reporter genes for bioluminescence imaging [12], and fluorescence reporter genes for fluorescence imaging [13]. Metabolic labelling has been applied, taking advantage of bacterial pathways to place specific labels within bacterial components [14, 15, 16]. Bacteria have also been labelled following a nanotechnology approach [17].

One limiting aspect in this field is the complexity harboured by the microbiota in terms of the number of diverse microorganisms and of spatial organization, especially regarding the gut microbiota [18]. Labeling techniques frequently only enable the study of a small subset of microorganisms. There are a few instances of attempts to image the whole microbial landscape [19]. Although the term "gut dark matter" is frequently used to describe how many gut bacteria cannot be cultivated, it may also be used to describe the enormous challenges associated with seeing these microbes in the intestines. Over the past several years, significant efforts have been made to create appropriate and adaptable chemical and biological methods for imaging gut microbes [20].

This review will highlight recent advances in using quantitative imaging approaches to characterize individual microbiomes and host-microbe interactions *in vivo* in preclinical and clinical settings.

2. Preclinical imaging of microbiota

2.1. Mouse microbiota models

There are two main types of animal models to investigate the role of the microbiota on physiology and disease, germ-free (GF) models and antibiotics treatment regimens, and each has strengths and limitations (Figure 1) [21].

Germ-free mice are raised in isolators without exposure to microorganisms to keep them free of bacteria, viruses, fungi, and eukaryotic pathogens. GF mice have been developed to accurately study the absence of microbiota or for the generation of gnotobiotic animals in which a defined microbiota can be transferred under stringent control. Studies with GF animals have validated the critical role of the microbiota in instructing the development and function of the immune system [22]. Setting up and maintaining these animal models requires highly specialized facilities, and the cost and expertise needed to manage them can be very challenging for the lab animal community. Moreover, GF animals are broadly impaired in the structural and functional development of the immune system, display an aberrant intestinal epithelial morphology and present gross physiological abnormalities, including an enlarged cecum and reduced gastrointestinal motility [23].

The antibiotics have been implemented to overcome some of the challenges associated with the GF animal model. Broad-spectrum antibiotic treatment can be applied to deplete the gut microbiota of mice of all genotypes and under different conditions. In mice, antibiotics can reduce bacterial populations that have been present since birth. Alternatively, other studies administer antibiotics to pregnant dams' drinking water to reduce the transfer of microorganisms from the mother to the

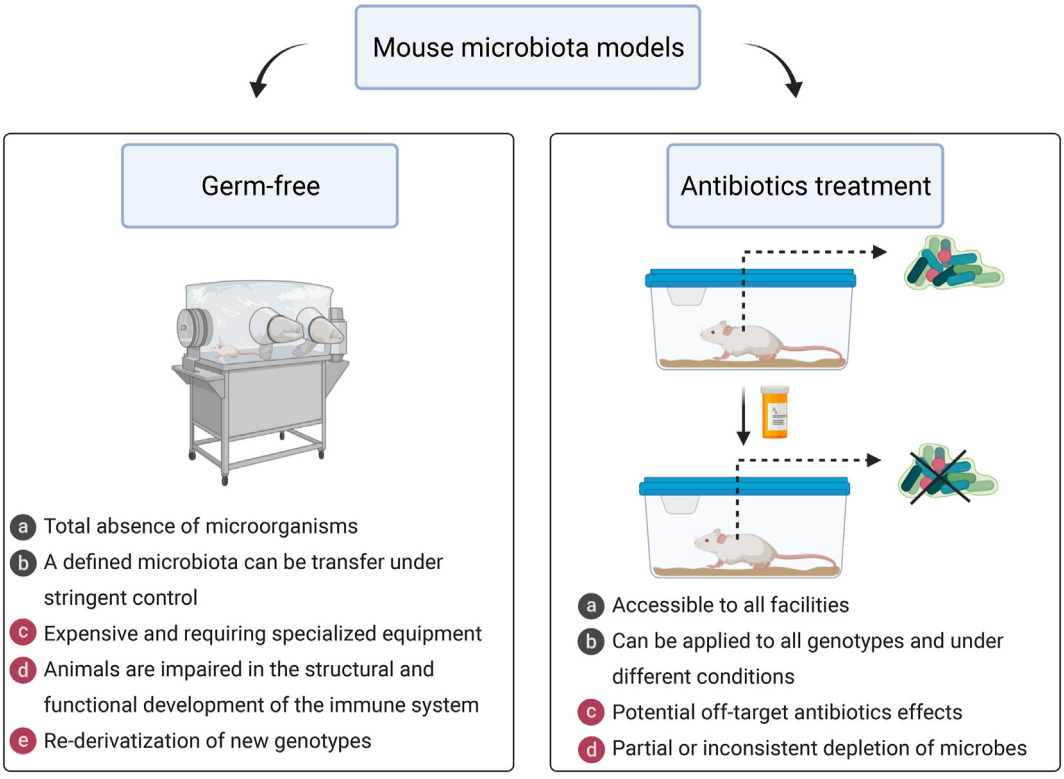

Figure 1. Mouse microbiota models. The advantages (black circle letters) and drawbacks (red circle letters) of germ-free and antibiotic-treated mouse model systems are compared. Germ-free mice are grown in isolators that completely isolate them from microorganisms in order to maintain them free of detectable bacteria, viruses, and eukaryotic germs. Germ-free mice allow researchers to explore the complete absence of germs or to create gnotobiotic animals that are only colonized by identified bacteria. However, the expense, effort, and expertise necessary to maintain these mice make them unavailable to many researchers. Germ-free animals are frequently compromised in many areas of development and early immunological education. Antibiotic therapy has developed as an alternative option to avoid some of these problems.

fetus, and then they keep the animal on the regimen during weaning to examine the impact of bacterial depletion early in development [24]. Different elements of the microbiota can be selectively eliminated by antibiotics owing to differences in their mechanisms of action. Individual antibiotics can be employed to alter the gut microbiota's composition in order to uncover bacterial groups that are relevant to various phenotypes. In contrast, the gut microbiota can be extensively depleted using a mixture of various antibiotic classes. Different regimens with varying antibiotic combinations, dosages, and lengths of treatment have been utilized by researchers [21].

However, while antibiotic treatment provides an accessible alternative to germ-free models, it is crucial to consider any potential off-target antibiotic effects and partial or inconsistent depletion of microbes [21]. Additionally, the high interlaboratory variability associated with antibiotics treatment regimens makes it more challenging to compare results than germ-free mouse studies.

In the light of these considerations, demonstrating causality and understanding how microbiota disorders contribute to immunological dysregulation and inflammatory diseases will require the broader adoption of animal models. Indeed, these animals can be subjected to rigorous controls for the host's genetic landscape, microbiota profile, individual strains of specific microbes, diet and antibiotic regimens.

2.2. Bacteria labelling and imaging approaches

There is a critical need for experimental models that can investigate the dynamic and physiologically significant interactions between the human host and the complex populations of aerobic and anaerobic bacteria. Current preclinical imaging of gut microbiota relies on models where the bacteria can be isolated, labelled, and re-administered (Figure 2). The visualization process employed to define bacterial biodistribution is based on the labelling method applied: optical imaging, which includes Bioluminescence Imaging (BLI) and fluorescence imaging, Magnetic Resonance Imaging (MRI), nuclear imaging techniques such as Positron Emission Tomography (PET) and Single-Photon Emission Computed Tomography (SPECT), and ultrasound. Future advances that would be a welcome improvement over the current approach include: i) the ability to label different targets at the same time in order to differentiate distinct bacterial strains; ii) targeting specific factors that are selectively produced by bacteria, such as bacterial toxins; iii) identification of bacterial species of interest, being able to provide insights into the causal relationship between dysbiosis and diseases and determine whether dysbiosis is truly causative or merely a consequence of inflammation [25]; iv) development of new methods for co-culturing a complex living human gut microbiome, including obligate anaerobes that require strict anaerobic conditions [26].

The labelling principles of the tracers for microbiota are reported in Figure 3. Different technologies can track genetically modified bacteria longitudinally, but each has its own limitations. For example, luciferases and most fluorescent proteins require oxygen generation, and the hypoxic environment in the intestine severely limits their use in imaging the microbiome *in vivo* [13]. Furthermore, *in vivo* bacterial imaging using bioluminescence detection frequently lacked adequate spatial resolution [27]. Bacteria modified with acoustic reporter proteins, which could create hollow nanostructures within bacteria and respond to ultrasound detection, demonstrated better spatial resolution and anaerobic environment adaptability [28]. Despite these benefits, the adoption of this technology, like other genetic engineering strategies, is limited to genetically modifiable bacteria, which now account for a very small proportion of gut bacteria. As a result, chemical labelling approaches have been developed. Metabolic labelling of bacteria allows the application of fluorescence imaging and nuclear imaging using unnatural amino acids, unnatural sugars, and stable isotopes. As a traditional method in chemical biology, metabolic labelling involves synthetic precursors or mimics of natural substrates that have been chemically labelled. These substances are then integrated into biomolecules by the

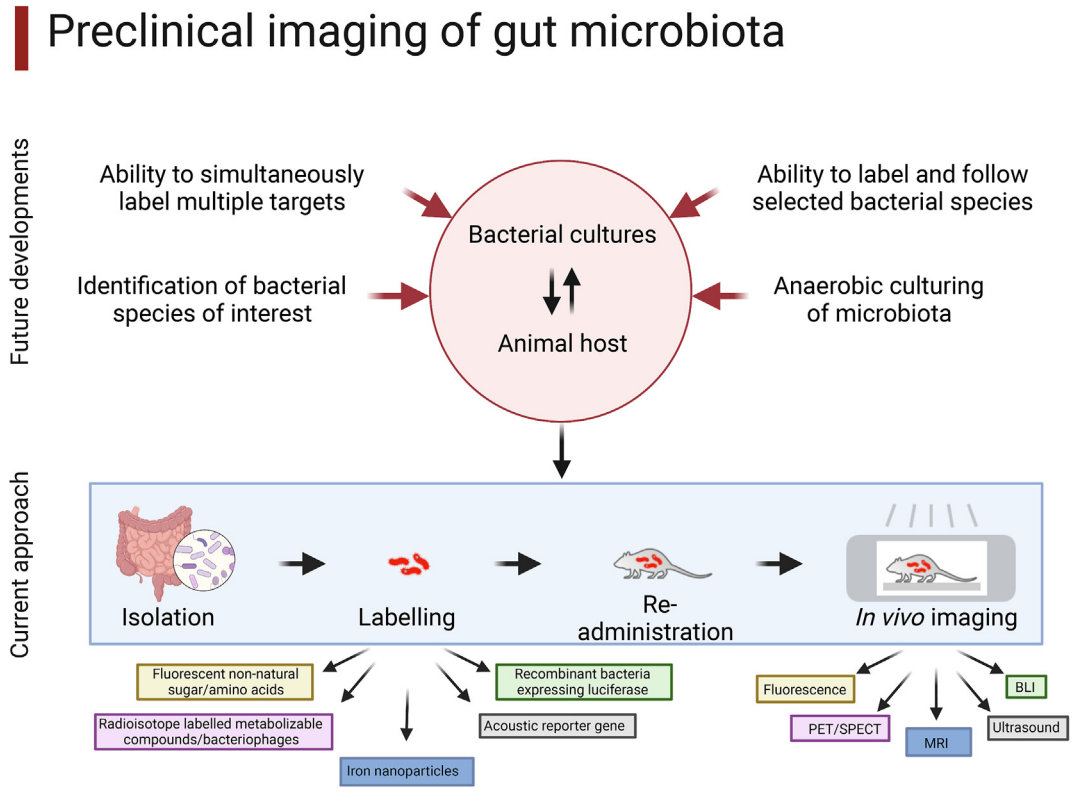

Figure 2. Preclinical imaging of microbiota: current approach and future developments. There is a great need for experimental models that can examine dynamic and physiologically relevant human host-microbiome interactions in complex populations of human aerobic and anaerobic microbiota. Current preclinical imaging of gut microbiota relies on models where the bacteria can be isolated, labelled, and re-administered.

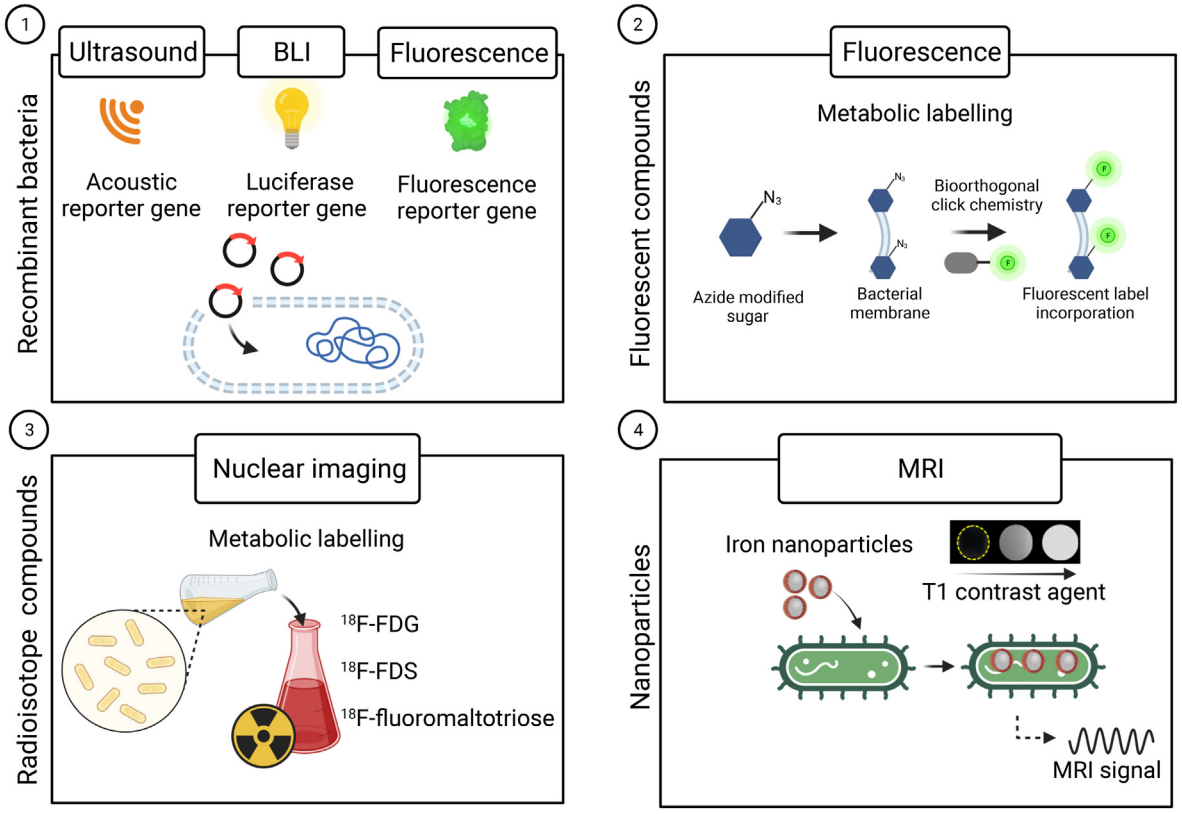

Figure 3. The labelling principles of the tracers for microbiota *in vivo* imaging. Advanced labelling methods raise the prospect of studying bacteria with different imaging techniques. 1) Reporter gene imaging is a key part of molecular imaging. A reporter protein can induce a specific signal that is detectable by an imaging device. Three reporter gene-based techniques have been successfully applied to bacteria: acoustic reporter genes to be detected with ultrasound imaging, luciferase reporter genes for bioluminescence imaging, and fluorescence reporter genes for fluorescence imaging. 2) Metabolic labelling takes advantage of bacterial pathways to place specific labels within bacterial components. When grown in the presence of a synthetic sugar or a D-amino acid variant elaborating an azido (-N3) group, diverse bacterial species are able to take up the synthetic molecule, incorporate it within the bacterial membrane, and successfully display this azido-modified structure on the cell surface. Accessible azide groups in this metabolically labeled membrane can then be covalently reacted to a fluorophore-linked alkyne to undergo cycloaddition, thus fluorescently labeling the membrane (biorthogonal click chemistry). 3) Metabolic labelling of bacteria with radioisotope-based compounds allows the application of nuclear imaging techniques such as PET and SPECT. 4) Bacteria can be labelled with iron nanoparticles, allowing visualization by MRI. PET: Positron Emission Tomography. SPECT: Single-Photon Emission Computed Tomography. MRI: Magnetic Resonance Imaging.

endogenous biosynthetic machinery of the bacteria. Bacteria can be labelled with iron nanoparticles, allowing visualization by MRI. As shown in Table 1, each labelling/modality offers its own unique benefits and intrinsic limitations.

2.3. Optical imaging

For optical imaging, a number of contrast mechanisms are provided, including light absorption, scattering, fluorescence, and bioluminescence (for a review, see Ntziachristos et al. [29]). Elegant methods for creating fluorescent molecular probes have been reviewed [30, 31]. After a single injection of the imaging agent, optical signals can be observed in tissues for several weeks. Controlled release of enzyme substrates can be used in BLI to prolong light emission during longitudinal studies.

BLI technology is emerging as a powerful tool for real-time monitoring of bacteria labelled by luciferase as a reporter gene. BLI systems emit visible light due to the luciferase-mediated oxidation of a luciferin substrate in cells with no background bioluminescence, thus allowing sensitive detection. *In vivo* imaging is commonly performed with endogenous ATP and requires only exogenous administration of the luciferase substrate. Gregor *et al.* reported an optimized *Photorabdus luminescence* lux operon, which encodes the genes necessary for luciferase and substrate production, significantly improving the brightness of bacterial bioluminescence [32].

Fluorescence tracers have emerged as a promising strategy for bacteria imaging in preclinical models. This method allows longitudinal and real-time visualization utilizing intravital two-photon microscopy and non-invasive whole-body optical imaging, the absence of ionizing radiations, and cost-effectiveness (Table 1). Conventional fluorescent labelling based on genetic techniques that target proteins is accessible, but its application is limited because fluorescent proteins require aerobic conditions, and most gut commensals are anaerobes [33]. As an alternative to proteins, bacterial-derived polysaccharides have been investigated primarily for chemical labelling approaches suitable for anaerobic bacteria, which represent the vast majority of the gut microbiota and are incompatible with traditional labelling methods. Moreover, bacterial-derived polysaccharides have been investigated for their pivotal role in host-microbe interaction. Over the last three decades, interactions of the intestinal commensal *Bacteroides fragilis* with the host immune system have been analysed in detail, identifying unique immunomodulatory effects of commensal-expressed polysaccharides (CPSs) [34].

Kasper's group reported successive breakthroughs in metabolic labelling combined with biorthogonal click chemistry that has proven successful for the selective *in vivo* imaging of bacteria independently of host factors and secondary pathologies (see Figure 3) [16, 35]. In a first study, the metabolic incorporation of a non-natural sugar, N-azidoacetylgalactosamine (GalNAz), was applied to tag and trace the CPSs of various commensal anaerobes, including *B. fragilis* [35]. In a follow-on study, a more versatile method was developed that uses non-natural fluorescent D-amino acids to specifically tag up to three prominent surface immunomodulatory macromolecules in live anaerobic commensal

Table 1. Advantages and disadvantages of common imaging modalities in preclinical imaging of microbiota.

Imaging modality	Labelling methods	Advantages	Disadvantages	Ref
BLI	Recombinant pathogens, genetically engineered to express a luciferase enzyme.	It provides temporal and spatial information regarding labelled bacteria and their metabolic activities.	It is necessary to administrate an exogenous substrate (i.e., luciferin).	[12]
		Bioluminescence signal is proportional to bacterial load.	Limited penetration depth.	
MRI	Iron nanoparticles (IONPs).	This cell labelling method can easily be transferred to other bacterial species.	Each cell division step continuously leads to a dilution of IONPs and their relative MRI signal.	[17, 39]
		It is independent from metabolic activity and oxygenic environment.		
		MRI can simultaneously track bacteria and provide information on the morphology of organs and the inflammatory response.		
		It is not subject to limited penetration depth.		
Fluorescence	Metabolic incorporation of i) non-natural sugar (GalNAz); ii) non-natural fluorescent D-amino acids (FDAA).	FDAA provides the ability to track simultaneously three bacterial components (PNG, LPS, CPS).	Each cell division step continuously leads to a dilution of fluorescence signal.	[71]
		Fluorescence signal can be followed by intravital two-photon microscopy and whole-body imaging.		
		High sensitivity.	Limited penetration depth.	
		Lifetime analyses improve specificity.		
Ultrasound	Acoustic reporter gene or ARG	Cells expressing ARG become ultrasound-invisible when high-pressure pulses are applied. Acoustic signals' sources can be verified and background can be subtracted using the ARG-based contrast's ability to be erased in situ.	ARG detection should be enhanced beyond the level seen in this initial investigation.	[11]
		Acoustic multiplexing can be performed using genetic variants of gas vesicles that collapse at different pressures.	It will be helpful to adapt ARGs to a wider variety of bacterial hosts.	
		Deep tissue penetration and high spatial resolution.	It might be necessary to modify the ARG cassette in order to express ARGs in Gram-positive species.	
			It will be important to maintain ARG construct stability.	

bacteria: peptidoglycan (PNG), lipopolysaccharide (LPS) and CPS [16]. The ability to simultaneously track three bacterial components involved in a complex immunomodulatory process will underpin further investigations of the progression of intestinal diseases, including inflammatory bowel disease and colorectal cancer.

Recent developments in the second near-infrared (NIR) window (NIR-II, 1000–1700 nm) fluorescence have shown that NIR-II imaging may achieve deep tissue penetration (up to 20 mm) with improved temporal and spatial resolution [36]. The design of a second near-infrared (NIR–II)–based approach for *in vivo* imaging of gut bacteria is reported in a recent study [37]. Using D-propargylglycine in gavage and then a click reaction with an azide-containing NIR-II dye, the gut microbiota of a donor mouse were efficiently labeled with NIR-II fluorescence on their peptidoglycan. With high spatial resolution and deep tissue penetration under NIR light, the bacteria could be located in the recipient mouse intestine.

2.4. Magnetic resonance imaging

MRI's characteristics enable the acquisition of high-resolution anatomical and functional images of multiple organ systems, making it well-suited to the molecular imaging objective, namely the imaging of processes at the cellular and subcellular levels [38]. However, detection of events at this level frequently necessitates nanomolar sensitivity, which precludes the use of standard gadolinium chelates as molecular MR imaging agents due to their micromolar sensitivity. The primary approach to conventional gadolinium chelates' insufficient sensitivity has been to create innovative MR contrast agents with much higher relaxivities. They include superparamagnetic iron oxide nanoparticles.

High-resolution MRI can trace bacteria while also providing information on organ morphology and the inflammatory response. Although specific bacteria can be detected only with specific probes, the presence of pathogens can be indirectly detected by MRI through the detection of oedema and modifications in local tissue parameters such as relaxation time, water content, or diffusivity [39].

A recent investigation into the longitudinal and transverse relaxation rates of MRI revealed notable variations across several bacterial strains [40]. Common commensal strains exhibit noticeably high MRI relaxation rates, which is partially explained by their high cellular manganese levels. Compared to other species, *Lactobacillus crispatus* exhibits particularly high values, about 10-fold more signal than the background signal in the relevant tissue, and a linear relationship between relaxation rate and the fraction of living cells.

Contrast-enhanced magnetic resonance imaging in microbiology is a relatively recent approach, with a limited number of reports in the scientific literature [41]. Among contrast-enhanced MRI applications for bacterial imaging, iron oxide nanoparticles designed to label *S. aureus* bacteria were described to track bacteria longitudinally during infection in a preclinical animal model [17]. The majority of investigations are carried out with the goal of creating tools for visualizing infections. However, the labelling approach described in these studies may be applied to additional bacterial species in the context of investigating the microbiota.

2.5. Ultrasound

Bourdeau and colleagues developed engineered gas vesicle gene clusters as the first reporter genes for ultrasound, enabling this widely used noninvasive imaging modality to visualize genetically modified bacteria within living animals [11]. When the eight chaperone and assembly factor genes from *B. megaterium* were co-expressed with the two primary structural genes from *Anabaena flos-aquae*, gas vesicles were created that rendered *E. coli* "visible" under ultrasound. The first acoustic reporter gene was a construct termed ARG1. A family of ARGs has been created (Table 1). Cells expressing ARGs become ultrasound-invisible when high-pressure pulses are applied. Therefore, acoustic signals' sources can be verified and background can be subtracted using the ARG-based contrast's ability to be erased *in situ*. A multiplexing approach can be applied with this technique. Indeed, by delivering acoustic pulses of progressively increasing amplitude and observing the disappearance of backscattered signal, gas vesicles with various critical collapse pressures may be discriminated against one another. The collapse of one group of gas vesicles is followed by that of another, and so forth. The contribution

of each population to the overall signal may then be calculated using a signal processing paradigm similar to spectral unmixing.

The sensitivity of ARG detection has to be increased above the level demonstrated in this preliminary investigation in order to be used in imaging a wider variety of cell types and biological contexts. Furthermore, it will be crucial to preserve ARG construct stability for studies including in vivo colonization.

2.6. Measurement of in vivo bacterial metabolic activity

PET and SPECT represent functional imaging approaches that can be used both as translational methods to measure *in vivo* bacterial metabolic activity.

Bacteria-specific imaging with radiotracers is based on variations in metabolism, structure, or mechanism between mammalian and bacterial cells. Small compounds targeting carbohydrate metabolism, bacterial folate biosynthesis, iron transport, D-amino acids, and antimicrobial peptides are among the radiotracers being developed for bacterial imaging.

The gut microbiota produces short-chain fatty acids (SCFA) primarily from the fermentation of indigestible carbohydrates, and SFCA is the primary nutrition for colonocytes, according to recent research comparing germ-free to conventionally raised mice [42]. As a functional imaging technique, intestinal 2-deoxy-2-18F-fluoro-D-glucose (^{18}F-FDG) absorption can be utilized to monitor the metabolic change in the human host that results from a decrease in gut bacterial load, which stimulates SCFA synthesis. Because it can collect at sites of sterile inflammation or other lesions, ^{18}F-FDG imaging has a low ability to distinguish sterile inflammation from bacterial burden, resulting in a high percentage of false positives [43, 44]. In a preclinical model, the phosphorylated analogue ^{18}F-FDG-6-P showed a specific uptake and retention by *S. aureus* through hexose phosphate transporters, absent in human cell membranes [43]. Additionally, 2-deoxy-2-^{18}F-fluoro-D-sorbitol (^{18}F-FDS) can be used to increase labelling specificity, with a higher affinity for Gram-negative bacteria [19]. Among Gram-negative bacteria, Enterobacteriaceae are the most typically expanded symbionts during disease states such as antibiotic treatment, IBD, colorectal cancer, obesity, and celiac disease [45]. ^{18}F-FDS can efficiently discriminate infections induced by *E. coli* or *K. pneumoniae* from sterile inflammation in mice models [46, 47]. It should be noted that ^{18}F-FDS has been effectively used as a gut microbiota tracer, allowing for an assessment of the entire gut bacterial composition rather than just a few species. Furthermore, PET-CT imaging in combination with ^{18}F-FDS has yielded encouraging results for clinical applications since the spatial resolution of the imaging improved as the animal models were extended from mice to hamsters [19].

Second-generation tracers for the maltodextrin transporter, i.e., 6″-^{18}F-fluoromaltotriose, were synthesized to image bacterial infections without targeting inflammatory lesions. These tracers improved pharmacokinetic profiles in preclinical models, and a wide variety of maltose analogues could be considered in tracer design [48, 49].

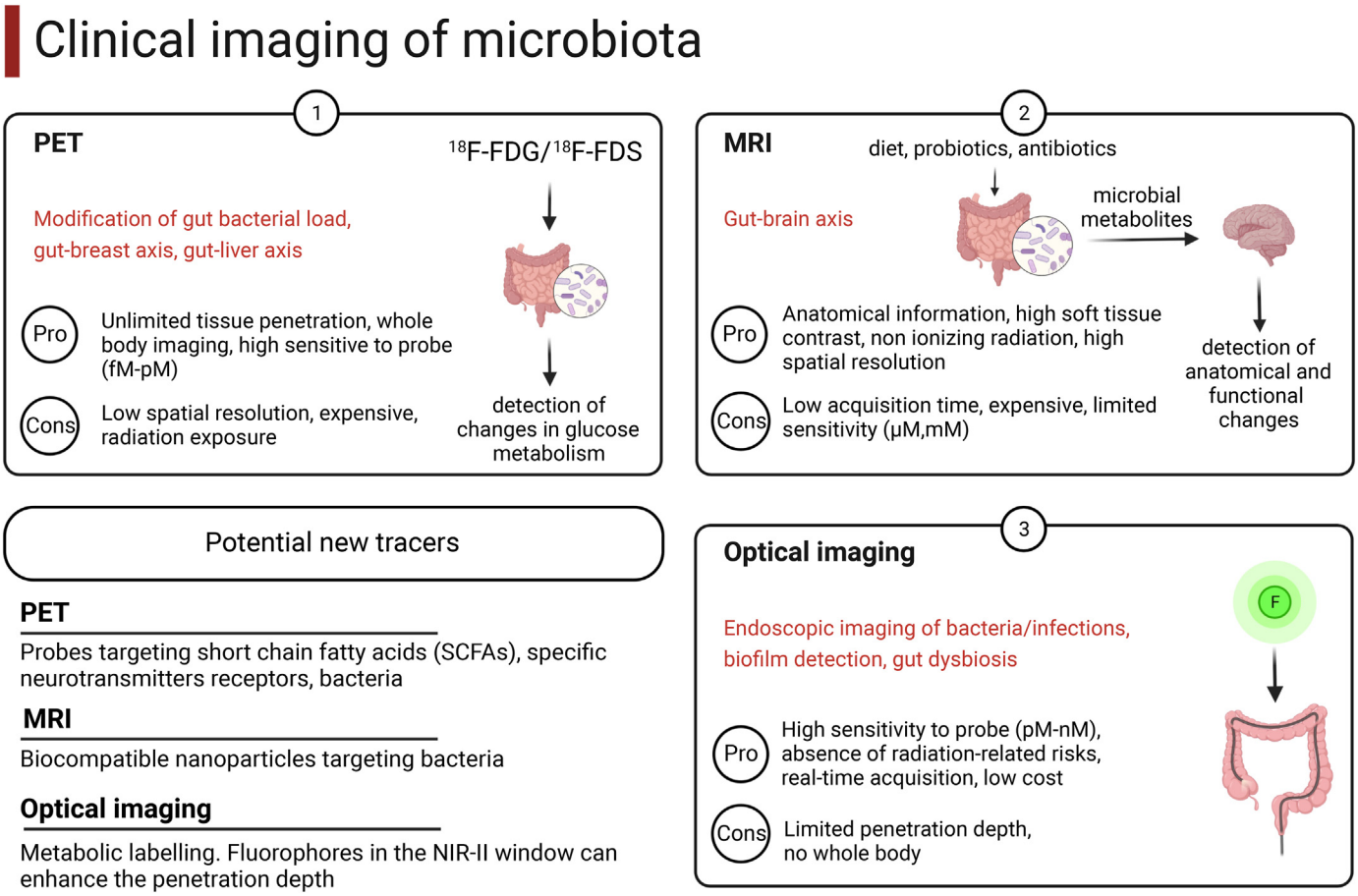

Figure 4. Clinical imaging of microbiota. 1) Some of the compounds tested in preclinical imaging may be readily applicable for PET imaging of gut microbiota. For instance, colonic ^{18}F-FDG or ^{18}F-FDS uptake may represent a novel imaging approach to capture the functional consequences of the microbiota-host interaction, providing information on gut bacterial load, gut-breast axis, and gut-liver axis. 2) We may explore bidirectional neurohormonal communication that integrates host gut and brain signaling using MRI's capacity to identify anatomical and functional changes. 3) Because optical imaging has limited tissue penetration capacity, it can be used in an endoscopic context. The chemical versatility of bacteria labelling is an advantage. Targeting SCFAs metabolism and neurotransmitter receptors can lead to the development of new potential PET tracers. The labelling of bacteria by nanoparticles holds potential for MRI. Dyes in the NIR-II window that provide better tissue penetration are attractive in optical imaging. PET: Positron Emission Tomography. MRI: Magnetic Resonance Imaging.

Preclinical PET-CT enables the assessment of the beneficial effects of gut-microbiota transplant. Among the recent studies, Wang et al. used metabolic labelling with ^{64}Cu and PET imaging to study the ability of *B. fragilis* transplanted *via* oral gavage to restore the tumour response to anti-PD-1 therapy in a mouse breast cancer model [50]. Recently, PET radiotracers specific to bacteria and not to mammalian cells have been identified in D-amino acids. These amino acids are not metabolized by mammalian tissues but are incorporated by living bacteria. In a mouse model of acute bacterial myositis and in rodent models of *P. aeruginosa* pneumonia, a modified D-amino acid was accumulated only by living microorganisms. These tracers could be used for clinical purposes to address a number of important human infections and, because they are sensitive to all or most species of bacteria, to study complex microbial environments [14, 15].

2.7. Clinical imaging of microbiota

Clinical imaging approaches to visualize bacteria *in vivo* were initially developed to support the diagnosis of infection [51]. These techniques include CT and MRI for anatomical/structural information and SPECT or PET for metabolic/functional data [39, 52]. Frequently, there is significant overlap in the imaging data of infectious and non-infectious conditions using these tools due to the contrast agents low functional specificity, which relies on secondary inflammatory changes to localize the disease [51]. To address these issues, approaches targeting bacteria-specific metabolic pathways emerged as an essential tool in clinical imaging, with applications ranging from bacterial infection diagnosis to measurement of *in vivo* bacterial metabolic activity in the microbiome study (Figure 4).

Some of the compounds tested in preclinical imaging may be readily applicable for PET imaging of gut microbiota. For instance, several features make the ^{18}F-FDS-based imaging method readily suitable for clinical applications, directly labelling bacteria in their natural intestinal niche [19]. ^{18}F-FDS is synthesized by a single-step chemical reaction from an FDA-approved agent (^{18}F-FDG) [53], safely tested in humans as an intravenous infusion [54]. In a human pilot study, no adverse effects were observed up to 24 h post-injection of ^{18}F-FDS. Rapid clearance from the circulation through the urinary system was noted, suggesting an excellent potential for appropriate and effective imaging of bacterial infections *in vivo* [55].

A decrease in intestinal bacterial load generated by broad-spectrum antibiotics can be tracked in healthy humans by measuring an increase in ^{18}F-FDG uptake caused by a shift in colonocyte metabolism from SCFA lipolysis to glycolysis. As a result, intestinal ^{18}F-FDG uptake could be a unique imaging method for capturing the functional outcome of the microbiota-host interaction [56]. In breast cancer patients, Yoon et al. showed a variation of the intestinal ^{18}F-FDG uptake in concomitance with the change in the abundance of Ruminococcaceae [57], reinforcing the hypothesis that crosstalk between microbiota and oestrogen synthesis occurs and that microbial dysbiosis may be associated with developing hormone-related breast cancer. In another study, in healthy male patients, Kang et al. reported a rise in intestinal ^{18}F-FDG uptake in parallel with a low relative abundance of the unclassified Clostridiales. They observed a lower intestinal ^{18}F-FDG uptake than the liver was associated with a high quantity of *Klebsiella* [58]. The significant correlation between background intestinal uptake and bacterial load implies that intestinal ^{18}F-FDG uptake could potentially serve as a marker of mucosal inflammation and impaired intestinal barrier function. Future studies could evaluate the effect of alternative options for using different tracers, such as SCFA, to image additional metabolic pathways.

The gut microbiota plays a central role in the gut-brain axis: a bidirectional neurohormonal communication that integrates the host gut and brain signalling. This bidirectional communication includes the triad microbiota-gut-brain, linked with neuropsychiatric disorders and neurologic diseases [59, 60, 61]. In this context, functional magnetic resonance imaging (fMRI) is a promising tool in the attempt to identify differences in brain function in patients affected by different pathological conditions, before and after chronic ingestion of probiotics, after treatment with drugs or behavioural interventions, or dietary interventions [62, 63, 64]. In addition, fMRI can highlight further the underlying ability of microbiota-derived metabolites to influence the brain [65]. The other functional neuroimaging modality is PET, which can directly sensitively measure physiological and neurological processes. With radiopharmaceuticals targeting specific neurotransmitters receptors, or straight the bacteria [66, 67], PET has the potential to investigate the influence of gut microbiota profiles on particular brain regions governed by diverse signalling neurochemical pathways, including dopamine and serotonin [68].

For the mapping of the complexity of the microbiomes, a versatile tool has been identified in high-phylogenetic-resolution microbiome mapping by fluorescence in situ hybridization (HiPR-FISH) [69], which exploits the labelling of bacteria by combining up to ten fluorophores. HiPR-FISH can have broad applicability in human health. In particular, in investigating complex microbiomes in the gut, in the oral cavity or on implanted devices. In addition, it could be helpful for studies on gut-related disorders such as inflammatory bowel diseases. Furthermore, HiPR-FISH could allow the understanding of the role of the microbiota in the initiation and progression of tumours, e.g., colorectal cancer [70].

3. Conclusions

With the exciting advances related to the influence of gut microbiota on human health, the need has arisen to model it effectively. Specifically, the development of small animal models allows elucidating the mechanisms that shape the diversification of bacterial communities and tracking its dynamic changes due to interaction with diet, genotype/epigenetic profile, and immune-metabolic function. In this context, the development of preclinical imaging methods to label, follow and study selected bacterial species in a diverse microbiota represents a key area of translational research. In the clinical setting, imaging approaches targeting bacteria-specific metabolic pathways have emerged as an essential tool, and nuclear molecular imaging can provide unique information on microbiota-derived pathological conditions.

Declarations

Author contribution statement

All authors listed have significantly contributed to the development and the writing of this article.

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Data availability statement

No data was used for the research described in the article.

Declaration of interest's statement

The authors declare no competing interests.

Additional information

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