

## CRISPR/CAS- DECODING LIFE- A MINI REVIEW

### Abstract

Since its first use in 1990, gene therapy has been a part of the ever-expanding arms of treatment modalities for various conditions. Though initial setbacks were recorded, making the results sub-optimal, advances in science have rekindled efforts in gene therapy with the use of re-engineered viruses, non-viral vectors, multiple checkpoints for immunogenic reactions and mutagenesis. Recently, the field is witnessing a paradigm shift wherein instead of introducing the therapeutic gene into a locus, a more risk-free solution is to precisely fix the existing genetic abnormalities in situ. This was made possible with the introduction of the CRISPR/ Cas system and previous systems such as ZFNs, and TALENs. This article reviews the use of CRISPR/Cas in dentistry and also sheds light on the other systems such as ZFNs and TALENs

**Keywords:** CRISPR/Cas systems, Periodontitis, Genome editing, Zinc finger nucleases.

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## I. INTRODUCTION

Gene therapy has provided wide expanses of therapeutic options for many patients with genetic diseases who previously had few treatment options since its first use 30 years before. It was in 1990 that gene therapy was used in the management of ADA-SCID. The results from this trial were suboptimal as one patient improved moderately while the other did not [1]. Yet it paved way for multiple gene therapy trials to follow. Gene therapy also saw a major setback when an 18-year-old succumbed to the trial after 4 days owing to multiple organ failure that was caused due to a stronger immune response by the adenovirus vector. It was also followed by tragic failures when several young children developed leukemia due to insertional oncogenesis from a gene therapy trial [2]. Advances in science have rekindled efforts in gene therapy with the use of re-engineered viruses, non-viral vectors, and multiple checkpoints for immunogenic reactions and mutagenesis. Recently, the field is witnessing a paradigm shift wherein instead of introducing the therapeutic gene into a locus, a more risk-free solution is to precisely fix the existing genetic abnormalities in situ. This was made possible with the introduction of the CRISPR/ Cas system and previous systems such as ZFNs, and TALENs.

Nucleases can be engineered to target a particular locus in a controlled manner and these utilize the cellular repair mechanism. There is a generation of nuclease induced double-stranded breaks (DSBs) that stimulates a repair mechanism by one of the two pathways: homology-directed repair (HDR) and non-homologous end joining (NHEJ). The DSBs are most often left with two incompatible DNA ends [3]. In NHEJ, the exonuclease or endonuclease activity exposes or generates small regions of microhomology following the end resection of 5' or 3' overhangs. After the modification of the DNA ends, the process of ligating them together takes place with little or no homology, generating deletions or insertions. This is in contrast to the HDR, where an undamaged DNA template is used on the sister chromatid or homologous chromosome to repair the break, which in turn leads to the reconstitution of the original sequence. In the initial stages of genome editing, the spotlight was on ZFN and Meganucleases to induce the DSBs. This was then followed by the introduction of TALEN. Much recently, the utilization of CRISPR-Cas9 has potentiated a transformative influence, which works by harnessing the adaptive immune system of prokaryotes.

- 1. Zinc Finger Nucleases:** Zinc finger nucleases (ZFNs) are a class of engineered DNA-binding proteins that have shown promise for inducing double-strand breaks in DNA at user-specified locations for targeted editing of the genome. Each ZFN is typically composed of three or four zinc-finger domains, as each module consists of about 18-36 amino acids [4]. ZFN function as dimers and via the zinc-finger DNA binding domain 9-18 bps are recognised by each monomer [5]. The formed FokI dimer precisely cleaves the target DNA after two separate ZFNs recognise and bind to particular locations on opposing DNA strands.
- 2. Talens:** An assembled FokI dimer precisely cleaves the target DNA after two separate TALENs recognise and bind to particular locations at opposing DNA strands. An off-target mutation is a major hurdle in the use of ZFN. To enhance the specificity, the creation of obligate heterodimeric ZFN architectures that works to prevent unwanted homodimerization due to the charge-charge repulsion. Another approach developed was to deliver them as proteins into cells as these proteins are cell permeable and can

minimise unwanted mutations. A nonspecific DNA-cleaving nuclease is coupled to an easily constructed DNA-binding domain in transcription activator-like effector nucleases (TALENs), which is frequently employed for effective gene editing. This allows TALENs to target virtually any sequence. It consists of repeat domains each approximately 34 residues in length [5] and through the Repeat variable diresidues (RVDs) i.e. amino acids residues present in 12 and 13 positions [6], they contact the DNA. The similarities between ZFN and TALENs include the modularity in form and function and FokI cleavage mediates its dimerisation. TALENs have advantages such as the reduced time needed to assemble the nuclease, and increased specificity yet their large size makes their entry into cells a concern [6]. Recent advances such as its delivery as protein and mRNA have helped to overcome these challenges.

- 3. Crispr-Cas:** The transforming phase in gene engineering happened during the 2000s when researchers suggested a new tool using the clustered regularly interspaced short palindromic repeats (CRISPR)-associated protein 9 (Cas9) system. CRISPR was a primitive concept that had been derived from the adaptive immune system of prokaryotes. These elements were seen in more than 40% of sequenced bacteria and 90% of archaea via computational analysis. The CRISPR defence mechanism protects bacteria from repeated viral attacks via three basic stages: adaptation (spacer acquisition), crRNA synthesis (expression), and target interference [7]. The spotlight surrounding this technique than the previously established techniques such as ZFN and TALENs was because the recognition of the site of interest is by an interaction of the RNA/DNA whereas for ZFN and TALENs it is through a protein and DNA. There are 6 types and 33 subtypes [8] of the CRISPR-Cas systems which have been grouped under two classes. The Class 1 system consists of the multiprotein crRNA-effector complexes consisting of types I, III, IV and the single protein-crRNA effector complexes comprising of types II, V, and VI are classified under the class 2 system [9]. In the adaptive immune system, the guide RNA targets the intruding viral DNA. In the case of gene editing, both the parts of guide RNA i.e. CRISPR RNA (crRNA) and transactivating CRISPR RNA (tracrRNA) are combined to form a single guide RNA (sg RNA), which directs the cas-9 to create DSBs upstream the PAM sequence by a localised DNA melting process leading to the formation of RNA-DNA hybrid. The NUC lobe of cas-9 consists of RuvC and HNH domain, which cleaves the complementary strand and the non-complimentary strand respectively. This can be then repaired by either of the two pathways such as Non-homologous end joining (NHEJ) and homology-directed repair (HDR) [10]. CRISPR-Cas can be a promising treatment modality for many diseases not limited to neurological, cardiovascular, cancer and dentistry. It can be applied right from diagnosis, drug discovery and therapeutics.

## II. CRISPR/CAS IN DENTISTRY

Oral squamous cell carcinoma (OSCC) is the most common type of oral cancer has long had a poor prognosis and only few excellent therapeutic choices. Six out of 14 (or 43%) of the OSCC cell lines responded to the introduction of Genome-scale CRISPR-Cas9 Knock-Out (GeCKO) libraries, according to Ludwig M et al 2017 research [11]. Using CRISPR/Cas9 technology, Huang et al 2017 examined the role of the p75NTR in human tongue squamous carcinoma cells. This research demonstrates the deletion of p75NTR inhibits a number of tumour-promoting characteristics in SCC-9 cells, suggesting that p75NTR is a promising target for the creation of revolutionary tongue cancer treatment

modalities [12]. *Streptococcus mutans* main virulence component, glucosyltransferases (Gtfs), uses sucrose to make extracellular polysaccharides (EPS), which results in the development of dental plaque biofilm. The first phase in the process of dental plaque formation is biofilm formation. Self-targeting CRISPR arrays with spacer sequences identifying with *gtfB* were created and cloned onto plasmids in a work by Gong T et al. To obtain the necessary mutations, this plasmid was converted into UA159 (self-targeting), leading to a decrease in the production of EPS and subsequently, the breakdown of biofilm formation. In order to manage xerostomia, Wang Z et al. enhanced the expression of the AQP1 gene using the CRISPR/Cas9 system by developing a gRNA sequence and a homology-directed repair (HDR) template for the endogenous promoter of the cytomegalovirus (CMV). He suggested that replacing the endogenous promoter may be used to treat salivary gland dysfunction[13]. It may be conceivable to create a viable mesenchymal stem cell-derived therapy for primary Sjogren's disease given that the CRISPR/Cas9 system has recently been utilised to target critical genes in various cell lines and species [14].

Gene therapy is now mostly utilised to relieve pain in animal models. Research has showed expressing the human preproenkephalin gene in a mouse model using a herpes simplex vector decreased trigeminal pain [15]. With enhanced vector genes systems in the future, gene therapy may provide the pain therapist with some ray of hope in the treatment of pain syndromes such as trigeminal neuralgia and temporomandibular joint problems [16, 17]. It was found that the affected person is incapable of feeling pain owing to some aberrations in this gene. The underlying genetic defect hinders the neural pathway's ability to transmit pain signals by controlling specific chemicals necessary for this process. Editing epigenetic markers that triggered this pathway is possible with CRISPR technology.

### **III. CRISPR/Cas IN PERIODONTOLOGY**

CRISPR-Cas9 recognises pertinent cellular pathways, thereby leading to insightful comprehension of the pathophysiology of periodontitis. With the use of the CRISPR/cas9 knockout plasmid, Zhang and colleagues investigated the effect of PTPN2 on human OKF6-TERT2 oral keratinocytes. They found that PTPN2 decreased periodontal inflammation in type 2 diabetes mellitus via dephosphorylate protein substrates in the JAK1/STAT3 signalling pathway after 25VD3 treatment in human oral keratinocytes and a mouse model of the disease [18]. Another study done by Xie et al., in 2019, looked at inflammatory response, used CRISPR to make two THP-1 lineage cell lines using lentiviral vectors. This enabled them to examine the deletion of mRNA-binding protein 1 for insulin-like growth factor 2 (IGF2BP1). TNF $\alpha$ , interleukin 1 beta, and interleukin 6 are just a few of the pro-inflammatory cytokines, that get reduced by IGF2BP1, IGF2BP1 over expression facilitated the induction of pro-inflammatory cytokines by LPS. Because LPS causes the nuclear translocation of p65-p53 and activates NF $\kappa$ B. By blocking the functions that LPS causes, over expression of IGF2BP1 promotes the inflammatory response, while decreased expression of IGF2BP1 can reduce the inflammatory reactions that cause alveolar bone loss. Additionally, a much-needed direction is shown by Burmistrz et al who demonstrated that 95% of clinical strains of *P. gingivalis*, a keystone pathogen implicated in chronic periodontitis have been found to harbour CRISPR arrays[19].

Six CRISPR/Cas types were found In *P.gingivalis* strains, while 12 other kinds were found in 46 different Porphyromonas species, according to Watanabe et al. Additionally, they proposed that *P. gingivalis* might use the CRISPR function to preferentially acquire

advantageous DNA sequences for its own survival and evolution[20]. This system might be involved in more activities than only acting as an "immune" system to protect against invaders, according to Chen and Osler. Understanding this mechanism better may even help to clarify why *P.gingivalis* is referred to as the "keystone pathogen" in periodontitis[21]. Zhou et al in their study on periodontitis and periodontally healthy patients concluded that more short direct repeats DRs were found in the Periodontal Disease group whereas Periodontally Healthy group possessed 33 more spacers than the former group. Additionally, in 386 of 444 phage species, the Periodontally Healthy group exhibited more spacer-contained readings. This finding implies that healthy individuals' microbiomes were better able to fend off phage invasion than patients with periodontitis. The evenness and diversity of oral bacterial populations were related to the defence potential of CRISPRs. Healthy individuals were more comparable to one another, had more spacers and fewer DRs than unhealthy individuals, which will help build a strong bacterial community that can fend off phage invasion [22]. These are a few researches that have already been carried out and more pathbreaking researches using this pioneering tool may open new arenas in dental treatment. Periodontitis may become easier to treat or prevent by comprehending the mechanisms driving the inflammatory reactions or understanding the microbes and modulating the microenvironment. This will also enable dental healthcare practitioners to effectively identify and understand the risk factors that put their patients at risk.

#### IV. CONCLUSION

The 21st century has seen astonishing developments in the field of science and technology. The use of CRISPR Cas has generated many hopes but it is with combined efforts from physicians, researchers, scientists and policymakers, that this groundbreaking technology can be put to use to treat a plethora of diseases and conditions. The modifications done via gene therapy could have an everlasting impact for generations to follow.

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