



CRISPR-Cas9 Gene Editing and Its Application in Endodontics: A Review

Ashish J Johnson ^a , Periya Samy R ^a , Tarun Kumar Singh ^{a*} , Aakash Gupta Gill ^a , Ikroop Gill ^a

^a Division of Conservative Dentistry and Endodontics, Department of Dentistry, All India Institute of Medical Sciences (AIIMS), Bathinda, Punjab, India

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*Corresponding author: Tarun Kumar Singh, Room No. 3027, C-Block, Ground Floor, AIIMS Bathinda, Punjab, India.

E-mail: drtarunkumarsingh@gmail.com

Abstract

Introduction: CRISPR-Cas9 gene-editing technology has revolutionized biomedical research, offering precise genomic manipulation. However, its translation into endodontics- specifically for biofilm disinfection and pulp-dentin regeneration- remains a subject of intense investigation and debate. **Materials and Methods:** A narrative review was conducted based on the electronic search of PubMed, Scopus, and Google Scholar from the year of (2000 to 2026) to identify studies investigating CRISPR-Cas9 applications in endodontic pathogens and dental pulp stem cells. **Discussion:** The review synthesizes current evidence on three frontiers: 1) Antimicrobial Precision, where CRISPR antimicrobials target resistance genes in *Enterococcus faecalis* biofilms; 2) Regenerative Endodontics, highlighting the engineering of stem cells to survive hypoxic, necrotic root canal environments; and 3) Host Modulation, utilizing gene editing to dampen periapical inflammation. Crucially, the review critically appraises the significant barriers to clinical adoption, including the lack of efficient intra-canal delivery systems, immunogenicity risks, and stringent regulatory hurdles for non-lethal dental conditions. **Conclusion:** While CRISPR-Cas9 holds transformative potential for personalized endodontics and tissue engineering, it remains a high-cost research tool at present. Clinical viability depends on the development of safe, non-viral delivery vectors and the establishment of regulatory pathways for localized dental gene therapies.

Keywords: Biofilms; CRISPR-Cas9; Endodontics; Gene Therapy; Regenerative Dentistry

Introduction

The genomes of prokaryotic species, including bacteria, contain a family of DNA sequences called 'clustered regularly interspaced short palindromic repeats' (CRISPR) genes [1]. A more sophisticated technique for editing an organism's DNA is CRISPR-associated protein 9 (CRISPR-Cas9) technology. It stems from the innate anti-viral and plasmid defense mechanisms of bacteria and archaea, which employ CRISPR RNA in conjunction with a plethora of Cas proteins to disrupt the DNA of intruding viruses or other foreign elements [2]. From the perspective of endodontics, CRISPR-Cas9 technology can be revolutionary in the treatment of dental-related diseases and disorders by targeting specific genes that play a crucial role in tooth development, host immune response, and disease susceptibility [3, 4].

The high level of precision inherent in CRISPR-Cas9 technology serves as a significant factor in enabling targeted revisions in particular genes or genomic sections [5]. This precision may reveal novel prospects for therapeutic interventions in genetic dental disorders; additionally, it can facilitate the modulation of immune responses when considering endodontic complications. Additionally, the CRISPR-Cas9 technique may lead to a deeper understanding of the genetic factors that cause various endodontic problems, which could ultimately result in the development of customized treatment plans for each patient [6, 7].

Several issues require discussion regarding the ethical implications and legal frameworks surrounding the application of CRISPR-Cas9 in the medical and dental fields. Future research in endodontics must consider both the risks and ethical implications of gene editing technology.

Despite these theoretical advantages, the application of CRISPR-Cas9 in endodontics faces unique anatomical and biological challenges not seen in other medical fields, such as the mineralization of the dentin-pulp complex and the polymicrobial nature of root canal infections. While the literature is replete with broad overviews of gene editing in dentistry, there is a scarcity of critical reviews that specifically bridge the gap between *in vitro* gene editing mechanisms and the clinical realities of root canal therapy. Therefore, this review aims to provide a critical appraisal of the current ‘state-of-the-art’ regarding CRISPR-Cas9 in endodontics, specifically evaluating its potential in biofilm elimination and pulp regeneration, while rigorously examining the biophysical, safety, and regulatory barriers that currently limit its chairside translation.

Materials and Methods

Search strategy and data sources

To ensure a comprehensive and reproducible review of the literature, an electronic search was conducted across three major databases: PubMed (MEDLINE), Scopus, and Google Scholar. The search spanned the period from January 1, 2000, to January 10, 2026, encompassing both early foundational studies and the most recent experimental breakthroughs.

Search terms and Keywords

A combination of Medical Subject Headings (MeSH) and free-text keywords was utilized to capture the multidisciplinary nature of the topic. The search strings included:

- “CRISPR-Cas9” OR “Gene Editing” AND “Endodontics” OR “Root Canal Treatment”
- “Regenerative Endodontics” AND “Dental Pulp Stem Cells” AND “Gene Therapy”
- “*Enterococcus faecalis*” AND “Biofilm” AND “CRISPR”
- “CRISPR Delivery Systems” OR “Nanoparticles” AND “Dentistry”

Eligibility criteria: Studies were selected based on the following strict criteria:

• Inclusion criteria:

- 1) Original research articles (*in vitro* and *in vivo* animal studies) investigating the application of CRISPR-Cas9 in endodontic pathogens, dental pulp regeneration, or tissue engineering;
- 2) Scoping and systematic reviews relevant to gene therapy in dentistry;
- 3) Regulatory guidance documents (e.g., FDA) published between 2016–2026;
- 4) Articles published in the English language.

• Exclusion criteria:

- 1) Studies focused solely on non-endodontic applications (e.g., orthodontics or caries prevention without pulpal involvement);
- 2) Editorials, conference abstracts, and personal opinion letters lacking experimental data;
- 3) Studies utilizing older gene-editing tools (ZFNs, TALENs) without comparison to CRISPR-Cas9.

Data selection and synthesis

Initial screening was performed based on titles and abstracts to remove duplicates and irrelevant topics. Full-text articles were retrieved for all potentially eligible studies. A total of 42 references were selected for the final qualitative synthesis.

Mechanism of Action

There are two primary categories of DNA repair pathways:

- a. **The route known as non-homologous end joining (NHEJ):** Although this route responds quickly to double-strand breaks (DSBs), it is prone to mistakes. When DSBs are detected, the DNA ends are repaired, kinases and ligases are activated, and the damaged ends are subsequently rejoined. Canonical NHEJ (C-NHEJ) and alternative NHEJ (alt-NHEJ) are the two subclasses of NHEJ. While alternative NHEJ is associated with larger deletions, the canonical version typically achieves precise end-joining or modest deletions (Fig. 1).
- b. **The homology-directed repair (HDR) pathway:** HDR uses a double-stranded donor template to allow for more accurate DNA repair. Depending on its sequence, this template can either introduce precise changes, such as point mutations or gene insertions, or restore the original DNA sequence. HDR repair events and alternative non-homologous end-joining (alt-NHEJ) are more likely to occur when C-NHEJ is inhibited. The applications of CRISPR/Cas9 in gene editing and biomedical research are based on these DNA repair processes.

The process of gene editing using CRISPR-Cas9 technology involves steps that facilitate the repair and modification of the desired genome [6]. The mechanism of gene editing (Fig. 2) is sensitive and should be performed with proper technology [7]. The steps include:

Step 1: Ribonucleoprotein Complex Assembly The process initiates with the synthesis of a single-guide RNA (sgRNA), a chimera designed to be complementary to the specific target DNA sequence. This sgRNA binds to the Cas9 endonuclease to form a catalytically active ribonucleoprotein (RNP) complex, changing the enzyme’s conformation to a DNA-surveillance state.

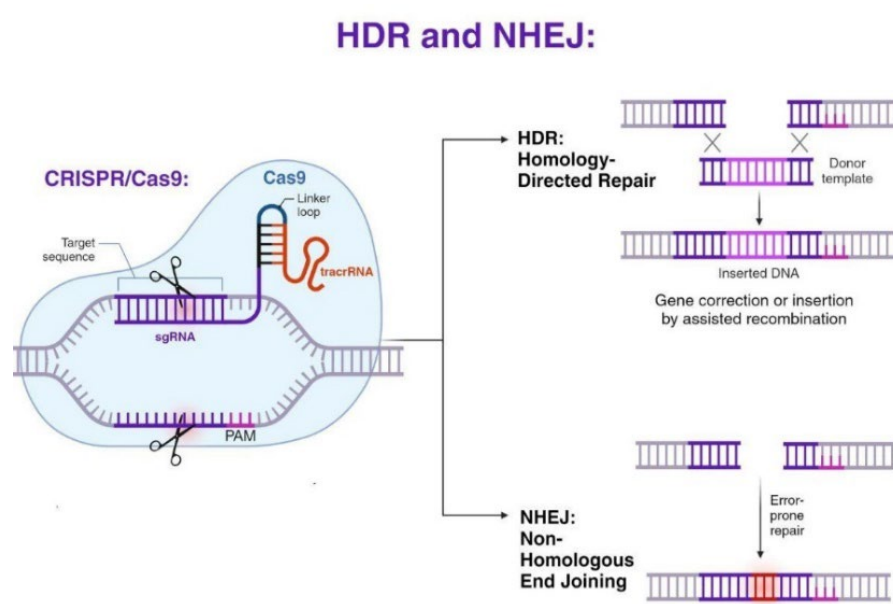


Figure 1. Schematic diagram of Non-Homologous End Joining (NHEJ) and homology-directed repair (HDR) pathway

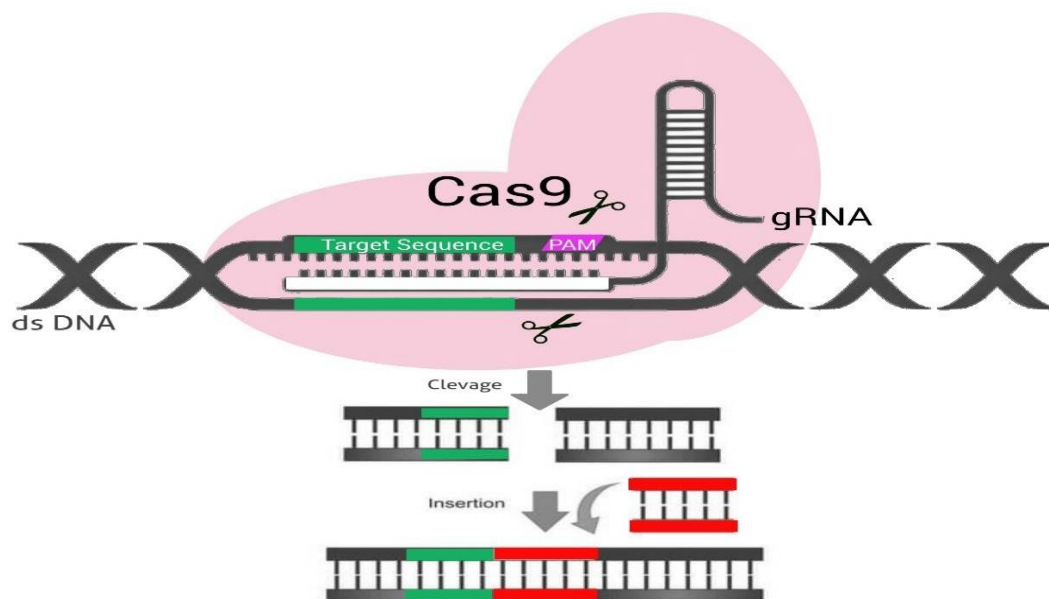


Figure 2. CRISPR Cas9 gene editing outline

Step 2: Target Recognition and Binding The RNP complex scans the genome for a specific protospacer adjacent motif (PAM) sequence (typically 5'-NGG-3' for *Streptococcus pyogenes* Cas9). Upon identifying the PAM, the Cas9 enzyme destabilizes the DNA double helix, allowing the sgRNA to invade and anneal to the complementary target strand *via* Watson-Crick base pairing. This "R-loop" formation serves as a critical checkpoint for specificity.

Step 3: Endonucleolytic Cleavage Stable DNA-RNA pairing triggers the activation of the Cas9 HNH and RuvC nuclease domains. The HNH domain cleaves the complementary DNA

strand, while the RuvC domain cleaves the non-complementary strand. This synchronized activity generates a site-specific double-strand break (DSB) approximately three base pairs upstream of the PAM sequence.

Step 4: DNA Repair and Genetic Modification The induction of a DSB activates the host cell's endogenous DNA repair machinery. In the absence of an exogenous template, the break is repaired *via* non-homologous end joining (NHEJ), an error-prone pathway that frequently introduces insertions or deletions (indels), leading to gene disruption. Conversely, if a homologous

donor template is provided, the cell utilizes the high-fidelity homology-directed repair (HDR) pathway to precisely insert or correct specific genetic sequences.

Discussion

Applications of CRISPR-Cas9 in endodontics

• Targeted gene editing to correct genetic mutations associated with endodontic diseases and genetic disorders.

Recent literature demonstrates the progression from theoretical potential to experimental validation for CRISPR- Cas9-mediated gene editing in endodontics. Notably, studies using animal models have shown that CRISPR/Cas9-mediated Fam83h knockout impairs the Wnt/ β -Catenin pathway, which is integral to tooth mineralization [8, 9].

In addition, correction of enamel defects resulting from LAMA3 mutations has been supported by both cellular and preclinical research, underscoring their clinical relevance [10].

Further, the facilitation of dentinogenesis using oxytocin in CRISPR/Cas9-modified rat dental pulp cells has advanced the scope for tissue engineering applications [11, 12].

Chitosan hydrogel-delivered base editor ABE8e has been shown to correct PAX9 mutations in dental pulp stem cells, representing a potential gene therapy for hypodontia [13].

Collectively, these experiments move beyond speculation, providing credible evidence for genetic correction and individualized approaches in endodontic disease management.

• Gene editing for the treatment of inflammation and infection in endodontics.

Experimental studies have utilized CRISPR-Cas9 techniques to modify pathogenic genes in *Streptococcus mutans*, a model organism for endodontic biofilms and infections [14-16]. These studies reveal the role of targeted gene knockout for attenuating microbial pathogenicity and evaluating antibiotic resistance mechanisms, facilitating prospects for novel antimicrobial therapies.

Recent findings also challenge the notion that regeneration is impossible in the presence of inflammation. Irfan *et al.* [17] utilized CRISPR-Cas9 to knock out the C5L2 receptor in dental pulp stem cells. They found that deleting this specific receptor allowed the cells to maintain their odontogenic differentiation capability even when exposed to high levels of TNF- α , a potent inflammatory cytokine found in apical periodontitis. This suggests a future clinical avenue where 'inflammation-resistant' cell lines could be used for pulp capping or regeneration in teeth with symptomatic irreversible pulpitis, bridging the gap between endodontics and vital pulp therapy.

• Restoration and regeneration of dental pulp through gene editing.

Research employing CRISPR in combination with induced pluripotent stem cells (iPSCs) has demonstrated restoration of tooth parenchymal and stromal tissues through reactivation of critical developmental signals in decellularized matrices [18-20].

Selective silencing of proinflammatory genes, such as those encoding IL-1 β , has shown efficacy in reducing pulp inflammation and promoting tissue repair [21-23], thereby establishing gene editing as a promising adjunct for regenerative endodontics.

A critical limitation of current regenerative endodontic procedures (REPs) is the survival of stem cells introduced into the necrotic, hypoxic root canal space. While general dental literature discusses tissue engineering broadly, the endodontic context requires addressing the hostile ischemic microenvironment of a failed root canal. Research indicates that dental pulp stem cells (DPSCs) are highly susceptible to apoptosis under hypoxic conditions, which frequently leads to the failure of the regenerative scaffold. CRISPR-Cas9 offers a transformative solution to this specific 'endodontic limitation' by enabling the genetic engineering of DPSCs prior to transplantation. By editing apoptotic pathways or enhancing the expression of survival factors in hypoxic conditions, CRISPR could generate 'super-charged' mesenchymal stem cells capable of surviving the initial ischemic phase of revascularization, thereby ensuring the formation of functional pulp-dentin complexes rather than mere fibrotic repair [24].

The combination of gene editing with single or multiple stem cell transplantation or tissue-engineered constructs holds substantial promise for enhancing the regenerative potential of the dental pulp. Studies have explored the synergistic effect of CRISPR-mediated gene editing with stem cell therapy, demonstrating improved outcomes in pulp regeneration for conditions such as pulpitis and necrosis [24-26]. Recent experimental research has specifically targeted genes integral to inflammatory pathways, showing that their knockdown can significantly improve tissue repair and functional restoration in endodontic contexts. These integrative approaches represent a significant advancement over traditional therapies, offering the potential for more robust and predictable regeneration of dental tissues.

Furthermore, the success of regenerative procedures is often compromised by the hypoxic microenvironment of the necrotic canal, which triggers apoptosis in transplanted or migrating stem cells. Addressing this, Kim *et al.* [27] recently demonstrated that CRISPR-Cas9 can be used to engineer DPSCs

to constitutively express Brain-Derived Neurotrophic Factor (BDNF). In their study, these CRISPR-edited DPSCs not only survived in inflamed, hypoxic tissue but also significantly enhanced dentin regeneration in injured teeth compared to wild-type cells. This provides the first concrete evidence that gene editing can 'armor' stem cells against the hostile conditions of a failed root canal.

• Prevention of antibiotic resistance

Endodontic infections are frequently polymicrobial and often display resistance to conventional antibiotic treatments, posing challenges to effective management. Cutting-edge studies have demonstrated that CRISPR provides a novel and highly precise method for controlling these infections by targeting and editing the essential genes of pathogenic bacteria within the root canal system. This precision, unmatched by broad-spectrum antibiotics, enables the selective removal of infection-causing organisms while preserving the native microbiota, thereby reducing the likelihood of resistance development. In recent preclinical models, CRISPR-based antimicrobials have been shown to disrupt biofilm formation, eliminate resistant bacterial populations, and contribute to more effective and sustainable infection control. Such targeted interventions not only promise improved treatment outcomes but are also a vital step forward in the fight against antibiotic resistance, a critical emerging issue in contemporary dental medicine [28].

While CRISPR-Cas9 offers a theoretical advantage in reversing antibiotic resistance by targeting specific genes such as *tetM* and *ermB* in *Enterococcus faecalis* (*E. faecalis*), the transition from planktonic models to the complex root canal environment presents significant hurdles. Unlike *in vitro* monocultures, endodontic infections are polymicrobial and encased in a dense extracellular polymeric substance (EPS) matrix that severely restricts the diffusion of high-molecular-weight delivery vectors. Recent experimental evidence suggests that while bacteriophage-mediated delivery can effectively penetrate biofilms, its narrow host range limits its utility against the diverse multispecies consortia found in failed root canals. Furthermore, the efficiency of plasmid curing, removing resistance genes without killing the bacteria, remains variable in biofilm phenotypes compared to planktonic states. Therefore, the clinical efficacy of CRISPR-based antimicrobials depends not merely on gene editing efficiency, but on developing non-viral delivery systems, such as lipid nanoparticles, capable of penetrating the apical delta and lateral canals without being neutralized by the necrotic tissue debris [29-31].

• Advancement of personalized and efficient treatment options in endodontics.

Precision medicine applications in dentistry have been bolstered by evidence supporting the use of sequence-specific antimicrobials delivered *via* RNA-guided nucleases, as demonstrated by early-phase experiments [32]. Nanotechnology-enabled CRISPR/Cas9 delivery systems for genome editing in cancer models may soon be translated into clinical protocols for dental diseases, based on recent innovations [33].

The concept of 'personalized endodontics' *via* CRISPR-Cas9 relies on a precise diagnostic-therapeutic pipeline that currently exceeds standard clinical capabilities. A realistic pathway to implementation would require rapid, chairside Next-Generation Sequencing (NGS) to identify the specific resistance profile of a patient's infection (*e.g.*, identifying a specific *E. faecalis* strain), followed by the chairside synthesis or selection of a guide RNA (gRNA) tailored to that strain. Currently, this workflow is cost-prohibitive and time-intensive. While 'one-size-fits-all' CRISPR antimicrobials targeting conserved bacterial sequences are more feasible, true personalization faces the 'delivery bottleneck': standardizing a delivery vehicle (viral or nanoparticle) that is safe, stable in irrigants like sodium hypochlorite, and regulatory-approved for intra-canal use. Until these logistical gaps between genomic diagnostics and chairside delivery are bridged, CRISPR remains a potent research tool rather than an immediate clinical alternative to mechanical instrumentation [34].

• Control of periapical pathogens and management of apical periodontitis.

Experimental modulation of genes responsible for periapical pathogen survival and virulence factors using CRISPR-Cas9 has established proof of concept for targeted disease management in periodontal tissues [35].

Beyond bacterial control, the persistence of apical periodontitis is often driven by a dysregulated host immune response to residual antigens. Standard root canal therapy removes the bulk of the infection but does not actively modulate the periapical inflammation. CRISPR-Cas9 presents a novel avenue for 'Host Modulation Therapy' (HMT) in endodontics by targeting the specific inflammatory cascades triggered by endodontic pathogens. For instance, *E. faecalis* is known to activate Caspase-1, leading to the hyper-secretion of Interleukin-1 β (IL-1 β) in macrophages, a key driver of bone resorption. By utilizing CRISPR to selectively silence the gene expression of proinflammatory cytokines (IL-1 β) or downregulate NLRP3 inflammasome activation in the periapical tissues, clinicians could potentially arrest bone destruction and accelerate periapical healing in refractory cases [22].

• **Restoration of dental pulp vitality and tissue engineering.** Cutting-edge studies utilizing CRISPR/Cas9 editing for lincRNA-ASAO and other pulp-repair-associated genes have documented improved outcomes in pulp vitality and dentinogenesis [36-38]. These enhancements, amplified by advanced growth factor delivery systems, promise longer-lasting and more cost-effective therapies for dental tissue regeneration.

Ultimately, the application of CRISPR in endodontics should progress beyond theoretical genetic correction to the functional regeneration of the pulp-dentin complex. Current calcium silicate-based sealers promote mineralization through passive chemical signaling; however, CRISPR enables active genetic instruction. Recent studies have demonstrated that CRISPR-Cas9 can be used to knock out negative regulators of the Wnt/ β -Catenin pathway (such as *Fam83h*), which is integral to tooth mineralization and dentin formation. Furthermore, the precise correction of genetic mutations, such as *PAX9*, in dental pulp stem cells using base editors has shown potential for restoring normal odontogenic differentiation. This suggests a future clinical reality where 'smart' biological sealers or scaffolds deliver CRISPR-Cas9 ribonucleoproteins to resident stem cells, actively triggering the deposition of reparative dentin to bridge perforations or reinforce immature roots.

Finally, the synergy between bioactive materials and gene editing represents a new frontier. While calcium silicate sealers passively promote mineralization, epigenome editing (using catalytically dead Cas9 or 'dCas9') offers a way to actively 'prime' resident cells to respond to these materials. As noted in a recent scoping review by Dziedzic *et al.* [34], the next generation of personalized therapy will likely involve identifying patient-specific inflammatory markers (*e.g.*, via nanopore sequencing) and deploying CRISPR-based epigenetic modulators to suppress local inflammation, thereby maximizing the bioactive potential of hydraulic silicate sealers.

Ethical considerations and regulatory framework

Gene editing's ethical ramifications in endodontics

The application of CRISPR-Cas9 in endodontics raises several intricate and nuanced ethical issues [39]. The possibility of altering a person's genetic composition raises ethical concerns about equity, informed consent, and the potential for unforeseen consequences for subsequent generations.

Regulatory framework for CRISPR-Cas9 in dental applications

Safety, effectiveness, and supervision must all be carefully considered when regulating gene editing technologies in dental

applications. Regulatory agencies play a crucial role in ensuring that the application of CRISPR-Cas9 in endodontics adheres to established standards and ethical principles. Various nations have implemented specific protocols for modifying specific cell types in accordance with their respective legal frameworks [40].

Drawbacks and challenges of CRISPR-Cas9 gene editing technology

Delivery systems

The most formidable barrier to clinical translation is the efficient delivery of CRISPR components into the complex, mineralized anatomy of the root canal system. While *in vitro* models often utilize electroporation or microinjection, these are clinically infeasible for endodontic therapy. The specific challenge lies in penetrating the dentinal tubules, which range in diameter from 2 to 5 μ m and are often clogged with necrotic debris and bacterial biofilms. Viral vectors (*e.g.*, AAV), though efficient, pose safety risks of insertional mutagenesis and are size-limited for the large Cas9 gene (~4.2 kb). Conversely, non-viral vectors, such as lipid nanoparticles (LNPs) or gold nanoparticles, offer a safer profile but have historically struggled to penetrate the dense extracellular polymeric substance (EPS) of mature biofilms. Without a delivery vehicle capable of diffusing through the apical delta and lateral canals to reach sessile bacteria, CRISPR treatment may fail to sterilize the canal as effectively as standard sodium hypochlorite irrigation [41].

Immunogenicity and safety profile

Safety concerns in a dental setting differ significantly from those associated with systemic gene therapies. A critical, often overlooked oversight is the immunogenicity of the Cas9 protein itself. Since the most common Cas9 variants are derived from *Staphylococcus aureus* or *Streptococcus pyogenes*, pathogens frequently encountered by humans, up to 78% of the population possesses pre-existing anti-Cas9 antibodies and T-cells. In an inflamed pulp (pulpitis), introducing a bacterial protein could theoretically trigger a hypersensitivity reaction or localized 'immune storm', exacerbating necrosis rather than promoting regeneration. Furthermore, the risk of off-target effects where Cas9 creates unintended double-strand breaks in the host genome carries profound liability. In life-threatening conditions like cancer, this risk is acceptable; however, in dentistry, where the alternative (root canal treatment or extraction) is safe and effective, even a negligible risk of oncogenic mutation in dental pulp stem cells renders *in vivo* application currently ethically unjustifiable [42].

Economic and regulatory feasibility

Finally, the economic disparity between current endodontic

standards and gene therapy is immense. The average cost of a non-surgical root canal treatment ranges from \$500 to \$1,500, offering a high survival rate (up to 97% over 8 years). In contrast, current gene therapy protocols exceed \$1 million per patient due to the complexity of vector manufacturing and the requirements for personalized sequencing. Regulatory agencies (FDA/EMA) classify CRISPR products as 'advanced therapy medicinal products,' requiring rigorous, multi-phase clinical trials that take decades. Unless a 'universal', off-the-shelf CRISPR antimicrobial is developed that requires no patient-specific sequencing, the technology will likely remain a high-cost research tool rather than a viable chairside alternative for the general dental population.

The clinical translation of CRISPR-Cas9 in endodontics is currently at a crossroads between experimental proof-of-concept and chairside reality. While *in vitro* studies demonstrate high precision, a realistic timeline for clinical adoption is estimated at 10–15 years, contingent on the development of non-viral, stable delivery vectors that can navigate the apical delta. Economically, the current million-dollar cost of gene therapy presents a significant barrier compared to the high success rate (up to 97%) and relative affordability of conventional root canal therapy. For CRISPR to be viable, it must transition from personalized sequencing to "off-the-shelf" antimicrobial or regenerative formulations that bypass patient-specific manufacturing costs. Furthermore, current *in vivo* evidence, such as the work by Kim *et al.* underscores that success in animal models relies heavily on "armoring" stem cells to survive the ischemic, hypoxic conditions of the root canal a critical step toward functional pulp-dentin regeneration rather than mere fibrotic repair [17, 27, 34].

Conclusion

The incorporation of CRISPR-Cas9 appears to be bringing the genetic backdrop of endodontic therapy closer to the future. To provide individualized treatment, CRISPR-Cas9 also enables gene editing to correct the genetic mutation that causes endodontic diseases and other dental ailments. Furthermore, the technology can be used to deactivate or eliminate specific genes that cause inflammation, root canal infections, and other endodontic disorders. Gene editing to enhance dental pulp stem cell therapy may improve regenerative outcomes in endodontics. This suggests that gene editing may lead to the development of more personalized and effective endodontic treatment options. Addressing the risks and challenges associated with gene editing technologies is also crucial.

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Conflict of interest

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Authors' contributions

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