# CRISPR/Cas-mediated targeted gene editing of *Streptococcus mutans*: A promising approach for precision dentistry for the prevention and management of caries (Review)

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**Abstract.** Dental caries, a prevalent chronic condition of global significance, presents substantial risks to oral health, including severe discomfort, tooth loss and impaired oral function if left untreated. The effective prevention and management of dental caries are paramount for maintaining optimal oral health and mitigating associated systemic health complications. Proactive strategies can reduce the need for extensive interventions, enhancing overall well-being. Recent advances in molecular biology have revolutionized genetic research and therapy. The CRISPR/Cas system, derived from bacterial immune systems, enables precise DNA modifications through targeted incisions across various disciplines. The CRISPR/Cas system is renowned for its unprecedented precision and efficiency in DNA modification, revolutionizing both genetic research and therapeutic applications. The present review discusses the potential application of CRISPR/Cas technology in precision dentistry, emphasizing how it provides a paradigm shift from conventional antimicrobial and fluoride-based strategies. Unlike broad-spectrum antimicrobials or fluoride, CRISPR enables the precise genetic targeting of *Streptococcus mutans* and other cariogenic bacteria, potentially reducing resistance development and microbiome disruption. Additionally, the present review discusses novel CRISPR-based approaches tailored for gene editing in *Streptococcus mutans*, which have yet to be widely explored in clinical research. It elucidates the mechanisms by which genes associated with microbial biofilm formation and caries susceptibility can be targeted and modified using CRISPR/Cas methodologies. Particular emphasis is placed on the utilization of CRISPR/Cas for gene silencing in *S. mutans*, a key cariogenic organism, to elucidate gene regulation mechanisms and their roles in bacterial survival and virulence. The present review also highlights recent advancements in gene editing through CRISPR/Cas, emphasizing its potential to enhance our understanding of caries pathogenesis and facilitate development of novel therapeutic approaches. By integrating current research findings and evaluating practical applications of CRISPR/Cas in dentistry, the present review describes the promising avenues of this cutting-edge approach in personalized preventive strategies and targeted treatments in dental care.

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## 1. Introduction

Dental caries is an infectious disease that affects the calcified tissues of the teeth, demineralizing the inorganic portion of the tooth and destroying its organic portion (1). The structure of both permanent and deciduous teeth, the pathogenicity of the oral bacteria community, and the oral habitats formed by saliva, sugar consumption and hereditary factors are all elements that contribute to this complex disorder (2). Initially, caries affects the tooth enamel, but can progress to the dentin and root canal, causing abscesses if left untreated. The metabolism of dietary carbohydrates by bacteria produces acids that contribute to the development of caries, while the response of the host to bacterial infection significantly affects the risk of developing caries. Therefore, food, host defense systems

and bacterial makeup all play a major role in determining the amount of tooth damage (3). One of the key processes in the development of caries is the adhesion of bacteria to teeth through the pellicle, which encourages the growth of biofilms. On the rough surfaces of poorly produced enamel, such as those observed in individuals with amelogenesis imperfecta, this adhesion is very frequent. The natural microbiome of healthy teeth forms a biofilm community that helps maintain tooth integrity by counteracting acid production from dietary carbohydrates. This can occur as a result of reactions, such as the urea or arginine synthesis of ammonia (4). Dental caries is linked to dysbiosis, a disrupted microbial equilibrium. In a healthy condition, the mouth microbiome and the host have a symbiotic connection (5). In caries, a diet high in sugar and acid creates an environment that promotes persistent acid production from carbohydrate metabolism. This environment supports the growth of highly acidogenic and aciduric bacteria, such as *Streptococcus mutans* (*S. mutans*) and *Lactobacillus* species, disrupting the pH balance of supragingival biofilms and tipping the demineralization-remineralization balance towards tooth mineral loss (6,7). This is corroborated by the ecological plaque theory, which demonstrates that in the oral cavity, at a healthy pH of 7.0, *Streptococcus gordonii* is predominant. Acid-resistant species including *S. mutans, Veillonella dispar* and *Lactobacillus casei*, on the other hand, become more prevalent at a lower pH of 4.5, suggesting a selection change in microbial populations under acidic circumstances that benefits these species (8). In 2019, the global average prevalence of dental caries in deciduous teeth was estimated at 43%, with 514 million children experiencing untreated caries (9,10). 2.3 billion individuals have untreated dental caries, with an age-standardized frequency of 29.4% in permanent teeth (11). Due to these high prevalence rates, extensive research has been conducted to target various etiological causes of caries, aiming to prevent and reduce the burden of the disease.

The management of dental caries has evolved to focus on both suppressing cariogenic bacteria and strengthening tooth resistance to demineralization. While maintaining oral hygiene is essential, it can be challenging, particularly for high-risk individuals, necessitating additional preventive measures. Early treatments relied on broad-spectrum antimicrobials, such as vancomycin and kanamycin to eliminate *S. mutans* and disinfect lesions (12,13). However, their limited penetration and non-specific bacterial suppression rendered them ineffective. Traditional approaches, including fluoride and broad-spectrum antimicrobials, provide some benefits, but lack precision, often leading to microbial resistance and the disruption of the oral microbiome. Although fluoride enhances enamel resistance and provides temporary antimicrobial effects, it does not selectively target *S. mutans*, limiting its efficacy in the long-term prevention of caries (14). In an effort to restore dental health, current strategies concentrate on restructuring the oral microbiota associated with caries by focusing on *S. mutans* or the larger acidogenic, acid-tolerant microbiota (15). Clinical investigations have demonstrated variable degrees of response to various therapies, including xylitol, tea and cranberry polyphenols, povidone-iodine, casein phosphopeptide-amorphous calcium phosphate fluoride and chlorhexidine (16-21). Fluoride, which can be found in a variety of forms, including toothpaste, gels, varnishes, mouth rinses, lozenges, and the fluorination of water, milk and salt, is still the most widely used preventative agent. Its use has notably decreased tooth decay. Fluoride functions by preventing the synthesis of extracellular polymeric substances (EPS) and carbohydrate metabolic processes of the bacteria, which lowers its adhesion and thereby cariogenic bacterium development. Long-term fluoride exposure lowers salivary *S. mutans* levels, reduces caries scores and prevents enamel demineralization. However, its antimicrobial effect on microbial communities tends to be minimal and short-lived. Various fluoride compounds, including sodium fluoride, stannous fluoride and sodium monofluorophosphate, are effective in dental products. Silver diamine fluoride (SDF) combines silver and fluoride, exerting marked antimicrobial and caries-arresting effects. SDF-treated lesions demonstrate increased hardness due to silver and fluorohydroxyapatite formation. SDF is a minimally invasive, cost-effective treatment for at-risk patients; however, its limitations highlight the need for improved prevention methods for caries (3).

Nanoparticles, such as silver nanoparticles (AgNPs) and nano silver fluoride (NSF) represent advanced antimicrobial delivery systems in dentistry. These nanoparticles help prevent bacterial adhesion, attachment and biofilm formation on teeth, and they are more effective at remineralizing dentin caries and less likely to cause tooth discoloration compared to SDF. The minute silver particles minimize toxicity by continually releasing ions, penetrating dentinal tubules and bacterial cells, and exerting a potent antibacterial action against *Lactobacilli* and *S. mutans* even at low concentrations (3). AgNPs in dental materials have the ability to modify the composition of biofilms and reduce the proliferation of *S. mutans* and *Candida albicans*, rendering NSF and AgNPs viable substitutes for SDF in the prevention of caries (22). Recent progress in second- and third-generation sequencing technologies has revealed the complexity of the healthy human microbiome, highlighting its roles in immune regulation, digestion and pathogen resistance (23). As a result, the 'one germ, one disease' paradigm has given way to an improved knowledge of dysbiosis and polymicrobial conditions. Traditional broad-spectrum antimicrobials can disrupt the microbiome, leading to issues, such as antibiotic resistance. There is a growing research focus on the more precise targeting of pathogens while preserving beneficial microbiota (3,24). Efforts to target *S. mutans*, the primary bacteria causing dental caries, have included immunization, bacteriophages, probiotics and small molecules; however, these methods face challenges in clinical application and effectiveness. Of note, specifically targeted antimicrobial peptides (STAMPs) provide precision in targeting pathogens. C16G2, a STAMP engineered to target and eliminate *S. mutans*, has demonstrated potential in decreasing *S. mutans* levels in biofilms and supporting a healthier oral microbiome (25). C16G2 is currently in phase II clinical trials (C3J16-V204-00). Other innovative approaches include pH-responsive antimicrobials that target acid-producing bacteria. Examples include quaternary pyridinium salt, acid-activated nanoparticles for farnesol delivery, and catalytic nanoparticles that produce free radicals to degrade biofilm and kill bacteria. These approaches have exhibited potential for more effective, targeted caries prevention (26-28). Although no currently approved targeted therapies for dental caries exist, these findings have advanced

the current understanding of the disease and have guided future research toward precision therapies to supplement existing fluoride treatments (3).

Prokaryotic genome sequences frequently include clustered regularly interspaced short palindromic repeats (CRISPR), which are identified by short direct repeats (DR) spaced by spacer sequences (29). Initially, the CRISPR/Cas system functioned as a part of the immune response in bacterium and archaea against phage infections and plasmid transfers (30). Recently, this system has gained prominence as a potent tool for genetic engineering, offering a straightforward and efficient method for genome manipulation with various research and clinical applications (31). CRISPR/Cas relies on the base pairing between RNA and DNA. Each CRISPR loci in bacterial genomes consists of a CRISPR gene array and a CRISPR-associated (Cas) gene array. While the Cas generates Cas proteins, which function as endonucleases, the CRISPR array encodes CRISPR RNA (crRNA), which controls DNA-binding specificity. The majority of human oral microbiota contain naturally occurring CRISPR loci (32). Research comparing CRISPR loci in dental plaque biofilms from healthy individuals and those with periodontitis has revealed that patients who are in good health exhibit a greater similarity in CRISPR sequences, indicating a bacterial colony that is steady and robust, and that is resistant to bacteriophage invasion (29). This suggests that the CRISPR system may contribute to the preservation of the equilibrium of the oral microbiome and may one day be utilized to modify the microbiome to treat diseases. Additionally, by altering host gene regulatory pathways, CRISPR may be used to control infectious conditions (33).

By contrast, CRISPR/Cas technology represents a paradigm shift by providing a highly specific, programmable method for gene editing within cariogenic bacteria, allowing for precise genetic modifications that could reduce bacterial virulence, disrupt biofilm formation, and enhance fluoride sensitivity. Unlike traditional antimicrobials, CRISPR can be tailored to selectively eliminate or modulate *S. mutans* populations without affecting beneficial oral microbiota, potentially preserving microbial balance while reducing caries risk. Moreover, recent advancements in CRISPR-based bacterial targeting, engineered bacteriophages, and host genome modifications present novel therapeutic avenues beyond conventional dental treatments. By directly altering genes associated with cariogenicity, CRISPR has the potential to address the root cause of dental caries rather than merely managing symptoms, marking a significant step toward precision dentistry (29-34). The present review explores the current applications and future prospects of CRISPR/Cas in the prevention of dental caries, highlighting its unique advantages over traditional approaches, potential limitations and the necessary steps for clinical translation. By integrating recent research findings, the present review aimed to provide insights into how CRISPR-mediated gene editing could revolutionize preventive and therapeutic strategies in dentistry, paving the way for truly targeted and personalized oral healthcare.

## 2. Data collection methods

The present review was conducted by analyzing peer-reviewed literature from the PubMed, Scopus and Web of Science databases, focusing on studies published between (2015-2024).

Key words included 'CRISPR/Cas AND dental caries', 'CRISPR/Cas9 AND *Streptococcus mutans*' and 'gene editing AND oral microbiome'. Articles were selected based on their relevance to precision dentistry, preclinical advancements and the potential translational applications of CRISPR. Studies solely discussing genetic modifications outside oral microbiology were excluded.

## 3. Genome editing utilizing CRISPR/Cas

The CRISPR/Cas system has become a ground-breaking tool for accurate genetic modification over the past 10 years. This method introduces targeted double-strand breaks (DSBs) in the host genome by using DNA sequence-specific endonucleases from simpler species. With great translational and therapeutic potential, CRISPR/Cas presents a promising avenue in the fields of oral and craniofacial research, for exploring gene functions, as well as developing gene therapies. Through Cas nuclease-mediated DNA cleavage and crRNA-guided DNA recognition, CRISPR/Cas functions as an adaptive immune system. Unique spacers are inserted between conserved repeating sequences in the CRISPR region. Cas nucleases break down invasive foreign DNA and incorporate it into the CRISPR region as novel spacers. The invasive DNA is subsequently targeted and cleaved by Cas nucleases owing to these spacers. Over 40 kinds of Cas proteins have been found, all of which are crucial for the synthesis of crRNA, the spacer integration process, and DNA fragmentation. Cas1, Cas2 and CSN2 are responsible for the acquisition of new spacers, whereas Cas9 is principally involved in DNA cleavage (33).

The CRISPR/Cas system addresses foreign DNA in three distinct phases: Adaptation (spacer acquiring), expression (crRNA translation), and interference (destruction and fragmentation of the foreign genome). With the aid of the Cas1-Cas2 protein complex, a section of foreign DNA is incorporated into the host chromosome during the adaptation phase, creating a new spacer component in the CRISPR structure. In the expression stage, the CRISPR array is translated into pre-crRNA and the Cas enzyme then processes it into mature crRNA. This is achieved by Cas6 in systems type I and III; and Cas6 enzyme in systems of Type II. Following this, in the interference phase, the crRNA-Cas complex targets and breaks down the foreign DNA/RNA. The protospacer-adjacent motif (PAM) directs the identification of target sequences, and certain nuclease domains initiate nuclease action. The variety and specificity of CRISPR/Cas systems across species is attributed to their utilization of distinct proteins and processes for the purpose of targeting and cleaving DNA or RNA (35). The most popular technology for gene editing is the class 2 CRISPR system Cas9 endonuclease from *Streptococcus pyogenes*. Its ease of use, affordability, efficacy and speedy genome-editing capabilities have revolutionized biological research (36).

Genome editing through CRISPR/Cas9 involves creating double-strand breaks (DSBs) in DNA, which are then repaired by physiological processes. In the native system, a complex consisting of transactivating crRNA (tracrRNA) and crRNA pairs directs Cas9 to the target DNA region. The partly complementary tracrRNA aids in the maturation of the crRNA. Cas9 requires a corresponding protospacer sequence and a brief PAM in order to effectively cleave at the target site, producing

DSBs. Researchers have developed guide RNAs (gRNAs) that combine crRNA and tracrRNA elements to streamline genome editing. CRISPR/Cas9 variants can recognize target sequences of 20-24 nucleotides along with 2-4 nucleotide PAM sequences, allowing for precise targeting of 22-29 nucleotide sequences in most genomes (37). By leveraging this versatile system, researchers can introduce targeted genetic changes with high precision, paving the way for advancements in various fields, including oral and craniofacial research, and offering promising avenues for therapeutic interventions.

## 4. Precision dentistry and its significance in dental caries

Historically, dental care has predominantly employed a 'one-size-fits-all' strategy for managing conditions, such as dental caries and periodontitis, treating all patients similarly irrespective of disease stage or individual variations. Traditionally, carious lesions were managed invasively by removing all affected tissue and filling cavities, often overlooking alternative management options or the underlying causes. Diagnostic methods were uniformly applied to all patients, utilizing visual-tactile evaluations and radiographs. By contrast, precision dentistry customizes therapy based on the biological, social and behavioral characteristics of an individual to predict the most effective, efficient and safe treatments, and to prevent disease onset and progression. Precision therapeutics for dental caries aim to provide personalized, targeted treatments addressing the specific causes and risk factors of each patient, to prevent, halt, or reverse caries with minimal intervention (38). One promising approach in precision dentistry involves using CRISPR/Cas technology (Fig. 1). This cutting-edge gene-editing method focuses on altering specific genetic elements linked to the development and progression of caries. Potential uses of CRISPR in precision dentistry for addressing dental caries include the following: Targeting cariogenic bacteria and preventing biofilm formation, modulating host factors, utilizing CRISPR technology to study the genes in *S. mutans* (39-52).

By leveraging these targeted interventions, precision dentistry can offer more personalized and effective treatment options for dental caries, ultimately improving patient outcomes.

*Targeting cariogenic bacteria and preventing biofilm formation.* *S. mutans* is strongly linked to the development of dental caries owing to its acid production, biofilm establishment and glucan synthesis capabilities (39). The study by Serbanescu *et al* (40) highlighted the CRISPR/Cas type II-A in *S. mutans* as a promising target for reducing virulence and controlling the spread of antibiotic resistance genes, suggesting that targeting this CRISPR system could help manage resistance in these bacteria. Chen *et al* (41) discovered that there may be a connection between CRISPR sites and cariogenic potential, since *S. mutans* strains with two CRISPR sites produced greater amounts of EPS and formed biofilms more frequently than strains without these sites (41). The deletion of Cas3, a key Cas protein in *S. mutans*, has been shown by Tang *et al* (42) to affect biofilm formation and enhance fluoride sensitivity, which in turn affects the competitiveness of bacterium in dual-species biofilms under fluoride treatment.

In order to treat dental caries, it is possible to improve fluoride efficacy and modify oral microecology by addressing the effects of the Cas3 gene on both biofilm production and fluoride resistance (42). Additionally, it has been shown that deleting the Csn2 gene in *S. mutans* alters colony morphology and heightens acid sensitivity, leading to the reduced expression of aciduricity-related genes. This genetic change affects *S. mutans* virulence and EPS formation, suggesting that targeting Csn2 could provide new strategies for addressing imbalances in the oral microbiota that can cause dental caries (43). Recent research has also examined the regulation of CRISPR system expression in *S. mutans* by the global regulators, CcpA and CodY, which are crucial for amino acid biosynthesis and glucose metabolism. This control influences the generation of (p)ppGpp during the stringent response, promoting metabolic activities and helping adaptation to environmental stress (44). Furthermore, by altering transcription products, such as crRNA and tracrRNA, which function in tandem with Cas proteins to accurately target and break DNA; CRISPR has been used as a self-targeting genome-editing tool. By modifying the genes coding for glucosyltransferases using CRISPR/Cas9, Gong *et al* (45) targeted virulence genes in *S. mutans*, significantly decreasing EPS production and preventing biofilm formation (Table I).

*Modulating host factors.* CRISPR technology offers the potential to modify patient genomes to influence susceptibility to dental caries by targeting specific genes for either knockout or upregulation. This approach can be used to enhance genes associated with a reduced risk of developing caries, such as those involved in stronger enamel formation or increased production of natural antimicrobial peptides in saliva. Conversely, CRISPR can disrupt genes that increase the risk of caries, including those related to enamel defects or saliva composition. It is essential to comprehend these hereditary elements and how they are regulated in order to combat tooth decay. With the use of homology-directed repair or suppressing deleterious mutations, CRISPR-Cas9 may be used to edit patient genomes for therapeutic purposes or to study these genes using knockout approaches (46-51). According to Shaffer *et al* (46), there may be a hereditary component to the increased susceptibility of the female population to dental caries compared to males. Moreover, it was discovered that individuals with the G-20A variant of the beta-defensin 1 (DEFB1) gene in saliva had a 5-fold higher decay-missing-filled teeth (DMFT) index and a significantly higher risk of developing dental caries. DEFB1 rs179946 (G-52A), on the other hand, was previously linked to lower DMFT scores (47). Active caries are more common in those with the TC and CC genotypes of Fok1 RFLP (rs2228570) of the vitamin D receptor gene polymorphisms, according to a previous case control study (48). In particular, the rs2228570 T allele was connected to increased caries activity, whereas the C allele was linked to caries-free status. The risk of developing caries was 2.814-fold higher in individuals with the TC genotype and 3.116-fold higher in those with the TT genotype. This demonstrates how hereditary variables affect the susceptibility of an individual to developing dental caries (29). A previous study demonstrated that in saliva, genetic variations in polymorphic acid proline-rich proteins influenced the bacterial adherence to the initially
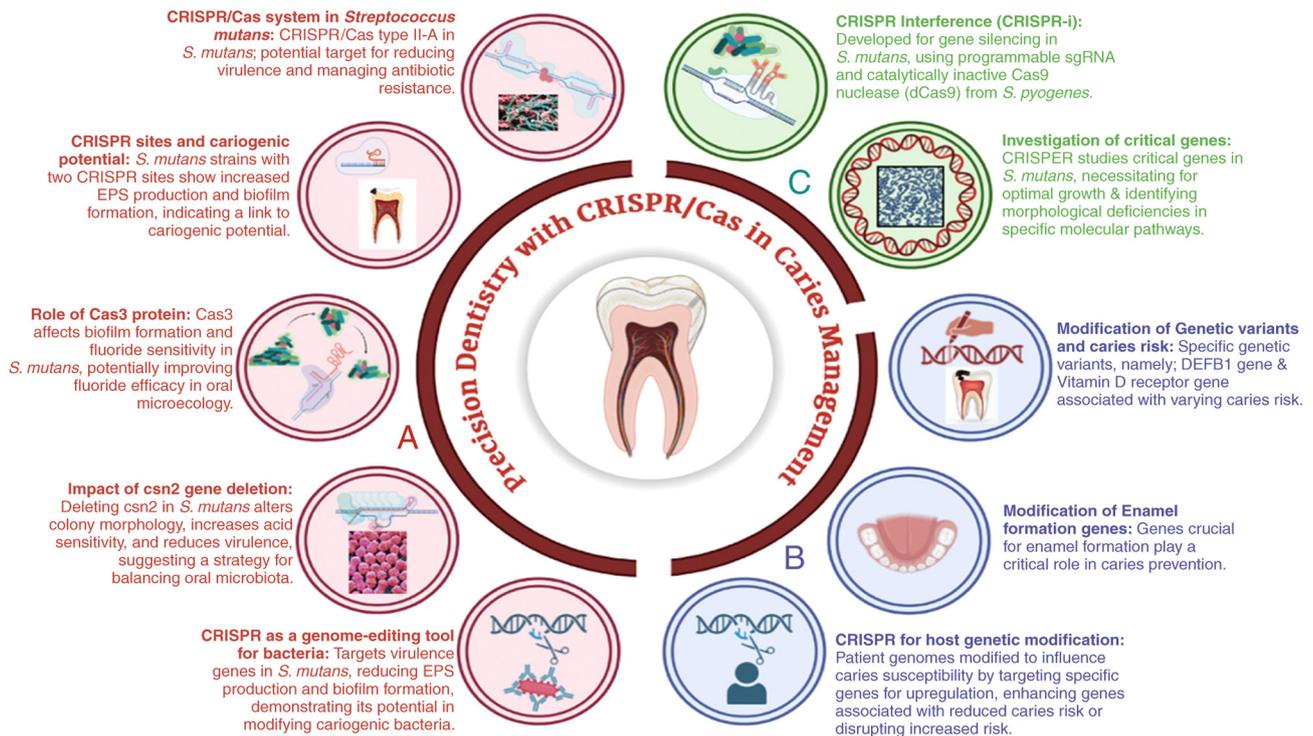
Figure 1. Applications of CRISPR/Cas in the management of caries. (A) Targeting cariogenic bacteria and preventing biofilm formation. (B) Modulating host factors. (C) Utilizing CRISPR technology to study the genes in *Streptococcus mutans*. EPS, extracellular polymeric substances; sgRNA, single-guide RNA; DEFB1, beta-defensin 1.

formed pellicle, and were hence linked to an increased risk of developing caries. This association was notably observed in Caucasians, who exhibited a higher *S. mutans* colonization due to these genetic variations (49). Proper enamel formation is essential for preventing tooth decay, with genes such as enamelin, amelogenin, matrix metalloproteinase 20 and kallikrein 4 playing critical roles in enamel development. Variants in these genes have been shown to be associated with enamel defects, such as amelogenesis imperfecta and varying susceptibility to caries (50). Additionally, the laminin subunit alpha 3 genetic mutation, which encodes laminin type V, has been linked to enamel defects due to impaired cell-to-cell attachment of ameloblasts, crucial for enamel formation (51).

*Use of CRISPR technology to investigate the genes in S. mutans.* Streptococcus species, including *S. mutans*, are implicated in various human infections, such as pneumonia and meningitis, and are prevalent in the oral cavity. *S. mutans*, in particular, is closely linked to dental caries (53). According to recent research, *S. mutans* possesses a large number of key genes that are necessary for its existence (54). While some of these genes are unique to *S. mutans*, the remainder are shared with various pathogenic *Streptococci*. A CRISPR interference (CRISPR-i) system for gene silencing has been created by researchers, utilizing a programmable single-guide RNA (sgRNA) and a catalytically inactive Cas9 nuclease (dCas9) from *Streptococcus pyogenes*. This repurposes the bacterial CRISPR/Cas system. Using this method, almost all of the critical genes in *S. mutans* have been investigated; the results indicate that these genes are necessary for the organism to grow to its full potential and show a range of morphological

deficiencies in genes associated with comparable molecular pathways (52). Specifically, CRISPR-i was used to investigate the role of L-rhamnose glycopolymers in *S. mutans* viability, pathogenicity and cell replication. Researchers observed structural anomalies, such as chromosome segregation issues, enlarged cells and changes in cell shape. In a wax worm model, rhamnose-glucose polysaccharide, or RGP, production was disrupted, which affected pathogenicity and cell replication This groundbreaking study represents the first application of CRISPRi in *S. mutans*, utilizing Cas9 and revealing the potential for developing advanced CRISPR-Cas tools (52). The research underscores the effectiveness of CRISPRi in studying essential genes and their functions, providing valuable insight into gene function and regulation without permanent genome alterations (52). Further research in this area could provide critical insights for developing an agent to inhibit the growth and proliferation of *S. mutans* within biofilms.

## 5. Limitations and concerns regarding CRISPR translation to clinical practice

CRISPR/Cas9 is favored for its simplicity compared to earlier genome-editing methods, mainly due to its use of ~20-bp crRNA sequences for targeting specific genetic sequences (36). Despite its benefits, the use of CRISPR/Cas9 in treating dental caries faces several challenges. The development and application of CRISPR/Cas technology are currently costly, which may limit its accessibility and widespread use in dental care (34). One of the key concerns in translating CRISPR to clinical dentistry is the potential for off-target mutations, where unintended genetic modifications could impact non-cariogenic

Table I. *In vitro* studies evaluating the effects of CRISPR/Cas on the virulence factors and cariogenic potential of *Streptococcus mutans*.

| Authors, year of publication | Nature of study | Gene/protein | Findings | (Refs.) |
|---|---|---|---|---|
| Serbanescu *et al*, 2015 | *In vitro* | CRISPR/Cas type II-A | Target for reducing virulence and antibiotic-resistance genes in *S. mutans* | (40) |
| Chen *et al*, 2017 | *In vitro* | CRISPR I CRISPR II | *S. mutans* strains with two CRISPR sites produced greater amounts of EPS and formed biofilms more frequently than strains without these sites | (41) |
| Tang *et al*, 2019 | *In vitro* | Cas3 | Deletion impact biofilm formation and enhance fluoride sensitivity | (42) |
| Zhang *et al*, 2020 | *In vitro* | Csn2 | Deleting in *S. mutans* altered colony morphology and heightened acid sensitivity | (43) |
| Kang *et al*, 2023 | *In vitro* | CcpA and CodY | Regulation of CRISPR system expression in *S. mutans*, promoting metabolic activities and helping adaptation to environmental stress | (44) |
| Gong *et al*, 2018 | *In vitro* | Glucosyltransferases (gtfs) | Self–targeting CRISPR arrays in *S. mutans* against gtfs, significantly lowering EPS production and preventing biofilm formation | (45) |
| Shields *et al*, 2020 | *In vitro* | CRISPR interference (CRISPR-i) | Gene silencing utilizing CRISPR/Cas from *S. pyogenes* to investigate the role of L-rhamnose glycopolymers in *S. mutans* | (54) |

EPS, extracellular polymeric substances.

bacteria or host genes (55). Previous studies have sought to improve CRISPR specificity by employing high-fidelity Cas9 variants (e.g., SpCas9-HF1) and base-editing techniques to minimize undesired changes (56-58). However, further validation is required before CRISPR can be safely applied in human oral microbiomes.

Delivering CRISPR/Cas components precisely to specific cells or tissues in the oral cavity remains challenging, as current methods may not efficiently target cariogenic bacteria or affected areas. Additionally, the introduction of these components could provoke an immune response, leading to inflammation or other adverse effects. The complexity of the oral microbiome further complicates treatment, as ensuring specificity to *S. mutans* without disrupting beneficial bacteria is difficult, potentially disturbing microbial balance or harming commensal bacteria. Individual genetic variability also influences the effectiveness and safety of CRISPR therapies, necessitating costly and extensive genetic screening. Furthermore, as with antibiotics, CRISPR-based treatments face the risk of bacterial resistance, which has been observed in laboratory studies. This raises concerns about their long-term efficacy and underscores the need for further research on genetic adaptation and escape mechanisms before clinical application (59,60).

The incomplete understanding of the genetic underpinnings of cariogenic bacteria and the complex interactions within the oral microbiome may hinder the development of effective CRISPR-based interventions. Integrating CRISPR/Cas therapies with existing preventive and therapeutic strategies for dental caries presents significant challenges, requiring the creation

of novel protocols and comprehensive training programs for dental professionals. Additionally, the clinical application of these advanced genetic technologies is complicated by ethical considerations and regulatory oversight. Unlike conventional treatments, such as antibiotics or fluoride, CRISPR involves direct genetic modification, necessitating stringent review by regulatory bodies such as the FDA, EMA and WHO. Issues of informed consent, long-term genetic effects and ethical debates surrounding somatic vs. germline modifications further complicate the approval process. Moreover, the current CRISPR/Cas9 technology faces technical limitations related to precision, efficiency and scalability, which need to be resolved to ensure its successful application in treating dental caries and other oral health conditions. Addressing these technical constraints is crucial for broader clinical implementation, while maintaining a balanced approach that considers ethical, regulatory and scientific challenges. As research progresses, carefully weighing the potential benefits of CRISPR in caries management against these concerns will be essential for advancing gene editing in dentistry while upholding rigorous scientific and ethical standards (61).

## 6. Future directions

CRISPR technology holds immense potential in dental care by identifying genetic biomarkers linked to caries susceptibility, thus enabling personalized prevention and early intervention strategies (Fig. 2). Thus far, studies have solely examined *S. mutans* as the causative pathogen. The oral cavity is a sophisticated ecosystem that is heavily populated by a diverse
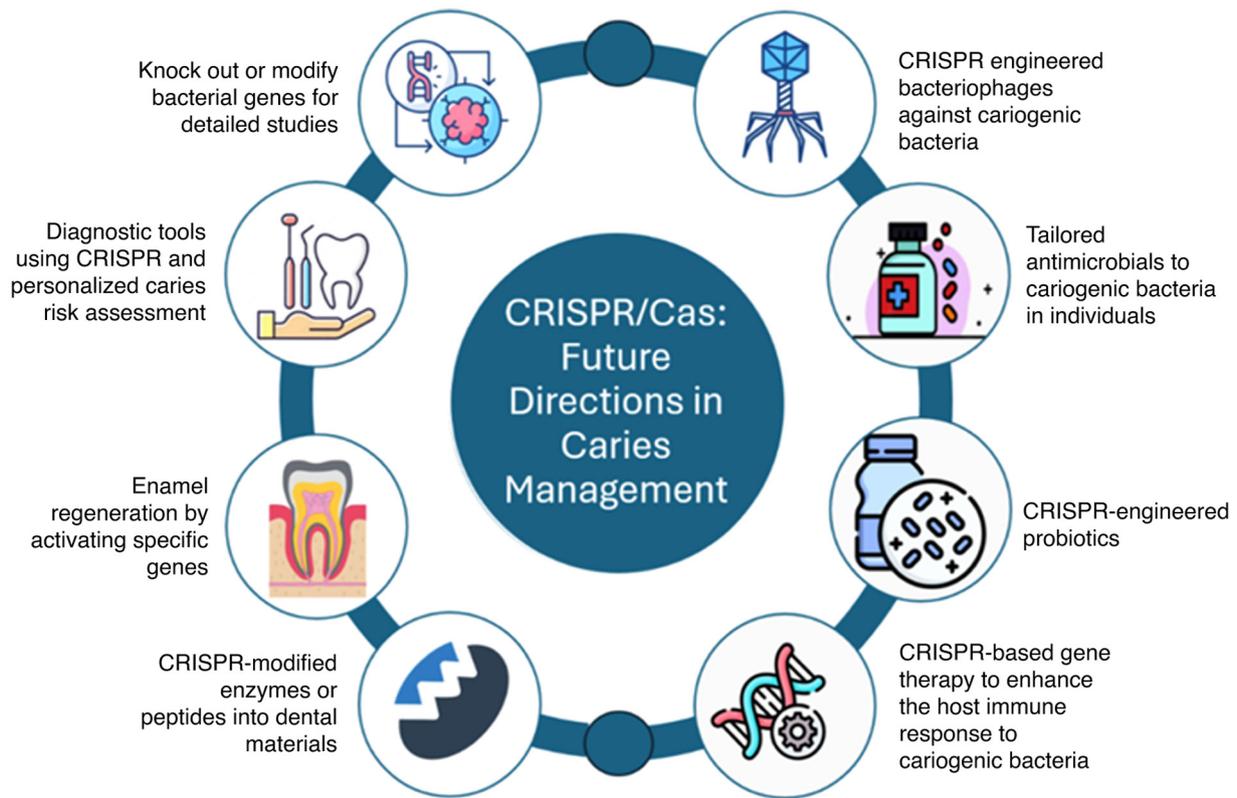
Figure 2. Future directions of CRISPR/Cas in the prevention and management of dental caries.

range of organisms, including viruses, bacteria, protozoa and fungi. These organisms live in tiny microbial niches on the tooth surface and other parts of the oral cavity. Furthermore, the oral and biofilm microenvironment conditions cause alterations in the biofilm. Therefore, caries cannot be identified by the mere existence of a biofilm, which is present on the tooth surface (62). Moreover, as caries is considered to have a multifactorial etiology, variables such as frequency and the amount of sugar in the diet can potentially shift the ecological balance in favor of dysbiosis. These factors need to be considered in subsequent research in order to develop novel and innovative treatment approaches.

Using the aforementioned technique, CRISPR-based antibiotics (63) can be created specifically targeting *S. mutans* or antibiotic-resistant bacteria, eliminating harmful bacteria, while preserving beneficial oral microbiota. Additionally, engineered bacteriophages (64) with CRISPR-Cas systems can selectively target *S. mutans*, minimizing side-effects and enhancing the prevention of caries. CRISPR can also be used to develop antimicrobials tailored to the genetic makeup of cariogenic bacteria in individuals, reducing resistance and preserving beneficial bacteria (64). Moreover, CRISPR-engineered probiotics (65) can outcompete harmful bacteria, promoting a balanced oral microbiome. CRISPR-based gene therapy (66) provides the potential to enhance the immune response of the host to cariogenic bacteria by modifying relevant genes. Utilizing CRISPR/Cas technology to knock out or modify genes in *S. mutans* and other oral bacteria allows for detailed studies of their roles in biofilm formation, acid production and virulence, which can enhance the current understanding of the molecular mechanisms that contribute to dental caries.

Diagnostic tools using CRISPR can detect cariogenic bacteria early, allowing for timely intervention. Furthermore, CRISPR can help analyze genetic markers for caries susceptibility, providing personalized risk assessments and preventive strategies. The technology can also promote enamel regeneration by activating specific genes, aiding in the repair of early carious lesions without invasive treatments. Lastly, incorporating CRISPR-modified enzymes or peptides (67) into dental materials can offer ongoing protection against cariogenic bacteria and enhance the durability of dental restorations. While CRISPR/Cas presents a groundbreaking approach for precision dentistry, it remains largely in preclinical stages for caries management. Current research is focused on *in vitro* and animal model studies, with significant technical, safety and regulatory hurdles yet to be addressed before clinical translation. Future research is warranted to prioritize delivery mechanisms, safety validation and long-term microbiome stability before CRISPR-based therapies can be integrated into routine dental care.

## 7. Conclusion

The CRISPR/Cas system presents a revolutionary approach to preventing and treating dental caries. By precisely targeting cariogenic bacteria, such as *S. mutans* and modulating host genetic factors, CRISPR/Cas technology can potentially alter the oral microbiome and enhance resistance to caries. Studying and editing specific genes in bacteria and hosts offers valuable insights into caries development and opens up new possibilities for treatment. However, challenges such as off-target effects, effective delivery methods, and ethical issues still need to be

addressed. Despite these hurdles, the ongoing advancements in CRISPR/Cas technology hold promise for precision dentistry, potentially transforming dental care into a more personalized, effective and minimally invasive practice. Further research and clinical trials are required in order to overcome the current limitations and aid in the realization of the full potential of CRISPR/Cas in managing dental caries.

## Acknowledgements

Not applicable.

## Funding

## Availability of data and materials

Not applicable.

## Authors' contributions

MP and PS conceptualized the study and performed the literature search and the selection of studies. AK and RB wrote the manuscript, and reviewed and edited the manuscript. All authors have read and approved the final manuscript. Data authentication is not applicable.

## Ethics approval and consent to participate

Not applicable.

## Patient consent for publication

Not applicable.

## Competing interests

The authors declare that they have no competing interests.

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