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Oral microbiome research from a public health perspective and implications for oral health

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Advancing oral microbiome research has revealed the association between oral microbiome composition and oral disease. However, much of the research has predominantly focused on comparing health and disease conditions, overlooking the potential dental public health implications. This article examines the evolution of oral microbial research from inception, advancement, and current knowledge of health-associated microbiota. Specifically, we focus on two key aspects: the impact of lifestyle and environmental factors on the oral microbiome and using the oral microbes as a therapeutic modality. The complex interaction of host intrinsic, environmental, and lifestyle factors affects the occurrence and development of the oral microbiota. The article highlights the need for ongoing research that embraces population diversity to promote health equity in oral health research and integrate public health practices into microbiome-based research. The implication of population-level interventions and targeted approaches harnessing the oral microbiome as an intervention, such as oral microbiome transplantation, should be further explored.

Keywords: *oral microbiome, dental public health, epidemiology, microbiota, research*

Introduction

The human body is considered a "superorganism", consisting of functionally, metabolically, and spatially interconnected microorganism and human cells that have co-evolved over thousands of years (Sleator, 2010). The microbes provide various traits the human body requires for evolution, highlighting the symbiotic relationship between humans and microbes. The human microbiome consists of an estimated $3.8*10^{13}$ bacterial cells and includes commensal bacteria inhabiting the oral cavity, the skin, and the intestinal mucosa (Cho *et al.*, 2021; Sender *et al.*, 2016). The updated expanded Human Oral Microbiome database (HOMD) includes 774 species in the aerodigestive tract (Chen *et al.*, 2010).

Joshua Lederberg, a Nobel Prize recipient, introduced the word "microbiome" (Dewhirst *et al.*, 2010), referring to the entire genome (genes) of microorganisms such as bacteria, archaea, lower or higher eukaryotes, viruses and their surroundings and microenvironment. The microbiome encompasses a broader spectrum, including microbial structural elements, metabolites and environmental conditions, microbial communities and the contiguous extension of the oral cavity is called the oral microbiome. In comparison, microbiota refers to various living microorganisms residing within a given environment.

The oral cavity harbours the second most varied site of microbial growth within the human body after the colon (Dewhirst *et al.*, 2010). Compared to the colon, the oral cavity is an open environment and is affected by intrinsic (pH of saliva, immune system) and extrinsic factors (e.g., lifestyle and environmental factors) and, over a longer time frame, genetic mutations and horizontal gene transfer (Consortium Human Microbiome Project, 2012; Dewhirst *et al.*, 2010). Oral microbiome research has mainly focused on common oral diseases, such as dental caries, gingivitis, periodontitis, and mucositis (Morrison *et al.*, 2023). Studies involving healthy participants are essential for defining the oral microbiota in health before correlating with the disease. However, there is a lack of research in understanding the factors associated with a healthy oral microbiome at a population level. It is important to understand the variations in communities and then implement the best practices for public health to support the development of health-associated microbiomes.

We provide an overview of oral microbiome research, the influence of host lifestyle, and environmental factors shaping the oral microbiome and concepts of health and disease through a public health lens, addressing the issues and challenges related to oral microbiome research in reducing oral health inequities. The application of health-associated oral microbiome as a public health intervention and personalised medicine is also described.

Timeline of oral microbiome research: shift from culture to molecular to next-generation sequencing

Figure 1 describes the timeline of oral microbiome research. The origin of microbes dates to the early 16th century. The development of the microscope and Antony Philips van Leeuwenhoek's subsequent detection of

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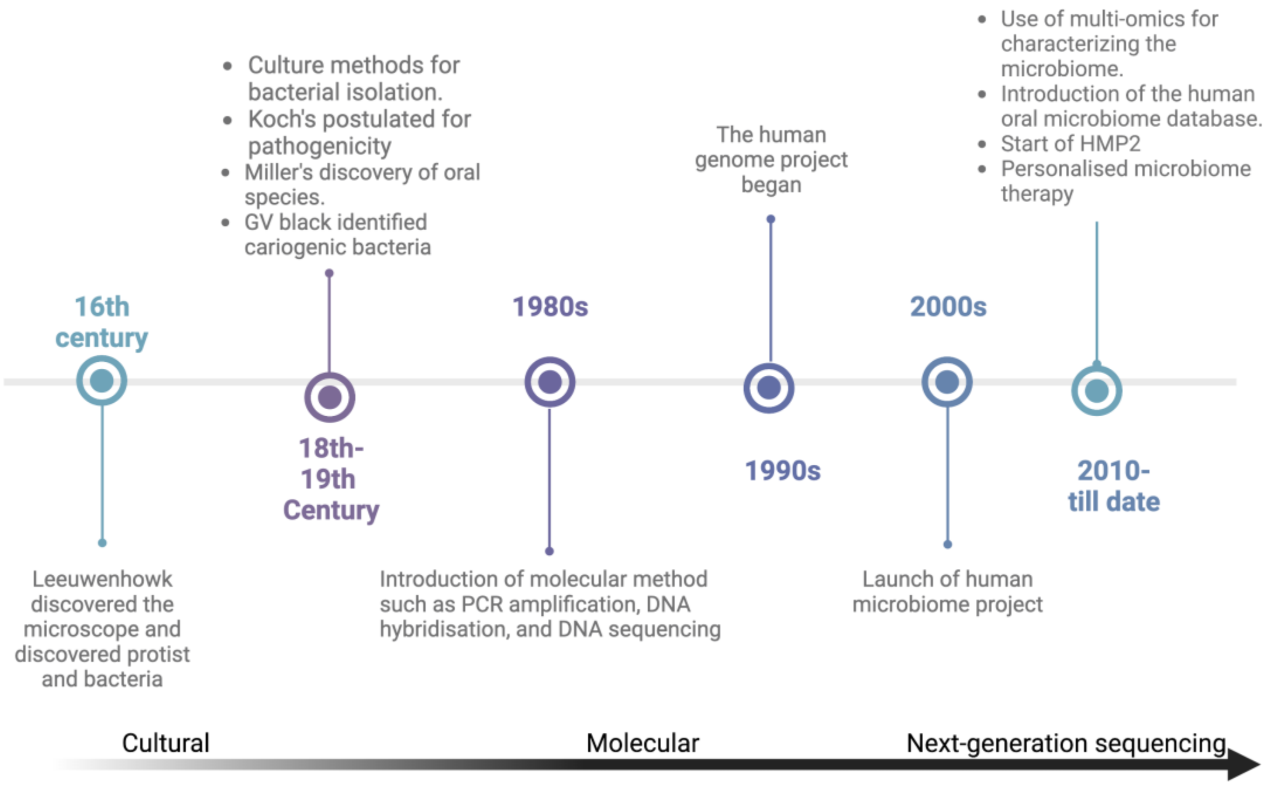

Figure 1*. Timeline of oral microbiome research. From cultural to molecular to next-generation sequencing.*

bacteria in pond water and dental plaque in the 1670s marked the beginning of the study of human microbiology (Lane, 2015). He was recognised as the 'father of microbiology' after discovering protists and bacteria, which he called animalcules. He was unknowingly, the first to draw dental plaque and the most abundant bacteria in the mouth, *Streptococcus* (Brock, 1961; Porter, 1976).

The 1800s saw the introduction of a culture-based approach that relies on developing viable and culturable bacteria to identify microbes with biochemical subtyping. Robert Koch, in 1881, is credited for developing the first microbial isolation techniques (Brock, 1961). Koch also formulated concepts for pathogenicity known as Koch's postulates, which was an important milestone in microbiology (Handelsman, 2004). WD Miller (1890), a dental scientist, collaborated with Koch and described the isolation of more than 100 species from the oral cavity and published a seminal work titled 'microorganisms of the human mouth'. At the same time, GV Black (1886) reported culturing and identifying bacteria from samples of carious teeth and *S. mutans* was identified as a contributor to dental caries. The emerging field of environmental microbiology has brought significant changes in the perspective of health and disease, and ground-breaking research highlighted the positive impacts microorganisms have on their hosts (Paster *et al.*, 2001).

In the twenty-first century, research shifted from focussing on a single species causing a disease to considering an overall change in the microbial community known as the ecological plaque hypothesis. The 1980s saw a revolution in oral microbiome research with the advent of DNA sequencing techniques, DNA hybridisation, and polymerase chain reaction (PCR), allowing the amplification and identification of specific genes in oral samples. Carl Woese and George E Fox (1977) utilised the 16S ribosomal RNA molecule as a phylogenetic marker and later used the primed 16S rRNA gene in high throughput amplicon sequencing (Woese and Fox, 1977). Two standard methods are used to study the microbiome: the targeted gene approach and the shotgun metagenomics approach.

The Human Genome Project (HGP) began in 1990, and the first sequence constituted about 90% of the human genome (Carrasco-Ramiro *et al.*, 2017). The human microbiome project (HMP) was launched as an extension of the HGP, and the term microbiome was popularised. The National Institute of Health established the HMP in 2007. The HMP was carried out in two phases and was completed in 2018 (Turnbaugh *et al.*, 2007). The first phase (HMP1) focused on characterising the microbial community of numerous human body sites from healthy adults and on specific diseases. The focus of the second phase was to promote an understanding of host-microbiome communication, including molecular mechanisms and immunity, using multi-omics approaches such as metabolome, proteome, transcriptome, epigenome, and genome. The human oral microbiome database (HOMD) was released as the first oral-specific web-based database with information on oral microbial species identified and based on 16S rRNA sequencing data.

Around the late 2000s, several researchers started using 16S rRNA genes and PCR amplification to study the oral microbiome concerning dental and root caries (Aas *et al.*, 2008; Preza *et al.*, 2008). Keijser *et al.* (2008) studied the bacterial richness of the commensal oral microbiome from saliva and plaque from a healthy adult population using a next-generation sequencing (Keijser *et al.*, 2008). Oral microbiome research has evolved after introducing metagenomics and meta-transcriptomics, revealing the microbial complexities. The current trend reflects a shift in the research focus from treatment to prevention, which may lead to targeted intervention in the future. Researchers have progressed beyond studying individual microbial communities to analysing the function of the oral microbiome in disease development and discovering microbial indicators and targets for various oral diseases (Sedghi *et al.*, 2021).

Oral microbiome in health

The oral environment provides favourable conditions for the proliferation of numerous microorganisms due to its optimal temperature, moisture levels, and host-derived nutrients like gingival crevicular fluid and salivary proteins (Kilian *et al.*, 2016). The construction of our microbial community during the postnatal period is crucial for the optimal morphological and functional development of the immune system as it begins immediately after birth (Belkaid and Hand, 2014). The commensal human microbiome includes ten times as many cells as humans. The increase in the relative abundance of single microbial taxa may or may not affect microbial function, but instead, it is the complex ecological interactions of multiple microbial taxa. Higher diversity has been associated with health (Nath *et al.*, 2022). These microbial communities are normal inhabitants of the oral cavity and perform a wide range of activities for the host's survival. Each individual possesses a distinct "microbial identity" characterised by their unique microbial population, almost as unique as their fingerprint. Despite their inter-individual differences, the fundamental function of the microbiome remains consistent across individuals.

The term "core microbiome" has mainly been applied to taxonomically defined microbial communities to identify groups of microorganisms that are particularly common in the host population (Hamady and Knight, 2009). Earlier, the core microbiome was determined to understand human biological functions, although this has been challenged over the years as rare taxa are equally important (Jousset *et al.*, 2017). The healthy core microbiome has been divided into five types: common core, temporal core, ecological core, functional core and host-adapted core (Risely, 2020). The common core is the most prevalent taxa (30%-95%) within the host population and has stability over time (detected in 70% of sampling events). The oral microbiome primarily consists of six phyla, which account for 96% of the taxa: *Firmicutes* (genera *Streptococcus* and *Granulicatella*), *Bacteroidetes* (genera *Prevotella*, *Capnocytophaga*, and *Porphyromonas*), *Proteobacteria* (genera *Neisseria* and *Haemophilus*), *Actinobacteria* (genera *Corynebacterium*, *Rothia*, and *Actinomyces*), *Spirochaetes*, and *Fusobacteria* (genus *Fusobacterium*). The phyla *Euyachaeota*, *Chlamydia*, *Chloroflexi, SR1*, *Synergistetes, Tenericutes*, and *Saccharibacteria* comprise the remaining 4% of the taxa (Benn *et al.*, 2018).

The overgrowth of pathobionts results in the pathogenicity of the local microbial community, resulting in inflammation and further increasing the growth of other pathogenic bacteria, exacerbating host immune responses and inflammatory sequelae. The microbiota (*Porphyromonas gingivalis, Tannerella forsythia, Treponema denticola* and *Aggregatibacter actinomycetemcomitans*) associated with periodontitis interferes with the host's signalling pathways, which disturbs the homeostasis, and once the host's innate immunity is weakened, leads to a change in the relative abundance of micro-organisms, causing inflammation and bone loss (Nath *et al.*, 2022; Relvas *et al.*, 2021). The oral microbiota of dental caries is associated with acid- and alkali-producing, acid-tolerant species such as *S. mutans*, *Actinomyces*, *Lactobacillus*, *Neisseria*, *Prevotella*, *Propionibacterium*, and *Scardovia* (Baker *et al.*, 2021).

The role of oral microbiome in dental public health.

The early investigations mainly focused on finding the pathogenic microbiota causing oral disease, neglecting the role of the host or environmental factors in maintaining a healthy oral microbiome. Recent research has shifted the focus towards host factors, their combined influence on the host-oral microbiome (Mukherjee *et al.*, 2021; Willis *et al.*, 2022) and encompassing the development of a balanced and healthy oral microbiome. The composition and function of the oral microbiome are strongly shaped by the host's lifestyle and environmental factors such as diet, antibiotic usage, education, housing conditions, and social network characteristics (Nearing *et al.*, 2020; Renson *et al.*, 2019). Table 1 describes the host, environmental factors affecting the oral microbiome and public health initiatives.

Diversity in populations is necessary to fully understand the role of the oral microbiome and the differences in geographic and socio-economic factors. However, the current literature relates primarily to euro-centric urban populations. Most studies have been conducted in the U.S. Some examples of large-scale population studies include the HMP (Chuong *et al.*, 2017) and the National Health and Nutrition Examination Survey oral microbiome study (Vogtmann *et al.*, 2023). The research's generalisability and representativeness have been questioned, with 80% of the population being privileged white groups with minimal race/ethnicity variability. For findings to be generalisable and to increase understanding of the global oral microbiome, research needs to be diversified, including among underprivileged minoritised populations (Nath *et al.*, 2021a). Large-scale population-based studies with socio-economically diverse samples that explore the socioeconomic, lifestyle and environmental factors are required to better understand the role of the human oral microbiome.

The body of evidence is also limited to variations in relation to structural inequities and oral microbiomes. The altered oral-gut microbiome can affect the gestational environment and be passed on from mother to offspring with intergenerational health impacts. Structural discrimination based on socio-economic status (SES), race, ethnicity, and gender can influence the microbiome's composition. The oral microbiome responds to these changing factors, potentially reflecting the impact of discrimination on individuals belonging to marginalised groups (Amato *et al.*, 2021). The differences in the oral microbiome among minoritised populations may reflect structural inequalities and exacerbate health inequities (Handsley-Davis *et al.*, 2020).

Table 1. Lifestyle and environmental factors affecting the oral microbiome.

Factors	*Effects on the microbiome*	*Public health recommendation*
Age	Older age is associated with lower relative abundance and diversity and even higher levels of *Prevotella* and *Veillonella* (Takeshita *et al.*, 2016).	Targeted programmes to maintain good oral hygiene and regular dental visits for older people for health-associated microbiome.
Social Factors	Income, education, race, ethnicity and housing are associated with disparities in dental caries and periodontal disease (Renson *et al.*, 2019). There are differentially abundant taxa among non-Hispanic white, non-Hispanic black, Asian, Mexican, and Puerto Rican ethnicities. (Consortium Human Microbiome Project, 2012)	Policies to reduce income inequality, improve access to dental services, and having a culturally safe environment for the minoritised and underprivileged population.
Diet	Highly processed modern Western diet can shift the oral microbiome. High intake of sugar and refined carbohydrates is associated with *Streptococcus*, *Actinomyces*, *Rothia*, *Scardovia*, *Veillonella*, and *Lactobacillus (*Angarita-Diaz et al., 2022).	Facilitating healthy eating, dietary recommendations for caries prevention, and improving access to fibre rich nutritious healthy food.
Physical activity	Lack of exercise and sedentary lifestyle can negatively impact the oral microbiome with lower alpha and beta diversity (Uchida *et al.*, 2021).	Encouraging regular exercise can improve the oral microbiome.
Smoking	*Streptococcus sobrinus* and *Eubacterium brachy* associated with smoking (Belstrøm *et al.*, 2014). Smoking linked to development of anaerobic oral environment and reduction of aerobic bacteria (Yu *et al.*, 2017).	Tobacco reduction policies.
Alcohol	Can impact bacterial cytotoxicity, as ethanol acts as a substrate for bacterial metabolism and cause disruptions in saliva-bacterium interactions (Fan *et al.*, 2018). Although moderate and regular red wine consumption has shown no changes in diversity (Sanz-Martin *et al.*, 2017).	Alcohol reduction strategies
Antibiotic usage	Reduces alpha diversity and relative abundance. Overuse can lead to drug resistant strains (Moraes *et al.*, 2020).	Antibiotic stewardship to reduce antimicrobial resistance and development of resistome.
Chronic health condition	Diabetes, rheumatoid arthritis (RA) are associated with inflammatory responses and the development of periodontal disease with more *Capnocytophaga*, *Porphyromonas* and *Pseudomonas* among diabetics, *Prevotella* and *Leptotrichia* and *Prevotella* among those with RA (Graves *et al.*, 2019).	Targeted health education about conditions caused by periodontitis. Professional tooth cleaning, oral hygiene instruction, motivation.
Saliva factors	Saliva flow influences which taxa can persist in mouth and which are cleared (Marsh *et al.*, 2016). Reduced salivary flow, lowered buffering and constant low pH increase prevalence of caries-associated microbiota.	Eating vegetables that stimulate saliva flow, such as celery and carrots. Chewing gums containing polyols and xylitol to increase salivary flow.
Oral hygiene	Brushing frequency and flossing have been associated with richer and even microbiome (Bertelsen *et al.*, 2022; Nath *et al.*, 2022).	Supporting brushing twice daily and flossing.
Fluoride exposure	Fluoride toothpaste and in water prevent demineralisation and promote remineralisation s.	Water fluoridation, use of fluoride toothpaste and regular application of fluoride varnish
Dental visits and treatment	Promotes screening for oral diseases. Preventive measure such as scaling, and fluoride treatment support the health-associated microbiome.	Widening and supporting access to care
Dental diseases	Dental caries and periodontal disease are associated with a disease related microbiome	Therapies may modulate the oral microbiome, including oral microbiome transplantation, prebiotics, probiotics, or antimicrobial peptides (Baker and Edlund, 2019; Nath *et al.*, 2021b).

Such findings can help to understand the oral microbiome and develop strategies to modify and shape it via individualised therapies, diet, drugs, or bacterial transplants. The microbiome data collected from dental public health surveys could be used for biomarker discovery for health conditions and disease, screening, and diagnosing oral health conditions.

Therapeutic applications of oral microbiome

For many decades, there was a belief that oral diseases such as dental caries and periodontitis were primarily caused by the increased mass accumulation of bacteria and plaque build-up rather than differences in the qualitative composition of oral bacteria. Traditional dentistry mainly focused on addressing this issue with mechanical plaque removal as the primary approach. Whilst scaling and root planing support plaque removal, they may be insufficient if there are underlying alterations or pathogenic factors within an individual's oral microbiome. The mechanical cleaning removes all the bacteria, including those that are beneficial. Eliminating the pathogenic bacteria and creating an environment supporting a healthy microbiome's growth is crucial. The focus should be shifted from repairing the damage (such as teeth restoration) to a preventive approach. Furthermore, the microbiome's quantity or quality dictates the disease's severity and how an individual responds to the altered microbiome (Kilian *et al.*, 2016). Prebiotics and probiotics have been used to modulate the oral microbiota. Probiotics are live microorganisms administered in small amounts to confer health benefits in the host. A combination of prebiotics and probiotics is known as synbiotics, and postbiotics are inactivated microorganisms and their components that benefit the host (Airola *et al.*, 2023). Arginine has been used in prebiotics to generate ammonia, which buffers dental acids produced by dental plaque (Bowen *et al.*, 2018) and promotes the growth of health-associated species. Several researchers have explored the use of *Streptococcus dentisani* and *Streptococcus* A12 in anti-cariogenic probiotics formulations (Baker and Edlund, 2019).

Oral microbiome transplantation as a potential dental public health intervention

As a daily life occurrence, the involuntary transmission of oral microorganisms from one individual to another occurs daily through saliva. Oral microbiome transplantation (OMT) is a therapeutic intervention like faecal microbiome transplantation (FMT) that involves the transfer of oral microbiota from a healthy donor to a recipient with either dental caries or periodontal disease (Figure 2A). Dewhirst and Hoffman proposed OMT (Nascimento, 2017); later on, Pozhitkov et al. (2015) hypothesised the transfer of health-associated microbiota for treating periodontitis. The initial protocol suggested the direct transfer of oral bacteria from a healthy donor to a recipient. They defined healthy donors as free of cariogenic bacteria such as *S. mutans* and producing a minimal pH fall in response to sugar challenge. The authors suggested the application of an antimicrobial agent such as sodium hypochlorite (NaOCl) followed by neutralisation by sodium ascorbate buffer before the transplantation. We proposed the conceptual process of OMT from benchtop to chairside (Figure 2B).

So far, there is no evidence of microbiota transfer among humans. OMT experiments have been tested using a canine model of naturally occurring periodontitis (Beikler *et al.*, 2021). There was a transient ecological shift in the oral microbiota composition among the recipient dogs, resembling the donor dogs with no adverse events. For long-lasting beneficial effects, a single transplant may be insufficient and requires multiple transplantations to sustain a pro-homeostatic alteration in the recipient's dysbiotic microbiota. Other authors have proposed similar concepts of microbial transfer. Payne and colleagues (2019) transferred a dysbiotic mouse oral microbiome, resulting in periodontitis and bone loss among healthy mice. Xiao et al. (2017) showed that the transfer of microbiota from rats with diabetes to germ-free mice caused a shift in bacterial composition, and

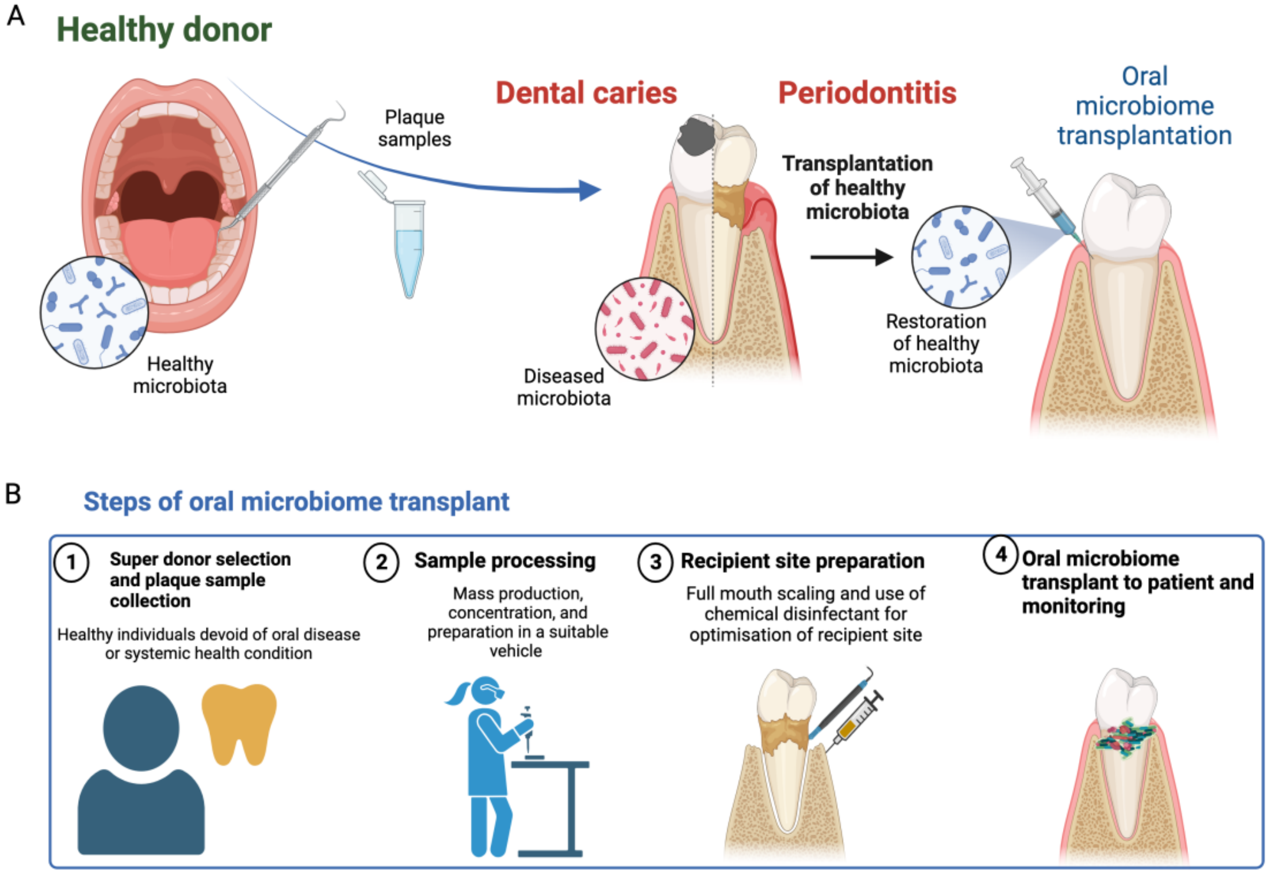

Figure 2. *Conceptual process of Oral Microbiome Transplantation. A) collecting plaque samples from healthy donors and transferring the microbiota to a patient with dental caries and periodontitis. B) Standardized steps in Oral microbiome Transplantation. Image created with BioRender.com.*

treatment with IL-17 antibody decreased the pathogenic activity of the diabetic mice. Xiao et al. (2021) reported the application of OMT for head and neck radiotherapy-induced oral mucositis in mice.

The donor screening, storage of collected microorganisms, microbiota delivery methods, and sustainability, safety and stability of the transplanted microbiota need further clarification and investigation before clinical trials. The oral microbiome transplants must be tailored for each recipient and dental condition. Because of low environmental pH, microbial diversity appears lower in dental caries. In contrast, periodontitis is associated with greater microbial diversity (Relvas *et al.*, 2021) in the early stages. As the disease advances, the diversity collapses, leading to the predominance of pathogenic species like the red complex bacteria. Therefore, the treatment aims to disrupt this dysbiosis, reduce the load of pathogenic bacteria, and establish a balanced oral microbiome. For OMT to succeed, the transplanted micro-organisms should endure the selective pressure of the oral environment, colonise the recipient site, compete with the disease microbiota for adhesion sites and nutrition, be sustained in the recipient site and modulate host response (Nath *et al.*, 2021b). The OMT might be beneficial in specific populations as it has the potential to cause a shift towards a healthy microbiome. It may support elderly people in long-term care facilities or those with a disability or dementia to prevent oral disease and improve the overall quality of life. Applying OMT might also benefit children as a preventive approach for early childhood caries. For populations residing in rural and remote locations, OMT could be beneficial where groups have limited access to dental services (Nath *et al.*, 2021b).

Conclusion

This article highlights public health measures to improve health-associated oral microbiota. An emphasis on continued research involving population diversity and minoritised underprivileged communities is required to enhance our understanding of the oral microbiome. Manipulating the oral microbiome purposefully is a promising area for managing dental caries and periodontitis, adopting an interdisciplinary approach that integrates oral health, microbiology, and public health with personalised medicine in tailoring towards an individual microbiome. Future research would benefit from a more natural selection process, with oral microbiome transplants considered.

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Conflict of interest

None declared.

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Author contribution statement

SN conceived the idea of the manuscript and drafted the manuscript. LSW, PZ, KK contributed to drafting and critically reviewed the manuscript. LMJ conceived the idea of the manuscript, contributed to drafting, and critically reviewed the manuscript. All authors read and approved the final version.

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