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Research Article

PERIOMICROBIOME IN ASSOCIATION WITH ONCOGENESIS

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ABSTRACT

The oral cavity hosts over 800 bacterial species, including key pathogens linked to periodontal disease like Streptococcus mutans and Porphyromonas gingivalis. This discussion examines how microbial infections and inflammation pathways contribute to cancer development. Pathogens disrupt immune responses and cause DNA methylation changes, associated with bacteria such as H. pylori and F. nucleatum. Periodontal disease-related inflammation connects to cancer through infection, inflammatory mediators, and risk factors. These pathogens independently increase oral squamous cell carcinoma risk in non-smokers and HPV-negative individuals, showing microbiome-induced inflammation's role in cancer. Disruption of the gingival barrier leads to inflammation and potential cancer, with epigenetic changes in periodontal tissues highlighting how infections and inflammation drive disease and cancer risk.

INTRODUCTION

The field of human micro-biome research has undergone a revolution in its approach toward understanding how microorganisms influence the physiology of their host.[1] the existence of microbes was first discovered in the 1700s while analyzing dental plaque under a microscope.[2] Over 250 oral species, including S. mutans, P. gingivalis, T. forsythia, and A. actinomycetemcomitans, are linked to dental caries and periodontal disease. Research focuses on understanding how microbial diversity, abundance, function, genetic factors, and ecological pressures influence oral health and disease.[1,3-7] Recent genetic research has clarified the roles of various oral microbiome members.[1] Pioneer oral microbes like S. mitis, S. sanguinis, S. gordonii, and S. salivarius excel in this niche by binding to tongue and cheek cells before teeth emerge and outcompeting other species.[8] Merging teeth develop a protective glycoprotein coat that triggers microbial colonization, forming dental plaque with acidic and anaerobic microenvironments.[1]

Recent data show that the oral microbiome, including bacteria like Clostridia and Prevotella sp., influences taste thresholds and dietary choices to support their persistence.[9, 10] Oral microbes have been found in the small intestines, lungs, heart, placenta, and brain, with well-documented links between oral microbiota, especially from periodontal disease, and systemic conditions like cardiovascular disease and hypertension, emphasizing the oral microbiome's importance in dental medicine.[1] New methods aim to increase oral cavity alkalinity and target pathogenic species like S. mutans, while supplements can significantly alter the oral microbiome's composition and metabolism.[11-14]

BRIEF OVERVIEW OF ONCOGENESIS:

Oral cancer, mainly OSCC from the oral mucosa, arises from genetics and factors like tobacco, alcohol, betel quid, and HPV. While incidence is rising, 15% of cases lack these major risk factors, prompting exploration of others. Disruption in the small intestine can lead to diseases like diabetes and inflammatory bowel disease. Hajishengallis et al. (2015) proposed a model of periodontal disease emphasizing anaerobic bacteria. With about 100 trillion microbes in the body, their interactions affect immune responses and health, and periodontitis is a recognized independent risk factor for oral carcinoma. Variation of oral microbiota is associated with human PDL stem cells link to proto-oncogenes and is considered a diagnostic biomarkers of human periodontal cancer[15] shown in figure 1.

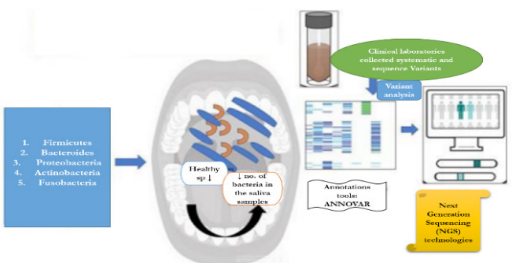

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Figure 1 The oral microbiome as a reliable diagnostic tool in the early detection of peridental treatment.

IMPORTANCE OF STUDYING THE RELATIONSHIP BETWEEN MICROBIOME AND CANCER

Eleven organisms (7 viruses, 3 platyhelminths, 1 bacterium) are recognized as cancer causes: EBV, HBV, HCV, KSV, HIV, HPV, HTLV, Opisthorchis viverrini, Clonorchis sinensis, Schistosoma haematobium, and Helicobacter pylori. They contribute to cancer through mechanisms like B cell differentiation, cell-cycle disruption, immune hyperactivation, T cell dysregulation, and direct oncogenesis, with KSV reducing apoptosis by interacting with oncogenic proteins. The 3 carcinogenic flatworm sp are associated with cholangiocarcinoma, hepatocellular carcinoma, and bladder cancer for S. haematobium, through induction of chronic inflammation that leads to oxidative stress and DNA toxicity.[16] Examples of microbiome-associated carcinogens are summarized below and depicted in Figure 2.

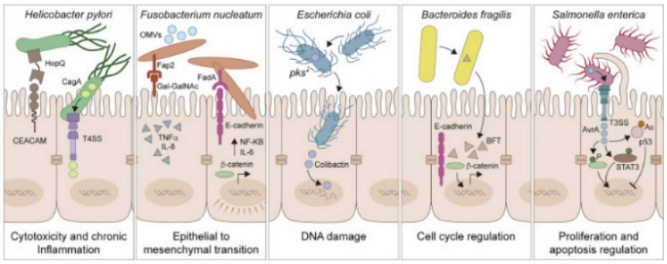

Figure 2 Microbial impacts on processes in epithelial cells[16]

The virome, mycobiome, and neoplastic parasitome can influence cancer. Dysbiosis contributes to cancer via bacterial interactions. Smoking and diet change oral pH, affecting microbial communities and byproducts. Oral dysbiosis with pathogens like S. mutans, F. nucleatum, and P. gingivalis is linked to dental caries, periodontitis, and OSCC, while GI microbiome shifts are related to GI cancer and CRC. Host-microbe interactions and specific microorganisms impact tumorigenesis in various ways.[16]

METHODS OF STUDYING THE PERIOMICROBIOME

Research on the oral microbiome could be crucial for early OSCC diagnosis.[17,18]

Next Generation Sequencing (NGS)[19] (Figure 3).

The main methods for microbial analysis are whole metagenome shotgun sequencing (WMS) and 16S rRNA amplicon sequencing. Both sequence microbial DNA and compare it to databases to determine organism abundance. In WMS, DNA is fragmented, and millions of short sequences are read and reassembled into full or partial genomes.[20] 16S rRNA sequencing, or 16S barcoding, is widely used in metagenomic studies for its scalability. The 16S rRNA gene, present in all bacteria and archaea, has conserved regions for universal primer-based sequencing and nine hypervariable regions (V1 to V9) that enable taxonomic identification by mapping reads to a database.[19]

16S sequencing lacks significant taxonomic resolution as compared to WMS sequencing, only permitting distinction up to the genus level. Alternatives to 16S sequencing have been proposed to improve resolution or to avoid bias due to the varying number of copies of the 16S gene in different species[21] (though there are methods to correct for it). The rpoB gene, being single-copy and more variable than 16S rRNA, offers deeper taxonomic resolution. Using rpoB alongside 16S sequencing enhances resolution. The FROGS database supports rpoB sequences, and multilocus sequence analysis (MLSA) with multiple housekeeping genes can better differentiate closely related organisms.[19] The 16S rRNA gene is the standard for microbiome marker gene analyses.

All of the marker gene techniques mentioned are useful when asking the question, "What microorganisms are present in a sample?" giving an overview of the microbial makeup across many samples. WMS sequencing allow for the detection of species or even strains, in addition to functional annotations of microbiome samples[22], only be predicted based on known full genome sequences when performing 16S sequencing. So, WMS gives insight into the functional potential of the microbiome, allowing researchers to ask the question "What can the microorganisms present actually do?".

Metagenome studies use metatranscriptomics and metabolomics, with metatranscriptomics using NGS to profile microbial gene expression through mRNA. Metaproteomics assesses microbial functional activity by cataloging protein abundances using protein extraction and mass spectrometry (MS/MS).[19] Metabolomics (Figure 3E), answers the question, "What are the microorganisms producing in a given sample?" The metabolome is the total set of small molecules produced by the microbiome (and the host) in a sample, and is a strong indicator of the health/ dysbiosis of a sample.[23] Metabolites are usually quantified using chromatography with mass spectrometry (MS) or nuclear magnetic resonance (NMR).

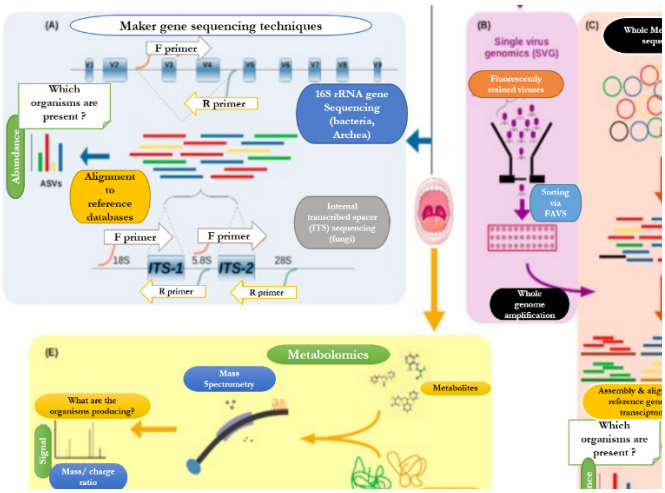

Figure 3. Schematics of microbiome study techniques include: (A) Marker gene sequencing targets specific genome regions to quickly and affordably identify organisms. (B) Single virus genomics (SVG) isolates individual viruses using fluorescence-activated virus sorting (FAVS), followed by whole genome amplification and sequencing. (C) Whole metagenome shotgun sequencing (WMS) fragments all DNA in a sample, sequences it, and assembles the data for mapping or de novo assembly. (D) Metatranscriptomics performs shotgun sequencing on mRNA for differential gene expression analysis. (E) Metabolomics and metaproteomics quantify microbiome-produced metabolites and proteins, respectively.

Metaproteomics faces computational issues and ambiguous

quantifications due to peptide redundancy. Metabolomics must distinguish between host and microbiome metabolites and link them to genes and pathways. Combining these techniques with other omics data is crucial for studying the bacteriome, though not fully comprehensive. For mycobiome classification, researchers use the ITS region, similar to the 16S rRNA gene for bacteria, providing comparable taxonomic resolution.[19] Studying the virome is challenging due to the absence of conserved marker regions like those in bacteria and fungi. The entire virome must be sampled and compared to known viral sequences, but current databases often lack comprehensive viral characterization, making it hard to classify new, unmatched viral sequences.[24] The challenge is the low proportion of viral nucleic acids among other microbes. Enrichment methods and single virus genomics (SVG), using fluorescence-activated viral sorting (FAVS) to isolate and sequence individual viruses, have been proposed to address this.[18]

Researchers must agree on collection and sequencing methods to ensure reproducibility. Some of these referenced studies have shown that the microbiome profile of a sample is not heavily influenced by the collection technique[25-26], these are focused on large-scale differences. As sequencing and databases improve, researchers will compare samples at finer scales, with minor technical variability from swabbing or rinse collection methods potentially affecting results.[19]

There are many data analysis methods and software packages available. The phyloseq[27] and microbiome packages for R offer a means to organize the data from sequencing experiments alongside any metadata, and provide a collection of tools and tutorials for calculations and plotting in typical microbiome analyses. This includes functions for calculating the α-diversity and β-diversity. The vegan R package offers multivariate tests like anosim and adonis for comparing microbiome compositions. Linear mixed effects models use lm, glm, lmer, or glmer functions, while PICRUSt and Tax4Fun reconstruct metagenomes from reference genome databases. Machine learning techniques have been implemented to attempt to predict disease based on the microbiome composition[28], and some investigators have made their code publicly available. The oral microbiome has been used in a classifier for colorectal cancer.

Compositional microbiome data and its analysis are improving. Vandeputte et al. introduced quantitative microbiome profiling (QMP) to address 16S copy number and sampling biases, while Gloor et al. recommended using centered log-ratio transformation for normalization, minimizing read depth effects.[19]

CHALLENGES IN STUDYING ORAL MICROBIOME IN RELATION TO CANCER

Sample allocation: Most microbiome studies use stool or oral samples as proxies for the gut microbiome. Improving accuracy in cancer-microbiome assessments may involve better computational inference from stool or direct, minimally invasive gut sampling. Assessing the human tumor microbiome remains more challenging.[16]

Data & resource availability: Optimizing microbiome research amid the scientific reproducibility crisis and addressing the lack of standardization in data acquisition and analysis methods are key challenges.[16]

Inter-individual microbiome variability: Biological variations from individual microbiome uniqueness challenge generalizing results and distinguishing signal from noise. Host factors like geography, age, and lifestyle, as well as underlying diseases, influence this variability. Personalized and disease-specific contributions may emerge, requiring new computational tools to capture and analyze these patterns over time.[16]

Correlation versus causation: A key challenge is moving from identifying associations to proving causality and mechanisms. One approach is transferring whole microbiomes or specific microbes into germ-free mice to study their impact on cancer. For example, CRC patient microbiomes in germ-free mice showed increased cell proliferation and inflammation, and fecal transfers from CRC patients to conventional mice enhanced polyps and dysplasia. Mono-colonization with enterotoxigenic B. fragilis or E. coli accelerated tumor development in mouse models.[16]

OVERVIEW OF MICROBIOME COMPOSITION

NORMAL ORAL MICROBIOTA COMPOSITION

There are 1,000 oral bacteria species across several phyla and divisions, including Actinobacteria, Bacteroidetes, and CPR members like GN02, SR1, and TM7, which affect oral microbiome structure and function, correlating with diseases like periodontitis and halitosis. Only TM7 had been cultured from the human oral cavity until November 2021.[29] Oral archaea, once thought to be only methanogens, now include non-methanogenic types found in inflamed tissues and biofilms. About 100 fungal species, including Aspergillus and Candida, are present in the oral cavity but make up only 0.004% of oral microorganisms. Recent studies identified Malassezia and Candida. The oral virome includes eukaryotic viruses and phages, which may help treat bacterial infections through lysis. Oral viruses are considered personal, persistent, and gender-specific.[29]

Core Oral Microbiota: The Human Microbiome Project expanded the core microbiome concept into five types: common, temporal, ecological, functional, and host-adapted, based on distribution and health effects. The oral core microbiome includes Actinobacteria, Bacteroidetes, Firmicutes, Fusobacteria, and Proteobacteria, with variations by niche and life stage. For instance, hormonal changes affect Campylobacter and Prevotella during the menstrual cycle, and pregnancy influences the abundances of Neisseria, Porphyromonas, and Treponema. Core oral microbiota should be defined by the host's life stage and oral ecological niche..[29]

Oral Microorganism Databases: Two main oral microbiome databases are HMP and HOMD, with HMP studying microbial communities across five body sites and eHOMD providing detailed data on 775 species, 687 from HOMD version 14.51.[29]

FACTORS INFLUENCING ORAL MICROBIOME DIVERSITY AND STABILITY

Host genetics: Host genetics affect the composition of certain bacterial species in later childhood, as shown by twin studies.[30] Host genetics and sex-specific differences influence microbiota changes linked to environmental exposures.[31-34] Exposure to heavy metals alters gut microbiome composition

variably due to interactions between host genes, environment, and microbiome. This variability, seen in caries microbiome studies with 30–60% heritability, is influenced by both direct and indirect factors. Environmental changes affect host epigenetics and microbiome health, with twin studies showing discordance in methylation despite genetic similarity. Studies within specific population structures are more effective than random ones, often using parent-offspring pairs and other genetic structures.[30]

Early life: NGS studies detected microbial changes up to 12 months before caries appeared in 3-year-olds and even before teeth emerged, linked to S. mutans. Microbial diversity starts within hours of birth, influenced by maternal sources, and stabilizes by adulthood. The first 1000 days impact gut microbiome development, while the oral microbiome evolves according to birth mode, early feeding practices, and antibiotic exposure. Relevant aspects of the oral microbiome trajectory are summarized in[30] Figure 4. The womb is a sterile environment[36] so the infant oral microbiome is first exposed to microbes through contact with the vagina/ uterus during delivery. It is primarily inoculated during the first 6 months with early feeding & is dominated by Streptococcus, Veillonella and Lactobacillus species.[30]

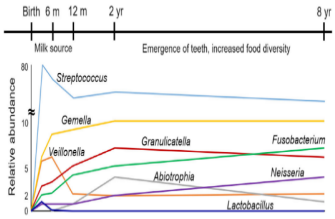

Figure 4. Key aspects & factors effecting the oral microbiome trajectory. Relative abundance plot derived from Dzidic et al.

Diet, a major factor in the microbiome trajectory with breastfed infants,[30] has higher abundances of Streptococcus & Veillonella species compared with formula fed infants. From 6 months to 2 years, emerging teeth provide surfaces for biofilm growth, and solid food intake increases microbial diversity by offering more nutrients.[37,38] This enables other species (Gemella, Granulicatella, Haemophilus and Rothia), present at low abundance from 3 months of age to increase in abundance with time. In later childhood, bacterial diversity increases, with factors like poor hygiene, sugar, smoking, and low SES affecting caries. NGS shows that smoking and sugar shift microbiome functions to anaerobic metabolism. Diet impacts microbiome composition, influencing taste and eating habits. Reducing sugar and improving oral hygiene help prevent caries.[30]

Saliva: Saliva reflects oral microbiota but is less representative than supragingival plaque. Firmicutes are dominant in saliva, while Actinobacteria and Fusobacteria are more common in plaque. Oral rinse fluid better represents site-specific microbiota. In a study of 997 Qatari individuals, the salivary microbiome mainly included Bacteroidetes and genera like Gemella, Haemophilus, and Streptococcus, differing from Japanese and Chinese populations. These variations may be due to genetic, dietary, and environmental factors. Salivary microbiome changes can serve as biomarkers for monitoring diseases and overall health.[29]

Saliva aids microbial adhesion and clearance through the acquired pellicle and contains defense proteins like immunoglobulins and antimicrobial peptides. Anxiety and depression can alter the abundance of Actinomyces and other bacteria via cortisol and C-reactive protein. Host-derived tsRNAs also regulate bacterial growth.[29]

Tooth Surfaces: The non-shedding tooth surface promotes bacterial growth and plaque formation, with distinct microbial compositions across its five areas: occlusal, proximal, supragingival, lingual, and labial. For example, Streptococcus spp. are present on the labial surface of incisors and cuspids in 40–70% of cases.[29]

Soft Tissue Surfaces: Streptococcus predominates in mucosal tissue, while the tongue hosts diverse microorganisms, including anaerobes like Actinomyces, Porphyromonas, Prevotella, Streptococcus, and Veillonella, along with Haemophilus, Leptotrichia, and Neisseria, and is linked to halitosis.[29]

The oral mucosa's immune functions influence microbiome composition by detecting pathogens through pattern recognition receptors, triggering inflammatory responses, such as recognizing Candida albicans and activating the IL17/ Th17 pathway, with deficiencies linked to increased fungal overgrowth in immunocompromised individuals.[29]

Disease States: Oral microorganisms impact systemic diseases like cardiovascular and digestive disorders, with variations in salivary microbiomes linked to obesity and conditions like primary sclerosing cholangitis. Inflammatory diseases such as diabetes, rheumatoid arthritis, and SLE show specific microbial changes, while oncological treatments like radiotherapy and chemotherapy alter oral microbiota, with more pronounced changes in bacteria than fungi.[29]

Endogenous and exogenous factors can shift the abundance and composition of oral microbes, causing dysbiosis.

Oral diseases: 16S rRNA gene sequencing shows that dysbiosis is crucial; supragingival plaque is linked to caries, while subgingival plaque causes periodontal disease, leading to inflammation and severe dysbiosis (Johnston et al., 2021).[29]

Biomarkers of periodontitis include S. mutans, red complex bacteria, and various other species, with increased microbial diversity indicating periodontal disease and dysbiosis linked to changes in dominant species. Oral cancer and periodontitis are associated with microbial changes and viral influences like HSV and EBV, which increase pathogenicity and inflammation.[29]

Interactions Between Bacteria and Fungus During Biofilm Formation: C. albicans is crucial for its virulence factors, including mycelial formation. In children with severe ECC, C. albicans boosts acidogenic and acid-tolerant bacteria, like S. mutans and Lactobacillus, and enhances Extracellular Polymeric Substances (EPS) production, which supports more caries-active S. mutans. S. mutans' Glucosyltransferase B (GtfB) binds to C. albicans, aiding ECM formation and mixed-species biofilms. Co-culturing S. mutans with C. albicans increases biofilm biomass, but Streptococcus pneumoniae Adhesin Protein (spaP) loss reduces C. albicans abundance in vivo, indicating AgI/II is vital for their biofilm formation. The

recognition of C.albicans by S.gordonii involves Agglutinin-Like Sequence 3 (Als3) and Streptococcal Surface Protein B (SspB) interaction, providing a new mechanism for the communication between fungi & bacteria. C. albicans interacts with A.naeslundii. The cross-kingdom dual-species biofilm formed by A.viscosus and C.albicans showed significantly enhanced cariogenic virulence.[29]

LINK BETWEEN ORAL MICROBIOME AND CANCER

EVIDENCE FROM EPIDEMIOLOGICAL STUDIES LINKING ORAL MICROBIOTA TO CANCER RISK

Accumulating evidence suggests a link between oral cavity bacterial microbiota & OSCC.[40] OSCC is caused by multiple simultaneous factors[41] and the contribution of bacteria is difficult to separate from them. Changes in oral bacterial composition often precede or accompany OSCC, visible in lesions like leukoplakia and OLP. Chronic inflammation from infections promotes cell proliferation and oncogene activation linked to OSCC. Periodontal disease and pathogens disrupt cell-signaling pathways, increasing cancer risk. Although studying specific bacteria's impact on carcinoma is complex, altered oral microbiota could predict OSCC. Elevated levels of P. gingivalis and F. nucleatum are found in OSCC, with high P. gingivalis antibody levels linked to orodigestive cancer mortality. Bacteria can colonize tumors due to decreased immune defense. Pathogenic microbiota changes, along with smoking and alcohol, contribute to cancer. Personalized medicine, such as phage therapy, may manage carcinogenic bacteria. Meanwhile, avoiding alcohol & tobacco products decreases the exposure to acetaldehyde production directly/ indirectly by oral cavity bacteria.[40]

MECHANISMS PROPOSED FOR HOW ORAL MICROBIOTA MAY INFLUENCE ONCOGENESIS

INFLAMMATION AND IMMUNE MODULATION

Extensive bacterial exposure is considered a prerequisite in periodontal tissue inflammation, where the microbial biofilm triggers both innate & adaptive immune responses destroying the supportive tooth structures.[42] However, it is the host response which dictates the disease phenotype.[43,44] The periodontal innate inflammatory response detects microbial pathogens at the epithelial boundary through neutrophils, macrophages, dendritic cells, natural killer cells, and monocytes. This process involves toll-like and nucleotide-binding oligomerization domain-like receptors. Early periodontium inflammation includes increased vascular permeability, PMN recruitment, and activation, regulated by prostaglandins and cytokines. The activated immune cells produce and release ROS and reactive nitrogen species in response to infection; thus, reaching a chronic stage when the APCs cells become involved and present the foreign antigens/ microorganisms to immunocompetent cells, expanding the antibody-secreting plasma cell population (Figure 5). The IL-37 gene, a recent addition to the IL-1 family, was studied for its anti-inflammatory effects by reducing pro-inflammatory cytokine production. Periodontal pathogens can disrupt immune activation, epigenetic remodeling, and gene expression. Research in 2007 using a murine model showed that C. rectus infection led to DNA hypermethylation of the insulin-like GF-2 gene and reduced its expression. The epigenetic modulation of the pro-inflammatory mediator TNF□α were also investigated.[40]

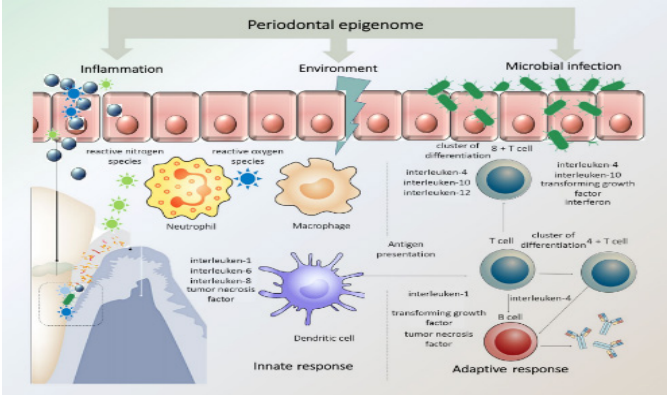

Figure 5. Periodontal epigenome in response to microbial infection, environmental stimuli and inflammation.

Studies of periodontal disease epigenetics in vitro, animal models, and clinical trials showed that P. gingivalis infection can cause TLR-2 promoter hypermethylation, leading to increased TLR-2 methylation in epithelial cells. This hypermethylation correlates with reduced TLR-2 mRNA expression in periodontitis patients. It was reported the same unmethylated TLR-4 and inconclusive methylation patterns for TLR-2 in both patients with periodontitis & healthy subjects.[40]

The positive correlation between several inflammatory markers, epigenome, and gene expression, has been reported not only for US but also for other populations.[40] In 1 study, the DNA CpG (Cytosine-phosphate-Guanine) methylation patterns collected from gingival biopsies taken from healthy patients and patients with aggressive periodontitis, showed higher methylation levels of CCL25 (associated with the chemotactic action of macrophages, thymocytes and dendritic cells.) and IL-17C (involved in the initiation or maintenance of the pro-inflammatory response) genes in healthy gingival tissue. In aggressive periodontitis P.gingivalis infection induce CCL25 gene expression supporting the upregulation of CCL25 through epigenetic alterations.[45]

The discrepancies in periodontal epigenome may be due to the level of inflammation in the tissue samples included, and/ or to the different dental biofilm compositions plus diverse virulence effects of different types of bacteria.[46] Yin and Chung found that P. gingivalis infection caused hypermethylation of IL-12A, TLR-2, CD276, ELA2, INHBA, and GATA3, and hypomethylation of STAT5A and ZNF287. F. nucleatum infection led to hypermethylation of MALT1 and hypomethylation of ELA2 and GATA3.

The number of genes linked with chromatin modification were different in epithelial cells infected with P.gingivalis.[40]

T. denticola induces hypomethylation of the MMP-2 promoter and chronic activation of pro-MMP2 in periodontal fibroblasts, contributing to tissue destruction. ECM-related genes such as MMP-25, COL4A1-A2, FANK1, and others show hypermethylation and reduced mRNA expression.1 study demonstrated DNA methyltransferase-1 upregulation and RUNX2 expression downregulation by the LPS in PDL cells, [40] thus, demonstrating the modulation of the inflammatory response through epigenetics affecting mineralized tissues, as seen in periodon-

Oral disease	Pathogens in traditional research	Oral microorganism with increased abundance from sequencing investigation	Oral microorganism with decreased abundance from sequencing investigation
Caries	**Genera**: *Actinomyces, Lactobacillus, Neisseria, Porphyromonas, Prevotella, Propionibacterium, Streptococcus* **Species**: *Actinomycesisraelii, A. viscosus, Lactobacillus acidophilus, L. casei, L. fermentum, Streptococcus mitis, S. mutans, S. sanguinis* **Others**: *Candida albicans*	**Genera**: *Bifidobacterium, Haemophilus, Legionella, Neisseria, Prevotella, Propionibacterium, Rothia, Shuttleworthia, Veronococcus***Species**: *Porphyromonas catoniae, Prevotellahisticola, S. mutans, Veillonelladispar* **Others***: C. albicans, EBV*	**Genera**: *Anaerosporobacter, Caldicoprobacter, Dysgonomonas, Hespellia, Proteiniphilum,* **Species**: *Capnocytophagagranulosa, Leptotrichiabuccalis,*
Periodontal disease	**Genera**: *Fusobacterium, Parvimonas, Prevotella***Species**: *Actinobacillus actinomycetemcomitans, A. viscosus, P. gingivalis, Tannerella forsythia, Treponemadenticola*, **Others**: *Cytomegalovirus, EBV, HSV-1*	**Genera**: *Desulfobulbus, Eubacterium, Filifactor, Fretibacterium, Parvimonas, Porphyromonas, Prevotella, Tannerella, Treponema,* **Species**: *Filifactoralocis, P. denticola, P. endodontalis, P. gingivalis, T. denticola, T. forsythia* **Others**: *Redondoviridae*	**Genera**: *Actinomyces, Capnocytophaga, Corynebacterium, Neisseria, Rothia, Streptococcus***Species**: *C. gingivalis, C. ochracea, Neisseria subflava, P. catoniae, Rothiaaeria, S. infantis, S. mitis, S. oralis, S. sanguinis*
Pulp periapical disease	**Genera**: *Actinomyces, Bacteroides, Enterococcus, Fusobacterium, Peptostreptococcus, Porphyromonas, Prevotella, Saccharomycodes, Streptococcus* **Species**: *Enterococcus faecalis, Peptostreptococcus micros, P. endodontalis, P. gingivalis, P. melaninogenicus*	**Genera**: *Aggregatibacter, Fusobacterium, Lactobacillus, Peptostreptococcus, Porphyromonas, Prevotella, Schwartzia, Slackia, Treponema* **Species***: Dialisterinvisus, E. faecalis, Fusobacteriumnucleatum, P. gingivalis, P. micros, T. denticule* **Others**: *C. albicans, HSV*	**Genera**: *Acinetobacter, Actinomyces, Corynebacterium, Granulicatella, Haemophilus, Leptotrichia, Staphylococcus, Streptococcus* **Species**: *N. subflava, P. melaninogenica, P. nanceiensis, R. mucilaginosa*
Oral cancer	**Species**: *F. nucleatum, P. gingivalis* **Others**: *HPV*	**Genera**: *Aggregatibacter, Alloprevotella, Capnocytophaga, Fusobacterium, Parvimonas, Peptostreptococcus, Porphyromona, Prevotella, Treponema***Species**: *Catonellamorbi, F. nucleatum, F. periodonticum, Haemophilus influenza, P. intermedia, Parvimonasmicra, S. constellatus, T. alocis, T. denticola***Others**: *Candida, Gibberella*	**Genera**: *Acitinomyces, Haemophilus, Lautropia, Porphyromonas, Rothia, Streptococcus, Veillonella***Species**: *A. odontolyticus, H. parainfluenzae, P. pasteri, S. mitis, S. oralis, V. parvula*
Recurrent oral ulce	**Genera**: *Streptococcus* **Species**: *Helicobacter pylori, S. sanguinis*	**Genera**: *Actinobacillus, Alloprevotella, Fusobacterium, Haemophilus, Porphyromonas, Prevotella, Vibrio*- **Species**: C. *gingivalis, C. sputigena, Escherichia coli, F. nucleatum, H. parahaemoliticus, H. parainfluenzae, N. flavescens, N. sicca***Others**: *C. albicans, Malassezia*	**Genera**: *Streptococcus, Veillonella* **Species**: *Gemellahaemolysans, S. oralis, S. salivarius, V. dispar* **Others**: *Cladosporium sp*.
Peri-implantitis	**Genera***: Fusobacterium, Parvimonas, Staphylococcus* **Species**: *P. gingivalis, T. denticola,*	**Genera**: *Eubacterium, Filifactor, Fretibacterium, Porphyromonas, Tannerella, Treponema***Species**: *A. cardiffensis, E. minutum, Eubacteriuminfirmum, Fretibacteriumfastidiosum, G. sanguinis, Kingelladenitrificans, L. hofstadii, P. gingivalis, P. intermedia, T. alocis, T. denticola, T. forsythia, T. maltophilum,*	**Genera**: *Actinomyces, Haemophilus, Rothia, Streptococcus, Veillonella***Species**: *A. cardiffensis, E. infirmum, R. dentocariosa, S. sanguinis, V. dispar*

titis.[42]

PRODUCTION OF CARCINOGENIC METABOLITES:

Ethanol's metabolites (acetaldehyde, hydroxyl ethyl radicals & hydroxyl radicals) are carcinogenic. The International Agency for Research on Cancer classified acetaldehyde associated with alcohol consumption as a Group 1 carcinogen in humans causing sister chromatid exchanges, point mutations, DNA adducts & hyperproliferation of epithelium. Certain bacteria (S.salivarius, S.intermedius, S.mitis and non-pathogenic Neisseria spp) and Candida spp. possess the alcohol dehydrogenase (ADH), catalysing the production of mutagenic amounts of acetaldehyde under aerobic/ microaerophilic conditions.[47]

Metabolic derivatives (organic acids, volatile sulfur compounds (VSC) & ROS) from periodontal pathogens induce DNA damage, mutagenesis, 2^0 hyperproliferation of the cells, metastasis and cancer progression. Microorganisms involved in alcohol metabolism to acetaldehyde can impact cancer development. VSC-producing periodontal pathogens (P.g, P.i, A.a and F.nucleatum) generate hydrogen sulfide, methyl mercaptan, dimethyl sulfide, and dimethyl disulphide, influencing epithelial cell proliferation & induces apoptosis through the activation of the mitochondrial pathway. They contribute to connective tissue breakdown and inflammation by stimulating the release of IL-1ß from mononuclear cells.[48]

ROS function as signals to regulate cell proliferation & survival. Low ROS levels fail to support proper cellular functioning by regulating numerous biochemical reactions. Excessive ROS damages cells, disrupts processes, and promotes cancer through angiogenesis, metastasis, and survival. P. gingivalis (P.g) stimulates ROS production, leading to JAK2 phosphorylation and increased IL-6 and IL-1ß. P.g NDK modulates ROS and antioxidant responses via P2X7/NADPH-oxidase. P.g-induced ferritinophagy and F. nucleatum also drive ROS production and inflammation in various cells, affecting cell proliferation and apoptosis through AKT/MAPK and NF-κB pathways. Okinaga et al. demonstrated that A.a induces IL-1β production in RAW 264 cells by generating ROS & cathepsin B. T.forsythia stimulates ROS inducing the expression of IL-24.[48]

EFFECTS OF HOST CELL SIGNALLING PATHWAYS

Induction of chronic inflammation: Chronic inflammation from periodontal pathogens (Fusobacterium, Porphyromonas, Prevotella) heightens cancer risk by disrupting stromal integrity, promoting invasion, and metastasis. These pathogens upregulate IL-1β, IL-6, IL-17, IL-23, TNF-α, CXC family, MMP-8, and MMP-9, which are linked to carcinogenesis. Elevated serum IL-6 and saliva IL-8 levels are associated with poorer OSCC prognosis. NF-κB activation is common in bacteria-associated tumors, with LPS triggering a strong immune response in infections. Pattern recognition receptors (PRRs) like TLRs, triggers the NF-κB signaling pathway and induces the production of inflammatory-associated cytokine contributing to the carcinogenesis process.[48]

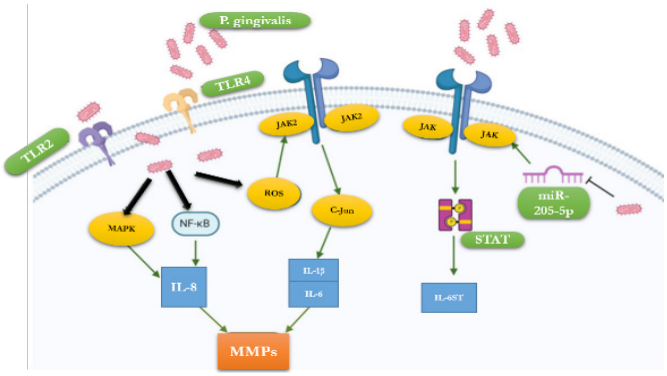

Figure 6. Mechanisms linking P.g and inflammation. Toll like receptor (TLR). Mitogen-activated protein kinases (MAPK). Interleukin (IL). Janus kinase (JAK). Signal transducer and activator of transcription (STAT)

P.g activates MAPK and NF-κB pathways, increasing IL-8 production, and stimulates the JAK/cJun axis, elevating IL-1β and IL-6 levels, triggering inflammatory responses through TLR4/TLR2 pathways. The host's defense mechanism activates inflammatory cascades and utilizes miRNA as alternative genetic inhibitors. P.g suppresses the expression of miR-205-5p, leading to the activation of JAK/STAT by upregulating IL6ST[48] (figure 6).

Inhibition of the host's immune system: P.g and F.nucleatum trigger proinflammatory and immunosuppressive responses, weakening anti-tumor immunity. A.a secretes CDT, disrupting host responses by affecting phagocytosis and cytokine balance. A.a outer membrane protein 29 (OMP29) inhibits CXCL-8, vital for tumor angiogenesis, and modulates apoptosis and inflammatory genes in Gene Expression and Chromatin Structure (GECs). The tumor microenvironment is crucial for immune escape, with high PD-L1 levels in OSCC, which P.g membrane fractions stimulate in SC cells and gingival keratinocytes.

Peptidoglycan (PDG) from P.g induces PD-L1 expression in OSCCs and colon cancer cells, dependent on NOD1 & NOD2, and the activation of RIP2 & MAPK signaling pathways.[48]

P.g reduces IFN-γ-induced release of CXCL9, CXCL10, and CXCL11 from epithelial cells by inhibiting chemokine gene transcription, which correlates with reduced IRF-1 and STAT1 levels. Its secreted nucleoside diphosphate kinase (NDK) promotes tumorigenesis by inhibiting ATP activation of purinergic receptor P2X7, thereby suppressing IL-1ß production in the epithelium. P.g, through Mfa1 and FimA fimbriae, fosters immunosuppression and oncogenic cell proliferation in myeloid-derived dendritic suppressor cells.[48]

Neutrophils engage in phagocytosis, bacterial killing, and digestion. P.g disrupts macrophage antimicrobial responses by affecting C5aR/TLR2 crosstalk and MyD88/PI3K signaling, leading to MyD88 degradation and impaired antigen presentation and T-cell activation. It also reduces neutrophil phagocytosis of P.g. Gingipains activate PAR-2, which boosts ROS levels and NETs formation. A.a induces NETs and MMP-8/9 release in neutrophils, aiding tissue destruction and disease progression[48] (FIGURE 7)

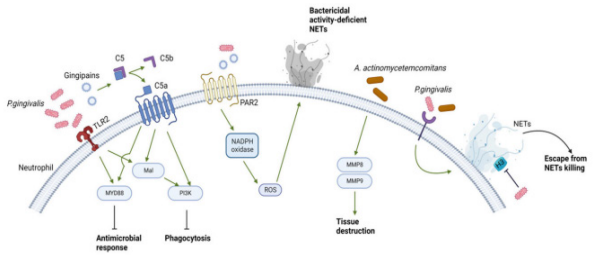

Figure 7 P.g and A.a escape the killing by neutrophils. Adapted from Ref.

Gingipains from P.g degrade CD14 on macrophages, hindering phagocytosis of infected cells. P.g's fimbria and sialidase help evade the immune system by targeting CXCR4 and CR3, activating PKA signaling to suppress TLR2 responses and reducing IL-12, which impacts T-cell responses. P.g inhibits T-cell antimicrobial responses by down-regulating IL-2 through prevention of PKC and p38 phosphorylation and AP-1 activation, while upregulating IL-10 to inhibit antigen presentation and T-cell activation. The increased IL-10 production activates PD-L1 on macrophages and PD-1 on CD4+ T cell surfaces, indicating multiple inhibitory mechanisms employed by P.g to evade the host's immune response.[48]

SPECIFIC MICROBIAL SPECIES IMPLICATED IN ONCOGENESIS

Key Microbial Species Associated With Oral Squamous Cell Cancer

Periodontitis-correlated taxa were significantly increased in the microbiota of the OSCC (Zhao et al., 2017), including P.g, F.nucleatum, P.aeruginosa, F.periodonticum, A.segnis, C.rectus, C.showae, P.stomatis, P.micros and C.morbi (Zhang et al., 2019). In the hypoxic tumor microenvironment, pathogenic periodontal bacteria abundance shifts notably, with the tumorous mucosa harboring saccharolytic and aciduric species. Host proteins may also be metabolized/ fermented into sulfides & nitrosamines by Firmicutes and Bacteroides, potentiating cell mutations.[15] Microbiota composition varies with sampling type and OSCC stage. In OSCC tissue, Solobacterium moorei, hydrogen sulfide producer F. naviforme, and N. flavescens increase significantly, potentially promoting invasion by enhancing ROS release and collagen degradation. F. periodonticum may collaborate with F. nucleatum in tumor progression, with F. nucleatum aiding P. gingivalis growth by creating a reducing environment. Interactions between bacteria can impact species proliferation, with P. gingivalis negatively affecting S. cristatus and S. intermedius. Dysbiosis fosters OSCC development, with Capnocytophaga gingivalis, Rothia mucilaginosa, and P. intermedia significantly enriched in the lining mucosa, tongue, and gingiva, respectively, where they secrete peptidases activated through PARs (Protease-Activated Receptors). Then, degrades host tissue like ECM, destruct host physical barriers and modulate host immune response, contributing to the onset & progression of tumors.[15] OSCC tumor microbiome analysis showed increased genes related to cell motility (bacterial chemotaxis, flagellar assembly), proinflammatory bacterial components (lipopolysaccharide biosynthesis), and metabolism of cofactors and vitamins. S. infantis was more abundant in smokeless tobacco non-consumers and in the buccal site compared to OSCC tumor sites. C. gingivalis, P. melaninogenica, S. mitis, F. periodonticum, P. tannerae, and P. intermedia were enriched in unstimulated saliva from OSCC patients. C. gingivalis, P. melaninogenica, and S. mitis showed diagnostic sensitivity and specificity of 80% and 82%, respectively, in OSCC saliva samples. Neisseria species plays an important role in alcohol-related carcinogenesis because they produce acetaldehyde.[15]

High levels of P. gingivalis in saliva are linked to advanced OSCC stages but lower recurrence rates. P. gingivalis in OSCC tissue may originate from the salivary microbiome. Increased abundance of Fusobacteria species in oral tongue samples of OSCC patients is associated with significantly increased programmed death-ligand 1 (PD-L1) expression and reduced abundance of Rothia and Streptococcus species— with lower α-diversity.[15]

Recent studies have linked F. nucleatum to clinical outcomes in malignancies. Variations in F. nucleatum across different intestinal segments can affect colorectal cancer prognosis. In esophageal cancer, F. nucleatum-positive patients show lower cancer-specific survival and higher cancer-specific mortality, partly due to increased CCL20 chemokine levels. Conversely, S. anginosis is associated more with esophageal cancer than oral cancer. Dysbiosis may contribute to the development of breast cancer via pathways related to immune modulation & the establishment of a tumour microenvironment most notably to oestrogen metabolism and the estrobolome, a phrase referring to a group of bacteria capable of regulating oestrogen circulation in the enterohepatic tract.[28] Thus, balancing a dysbiotic microbiota with antibiotic medication, which often raises the risk of breast cancer, can be beneficial before or after a cancer diagnosis. F.nucleatum , periodonticum, S.salivarius, Porphyromonas and Lactobacillus subspecies are linked to the diagnosis of HNSCC. F. nucleatum (polymorphum), Campylobacter subspecies, P. aeruginosa, and Porphyromonas are termed "mobile microbiomes." Modifying pH environments and using probiotics like S. dentisani or Streptococcus A12, along with antimicrobial peptides, could help combat oral cancer .Oral antibiotics should be studied more closely since they may affect the gut microbiota, boosting immunological dysbiosis & decreasing the quantity of probiotic bacteria, favouring the development of OSCC (Wei et al., 2022).[15]

CLINICAL IMPLICATIONS AND CHALLENGES

Therapeutic Implications Targetting The Microbiome For Case Prevention And Treatment

Antibiotic Treatments: Oral antibiotics targeting caries can disrupt bacterial signaling systems (Cui et al., 2019). For instance, sulfated vizantin inhibits extracellular glucosyltransferase release, boosts cell-associated glucosyltransferase, and prevents S. mutans biofilm maturation (Oda et al., 2020).

Walkmycin C (an HK inhibitor) inhibits the biofilm formation & acid resistance of S.mutans (Eguchi et al., 2011).[29] Moreover, the combination of amoxicillin & metronidazole exerted greater antimicrobial effects on subgingival biofilms of bacterial species in vitro (Soares et al., 2015). Amoxicillin + metronidazole + SRP was superior to SRP alone in reducing the PD & CAL, and clinical improvement in the subgingival

region & saliva, especially in type 2 diabetic patients or aggressive periodontitis.[29]

However, the development of antibiotic resistance has been accelerated by the abuse of antibiotics (Loffler and Bohmer, 2017) and the discovery of other unique antibiotics that are specific to pathogens is urgently required (Singh et al., 2017).[29]

Periodontal Interventions: This therapy, involving supra- and sub-gingival scaling and root planing, targets periodontal pathogens and enhances oral health. It changes the oral microbiome's composition and interactions (Zhang et al., 2021a). Combined with systemic antibiotics, it reduces A. actinomycetemcomitans and F. alocis while increasing Streptococcus, Rothia, and Prevotella spp. This combined approach improves plaque control compared to antibiotics alone. Thus, periodontal intervention therapy remains an essential technique for the treatment of oral diseases.[29]

Photodynamic Therapy: This therapy uses light to activate a photosensitizer, generating reactive oxygen species that oxidize bacterial lipids and kill bacteria. A trial showed that scaling and root planing with photodynamic therapy (PDT) reduced GI, PD, CAL, and counts of A. actinomycetemcomitans and P. gingivalis more than scaling and root planing alone. PDT with photobiomodulation also shortened oral mucositis remission from 15 to 11 days.

Clinical trials of pulpitis (da Mota et al., 2015), periapical periodontitis, oral leukoplakia, peri-implantitis, and adverse biofilm changes caused by orthodontic brackets showed that PTD was more effective than traditional treatments.[29]

Probiotic Therapy: Probiotics are non-pathogenic microorganisms with preventive and therapeutic effects on oral infections. They release bacteriocins to combat dental plaque, produce glucanase and urease to counteract plaque formation and saliva acidity, and influence the virulence of S. mutans by modulating acid tolerance, EPS production, and quorum sensing genes. Probiotics also inhibit the effects of IFN-γ and IL-10 (Wasfi et al., 2018). In chronic periodontitis, they reduce pathogenic complexes, TNF-α, and IL-1β (Invernici et al., 2018). Probiotics like L. casei and L. rhamnosus reduce C. albicans in oral candidiasis, and B. subtilis and S. thermophilus inhibit its growth and biofilm formation. These are proved to reduce the prevalence of oral candidiasis especially in frail elderly. Lastly, S.parasanguinis directly inhibits the activity of GTF, which can prevent C. albicans binding to glucan.[29]

Quorum Quenching Therapy: QQ is an alternative treatment for oral infections that maintains oral microflora balance and inhibits biofilm formation without eradicating bacteria. For instance, D-galactose disrupts the AI-2 QS system, reducing biofilm formation. Furanone compounds and D-ribose are QS inhibitors that mitigate bacterial infection and periodontal tissue damage from F. nucleatum and P. gingivalis. Inhibiting the peptidase domain of the ATP-binding box transporter ComA effectively blocks the streptococcal QS pathway, causing phenotypic or behavioral changes. Thus, QS inhibitors/ QQ is a promising method for controlling oralbiofilm-related diseases.[29]

Phage Therapy: Phage therapy, utilizing strictly lytic phages, is a novel approach for treating multi-drug-resistant bacteria and biofilms in oral infections. For example, T4 Rnl1 phage shows antimicrobial activity against S. mutans, altering its extracellular polysaccharide structure. Phage φAPCM01 reduces S. mutans biofilm metabolism and live cells by 5 log CFU/mL after 24 hours, while Siphoviridae phages cut F. nucleatum biofilm biomass by 70%. Phages targeting Actinobacteria, Firmicutes, and Fusobacteria offer effective alternatives or adjuncts to broad-spectrum antibiotics. Thus, the construction of phage libraries against oral pathogens offers excellent opportunities for more-personalized dentistry and oral microbiome engineering.[29]

Other Therapies: Fluoride nanophase materials and natural extract-based OH products prevent caries by promoting enamel remineralization, reducing harmful microorganisms, and disrupting carbohydrate fermentation. Nanoparticles (NPs) enhance drug efficacy by attaching to bacterial cell walls, disrupting biofilms, and releasing ions that damage cells. They also improve drug solubility and stability. Recent advancements include Dimethylaminododecyl methacrylate with pH-dependent antifungal effects, Thymus capitatus oil affecting S. mutans and C. albicans, Citrus limon var. pompia extract targeting S. mutans, and catechol inhibiting A. actinomycetemcomitans, P. gingivalis, and P. intermedia.

These data provide promising methods for the clinical treatment of caries, periodontitis, pulpitis, oral mucosal diseases & halitosis.[29]

FUTURE DIRECTIONS AND RESEARCH NEEDS

In this review, we have summarized recent studies of the communities of oral microbiota and the endogenous & exogenous factors that influence its composition (Fig 8). Advances in metagenomics, metaproteomics, and metabonomics have enhanced our understanding of oral microbiota communities. Increasing evidence supports the notion that various internal & external factors cause the dysbiosis of the oral microbiota, contributing to oral & systematic diseases.[29]

Therefore, more attention must be paid to mechanisms underlying the interactions among oral microorganisms within these communities & their interactions with their host, rather than merely identifying the composition of the oral microbiome. Current studies still focus on[29] Cancer, characterized by its multi-step & slowly progressing, involving a complex interplay of genetic & environmental factors. Periodontal pathogens influence chronic inflammation, immune response, cell invasion, proliferation, anti-apoptotic activity, and carcinogenic substances, all of which precede cancer development. While linking periodontal pathogens directly to carcinoma is challenging, pathogens like P. gingivalis, F. nucleatum, A. actinomycetemcomitans, T. forsythia, and T. denticola affect multiple signaling pathways contributing to cancer. Further research into these mechanisms may reveal key pathways for diagnosis, prevention, and treatment.

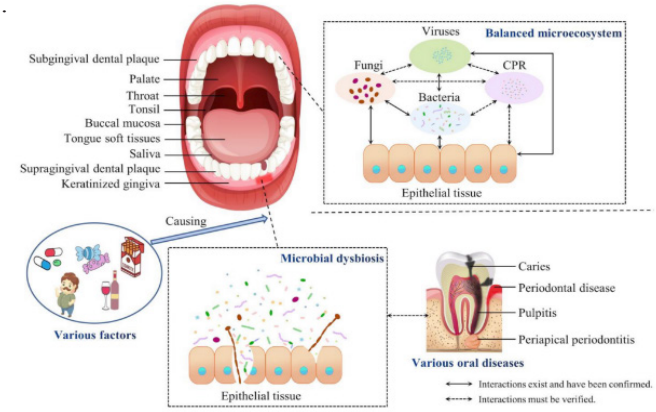

Figure 8. Compositions of the balanced oral microbiota and during dysbiosis. The oral cavity is divided into 9 niches. The communities of oral microorganisms & their interactions with the host maintain the oral microecosystem in a dynamic balance. However, various factors cause the dysbiosis of the oral microbiota. CPR, candidate phyla radiation.

These insights enhances the efficacy of treatment & contribute to advancements in survival outcomes. We have also highlighted recent promising therapeutic strategies for oral diseases. Few studies on these strategies' impact on oral dysbiosis show consistent results due to limitations in sampling procedures and challenges in enrolling subjects with varied clinical traits. To adequately mimic both the host & microbial behavior during the therapeutic process, an effective approach/ model is required to analyze the shifts in compositions of the microbial communities.[29] Thus, using the oral microbiome as a reliable diagnostic tool has emerged as an important non-invasive option in the early detection of periodontal treatment.

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