

Molecular Imaging of the Human Microbiome: Biotechnological Probes and Translational Applications in Inflammation and Oncology

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Abstract

Background: The human microbiome has an important role in physiological balance, immunological control, metabolism, and disease development. Although microbial changes have been related to inflammatory diseases and cancer, conventional culture and sequencing methods are limited to analysing specimens outside the body, lacking the spatial dynamics and metabolic activity of microorganisms in living tissues in real time. In vivo molecular imaging overcomes these limitations by allowing non-invasive visualisation of biological processes with microbiome-targeted radiological probes.

Objective: This review assesses radiological molecular imaging techniques and biotechnological probes for in vivo mapping of the human microbiome. It describes the technological, biological, and clinical translation obstacles and evaluates their usefulness in investigating host-microbe interactions spanning inflammatory and oncological disorders.

Methods: The microbiome, host-microbe interactions, molecular imaging, radiotracers, PET, SPECT, and associated diseases were the subjects of a systematic literature search across scientific databases. The imaging techniques, biological targets (such as microbial metabolism, enzymes, and transport systems), and clinical limits of experimental, translational, and review papers were assessed.

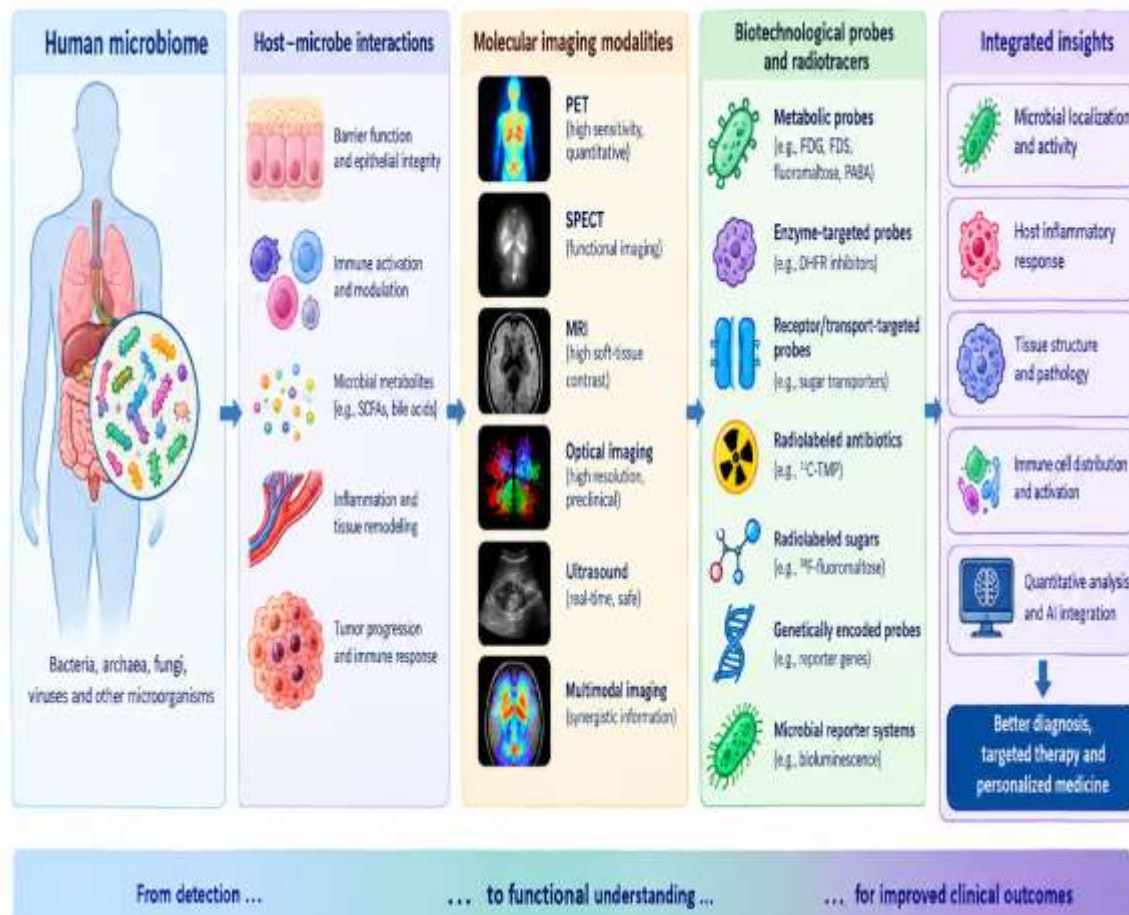
Conclusion: Biotechnology, microbiology, radiology, nuclear medicine, and precision medicine are all connected via microbiome molecular imaging. For non-invasive mapping of host-microbe interactions in inflammation and cancer, radiological probes have a great deal of promise. Improvements in probe specificity, sensitivity, safety, and quantification as well as integration with sequencing, metabolomics, multimodal imaging, and artificial intelligence are necessary to translate these techniques to clinical practice.

Keywords: Human microbiome; interactions between hosts and microbes; molecular imaging; radiological imaging; PET; SPECT; radiotracers; biotechnological probes; Microbiological imaging, tumour microenvironment, inflammation, and cancer.

Introduction

The human body is home to a wide variety of microorganisms known as the human microbiota. Numerous microorganisms, including bacteria, viruses, fungi, and archaea, can be found in the gastrointestinal tract, oral cavity, skin, respiratory tract, genitourinary system, and other anatomical locations. The collective term for these microbial populations' genetic and functional characteristics is "human microbiome." Rather than being passive inhabitants, microbes are essential for several physiological processes, including as food metabolism, immune system development, defence against pathogenic organisms, and the maintenance of the integrity of the epithelial barrier. The microbiome's significance in human physiology and disease biology is thus becoming increasingly recognised. [1–5].

Figure 1. Molecular Imaging Modalities for the Human Microbiome {Ogawa M, Takakura H. In vivo molecular imaging for biomedical analysis and therapies. Anal Sci. 2018;34(3):273–281. doi:10.2116/analsci.34.273}



The human body can therefore be considered a complex host-microbe ecosystem, in which microbial communities continuously interact with host tissues. Microbial metabolites, surface molecules, and secreted products can influence epithelial and immune cells, while host factors such as nutrient availability, mucus production, antimicrobial peptides, and immune responses regulate microbial survival and activity [2,4,6]. These interactions are particularly important at mucosal surfaces, where the immune system must maintain tolerance toward beneficial microorganisms while responding to potentially harmful organisms. Microbial products can activate pattern-recognition receptors and downstream signalling pathways, thereby influencing both innate and adaptive immune responses [6-10]. At the same time, changes in the host environment caused by inflammation, metabolic alterations, or tissue damage can modify microbial composition and behavior [10-12].

Human Microbiome and Its Biological Significance

The human microbiome is a dynamic ecosystem whose composition and function can vary according to anatomical location, age, diet, medication exposure, environmental conditions, genetics, and disease status. The gastrointestinal tract represents one of the most diverse and metabolically active microbial environments [3,4]. Gut microorganisms produce numerous metabolites and microbial products that can influence epithelial integrity, immune-cell activity, energy metabolism, and systemic physiological processes [1,2,13,14].

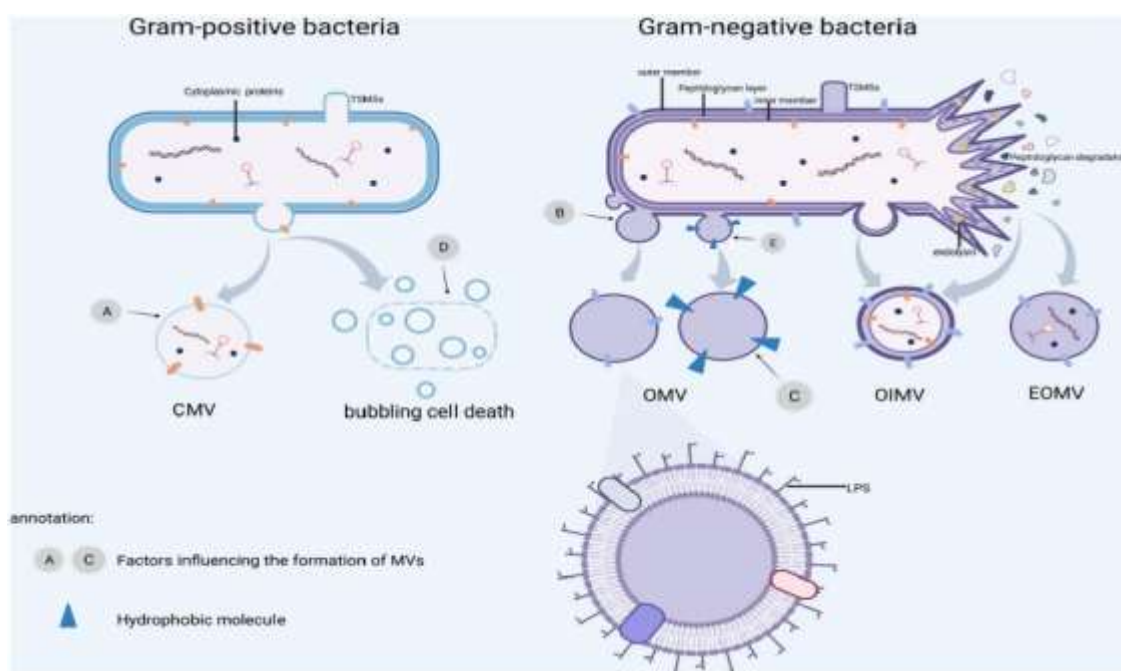
Advances in metagenomics, metatranscriptomics, metabolomics, high-throughput sequencing, and related omics technologies have greatly expanded our knowledge of microbial composition and function [5,10]. These techniques have demonstrated that alterations in microbial diversity and community structure, a condition known as dysbiosis, are associated with a number of clinical diseases. Microbial composition alone, however, cannot fully account for biological activity. Even within the same anatomical location, microbes' metabolic activity, geographic distribution, and interactions with host tissues may differ. Therefore, in order to completely understand the microbiome, it is necessary to know not only which microbes are present but also where they are found, if they are active, and how they interact with the host. [1,5].

Microbiome in Inflammatory Disease

It is becoming more well acknowledged that inflammation and the microbiota have a reciprocal interaction. Immune-cell activation, inflammatory signalling, microbial metabolite synthesis, and the integrity of the epithelial barrier can all be impacted by changes in microbial makeup or function. This interaction is especially important in conditions that cause inflammation of the mucosa, like inflammatory bowel disorders, where disturbance of the host-microbiome link may lead to tissue damage and ongoing immune activation [5–12].

Microbial components and metabolites can influence inflammatory pathways through interactions with host receptors and immune-signalling networks [6,7]. Conversely, inflammation can alter the local tissue environment by changing oxygen availability, nutrient concentrations, mucus composition, and antimicrobial activity. These changes may favour certain microbial populations and further modify microbial behaviour. Thus, microbial alterations may act as a cause, consequence, or amplifier of inflammation [4,5,10].

Figure.2 Biogenesis and structural diversity of bacterial membrane vesicles (MVs) in Gram-positive and Gram-negative bacteria (Kulp et al., 2015; Orench-Rivera and Kuehn, 2016; Roier et al., 2016)



Conventional imaging can demonstrate anatomical abnormalities and inflammatory changes, but it generally cannot determine whether an observed signal is caused by host inflammation, microbial activity, or both [18,21]. For example, ^{18}F -fluorodeoxyglucose (^{18}F -FDG) PET detects increased glucose metabolism but is not specific to microorganisms because ^{18}F -FDG can accumulate in metabolically active inflammatory and malignant cells [6]. This limitation has encouraged the development of more selective molecular imaging strategies capable of targeting microbial metabolic pathways or bacterial-specific molecular characteristics [21,22,27].

Microbiome and Cancer

The microbiome–cancer relationship has become an important area of biomedical research [5,6]. Microorganisms may influence different stages of carcinogenesis through chronic inflammation, epithelial barrier disruption, immune modulation, production of biologically active metabolites, genomic instability, and changes in the tumour microenvironment. Microbial communities may also influence responses to chemotherapy, radiotherapy, targeted therapies, and immunotherapy [7–9].

The tumour microenvironment is a complex biological system containing malignant cells, immune cells, stromal components, extracellular matrix, blood vessels, metabolites, and microorganisms. Microorganisms associated with tumours may interact directly or indirectly with these components and potentially influence tumour progression and

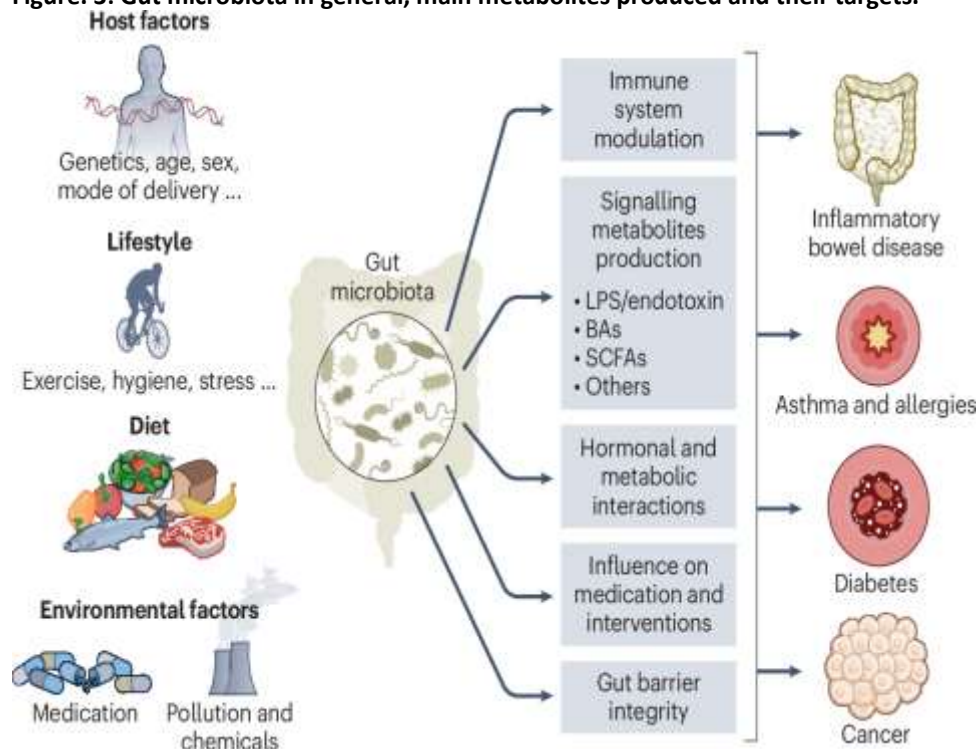
treatment response. This has led to increasing interest in the microbiome–cancer axis, which links microbial activity with tumour biology and host immunity [7–9].

Despite considerable progress, much of the available information regarding tumour-associated microorganisms is obtained from tissue sampling, sequencing, culture-independent molecular techniques, and other ex-vivo methods. These approaches can identify microbial signatures but provide limited information about the precise spatial distribution and real-time activity of microorganisms within tumours or surrounding tissues. Molecular imaging may therefore provide complementary information by allowing microbial distribution and activity to be investigated within living systems [17,18,20].

Limitations of Conventional Microbiome Assessment

Although sequencing and other omics-based methods have transformed microbiome research, they have several limitations. Conventional investigations generally depend on samples such as stool, saliva, mucosal material, blood, or tissue biopsies. While these specimens provide valuable information regarding microbial composition and genetic potential, they may not accurately represent the spatial heterogeneity of microorganisms within complex biological environments [5].

Figure. 3: Gut microbiota in general, main metabolites produced and their targets.



Limitations of Conventional Microbiome Studies (Lynch SV, Pedersen O. The human intestinal microbiome in health and disease. N Engl J Med.)

An additional challenge is distinguishing microbial presence from microbial activity [1,5]. Detection of microbial DNA or RNA does not necessarily demonstrate that the corresponding microorganism is metabolically active at the time of sampling. Similarly, relative abundance measurements may not accurately represent the absolute microbial burden or its functional contribution to disease. Bulk sequencing can also make it difficult to determine the spatial relationship between microorganisms and specific host cells or pathological regions.

The diversity of the human microbiome further complicates the development of universal imaging biomarkers. Different microbial species have distinct metabolic pathways, distributions, and biological behaviours, while many microorganisms—particularly anaerobic organisms—are difficult to culture or manipulate experimentally. Microbial populations may also vary substantially between individuals and anatomical sites. These factors make selective and broadly applicable imaging probes difficult to develop [5,21,28].

Need for In-Vivo Molecular Imaging

These limitations have created a need for approaches that can visualize microorganisms and their biological activity directly within living systems. In-vivo molecular imaging provides a potential complementary strategy because it can offer spatial and functional information and, in appropriate settings, permit repeated assessment over time. Unlike conventional sequencing, which generally represents a molecular snapshot from a collected specimen, imaging may provide information about microbial distribution, persistence, migration, and activity within the host [5,10].

Among the available molecular imaging approaches, positron emission tomography (PET) and single-photon emission computed tomography (SPECT) are particularly promising because radiotracers can be designed to target specific biological pathways. Experimental microbial-imaging probes include ^{18}F -fluorodeoxysorbitol (^{18}F -FDS), ^{18}F -fluoromaltose, ^{11}C -para-aminobenzoic acid (^{11}C -PABA), and radiolabelled trimethoprim derivatives. These probes aim to exploit bacterial metabolism, transport mechanisms, enzymatic activity, or molecular targets to provide greater specificity than conventional metabolic imaging [6,11].

However, direct imaging of microorganisms remains technically challenging. An effective imaging probe should provide high target specificity and sensitivity, favourable pharmacokinetics, low non-specific uptake, and adequate signal-to-background contrast while maintaining acceptable safety and clinical feasibility. Probe performance may also vary according to microbial species, bacterial burden, metabolic state, and tissue environment. Therefore, molecular imaging of the microbiome should currently be considered a promising and developing field rather than an established routine clinical approach [5,6].

Overall, integrating microbiome science with radiochemistry, biotechnology, molecular imaging, and precision medicine may provide a more comprehensive understanding of host–microbe interactions. The ability to determine not only which microorganisms are present, but also where they are located and what they are doing, could complement sequencing and other omics technologies. Such approaches may be particularly valuable in inflammatory and oncological diseases, where spatial microbial activity and host responses are closely interconnected.

Review Methodology

This review was conducted as a structured literature review to summarize current evidence on molecular imaging of the human microbiome, with particular emphasis on microbial-targeted probes, radiotracers, imaging of microbial activity, and their potential applications in inflammatory and oncological diseases. The review focused on studies that provided information on microbial localization, metabolic activity, host–microbe interactions, imaging specificity, and the potential translation of emerging technologies into clinical practice.

Literature Search

A literature search was conducted using major biomedical and multidisciplinary databases, including PubMed/MEDLINE, Scopus, Web of Science, and ScienceDirect. Google Scholar was additionally used to identify relevant publications that might not have been retrieved through the primary databases. Reference lists of important review articles and relevant primary studies were also screened manually to identify additional publications.

The search covered literature related to the human microbiome, microbial imaging, molecular imaging, bacterial imaging, radiotracer development, molecular probes, PET, SPECT, multimodal imaging, inflammation, cancer, and tumor-associated microbiota. Foundational studies were also considered when they were important for explaining the biological basis or development of a particular imaging approach.

Search Strategy

Keywords were combined using Boolean operators such as AND and OR, with minor modifications according to the indexing system of each database.

The main search concepts included:

“human microbiome” OR “human microbiota”,
“host–microbe interaction”,
“microbial imaging” OR “bacterial imaging”,
“molecular imaging” OR “in vivo imaging”,
“molecular probes” OR “radiolabeled probes” OR “radiotracers”,
“PET” OR “positron emission tomography”,

“SPECT” OR “single-photon emission computed tomography”,
“microbiome and inflammation”,
“microbiome and cancer”, and
“tumor-associated microbiota”.

The search strategy was designed to capture both established and emerging imaging technologies rather than restricting the review to a single imaging modality.

Eligibility Criteria

Studies were included when they were relevant to microbiome or microbial molecular imaging and provided meaningful information regarding an imaging modality, biological target, probe, radiotracer, or imaging application. Research articles, reviews, methodological studies, and relevant translational or preclinical studies were considered. Particular attention was given to studies investigating in-vivo visualization of microorganisms or microbial activity, microbial-targeted molecular probes, radiotracers, and imaging approaches relevant to inflammation, infection-associated pathology, cancer, or the tumour microenvironment. Studies involving human participants, clinically relevant translational models, or established experimental models with potential relevance to human applications were considered. Publications were generally restricted to English-language scientific literature with sufficient methodological information for evaluation.

Studies were excluded when they were unrelated to microbial or microbiome imaging, focused solely on conventional microbiome profiling without an imaging component, lacked adequate methodological information, or were primarily concerned with conventional radiological imaging without a molecular or microbiological objective. Duplicate publications, unsuitable conference material, non-peer-reviewed publications, and studies involving unrelated environmental or animal microbiomes without meaningful translational relevance were also excluded.

Study Screening and Selection

Retrieved records were assessed in a stepwise manner. Duplicate records were removed, followed by screening of titles and abstracts. Publications considered potentially relevant were then assessed by reviewing their full text against the predefined eligibility criteria.

The overall selection process followed the sequence:

Database searching → Duplicate removal → Title and abstract screening → Full-text assessment → Final study selection

The review was structured to maintain transparency in study identification and selection. Where complete search and screening records are available, these can be presented using a PRISMA 2020 flow diagram. Because the available evidence is heterogeneous and includes preclinical, methodological, translational, and clinical studies, the review was treated as a structured literature review rather than a formal systematic review or meta-analysis.

Data Extraction

Relevant information from the selected publications was organized using a predefined data-extraction framework. The extracted variables included author and publication year, study design, experimental or clinical model, microorganism or microbial group, disease condition, imaging modality, molecular target, probe or radiotracer, radionuclide where applicable, mechanism of uptake, principal imaging findings, and reported limitations.

Additional information regarding imaging specificity, sensitivity, spatial or functional information, biological significance, and potential clinical translation was recorded when available. The extracted data were subsequently grouped into thematic categories according to the type of imaging technology, biological target, and intended application.

Quality and Evidence Considerations

The quality and relevance of the available evidence were considered during synthesis. Assessment focused on study design, methodological transparency, experimental controls, probe characterization, target specificity, reproducibility, imaging performance, and the degree of validation against microbiological or molecular reference methods.

For preclinical studies, particular consideration was given to probe specificity, biodistribution, pharmacokinetics, appropriate controls, microbial validation, and potential safety or translational limitations. For clinical or

observational studies, attention was given to study population, comparator groups, outcome assessment, methodological limitations, and possible sources of bias.

Because the literature encompasses different study designs and evidence levels, a single standardized quality score was not applied universally. Instead, methodological strengths and limitations were interpreted according to the nature of each study.

Data Synthesis

Due to substantial heterogeneity in microorganisms, imaging modalities, biological targets, experimental models, and outcome measures, a quantitative meta-analysis was not considered appropriate. Instead, findings were synthesized thematically and comparatively.

The synthesis focused on major areas including microbial metabolic imaging, enzyme- and transporter-targeted probes, genetically encoded reporter systems, PET and SPECT radiotracers, multimodal imaging, host–microbe interactions, inflammatory diseases, cancer, technical limitations, and clinical translation.

This approach allowed different imaging strategies to be compared according to their biological target, specificity, imaging performance, advantages, limitations, and translational potential.

Reporting Framework

The reporting of the review was informed by the principles of the PRISMA 2020 framework, particularly for describing literature identification, screening, eligibility assessment, and study inclusion. A PRISMA-style flow diagram may be included when complete search and screening numbers are available.

Overall, the methodological framework followed the sequence:

Research question → Literature search → Study identification → Screening → Eligibility assessment → Data extraction → Quality consideration → Thematic synthesis → Conclusions

The final synthesis was organized around the central objective of determining how molecular imaging and biotechnological probes can complement conventional microbiome analysis by providing information about microbial localization, activity, host–microbe interactions, and disease-associated microbial changes. This framework also enabled consideration of the current limitations of microbial imaging and the prospects for future clinical translation.

Discussion

The transition from traditional, ex vivo taxonomic profiling to dynamic, in vivo molecular imaging represents a critical paradigm shift in human microbiome research. Over the past two decades, high-throughput sequencing and metabolomics have significantly deepened our understanding of microbial diversity and dysbiosis. However, these approaches rely on collected biological samples—such as stool, saliva, or tissue biopsies—which inherently destroy the spatial architecture of tissues and offer only static snapshots of microbial composition. They fail to provide real-time information regarding where microorganisms reside, whether they are metabolically active, or how they continuously interact with neighboring host cells in intact living organisms.

In vivo molecular imaging using targeted radiological probes, particularly through Positron Emission Tomography (PET) and Single-Photon Emission Computed Tomography (SPECT), effectively addresses these diagnostic limitations. By visualizing microbial processes directly within living tissue, nuclear imaging provides both high sensitivity and spatial biogeography, allowing researchers to track the dynamic evolution of host–microbe interactions over time.

A major clinical contribution of microbiome-targeted molecular imaging is its capability to distinguish active bacterial involvement from non-specific, sterile host inflammation. Conventional imaging agents, such as ^{18}F -FDG, measure glucose hypermetabolism and accumulate heavily in activated host immune cells (neutrophils and macrophages) as well as malignant cells. Consequently, an elevated ^{18}F -FDG signal indicates host metabolic activity but cannot verify whether bacteria are present or driving the disease process.

In contrast, selective radiotracers target biochemical features unique to prokaryotes:

- **Substrate Transport Systems:** Probes like ^{18}F -FDS (targeting the sorbitol-specific phosphotransferase system in Gram-negative Enterobacteriaceae) and ^{18}F -Fluoromaltose (targeting bacterial ABC transporters) enter bacterial cells exclusively, leaving mammalian cells unlabeled.
- **Prokaryotic Metabolic Enzymes:** Radiotracers such as ^{11}C -PABA exploit the de novo bacterial folate synthesis pathway, while ^{11}C -Trimethoprim selectively binds bacterial dihydrofolate reductase. Because human cells lack

these enzymatic pathways or possess distinct structural variants, uptake confirms both the presence and metabolic viability of intact bacterial cells.

In oncology, mapping intratumoral microbes provides essential diagnostic clarity. Intratumoral bacteria colonize hypoxic, necrotic tumor environments and are known to directly influence tumorigenesis, modulate local immune suppression, and degrade chemotherapeutic agents (such as the enzymatic inactivation of gemcitabine by bacterial cytidine deaminase). Non-invasive radiological mapping enables the precise localization of these microbial niches within the tumor microenvironment, laying the foundation for targeted antimicrobial or microbial-guided therapies.

Limitations

Despite compelling preclinical results, several significant biological, technical, and chemical limitations currently hinder the routine clinical translation of microbiome-targeted radiotracers:

- **Low Biomass Signal & Sensitivity Thresholds:** Outside the gastrointestinal lumen, bacterial density within solid host tissues, inflammatory lesions, or tumors is often low. Detecting these low-abundance populations requires probes with exceptionally high binding affinity and rapid target uptake to achieve an acceptable signal-to-background ratio above baseline tissue noise.
- **Metabolic Plasticity & Phenotypic Variation:** Prokaryotic metabolism is highly dynamic and context-dependent. Bacteria alter gene expression, substrate transporter activity, and enzyme production in response to local microenvironmental conditions, such as hypoxia, nutrient starvation, or host inflammatory responses. This phenotypic plasticity can lead to variable radiotracer accumulation, potentially causing false-negative imaging results even when target bacteria are present.
- **Taxonomic Diversity vs. Probe Specificity:** The human microbiome comprises thousands of distinct species spanning different domains, phyla, and metabolic profiles. Developing a single universal radiotracer that broadly visualizes all microbes without sacrificing specificity remains unfeasible. Conversely, highly specific probes (e.g., targeting a single bacterial genus) may miss clinically relevant coinfections or broader dysbiotic shifts.
- **Pharmacokinetics, Excretion, and Dosimetry:** Ideal PET/SPECT radiotracers must demonstrate rapid clearance from non-target host tissues while maintaining sufficient stability in vivo. Probes cleared primarily through renal or hepatobiliary routes generate strong background signals in the kidneys, bladder, liver, and intestines, obscuring underlying microbial uptake within nearby abdominal and pelvic organs. Furthermore, repeated radiation exposure limits the frequency of longitudinal PET/SPECT scanning in human subjects.

Future Directions

To overcome these biological and technical barriers, future research must focus on advanced probe chemistry, multi-modal imaging technology, and multi-scale data integration:

- **Development of Next-Generation Broad-Spectrum & Taxon-Specific Probes:** Future probe synthesis should aim to expand the library of clinically approved radiotracers. Priorities include refining broad-spectrum bacterial probes that target highly conserved prokaryotic structures (e.g., cell wall components or universally essential bacterial transporters) alongside specialized probes targeting specific pathobionts implicated in gut mucosal inflammation or cancer.
- **Hybrid and Multimodal Imaging Platforms:** Integrating high-sensitivity nuclear imaging (PET/SPECT) with high-resolution anatomical and functional imaging (PET/MRI or PET/CT) provides a comprehensive diagnostic platform. PET/MRI is particularly promising for mucosal and soft-tissue applications, as it combines soft-tissue contrast with minimal additional radiation exposure, allowing precise coregistration of microbial metabolic signals within complex host tissue structures.
- **Integration with Multi-Omics and Artificial Intelligence:** Combining non-invasive in vivo spatial imaging with ex vivo metagenomics, single-cell RNA sequencing, and metabolomics will yield a holistic, multi-scale understanding of human disease. Implementing machine learning and artificial intelligence (AI) algorithms can assist in coregistering complex imaging datasets with deep sequencing profiles, establishing predictive models that connect localized microbial metabolic signals to overall disease progression and treatment outcomes.

Conclusion

Molecular imaging of the human microbiome represents a major frontier at the intersection of biotechnology, microbiology, radiology, nuclear medicine, and precision healthcare. Moving beyond the constraints of static ex vivo

profiling—such as metagenomics and tissue biopsies—targeted radiological imaging allows for the non-invasive, real-time visualization of microbial spatial distribution, viability, and metabolic activity within living host tissues.

Microbiome-targeted radiotracers and probes hold immense clinical potential for differentiating active bacterial colonization from non-specific host inflammation and for mapping microbial niches within the complex tumor microenvironment. Nevertheless, translating these novel probes into routine clinical practice requires addressing critical challenges, including optimizing target specificity, sensitivity in low-biomass environments, signal-to-background contrast, and radiotracer pharmacokinetics.

Ultimately, integrating selective molecular imaging with high-throughput multi-omics (metagenomics and metabolomics), multimodal imaging platforms (PET/MRI and PET/CT), and artificial intelligence will provide a comprehensive diagnostic framework. This multi-scale approach will transition microbiome research from observational profiling to functional, spatially resolved clinical diagnostics, paving the way for personalized therapeutic interventions in inflammatory and oncological diseases.

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