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Genome Editing Tool: CRISPR A Comprehensive Review

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Abstract: *CRISPR technology is a modern gene-editing tool that allows scientists to make precise changes in DNA. It works like a pair of scissors that can cut and modify specific genes. In the medical field, CRISPR has opened new possibilities for understanding and treating many diseases. It is being used in areas such as cancer research, genetic disorder correction, infectious disease control autoimmune disease, and drug development. Scientists are also exploring its use in organ transplantation and improving immune therapies. This technology provides hope for creating personalized treatments and curing diseases that were once thought to be untreatable. However, there are still challenges related to safety, ethics, and accuracy that need to be addressed. This review highlights the various medical applications of CRISPR technology and its potential to transform the future of medicine.*

Keywords: CRISPR Technology, Gene Editing method, DNA, Guide RNA, CAS 9

I. INTRODUCTION

CRISPR-Cas technology is one of the biggest discoveries in modern science. It allows scientists to edit genes, meaning they can add, remove, or change parts of DNA in living organisms. The full form of CRISPR is Clustered Regularly Interspaced Short Palindromic Repeats, and it was first found in bacteria as a natural defense system against viruses [1]. This system works with a special enzyme called Cas9 and a small piece of guide RNA (gRNA). The guide RNA helps Cas9 find the exact place on the DNA where the cut should be made. Once the DNA is cut, the cell's repair system fixes it either by joining the ends together (which can disable a gene) or by inserting a new piece of DNA (which can correct or change a gene) [2]. Earlier gene-editing methods like zinc-finger nucleases (ZFNs) and TALENs were effective but difficult and costly to design. CRISPR-Cas9, on the other hand, is faster, cheaper, and easier to use, which is why it quickly became a favorite tool for researchers all over the world [3]. Today, CRISPR is used in many areas of science and medicine. It helps scientists study genetic diseases, create new treatments for cancer, improve crops and livestock, and even develop rapid tests for infections such as COVID-19 [4]. Researchers are also exploring its use in drug discovery and personalized medicine. However, there are still some challenges and ethical questions. Sometimes, CRISPR may cut the wrong DNA section, leading to off-target effects. There are also concerns about using it in human embryos, and about how to make sure this technology is used safely and fairly for everyone [5]. In this review, we will discuss how CRISPR works, its different types, and its applications in human health, agriculture, and pharmaceuticals. We will also look at its current limitations, ethical issues, and future possibilities.

Historical Background & Discovery of CRISPR:

The story of CRISPR began more than 30 years ago, long before scientists knew it could change the future of genetics. In 1987, a Japanese scientist named Yoshizumi Ishino and his team at Osaka University accidentally discovered some strange repeated DNA sequences while studying a bacterial gene called iap in Escherichia coli. They didn't know what

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those sequences did, but they recorded them carefully [6]. A few years later, researchers found similar repeating DNA patterns in other microorganisms like Haloferax and Mycobacterium tuberculosis. In 2002, Dutch scientist Ruud Jansen gave these repeats their official name CRISPR, which stands for Clustered Regularly Interspaced Short Palindromic Repeats [7]. He also noticed that these repeats were always found next to CRISPR-associated (Cas) genes, suggesting they might work together. The real breakthrough came in 2005, when independent teams led by Francisco Mojica, Alexander Bolotin, and Rodolphe Barrangou realized that the sequences between the repeats matched fragments of virus DNA. This meant that bacteria were actually keeping a "memory" of viruses that had infected them before a form of natural immunity [8,9]. In 2012, scientists Jennifer Doudna and Emmanuelle Charpentier made history by showing that CRISPR could be used as a programmable gene-editing tool in the lab. They designed a simple system with two key parts Cas9 enzyme and a guide RNA that could cut any DNA sequence chosen by scientists [3]. This discovery changed the field of biology forever and earned them the Nobel Prize in Chemistry in 2020. Since then, CRISPR has become one of the most powerful tools in biotechnology. It is now used to study genes, treat diseases, improve crops, and even develop diagnostic tools for infections like COVID-19. From a random discovery in bacteria to a revolutionary tool for life sciences, CRISPR's journey shows how curiosity-driven research can lead to major scientific advances.

Function of CRISPR as an Adaptive Immune System:

Bacteria and archaea live in environments full of viruses (called bacteriophages) that can infect and destroy them. To survive, these microorganisms have developed a natural defense system known as CRISPR/Cas, which works like an adaptive immune system meaning it can "remember" past infections and protect against them in the future [9]

The CRISPR-Cas system works in three main stages:

(1) Adaptation
(2) Expression
(3) Interference

1. Adaptation (Spacer Acquisition)

When a virus infects a bacterium, the CRISPR system captures small pieces of the virus's DNA, called spacers, and stores them in a special region of the bacterial genome called the CRISPR array [10]. These spacers act like a genetic memory card, allowing the bacteria to recognize the same virus if it attacks again. Each new infection adds more spacers, building a library of viral DNA sequences that represent previous threats.

2. Expression (CRISPR RNA Formation)

When the bacteria detect danger, the CRISPR region is transcribed into a long RNA molecule called pre-crRNA, which contains all the stored viral sequences. This RNA is then cut into smaller pieces known as crRNAs (CRISPR RNAs), each containing one viral spacer sequence. These crRNAs combine with special proteins called Cas (CRISPR-associated) proteins to form a CRISPR-Cas complex [11].

3. Interference (Target Destruction)

When the same virus tries to infect the bacterium again, the CRISPR-Cas complex uses the crRNA as a guide to search for matching DNA sequences in the virus. Once the match is found, the Cas enzyme (like Cas9) cuts the viral DNA at the exact spot, destroying it before it can harm the cell [12]. This precise defense mechanism helps bacteria neutralize viruses quickly and also store new genetic memories for future protection. In simple terms, CRISPR works like a biological "immune memory" system in bacteria. It learns from past infections, remembers the viral DNA, and uses that information to fight off similar attacks in the future. Scientists later realized that this natural defense system could be reprogrammed to edit genes in humans, animals, and plants, making CRISPR-Cas9 one of the most revolutionary tools in modern science.

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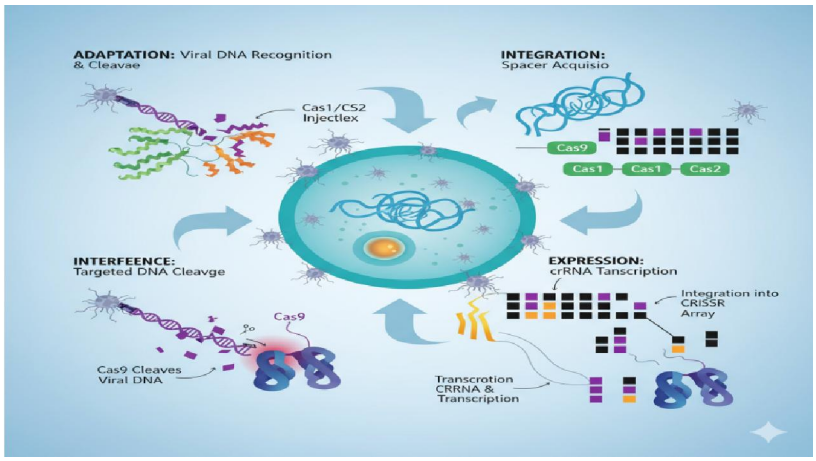

Fig 1: Function of CRISPR in Adaptive Immune System

Mechanism of CRISPR/Cas9 System

The CRISPR/Cas9 system is one of the most powerful tools in modern molecular biology. Originally discovered as a bacterial immune mechanism, scientists have now repurposed it as a programmable gene-editing system that can accurately modify DNA in almost any organism. [3] It works by using a Cas9 enzyme guided by a short piece of RNA to find and cut a specific DNA sequence just like using a "molecular scissor" directed by GPS.

Components of the CRISPR/Cas9 System

The CRISPR–Cas9 system mainly consists of two key components:

a) Cas9 Protein

Cas9 is a nuclease enzyme derived from the bacterium Streptococcus pyogenes. It acts as a molecular scissor that cuts both strands of DNA at a target site. The enzyme recognizes a short DNA sequence known as the PAM (Protospacer Adjacent Motif), which helps ensure that only the correct DNA is cut [13]. Cas9 has two important cutting regions, called HNH and RuvC domains. The HNH domain cleaves one DNA strand complementary to the guide RNA. The RuvC domain cuts the opposite strand. Together, they generate a double-strand break (DSB) that triggers the cell's DNA repair mechanisms.

b) Guide RNA (gRNA)

The guide RNA is a synthetic fusion of two naturally occurring RNAs found in bacteria:

•crRNA (CRISPR RNA): contains the 20-nucleotide sequence that matches the target DNA.

•tracrRNA (trans-activating CRISPR RNA): helps Cas9 bind and stabilize the crRNA.

In lab systems, these two are combined into a single guide RNA (sgRNA), which simplifies the process and allows precise targeting of almost any gene [14]

Mechanism of Action:

Recognition, Binding, and Cleavage

The CRISPR/Cas9 system follows three main steps to edit genes:

Step 1: Recognition

The guide RNA directs Cas9 to a specific DNA sequence by base pairing with the target site. Cas9 first scans the genome to locate a nearby PAM sequence (5'-NGG-3'). Without this PAM site, Cas9 will not bind or cut, which prevents unwanted DNA breaks [15]

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Step 2: Binding

Once the correct PAM site is found, the guide RNA binds to its complementary DNA strand through Watson-Crick base pairing. This interaction unwinds the DNA double helix and activates Cas9's cutting domains. [14]

Step 3: Cleavage

Cas9 cuts both DNA strands, creating a double-strand break (DSB).

The cell then repairs this break in one of two ways: [13,14]

•Non-Homologous End Joining (NHEJ): directly reconnects DNA ends but may introduce small insertions or deletions (useful for gene knockout).

•Homology-Directed Repair (HDR): uses a supplied DNA template to precisely insert or correct a gene sequence (useful for gene correction or addition).

This simple yet powerful process allows researchers to turn genes on or off, correct mutations, or even insert entirely new DNA sequences.

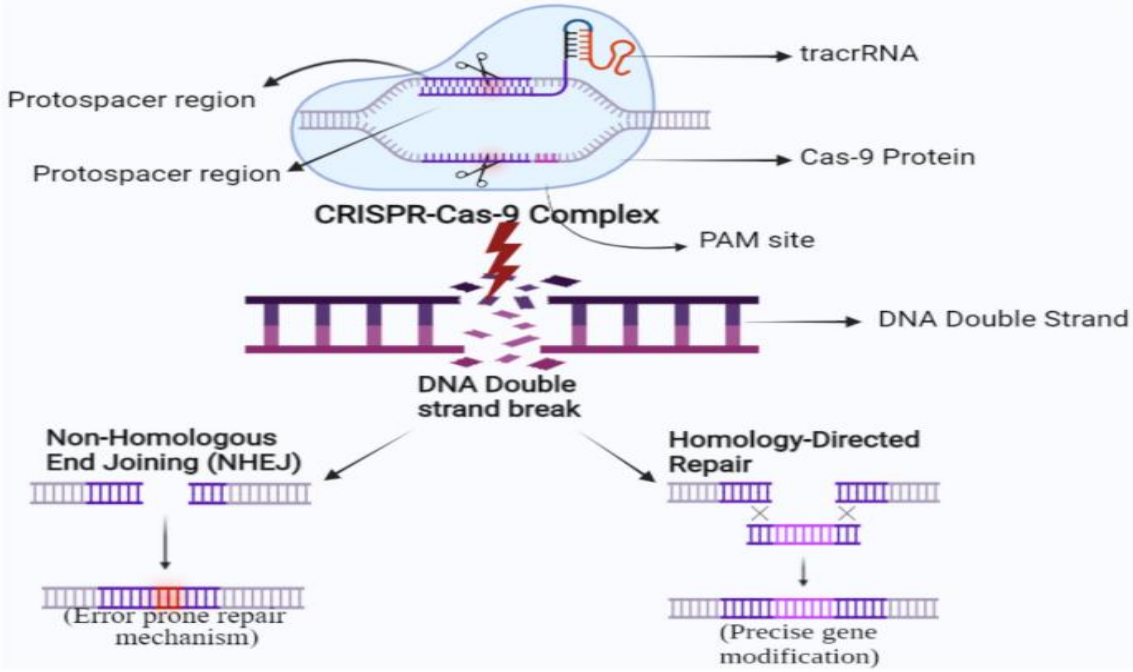

Fig:2 Mechanism of action of CRISPR [16]

Types of CRISPR system

CRISPR systems are broadly classified into two main classes based on their effector proteins [17] :

Table 1: Types of CRISPR system

Property	Cas9	Cas12a	Cas13a
Cas effector	Class-II and Type-2	Class II-Type-5-A	Class II-Type-6
Size (AA)	1000–1600 AA	1200–1300 AA	1000–1200 AA
DNA Nuclease Domain	RuvC and HNH	RuvC only	HEPN × 2
Guide RNA	crRNA and tracrRNA	crRNA only	crRNA only
Spacer Length	20 nt	23 nt	24 nt

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Cut Type	Blunt end cuts	5 nt staggered end cut	Near U or A
Pattern of Cleavage	Cis	Cis	Trans
PAM Sequence	3′-NGG (G-rich)	5′-TTTV (T-rich)	Not required
pre-crRNA Processing	Required intrinsic RNase activity	Requires host RNase-III and crRNA	Requires host RNase-III and crRNA
Target Nucleic Acids	dsDNA	ssDNA, dsDNA	Only ssRNA
Mechanism of Gene Editing	NHEJ and HDR	Cis and Trans-cleavage activity	Cis and Trans-cleavage activity
Major Applications	Gene editing, Multiplex editing	Nucleic acid detection, RNA editing	RNA editing, Nucleic acid detection, Methylation status of DNA

The Class II system (especially Cas9) is most widely used in biotechnology because of its simplicity and efficiency it only needs one protein and one guide RNA.

Base and Prime Editing Advances

Although CRISPR–Cas9 is highly accurate, its double-strand breaks can sometimes cause unwanted mutations. To overcome this, scientists have developed next-generation CRISPR tools:

a) Base Editing

Base editors can change a single DNA base (A, T, G, or C) without cutting both DNA strands. For example, cytosine base editors (CBEs) and adenine base editors (ABEs) can precisely convert C→T or A→G. This allows correction of point mutations responsible for many genetic diseases [18].

b) Prime Editing

Prime editing, developed in 2019, is an even more flexible method. It uses a modified Cas9 (nickase) fused to a reverse transcriptase enzyme and a special prime editing guideRNA(pegRNA). This system can insert, delete, or replace specific DNA sequences without creating double-strand breaks or requiring donor DNA templates [19]. Together, base and prime editing represent the next generation of CRISPR technologies, offering safer and more precise gene correction methods.

CRISPR/Cas 9 and Autoimmune Disease

Autoimmunity typically arises from a widespread immune response due to inadequate regulation of the immune system. For genetic modification in living organisms using CRISPR-Cas9, both sgRNA and Cas9 need to enter the cell nucleus to make edits at the designated DNA location. There are two primary methods for delivering CRISPR-Cas9: viral vectors and lipid nanoparticles.[20] Among the viral vectors, the adeno-associated virus (AAV) is the most common choice, as it has a low chance of integrating into unrelated genes and has proven to be safe. Because AAV is relatively small, the larger SpCas9 from Streptococcus pyogenes is not often used despite its effectiveness; instead, smaller Cas9 proteins from various bacterial sources are utilized. For instance, Cas9 from Staphylococcus aureus (SaCas9) or Campylobacter jejuni (CjCas9) can be combined with sgRNA in AAV for effective in vivo gene editing.[20] The protein PCSK9, which modulates low-density lipoprotein (LDL) and is a significant contributor to atherosclerosis and coronary artery disease, was successfully knocked out in liver cells with SaCas9 using the non-homologous end joining (NHEJ) method, which led to a noticeable reduction in LDL levels. Another investigation indicated that delivering meganuclease targeting PCSK9 via AAV resulted in lower serum cholesterol levels. [20]

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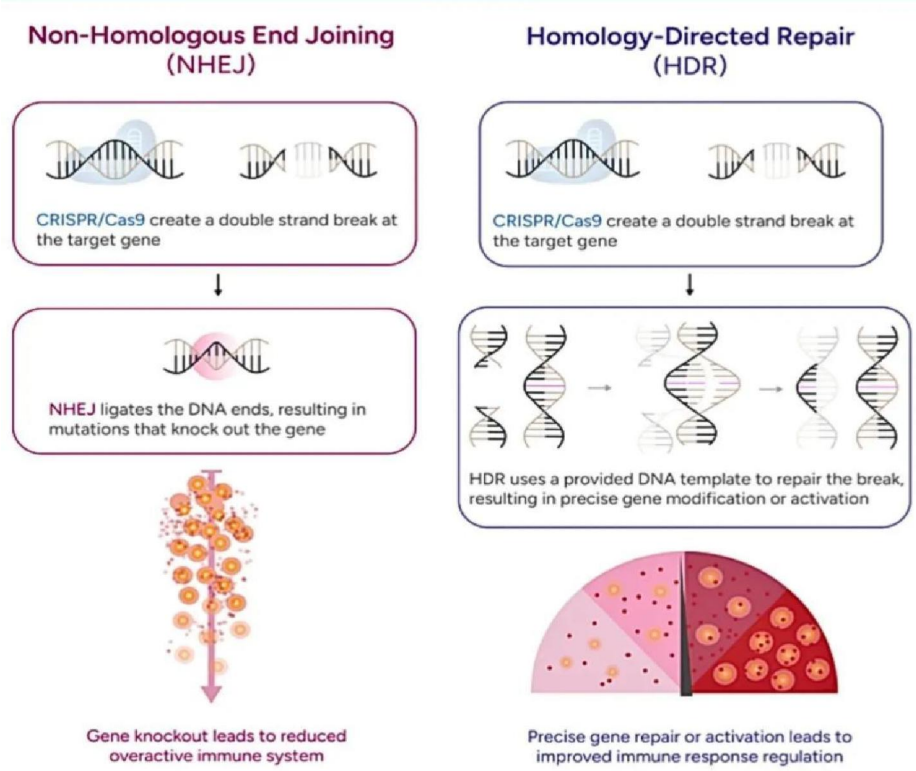

Fig:3 Mechanism of CRISPR in Autoimmune disease [21]

Application of CRISPR for Autoimmune related Diseases

1. Rheumatoid Arthritis (RA)

Use: CRISPR is used to study and modify genes responsible for inflammation such as PTPN22, TNF-α, and IL-1β. [22]

Application: Scientists use CRISPR to knock out inflammatory genes in immune cells and synovial fibroblasts to reduce joint inflammation. [22]

Future therapy: CRISPR-engineered T regulatory (Treg) cells are being explored to suppress the autoimmune response in RA. [22]

2. Inflammatory Bowel Disease (IBD)

Use: CRISPR helps to identify genetic mutations like NOD2, IL23R, and ATG16L1 that cause Crohn's disease and ulcerative colitis. [24]

Application: CRISPR-edited intestinal organoids (lab-grown gut tissues) are used to test treatments and repair defective gut-lining genes. [24]

Goal: Restore gut immune balance and repair the intestinal barrier. [24]

3. Systemic Lupus Erythematosus (SLE)

Use: CRISPR targets genes that lead to the overproduction of autoantibodies, such as TLR7 and BAFF. [23]

Application: It helps create engineered CAR-T cells that destroy autoreactive B cells causing lupus. [23]

Outcome: Studies show CRISPR-modified CAR-T therapy can induce long-term remission in severe lupus cases. [23]

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4. Multiple Sclerosis (MS)

Use: CRISPR is used to edit genes linked to the loss of myelin (the nerve coating) and to study immune cell attacks on the nervous system. [22]

Application: It helps modify T cells to reduce their autoreactivity and increase the activity of protective Treg cells. [22]

Goal: Prevent immune cells from attacking nerve cells and reduce disease progression. [22]

5. Type 1 Diabetes Mellitus (T1DM)

Use: CRISPR is applied to edit stem-cell–derived islet cells so they can evade immune attack. [25]

Application: Scientists knock out HLA genes and insert "don't-eat-me" signals like CD47, creating immune-protected insulin-producing cells. [25]

Success: In 2024–25, gene-edited islet cell transplants showed promising results in humans patients produced insulin without strong immunosuppressive drugs. [25]

6. Psoriasis

Use: CRISPR targets inflammatory cytokines like IL-17, IL-23, and TNF-α involved in excessive skin cell growth. [26]

Application: In preclinical studies, CRISPR knocked out inflammation-related genes in keratinocytes, reducing psoriasis-like skin lesions in mice. [26]

Goal: Develop topical CRISPR-based creams or nanoparticle therapies to calm inflammation at the skin level. [26]

Biosensor in CRISPR

In a biological sample, biomolecule identification may offer details regarding the history and probability of any potential or existing complication. As a result, the word biosensor, which implies the ability to identify and analyze any biomolecule, which comes with it. into existence. [27] Biosensors are made to be very selective and their aim's accuracy in any biological specimen, including serum, plasma, blood, urine, cell extract, and other bodily fluids. A biosensor instrument may be able to consist of a number of components that are skilled at identifying signal processing, transduction, and cation. As a result, possibly offering speed, real-time measurement, simplicity of use, and molecular analysis accuracy. [27] In recent years, biosensors have given a surprising number of therapeutic uses in the identification and initial diagnosis of a range of disease. [27]

Mechanism of CRISPR based biosensor

The fundamental concept behind CRISPR-based biosensors primarily relies on two processes, namely (a) cleavage-based and (b) binding-based. There are three steps involved: (i) signal amplification, (ii) signal trans induction, and (iii) signal reporting. Using CRISPR/Cas effector proteins can bind the target analyte in Cas biosensing systems target nucleic acids for their unique identification. These effectors divided enzyme or split fluorescent is combined with protein. The Cas effector protein's enzymatic activity starts this makes the signals generated easier to detect. [28] Nuclease the main effector protein employed was inactivated Cas9 (dCas9). Reinsertion of is caused by the binding of dCas9 to a target nucleic acid. The cleaved fluorescent protein or enzyme generates the detection signals. [29] Instances are a paired dCas9 (PC) an RCH technique (rolling circle) and the reporter system [30] split horseradish peroxidase, CRISPR-Cas9, and amplification). The CRISPR/Cas biosensing based on cleavage, on the other hand, Cas effector proteins, which have collateral activity, are used in these methods nucleic acids on target. These effector proteins create a ternary complex the target nucleic acid, the complex, and the crRNA (or sgRNA). The reporter molecule, which might be either ssRNA or ssDNA, goes through trans-cleavage to produce little fragments. [31] This activity of collateral cleavage is induced by the ternary complex and aimed at nucleic acid.

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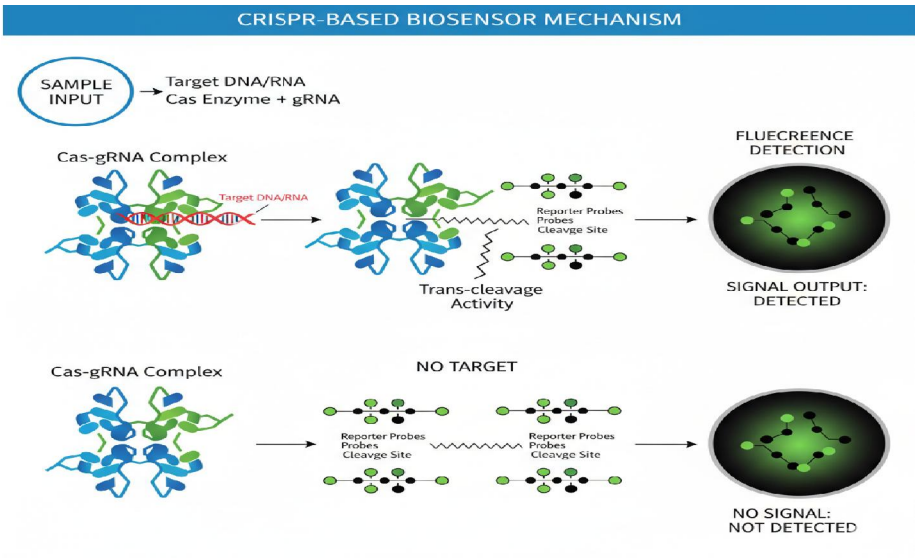

Fig4: CRISPR based biosensor mechanism

CRISPR/Cas12a system with a digital microfluidic chip (DMF)

By combining the CRISPR/Cas12a system with a digital microfluidic chip (DMF), created a quick, sensitive, and automated approach for identifying bacterial pathogens. The detection limit of this assay in the case of Staphylococcus aureus was 32 CFU mL^{1}. With this assay, one may do the complete detection process using a small amount (6.6 µL) of reagent in under 55 minutes, demonstrating an outstanding level of sensitivity and specificity. Eventually, due to its superior sensitivity and reliability, this platform may be used successfully to a variety of human samples, including milk and urine. This method allows for the sequential execution of multiple biochemical experiments on a DMF chip, such cell lysis, RPA amplification, and Cas12a trans-cleavage, without the need for manual operations, thanks to the fluid handling of the droplets via automation. [32]

CRISPR Delivery Methods

The efficiency and safety of CRISPR/Cas9 genome editing largely depend on the method used to deliver the Cas9 and guide RNA components into target cells. Delivery strategies can be broadly categorized as viral, non-viral, and physical methods.

1. Viral delivery systems

such as adeno-associated viruses (AAV), lentiviruses, and adenoviruses are among the most widely used approaches for in vivo genome editing because of their high transduction efficiency and stable expression of the CRISPR components. However, they present limitations such as restricted cargo capacity (especially in AAV) and possible immune responses, which can complicate therapeutic applications [33].

2. Non-viral delivery systems

It have been developed to overcome the drawbacks of viral vectors. These include lipid nanoparticles (LNPs), polymeric carriers, and inorganic nanoparticles that can encapsulate plasmid DNA, mRNA, or Cas9 ribonucleoprotein (RNP) complexes. Such systems are less immunogenic and can enable transient expression of CRISPR components, reducing off-target effects [34].

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3. Physical delivery methods,

It including microinjection, electroporation, and hydrodynamic injection, provide direct entry of CRISPR reagents into cells or embryos. These methods are particularly effective for ex vivo genome editing but are less suitable for systemic in vivo applications due to cell damage and limited tissue targeting [34].

Ethical and Safety Issues in CRISPR/Cas9 Genome Editing

The advent of CRISPR/Cas9 technology has revolutionized the field of gene editing, offering the potential to correct hereditary disorders and modify genetic material in plants and animals. Despite these promising benefits, the technology presents profound ethical and safety challenges that demand careful consideration. A key ethical dilemma lies in germline genome editing, where changes made to DNA can be inherited by future generations. Such interventions raise complex questions about human autonomy and the rights of individuals who will be affected by these edits but cannot give consent [35]. Concerns have also been expressed regarding the possibility of genetic enhancement, where CRISPR might be misused to create "designer babies" with desired traits, potentially leading to social inequality and moral conflicts [36]. From a safety perspective, CRISPR/Cas9 is not without limitations. One major risk involves off-target mutations, where unintended sections of the genome are edited, potentially triggering harmful genetic alterations or tumor formation [36]. Another concern is mosaicism, a phenomenon where not all cells exhibit the intended modification, reducing the reliability and consistency of therapeutic outcomes [37]. The ethical debate also encompasses the use of CRISPR in human embryos and disease models. While such research can significantly enhance medical understanding, it raises sensitive questions about the moral status of embryos and the ethical boundaries of experimentation [37]. Furthermore, the absence of standardized global regulations allows discrepancies in research practices across countries, increasing the risk of misuse or unethical applications [35,36]. In summary, although CRISPR/Cas9 holds transformative potential in science and medicine, it is crucial to implement robust ethical frameworks and safety protocols. Encouraging transparent public dialogue, establishing global bioethical standards, and promoting responsible scientific use can help ensure that progress in genome editing aligns with human values and societal welfare [35–37].

Limitations and Challenges of CRISPR/Cas9 Genome Editing

Despite the revolutionary potential of CRISPR/Cas9 technology in treating genetic disorders, improving crops, and advancing biomedical research, several technical and ethical challenges still limit its widespread clinical application. One of the foremost limitations is the off-target effect, where CRISPR mistakenly cuts DNA sequences similar but not identical to the target site. Such unintended edits can lead to unwanted mutations or activation of oncogenes, posing serious safety risks for therapeutic use [38]. In addition, the editing efficiency varies significantly depending on cell type, chromatin structure, and the delivery method employed, making it difficult to achieve consistent and precise outcomes in living organisms [39]. Another major challenge lies in delivering the CRISPR components Cas9 nuclease and guide RNA efficiently into target cells or tissues. Viral vectors, though effective, can trigger immune responses or integrate unpredictably into the host genome, while non-viral carriers such as lipid nanoparticles or electroporation often face limitations in tissue targeting and stability [40]. Immunogenicity also remains a concern, as the Cas9 protein originates from bacteria, and exposure may provoke an adaptive immune response in humans, reducing the safety and durability of genome editing treatments [40]. Furthermore, the long-term stability and heritability of edits have not yet been fully studied, leaving uncertainty about the potential for delayed or transgenerational side effects [38,39]. Beyond these biological hurdles, regulatory and ethical issues complicate clinical translation. Global standards on human genome editing remain fragmented, increasing the risk of misuse or uneven research oversight [39]. Addressing these challenges requires continued refinement of delivery systems, development of more precise gene-editing variants such as base and prime editors, and the establishment of unified ethical and regulatory frameworks to ensure responsible use of this transformative technology [38–40].

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Future Prospects of CRISPR Technology

The CRISPR/Cas9 system is anticipated to advance well beyond its current applications in gene editing, moving toward smarter, safer, and more flexible technologies. Recent research highlights the potential of integrating artificial intelligence (AI) and machine learning algorithms into CRISPR design to enhance guide RNA accuracy, minimize off-target modifications, and boost overall editing precision [41]. This fusion of AI and biotechnology could accelerate the clinical translation of CRISPR-based therapies, making genome editing more tailored and reliable for diverse patient needs. In medical science, CRISPR is steadily transitioning from laboratory research to clinical implementation for treating inherited and multifactorial diseases. Ongoing trials have already demonstrated encouraging outcomes in correcting mutations linked to disorders such as sickle cell anemia, muscular dystrophy, and several forms of cancer. Nevertheless, key hurdles remain, including achieving targeted delivery, preventing immune reactions, and ensuring the stability of edited sequences. Future innovations are likely to emphasize advanced delivery mechanisms, next-generation editing tools like base and prime editing, and robust ethical and regulatory frameworks for safe clinical use [42,44]. Apart from therapeutic applications, CRISPR is also reshaping diagnostics. Novel Cas12 and Cas13 systems have been utilized in portable biosensor platforms, enabling rapid, affordable, and ultrasensitive detection of pathogens and biomarkers at the point of care [30]. The convergence of CRISPR with nanotechnology further improves sensor precision, suggesting future possibilities for personalized monitoring and preventive healthcare. In the agricultural and environmental sectors, CRISPR is proving to be a transformative force by generating pest-resistant and climate-adaptive crops while reducing dependence on harmful agrochemicals. Additionally, CRISPR-based gene drives are being investigated for sustainable pest population control, with the goal of achieving ecological balance and improved food security [45]. Overall, the next decade is expected to position CRISPR as a multifunctional platform uniting therapeutic, diagnostic, and biotechnological innovation. With continued refinement and ethical oversight, this technology is poised to redefine biological research and support a more sustainable and health-conscious future.

II. CONCLUSION

The emergence of CRISPR-Cas9 has revolutionized modern genetic engineering by offering a precise, rapid, and cost-effective tool for targeted genome modification. Its diverse applications across healthcare, agriculture, and biotechnology have paved the way for innovative treatments of genetic diseases, enhanced crop varieties, and progress in synthetic biology. However, issues such as unintended gene edits, ethical implications, and challenges in efficient delivery remain areas of concern. Ongoing scientific exploration and responsible utilization of this technology are vital to maximizing its benefits while minimizing risks. With continuous advancements and interdisciplinary cooperation, CRISPR is poised to redefine the future of medicine, food production, and biological innovation, fostering a more advanced and sustainable global landscape.

REFERENCES

1. Redman M, King A, Watson C, et al. What is CRISPR/Cas9? Archives of Disease in Childhood - Education and Practice Edition. 2016; 101(4):213–215.
2. Hsu PD, Lander ES, Zhang F et al. Development and applications of CRISPR-Cas9 for genome engineering. Cell. 2014; 157(6):1262–1278.
3. Jinek M, Chylinski K, Fonfara I, et al. A programmable dual-RNA-guided DNA endonuclease in adaptive bacterial immunity. Science. 2012; 337(6096):816–821.
4. Zhang Y, et al. CRISPR/Cas9 editing in plants: a review. Nature Biotechnology. 2018; 36(2):123–131.
5. Ishii T, Araki M. Gene editing and human future(ethical aspects of CRISPR-Cas9).Reproductive Biology and Endocrinology. 2017; 15(1):49.
6. Ishino Y, Shinagawa H, Makino K,et al. Nucleotide sequence of the iap gene, responsible for alkaline phosphatase isozyme conversion in Escherichia coli, and identification of the gene product. J Bacteriol.1987;169(12):5429-5433.

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7. Jansen R, Embden JD, Gaastra W, et al. Identification of genes that are associated with DNA repeats in prokaryotes. Mol Microbiol.2002;43(6):1565-1575.
8. Mojica FJM, Díez-Villaseñor C, García-Martínez J, et al.Short motif sequences determine the targets of the prokaryotic CRISPR defence system. Mol Microbiol. 2005;36(1):244-246.
9.Barrangou R, Fremaux C, Deveau H, et al. CRISPR provides a acquired resistance againt viruses in prokaryotes. Science.2007;315(5819):1709-1712.
10. Marraffini LA, Sontheimer EJ. CRISPR interference: RNA directed adptive immunity in bacteria and archaea. Nat Rev Genet.2010;11(3):181-190.
11.Bhaya D, Davison M, Barrangou R. CRISPR-Cas systems in bacteria and archaea: versatile small RNAs for adaptive defense and regulation. Annu Rev Genet. 2011; 45:273-297.
12. Makarova KS, Haft DH, Barrangou R,et al. Evolution and classification of the CRISPR-Cas systems. Nat Rev Microbiol.2011;9(6):467-477.
13. Anders C, Niewoehner O, Duerst A, et al. Structural basis of PAM-dependend target DNA recognition by the Cas9 endonuclease.Nature. 2014;513(7519):569-573.
14. Cong L, Ran FA, Cox D,et al. Multiplex genome engineering using CRISPR/Cas systems. Science.2013;339(6121):819-823.
15. Sternberg SH, Redding S, Jinek M, et al. DNA interrogation by the CRISPR RNA-guided endonuclease Cas9.Nature.2014;507(7490):62-67.
16.https://www.autoimmuneinstitute.org/wp-content/smush-webp/2024/12/Gene-Editing-Mechanisms-in-Autoimmune-Disease-GAI-1.jpg.webp Assest on 6 Oct 2025
17. Makarova KS, Wolf YI, Alkhanbashi OS,et al. An updated evolutionary classification of CRISPR-Cas systems. Nat Rev Microbiol. 2015;13(11):722-736.
18. Komor AC, Kim YB, Packet MS, et al. Programmable editing of a target base in genomic DNA without double-stranded DNA cleavage. Nature. 2016;533(7603):420-424.
19. Anzalone AV, Randolph PB, Davis JR, et al. Search and replace genome editing without double-strand breaks or donar DNA.Nature.2019;576(7785):149-157.
20.Lee M.H, Shin J.I, Yang J.W, et al. Genome Editing Using CRISPR-Cas9 and Autoimmune Diseases: A Comprehensive Review. Int. J. Mol. Sci. 2022,;23,(1337): 1-13.
21.https://link.springer.com/article/10.1007/s11033-022-07880-6#auth-Darakhshan-Javaid-Aff1. Assets on 6 Oct 2025
22. Zhang M-L, Li H-B and Jin Y, Application and perspective of CRISPR/Cas9 genome editing technology in human diseases modelling and gene therapy.Front. Genet. 2024;15:1364742:1-14
23. Zhou J, Lei B, Shi F et al. CAR T-cell therapy for systemic lupus erythematosus: current status and future perspectives. Front. Immunol. 2024;15:1476859:1-22
24. Appiah, J.K.; Hayat, U.;Garg, N.; Asante et al.Emerging Therapies in Inflammatory Bowel Disease: A Comprehensive Review. J. Clin. Med. 2025;14(17), 6119:1-20
25. Karim E. Shalaby and Essam M. Abdelalim Hypoimmune stem cells and islets: hype or a true breakthrough in diabetes treatment? Cellular & Molecular Biology Letters 2025; 30:112:1-33
26. Gexiao Xu,Guanyi Ma,Jiachen Sun et al.Comprehensive analysis of pyroptosis-related genes in psoriasis and targeted gene editing of CASP1 and CASP5 using lipid nanoparticles to alleviate skin inflammation . Font Bioeng. Biotechnol. 2025;13:1639869:1-14
27. Haixiang Yu, Obtin Alkhamis, Juan Canoura Advances and Challenges in Small-Molecule DNA Aptamer Isolation, Characterization, and Sensor Development. Angew Chem Int Ed Engl. 2021; 60(31): 16800–16823.
28. H. Li, Y. Xie, F. Chen, et al. CRISPR for companion diagnostics in low-resource settings Chem. Soc. Rev., 2024, 24(20):4717-4740.
29. M. Azhar, R. Phutela, M. Kumar, et al. Rapid and accurate nucleobase detection using FnCas9 and its application in COVID-19 diagnosis Biosens. Bioelectron., 2021,183, 113207:1-8

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30.Sahel DK, Giriprasad G, Jatyan R, et al. Next-generation CRISPR/Cas-based ultrasensitive diagnostic tools: current progress and prospects. RSC Adv. 2024; 14:32411–32435.
31.Lee, J.E.; Pack, S.P. Recent Progress in DNA Biosensors: Target-Specific and Structure-Guided Signal Amplification. Biosensors 2025,15, 476:1-22
32. Lianyu Lu, Huimin Zhang, Fanghe Lin, et al. Sensitive and automated detection of bacteria by CRISPR/Cas12a-assisted amplification with digital microfluidics. Sen. Actuators, B, 2023, 381, 133409:1-8
33. Wang D, Zhang F, Gao G. CRISPR-Based Therapeutic Genome Editing: Strategies and In Vivo Delivery by AAV Vectors. Cell. 2020;181(1):136-150
34.Hu Y, Zhong L, et al. Lipid-Nanoparticle-Based Delivery of CRISPR/Cas9 Genome Editing Systems. Mol Pharm. 2022;19:2284-2297.
35. Rodriguez E. Ethical issues in genome editing using CRISPR/Cas9 system. J Clin Res Bioeth. 2016;7(2):1–6.
36. Lorenzo D, Esquerda M, Palau F, et al. Ethics and genomic editing using the CRISPR-Cas9 technology:challenges and conflicts. NanoEthics. 2022;16(3):313–321.
37. D'Souza RF, Mathew M, Surapaneni KM. A scoping review on the ethical issues in the use of CRISPR-Cas9 in the creation of human disease models. J Clin Diagn Res. 2023;17(12):JE01–JE08.
38. Wang H, La Russa M, Qi LS. CRISPR/Cas9 gene editing in human cells and its potential clinical applications: applications, limitations, and implications for disease treatment. Front Med.2017;11(5):463–471.
39. Zhang Y, Li D, Zhang Y,et al. CRISPR/Cas: advances, limitations, and applications for precision medicine. Front Med. 2021;8:649896.
40. Rahman M, Li J, Bian Y, Li Z. Strategies to overcome the main challenges of the use of CRISPR/Cas9 as a replacement for cancer therapy. Mol Cancer. 2022;21(1):64.
41 Ahn H, Lee J, Kim H. Artificial intelligence meets CRISPR: Optimizing gene editing with deep learning approaches. Front Bioeng Biotechnol. 2024;12:1335901.
42. Li H, Zhang F. The future of CRISPR genome editing: Advancements and challenges. Mol Cancer. 2023;22(1):54.
43. Ghosh S, Dutta S, Kumar R. CRISPR-based diagnostic tools: Emerging applications for rapid and sensitive pathogen detection. RSC Adv. 2024;14(48):32341–32358.
44. O'Brien MA, Xu W, Wang L. Clinical translation of CRISPR therapeutics: Opportunities and ongoing challenges. Int J Mol Sci. 2024;25(24):13321.1-8
45. Sharma P, Singh R, Gupta VK. CRISPR-Cas systems in agriculture: Towards sustainable crop improvement and pest management. Insects. 2024;15(9):653.1-6