



Review Article

CRISPR-Based Genome Editing in Oral and Maxillofacial Medicine: Bridging *in Vitro* and Animal Models to Clinical Translation

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ABSTRACT

The emergence of clustered regularly interspaced short palindromic repeats (CRISPR)/Cas genome-editing technology represents fundamental changes with significant implications for oral and maxillofacial medicine. The present study aimed to synthesize current evidence from fundamental *in vitro* studies, engineered animal models, and emerging clinical trials to critically evaluate the potential applications and challenges of this biotechnology. The current review explored the transformative effects of CRISPR-Cas9 in key issues, including developing animal models for oral cancer and hereditary syndromes, *ex vivo* cell engineering for immunotherapies such as Chimeric antigen receptor (CAR) T-cell (CAR-T cells) for head and neck cancers, regenerative strategies using CRISPR-enhanced induced pluripotent stem cells (iPSCs) for salivary gland and enamel repair, and rapid diagnostic platforms for oral pathogens. Although preclinical data from murine models and organoid systems offered considerable potential for target validation and mechanistic understanding, their adoption in clinical settings is constrained by significant limitations. These limitations included the lack of tissue-specific delivery vectors, including standard lipid nanoparticles or viral vectors, unresolved off-target effects, long-term safety concerns, and complex ethical and regulatory challenges. The most immediate clinical impact was anticipated in two key areas, including CRISPR-based diagnostic tools such as the SHERLOCK platform, used for identifying SARS-CoV-2 variants or drug-resistant tuberculosis, and *ex vivo* cellular therapies being tested in controlled trials for specific diseases. The current findings indicated that integrating CRISPR into personalized oral healthcare required coordinated efforts to overcome translational barriers, conduct thorough clinical validation, and develop standardized safety and efficacy criterion specific to dental and maxillofacial outcomes.

1. Introduction

Recent advancements in genetic engineering have significantly transformed diagnostic and therapeutic methods for a wide range of disorders, including oral and maxillofacial disorders in particular¹. The circumstances

affecting the oral and maxillofacial area pose a significant threat to public health worldwide. More than 3.5 billion people worldwide are affected by oral disorders, including untreated dental caries, severe periodontitis, and total tooth

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loss, which negatively affect the functioning of the orofacial region and patients' well-being². Furthermore, oral disorders are associated with an increased risk of systemic disease, underscoring the need to develop tailored treatment options³. The introduction of clustered regularly interspaced short palindromic repeats (CRISPR) technology, a highly selective and cost-effective gene-editing platform, stands out as a revolutionary innovation with significant promise for advancing medical practice⁴⁻⁶.

CRISPR/Cas systems enable the correction of disease-associated genetic variations, the downregulation of harmful gene expression, and the refinement of diagnostic techniques⁷, all made possible by their fundamental ability to precisely modify genomes. Thus, CRISPR/Cas systems have sparked significant interest across a variety of medical subspecialties, including oral pathology, maxillofacial surgery, and oral medicine⁸. A wide range of diseases is now being investigated for their potential translational applications. These ailments include hereditary craniofacial abnormalities, oral cancers, autoimmune pathologies, and microbial infections. Several of these conditions have been treated in the past with limited long-term success⁹. CRISPR technology's main advantage is the Cas9 endonuclease, guided by RNA, can precisely induce targeted DNA double-strand breaks¹⁰. These targeted breaks are then repaired by cellular mechanisms, such as homology-directed repair or non-homologous end joining^{11,12}. This mechanism can result in precise gene correction or inactivation. However, some issues persist, including the possibility of off-target genomic modifications, accidental insertions or deletions, and inconsistent DNA repair fidelity¹¹. Currently, these technological challenges, compounded by ongoing ethical debates and regulatory concerns, are the primary impediments to the technology's broad clinical use. In oral and maxillofacial medicine, the development of novel, patient-specific treatment methods remains a primary priority.

Beyond its direct therapeutic potential, CRISPR is a powerful tool for creating sophisticated animal models of oral cancer¹³. It is feasible to develop precise genetically engineered mouse models (GEMMs) that completely reproduce human disease-causing mutations by employing targeted knock-in or knock-out of specific genes, such as *TP53*, *NOTCH1*, and *CDKN2A*¹⁴. These models may be generated using targeted knock-in or knock-out techniques. Compared to the conventional xenograft systems, these CRISPR-engineered models provided improved preclinical insights by more accurately reproducing the native tumor microenvironment, natural carcinogenesis, and treatment response¹⁵⁻¹⁸. The aim of the current study was to critically examine the therapeutic and diagnostic potential of CRISPR/Cas for diverse oro-facial disorders by integrating basic findings and animal models, while also addressing the key translational challenges to its clinical application.

2. Fundamentals of gene editing in medicine and biomedical studies

The CRISPR/Cas9 system has become the dominant technology in genome engineering due to its remarkable effectiveness,

versatility, and relative simplicity⁵. There are several important benefits of CRISPR/Cas9 compared to the older gene-editing systems, such as zinc finger nucleases (ZFNs) and transcription activator-like effector nucleases (TALENs)¹⁹. One of these advantages is precisely targeting genomic regions with a single guide RNA molecule^{19,20}. Additionally, the complexity of the process is reduced, which is another advantage²⁰. The CRISPR/Cas9 system, originally discovered in *Streptococcus pyogenes*, has been primarily used in translational and clinical applications²¹. The CRISPR-Cas9 system operates through a guide RNA (gRNA), which directs the Cas9 endonuclease to a genomic region complementary to the target sequence, thereby generating a site-specific double-strand break^{21,22}. This is accomplished by guiding the Cas9 system to the target area. The two main mechanisms responsible for repairing the lesions are error-prone non-homologous end joining (NHEJ), which often results in insertions or deletions, and high-fidelity homology-directed repair (HDR), which allows effective genetic correction when a donor DNA template is available²³⁻²⁵. CRISPR systems are categorized into six primary types (I–VI), characterized by unique structural and functional properties²⁶. Among these, the Type II Cas9 enzyme remains the predominant tool in biomedical studies, mainly because of its flexible operations and steady activity pattern (Figure 1).

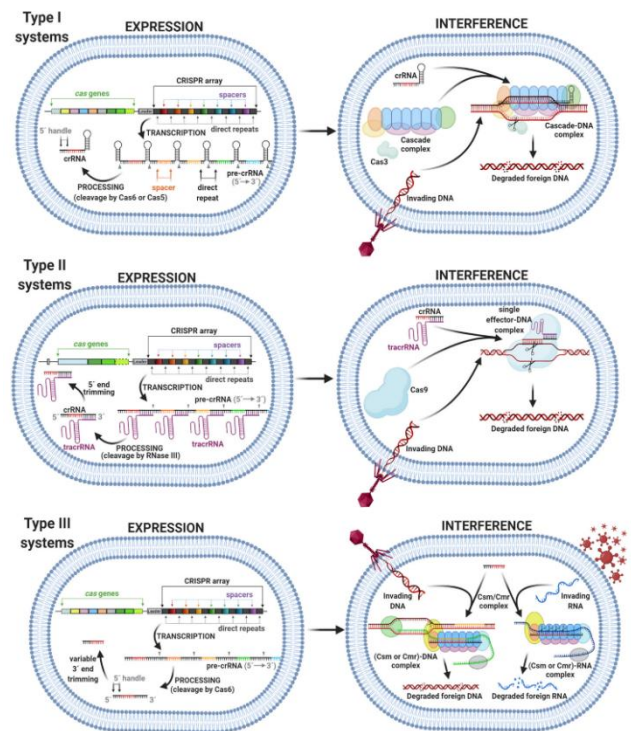


Figure 1. CRISPR expression and interference steps in type I, type II, and type III systems (Source: MDPI Copyright, 2021; Nidhi et al.²⁷)

Concurrently, alternative Cas effector proteins, including Cas12 (Type V) and Cas13 (Type VI), are gaining significant attention for their specialized applications in DNA and RNA modification, respectively²⁸⁻³⁰. Figure 2 compares the key features, such as PAM/PFS requirements and DSB mechanisms, of three CRISPR systems (Cas9, Cas12, and Cas13).

	Cas9	Cas12	Cas13
Target	DNA	DNA	RNA
Protospacer restrictions	PAM	PAM	PFS
Cut	Blunt-ended DSB	Sticky-ended DSB	Degraded RNA
Spacer size	16-20 nt	16-25 nt	25-35 nt
Characteristics	No collateral cleavage No secondary structure restrictions	Collateral cleavage No secondary structure restrictions	Collateral cleavage Secondary structure restrictions
Use	Gene editing Nucleic acid detection	Gene editing Nucleic acid detection	RNA knockdown Nucleic acid detection

Figure 2. Comparison of the properties of CRISPR/Cas9, /Cas12, and/Cas13 systems. PAM: Protospacer adjacent motif, PFS: Protospacer flanking site, DSB: Double-strand break (Source: MDPI Copyright, 2022; Nidhi et al.²⁷)

Due to the intricate mechanistic details of enzyme specificity, such as the functions of the *HNH* and *RuvC*

Table 1. Essential elements of therapeutic CRISPR-Cas9 editing

Component/mechanism	Core function	Relevance in clinical and translational applications
Single-guide RNA (sgRNA)	Provides sequence specificity by guiding the Cas9 endonuclease to its complementary genomic target locus	Governs the accuracy and specificity of the intervention, directly influencing target engagement and the risk of off-target effects
Cas9 Endonuclease	Executes a double-strand break in the DNA duplex at the site specified by the sgRNA	Serves as the molecular effector for genetic modifications, facilitating either disruptive (knock-out) or corrective (knock-in) editing
Protospacer adjacent motif (PAM)	A short, conserved nucleotide sequence adjacent to the target site that is essential for Cas9 recognition and cleavage	Defines the editable genomic space and is a critical parameter for gRNA design to minimize off-target activity
Non-homologous end joining (NHEJ)	A dominant, error-prone cellular repair pathway for double-strand breaks, often resulting in small insertions or deletions (indels)	Exploited to disrupt gene function by frameshift mutations, applicable for silencing disease-causing alleles.
Homology-directed repair (HDR)	A high-fidelity repair pathway that uses an exogenous DNA template to edit the sequence at the break site precisely	Enables precise gene correction or insertion, forming the basis for therapies to rectify specific pathogenic mutations

3. Clinical trials for CRISPR-based gene editing biological therapies

In clinical medicine, gene editing using CRISPR/Cas9 has the potential to be a substantial therapeutic tool for treating a wide range of diseases caused by genetic changes. Monogenic genetic illnesses, cancer immunotherapy, different inherited immunodeficiencies, and certain viral infections are also included in this category^{33,34}. There is a growing number of clinical studies being conducted, and it is anticipated that the outcomes of these trials will guide the practical integration of therapeutics. A wide variety of diseases are being examined, including both systemic and hematological cancers. Cancers are often treated with cutting-edge methods, such as chimeric antigen receptor (CAR) T-cell therapy³⁵. Therapeutic gene editing using CRISPR/Cas systems has been broadly explored for a diverse array of human diseases, encompassing multiple malignancies such as renal cell carcinoma, nasopharyngeal carcinoma with *PDL-1* knockout, advanced-stage solid

nuclease domains³¹, a simplified schematic overview of the CRISPR/Cas9 mechanism and its key domain activities is provided in Figure 3, with complementary details summarized in Table 1.

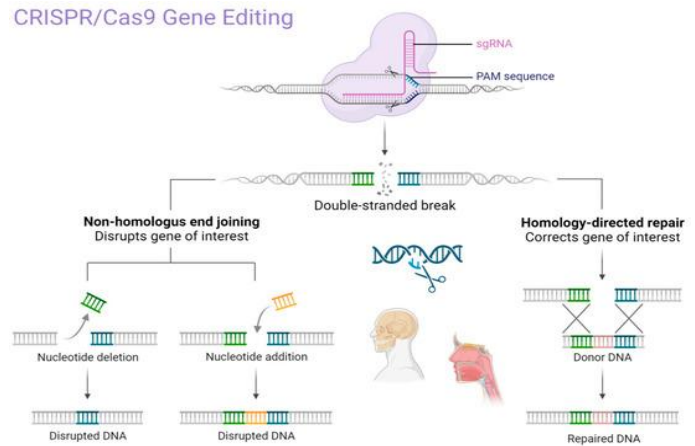


Figure 3. Schematic overview of the CRISPR/Cas9 mechanism gene editing technology (Source: MDPI Copyright, 2025; Dziedzic et al.³²)

tumors, and esophageal cancers^{35,36}. Hematologic disorders such as sickle cell disease and transfusion-dependent thalassemia³⁷, metabolic disorders³⁸, genetic and neurological conditions, including neurofibromatosis type 1^{37,39}, Rett syndrome with associated craniofacial manifestations⁴⁰, Duchenne muscular dystrophy, and retinal diseases have been treated with the CRISPR/Cas system³⁷. CRISPR/Cas-based therapeutic applications have been extended to amyloidosis and numerous metabolic, endocrine, and autoimmune disorders³⁷, oral diseases⁴¹, infectious diseases such as HIV-1 (via modified CD34+ cells) and HPV-related cervical intraepithelial neoplasia^{42,43}, and also to diagnostic and therapeutic strategies for emerging viral diseases⁴⁴. Table 2 provides a comprehensive compilation of present and projected clinical uses of CRISPR, with a particular focus on oral and maxillofacial medicine. Table 3 provided an overview of promising preclinical achievements in oral medicine. The results demonstrated effectiveness across a variety of domains, including the prevention of tumor development in mouse models of oral

cancer, the regeneration of enamel and bone tissue, and the targeted manipulation of the periodontal microbiota^{38,40,45}.

Table 2. Current and emerging CRISPR-Cas applications in oral and maxillofacial medicine

Condition/ clinical domain	CRISPR-based therapeutic or diagnostic strategy	Current development stage	References
Oral squamous cell carcinoma (OSCC)	Disruption of oncogenes (<i>TP53</i> , <i>PD-1</i>) and development of engineered CAR-T cell immunotherapies	Advanced preclinical models and early-phase clinical trial initiatives	(35)
Heritable craniofacial disorders such as amelogenesis, dentinogenesis imperfecta	Precise gene correction strategies using CRISPR-Cas9 in patient-derived iPSCs and animal models	Proof-of-concept established in preclinical models	(36,37)
Chronic periodontitis	Targeting host inflammatory pathways and disrupting pathogenic biofilm formation	Exploratory stage, primarily in <i>in vitro</i> and animal model systems	(38,39)
Salivary Gland dysfunction and regeneration	Generation of patient-specific organoids and guided differentiation of iPSCs for tissue repair	Active preclinical investigation in disease modeling and regenerative approaches	(40,41)
Persistent oral viral infections such as HPV, HIV	Direct cleavage of integrated viral genomes and engineering of virus-resistant cells	Ongoing preclinical development with translational trials in planning phases	(42,43)
Rapid pathogen detection, such as SARS-CoV-2, EBV in oral samples)	Deployment of CRISPR-based diagnostics such as SHERLOCK, DETECTR for point-of-care testing	Technology prototypes validated; clinical evaluation for regulatory approval in progress	(44)

CAR-T cells: Chimeric antigen receptor T-cell, EBV: Epstein-Barr virus, iPSCs: induced pluripotent stem cells

Table 3. Preclinical CRISPR models and their therapeutic outcomes

Experimental model / system	CRISPR target	Primary biological outcome	References
Murine oral cancer xenografts and GEMMs	Tumor-related genes such as <i>TP53</i> , <i>CD44</i> , <i>HuR</i> , <i>PD-1</i>	Significant tumor growth inhibition and increased sensitivity to conventional chemotherapy	(45)
Induced pluripotent stem cell-derived ameloblast lineages	Enamel matrix genes such as <i>AMELX</i> , <i>ENAM</i> , <i>FAM83H</i>	Successful differentiation into ameloblast-like cells and partial biomimetic enamel matrix deposition	(36)
Salivary gland organoid co-culture systems	Pluripotency and differentiation regulators such as <i>SOX2</i> , <i>NANOG</i> , <i>AQP5</i>	Enhanced epithelial cell proliferation and functional maturation in engineered salivary gland tissue	(40)
Periodontal pathogen model (<i>Porphyromonas gingivalis</i>)	Bacterial virulence factor, such as <i>cas3</i>	Attenuation of pathogenicity, leading to reduced biofilm formation and host inflammatory response	(38)
Craniofacial bone defect models (mouse)	Osteogenic signaling pathways such as <i>BMP</i> , <i>RUNX2</i>	Accelerated bone mineralization and improved quality of new bone formation in critical-sized defects	(45)

GEMMs: Genetically engineered mouse models

4. Prospects for gene-editing biotechnologies in personalized oral medicine

In the field of oral medicine, the data available for the direct clinical use of CRISPR-Cas systems remains limited. The majority of available information consists of preliminary studies on diagnostics, disease screening, and therapeutic control^{8,46,47}. When it comes to the management of viral infections, the treatment of head and neck malignancies, the control of periodontitis, and the facilitation of tissue regeneration, early efforts primarily focus on these areas. Although there is a vast theoretical space for the use of CRISPR-based gene-editing biological treatments (GEBTs) in this sector, the available evidence is inadequate to clearly forecast their precise future purposes or the timescale for their assimilation into routine clinical practice⁴⁸. CRISPR technology, on the other hand, has the inherent capability for precise genomic intervention, suggesting that it could emerge as a viable therapeutic alternative for certain hereditary or autoimmune oral conditions⁴⁸.

In the field of oral and maxillofacial medicine, the potential applications of gene-editing biotechnologies encompass several promising pathways. For instance, the development of personalized screening protocols and gene-targeted therapies for head and neck cancers and

immune-mediated pathologies, in particular hematologic malignancies such as lymphoma, leukemia, and multiple myeloma, as well as systemic conditions such as AIDS that manifest oral symptoms^{49,50}. Additionally, CRISPR provided a platform for developing patient-specific therapeutics for cancers originating in the craniofacial complex, including new anticancer vaccines⁵¹.

Another major possibility is the development of individualized treatments for genetic and developmental diseases that affect the structure of the craniofacial region⁴⁶. In regenerative surgery, gene-edited xenotransplants have the potential to enhance biocompatibility and functional outcomes in complex reconstructions⁵². Furthermore, CRISPR/Cas9 has the potential to assist in converting somatic cells into induced pluripotent stem cells (iPSCs), which may then be used to create craniofacial tissue⁵. The use of CRISPR-based quick diagnostic platforms has the potential to improve the detection of oral infections. This technique can enable the accurate and timely identification of viral and bacterial agents responsible for infection⁵³.

5. Primary head and neck cancers and oral manifestations of systemic malignancies

The development of therapeutic vaccinations is an

example of a forward-looking use of CRISPR technology in oral oncology. CRISPR technology is particularly advantageous for treating cancers associated with human papillomavirus in the head and neck. The use of CRISPR technology is still in the early stages of investigation and lack substantial clinical validation, despite a solid conceptual base⁵⁴. When used in a targeted manner, these vaccines have the potential to provide a refined therapeutic approach for certain oral neoplasms. Although current CRISPR-based GEBTs are largely used to treat hematopoietic diseases such as leukemia and multiple myeloma, their widespread implementation may help alleviate oral difficulties and reduce the unpleasant effects associated with traditional chemotherapy⁵⁴.

Focusing directly on the oncogenic drivers within head and neck tumors is currently being explored. CRISPR/Cas9 is a technology that can remove cancer stem cells defined by mutations in the *TP53* gene⁵⁵. This technique gives a paradigm-shifting opportunity for gene therapy. As an additional application, the platform is being exploited to adjust the production of oncogenic microRNA and to overcome chemoresistance by altering genes such as *CD44*⁵⁶. Furthermore, it has been established that reducing urokinase plasminogen activator receptor (uPAR) levels via CRISPR can hinder tumor progression in model systems³⁵. Most preclinical studies have supported these methods, and several clinical studies are now underway to evaluate their therapeutic potential³⁵.

Additionally, CRISPR/Cas9 provided a synergistic technique that may enhance the effectiveness of traditional cancer therapies. The development of drug resistance is a significant challenge in oncology⁵⁶. This resistance is mediated by genes involved in drug efflux, DNA damage repair, apoptotic evasion, and pro-survival signaling. Targeting these resistance pathways with CRISPR has shown potential to re-sensitize tumors to pharmacological treatments⁵⁷. Thus, CRISPR/Cas9 cannot be overstated when it comes to head and neck squamous cell carcinoma (HNSCC), a rapidly progressing cancer that often results in adverse outcomes¹³. For instance, the RNA-binding protein *HuR*, encoded by *ELAVL1* and implicated in tumor growth and treatment resistance, has been successfully inactivated using CRISPR/Cas9. By coupling *HuR* deletion with the chemotherapeutic epirubicin, numerous cell death mechanisms, including apoptosis, necroptosis, and autophagy, were synergistically activated in SAS cell line models, resulting in increased cytotoxicity⁴⁵. These results were supported by earlier studies demonstrating that the combination of nanoparticle-delivered, *HuR*-targeted CRISPR components with epirubicin improved therapeutic efficacy while preserving a high safety profile in mouse models⁵⁸⁻⁶¹. Figure 4 illustrated the use of CRISPR-based genetic screening methods to systematically identify genes crucial for cell viability and those mediating resistance to therapeutic agents.

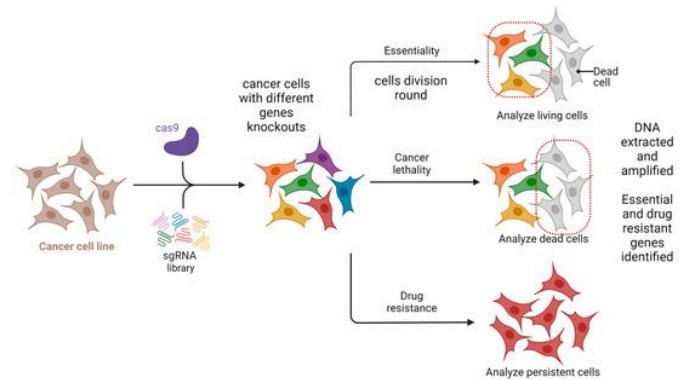


Figure 4. CRISPR-based screening for essentiality and drug resistance (Source: MDPI Copyright, 2025; Dziejcz et al.³²)

6. CRISPR-engineered xenotransplantation for oral and maxillofacial reconstruction

New advancements in gene-editing technologies, notably in translational models utilizing pigs and humans, have brought to light the promise of CRISPR-Cas systems in xenotransplantation⁶²⁻⁶⁵. According to the findings of a preliminary study, certain genetic alterations can reduce the immunological barriers between species, thereby improving the biocompatibility of animal-derived tissues for human implantation⁶⁶⁻⁶⁸. The main concept of the preliminary studies was to use CRISPR to reduce essential xenogeneic immune responses, thereby facilitating transplant acceptance despite considerable genetic differences. The reconstruction of craniofacial skeletal defects following trauma or tumor ablation, complex composite tissue flap transfers, salivary gland replacement, and the use of bioengineered xenogeneic bone matrices for alveolar or facial ridge augmentation are all examples of potential applications in maxillofacial surgery⁶⁷. The development of technologies for elective and quality-of-life treatments in maxillofacial surgery is expected to progress more slowly. Thus, the majority of recent studies in xenotransplantation have focused on addressing significant organ shortages in life-threatening situations.

7. CRISPR/Cas9 applications in organoid models and tissue engineering for oral diseases

Excision by surgical means, prosthetic implantation, or autologous grafts are the primary methods used in most modern therapeutic approaches for maxillofacial and oral diseases⁶⁹. Often, these methods are insufficient to fully restore the body's natural beauty and physiological function. Organoid technology has thus emerged as a critical area in regenerative medicine, with particular emphasis on pluripotent stem cells and customized biomaterial scaffolds, such as collagen-based biopolymers and poly(lactic-co-glycolic acid) hydrogels⁷⁰. The formation of three-dimensional organoid structures is significantly influenced by external morphogenic stimuli that direct stem cell

differentiation toward specific lineages^{15,16}. The CRISPR/Cas9 systems can precisely initiate this process by activating or suppressing critical genetic variations. This technique enables the orchestration of cellular differentiation and the formation of iPSCs⁷¹⁻⁷⁴. Ono et al.⁴⁰ explored the potential of salivary gland cells to regenerate using iPSCs. When embryonic submandibular gland cells and mouse-iPSCs were co-cultured, a synergistic effect was observed, significantly enhancing mutual differentiation^{75,76}. Morphological changes toward more complex structures resembling bone marrow were observed and served as proof, and they were compared with monoculture controls⁷⁶. Furthermore, molecular studies indicated that more complex, bone marrow-like structures were associated with reduced expression of pluripotency genes. (*Sox2*, *c-Myc*, *Nanog*) and the overexpression of differentiation markers (*Klf4*, *Aqp5*), thereby increasing the capacity of salivary gland cells to regenerate⁷⁵. Induced pluripotent stem cells generated using CRISPR/Cas9 have the potential to significantly expand therapeutic options for salivary gland diseases, according to these results⁷².

Moreover, a previous study by Arakaki et al.⁷⁷ indicated that iPSC co-culture for enamel regeneration was beneficial. Co-culturing mouse-iPSC with dental epithelial cells promoted differentiation into ameloblast-like cells, which are necessary for enamel matrix production⁷⁷. It is important to note that the generated cells, although exhibiting a stromal-like shape, lacked the distinctive molecular markers of mature ameloblasts (such as amelogenin) and epithelial cells (such as *p63* and *cytokeratin 14*)⁷⁷. Despite regenerated tissues having structural similarity to their native counterparts, molecular differences may persist. There is a lack of direct evidence on the use of CRISPR/Cas9 for the regeneration of maxillofacial tissue. Reprogramming somatic cells into patient-specific iPSCs and correcting harmful mutations in hereditary oral disorders are only two examples of how this technique provided significant potential to advance regenerative medicine.

8. CRISPR/Cas as a transformative tool for animal and laboratory modeling of oral and maxillofacial diseases

The development of CRISPR/Cas genome-editing technology has profoundly transformed the modeling of oral and maxillofacial diseases, allowing for a level of genetic precision previously unattainable⁸. With CRISPR, it is now possible to construct intricate genetically modified animal models and sophisticated *in vitro* systems that reliably reproduce human disease genotypes and phenotypes^{78,79}. The CRISPR/Cas technique is particularly significant for a sector that encompasses a wide range of challenging conditions, including oral squamous cell carcinoma (OSCC), hereditary craniofacial syndromes, and autoimmune oral maladies⁸⁰.

The production of realistic genetically engineered mouse models, which are often referred to as GEMMs, is

one of the most essential applications. Using CRISPR, knock-in and knock-out models can be efficiently generated that incorporate specific human-relevant mutations⁸¹. For instance, to explore the initiation, progression, and metastasis of ovarian squamous cell carcinoma within an immunocompetent native environment, it is feasible to construct mouse models with combinatorial mutations in key driver genes such as *TP53*, *NOTCH1*, and *CDKN2A*⁸². This approach provided the opportunity to investigate the progression of OSCC beyond the initial stage. By introducing orthologous mutations into the mouse genome, CRISPR plays a crucial role in modeling monogenic craniofacial defects, such as cleidocranial dysplasia⁸³. Also, CRISPR-engineered models represented a major improvement over established xenograft. Unlike xenografts, which may form in immunocompromised hosts, GEMMs can drive carcinogenesis within an intact immune system and within genuine tissue stroma⁸⁴. This contrasts with xenografts, which can develop in immunocompromised hosts. However, it is now feasible to conduct physiologically relevant investigations, such as studies on tumor-microenvironment interactions, angiogenesis, immune evasion, and spontaneous metastatic dissemination. As a result, therapeutic testing, particularly for immunotherapies, yields more predictive information and supports translational studies.

Furthermore, CRISPR/Cas technology serves as the basis for the next generation of sophisticated laboratory models that go beyond whole organisms. To conduct conclusive causal investigations of particular mutations on cellular behavior, drug resistance, and invasive potential, isogenic cell line pairs can be created that vary only in a single CRISPR-modified gene^{85,86}. In addition, CRISPR is crucial for generating iPSCs from patients. Genetically modified iPSCs have the potential to differentiate into sophisticated, three-dimensional organoids of oral tissues, such as dental epithelium or salivary glands³². Such disease-in-a-dish models are useful platforms for individualized drug screening and for dissecting the molecular cascades underlying genetic diseases in a human cellular setting³². CRISPR-based functional genomic screenings using comprehensive gRNA libraries are now being implemented in oral cancer cell lines as a complementary approach to these established techniques⁵. In the process of methodically identifying genes important for cell survival, tumor development, or treatment response, new therapeutic targets and resistance mechanisms were also identified. Consequently, CRISPR/Cas technology is the foundation for contemporary experimental pathology in oral medicine. The acceleration of deconvolving disease processes and the development of targeted therapeutics is achieved by providing models that are resilient, precise, and physiologically appropriate. These models range from modified animals to human organoids grown from iPSCs. Additionally, the combination of CRISPR-based models with single-cell omics and advanced imaging is paving the way for a new era of personalized, predictive investigation in oral and maxillofacial health.

9. Conclusion

CRISPR-based treatments are not yet ready to be integrated into standard oral and maxillofacial care because the evidence base remains fragmented and inconsistent. The primary clinical uses of CRISPR are expected to be in CRISPR-based diagnostics and *ex vivo* cellular therapies, both implemented through strict, protocol-based frameworks. Conversely, *in vivo* therapeutic genome editing should remain under rigorous examination through clinical investigations. CRISPR/Cas technique requires standardizing safety evaluations for off-target effects, defining clinically relevant functional goals for oral-facial outcomes, and developing cost-efficient manufacturing processes to enable equitable access. Key limitations included a lack of targeted delivery mechanisms for oral tissues, a scarcity of evidence on long-term safety and efficacy, and the absence of preclinical models that accurately capture the multidimensional nature of clinical diseases. The rapid deployment of healthcare solutions is further hampered by persistent ethical dilemmas, significant financial constraints, and continually evolving regulatory frameworks. Although CRISPR technology has potential applications in diagnostics, regenerative medicine, and oral cavity cancer, these areas remain largely exploratory and experimental for now. Three critical prerequisites must be met before considering integration into routine care, including generating robust clinical data, addressing the technique's core limitations, and conducting a realistic assessment of its practical viability.

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Declarations

Availability of data and materials

All datasets are available upon request from the corresponding author.

Authors' contributions

All authors contributed to the study's conceptualization and methodology. Fatemeh Keikha conceived and designed the study, and Marziyeh Saki drafted the manuscript. Farshad Shahkhah, Mehran Bahmani, Diana Abbasi, Faezeh Sadat Moazzeni, and Amin Shahinzadeh performed data collection and revised the manuscript. All authors read and approved the final edition of the manuscript before publication in the present journal.

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The authors declared that they have no competing interests.

Ethical considerations

All the authors confirmed that ethical concerns, such as plagiarism, permission to publish, research misconduct, data fabrication or falsification, duplicate submissions, and redundant publication, have been thoroughly reviewed. No AI-assisted technologies were used in the generation of this manuscript.

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