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Dental Caries As A Biofilm-Mediated Disease: Current Concepts And Emerging Therapies

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Abstract

Background: Dental caries is a globally prevalent biofilm-mediated disease driven by ecological shifts within the oral microbiome. Frequent sugar intake, inadequate salivary buffering, and biofilm maturation promote dominance of acidogenic and aciduric bacteria that demineralize enamel.

Methods: A comprehensive review of contemporary concepts in caries etiology, biofilm ecology, diagnostic approaches, preventive strategies, and emerging therapies was synthesized from current literature.

Results: Evidence indicates that cariogenic dysbiosis results from environmental pressures rather than infection by a single pathogen. Key determinants include EPS-rich biofilm architecture, repeated low pH episodes, host factors such as saliva and enamel integrity, and dietary behavior. Mechanical plaque control and fluoride remain foundational, while adjunctive measures antimicrobials, probiotics, bioactive materials, and smart anti-biofilm technologies show growing promise in modulating biofilm virulence and enhancing remineralization.

Conclusions: Viewing caries as a reversible, ecologically driven biofilm disorder emphasizes prevention, risk assessment, and minimally invasive care. Emerging biofilm-targeted and microbiome-modulating therapies may significantly enhance future caries management.

Keywords Dental caries; biofilm-mediated disease; oral microbiome; dysbiosis; extracellular polymeric substances; Streptococcus mutans; fluoride; dietary sugars; antimicrobial therapy; emerging therapies.

Introduction

Dental caries remains one of the most prevalent chronic diseases worldwide, affecting billions of individuals across all age groups and socioeconomic strata. The global burden of dental caries is staggering, with recent epidemiological studies estimating that over 2.2 billion people suffer from caries of permanent teeth and more than 500 million children are affected by caries in deciduous teeth. This makes dental caries the third most common preventable disease globally, trailing only behind cardiovascular diseases and diabetes in terms of prevalence. The disease is not only a major cause of pain, tooth loss, and impaired quality of life but also imposes a substantial economic burden, with direct and indirect costs reaching hundreds of billions of dollars annually. Despite advances in oral health care and preventive strategies, the incidence of caries continues to rise in many regions, particularly in low- and middle-income countries where access to dental care is limited. The persistence of untreated caries reflects not only a microbial problem but also social, economic, and systemic barriers to care, highlighting the need for comprehensive public health interventions to address this silent epidemic (Li et al., 2025).

For much of the 20th century, dental caries was conceptualized as an infectious disease, with the primary focus on specific microorganisms most notably Streptococcus mutans as the causative agents. This paradigm was rooted in the pioneering work of Willoughby Miller in the late 19th century, who proposed the "chemo-parasitic theory" of caries, suggesting that acid production by oral bacteria led to enamel demineralization. The mid-20th century saw the rise of the "infectious disease model," which emphasized the transmissibility of cariogenic bacteria, particularly from mother to child, and the importance of targeting these pathogens through antimicrobial agents and vaccines. However, this model proved inadequate, as attempts to eradicate S. mutans or develop effective vaccines failed to significantly reduce caries rates. The limitations of the infectious disease model became increasingly apparent as researchers recognized that caries development was not solely dependent on the presence of specific pathogens but rather on the complex interactions within the oral microbial community. This shift in understanding paved the way for the biofilm-mediated disease model, which views caries as a multifactorial process driven by the dynamic interplay between host, diet, and the microbial biofilm (Giacaman et al., 2022).

The transition from an infectious disease model to a biofilm-mediated disease model has fundamentally transformed our understanding of dental caries pathogenesis and management. The biofilm concept recognizes that caries is not simply the result of infection by a single pathogen but rather the outcome of ecological imbalances within the oral microbial community. Dental biofilms, or plaque, are complex, structured communities of microorganisms embedded in a self-produced extracellular matrix that adheres to tooth surfaces. These biofilms are highly dynamic, with microbial composition and metabolic activity influenced by factors such as diet, oral hygiene, and host immune responses. The biofilm matrix provides protection to resident microorganisms, enhancing their resistance to antimicrobial agents and mechanical removal. This resilience makes caries prevention and treatment more challenging, as traditional approaches targeting individual pathogens are often ineffective against the biofilm as a whole. Understanding the biofilm concept is crucial for developing effective preventive strategies, such as promoting a healthy oral microbiome, controlling dietary sugars, and improving oral hygiene practices. Moreover, the biofilm model has led to the development of novel therapeutic approaches, including anti-biofilm agents, probiotics, and microbiome-modulating therapies, which aim to restore ecological balance and prevent caries progression (Meyer et al., 2021).

The global impact of dental caries extends far beyond the oral cavity, affecting overall health, quality of life, and socioeconomic well-being. Untreated caries can lead to severe odontogenic infections, which may become life-threatening without timely intervention. The disease is particularly burdensome for vulnerable populations, including children, the elderly, and those with limited access to dental care. In low-resource settings, the lack of preventive and restorative services exacerbates oral health disparities, perpetuating cycles of poverty and inequality. The economic burden of caries is substantial, with direct costs related to dental treatments and indirect costs associated with lost productivity and reduced quality of life. Despite its overwhelming prevalence, oral health is often excluded from broader public health strategies, reinforcing the crisis. Addressing the global burden of dental caries requires a multifaceted approach that integrates preventive care, public health education, and policy interventions to ensure equitable access to oral health services (Li et al., 2025).

The evolution of caries management has mirrored the changing understanding of its etiology. Early approaches focused on mechanical removal of carious lesions and restoration of damaged teeth, with little emphasis on prevention. The infectious disease model led to the development of antimicrobial therapies and vaccines, but these strategies were largely unsuccessful due to the complexity of the oral microbiome. The biofilm-mediated disease model has shifted the focus to ecological management, emphasizing the importance of maintaining a healthy oral microbiome and preventing ecological imbalances that favor cariogenic bacteria. Modern caries management strategies include dietary counseling, fluoride application, antimicrobial mouth rinses, and the use of probiotics to promote beneficial microbial communities. Additionally, advances in biofilm research have led to the development of novel therapeutic agents that target the biofilm matrix and disrupt microbial interactions, offering new avenues for caries prevention and treatment (Spatafora et al., 2024).

The role of biofilm in caries pathogenesis is central to the modern understanding of the disease. Dental biofilms are highly organized microbial communities that form on tooth surfaces and are characterized by the production of extracellular polymeric substances (EPS) that provide structural integrity and protection to resident microorganisms. The biofilm matrix facilitates the retention of acid-producing bacteria, such as S. mutans, and creates a microenvironment conducive to enamel demineralization. The dynamic nature of biofilms allows for rapid adaptation to changes in diet and oral hygiene, making them resilient to conventional antimicrobial therapies. The biofilm model also highlights the importance of microbial interactions, including competition and cooperation, in shaping the composition and metabolic activity of the oral microbiome. Understanding these interactions is essential for developing effective preventive and therapeutic strategies that target the biofilm as a whole rather than individual pathogens (Chen et al., 2020).

Etiology and Pathogenesis

Dental caries arises from a dynamic imbalance between demineralization and remineralization at the tooth–biofilm–saliva interface, driven by frequent exposure of dental plaque to fermentable carbohydrates and the resulting acidogenic and aciduric microbiota. The contemporary view defines caries as a non-communicable, biofilm-mediated disease in which dysbiosis of the tooth-associated microbiome, rather than infection with a single pathogen, leads to microenvironmental acidification, subsurface enamel dissolution, and eventual cavitation when the cumulative mineral loss exceeds reparative capacity. Acid production by bacteria such as Streptococcus mutans, Lactobacillus spp., Scardovia spp., and bifidobacteria lowers the pH within the plaque matrix below the critical threshold for hydroxyapatite dissolution (approximately pH 5.5), while the extracellular polymeric substance (EPS) matrix impedes diffusion of salivary buffers and concentrates organic acids at the enamel surface. Salivary flow, buffering capacity, fluoride exposure, and host immune responses modulate the balance between pathological and protective factors, such that caries reflects the cumulative effect of biofilm composition, dietary habits, tooth susceptibility, and time, consistent with modern caries risk models (Peterson et al., 2014).

Biofilm formation on tooth surfaces begins within minutes after cleaning through the adsorption of salivary proteins, glycoproteins, and lipids, forming the acquired pellicle that provides specific receptors for microbial adhesion. Early colonizers predominantly Gram-positive facultative cocci such as Streptococcus sanguinis, Streptococcus mitis, Streptococcus oralis, and actinomyces (Actinomyces viscosus, A. naeslundii) attach via adhesin–receptor interactions and reversible physicochemical forces, and then proliferate to create initial microcolonies. As these primary colonizers metabolize dietary carbohydrates and salivary substrates, they modify local redox potential, oxygen tension, and nutrient availability, enabling the sequential recruitment of bridging organisms such as Fusobacterium nucleatum and a wider array of secondary and late colonizers, including obligate anaerobes that integrate into the maturing plaque.

This process, often described as autogenic succession, leads to a structurally and functionally heterogeneous biofilm with spatial stratification of aerobic species near the surface and anaerobes deeper in the plaque, establishing the ecological framework within which cariogenic dysbiosis can develop under frequent sugar exposure (Chen et al., 2020).

Early colonizers are typically pioneer streptococci from the mitis group and actinomyces species that are adapted to adhere directly to pellicle components via antigen I/II adhesins, fimbriae, and other surface proteins, forming a cohesive basal layer on enamel. Their metabolism initially maintains a relatively neutral pH through limited acid production balanced by alkali-generating pathways, and they engage in extensive coaggregation interactions with each other and with bridging organisms, establishing a scaffold for further biofilm accretion. Late colonizers, which include more fastidious anaerobes such

as Prevotella, Veillonella, Porphyromonas, and other Gram-negative rods, typically do not bind pellicle directly but attach to established early colonizers or to EPS structures, contributing to increased biofilm thickness, community complexity, and environmental heterogeneity. Although some late colonizers are more strongly associated with periodontal disease, their presence in supragingival plaque influences nutrient fluxes, interspecies signaling, and redox gradients, indirectly modulating cariogenic potential and illustrating the continuum between health-associated and disease-associated oral biofilm communities (Chen et al., 2021).

Classical models of dental plaque development describe a sequence from pellicle formation and initial bacterial adhesion to biofilm maturation and eventual dispersal, each stage characterized by distinct structural and functional features. In the initial stage, an acellular acquired pellicle forms on the clean tooth, followed by reversible and then irreversible attachment of early colonizers through adhesin-mediated binding, leading to the formation of discrete microcolonies embedded in nascent EPS. During the maturation phase, microcolonies grow tridimensionally, coalesce, and develop complex architectures with channels and gradients, while microbial diversity often decreases as more competitive, acid-tolerant taxa predominate under cariogenic conditions, generating a robust biofilm that is mechanically and chemically resilient. In the dispersion stage, single cells or aggregates detach due to shear forces, enzymatic degradation of matrix components, or active microbial processes, seeding new sites on tooth surfaces and perpetuating the cycle of plaque development and potential caries initiation when local ecological pressures favor acidogenic and aciduric species (Chen et al., 2021).

The healthy supragingival biofilm is a diverse, relatively stable ecosystem dominated by commensal streptococci, actinomyces, and other taxa that contribute to colonization resistance, nitrate reduction, and maintenance of near-neutral pH through alkali-generating pathways such as arginine deiminase and urease activity. Dysbiosis in the context of dental caries is not simply the overgrowth of a single pathogen but a community-level shift toward acidogenic and aciduric organisms driven by ecological perturbations, particularly frequent intake of fermentable carbohydrates that repeatedly lower plaque pH and select for acid-tolerant species. This ecological plaque hypothesis posits that environmental changes (low pH episodes) alter gene expression and competitive fitness within the biofilm, leading to reduced microbial diversity and dominance of cariogenic consortia enriched in Streptococcus mutans, Lactobacillus, Scardovia, Bifidobacterium, and related taxa that collectively enhance EPS production, acidification, and enamel demineralization. Advanced molecular profiling and metatranscriptomic analyses have confirmed that functional shifts in carbohydrate metabolism, stress response, and matrix biosynthesis pathways accompany taxonomic changes, supporting the view of caries as a dysbiosis-associated, biofilm-driven disease process rather than a classical infection (Nath et al., 2025).

Under conditions of infrequent sugar exposure and effective salivary buffering, commensal streptococci and other health-associated taxa maintain a relatively neutral pH and limit the expansion of highly acidogenic species through competition for adhesion sites and nutrients. When dietary patterns involve frequent consumption of sucrose and other fermentable carbohydrates, repeated acidification events create a selective bottleneck within the biofilm, suppressing acid-sensitive commensals while favoring bacteria capable of both producing and tolerating low pH, such as S. mutans and certain lactobacilli. Over time, this selection pressure reduces overall microbial diversity and leads to the emergence of a cariogenic community with enhanced expression of genes involved in glycolysis, lactic acid production, EPS synthesis, and acid tolerance mechanisms, including membrane ATPases and stress response regulators. The transition from a symbiotic to a dysbiotic biofilm is thus driven by environmental pressures rather than exogenous infection, and is potentially reversible in early stages through interventions that reduce sugar frequency, increase fluoride exposure, and promote alkali-generating commensals, highlighting opportunities for ecological approaches to caries management (Cunha-Cruz et al., 2013).

Streptococcus mutans is considered a key contributor to cariogenic biofilms due to its potent acidogenicity, aciduricity, and ability to synthesize large amounts of insoluble glucan EPS from sucrose via multiple glucosyltransferases, which promote firm adhesion to enamel and co-adhesion with other bacteria. This species possesses diverse carbohydrate uptake systems and regulatory networks that enable rapid fermentation of sugars to lactic acid, as well as robust stress tolerance mechanisms that allow survival and continued metabolism at low pH values that inhibit many competing organisms. Lactobacilli, while less important in initial colonization of sound enamel, are strongly associated with deep dentinal caries and lesion progression due to their extreme acidogenicity and capacity to thrive in acidic, low-oxygen niches within advanced lesions, often in synergy with S. mutans and bifidobacteria that enhance EPS production. Other acidogenic taxa, including Scardovia wiggsiae, Veillonella spp., and bifidobacteria, contribute to lactic and other organic acid production, cross-feeding, and niche specialization within the cariogenic consortium, underscoring the polymicrobial nature of caries-associated dysbiosis (Chen et al., 2020).

The central metabolic feature of cariogenic biofilms is rapid fermentation of dietary sugars through glycolytic pathways to organic acids, primarily lactic acid, which accumulate within the EPS-rich matrix and lower pH at the tooth surface. S. mutans and other lactic acid bacteria employ multiple phosphoenolpyruvate-dependent phosphotransferase systems and other sugar transporters to efficiently import sucrose, glucose, fructose, and other carbohydrates, which are then metabolized to ATP and acids, while F-ATPase and other proton-extruding systems maintain intracellular pH homeostasis under acidic external conditions. Sucrose is uniquely cariogenic because it serves both as a fermentable substrate and as a precursor for extracellular glucans synthesized by glucosyltransferases and fructans produced by fructosyltransferases, creating an adhesive, diffusion-limiting EPS scaffold that enhances biofilm cohesion and retention on tooth surfaces. These EPS not only provide structural integrity but also establish diffusion gradients that permit rapid penetration of sugars while retarding the outward diffusion of acids and limiting access of salivary buffers and antimicrobials, thereby amplifying demineralizing conditions within deep plaque layers (Zhu et al., 2022).

The biofilm matrix, composed of polysaccharides, proteins, lipids, extracellular DNA, and host-derived components, is now recognized as a critical determinant of cariogenic potential, often referred to as the biofilm "matrixome." In cariogenic biofilms, the EPS fraction is enriched in water-insoluble glucans with strong adhesive and cohesive properties that promote three-dimensional architecture, microcolony formation, and mechanical stability under shear forces from saliva and mastication. The dense, heterogeneous matrix fosters the creation of localized microenvironments with steep gradients in pH, oxygen, and nutrients, allowing acidogenic and aciduric bacteria to occupy protected niches adjacent to enamel where acids accumulate and remineralizing ions are depleted. Matrix components also mediate quorum sensing, horizontal gene transfer, and cooperative interactions among community members, contributing to antimicrobial tolerance and resilience that challenge conventional chemotherapeutic approaches and inform the development of matrix-targeted anti-biofilm strategies (Zhu et al., 2022).

Extracellular polymeric substances, particularly glucans derived from sucrose, are central to the virulence of cariogenic biofilms because they provide binding sites for bacterial adhesins, trap dietary sugars, and form a viscoelastic network that anchors biofilm to tooth surfaces. EPS-rich matrices increase the porosity and tortuosity of diffusion pathways, facilitating inward diffusion of low-molecular-weight carbohydrates while impeding outward diffusion of organic acids and penetration of antimicrobial agents, thus maintaining acidic microdomains conducive to demineralization. Experimental models have shown that the amount, insolubility, and spatial distribution of EPS correlate strongly with lesion severity, and that co-cultures of S. mutans with lactobacilli or bifidobacteria can synergistically enhance insoluble EPS production and caries lesion progression. These observations underscore EPS as an attractive target for novel therapeutics such as glucosyltransferase inhibitors, matrix-degrading enzymes, and smart materials designed to disrupt cariogenic biofilm architecture without broadly eradicating beneficial commensals (Santos et al., 2021).

Beyond its role in adhesion, the biofilm matrix confers protection against mechanical, chemical, and biological insults, rendering cariogenic plaque recalcitrant to routine oral hygiene and antimicrobial agents. The viscoelastic EPS network absorbs shear forces generated by saliva flow and mastication, reducing detachment of bacterial aggregates and enhancing persistence on tooth surfaces, particularly in stagnation areas such as pits, fissures, and proximal surfaces. Matrix components can sequester and inactivate antimicrobial molecules, limit their diffusion, and create refuges where cells enter slow-growing or persister states less susceptible to chemotherapeutic agents and environmental stresses, including fluctuations in pH and oxidative stress. Additionally, the matrix modulates exposure of bacterial antigens to host immune factors and may bind host proteins such as immunoglobulins and complement, thereby influencing immune recognition and inflammatory responses within the oral cavity (Jakubovics et al., 2021).

Cariogenic biofilms exhibit pronounced spatial heterogeneity, with steep pH gradients that reflect localized acid production at sites of intense carbohydrate metabolism and limited buffer penetration. Within EPS-rich microcolonies adjacent to enamel, pH can fall well below the critical threshold for enamel dissolution and remain low for extended periods after sugar intake, even as bulk salivary pH returns toward neutrality, due to restricted diffusion and ongoing fermentation of retained carbohydrates. At the same time, the biofilm contains aqueous channels and pores that facilitate the convective and diffusive transport of nutrients, signaling molecules, and metabolic byproducts, supporting metabolic cross-feeding and niche specialization among community members. This microenvironmental complexity allows acidogenic bacteria to occupy protected low-pH zones while other taxa exploit alternative substrates or higher pH regions, collectively sustaining a dysbiotic, cariogenic state that is difficult to reverse without modifying both diet and biofilm structure (Jakubovics et al., 2021).

Clinical Manifestations and Diagnosis

Clinically, dental caries manifests along a continuum from non-cavitated subsurface enamel demineralization, often visible as white spot lesions, to cavitated lesions that extend into dentin and, in advanced cases, involve the pulp and periapical tissues. Early enamel caries typically presents as chalky, opaque areas with intact surface integrity on smooth surfaces or along gingival margins, while progression into dentin produces soft, discolored lesions detectable with visual-tactile examination and radiographs, and may be accompanied by sensitivity to thermal or osmotic stimuli. Modern concepts emphasize lesion activity assessment distinguishing active, progressing lesions from inactive, arrested ones based on surface texture, plaque stagnation, and patient-level risk factors, which guides decisions between non-invasive remineralization strategies and operative intervention (Karlsson, 2010).

Caries risk assessment integrates clinical, behavioral, and biological factors to estimate a patient's likelihood of developing new lesions or experiencing progression, forming the basis for individualized preventive and therapeutic plans. Structured tools such as CAMBRA and ADA caries risk assessment forms consider past caries experience, visible plaque, dietary habits (especially frequency of sugar intake), salivary flow and buffering capacity, fluoride exposure, socio-behavioral factors, and special healthcare needs to stratify patients into low, moderate, or high risk categories. These tools reflect the understanding of caries as a biofilm-mediated, multifactorial disease, highlighting modifiable determinants such as diet, oral hygiene, and fluoride use, and are increasingly complemented by chairside tests for salivary mutans streptococci and lactobacilli, pH, and buffering that provide additional biological insight into caries risk(Cunha-Cruz et al., 2013).

Emerging Diagnostic Technologies (QLF, OCT, Molecular)

Emerging diagnostic modalities aim to detect early caries lesions and monitor biofilm changes with higher sensitivity, specificity, and objectivity than traditional visual-tactile and radiographic methods. Quantitative light-induced fluorescence (QLF) measures changes in tooth autofluorescence associated with mineral loss, providing quantitative indices of lesion depth and area, and can also assess red fluorescence from dental plaque, which correlates with bacterial composition and caries risk. Optical coherence tomography (OCT) offers high-resolution, cross-sectional imaging of enamel and dentin based on backscattered near-infrared light, enabling non-invasive visualization of subsurface demineralization and lesion progression over time. In parallel, molecular approaches including qPCR, 16S rRNA gene sequencing, and metagenomic or metabolomic profiling of plaque and saliva are being explored to identify microbial and functional signatures of cariogenic biofilms, potentially allowing more precise prediction of disease risk and response to therapy (Miyamoto et al., 2020).

Host factors such as tooth morphology, enamel composition, saliva quantity and quality, immune responses, and genetic determinants interact with environmental influences like diet, fluoride exposure, and oral hygiene to shape the risk and pattern of caries development. Deep pits and fissures, developmental defects, and areas of plaque stagnation create microhabitats where biofilm can mature and acids accumulate, while systemic conditions or medications that reduce salivary flow compromise natural clearance and buffering, predisposing to rampant caries. Environmental exposures, particularly frequent consumption of sugars and acidic beverages, low fluoride availability, and inadequate mechanical plaque control, amplify the cariogenic potential of biofilms and can override inherent host resistance, especially in susceptible individuals such as young children, older adults with hyposalivation, and medically compromised patients (Nath et al., 2025).

Saliva exerts multiple protective effects against caries through mechanical cleansing, buffering of plaque acids, provision of calcium, phosphate, and fluoride for remineralization, and delivery of antimicrobial proteins such as lysozyme, lactoferrin, peroxidases, and immunoglobulins. Adequate unstimulated and stimulated salivary flow rates are critical for maintaining pH homeostasis and diluting fermentable substrates, whereas hyposalivation from Sjögren's syndrome, head and neck radiotherapy, polypharmacy, or dehydration markedly increases caries risk, particularly on smooth surfaces and root surfaces. Variations in salivary composition, including buffering capacity, concentrations of bicarbonate, calcium, phosphate, and fluoride, and levels of secretory IgA, influence the susceptibility of individuals to caries, underscoring saliva as a key modulator of the biofilm microenvironment and a target for preventive strategies such as sialogogues and remineralizing agents (Lewis, 2018).

Dietary sugars and fermentable carbohydrates are indispensable drivers of cariogenic biofilm metabolism, with both the amount and, more importantly, the frequency of intake determining the pattern of plaque pH fluctuations and net demineralization. Sucrose is particularly harmful because it fuels acid production and serves as a substrate for EPS synthesis, whereas frequent snacking on sugary or starchy foods, consumption of sugar-sweetened beverages, and nocturnal feeding maintain low pH conditions that select for aciduric species and overwhelm salivary buffering and remineralization. Epidemiological and experimental data consistently show that high sugar and refined carbohydrate intake is associated with increased abundance of Streptococcus mutans, lactobacilli, and other acidogenic taxa, reduced microbial diversity, and greater caries experience, while dietary patterns low in free sugars and rich in fibrous foods are linked to lower caries risk. Public health recommendations to limit free sugar intake to less than 10% (ideally 5%) of total energy, combined with fluoride use and good oral hygiene, reflect the central role of diet in modulating biofilm composition and caries risk (Lewis, 2018).

Genetic factors influence susceptibility to dental caries through effects on enamel structure and composition, salivary flow and constituents, taste preferences (including sweet taste sensitivity), and immune responses to oral microbiota. Polymorphisms in genes related to enamel formation (such as amelogenin and enamelin), salivary proteins, innate immune receptors, and cytokines have been associated in some studies with caries experience, although findings are heterogeneous and gene–environment interactions are complex. Host immune responses, particularly salivary IgA directed against S. mutans and other cariogenic bacteria, can modulate colonization and biofilm composition, while systemic or local immunodeficiencies may predispose to more severe or atypical caries patterns, highlighting the importance of integrating biological susceptibility into risk assessment and personalized preventive strategies ("Oral and Dental Manifestations in Noonan Syndrome," 2019).

Caries development can be modulated at multiple levels by interventions that alter host–microbe interactions, sugar metabolism, and environmental conditions within the biofilm, shifting the balance from demineralization toward remineralization. Fluoride remains the cornerstone of caries prevention by enhancing remineralization of early lesions, forming fluorapatite-like mineral less soluble in acid, and inhibiting bacterial enolase, thereby reducing glycolytic acid production, especially under acidic conditions. Additional strategies include promoting alkali-generating bacteria through arginine-containing dentifrices or probiotics, using antimicrobial agents with substantivity (such as chlorhexidine) in high-risk cases, and developing novel therapeutics that target specific virulence traits such as glucosyltransferases, EPS matrix integrity, or quorum sensing pathways, thereby attenuating cariogenicity without broadly disrupting commensal flora (Zhu et al., 2022).

Host–microbe interactions within the dental biofilm are mediated through salivary pellicle components, pattern-recognition receptors, and immune effectors that recognize microbial-associated molecular patterns and shape community composition. Salivary proteins such as mucins, proline-rich proteins, and agglutinins influence bacterial adhesion and aggregation, while secretory IgA can inhibit colonization by blocking adhesins or promoting clumping and clearance of cariogenic species. Epithelial and immune cells respond to biofilm-derived signals by producing cytokines and antimicrobial peptides that can modulate microbial growth and virulence, whereas in turn, bacteria adjust their gene expression in response to host-derived signals, creating a bidirectional dialogue that determines whether the biofilm remains symbiotic or becomes dysbiotic and cariogenic (Peterson et al., 2014).

Repeated exposure to fermentable sugars imposes acidogenic stress on dental biofilms, selecting for organisms with robust acid tolerance mechanisms and reshaping community function toward enhanced carbohydrate catabolism. S. mutans responds to low pH by upregulating F-ATPase activity, altering membrane fatty acid composition, and activating stress response regulators that maintain intracellular pH and protein integrity, enabling continued acid production in environments that inhibit less acid-tolerant commensals. Other acidogenic and aciduric species deploy similar adaptive responses, while alkali-generating pathways such as arginine deiminase and urease, which could counteract acidification, are often underrepresented or downregulated in cariogenic biofilms dominated by aciduric taxa. This metabolic reprogramming under acidogenic stress thus perpetuates a self-reinforcing cycle of low pH, enamel demineralization, and dysbiosis, providing a rationale for interventions that reduce sugar frequency, enhance alkali production, or directly inhibit acidogenic pathways (Lemos et al., 2019).

Environmental parameters within the oral cavity including pH, redox potential, carbohydrate availability, oxygen tension, and exposure to fluoride or antimicrobials profoundly influence microbial gene expression and, consequently, biofilm behavior and cariogenicity. Transcriptomic studies of dental plaque have shown that under low pH and high-sugar conditions, genes involved in glycolysis, acid tolerance, EPS biosynthesis, and stress responses are upregulated in cariogenic taxa, while functions associated with amino acid metabolism and alkali generation may be downregulated, reflecting adaptation to an aciduric lifestyle. Fluoride and other environmental modifiers can alter expression of metabolic enzymes, transporters, and regulatory networks, attenuating virulence in some species, while sub-inhibitory concentrations of antimicrobials or host-derived factors may trigger biofilm-specific responses, including increased EPS production and efflux pump activity. Understanding how environmental cues shape microbial gene expression within dental biofilms is crucial for designing ecological interventions and smart materials that bias the system toward health-associated community configurations and reduced caries risk (Wei et al., 2024).

Mechanical Biofilm Control

Mechanical biofilm control remains the cornerstone of contemporary caries prevention, acting directly on the structured, polymicrobial biofilm that drives the ecological dysbiosis underlying dental caries. Regular toothbrushing with a fluoride dentifrice mechanically disrupts supragingival plaque biofilms on buccal, lingual, and occlusal surfaces, reducing biofilm biomass and the reservoir of acidogenic and aciduric organisms such as Streptococcus mutans and Lactobacillus spp., while simultaneously delivering fluoride to promote remineralization and inhibit demineralization at active caries sites. Systematic evaluations indicate that, under controlled conditions, manual toothbrushing can achieve plaque score reductions in the order of 50%–60%, whereas powered brushes may achieve somewhat greater biofilm disruption, although effectiveness in daily life is highly dependent on user technique, duration, and frequency of brushing. Despite this, mechanical plaque control alone cannot reliably reach interproximal and other stagnation sites where biofilm is thicker, more anaerobic, and more acidogenic, and where caries lesions commonly develop, particularly in high-risk individuals. Consequently, contemporary guidelines frame toothbrushing not as a stand-alone intervention but as one component of a multimodal strategy that integrates interdental cleaning, dietary control, topical fluorides, and risk-based professional care to maintain biofilm in a less cariogenic state rather than attempting complete microbial eradication (Keller et al., 2022).

Interdental cleaning is a critical adjunct to toothbrushing because biofilm in approximal areas accumulates more rapidly, exhibits higher acidogenicity, and is more strongly associated with both proximal caries and periodontal breakdown than biofilm on other surfaces. Floss, interdental brushes, and oral irrigators are all capable of disrupting interdental biofilm when used correctly; controlled studies and systematic reviews suggest that interdental brushes in particular can achieve substantial additional reductions in plaque and gingival inflammation when used alongside toothbrushing, often outperforming floss in patients with open embrasures or attachment loss. Nevertheless, population-level data reveal that daily interdental cleaning behaviors show chronically low adherence, largely because of time constraints, perceived difficulty, discomfort, and limited manual dexterity, especially among children, older adults, and individuals with disabilities, which constrains the real-world impact of interdental biofilm control on caries incidence. Emerging auto-cleaning and powered interdental devices aim to reduce technique sensitivity and improve patient compliance, but current evidence suggests that, while some devices can approach or exceed the plaque removal efficacy of conventional brushing and floss in the short term, their effectiveness is contingent on adequate fit, device design, and consistent use. In caries-susceptible individuals, personalized instruction, device selection tailored to embrasure morphology and dexterity, and behavioral support to improve motivation are increasingly emphasized as essential complements to mechanical tools themselves (Keller et al., 2022).

Professional mechanical cleaning (prophylaxis and periodontal debridement) serves as a periodic, high-intensity intervention that removes tenacious supragingival and subgingival biofilm and calculus deposits beyond what patients typically achieve at home, thereby resetting the biofilm to a less mature, less pathogenic state. In the context of caries prevention, professional polishing and prophylaxis facilitate the removal of thick, mature biofilms and surface stains, improving access for fluoride, sealants, and other topical agents while enhancing patient comfort and esthetics, which may in turn positively influence oral hygiene behaviors. Periodontal instrumentation that reduces gingival inflammation and pocket depth also modifies ecological niches that can harbor acidogenic or aciduric species, contributing indirectly to a more balanced supragingival microbiota that is less predisposed to sustained acidification after sugar exposures. However, clinical and epidemiological data indicate that professional cleaning at typical recall intervals (e.g., 3–12 months) has limited impact on long-term caries outcomes unless accompanied by effective daily supragingival biofilm control and risk-based fluoride use, because biofilm structure and microbial composition return to pre-treatment patterns within days to weeks after debridement. Accordingly, current caries-management-by-risk (CAMBRA) and minimally invasive dentistry concepts position professional mechanical cleaning primarily as a supportive measure for risk assessment, patient education, and facilitation of biofilm-targeted chemotherapeutic and remineralization strategies rather than as a stand-alone caries control technique (Keller et al., 2022).

Despite its central role, mechanical control of dental biofilm exhibits several inherent limitations that are particularly relevant when caries is conceptualized as a biofilm-mediated, behaviorally driven, and socially patterned disease. First, mechanical methods are intrinsically technique-sensitive: their efficacy depends on brushing duration, bristle positioning, contact pressure, coverage of all tooth surfaces, and appropriate selection and use of interdental devices, which many patients fail to master or sustain without continuous reinforcement. Second, mechanical disruption does not selectively target cariogenic taxa; rather, it reduces overall biofilm mass, which is beneficial but may be insufficient to shift a dysbiotic community back to health in the presence of frequent fermentable carbohydrate intake and low salivary buffering, especially in high-risk groups such as xerostomic patients, orthodontic patients, and young children consuming sugar-sweetened beverages. Third, social determinants including education, income, health literacy, and access to professional care profoundly influence the adoption and maintenance of effective oral hygiene habits, leading to persistent inequalities in caries experience despite wide availability of toothbrushes and fluoride dentifrices. Finally, from a strictly microbiological perspective, mechanical disruption does not fully address the recalcitrance of biofilm-embedded bacteria, which display up to 1,000- to 1,500-fold increased tolerance to antimicrobial agents compared with their planktonic counterparts, suggesting that additional strategies are needed to modulate biofilm ecology and virulence rather than relying solely on physical removal (Wu et al., 2025).

Chemical Control and Antimicrobials

Fluoride remains the most extensively studied and widely implemented chemotherapeutic agent in caries prevention, acting at multiple levels to counteract the demineralizing challenge posed by organic acids produced within cariogenic biofilms. At the enamel interface, low concentrations of fluoride in plaque fluid promote remineralization by facilitating the deposition of fluoridated hydroxyapatite or fluorapatite in subsurface lesions, which is more resistant to acid dissolution, while also reducing the critical pH at which demineralization occurs. At the microbial level, fluoride inhibits key enzymes involved in carbohydrate metabolism, including enolase and proton-translocating F-ATPase, thereby attenuating acid production and reducing the ability of cariogenic bacteria to maintain cytoplasmic pH homeostasis under acidic conditions. Topical delivery via fluoride-containing toothpastes, varnishes, gels, and mouthrinses allows sustained availability of fluoride ions in the plaque–saliva–enamel interface, with multiple clinical trials and meta-analyses demonstrating significant reductions in caries incidence across age groups, particularly when high-risk individuals receive additional professionally applied formulations. In the biofilm-mediated caries paradigm, fluoride is viewed less as a traditional antimicrobial and more as an ecological modulator that favors mineral repair and selectively disadvantages the most acidogenic, aciduric members of the community without eradicating commensal taxa (Luo et al., 2024).

Beyond fluoride, a range of antimicrobial agents has been deployed to chemically suppress cariogenic biofilms, with chlorhexidine representing the prototypical broad-spectrum antiseptic in dental practice. Chlorhexidine, a bis-biguanide cationic molecule, exerts bacteriostatic effects at low concentrations by disrupting cell membrane integrity and bactericidal effects at higher concentrations through cytoplasmic precipitation and cell lysis, displaying pronounced substantivity by binding to oral surfaces and being gradually released over time. Mouthrinses, gels, and varnishes containing chlorhexidine can substantially reduce planktonic bacterial counts and short-term biofilm vitality; experimental biofilm studies show marked decreases in viable biomass following brief rinsing, and clinical protocols have targeted S. mutans suppression in high-risk individuals using chlorhexidine varnishes or rinses as an adjunct to fluoride. Essential oils (e.g., thymol, eucalyptol, menthol) and other phenolic compounds used in commercial mouthrinses also exhibit membrane-disruptive and enzyme-inhibitory properties, reducing plaque mass and gingival inflammation, and in some formulations modestly decreasing mutans streptococci counts, while offering an alternative for patients who cannot tolerate chlorhexidine. Recent in vitro and in vivo studies have expanded interest in plant-derived essential oils and phytochemicals with targeted anti-S. mutans activity, including inhibition of glucosyltransferases, disruption of extracellular polymeric substances, and downregulation of biofilm-associated virulence genes, positioning these natural agents as promising adjuncts for biofilm modulation in caries prevention (Poppolo Deus & Ouanounou, 2022).

However, reliance on chemical antimicrobials for caries control within a biofilm-dominated ecosystem raises important limitations and resistance concerns that are increasingly recognized in the literature. From a biofilm biology standpoint, bacteria embedded in extracellular polymeric matrices exhibit dramatically increased tolerance to antiseptics and antibiotics, as diffusion barriers, altered metabolic states, and persister cells collectively reduce agent penetration and efficacy; as a result, clinically achievable concentrations of mouthrinse antimicrobials rarely eradicate biofilm but instead transiently reduce viable counts, allowing rapid regrowth once use is discontinued. Chronic or repeated exposure to sublethal concentrations of fluoride, chlorhexidine, and other antimicrobials has been associated in experimental models with selection of strains exhibiting increased minimal inhibitory concentrations, efflux pump upregulation, altered membrane composition, or enzymatic detoxification mechanisms, raising concerns about the potential contribution of oral antiseptic overuse to antimicrobial resistance and cross-resistance to medically important antibiotics. Clinically, long-term chlorhexidine use is constrained by well-documented adverse effects, including tooth and tongue staining, taste alteration, mucosal irritation, increased calculus formation, and, in rare cases, hypersensitivity reactions, which limit patient acceptance and justify short-term, targeted application rather than continuous daily use. Even with essential oils and other “natural” antimicrobials, the risk of mucosal irritation, allergic reactions, and disruption of the commensal microbiota necessitates careful formulation and evidence-based dosing, while the ecological rationale in modern cariology favors strategies that rebalance the microbiome rather than broadly suppressing microbial load. Consequently, current concepts advocate the integration of chemotherapeutic agents particularly fluorides and short-term antiseptics into a broader, risk-based, minimally invasive care model that emphasizes mechanical biofilm control, dietary modification, salivary support, and emerging microbiome-modulating therapies instead of a purely biocidal approach(Wu et al., 2025).

Dietary Interventions

Dietary modification is a fundamental pillar of caries prevention because frequent intake of fermentable carbohydrates, especially sucrose, provides the ecological driver for selection and dominance of acidogenic and aciduric species within dental biofilms. Sucrose uniquely supports both acid production and synthesis of extracellular polysaccharides via bacterial glucosyltransferases, enhancing biofilm cohesion, porosity, and retention of organic acids, thereby prolonging periods of low pH at the enamel surface and tipping the demineralization–remineralization balance toward net mineral loss. Modern diets rich in ultra-processed foods, sugar-sweetened beverages, and between-meal snacks result in repeated Stephan curves throughout the day, with plaque pH frequently dropping below the critical threshold for enamel demineralization, particularly in individuals with reduced salivary flow or buffering capacity. Population-based and interventional studies consistently link high frequency, rather than simply high quantity, of sugar exposure to increased caries risk, leading to evidence-based recommendations that emphasize limiting between-meal sugar intake, substituting noncariogenic snacks, and confining sugary foods and drinks to main meals where salivary flow is higher and fluoride exposure can be optimized. In this biofilm-centered framework, dietary counseling is not only a behavioral message but an ecological intervention that reduces the selective pressure favoring cariogenic consortia and supports the persistence of a more diverse, less aciduric microbial community (Luo et al., 2024).

The incorporation of sugar alternatives, prebiotics, and probiotics into caries-preventive strategies reflects a shift from purely subtractive dietary advice toward positive, microbiome-modulating interventions. Non-fermentable or low-fermentable sugar substitutes such as xylitol and certain low-intensity sweeteners can reduce acidogenic potential by providing sweetness without serving as efficient substrates for cariogenic bacteria; meta-analytic data suggest that consumption of sugar substitutes can significantly lower levels of cariogenic microorganisms in plaque and saliva, although effect sizes and clinical relevance vary by study design, dose, and background fluoride exposure. In parallel, prebiotics selective substrates that favor the growth or activity of beneficial oral microbes are being explored as a means to promote health-associated taxa that compete with S. mutans and other cariogenic species, thereby shifting biofilm composition toward a more homeostatic state under cariogenic challenges. Probiotics, delivered via dairy products, lozenges, tablets, or oral care vehicles, introduce exogenous beneficial strains (e.g., Lacticaseibacillus rhamnosus, various Lactobacillus and Bifidobacterium spp.) that can inhibit mutans streptococci through competitive adhesion, bacteriocin production, pH modulation, and immune regulation, with in vitro, animal, and human studies increasingly demonstrating reductions in S. mutans colonization, biofilm formation, and caries scores. Recent systematic and narrative reviews highlight that probiotic-supplemented milk and other formulations may provide clinically meaningful adjunctive benefits in caries prevention and management, particularly in children and high-risk populations, although optimal strains, doses, delivery vehicles, and treatment durations remain active areas of research. Collectively, these dietary and microbiome-directed approaches embody the current conceptualization of dental caries as a diet- and biofilm-mediated, reversible ecological shift, underscoring the need to integrate sugar restriction, sugar substitutes, and targeted microbial modulation with established mechanical and chemical control measures in comprehensive, patient-centered caries management (Zhang et al., 2024).

Emerging Therapies and Future Directions

Emerging therapies for dental caries, a biofilm-mediated disease, are increasingly focusing on biofilm-targeted strategies due to the complex structure and protective nature of the biofilm environment. Anti-biofilm peptides and enzymes represent a promising avenue by selectively disrupting biofilm formation and viability without broadly killing bacteria, thereby reducing the risk of resistance. These peptides often operate at acidic pH typical of cariogenic biofilms, targeting bacterial membranes and extracellular polymeric substances (EPS), which are essential for biofilm integrity. Enzymatic degradation of biofilm matrix components enhances drug penetration and bacterial eradication, offering a dual mechanism of action. Nanoparticles and hydrogels are being developed as targeted delivery systems to localize anti-biofilm agents effectively and provide controlled release triggered by environmental cues like pH. Such "smart" delivery enhances therapeutic precision and reduces cytotoxicity to surrounding tissues, improving clinical outcomes (Zhang et al., 2024).

Modulation of the oral microbiome is another innovative therapeutic direction aimed at restoring ecological balance rather than outright microbial eradication. Probiotics, prebiotics, and synbiotics deploy beneficial microorganisms or substrates that support their growth, inhibiting cariogenic bacteria such as Streptococcus mutans and disrupting biofilm formation. These biointerventions foster a healthy oral microbial environment, regulate immune responses, and may contribute to long-term caries prevention. The novel exploration of fecal microbiota transplantation (FMT) to the oral cavity, while mostly experimental at this stage, holds potential for resetting dysbiotic oral microbiota by introducing complex, healthy bacterial communities, though clinical validation is still required (Al Muhandir et al., 2024).

Remineralization technologies continue to evolve to repair early enamel lesions and restore mineral loss caused by acidogenic biofilms. Casein phosphopeptide-amorphous calcium phosphate (CPP-ACP) acts as a bioactive material releasing calcium and phosphate ions to supersaturate the enamel surface, thus fostering mineral deposition. Bioactive glasses and smart materials with nanoscale particles improve mineral penetration and hydroxyapatite formation, enhancing enamel hardness and durability better than traditional fluoride treatments. These materials show significant potential to reverse early caries non-invasively and to be integrated into daily dental care products (Taha et al., 2017).

Genetic and host response modulation holds a future promise for personalized caries prevention and management. Editing host susceptibility genes related to enamel formation or immune response may reduce vulnerability to caries. Immunomodulatory therapies aim to adjust the host immune environment to better control the pathogenic biofilm without excessive inflammation or tissue damage. These approaches are in early research stages but align with the precision medicine paradigm seeking targeted interventions based on individual genetic and immune profiles (Liu et al., 2018).

Future research priorities in dental caries emphasize overcoming challenges inherent in biofilm research, such as the complex interactions within polymicrobial communities and host factors. There is a critical need for long-term, well-designed clinical human studies to validate emerging therapies' safety and efficacy. Integration of multi-omics approaches (genomics, proteomics, metabolomics), systems biology, and precision medicine technologies promises to deepen understanding of caries pathogenesis and tailor interventions to individual risk profiles. These innovative frameworks could revolutionize caries management by shifting from generic treatments to highly personalized, effective therapies (Liu et al., 2018).

Conclusion

Dental caries is now understood as a biofilm-mediated, diet-driven, multifactorial disease rooted in ecological imbalance rather than infection by a single pathogen. Cariogenic dysbiosis emerges when frequent sugar exposure and inadequate oral hygiene shift the oral microbiome toward acidogenic and aciduric species capable of persistent enamel demineralization. Effective prevention and management require integrating mechanical biofilm control, fluoride use, dietary modification, and risk-based interventions. Emerging strategies such as anti-biofilm agents, microbiome modulation, smart delivery systems, and advanced remineralization technologies offer promising avenues for targeted, minimally invasive, and personalized caries care. Future progress depends on robust clinical research and the integration of multi-omics approaches to better understand and modulate the complex host–biofilm interactions that drive caries.

References

1. Al Muhandir, S. A., Awaji, Yahya Mazyad, Abdu Safhi, Hossen Yahya, Daghriri, Khansa Ali Ahmed, Oqayshi, Sameer Jubran, Al Othman, Bayan Hassan, Fathuldeen, Abeer Qassim Khmjan, Hothan, Ahmad Saleh, Holba, Hesham Adnan Ahmed, Abusaida, F. M., Zakan, M. M., Humdi, I. A. A., Alshafeey, M. A. M., Kubaisi, K. R., & Tubaiqi, S. M. (2024). Dental Caries: Biochemical Aspects, Management and Treatment Using Probiotics and Prebiotics-An Updated Review. Egyptian Journal of Chemistry, 67(13), 1977–1990. https://doi.org/10.21608/ejchem.2025.342113.10969
2. Chen, X., Daliri, E. B.-M., Kim, N., Kim, J.-R., Yoo, D., & Oh, D.-H. (2020). Microbial Etiology and Prevention of Dental Caries: Exploiting Natural Products to Inhibit Cariogenic Biofilms. Pathogens, 9(7), 569. https://doi.org/10.3390/pathogens9070569
3. Chen, X., Daliri, E. B.-M., Tyagi, A., & Oh, D.-H. (2021). Cariogenic Biofilm: Pathology-Related Phenotypes and Targeted Therapy. Microorganisms, 9(6), 1311. https://doi.org/10.3390/microorganisms9061311
4. Cunha-Cruz, J., Scott, J., Rothen, M., Mancl, L., Lawhorn, T., Brossel, K., & Berg, J. (2013). Salivary characteristics and dental caries: Evidence from general dental practices. Journal of the American Dental Association (1939), 144(5), e31–e40. https://doi.org/10.14219/jada.archive.2013.0159
5. Giacaman, R. A., Fernández, C. E., Muñoz-Sandoval, C., León, S., García-Manríquez, N., Echeverría, C., Valdés, S., Castro, R. J., & Gambetta-Tessini, K. (2022). Understanding dental caries as a non-communicable and behavioral disease: Management implications. Frontiers in Oral Health, 3. https://doi.org/10.3389/froh.2022.764479
6. Jakubovics, N. S., Goodman, S. D., Mashburn-Warren, L., Stafford, G. P., & Cieplik, F. (2021). The dental plaque biofilm matrix. Periodontology 2000, 86(1), 32–56. https://doi.org/10.1111/prd.12361

7. Karlsson, L. (2010). Caries Detection Methods Based on Changes in Optical Properties between Healthy and Carious Tissue. International Journal of Dentistry, 2010, 270729. https://doi.org/10.1155/2010/270729
8. Keller, M., Keller, G., Eller, T., Sigwart, L., Wiesmüller, V., Steiner, R., Offermanns, V., & Kapferer-Seebacher, I. (2022). Cleansing efficacy of an auto-cleaning toothbrushing device with nylon bristles: A randomized-controlled pilot study. Clinical Oral Investigations, 27(2), 603–611. https://doi.org/10.1007/s00784-022-04755-9
9. Lemos, J. A., Palmer, S. R., Zeng, L., Wen, Z. T., Kajfasz, J. K., Freires, I. A., Abranches, J., & Brady, L. J. (2019). The Biology of Streptococcus mutans. Microbiology Spectrum, 7(1), 10.1128/microbiolspec.gpp3-0051–2018. https://doi.org/10.1128/microbiolspec.gpp3-0051-2018
10. Lewis, M. (2018). Dental Disease, Defects, and Variations in Dental Morphology. In Paleopathology of Children (pp. 67–89). Elsevier. https://doi.org/10.1016/B978-0-12-410402-0.00004-7
11. Li, X., Li, R., Wang, H., Yang, Z., Liu, Y., Li, X., Xue, X., Sun, S., & Wu, L. (2025). Global Burden of Dental Caries from 1990 to 2021 and Future Projections. International Dental Journal, 75(5), 100904. https://doi.org/10.1016/j.identj.2025.100904
12. Liu, Y., Ren, Z., Hwang, G., & Koo, H. (2018). Therapeutic Strategies Targeting Cariogenic Biofilm Microenvironment. Advances in Dental Research, 29(1), 86–92. https://doi.org/10.1177/0022034517736497
13. Luo, S.-C., Wei, S.-M., Luo, X.-T., Yang, Q.-Q., Wong, K.-H., Cheung, P. C. K., & Zhang, B.-B. (2024). How probiotics, prebiotics, synbiotics, and postbiotics prevent dental caries: An oral microbiota perspective. NPJ Biofilms and Microbiomes, 10, 14. https://doi.org/10.1038/s41522-024-00488-7
14. Meyer, F., Enax, J., Epple, M., Amaechi, B. T., & Simader, B. (2021). Cariogenic Biofilms: Development, Properties, and Biomimetic Preventive Agents. Dentistry Journal, 9(8), 88. https://doi.org/10.3390/dj9080088
15. Miyamoto, N., Adachi, T., Boschetto, F., Zanocco, M., Yamamoto, T., Marin, E., Somekawa, S., Ashida, R., Zhu, W., Kanamura, N., Nishimura, I., & Pezzotti, G. (2020). Molecular Fingerprint Imaging to Identify Dental Caries Using Raman Spectroscopy. Materials (Basel, Switzerland), 13(21), 4900. https://doi.org/10.3390/ma13214900
16. Nath, S., Zilm, P., Jamieson, L., Santiago, P. H. R., Ketagoda, D. H. K., & Weyrich, L. (2025). The influence of diet, saliva, and dental history on the oral microbiome in healthy, caries-free Australian adults. Scientific Reports, 15(1), 18755. https://doi.org/10.1038/s41598-025-03455-0
17. Oral and Dental Manifestations in Noonan Syndrome. (2019). In Noonan Syndrome (pp. 135–158). Academic Press. https://doi.org/10.1016/B978-0-12-815348-2.00009-8
18. Peterson, S. N., Meissner, T., Su, A. I., Snesrud, E., Ong, A. C., Schork, N. J., & Bretz, W. A. (2014). Functional expression of dental plaque microbiota. Frontiers in Cellular and Infection Microbiology, 4, 108. https://doi.org/10.3389/fcimb.2014.00108
19. Poppolo Deus, F., & Ouanounou, A. (2022). Chlorhexidine in Dentistry: Pharmacology, Uses, and Adverse Effects. International Dental Journal, 72(3), 269–277. https://doi.org/10.1016/j.identj.2022.01.005
20. Santos, V. R. D., Valdez, R. M. A., Danelon, M., Souza, J. A. S., Caiaffa, K. S., Delbem, A. C. B., & Duque, C. (2021). Effect of S. mutans combinations with bifidobacteria/lactobacilli on biofilm and enamel demineralization. Brazilian Oral Research, 35, e030. https://doi.org/10.1590/1807-3107bor-2021.vol35.0030
21. Spatafora, G., Li, Y., He, X., Cowan, A., & Tanner, A. C. R. (2024). The Evolving Microbiome of Dental Caries. Microorganisms, 12(1), 121. https://doi.org/10.3390/microorganisms12010121
22. Taha, A. A., Patel, M. P., Hill, R. G., & Fleming, P. S. (2017). The effect of bioactive glasses on enamel remineralization: A systematic review. Journal of Dentistry, 67, 9–17. https://doi.org/10.1016/j.jdent.2017.09.007
23. Wei, Y., Dang, G., Ren, Z., Wan, M., Wang, C., Li, H., Zhang, T., Tay, F. R., & Niu, L. (2024). Recent advances in the pathogenesis and prevention strategies of dental calculus. Npj Biofilms and Microbiomes, 10(1), 56. https://doi.org/10.1038/s41522-024-00529-1

24. Wu, Z., Zhang, X., Wang, L., Sui, J., Yu, M., Bao, B., Qian, J., Yi, G., Ma, Y., & He, X. (2025). Antimicrobial and anti-biofilm activity of essential oils extracted from Clausena lansium (Lour.) Skeels and their main constituents against Streptococcus mutans. Frontiers in Microbiology, 16, 1612681. https://doi.org/10.3389/fmicb.2025.1612681
25. Zhang, J., Wang, Q., & Duan, Z. (2024). Preventive effects of probiotics on dental caries in vitro and in vivo. BMC Oral Health, 24, 915. https://doi.org/10.1186/s12903-024-04703-x
26. Zhu, J., Chu, W., Luo, J., Yang, J., He, L., & Li, J. (2022). Dental Materials for Oral Microbiota Dysbiosis: An Update. Frontiers in Cellular and Infection Microbiology, 12, 900918. https://doi.org/10.3389/fcimb.2022.900918