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A Narrative Review

Biofilm Formation in Dental Caries and Advancements in Biofilm-Targeted Therapies: A Decade of Progress

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ABSTRACT

Background: Dental caries is a widespread chronic disease globally, driven by the interaction of dietary sugars, host factors, and cariogenic bacteria such as *Streptococcus mutans* (S. mutans), alongside early colonizers like *Streptococcus sanguinis* and biofilm-initiating species such as *Actinomyces*. The formation of bacterial biofilms plays a central role in disease development by creating adhesive microbial communities that protect bacteria and facilitate acid production, leading to enamel demineralization. Traditional antimicrobial strategies often struggle to eliminate resilient biofilms, posing significant challenges for effective caries management. **Objective**: This review explores the structural components and functional dynamics of cariogenic biofilms, including acid tolerance, resistance mechanisms, and extracellular polymeric substance production. It integrates advancements in biofilm-targeted therapeutic strategies that emerged between 2015 and 2025. **Methods**: The review focuses on microbiological and experimental research published within this decade, analyzing strategies involving small molecule inhibitors, probiotics, bacteriocins, and enzymatic degradation techniques that specifically target S. mutans biofilms while maintaining oral microbiota balance. **Results**: Small molecule inhibitors such as ZY354, 3F1, and LCG-N25, along with probiotics like *Lactobacillus reuteri* and bacteriocins derived from *Enterococcus faecalis* (enterocins), have demonstrated significant inhibition of S. mutans biofilms. Enzymatic matrix degradation approaches have shown effectiveness in enhancing antimicrobial penetration and fluoride uptake, offering a promising addition to traditional therapies. Findings suggest that these emerging therapies can significantly reduce biofilm resilience and lower the incidence of dental caries. **Conclusion**: This decade-long analysis highlights the potential of precision-targeted interventions to advance dental caries prevention and management by improving biofilm control and supporting the maintenance of a healthier oral microbiome.

Keywords: Bacteriocins, Biofilm, Dental caries, Probiotics, *Streptococcus mutans*, Therapeutic

INTRODUCTION

Bacterial infection by *Streptococcus mutans* (S. mutans) produces tooth decay, better known as cavities, which results in the demineralization of enamel leading to cavities. S. mutans is a key bacterium associated with dental caries because of its ability to produce acids from fermentable carbohydrates (1). This process is often triggered by the consumption of sugars, which are fermented by normal oral bacteria to produce acids. Acid then damages the tooth structure, creating cavities (2). Dental caries is a leading infectious disease worldwide that affects individuals of every age group and requires proper management to protect oral health (3). Children who frequently snack outside school settings under the influence of environmental factors alongside social influences tend to develop unique eating habits and oral health practices (4). The role of S. mutans in the development of dental caries underscores the importance of preventive strategies, dietary control, and early intervention in maintaining optimal oral health across all age groups (5).

BIOFILM FORMATION AND STRUCTURAL COMPLEXITY

Initiation of Biofilm Development:

The formation of dental biofilms occurs when pioneer species, known as S. mutans, attach to the acquired enamel pellicle. Bacteria attach to glycoproteins in saliva through surface molecules known as antigen I/II, thus creating a base layer of biofilms (5). The biofilm community begins to diversify through bacterial co-aggregation between oral bacteria such as *Streptococcus sanguinis* and *Actinomyces* species (6). Biofilms gain their cohesive strength through the production of extracellular polymeric substances (EPS), with glucans as the main component that bacteria synthesize through glucosyltransferases using dietary sucrose (7). The matrix provides bacterial solid placement on tooth surfaces while simultaneously binding nutrients and protecting microorganisms from environmental challenges (8).

The Role of the Extracellular Matrix:

Cariogenic biofilms contain thick EPS-rich materials that exhibit distinct features. Repeated biofilm production of glucans, fructans, and extracellular DNA leads to a matrix that blocks antimicrobial diffusion, thus enabling bacterial pH regulation for enamel dissolution (8). Through the glycolytic conversion of sucrose, S. mutans generates lactic acid, which leads to enamel degradation by producing localized acid drops. Anionic properties in the bacterial matrix enhance the binding of calcium ions, while worsened hydroxyapatite loss occurs (9). Biofilm cells maintain an intact structure with EPS barriers that prevent fluoride permeation with high effectiveness (10).

PROTECTIVE MECHANISMS OF BIOFILMS AGAINST ANTIMICROBIALS

Reduced Penetration of Therapeutic Agents:

EPS forms a dense protective layer that hinders the passage of chlorhexidine and fluoride into the biofilm structure. Biofilm-embedded S. mutans cells demonstrate resistance to fluoride, which is 10–1,000-fold stronger than that of free-floating planktonic cells, partly because fluoride ions find it harder to penetrate (10). Cationic antimicrobial peptides are neutralized in the presence of negatively charged glucans within the matrix structure (8).

Metabolic Heterogeneity and Persister Cells:

Biofilms contain metabolically dormant persisters that endure antimicrobial resistance by decreasing the transport systems and shutting down ATP production. Stress-resistant cells within biofilms start growing again after the stress factor disappears, ultimately causing biofilm regrowth (8). Microenvironmental conditions in biofilms, characterized by oxygen gradients, enable the survival of anaerobic bacteria, which produces additional challenges during treatment (6).

Acid Tolerance and Stress Response Pathways:

S. mutans maintains its survival in acidic solutions through strong stress response mechanisms. Through the activity of F1F0-ATPase, bacterial cells pump protons outward to maintain intracellular pH at steady levels, alongside the SigX regulon's functions in gene regulation for acid tolerance (7). Through adaptations, biofilm bacteria can remain alive under pH changes that would destroy free microbes and keep dental caries active despite the buffering nature of saliva (8).

CHALLENGES IN BIOFILM ERADICATION

Mechanical Disruption and Incomplete Removal:

The mechanical action of brushing and flossing breaks down biofilms and reaches most of the dental surfaces; however, bacteria persist in deep pits, gaps, and beneath the gums. Biofilm remnants left on the tooth surface after brushing quickly replace themselves because of rapid bacterial recolonization, thus requiring intensive mechanical cleaning to remove them. Oratest analysis showed that incomplete plaque removal by children during brushing leads to biofilm rebound within one day, which increases the risk of tooth decay (11).

Limitations of Conventional Antimicrobials:

Resistance mechanisms found in biofilms prevent the successful use of topical fluorides and antiseptic chlorhexidine. The ability of fluoride to remineralize enamel has an opposing effect against biofilm acidic conditions that promote enamel demineralization (10). Broad-spectrum antimicrobial agents disrupt oral commensal bacteria, making dysbiosis difficult to control and causing poor health outcomes in patient populations (9).

Socioeconomic and Behavioural Factors:

The condition of biofilm virulence worsens in Pakistani rural areas because the population lacks access to proper oral care, does not use fluoridated toothpaste, and consumes high amounts of sugar (6). People with low health literacy may fail to recognize the importance of biofilm control because of their limited educational access (12).

ADVANCES IN BIOFILM-TARGETED THERAPIES (2015–2025)

Current research has examined how to maintain oral bacteria in equilibrium while creating new methods to manage dental plaque. According to Hernández (13), the therapeutic strategy for oral microbiota balance consists of the use of dentifrices and rinses during tooth brushing. To prevent caries, researchers have concluded that healthy biofilms require maintenance of their structure along with homeostasis while targeting critical bacterial characteristics related to attachment capabilities, nutritional exchanges, and information sharing. Researchers identified a deficit in current solutions that maintain oral microbiota equilibrium and supported the ongoing development of available products to fight dental caries. Smart nanotechnology applications for the targeted drug delivery of cariogenic pathogens while modifying biofilm pH has been the subject of research conducted by Liu (14). Their research confirmed that the biofilm structure presents an opportunity to enhance anticaries methods with better drug performance under acidic conditions through smart nanotechnology applications. Cai (15) explained that studying the microbial relationships between

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oral microorganisms provides the knowledge needed to create treatments targeting cariogenic biofilms. Studies indicate that disease states are directly related to microbiota diversity found in biofilms, making therapeutic interventions for virulent biofilm formation a promising approach for new treatments. Jiao (16) presented three antimicrobial approaches: antimicrobial photodynamic therapy, cold atmospheric plasma, antimicrobial agent release, and contact-killing strategies. Researchers have found promising potential in innovative antimicrobial biofilm control approaches; however, existing research and clinical trials that follow a structured methodology are necessary to create dependable dental materials with antimicrobial properties. Figure 1 illustrates the progressive development of biofilm-targeted therapeutic approaches for dental caries prevention, highlighting the use of LCG-N25, 3F1, and ZY354 as small molecule inhibitors, along with enterocins and *Lactobacillus reuteri*, as well as enzymatic matrix degradation strategies. These approaches aim to prevent biofilm formation, enhance antimicrobial activity, and support the maintenance of a balanced oral microbiota.

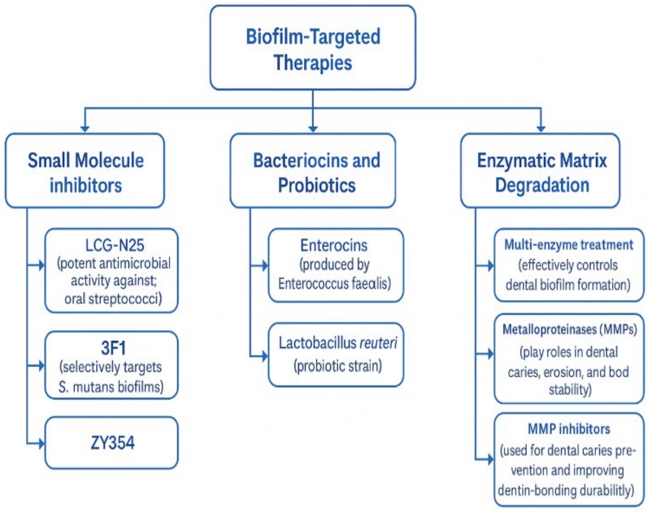

Figure 1: Biofilm Targeted Therapies

Table 1: Small Molecule Inhibitors in Biofilm-Targeted Therapies

Ref.	Year	Methods	Findings	Practical Applications
17	2017	Development of a chemical agent inhibiting enzyme function of *S. mutans*; tested on experimental rats consuming a sugar diet.	Small molecule effectively prevents dental caries in preclinical models; blocks *S. mutans* biofilm formation.	Shows potential for future therapeutic drug development for dental caries prevention.
9	2017	Biofilm dispersion assay; small molecule screening; dental caries prevention model in live rats.	Selective agent 3F1 effectively inhibits *S. mutans* biofilms without disturbing the oral microbiome.	Enables selective biofilm targeting, preserving microbial balance and preventing caries.
18	2017	In silico screening of small molecule library; binding analysis via OctetRed.	Identified lead compounds selectively inhibiting *S. mutans* biofilm formation and virulence factors.	Supports development of selective, non-toxic anti-virulence therapeutics for dental caries.
19	2019	Cytotoxicity evaluation on human oral cells and macrophages; antimicrobial testing against oral streptococci.	ZY354 shows low toxicity, suppresses EPS production, reduces *S. mutans* abundance, and inhibits demineralization.	Demonstrates promise for safe clinical application in anticaries treatment.
20	2019	Emphasis on mechanical methods including brushing, flossing, and use of mouthwashes; dietary sugar replacement with xylitol.	Mechanical approaches remain essential but difficult to implement universally.	Infection prevention strategies should complement chemical biofilm-targeted approaches.
21	2021	Crystal violet staining, colony-forming unit assays, fluorescence staining, electron microscopy, and biofilm metabolism analysis.	LCG-N25 exhibits strong antimicrobial activity against oral streptococci, with no cell toxicity or resistance induction.	Presents potential to strengthen current methods in dental caries management.
22	2022	Automated screening of antimicrobial small molecules; in vitro antibiofilm activity assays.	Identified six small molecule inhibitors of *S. mutans* glycosyltransferases with effective antibiofilm properties.	Offers candidates for future development of novel antibiofilm therapeutics for caries prevention.
23	2024	Investigations using antimicrobial peptides, probiotics, nanoparticles, and non-thermal plasma therapies.	Research highlights need for innovative methods to inhibit *S. mutans* biofilm formation.	Specialized biofilm-blocking approaches should be implemented to enhance caries prevention and oral health outcomes.

Small Molecule Inhibitors:

Small molecule inhibitors, such as LCG-N25, ZY354, and 3F1, have received considerable attention in the advancement of biofilm-targeted therapies. Table 1 presents a summary of key developments in small molecule inhibitors from 2015 to 2025.

Probiotics and Bacteriocins:

Research has demonstrated that *Lactobacillus reuteri* competes with harmful bacteria for their adherent sites to modify the biofilm structure (24). Antimicrobial peptides, known as enterocins, which originate from *Enterococcus faecalis*, restrict S. mutans expansion by destroying cell membranes (12). Laboratory research demonstrated that enterocins decrease

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biofilm biomass by 70% over 12 hours, representing a promising alternative to antibiotics (12). Table 2 summarizes key developments in biofilm-targeted therapies from 2015 to 2025, emphasizing the use of probiotics and bacteriocins, such as enterocins, as emerging strategies for biofilm prevention and disruption.

Table 2: Probiotics and Bacteriocins in Biofilm-Targeted Therapies

Ref.	Year	Methods	Findings	Practical Applications
12	2018	Isolation of *S. mutans* and *E. faecalis* from oral samples; spot-on-lawn assay and time-kill testing for inhibitory effects.	Enterocins exhibited strong bactericidal activity against *S. mutans* biofilms.	Presents a promising alternative to traditional fluoride-based treatments for dental caries control.
25	2018	Biofilm assessment using fluorescence microscopy, cell viability, and metabolic activity assays.	Bacteriocins demonstrated effective biofilm inhibition against cariogenic microorganisms.	Potential bio-therapeutic agents pending further validation through clinical trials.
26	2022	Crystal violet staining and scanning electron microscopy for biofilm inhibition analysis.	*Enterococcus faecium* DB1 strain produced substances that significantly inhibited *S. mutans* biofilm formation.	Offers a safe and natural alternative to conventional cariostatic agents.
27	2023	Review of the effects of Lactobacillus strains and analysis of commercial probiotic formulations for dental caries.	*Lactobacillus* probiotics showed strong antagonistic activity against *S. mutans* in vitro and in marketed products.	Supports the use of probiotic supplements as safer adjuncts to traditional caries prevention methods.
23	2024	Comprehensive review of antimicrobial peptides, probiotics, nanoparticle-based therapies, and non-thermal plasma strategies targeting biofilm regulation.	Highlighted promising biofilm-inhibition approaches for controlling *S. mutans* colonization and caries development.	Encourages the development and clinical integration of biofilm-targeted strategies for enhancing oral health prevention.

Table 3: Enzymatic Matrix Degradation in Biofilm-Targeted Therapies

Ref.	Year	Methods	Findings	Practical Applications
29	2015	Investigation of the effects of matrix metalloproteinase (MMP) inhibitors combined with therapeutic resin blends and primers on dentin durability.	MMP inhibitors significantly enhance the durability of dentin-bonded interfaces and improve restoration longevity.	Emphasizes the role of MMP inhibition in preventing caries progression and enhancing restorative success.
30	2015	Experimental inhibition of MMP and cathepsin activities using dental agents, chlorhexidine, synthetic inhibitors, and cross-linkers.	Inhibition of collagen degradation improves the mechanical stability of dentinal restorations.	Highlights the importance of preserving collagen structure for durable restorative outcomes.
31	2017	Application of enzymatic treatments combined with fluoride-containing products and biocides like chlorhexidine in oral care.	Enzymes contribute both to disease prevention and tissue healing in biofilm-related oral conditions.	Multi-enzyme formulations offer enhanced protection against dental biofilms and promote oral health maintenance.
28	2020	Assessment of metalloproteinase (MMP) activity as diagnostic indicators for pulp and periapical inflammation.	MMP expression correlates strongly with tissue inflammation and disease progression in dental pulp and periapical regions.	MMPs serve as useful biomarkers for early diagnosis and management of dental caries and erosion.
32	2024	Quantitative evaluation of biofilm regulation through enzymatic treatment during early and mature biofilm stages using crystal violet staining.	Application of multi-enzyme therapy significantly reduces biofilm formation on dental surfaces.	Supports multi-enzyme therapy as a promising approach for caries prevention and biofilm management.

Enzymatic Matrix Degradation:

The enzymes dextranase and mutanase destroy biofilm glucans, which damages EPS matrix structures, thus allowing better penetration of antiseptic agents. Current clinical trials show that enzymatic pretreatment leads to a 40% increase in fluoride effectiveness; however, research on prolonged safety implications is needed (8). The extracellular matrix is a target for host matrix metalloproteinases (MMPs) in both restorative dentistry treatments and endodontic therapies (28). Table 3 shows the mechanisms for targeting biofilm literature from 2015 to 2025 for enzymatic matrix degradation

CONCLUSION

In dental caries, the development of *Streptococcus mutans* is closely linked to biofilm formation, as bacterial persistence enables continuous acid production that demineralizes the enamel surface. Research targeting biofilms aims to promote more effective therapeutic strategies. Recent advances, including the development of small-molecule inhibitors, probiotics (*Lactobacillus reuteri*), and bacteriocin-based inhibitors (enterocins), have shown success in targeting S. mutans biofilms to reduce caries progression. This review highlights therapeutic advancements in biofilm management over the past decade, illustrating the evolving direction of dental caries treatment. The integration of these innovative therapies, alongside emerging technologies in nanotechnology and microbiome management, offers a more targeted and effective approach to dental caries prevention.

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