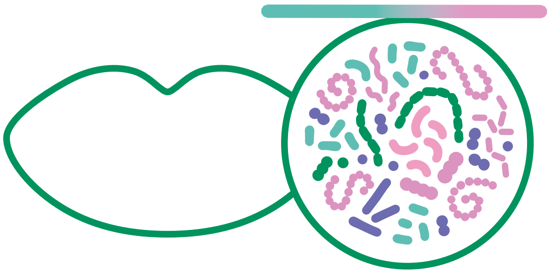


RETHINKING ORAL HEALTHCARE: **EXPLORING THE RESILIENCE AND MODULATION OF THE ORAL MICROBIOME**

This white paper explores how oral care professionals can support both oral and systemic health by promoting a balanced oral microbiota through more holistic, microbiome-friendly strategies.

April 2025
GUM is a registered trademark of SUNSTAR Suisse SA.

AN ORAL HEALTH WHITE PAPER SERIES | N° 4

RETHINKING ORAL HEALTHCARE: EXPLORING THE RESILIENCE AND MODULATION OF THE ORAL MICROBIOME

TABLE OF CONTENTS

© SUNSTAR Suisse SA.

ABSTRACT

Our **understanding of the oral microbiota** — and its role in both oral and systemic health — is **still evolving**. **Traditional oral care practices**, though effective in reducing plaque and disease, **may not fully address the complexity of the oral ecosystem**. As a result, they may unintentionally disrupt microbial balance, with possible implications for oral and systemic health.

Most oral microbes are not pathogenic and are, in fact, essential to maintaining oral health. Attempting to eliminate all microbes is neither feasible nor without risk. **A more effective approach supports a balanced oral microbiota** — one that fosters beneficial microbes while limiting the growth of pathogenic ones.

Disruptions to this balance, particularly in susceptible individuals, can lead not only to caries and periodontitis but may also elevate the risk and severity of systemic conditions such as diabetes and obesity.[2] **Restoring microbial balance is therefore key — not only for oral health, but for broader health outcomes.**

Emerging adjunctive strategies, including biological modulation through diet, lifestyle, prebiotics, probiotics, and synbiotics, **offer a promising complement to traditional oral hygiene**. These approaches can help promote microbial resilience and shift the oral microbiota towards a healthier, more stable state — minimizing the risk of disease at both the oral and systemic level.

This white paper explores the science behind oral microbiota balance and presents a path forward for more holistic, microbiome-friendly oral care.

This white paper was developed by the GUM® brand with the kind collaboration of Prof. Egija Zaura, professor of Oral Microbial Ecology at the Academic Centre for Dentistry Amsterdam (ACTA - The Netherlands) and Prof. Dr. Wim Teughels, professor in Periodontology & Oral microbiome in the Department of Oral Health Sciences at KU Leuven (Belgium).

The GUM® brand is dedicated to elevating oral care routines into **mindful and enjoyable self-care rituals** that empower healthier lives, for longer.

IMPORTANCE OF THE ORAL MICROBIOTA

THE ORAL MICROBIOTA IS A COMPLEX AND DYNAMIC ECOSYSTEM

A healthy oral environment is dependent on the microbiota that resides in the mouth.[2] This microbial community (also known as the oral microbiome when considered within its environment[3]) includes bacteria, fungi, viruses, protozoa, and archaea. Although the oral microbiome consists of a dynamic population, it is normally remarkably stable. It is however constantly exposed to different environmental stimuli, including diet, oral hygiene practices, smoking, salivary flow, and medications.[4] The most commonly studied oral microbes are bacteria. Currently more than 700 different bacterial species have been identified in the oral microbiota.[5] However, not all species are present in the same mouth at the same time. On average, a healthy human oral cavity contains over 200 bacterial species,[6] particularly belonging to the genera *Streptococcus, Actinomyces, Neisseria, Rothia, and Veillonella.*[7,8]

In a healthy mouth, the microbiota has many beneficial roles. For example, certain bacterial strains:

- **Maintain balance within the microbiota** by helping to keep harmful bacteria in check and preventing overgrowth that can lead to caries, periodontal diseases, oral candidiasis, and bad breath.[9]
- **Support periodontal health** by helping to regulate inflammation and the immune response, thus helping to prevent gingivitis and periodontitis.[10]
- **Neutralize acids** by maintaining a healthy pH balance in the mouth (facilitated by saliva), preventing excessive acid buildup that can demineralize the enamel.[11]

THE ORAL MICROBIOME AND MICROBIAL BALANCE IN ORAL HEALTH

Functions of the oral microbiota that help maintain good oral health[1,7]

Supports immune balance – Regulates pro- and anti-inflammatory processes

Protects against infections – Helps prevent disease development

Detoxifies chemicals – Processes environmental toxins

Strengthens barriers – Maintains mucosal defense

Aids digestion – Supports nutrient breakdown

Blocks harmful microbes – Prevents pathogen overgrowth

Oral health is influenced by the balance of microbial strains within the oral microbiota — some are beneficial by helping to protect against inflammation, while others contribute to periodontal diseases or caries development. Maintaining a dynamic equilibrium within the oral microbiota, to avoid that opportunistic pathogens take over, and between the microbiota and the host is therefore essential for oral health.[12,13]

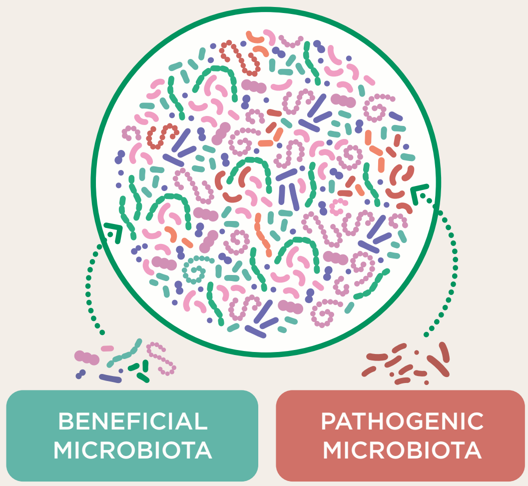


Figure 1: The oral microbiome and microbial balance in oral health

THE ORAL MICROBIOTA EVOLVES OVER A LIFETIME

The oral microbiota is acquired at birth through maternal transmission, and develops and diversifies from the first feeding onwards.[14-16] Changes in the resident microbiota continue throughout life, depending on teeth eruption and dental treatments such as orthodontic appliances,[17] but also with variations that reflect different diets, lifestyles, environments, age, medication use, hormonal changes, and oral hygiene regimens *(Figure 3)*.[14-16] The hard and soft oral surfaces (such as teeth, gingiva and tongue) each contain different microbiota, with their own distinct characteristics.[1,4,18,19] Furthermore, each person has their own unique microbial identity.[13]

The majority of oral bacteria can survive without oxygen. Oxygen-tolerant strains thrive on supragingival surfaces and exposed areas of the oral mucosa, where they rely on saliva as a primary nutrient source. In contrast, oxygen-intolerant bacteria accumulate in the gingival sulcus and subgingival niches, where they are exposed to gingival crevicular fluid, as well as in the deep grooves of the tongue.[20,21]

BACTERIAL RESIDENCE

Oxygen-tolerant bacteria thrive on exposed surfaces, relying on saliva

Oxygen-intolerant bacteria colonize subgingival areas, where they interact with crevicular fluid, and tongue grooves

Figure 2: Bacterial residence

THE ORAL MICROBIOTA EVOLVES THROUGH LIFE — AND CAN RESET WITH AGING

The maternal microbiome does not transfer directly to the fetus but primes the fetal immune system for microbial exposure after birth. **A balanced maternal oral microbiome is essential.**

Microbes colonize the infant's mouth through vaginal delivery, skin contact, and breastfeeding.

Once established, **oral microbial communities form a stable, interdependent balance** with host tissues. Tooth eruption and replacement alter microbial habitats, requiring management for oral health.

The oral microbiome evolves with genetics, systemic factors, and modifiable influences like diet, hygiene, behaviors (alcohol, tobacco), medications, and stress.

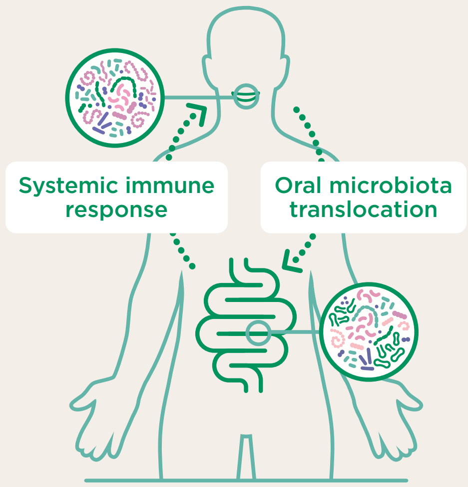
Hormonal changes (puberty, pregnancy, menopause) and aging can impact microbial balance, requiring proactive care.

Figure 3: Oral microbiota evolution through age

THE ORAL MICROBIOTA IMPACTS ORAL AND SYSTEMIC HEALTH

There is a highly dynamic, bidirectional relationship between the oral microbiota and the physiologic, metabolic, and immunologic functions that occur in the mouth.[1,7] This balance can be influenced by numerous internal and external factors.[24]

THE ORAL-GUT CONNECTION: A TWO-WAY MICROBIAL RELATIONSHIP



The oral microbiota is the second most diverse after the gut.[4] In fact, there is a direct connection between the oral and gut microbiota (known as the gum-gut axis). Beneficial or harmful factors that alter the oral microbiota have been linked to corresponding changes in the gut microbiota.[22,23]

Figure 4: The oral-gut connection

Beneficial factors.

Sufficient volume of high-quality saliva, effective oral hygiene, and robust immune function foster a balanced oral microbiota that is able to maintain good oral health and is beneficial to the host — the microbiota is in eubiosis.[25]

Detrimental factors.

Disruptions caused by poor oral hygiene, chronic stress, medication use, smoking, poor nutrition, dry mouth, etc. disrupt the microbial balance, which is detrimental to the host — the microbiota is led into dysbiosis. This imbalance weakens the oral ecosystem, increasing the risk of oral diseases such as caries and periodontitis. Dysbiosis triggers localized inflammation in the oral cavity, which can contribute to chronic, low-grade systemic inflammation. This systemic inflammation has been linked to conditions such as diabetes, cardiovascular disease, and obesity.[26]

A FRAGILE BALANCE: EUBIOSIS AND DYSBIOSIS

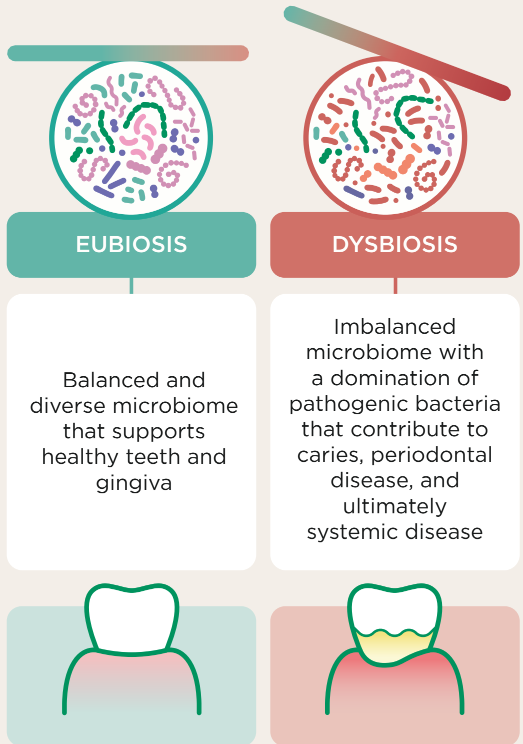


Figure 5: Eubiosis and dysbiosis

- **Balance within the oral microbiota has an impact on health**
- **Whether the oral microbiota remains in eubiosis or becomes dysbiotic depends on its resilience to change.**

RESILIENCE OF THE ORAL MICROBIOTA

WHAT IS MICROBIOTA RESILIENCE, AND WHY DOES IT MATTER?

Resilience in any biological system refers to its ability to recover from challenges and return to its pre-challenge state, which can have both positive and negative implications. Ideally, resilience serves as a protective factor, enabling the system to maintain a healthy environment. However, resilience can also sustain pathological states, making it harder to restore balance once disrupted.

For example:

- The human oral microbiota has coevolved with its host to thrive in the unique environment of the mouth. Although its primary goal is to grow and reproduce, it has developed mutually beneficial mechanisms that support both its own survival and the host's oral and systemic health—for example, by modulating inflammation and protecting against disease.[4] Accordingly, the microbiota has also developed remarkable resilience to cope with the influx of factors that can potentially influence its complex, diverse composition and functions, and thereby to maintain eubiosis.[19]
- Yet resilience also applies to pathogenic states, where poor oral hygiene and other factors disrupt the balance of the oral microbiota, allowing potentially pathogenic bacteria to dominate microbial communities (i.e., dysbiosis) — giving rise to a stable microbial community that is resilient against a return to good health.[19] In the case of caries, for example, dysbiosis favors acidogenic (acid-producing) and aciduric (acid-tolerant) microbes that thrive in low pH environments, leading to enamel demineralization. For periodontal diseases, dysbiosis is characterized by an increase in anaerobic and proteolytic organisms, which can evade or subvert host immune responses, resulting in tissue destruction and chronic inflammation. These distinct microbial shifts underscore the complexity of dysbiosis and its role in different oral diseases.[19,28,29]

To maintain oral and systemic health, it is vital that the oral microbiota is in a balanced, stable state (i.e., eubiosis) that is resilient to adverse changes in the local environment, such as acid stress or inflammation.

THE DOUBLE-EDGED SWORD OF RESILIENCE

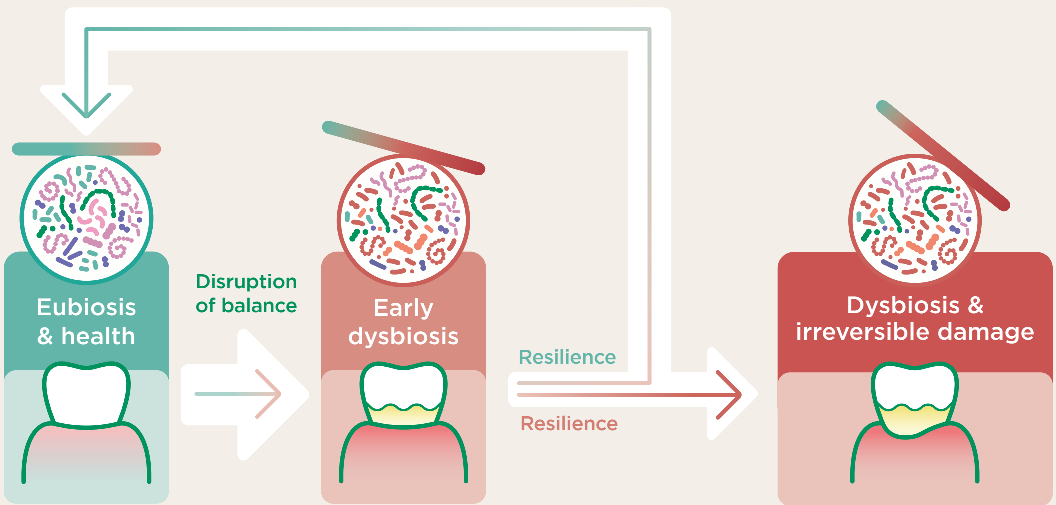


Figure 6: Resilience allows the microbiota to recover from disruptions and maintain balance. Resilience can also sustain disease states, making recovery difficult

KEY FACTORS THAT SHAPE MICROBIOTA RESILIENCE

Several **key factors can affect the resilience of the microbiota:**
- **the biofilm**
- **the saliva**
- **the immune status of the host**

INFLUENCERS OF RESILIENCE

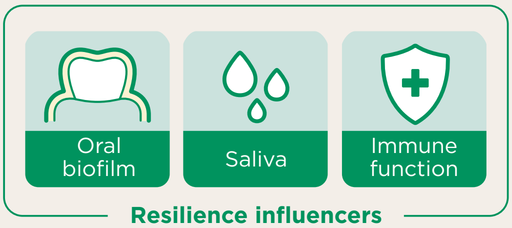


Figure 7: Key factors affecting the resilience of the microbiota

THE DUAL ROLE OF THE ORAL BIOFILM: ESSENTIAL FOR BALANCE, RISKY WHEN UNCHECKED

The oral biofilm is essential for resilience of the microbiota — but can cause problems. The numerous microbes within the mouth do not live as solitary cells, but instead form complex, functionally organized communities within extracellular biological matrix known as biofilms (such as dental plaque). These form in various st , as outlined in *Figure 9*.

The biofilm adheres to oral surfaces, including the teeth, supra- and subgingival surfaces, tongue, cheeks, lips, and the hard and soft palate and accumulate in gingival sulcus.[1,31] Especially, the non-shedding nature of teeth provides a stable surface where microbial communities can persist, thrive, and form thick biofilms.[1] The diverse conditions at each oral habitat (such as acidity, nutrient and oxygen availability, host defenses, oral hygiene, etc.) will determine the resulting type of microbiota that will reside at that site.[4]

BIOFILM NICHES

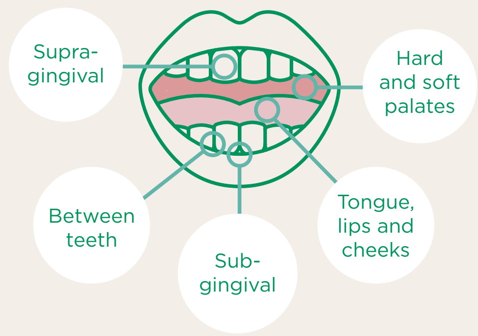


Figure 8: Biofilm niches

Biofilms provide a physical barrier that **protects microbes** against external insults and maintains an **optimal environment** for the resident microbial cells.

- Although the biofilm provides a protective environment for the oral microbiota, excessive **biofilm accumulation** on and between teeth **can be problematic**. Such build-up increases the number of microbes at the site, promoting biofilm maturation and enabling the outgrowth of potentially pathogenic bacteria that may trigger gingival inflammation.[31,32]

- As the biofilm matures, its composition can shift towards an unbalanced state — dysbiosis. In this state, **the reduced quantity of beneficial microbes creates conditions that favor the proliferation of potentially pathogenic bacteria, ultimately contributing to oral diseases such as gingivitis and periodontitis**.

Quorum sensing: How bacteria communicate, cooperate, and compete

Within the biofilm, bacteria from different species communicate with each other and coordinate their behavior and functions, in a process known as **quorum sensing**. This allows them to:[16,33,34]

- Colonize and interact with the host.
- Regulate biofilm formation.
- Quickly adapt to changes in the environment (e.g., nutrient availability, the presence of toxic compounds, dry conditions, etc.).
- Resist against colonization by competitor bacteria through various mechanisms (such as preventing their adhesion and reproduction).

This cell-to-cell communication permits **functional redundancy** within the biofilm — i.e., when multiple bacterial species perform similar roles, one species can replace another without affecting the overall function of the biofilm. **Thus, functional redundancy allows the biofilm to be stronger than each species individually — an important factor in the resilience of the microbiota.**[19]

Yet quorum sensing also enables pathogenic bacteria to compete with commensals and become more tolerant of host defenses and antimicrobial agents. For example, pathogenic bacteria use quorum sensing to delay bacterial production of tissue-damaging virulence factors until the pathogenic bacteria have accumulated enough to overwhelm host defense mechanisms; they also use it to form their own biofilms to protect against antibiotics and immune cells, adjust their metabolism to use resources from the commensal bacteria, and activate genes that make them more resistant to antibiotics.[33,35,36]

It is important to recognize that if the biofilm is allowed to mature *(Figure 9)*, dysbiotic microbial communities can become resilient — this can contribute to inflammation and periodontal diseases, which can themselves contribute to more serious systemic diseases.[37-39] Therefore, it is important to continuously maintain the biofilm at an immature, more balanced stage.

BIOFILM MATURATION AND DYSBIOSIS

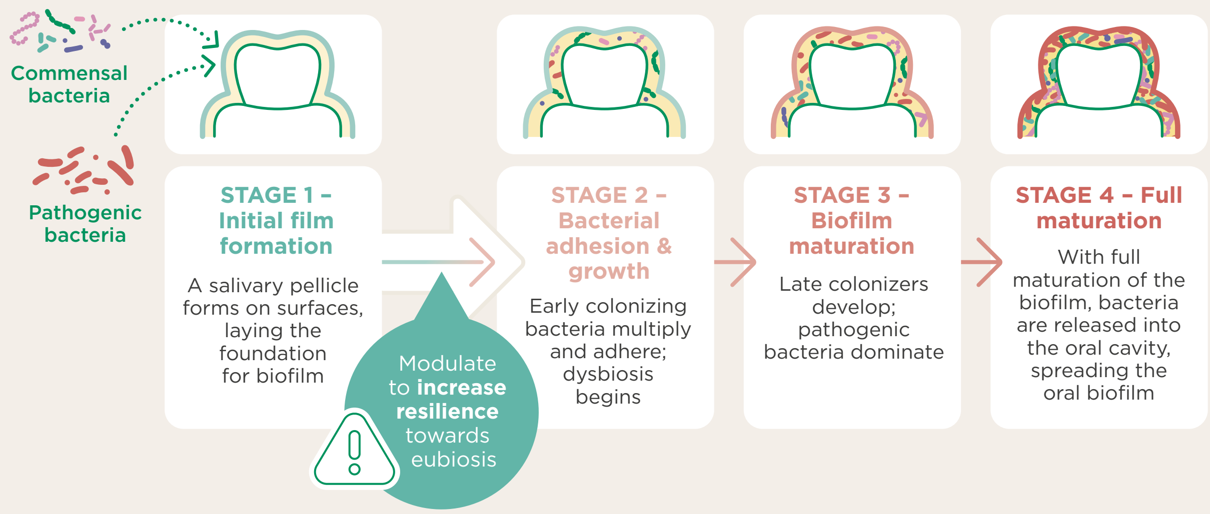


Figure 9: Stages of biofilm formation. To prevent dysbiosis and instead maintain eubiosis, it is important to disrupt the biofilm maturation at an early stage of formation (before stage 2).[30]

BIOFILM MATURATION IMPACTS ORAL & SYSTEMIC HEALTH

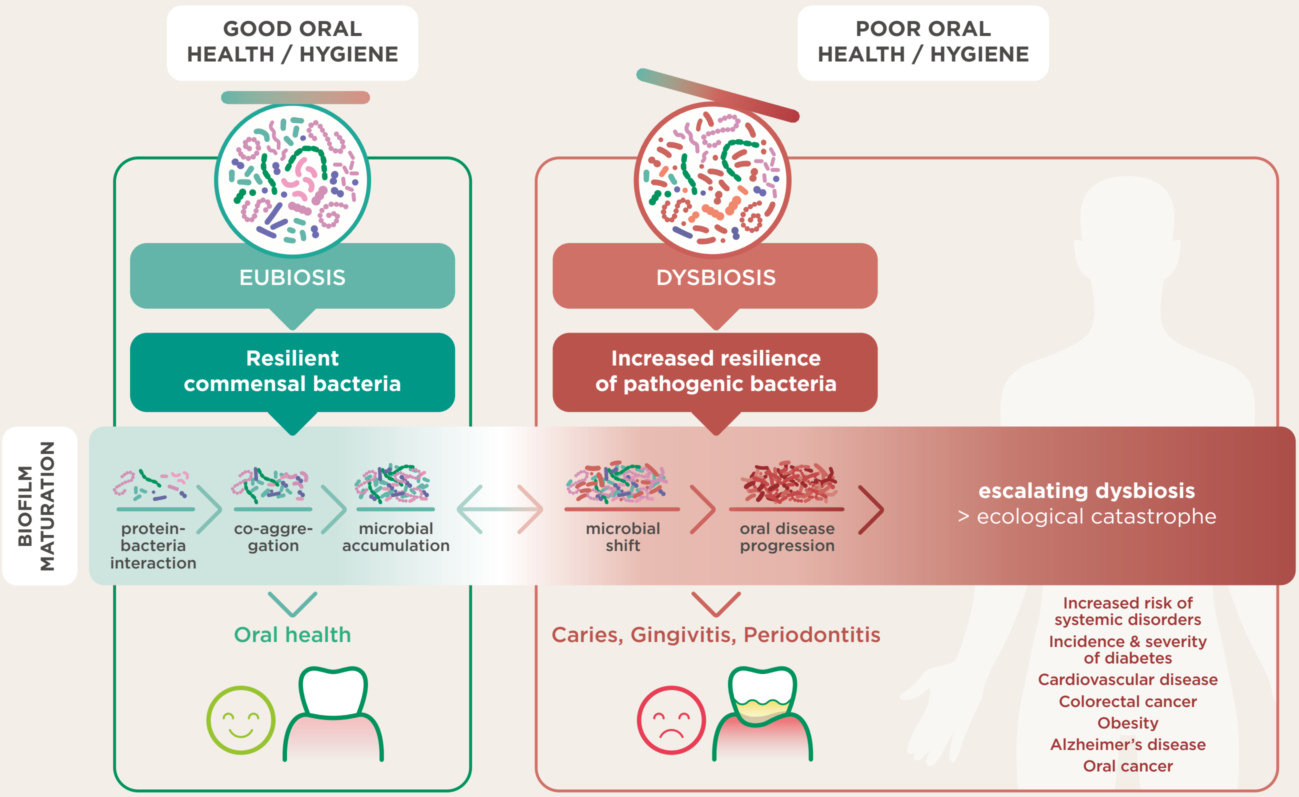


Figure 10. Resilience of the bacteria in oral biofilm can either be beneficial for the oral microbiota and the host — or beneficial for the dysbiotic oral microbiota and detrimental to the host.[1,4,15,16,21]

SALIVA AS A KEY REGULATOR OF MICROBIAL BALANCE AND ORAL HEALTH

To maintain a balanced oral microbiota, the microbes need to be kept warm, moist, and well nourished.[19] Saliva has key roles in maintaining the health of microbial communities:

- It provides moisture to prevent bacteria from becoming desiccated, as well as nutrients such as vitamins, glycoproteins, amino acids, urea, and bicarbonate for microbial growth and stability.[1,19,24] All these nutrients are in complex polymer forms. Breaking down these salivary components to simple energy sources requires concerted interactions among many different microbial species. By providing complex instead of simple energy sources, saliva contributes to the maintenance of a stable microbial community where different species need each other and have to collaborate. This prevents single species from having an advantage and dominating the rest.
- The continuous flow of saliva clears dietary carbohydrates and microorganisms from the oral cavity, delivers a range of immune factors (including antibodies, antimicrobial proteins, and peptides) that help to prevent infections, facilitates the transport and dispersion of microbes across different oral niches, and prevents excessive microbial accumulation.[40,41]
- Saliva contains antimicrobial components such as lysozyme, lactoferrin, and peroxidases, which help to regulate microbial populations and inhibit pathogenic overgrowth.[40,41]

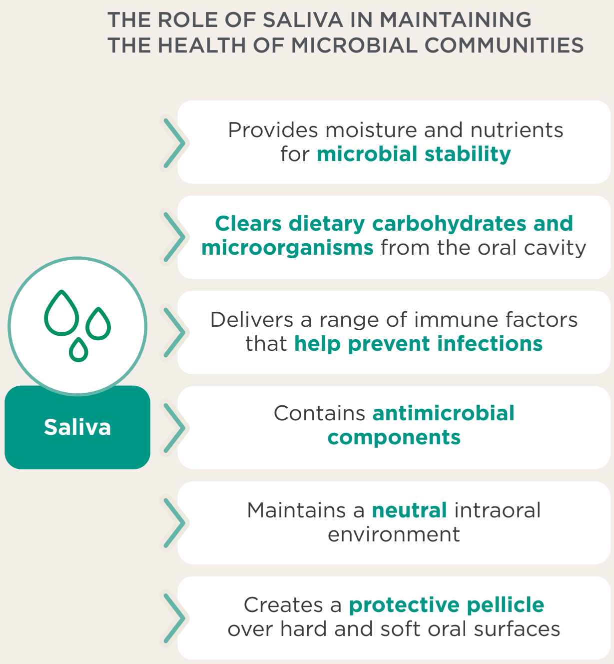


Figure 11. The role of saliva in maintaining the health of microbial communities

- Salivary fluid maintains a neutral intraoral environment to a) prevent acid conditions that lead to aciduric bacteria from becoming dominant[42] and b) counteract the acids produced by bacterial fermentation of carbohydrates, thus reducing the risk of tooth demineralization. This ability (known as its buffering capacity) is provided by its bicarbonate, phosphate, and protein systems that allow saliva to resist changes in pH.[19,41]
- Saliva creates a protective pellicle over hard and soft oral surfaces—this film contains receptors that allow bacterial attachment and colonization, but also proteins that prevent non-oral microorganisms from adhering to the oral surfaces.[41]

The dynamic interplay between saliva and the oral microbiota helps to preserve eubiosis and resist environmental stresses.

If saliva production or quality is reduced (for example, by not drinking enough fluids or regularly eating salty and sugary foods, or because of medications, underlying illness, or anxiety and stress), the mouth can become dry (hyposalivation). This increases the number of certain microorganisms within the oral microbial communities and alters the microbiota composition—including colonization by non-oral bacteria (such as coliforms and *Staphylococcus aureus*)[43] and fungi. Hyposalivation can lead to oral fungal infections, halitosis, dental caries, periodontitis, and systemic infections.[44] Existing systemic diseases can also have an adverse effect on the quality or quantity of saliva; diabetes, for example, raises glucose levels in saliva (and oral tissues) and increases the risk for hyposalivation, affecting bacterial nutrition and the subsequent composition of the oral biofilm.[24]

Saliva is one of the major influencing factors of resilience within the oral microbiota.[19]

IMMUNE FITNESS SHAPES SUSCEPTIBILITY TO DYSBIOSIS AND ORAL DISEASE

The pro- and anti-inflammatory activities of resident bacteria are crucial for maintaining oral health.[1] In areas where the biofilm has not been regularly removed, it matures and becomes dysbiotic. As a result, resilient dysbiotic microbial communities accumulated in gingival sulcus, supra- and subgingival surfaces, and on teeth induce a pathologic and dysfunctional inflammatory response that can induce a self-feeding cycle of escalating dysbiosis — an ‘ecological catastrophe’.[45,46] For example, the inflammatory reactions caused by dysbiosis can lead to[46]:

- Increased gingival crevicular fluid — this may contain nutrients to support the growth of pathogenic bacteria and immune factors that further exacerbate inflammation, change the local pH to favor the growth of pathogenic bacteria, block quorum sensors that inhibit the growth of certain species, leading to thicker and more robust biofilms.

- Increased capillary permeability, which allows fluid produced during inflammation to reach the inflamed pockets, providing essential nutrients for pathogenic bacteria, and easier access and translocation for bacteria and their byproducts to the systemic circulation and distant body parts, contributing to low grade systemic inflammation.

- Decreased oxygen availability, which promotes the growth of anaerobic, often pathogenic bacteria.

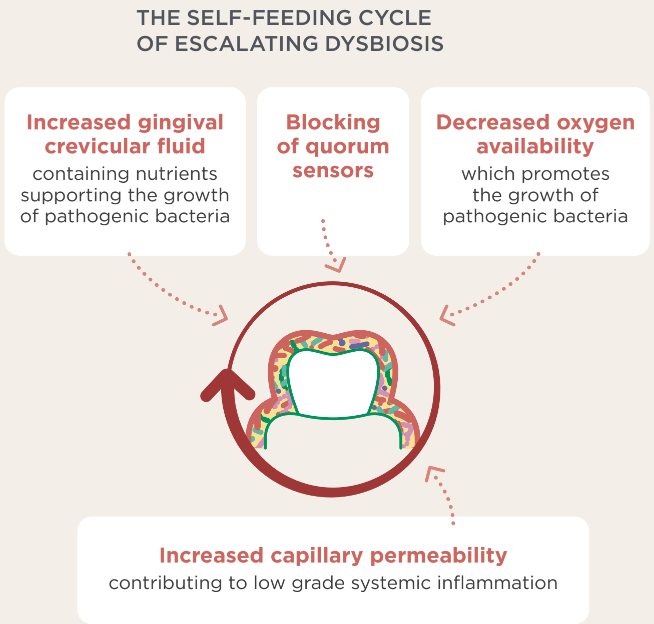


Figure 12. The self-feeding cycle of escalating dysbiosis

This escalating inflammatory response results in tissue damage, contributes to oral diseases such as periodontitis, and can increase the risk of chronic systemic diseases (e.g., diabetes, obesity, cardiovascular disease, inflammatory bowel disease, among others).[15,38,45] In addition to its impact on the inflammatory response, dysbiosis in the oral microbiota disrupts numerous other immune mechanisms throughout the body, which can lead to more serious systemic problems if eubiosis cannot be reestablished.[24,38,45]

Susceptibility to oral and systemic disorders is very much dependent on the immune status of an individual.[19,28] If a host's 'immune fitness' (i.e., adequate functioning of the immune system) is suboptimal (such as in people with diabetes or obesity), this will impair their ability to resolve the ongoing inflammation triggered by dysbiosis in the biofilm and increase their risk of systemic disease.[45,47]

Variations in caries and periodontal disease severity can occur even under identical environmental conditions due to differences in innate immune defence mechanisms, which also impact periodontal inflammation.

MODULATION OF THE ORAL MICROBIOTA

SHIFTING THE MICROBIOTA TOWARD BALANCE

Modulating the oral microbiota towards a state of eubiosis and good health while improving its resilience in this state is essential. Several actions can be taken to ensure that the microbial communities strengthen the health of the host and do not cause disease, as outlined below.

1) Prevent the dental biofilm from maturing and becoming dysbiotic.[19]

2) Maintain good levels and quality of saliva and prevent dry mouth (for example, by regularly hydrating, avoiding sugary foods and drinks, avoiding mouth breathing, reducing stress[48]).

3) Support immune fitness (e.g., through good nutrition, regular physical exercise, healthy sleeping, avoiding smoking, and by minimizing stress).

4) As in the gut, it may be beneficial to support the oral microbiota by restoring the microbial balance (such as through the use of pre- or probiotics).

Together these modulatory activities will help to control *against* factors that favor dysbiosis and instead direct the oral microbiota *towards* a resilient eubiosis.[24]

MECHANICAL MODULATION: DISRUPTING HARMFUL BIOFILMS

Regular brushing and interdental cleaning prevent biofilm maturation

Good oral hygiene is vital to prevent the biofilm from maturing. It is important to regularly disrupt biofilm growth at an early stage, before the biofilm can mature and become dysbiotic to the host *(stage 2, Figure 9)*, in which more resilient and potentially pathogenic microbiota will dominate *(stages 3-4)*.[30]

Mechanically disrupting plaque biofilm is essential for good oral health. It helps to keep the biofilm in an immature state, dominated by early plaque-forming bacteria (particularly streptococci).[19] These microorganisms help to maintain oral health by:

- Preventing colonization by harmful bacteria
- Modulating the immune system to enable bacterial survival

However, if left undisturbed, **biofilm will mature and can contribute to caries, periodontal diseases, halitosis, and systemic inflammatory conditions.**[49]

Cleaning techniques should target hard-to-reach areas to prevent dysbiosis

Brushing teeth at least twice a day for two minutes each time is important. A good technique using a brush with an appropriate design (e.g., with tailored bristles designed to reach supra- and subgingival areas) is needed to disrupt the biofilm (the tongue, which contains its own biofilm, should also be cleaned at regular intervals).[31,50,51] However, brushing alone has limited efficacy for removing biofilm from between the teeth. Using rubber interdental tools, brushes, or floss in combination with toothbrushing has been shown to be more beneficial at reducing gingival inflammation than toothbrushing alone, and is an essential component of oral hygiene practices.[52-54] Yet these cleaning techniques are not always performed correctly; additional methods can be required to ensure biofilm is disrupted before it can mature.[24]

CLEANING HARD-TO-REACH AREAS IS KEY

CHEMICAL MODULATION: THE ROLE OF TOOTHPASTE AND MOUTHWASH

Fluoride helps reinforce enamel and regulate microbial activity

For greater control over biofilm formation, toothbrushing should be combined with toothpaste that contains fluoride. Fluoride helps to strengthen tooth enamel by making it more resistant to demineralisation by acids, and has antibacterial properties that directly decrease the production of acid by bacteria, reduce acid tolerance (by increasing the permeability of cell membranes), and inhibit enzyme activity.[19]

Antimicrobial mouthwashes can reduce plaque but must be used strategically

Another strategy is to chemically disturb biofilm maturation, particularly in hard-to-reach areas, by using mouthwashes—these can contain a variety of antimicrobial agents (e.g. chlorhexidine gluconate, cetylpyridinium chloride, essential oils) designed to kill or inhibit bacterial growth *(stage 1, Figure 9)*.[55] Mouthwashes should not be used immediately after brushing however, to avoid washing away the concentrated fluoride left by toothpaste on the teeth. Studies have shown that mouthwashes can significantly reduce the amount of plaque and gingival inflammation, and may be effective anti-biofilm and anti-gingivitis agents.[55] Additionally, it should be noted that antimicrobial mouthwashes such as chlorhexidine are best reserved

In recent years, it has become increasingly clear that a more effective and holistic approach to good oral hygiene is needed to realize two key outcomes:

- **Reduce bacterial adhesion on dental surfaces** and **maintain the bacterial biofilm in an early developmental stage** and
- **Maintain eubiosis** and **prevent dysbiotic shifts in oral biofilms**.

It is possible that this could be achieved using biological modulation as an adjunct to current approaches aimed at improving oral health — a strategy that could represent the driving factor for achieving oral eubiosis, thus better supporting good oral and systemic health.

for short-term use in acute situations, such as post-surgical care, to minimize biofilm build-up and bacterial load and hence, to reduce the risk of complications.

Quorum sensing in the oral biofilm means that bacteria can become tolerant to antimicrobials used in mouthwashes.[33,35,36] The presence of dead bacteria within the oral biofilm after using a mouthwash may also increase the virulence of anaerobic bacteria that contribute to periodontitis.[56] Mouthwashes may also be ineffective against biofilm that has already become mature.[19] Furthermore, mouthwashes may inhibit or kill commensal species, taking away their beneficial roles and providing niches for colonisation of non-oral microbes or the establishment of pathogenic species.

Given these advantages and disadvantages, antimicrobial mouthwashes should: (a) generally be used only for short-term purposes, (b) always serve as an adjunct to mechanical cleaning, (c) be prescribed for specific indications determined by an oral healthcare professional (i.e., they are unnecessary for individuals with a healthy mouth), and (d) ideally be administered after a thorough professional cleaning.[57]

It should be noted that antimicrobial mouthwashes may be necessary after surgery to reduce the risk of developing surgical complications and/or poor wound healing.[58]

BIOLOGICAL MODULATION: SUPPORTING BENEFICIAL BACTERIA

Despite the various mechanical and chemical steps that can be taken to disrupt the oral biofilm, biofilm regrows within a few hours after cleaning.[24,39]

For microbiota that have survived mechanical and chemical cleaning, certain activities are essential to rebuild and reorganize the biofilm, including bacterial adhesion, nutrition, and coaggregation and communication.[24] Modulating any of these activities may help to guide microbiota towards a more balanced composition. Examples of how to achieve this and modulate the oral microbiota are outlined below.

Diet & lifestyle: building a microbiota-friendly environment

Both diet and the oral environment influence the balance, functions, and virulence of the oral microbial community,[59,60] while also affecting the host. Modulating these aspects could have a positive impact on establishing eubiosis. For example:

- The microbiota relies on saliva and gingival crevicular fluid for nutrition, and will utilize any sugars and proteins present in these fluids. Higher sugar levels after consuming sucrose and starch-rich foods or fermentable sugar-containing drinks for example will increase biofilm growth and especially survival of acidogenic and aciduric microbiota — leading to a higher risk of gingival inflammation and caries development.[48,60,61] Reducing the intake of sugar could help to reduce these risks.[60]
- Healthy, vitamin and fibre-rich foods may also support a healthier oral ecosystem. They induce less inflammation and

hence have a beneficial systemic effect overall.[60]

- Smoking is known to alter the ecology of the oral microbiota into dysbiosis by increasing the acidity of saliva, depleting oxygen, influencing bacterial adherence to mucosal surfaces, and impairing host immunity.[62] As smoking-related changes to the oral microbiota are not permanent, smoking cessation could help to restore eubiosis.

Prebiotics: feeding the right microbes

A prebiotic is defined as a "substrate that is selectively utilized by host microorganisms conferring a health benefit", i.e., a substance that serves as nutrient for beneficial microorganisms.[63]

In the oral ecosystem, prebiotics have the capacity to improve the microbial environment, encourage the growth of beneficial microbiota, and inhibit microbial adhesion or have antimicrobial properties that prevent pathogenic microbes from becoming established.[64]

Prebiotics tailored for oral health are less common than those for gut health.

Prebiotics specific for oral health are rare and an effective prebiotic in one body site (such as the gut) may have no effect elsewhere in the body (such as the oral cavity).[63] Those that have been shown to have potential to maintain a balanced oral microbiota include sugar alcohols (e.g., xylitol, sorbitol), arginine (an amino acid), and nitrate (a naturally occurring compound found in many fruits and vegetables such as red beet or spinach).

Select prebiotics like xylitol, arginine, and nitrate may promote oral eubiosis

Xylitol may lower the risk of dental caries via several mechanisms. These include enhancing remineralization of tooth enamel, reducing the level of caries-associated bacterium *Streptococcus mutans* in saliva, and raising the pH of dental plaque (i.e., reducing the acidity) by increasing saliva production and reducing the numbers of acid-producing bacteria.[65] However, xylitol loses its effect in the presence of fructose which is high in fruits or sucrose (both are also often added to processed foods).[64]

Arginine is actively metabolised by Arginine Deiminase System - an enzyme complex in some oral bacteria - leading to ammonia production which in turn leads to pH increase. This restoration of a neutral pH removes selective pressure on acid-tolerant microorganisms, hence, **mitigating the demineralization of tooth enamel.[64] Arginine may also work in synergy with fluoride to help prevent caries.[66]**

When nitrate encounters oral bacteria, particularly in acidic conditions, it is reduced to nitrite and nitric oxide — a gas with many physiological properties including antimicrobial activity against anaerobic bacteria that are associated with periodontitis; this action could be beneficial when the pH drops because

MICROBIOTA SUPPLEMENTS

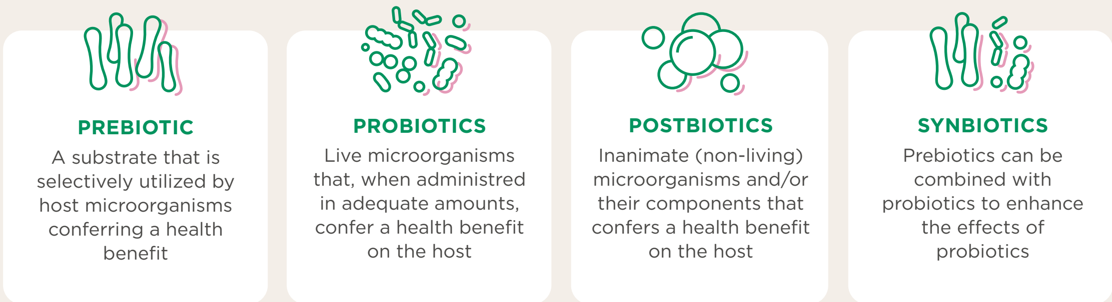


Figure 13. Microbiota supplements

of sugar fermentation. Nitrate is also broken down to ammonia in the presence of bacteria, which can increase the pH. Similarly, urea is metabolized by some oral bacteria to produce ammonia and subsequently reduce acidity within the biofilm. These actions improve the oral environment and encourage colonization by microbes related to oral health and restrict the growth of pathogenic bacteria associated with tooth decay and periodontitis.[64,66,67] Therefore, **increasing consumption of nitrate could modulate the composition and activity of the oral microbiota, helping to prevent or reduce bacterial dysbiosis and stimulating eubiosis.** In addition to modulating the oral microbiota, a further benefit of nitrate is the key role that bacterial nitrate reduction has in the maintenance of cardiovascular health: nitric oxide is involved in vasodilatation and thus has the potential to reduce blood-pressure.[19]

Probiotics: Rebalancing the microbiota

Probiotics are "live microorganisms that, when administered in adequate amounts, confer a health benefit to the host".[68] Although well-recognized for their benefits within the gut, using probiotics to improve oral health may be a novel concept for the general population. Yet for over 20 years,[66] probiotics have been extensively tested in the prevention or treatment of oral diseases, and research interest has significantly increased in the last 5-10 years.

In the oral cavity, probiotics have several potential actions, each with their own complex mechanisms *(Figure 14)*[24,64,69,70]:

Probiotics can modulate immune responses, strengthen the oral barrier, and inhibit pathogens.

1) Modulation of host immune response: Probiotics play a role in host innate and adaptive immune responses by modulating immune cells such as dendritic cells, macrophages, and B and T lymphocytes and fostering their function, and hence, that of the immune system.[71]

2) Another mode of action of probiotics is the **strengthening of the mucosal barrier function**, by promoting the production of tight junction proteins between the epithelial cells[72] and hence protecting from penetration by bacteria such as *Porphyromonas gingivalis (P. gingivalis)* and their toxins.[73]

3) Reduction of inflammation: By annihilating pro-inflammatory mediators (such as TNF-α, IL-1β),[74] probiotics can reduce the inflammatory response.

MECHANISMS OF ACTION OF PROBIOTICS IN THE ORAL MICROBIOTA

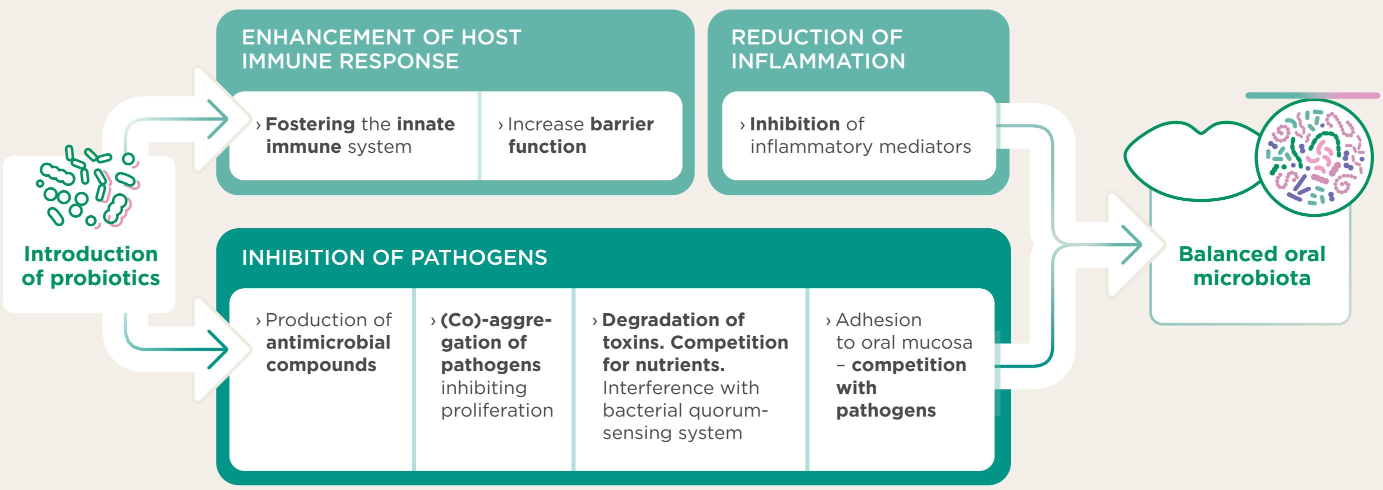


Figure 14. Mechanisms of action of probiotics in the oral microbiota

4) Direct inhibition and destruction of pathogens (e.g., via the production of antimicrobial substances, and activation of 'attack' immune cells) and of their by-products, such as gingipains (produced by *P. gingivalis*, gingipains are a group of proteolytic enzymes involved in periodontal disease, with a leading role in tissue destruction and immune evasion).[75]

5) Competitive microbial adhesion/ colonization and prevention of coaggregation (through competition for nutrients and binding sites on host cell surfaces), leading to indirect removal of pathogens and inhibition of pathogenic microbial biofilm.

Not all probiotic strains are equally effective — strains specific for the oral cavity show the most promise

Although these mechanisms concern the oral cavity, beneficial systemic effects of the probiotics are also to be expected, such as a systemic reduction of the inflammatory state.

It is important to note that the specific mechanisms of action differ between bacterial probiotic strains.[76] For oral health, most bacterial strains that have been evaluated belong to the *Streptococcus*, *Lactobacillus*, *Bifidobacterium*, and *Bacillus* genera.[69,76] The best described probiotic is *Limosilactobacillus reuteri (L. reuteri)*.

> **Using bacterial strains that are specific to the oral cavity, and even to each distinct microbial habitat within the mouth, are likely to have a more targeted and effective therapeutic effect than those that have been highly studied but may be more specific to the gut (e.g., certain *Lactobacillus* strains).**[19,66,70]

The conflicting results observed in clinical trials on probiotics for oral health can largely be attributed to the use of different bacterial strains. Additional factors may also play a role, such as insufficient dosage, product quality (e.g., non-viable bacteria), incorrect application methods (e.g., timing in relation to brushing, eating, or drinking), or lack of mechanical suppression of the oral microbiota.[76-78]

Despite these study design limitations, existing research has shown that targeting the oral biofilm with probiotics can[64,70,76,77]:

- Modulate the balance of oral microbiota.
- Create a biofilm that reduces the levels and/or inhibits the functions of oral pathogens.
- Reduce the prevalence of *Candida albicans*.
- Reduce the numbers of bacteria that cause halitosis (bad breath). And perhaps most significantly, prevent the demineralization of enamel, inhibit the formation of dental caries, lower the risk of periodontitis, and improve the outcomes of non-surgical periodontal therapy[79] and peri-implant mucositis treatment.[80]

Synbiotics: a synergistic approach

For greater therapeutic effects, prebiotics can be combined with probiotics to form synbiotics.[64] In the oral cavity, **prebiotics** (such as xylitol, arginine, and nitrate) can **enhance the ability of probiotics** to **reduce caries-associated microbiota**, maintain a high pH, or regulate the inflammatory response.[22,64,67,69,81] Glycerol in combination with the probiotic *L. reuteri* is an example of synbiotic that can improve the effectiveness of *L. reuteri*.[81]

> **This novel, synergistic approach to oral health is still in its infancy, but could lead to innovative, effective microbial products that might combat dysbiosis more effectively.**[2,64]

FUTURE DIRECTIONS

EXPANDING THE ROLE OF ORAL HEALTHCARE PROFESSIONALS

It is important for oral care professionals to be able to explain to patients how resilience within the oral microbiota can have either a positive or negative impact on their oral and general health. Providing advice on good oral hygiene techniques and a healthy diet and lifestyle—and how these will modulate their oral microbiota towards eubiosis — are the first essential steps towards self-care. Guidance with respect to the additional use of pre-, pro-, and synbiotics may further improve the oral health of their patients.

ADVANCING RESEARCH AND INNOVATION

Further research will drive the development of new products, along with treatment and preventive protocols, that selectively target pathogenic bacteria, utilize oral-specific microbial strains instead of gut strains, and promote oral eubiosis to support overall health. This requires a deeper understanding of the characteristics and interrelationships among various beneficial and dysbiotic microbial populations, and how their resilience toward dysbiosis can affect the physiologic mechanisms that keep the body in balance (homeostasis).[24,64,69,76-78] It is also likely that the delivery method used (in lozenges, mouthwashes or toothpastes) will have an impact on efficacy.[82]

In addition to disease prevention, further research into treatment options is necessary, e.g to use pre- and/or probiotics to reduce alveolar bone loss and attachment loss in periodontitis.[74,83] Co-supplementing probiotics with micronutrients may also be beneficial; for example, in the gut, B vitamins have been shown to improve the colonization of probiotics,[84] while vitamin D may help to regulate their anti-inflammatory, immunomodulatory, and anti-infective benefits.[85] It is possible that research in the oral cavity may reveal similar benefits. Recognizing the need for personalized treatment plans will also become increasingly important, which may help to overcome the different behaviors, age, medication use, health status, genetics, and associated risks of caries and periodontal diseases between patients. All of these factors, including assessment of clinical and microbial endpoints, need to be investigated in future trials to fully evaluate their impact on oral and systemic health.[78]

CONCLUSION

Dysbiosis within the oral microbiota can lead to dental caries, gingival inflammation, and periodontitis — unless the system can be modulated towards eubiosis. If dysbiotic microbial communities are allowed to become resilient, the subsequent effects on immune and metabolic functions can contribute to not only oral, but also systemic diseases.

Empowering self-care for lasting health

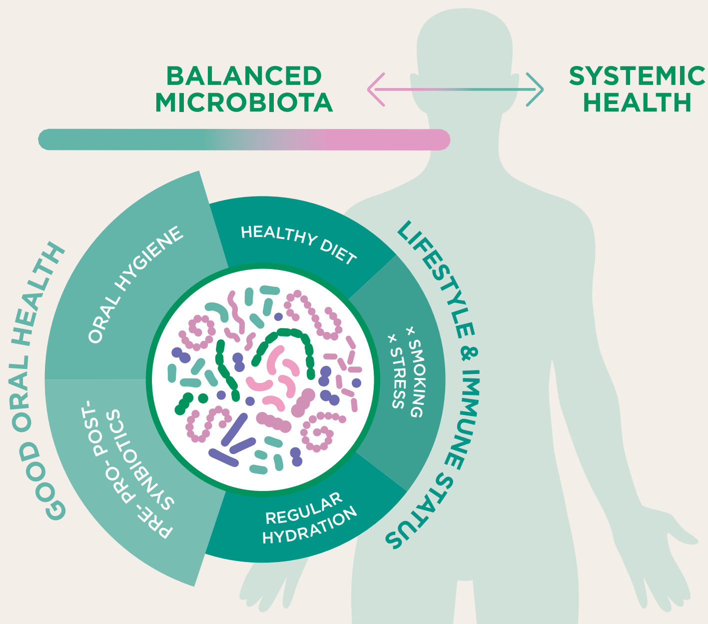
A fundamental principle in modulating the oral microbiota is self-care. Seemingly simple oral hygiene instructions given to patients can have significant benefits at the microbiological level, potentially even impacting systemic health. In addition to professional cleaning, oral health practitioners have a vital role in providing information to their patients about the following concepts:

1) The positive and potentially negative effects of the oral microbiota and why ensuring conditions that support eubiosis are important for oral and systemic health.

2) The benefits of maintaining a balanced microbiota and improving immune status using a holistic approach that includes *a)* good oral hygiene techniques, *b)* consuming a healthy diet that minimizes sugars and contains plenty of fruits and vegetables, *c)* limiting smoking, and stress, *d)* ensuring regular hydration.

3) The potential of pre-, pro-, and synbiotics to restore or maintain balance within the oral microbiota — exciting therapeutic prospects in the ongoing effort to further improve oral and systemic health, which could help to control factors that favor dysbiosis.

EMPOWERING SELF-CARE FOR LASTING HEALTH



GLOSSARY

Acidogenic: acid producing (bacteria).

Aciduric: able to grow in an acidic environment.

Aerobic: can survive and grow in an oxygenated environment.

Anaerobic: oxygen-intolerant, unable to grow in environments where there is oxygen.

Archaea: a group of single-celled prokaryotic microorganisms that are similar to — but evolutionarily distinct from — bacteria.

Dysbiosis: a state in which the balance within the microbiota, and between the microbiota and its host, is disrupted – potentially leading to harm and contributing to disease.

Eubiosis: a state of beneficial balance within the microbiota and between the microbiota and its host.

Functional redundancy: in this case, when multiple bacterial species in an ecosystem (the biofilm) perform similar roles, one species is able to replace another without affecting the function of the biofilm.

Oral microbiota: the community of microorganisms that live in the mouth; also known as the microbiome.

Postbiotics: inanimate (non-living) microorganisms and/or their components that confers a health benefit on the host.

Prebiotics: a substrate that is selectively utilized by host microorganisms conferring a health benefit. Oral prebiotics, less common, may work differently in the oral cavity.

Probiotics: live microorganisms that, when administered in adequate amounts, confer a health benefit on the host.

Proteolytic: proteolytic bacteria produce enzymes that break down proteins.

Quorum sensing: the process of cell-to-cell communication between bacteria that allows them to coordinate their behavior and functions (based on the number of bacteria in the population) and thereby adapt to the local environment.

Substrate: a substance that (in this case) bacteria use as nutrients for energy and growth.

Symbiosis: harmonious coexistence between the oral microbiota and the host, with microorganisms providing health benefits to the host.

Synbiotics: a combination of prebiotics and probiotics that work together to promote the growth and activity of beneficial bacteria, enhancing overall health.

Virulence factors: molecules and properties of molecules that help bacteria and other microorganisms to cause disease by, for example, attaching to host cells, evading the immune system, and competing for nutrients.

REFERENCES

1 Kilian M, Chapple ILC, Hannig M, *et al.* The oral microbiome – an update for oral healthcare professionals. British Dental Journal 2016;221(10):657-666. doi: 10.1038/sj.bdj.2016.865.

2 Giordano-Kelhoffer B, Lorca C, March Llanes J, *et al.* Oral Microbiota, Its Equilibrium and Implications in the Pathophysiology of Human Diseases: A Systematic Review. Biomedicines 2022;10(8). doi: 10.3390/biomedicines10081803.

3 National Human Genome Research Institute (2025). Microbiome. https://www.genome.gov/genetics-glossary/Microbiome, accessed: 4 Feb 2025.

4 Baker JL, Mark Welch JL, Kauffman KM, *et al.* The oral microbiome: diversity, biogeography and human health. Nat Rev Microbiol 2024;22(2):89-104. doi: 10.1038/s41579-023-00963-6.

5 eHOMD (2024). Expanded Human Oral Microbiome Database V3.1. https://www.homd.org/, accessed: 25 Nov 2024.

6 Campbell K. Oral microbiome findings challenge dentistry dogma. Nature 2021. doi: 10.1038/d41586-021-02920-w.

7 Deo PN, Deshmukh R. Oral microbiome: Unveiling the fundamentals. J Oral Maxillofac Pathol 2019;23(1):122-128. doi: 10.4103/jomfp.JOMFP_304_18.

8 Lu M, Xuan S, Wang Z. Oral microbiota: A new view of body health. Food Science and Human Wellness 2019;8(1):8-15. doi: https://doi.org/10.1016/j.fshw.2018.12.001.

9 Baty JJ, Stoner SN, Scoffield JA. Oral Commensal Streptococci: Gatekeepers of the Oral Cavity. J Bacteriol 2022;204(11):e0025722. doi: 10.1128/jb.00257-22.

10 Moreno CM, Boeree E, Freitas CMT, *et al.* Immunomodulatory role of oral microbiota in inflammatory diseases and allergic conditions. Front Allergy 2023;4:1067483. doi: 10.3389/falgy.2023.1067483.

11 Burne RA, Marquis RE. Alkali production by oral bacteria and protection against dental caries. FEMS Microbiol Lett 2000;193(1):1-6. doi: 10.1111/j.1574-6968.2000.tb09393.x.

12 Hu W, Chen S, Zou X, *et al.* Oral microbiome, periodontal disease and systemic bone-related diseases in the era of homeostatic medicine. Journal of Advanced Research 2024. doi: https://doi.org/10.1016/j.jare.2024.08.019.

13 Bostanghadiri N, Kouhzad M, Taki E, *et al.* Oral microbiota and metabolites: key players in oral health and disorder, and microbiota-based therapies. Frontiers in Microbiology 2024;15. doi: 10.3389/fmicb.2024.1431785.

14 Kaan AMM, Kahharova D, Zaura E. Acquisition and establishment of the oral microbiota. Periodontol 2000 2021;86(1):123-141. doi: 10.1111/prd.12366.

15 Joseph S, Curtis MA. Microbial transitions from health to disease. Periodontology 2000 2021;86(1):201-209. doi: https://doi.org/10.1111/prd.12377.

16 Sedghi L, DiMassa V, Harrington A, *et al.* The oral microbiome: Role of key organisms and complex networks in oral health and disease. Periodontology 2000 2021;87(1):107-131. doi: https://doi.org/10.1111/prd.12393.

17 Contaldo M, Lucchese A, Lajolo C, *et al.* The Oral Microbiota Changes in Orthodontic Patients and Effects on Oral Health: An Overview. J Clin Med 2021;10(4). doi: 10.3390/jcm10040780.

18 Marsh PD, Head DA, Devine DA. Dental plaque as a biofilm and a microbial community—Implications for treatment. Journal of Oral Biosciences 2015;57(4):185-191. doi: https://doi.org/10.1016/j.job.2015.08.002.

19 Wade WG. Resilience of the oral microbiome. Periodontology 2000 2021;86(1):113-122. doi: 10.1111/prd.12365.

20 Valm AM. The Structure of Dental Plaque Microbial Communities in the Transition from Health to Dental Caries and Periodontal Disease. Journal of Molecular Biology 2019;431(16):2957-2969. doi: https://doi.org/10.1016/j.jmb.2019.05.016.

21 Li X, Liu Y, Yang X, *et al.* The Oral Microbiota: Community Composition, Influencing Factors, Pathogenesis, and Interventions. Frontiers in Microbiology 2022;13. doi: 10.3389/fmicb.2022.895537.

22 Van Holm W, Zayed N, Lauwens K, *et al.* Oral Biofilm Composition, Dissemination to Keratinocytes, and Inflammatory Attenuation Depend on Probiotic and Synbiotic Strain Specificity. Probiotics and Antimicrobial Proteins 2024. doi: 10.1007/s12602-024-10253-z.

23 Maki KA, Kazmi N, Barb JJ, *et al.* The Oral and Gut Bacterial Microbiomes: Similarities, Differences, and Connections. Biol Res Nurs 2021;23(1):7-20. doi: 10.1177/1099800420941606.

24 Hernández P, Sánchez MC, Llama-Palacios A, *et al.* Strategies to Combat Caries by Maintaining the Integrity of Biofilm and Homeostasis during the Rapid Phase of Supragingival Plaque Formation. Antibiotics (Basel) 2022;11(7). doi: 10.3390/antibiotics11070880.

25 Santacroce L, Passarelli PC, Azzolino D, *et al.* Oral microbiota in human health and disease: A perspective. Exp Biol Med (Maywood) 2023;248(15):1288-1301. doi: 10.1177/15353702231187645.

26 Thomas C, Minty M, Vinel A, *et al.* Oral Microbiota: A Major Player in the Diagnosis of Systemic Diseases. Diagnostics (Basel) 2021;11(8). doi: 10.3390/diagnostics11081376.

27 Zemouri C, Ofiteru ID, Jakubovics NS. Future directions for studying resilience of the oral ecosystem. British Dental Journal 2020;229(12):769-773. doi: 10.1038/s41415-020-2407-8.

28 Rosier BT, Marsh PD, Mira A. Resilience of the oral microbiota in health: mechanisms that prevent dysbiosis. J Dent Res 2018;97(4):371-380. doi: 10.1177/0022034517742139.

29 Esberg A, Haworth S, Kuja-Halkola R, *et al.* Heritability of oral microbiota and immune responses to oral bacteria. Microorganisms 2020;8(8):1126. doi: 10.3390/microorganisms8081126.

30 Rath S, Bal SCB, Dubey D. Oral Biofilm: Development Mechanism, Multidrug Resistance, and Their Effective Management with Novel Techniques. Rambam Maimonides Med J 2021;12(1). doi: 10.5041/rmmj.10428.

31 Jakubovics NS, Goodman SD, Mashburn-Warren L, *et al.* The dental plaque biofilm matrix. Periodontology 2000 2021;86(1):32-56. doi: https://doi.org/10.1111/prd.12361.

32 Berger D, Rakhamimova A, Pollack A, *et al.* Oral biofilms: development, control, and analysis. High Throughput 2018;7(3). doi: 10.3390/ht7030024.

33 Li Y-H, Tian X. Quorum Sensing and Bacterial Social Interactions in Biofilms. Sensors 2012;12(3):2519-2538. doi.

34 Akimbekov NS, Digel I, Yerezhepov AY, *et al.* Nutritional factors influencing microbiota-mediated colonization resistance of the oral cavity: A literature review. Frontiers in Nutrition 2022;9. doi: 10.3389/fnut.2022.1029324.

35 Deep A, Chaudhary U, Gupta V. Quorum sensing and Bacterial Pathogenicity: From Molecules to Disease. J Lab Physicians 2011;3(1):4-11. doi: 10.4103/0974-2727.78553.

36 Vila T, Sultan AS, Montelongo-Jauregui D, *et al.* Oral Candidiasis: A Disease of Opportunity. Journal of Fungi 2020;6(1):15. doi.

37 Bui FQ, Almeida-da-Silva CLC, Huynh B, *et al.* Association between periodontal pathogens and systemic disease. Biomedical Journal 2019;42(1):27-35. doi: https://doi.org/10.1016/j.bj.2018.12.001.

38 Jia G, Zhi A, Lai PFH, *et al.* The oral microbiota – a mechanistic role for systemic diseases. British Dental Journal 2018;224(6):447-455. doi: 10.1038/sj.bdj.2018.217.

39 Kurtzman GM, Horowitz RA, Johnson R, *et al.* The systemic oral health connection: Biofilms. Medicine (Baltimore) 2022;101(46):e30517. doi: 10.1097/md.0000000000030517.

40 Vila T, Rizk AM, Sultan AS, *et al.* The power of saliva: Antimicrobial and beyond. PLoS Pathog 2019;15(11):e1008058. doi: 10.1371/journal.ppat.1008058.

41 Lynge Pedersen AM, Belstrøm D. The role of natural salivary defences in maintaining a healthy oral microbiota. J Dent 2019;80 Suppl 1:S3-s12. doi: 10.1016/j.jdent.2018.08.010.

42 Zhu J, Chu W, Luo J, *et al.* Dental Materials for Oral Microbiota Dysbiosis: An Update. Front Cell Infect Microbiol 2022;12:900918. doi: 10.3389/fcimb.2022.900918.

43 Hayashi Y, Saito T, Ohshima T, *et al.* Alterations of the oral microbiota and oral clinical findings in dry mouth. Journal of Oral Biosciences 2015;57(4):171-174. doi: https://doi.org/10.1016/j.job.2015.07.001.

44 Kozak M, Pawlik A. The Role of the Oral Microbiome in the Development of Diseases. Int J Mol Sci 2023;24(6). doi: 10.3390/ijms24065231.

45 Liu Y, Qv W, Ma Y, *et al.* The interplay between oral microbes and immune responses. Frontiers in Microbiology

2022;13. doi: 10.3389/fmicb.2022.1009018.

46 Loos BG, Van Dyke TE. The role of inflammation and genetics in periodontal disease. Periodontol 2000 2020;83(1):26-39. doi: 10.1111/prd.12297.

47 Cekici A, Kantarci A, Hasturk H, *et al.* Inflammatory and immune pathways in the pathogenesis of periodontal disease. Periodontol 2000 2014;64(1):57-80. doi: 10.1111/prd.12002.

48 Angarita-Díaz MdP, Fong C, Bedoya-Correa CM, *et al.* Does high sugar intake really alter the oral microbiota?: A systematic review. Clinical and Experimental Dental Research 2022;8(6):1376-1390. doi: https://doi.org/10.1002/cre2.640.

49 Bloch S, Hager-Mair FF, Andrukhov O, *et al.* Oral streptococci: modulators of health and disease. Front Cell Infect Microbiol 2024;14:1357631. doi: 10.3389/fcimb.2024.1357631.

50 Glenny A-M, Walsh T, Iwasaki M, *et al.* Development of Tooth Brushing Recommendations Through Professional Consensus. International Dental Journal 2024;74(3):526-535. doi: https://doi.org/10.1016/j.identj.2023.10.018.

51 Axe A, Mueller WD, Rafferty H, *et al.* Impact of manual toothbrush design on plaque removal efficacy. BMC Oral Health 2023;23(1):796. doi: 10.1186/s12903-023-03518-6.

52 Worthington HV, MacDonald L, Poklepovic Pericic T, *et al.* Home use of interdental cleaning devices, in addition to toothbrushing, for preventing and controlling periodontal diseases and dental caries. Cochrane Database Syst Rev 2019;(4). doi: 10.1002/14651858.CD012018.pub2.

53 Graziani F, Palazzolo A, Gennai S, *et al.* Interdental plaque reduction after use of different devices in young subjects with intact papilla: A randomized clinical trial. Int J Dent Hyg 2018;16(3):389-396. doi: 10.1111/idh.12318.

54. Ng E, Lim LP. An Overview of Different Interdental Cleaning Aids and Their Effectiveness. Dentistry Journal 2019;7(2):56. doi.

55 Takenaka S, Ohsumi T, Noiri Y. Evidence-based strategy for dental biofilms: Current evidence of mouthwashes on dental biofilm and gingivitis. Jpn Dent Sci Rev 2019;55(1):33-40. doi: 10.1016/j.jdsr.2018.07.001.

56 Rodriguez Herrero E, Boon N, Pauwels M, *et al.* Necrotrophic growth of periodontopathogens is a novel virulence factor in oral biofilms. Scientific Reports 2017;7(1):1107. doi: 10.1038/s41598-017-01239-9.

57 Brookes ZLS, McCullough M, Kumar P, *et al.* Mouthwashes: Implications for Practice. Int Dent J 2023;73 Suppl 2(Suppl 2):S98-s101. doi: 10.1016/j.identj.2023.08.013.

58 Romero-Olid MN, Bucataru E, Ramos-García P, *et al.* Efficacy of Chlorhexidine after Oral Surgery Procedures on Wound Healing: Systematic Review and Meta-Analysis. Antibiotics (Basel) 2023;12(10). doi: 10.3390/antibiotics12101552.

59 Buduneli N. Environmental factors and periodontal microbiome. Periodontol 2000 2021;85(1):112-125. doi: 10.1111/prd.12355.

60 Santonocito S, Giudice A, Polizzi A, *et al.* A Cross-Talk between Diet and the Oral Microbiome: Balance of Nutrition on Inflammation and Immune System's Response during Periodontitis. Nutrients 2022;14(12). doi: 10.3390/nu14122426.

61 Lundtorp Olsen C, Markvart M, Vendius VFD, *et al.* Short-term sugar stress induces compositional changes and loss of diversity of the supragingival microbiota. J Oral Microbiol 2023;15(1):2189770. doi: 10.1080/20002297.2023.2189770.

62 Wu J, Peters BA, Dominianni C, *et al.* Cigarette smoking and the oral microbiome in a large study of American adults. The ISME Journal 2016;10(10):2435-2446. doi: 10.1038/ismej.2016.37.

63 Gibson GR, Hutkins R, Sanders ME, *et al.* Expert consensus document: The International Scientific Association for Probiotics and Prebiotics (ISAPP) consensus statement on the definition and scope of prebiotics. Nat Rev Gastroenterol Hepatol 2017;14(8):491-502. doi: 10.1038/nrgastro.2017.75.

64 Luo S-C, Wei S-M, Luo X-T, *et al.* How probiotics, prebiotics, synbiotics, and postbiotics prevent dental caries: an oral microbiota perspective. npj Biofilms and Microbiomes 2024;10(1):14. doi: 10.1038/s41522-024-00488-7.

65 Nayak PA, Nayak UA, Khandelwal V. The effect of xylitol on dental caries and oral flora. Clin Cosmet Investig Dent 2014;6:89-94. doi: 10.2147/ccide.S55761.

66 Yu X, Devine DA, Vernon JJ. Manipulating the diseased oral microbiome: the power of probiotics and prebiotics. J Oral Microbiol 2024;16(1):2307416. doi: 10.1080/20002297.2024.2307416.

67 Rosier BT, Buetas E, Moya-Gonzalvez EM, *et al.* Nitrate as a potential prebiotic for the oral microbiome. Sci Rep 2020;10(1):12895. doi: 10.1038/s41598-020-69931-x.

68 Hill C, Guarner F, Reid G, *et al.* Expert consensus document. The International Scientific Association for Probiotics and Prebiotics consensus statement on the scope and appropriate use of the term probiotic. Nat Rev Gastroenterol Hepatol 2014;11(8):506-514. doi: 10.1038/nrgastro.2014.66.

69 Amargianitakis M, Antoniadou M, Rahiotis C, *et al.* Probiotics, Prebiotics, Synbiotics and Dental Caries. New Perspectives, Suggestions, and Patient Coaching Approach for a Cavity-Free Mouth. Applied Sciences 2021;11(12):5472. doi.

70 Chugh P, Dutt R, Sharma A, *et al.* A critical appraisal of the effects of probiotics on oral health. Journal of Functional Foods 2020;70:103985. doi: https://doi.org/10.1016/j.jff.2020.103985.

71 Mazziotta C, Tognon M, Martini F, *et al.* Probiotics Mechanism of Action on Immune Cells and Beneficial Effects on Human Health. Cells 2023;12(1). doi: 10.3390/cells12010184.

72 Takeuchi H, Sasaki N, Yamaga S, *et al. Porphyromonas gingivalis* induces penetration of lipopolysaccharide and peptidoglycan through the gingival epithelium via degradation of junctional adhesion molecule 1. PLoS Pathog 2019;15(11):e1008124. doi: 10.1371/journal.ppat.1008124.

73 Albuquerque-Souza E, Balzarini D, Ando-Suguimoto ES, *et al.* Probiotics alter the immune response of gingival epithelial cells challenged by Porphyromonas gingivalis. J Periodontal Res 2019;54(2):115-127. doi: 10.1111/jre.12608.

74 Nguyen T, Brody H, Radaic A, *et al.* Probiotics for periodontal health—Current molecular findings. Periodontology 2000 2021;87(1):254-267. doi: https://doi.org/10.1111/prd.12382.

75 Park JA, Lee GR, Lee JY, *et al.* Oral Probiotics, *Streptococcus salivarius* K12 and M18, Suppress the Release of Volatile Sulfur Compounds and a Virulent Protease from Oral Bacteria: An In-Vitro Study. Oral Health Prev Dent 2023;21:259-270. doi: 10.3290/j.ohpd.b4328987.

76 Beattie RE. Probiotics for oral health: a critical evaluation of bacterial strains. Frontiers in Microbiology 2024;15. doi: 10.3389/fmicb.2024.1430810.

77 Lundtorp-Olsen C, Markvart M, Twetman S, *et al.* Effect of Probiotic Supplements on the Oral Microbiota—A Narrative Review. Pathogens 2024;13(5):419. doi.

78 Inchingolo F, Inchingolo AM, Malcangi G, *et al.* The Benefits of Probiotics on Oral Health: Systematic Review of the Literature. Pharmaceuticals (Basel) 2023;16(9). doi: 10.3390/ph16091313.

79 Li J, Zhao G, Zhang HM, *et al.* Probiotic adjuvant treatment in combination with scaling and root planing in chronic periodontitis: a systematic review and meta-analysis. Benef Microbes 2023;14(2):95-108. doi: 10.3920/bm2022.0056.

80 Herrera D, Berglundh T, Schwarz F, *et al.* Prevention and treatment of peri-implant diseases-The EFP S3 level clinical practice guideline. J Clin Periodontol 2023;50 Suppl 26:4-76. doi: 10.1111/jcpe.13823.

81 Van Holm W, Verspecht T, Carvalho R, *et al.* Glycerol strengthens probiotic effect of *Limosilactobacillus reuteri* in oral biofilms: A synergistic synbiotic approach. Molecular Oral Microbiology 2022;37(6):266-275. doi: https://doi.org/10.1111/omi.12386.

82 Duane B, Yap T, Neelakantan P, *et al.* Mouthwashes: Alternatives and Future Directions. International Dental Journal 2023;73:S89-S97. doi: https://doi.org/10.1016/j.identj.2023.08.011.

83 Hathaway-Schrader JD, Novince CM. Maintaining homeostatic control of periodontal bone tissue. Periodontology 2000 2021;86(1):157-187. doi: https://doi.org/10.1111/prd.12368.

84 Sang L, Yang S, Zhu Y, *et al.* The combined use of B vitamins and probiotics promotes B vitamin absorption and increases Akkermansia abundance. Food & function 2024. doi.

85 Abboud M, Rizk R, AlAnouti F, *et al.* The Health Effects of Vitamin D and Probiotic Co-Supplementation: A Systematic Review of Randomized Controlled Trials. Nutrients 2020;13(1). doi: 10.3390/nu13010111.