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CRISPR : A CRISPY WAY TO DEAL WITH ORAL DISEASES

Dental Science

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ABSTRACT

CRISPR, an acronym for Clustered Regularly Interspaced Short Palindromic Repeats, refers to a sophisticated, programmable system capable of editing, silencing, or regulating gene expression. Among the various genome-editing platforms available, the CRISPR-Cas9 (CRISPR-associated protein 9) system has emerged as the most widely adopted due to its precision, efficiency, and versatility in introducing sequence-specific modifications. This advanced technology holds significant promise for a broad spectrum of biomedical applications, including transformative advancements in oral healthcare. Furthermore, the scope of gene editing has expanded beyond targeted modifications to include genome-wide functional screening for both gene loss-of-function and gain-of-function studies. This review provides an overview of the CRISPR-Cas9 genome editing system and its emerging applications in the field of dentistry.

KEYWORDS

Cas 9, CRISPR, Genetic Tool, Genome Editing

INTRODUCTION

Over the past six decades, advances in technologies for DNA synthesis and manipulation have played a pivotal role in the progress of molecular biology. This transformative era began with the elucidation of the DNA double helix structure and advanced significantly with the development of solid-phase DNA synthesis techniques, which facilitated genome analysis and characterization. However, this process is labor-intensive and requires highly specialized laboratories equipped with advanced molecular biology tools.[1]

In 1987, Japanese researchers first identified unusual repeat sequences within the iap gene region[2], though their function remained unclear. By 2000, these sequences were found across multiple bacterial species, yet their biological role was still undetermined.[3] A significant breakthrough occurred when these sequences were linked to bacterial adaptive immunity against viral infections, spurring a rapid expansion in research on the CRISPR-Cas9 system.[4]

Based on the structural and functional properties of associated Cas proteins, CRISPR systems are broadly classified into two classes: Class I (types I, III, and IV), which involve multi-protein effector complexes, and Class II (types II, V, and VI), which utilize a single, multifunctional Cas protein. Among these, the Class II type II CRISPR/Cas9 system has gained prominence due to its structural simplicity and high efficacy in genome editing applications.[5]

CRISPR, or Clustered Regularly Interspaced Short Palindromic Repeats, represents a revolutionary gene-editing platform derived from a naturally occurring bacterial immune mechanism. This system comprises two key components: a guide RNA (gRNA) that directs the Cas9 endonuclease to a specific genomic sequence, and the Cas9 protein, which introduces a double-strand break at the targeted site. This precise targeting mechanism allows for efficient and customizable genome modification in living organisms.[6]

Prior to the development of CRISPR/Cas9, genome editing relied on tools such as meganucleases, zinc finger nucleases (ZFNs), and transcription activator-like effector nucleases (TALENs). Despite their contributions, these technologies were hindered by challenges such as limited target specificity, complex design protocols, and difficulties in cellular delivery, particularly in the case of TALENs due to their large molecular size.[7,8,9]

In prokaryotes, CRISPR systems serve as a defense mechanism by targeting and breaking down foreign genetic material, such as plasmids and bacteriophages, providing adaptive immunity. The Cas9 enzyme, directed by a synthetic guide RNA, can be customized to identify and cut precise DNA sequences, enabling gene suppression, activation, modification, or substitution. Owing to its accuracy and programmable nature, Cas9 is often described as a “DNA targeting system”.[10]

The potential of CRISPR/Cas9 extends to various fields, including the treatment of genetic disorders. Recent literature highlights its applicability in addressing oral diseases rooted in genetic mutations. Given its robust and targeted gene-editing capability, CRISPR technology holds considerable promise for advancing oral healthcare.[11,12]

This review aims to provide a comprehensive overview of the major genome-editing nucleases, with a particular emphasis on the applications, mechanisms, and clinical implications of the CRISPR/Cas9 system in dentistry.

Mechanism Action of CRISPR-Cas 9 Technology (Fig . 1)

The CRISPR–Cas9 system, a naturally occurring adaptive immune mechanism in bacteria, enables sequence-specific defense against viral pathogens. Upon primary infection by a novel bacteriophage, Cas enzymes integrate short fragments of the viral genome, known as spacers, into the CRISPR locus of the bacterial chromosome. This genomic integration serves as a molecular memory, facilitating adaptive immunity against future infections by the same virus.[9]

During reinfection, the CRISPR array is transcribed into a precursor CRISPR RNA (pre-crRNA), which, in conjunction with the trans-activating crRNA (tracrRNA), is processed into mature guide RNAs (gRNAs). These gRNAs associate with the Cas9 endonuclease to form a ribonucleoprotein complex that recognizes and binds to complementary sequences within the invading viral DNA, directing site-specific cleavage and inactivation of the pathogen.[9]

Cas1 and Cas2 are universally conserved core components of the CRISPR-Cas system. These proteins form a stable heterodimeric complex essential for the acquisition of new spacers during the adaptation phase, thereby maintaining the dynamic and heritable nature of CRISPR-mediated immunity.[9]

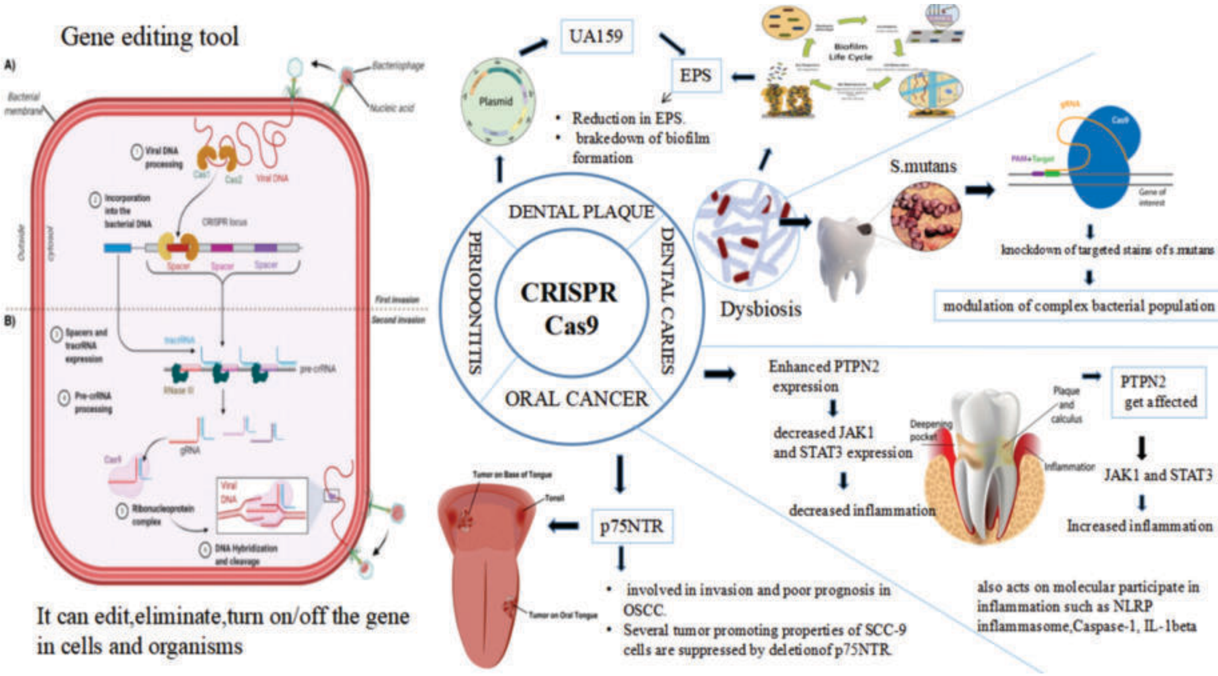

Figure 1 : Mechanism of Action & Application of CRISPR

Applications of CRISPR in Dentistry:

1. Dental Caries:

Dental caries is predominantly associated with the activity of Streptococcus mutans, a key cariogenic microorganism responsible for forming pathogenic biofilms on tooth surfaces. This microbial dysbiosis contributes to the demineralization of enamel, ultimately leading to the development of carious lesions. While conventional antimicrobial interventions—such as broad-spectrum antibiotics, targeted antimicrobial peptides (e.g., C16G2), and lytic bacteriophages—have demonstrated partial efficacy, they often lack specificity and control over microbial community dynamics.[13,14]

To address these limitations, current research is increasingly focused on employing RNA-guided nucleases (RGNs), particularly CRISPR/Cas systems, to engineer precision antimicrobial strategies. These systems enable the selective targeting and disruption of specific bacterial strains based on unique genetic signatures, offering a refined approach to modulating the oral microbiome.[15]

The CRISPR/Cas platform represents a promising tool for the prevention and treatment of dental caries through targeted gene editing of S. mutans. By inactivating genes involved in acidogenesis and biofilm formation, CRISPR can attenuate the cariogenic potential of S. mutans, thereby mitigating enamel demineralization and lesion progression. Furthermore, this technology can be harnessed to upregulate genes involved in enamel remineralization, promoting the natural repair of incipient lesions. In addition, CRISPR constructs can be engineered to deliver antimicrobial peptides specifically against S. mutans, enhancing microbial clearance while preserving the balance of the oral microbiota.[16,17]

Overall, CRISPR-based approaches offer a precise, customizable, and potentially transformative solution for the targeted control of cariogenic bacteria, paving the way for next-generation strategies in dental caries prevention and therapy.

2. Dental Plaque

One of the primary virulence factors of Streptococcus mutans is the enzyme family glucosyltransferases (Gtfs), which catalyze the conversion of dietary sucrose into extracellular polysaccharides (EPS). These EPS play a critical role in the initial stages of dental plaque development by facilitating the formation of biofilms on tooth surfaces.[15]

In a study conducted by Gong T et al., a CRISPR-based approach was employed to disrupt this biofilm-forming capability. Specifically, self-targeting CRISPR arrays were engineered to contain spacer sequences complementary to the gtfB gene, a key gene involved in EPS synthesis. These constructs were cloned into plasmids and subsequently introduced into the S. mutans UA159 strain, generating self-targeting mutants. The introduction of these CRISPR constructs led to a significant reduction in EPS production and effectively impaired biofilm formation, demonstrating the potential of CRISPR-mediated gene targeting in disrupting key virulence mechanisms of cariogenic bacteria.

3. Gingivitis and Periodontitis

Porphyromonas gingivalis, a Gram-negative, obligate anaerobic rod-shaped bacterium, is recognized as a keystone pathogen in the pathogenesis of microbial dysbiosis associated with chronic periodontitis. Notably, approximately 95% of clinical isolates of P. gingivalis have been shown to contain CRISPR arrays, suggesting that its endogenous CRISPR-Cas immune system may contribute to its ability to survive and adapt within the complex microbial environment of the periodontal pocket—an ecological niche often dominated by bacteriophages.[18]

This endogenous CRISPR activity is hypothesized to influence the composition and stability of the periodontal microbiome, potentially offering a natural mechanism for microbial modulation. As such, CRISPR-based technologies hold significant promise as targeted tools for preventing biofilm formation and, by extension, mitigating the progression of periodontitis in clinical dental settings.[18]

Beyond microbial modulation, the CRISPR-Cas9 system also facilitates functional genomic studies by enabling the precise knockout of genes to elucidate their roles in disease mechanisms. Gene knockout strategies, which involve the targeted inactivation or deletion of specific genes, are instrumental in identifying molecular pathways involved in inflammatory responses in periodontal tissues.[9]

For example, a study by Zhang et al. employed CRISPR-Cas9 to knock out the PTPN2 gene, which encodes a protein involved in the vitamin D receptor (25VD3)-mediated signaling pathway within gingival epithelial cells. Loss of PTPN2 resulted in enhanced phosphorylation of key transcription factors, JAK1 and STAT3, which are central to the inflammatory cascade. The increased phosphorylation correlated with heightened periodontal inflammation, underscoring PTPN2's role as a negative regulator of this pathway. The PTPN2 protein mediates its anti-inflammatory effects by dephosphorylating JAK1 and STAT3, thereby dampening the downstream inflammatory signaling.[19]

Additionally, another study highlighted the protective function of intracellular superoxide dismutase 2 (SOD2) in periodontal tissues. SOD2 was found to attenuate inflammation by inhibiting the NLRP3 inflammasome/caspase-1/IL-1β axis, a key inflammatory signaling pathway.[9]

These findings demonstrate how CRISPR-Cas9 can be used to dissect the molecular underpinnings of periodontitis and identify potential therapeutic targets for controlling excessive inflammatory responses in periodontal disease.

4. Oral Cancer

Oral cancer encompasses malignant neoplasms originating within the oral cavity, including the lips, tongue, gingiva, buccal mucosa, and the hard and soft palate. Among these, oral squamous cell carcinoma (OSCC) is the most prevalent subtype of head and neck cancer (HNC), characterized by a poor prognosis and low overall survival rates. The development of oral cancer is closely associated with both genetic and epigenetic alterations that drive tumorigenesis and disease progression.[11]

The CRISPR-Cas9 gene-editing system has emerged as a powerful and precise tool in oncology research, offering significant potential for elucidating the molecular underpinnings of oral cancer by targeting and modifying specific genes implicated in carcinogenesis. Its application enables the identification and functional validation of oncogenes and tumor suppressor genes, thereby facilitating the discovery of novel therapeutic targets.

In a study conducted by Huang et al., the tumor-promoting role of the p75 neurotrophin receptor (p75NTR) gene in human tongue carcinoma was investigated. Using CRISPR-Cas9-mediated gene knockout, suppression of p75NTR led to a marked inhibition of multiple malignant phenotypes in SCC-9 cells, indicating its potential as a therapeutic target in OSCC.[20]

Similarly, Kim et al. identified the heat shock protein HSPA5 (also known as GRP78/BiP) as a key regulator of tumor progression and cellular survival in HNC. Their findings demonstrated that HSPA5 supports cancer cell viability through the maintenance of lysosomal function. Targeting the MUL1-HSPA5 regulatory axis may thus provide a novel approach to impair lysosomal activity and reduce tumor survival in HNC.[21]

Further, a comprehensive genome-wide CRISPR-Cas9 screening study by Chai et al. across 21 Asian OSCC cell lines identified multiple genetic dependencies in OSCC. Notably, cells exhibiting a WWTR1 (TAZ) dependency signature showed enhanced responsiveness to immunotherapeutic interventions. The study highlighted YAP1 and WWTR1—two transcriptional co-activators of the Hippo signaling pathway—as high-priority therapeutic targets for OSCC management.[22]

Collectively, these findings underscore the utility of CRISPR-Cas9 in dissecting the molecular landscape of oral cancer and in advancing precision medicine approaches for its treatment.

Future of CRISPR in Dentistry

CRISPR technology is poised to bring significant advancements to the field of dental medicine through its unparalleled precision in gene editing. Its capacity to target and modify specific genetic sequences opens avenues for developing personalized therapeutic strategies tailored to the individual genetic makeup of patients, particularly for conditions such as periodontal disease and dental caries. This gene-specific approach has the potential to improve treatment efficacy while minimizing adverse effects.

Furthermore, CRISPR holds transformative potential in the area of regenerative dentistry. By enabling targeted genetic modifications, it may facilitate the regeneration and repair of dental tissues, including enamel, dentin, and periodontal ligaments, which are currently challenging to restore using conventional methods. In parallel, CRISPR-based diagnostic platforms are expected to enhance early detection of oral diseases and microbial dysbiosis, allowing for more timely and effective clinical interventions.

Despite these promising developments, the routine integration of CRISPR into dental practice will necessitate addressing several challenges. These include regulatory and ethical considerations, the development of safe and efficient gene delivery systems, and establishing standardized clinical protocols. Nonetheless, ongoing research and technological progress suggest that CRISPR-based innovations could redefine precision dentistry, improving both preventative and therapeutic outcomes in the near future.

SWOT Analysis

1. Strengths

- CRISPR-Cas9 enables precise, site-specific genome editing, making it highly suitable for targeting genes associated with dental pathologies such as periodontitis and dental caries.
- The technology supports the development of personalized therapeutic strategies tailored to individual genomic profiles, thereby enhancing treatment specificity and clinical efficacy.
- CRISPR can selectively target both pathogenic microorganisms (e.g., Streptococcus mutans) and host-related genes, expanding its functional scope in oral disease management.
- Continuous advancements in CRISPR-based platforms are enhancing gene editing accuracy, improving diagnostic capabilities, and fostering novel therapeutic interventions in dentistry.
- It serves as a robust tool for elucidating the molecular mechanisms of oral diseases, facilitating translational research and innovative therapeutic discoveries.

2. Weaknesses

- Off-target effects remain a concern, as CRISPR may introduce unintended genomic alterations, posing potential safety and efficacy risks.
- Efficient and targeted delivery of CRISPR components to oral tissues and cells remains technically challenging and necessitates further refinement.
- Ethical considerations surrounding permanent genetic alterations and their heritable implications continue to pose dilemmas in clinical and research contexts.
- Regulatory frameworks are often complex and inconsistent across jurisdictions, potentially impeding the clinical implementation and global integration of CRISPR-based dental applications.

3. Opportunities

- There is significant potential to develop prophylactic interventions by targeting genetic risk factors for oral diseases prior to clinical onset.
- Multidisciplinary collaboration among dental researchers, geneticists, and molecular biologists can expedite the translational application of CRISPR in oral health.
- CRISPR opens avenues for the development of novel treatment modalities targeting complex or refractory oral conditions that lack effective current therapies.
- The emergence of CRISPR-driven diagnostic and therapeutic tools presents promising commercialization opportunities within the evolving dental biotechnology sector.

4. Threats

- The rapid evolution of competing gene editing technologies (e.g., base editing, prime editing) may challenge CRISPR's dominance and clinical utility.
- Public apprehension regarding genetic manipulation and safety concerns may hinder widespread acceptance and adoption in dental practice.
- Legal conflicts over CRISPR patents and intellectual property rights may restrict research collaboration and delay product development.
- The high cost associated with CRISPR research, clinical validation, and implementation may limit its accessibility, particularly in resource-constrained dental care settings.

CONCLUSION

CRISPR-Cas9 technology offers a novel and precise approach for the therapeutic management of oral diseases, including periodontal disease and dental caries, by enabling targeted modification of genes implicated in disease pathogenesis. Through site-specific genome editing, CRISPR has the potential to regulate or disrupt genes involved in critical processes such as enamel development and host inflammatory responses. Additionally, it presents a viable strategy for the selective elimination of pathogenic microorganisms, notably Streptococcus mutans, a key etiological agent in dental caries. However, despite its considerable promise, the clinical translation of CRISPR-Cas9 in dentistry is currently limited by concerns related to off-target genomic alterations and the unpredictability of long-term gene expression changes. These limitations underscore the need for extensive preclinical research and refinement of delivery and targeting systems prior to routine clinical implementation.

REFERENCES

1. Doudna, J. A., & Charpentier, E. (2014). The new frontier of genome engineering with CRISPR-Cas9. Science, 346(6213), 1258096.
2. Ishino, Y., Shinagawa, H., Makino, K., Amemura, M., & Nakata, A. (1987). Nucleotide

sequence of the iap gene, responsible for alkaline phosphatase isozyme conversion in Escherichia coli, and identification of the gene product. Journal of bacteriology, 169(12), 5429-5433.
3. Mojica, F. J., Díez-Villaseñor, C., Soria, E., & Juez, G. (2000). MicroCorrespondence. Molecular microbiology, 36(1).
4. Bales, L. E. (2022). CRISPR-Cas in the Field of Dentistry: A Comprehensive Collection of the Potential Uses of CRISPR-Cas9 in Dental Health Care.
5. Liu, Z., Dong, H., Cui, Y., Cong, L., & Zhang, D. (2020). Application of different types of CRISPR/Cas-based systems in bacteria. Microbial cell factories, 19(1), 172.
6. Kondody, R. T., Nambiar, M., Shetty, N., & RNG, R. (2023). Prospects of CRISPR-CAS 9 Gene Editing Technology in Dentistry: A Review. RGUHS Journal of Medical Sciences, 13(1).
7. Silva, G., Poirot, L., Galetto, R., Smith, J., Montoya, G., Duchateau, P., & Pâques, F. (2011). Meganucleases and other tools for targeted genome engineering: perspectives and challenges for gene therapy. Current gene therapy, 11(1), 11-27.
8. Gupta, R. M., & Musunuru, K. (2014). Expanding the genetic editing tool kit: ZFNs, TALENs, and CRISPR-Cas9. The Journal of clinical investigation, 124(10), 4154-4161.
9. Barbour, A., Glogauer, J., Grinfeld, L., Ostadsharif Memar, R., Fine, N., Tenenbaum, H., & Glogauer, M. (2021). The role of CRISPR‐Cas in advancing precision periodontics. Journal of Periodontal Research, 56(3), 454-461.
10. Horvath, P., & Barrangou, R. (2010). CRISPR/Cas, the immune system of bacteria and archaea. Science, 327(5962), 167-170.
11. Torre, L. A., Bray, F., Siegel, R. L., Ferlay, J., Lortet‐Tieulent, J., & Jemal, A. (2015). Global cancer statistics, 2012. CA: a cancer journal for clinicians, 65(2), 87-108.
12. Garhnayak, M., Rath, S., Garhnayak, L., & Panda, S. (2023). CRISPR in dentistry: a boon or bane. Microbes and Infectious Diseases, 4(4), 1178-1182.
13. Gao, L., Xu, T., Huang, G., Jiang, S., Gu, Y., & Chen, F. (2018). Oral microbiomes: more and more importance in oral cavity and whole body. Protein & cell, 9(5), 488-500.
14. He, X., Lux, R., Kuramitsu, H. K., Anderson, M. H., & Shi, W. (2009). Achieving probiotic effects via modulating oral microbial ecology. Advances in dental research, 21(1), 53-56.
15. Gong, T., Tang, B., Zhou, X., Zeng, J., Lu, M., Guo, X., ... & Li, Y. (2018). Genome editing in Streptococcus mutans through self‐targeting CRISPR arrays. Molecular Oral Microbiology, 33(6), 440-449.
16. Citorik, R. J., Mimee, M., & Lu, T. K. (2014). Sequence-specific antimicrobials using efficiently delivered RNA-guided nucleases. Nature biotechnology, 32(11), 1141-1145.
17. He, X., Lux, R., Kuramitsu, H. K., Anderson, M. H., & Shi, W. (2009). Achieving probiotic effects via modulating oral microbial ecology. Advances in dental research, 21(1), 53-56.
18. Chen, T., & Olsen, I. (2019). Porphyromonas gingivalis and its CRISPR-Cas system. Journal of Oral Microbiology, 11(1), 1638196.
19. Mlachkova, A., Popova, C., & Doseva, V. (2020). Presence of IL-8 gene polymorphism and IL-8 serum levels in patients with chronic periodontitis-Literature review. Folia Medica, 62(2), 253-257.
20. Huang, P., Tong, D., Sun, J., Li, Q., & Zhang, F. (2017). Generation and characterization of a human oral squamous carcinoma cell line SCC-9 with CRISPR/Cas9-mediated deletion of the p75 neurotrophin receptor. Archives of Oral Biology, 82, 223-232.
21. Kim, S. Y., Kim, H. J., Kim, H. J., Kim, D. H., Han, J. H., Byeon, H. K., ... & Kim, C. H. (2018). HSPA5 negatively regulates lysosomal activity through ubiquitination of MUL1 in head and neck cancer. Autophagy, 14(3), 385-403.
22. Chai, A. W. Y., Yee, P. S., Price, S., Yee, S. M., Lee, H. M., Tiong, V. K., ... & Cheong, S. C. (2020). Genome-wide CRISPR screens of oral squamous cell carcinoma reveal fitness genes in the Hippo pathway. Elife, 9, e57761.