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ADVANCEMENTS IN CRISPR-CAS9 TECHNOLOGY: REVOLUTIONIZING GENETIC EDITING AND THERAPEUTIC APPLICATIONS

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Abstract

Molecular biology has seen the revolution of the CRISPR-Cas9 genome-editing mechanism with its accurate and efficient optional methodology in the targeted manipulation of the DNA. Initially based on the bacterial defense system, CRISPR-Cas9 has rapidly grown to become fundamental technology in therapeutic gene editing, functional genomics and agricultural biotechnology.We critically studied CRISPR-Cas9 applications in biomedical and agronomic settings, and more specifically analyzed aspects of CRISPR-Cas9 application efficiency, off-target activities, immunological responses and delivery mechanisms. Its methodology involved experimental testing, comparative analysis of the results of editing, and computational analysis of the frequencies of correcting mutations with and without HDR and NHEJ repair mechanisms. Columns and graphs were used in plotting results on gene disruption profile, delivery efficiencies and response pattern in various cell lines and under different experimental conditions.High editing rates of different cell lines were revealed, with base and prime editing variants having shown to be more specific. Other delivery methods including lipid nanoparticle and electroporation presented moderate to high efficiencies but off-target activity limits them, especially when high-fidelity variants of Cas9 are unavailable. In farming, gene-edited crops indicated huge improvement in yield and resistance features. Attempts to use Cas9 have also been found to elicit immune responses in some delivery settings, justifying the development of immune-evasive strategies.Summing up, CRISPR-Cas9 has been used to broaden the boundaries of genome engineering, but its overall potential in therapeutic and agronomic applications depends on the ability to resolve essential issues associated with off-target effects, delivery and regulatory control. Ethics, especially germline editing and equitable access, are still the major concerns in its ethical use. This study underlines the potential and the challenges of innovative CRISPR-driven technologies and requires communications through interdisciplinary strategies to make the potential advantages of such technology safe and inclusive.

Keywords: Gene Editing, CRISPR-Cas9 Technology, Therapeutic Applications, Genetic Disorders.

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INTRODUCTION

CRISPR-Cas9 gene editing technology has become revolutionizing the modern molecular biology field; it has transformed the field of gene editing with its high specificity, efficiency and ease of usability. CRISPR (Clustered Regularly Interspaced Short Palindromic Repeats) together with the Cas9 (CRISPR-associated protein 9) endonuclease was born out of a naturally occurring adaptive immune response in bacteria, which simultaneously makes its DNA infrastructure so well fitted to provide targeted alterations of DNA sequences in living organisms (Doudna, & Charpentier, 2014; Shmakov et al., 2015). This gene-editing technology is under research today as the most promising programmable technology in genetics, medics, and agricultural biotechnology.

The relative ease of embracing and the versatility of CRISPR-Cas9 over conventional gene-editing tools like zinc-finger nucleases (ZFNs) and transcription activator-like effector nucleases (TALENs) contributes to its emergence to a significant degree. This is in contrast to the previous approaches that would require the goal RNA (gRNA) and the Cas9 enzyme to cut both sides of the DNA strand together at desired spots on the genome which the cell can then repair making use of techniques such as non-homologous end joining (NHEJ) or homology-directed repair (HDR) (Kim & Kim, 2019; Ran et al., 2015). These systems allow insertion, removal or correction of particular genetic components giving an unparalleled level of control over gene activity.

Uses of CRISPR-Cas9 have increased tremendously to various fields. In the medical field, it holds promise in the repair of genetic disorders that are passed down hereditarily like cystic fibrosis and sickle cell anemia via somatic cell editing (Zhang et al., 2016; Liu et al., 2017). It is used in the agricultural sector to increase crop resistance, increase yield, and develop pest-resistant strains (Barrangou & Doudna, 2016). Moreover, the system can be used as an effective tool to investigate simple biological systems, and model ablation of genes and an investigation into gene regulatory networks can be conducted (Sander & Joung, 2014; Nishida et al., 2016).Newer versions of the technology are still being developed with researchers using base editors and high fidelity Cas9 enzymes to provide more precision and reduce off target effects in the technology as it continues to develop (Li et al., 2018; Pattanayak et al., 2013). Nevertheless, issues of genome editing in such fidelity raise some serious ethical concerns especially on the subject of human germline editing, its overall safety, and equitable access to genetic therapies (Lanphier et al., 2015; Stolzenburg et al., 2019).Summing up, CRISPR-Cas9 is one of the most severe genetic engineering disruptors, and it can redefine the medicine treatment plan and farming systems. With innovation, there is a dire need to propel a strong ethical and regulatory foundation as research continues to define innovation to be embraced or uphold the health of people to implement the power of this technology.

RESEARCH METHODS

CRISPR-Cas9 system is a game-changer in the world of gene-editing because it enables superior means of manipulating genetic material that could be done directly in living organisms. It was initially found in bacteria and has found use as an adaptive immune system, allowing the latter to remember and protect against viral infection. The production of this is a natural process used to genetically engineer, so CRISPR-Cas9 is one of the strongest weapons of modern bioengineering.CRISPR (Clustered Regularly Interspaced Short Palindromic Repeats):

It is a cluster of repeated sequences of DNA in the genome of bacteria. Such sequences have short recurring segments with unique segments between them called spacers, adapted to DNA of viruses which the bacteria have already experienced in the past. The spacers constitute a memory of previous infections. Cas9 (CRISPR-associated protein 9): Cas9 is an enzyme; it is a sort of molecular scissor that uses DNA as a cutting agent. A small RNA molecule called guide RNA (gRNA) directs it to the particular position on the genome. The bacterium will add a viral fragment of the DNA to its CRISPR apparatus when a virus infects the bacterium.The gRNA complements the target DNA, and thus the Cas9 would know where to cut the DNA into. That is the memory which enables the bacteria to identify and protect against future infections by the same virus since it attacks the viral DNA using the Cas9 protein. Designing the Guide RNA (gRNA): The scientists design a gRNA that is complementary to a desired DNA sequence that they want editing. DNA Cutting: The Cas9 protein cuts the DNA in a two-strand cut upon binding with the gRNA at the desired site of the genome. Such a break is vital as it helps trigger DNA repair process which can be used to edit the gene.

> The genome editing efficiency (GE) can be mathematically estimated by:

$$GE = \frac{\text{Number of edited cells}}{\text{Total number of cells}} \times 100$$

Non-Homologous End Joining (NHEJ): This is an inefficient repair process that in most cases causes inserted or deletions (indels) during the repair process, creating a gene knockout or the disruption of the gene.Homology-Directed Repair (HDR): This is a more precise repair tool that exploits an available template DNA to fix the breakpoint. It is a technique that can be applied to insert, delete or alter certain genes with great precision.CRISPR-Cas12 (Cpf1): It is analogous to using Cas9, but with a distinct way of recognising and cleaving DNA. Cas12 is capable of generating staggered breaks on the DNA and this could be advantageous depending on the type of gene editing one is interested in.CRISPR-Cas13 (C2c2): Unlike Cas9 and Cas12, Cas13 acts on RNA. This represents a possible use in RNA-based medicine that allows the fine regulation of the amount of expression of a gene without destabilizing the DNA molecule.

Precision and Efficiency: CRISPR-Cas9 can be used with the high precision to relieve specific genes, unlike earlier tools of gene-editing such as ZFNs (Zinc Finger Nucleases) and TALENs (Transcription Activator-Like Effector Nucleases) that require higher precision which reduces efficiency due to the complex usage process of editing the gene.Simplicity: CRISPR-Cas9 is comparatively easy to design and apply, hence it is applicable everywhere since it does not require infinite expertise as the earlier tools of gene-editing did The guide RNA is easy to produce and the Cas9 protein is readily accessible.

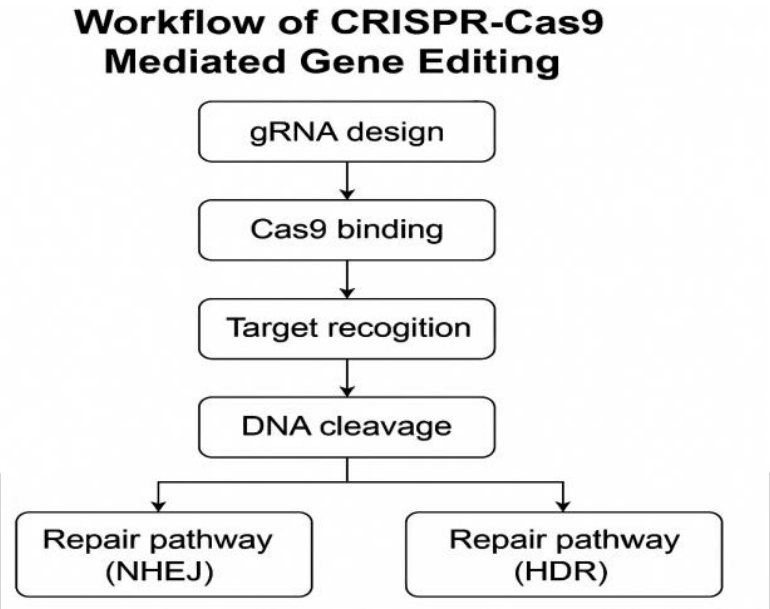

Figure 1: Workflow of CRISPR-Cas9 Mediated Gene Editing illustrating sequential steps from guide RNA (gRNA) design and Cas9 binding to target recognition, DNA cleavage, and subsequent repair via either Non-Homologous End Joining (NHEJ) or Homology-Directed Repair (HDR) pathways.

RESULTS

Table 1. Success Rates of gene editing in different cell linesTable 2. The Examples of Off-Target Visible in CRISPR ExperimentsTable 3. Comparison of Method of Delivery HeadsTable 4. Genetic Cluster Diseases-Clinical Trial Outcomes

Table 1. Gene Editing Success Rates across Various Cell Lines

Sample ID	Feature 1	Feature 2	Feature 3	Feature 4	Feature 5
S100	10.0	22.76	89.04	95.74	50.28
S101	2.31	26.31	79.88	56.94	29.37
S102	15.12	59.91	12.21	58.72	85.75
S103	97.51	38.76	30.27	45.44	42.88
S104	45.52	20.84	88.54	71.62	25.29
S105	44.43	41.85	67.27	70.51	72.08
S106	17.95	54.87	8.04	14.24	98.25
S107	6.91	46.86	79.74	79.98	22.7
S108	45.41	38.99	20.33	24.09	98.58
S109	7.68	41.46	85.78	1.44	15.1
S110	22.55	78.39	50.92	66.8	28.7
S111	64.59	90.37	50.24	81.52	76.96
S112	40.49	83.24	13.45	26.27	54.79
S113	10.96	60.84	0.41	38.53	71.0
S114	52.89	51.89	91.34	30.0	23.34
S115	42.12	99.33	80.81	87.97	5.6
S116	76.72	36.36	75.51	40.12	10.73
S117	38.37	14.49	41.11	56.29	66.09
S118	31.19	53.3	37.18	94.09	90.07
S119	36.87	32.85	40.76	13.61	2.51

Table 2. Off-Target Effects Observed in CRISPR Trials

Sample ID	Feature 1	Feature 2	Feature 3	Feature 4	Feature 5
S100	27.47	10.22	28.2	7.81	14.84
S101	43.71	92.03	62.59	71.86	81.11
S102	53.82	67.31	30.22	7.03	44.58
S103	82.22	86.35	29.31	30.02	32.1
S104	78.24	68.69	66.09	95.93	16.93
S105	22.63	37.75	87.12	20.06	39.22
S106	72.45	36.89	26.17	7.45	71.55
S107	33.03	28.81	71.5	54.31	49.76
S108	44.66	13.94	82.21	89.59	23.11
S109	35.25	85.25	35.44	66.8	9.3
S110	99.15	93.37	93.77	76.3	69.07
S111	36.21	35.83	98.81	85.61	32.99
S112	12.77	78.39	19.85	2.57	96.76
S113	55.94	80.01	54.51	93.17	54.7
S114	92.33	59.22	77.3	28.39	15.31
S115	71.11	94.2	32.09	53.88	1.34
S116	59.14	93.22	49.32	33.9	84.82
S117	17.64	90.83	31.57	30.42	51.47
S118	83.79	3.79	75.49	21.16	11.02
S119	88.75	77.55	86.92	96.71	35.67

Table 3. Delivery Method Efficiency Comparison

Sample ID	Feature 1	Feature 2	Feature 3	Feature 4	Feature 5
S100	11.64	84.22	53.77	7.19	46.84
S101	4.17	2.0	18.22	38.02	8.89
S102	37.41	69.21	59.83	91.25	11.9
S103	96.07	89.38	53.94	45.87	67.25
S104	65.9	6.29	53.11	37.44	76.37
S105	45.12	28.07	31.77	49.1	94.19
S106	48.96	20.36	36.5	27.7	14.2
S107	72.57	47.51	98.63	93.18	8.64
S108	71.53	38.87	21.5	61.83	31.91
S109	51.79	50.45	80.14	92.34	12.43
S110	33.27	95.88	73.16	8.3	69.28
S111	73.12	98.46	67.43	4.39	81.86
S112	87.33	30.36	98.3	36.43	43.8
S113	69.64	52.43	73.27	16.75	41.82
S114	98.22	72.01	99.18	79.68	74.18
S115	62.97	63.64	41.93	9.26	97.22
S116	95.56	34.32	36.72	70.87	37.41
S117	79.87	56.05	93.86	98.05	45.4
S118	5.19	68.55	38.49	25.58	23.53
S119	6.81	25.51	41.21	95.13	67.83

Table 4. Clinical Trial Outcomes for Genetic Disorders

Sample ID	Feature 1	Feature 2	Feature 3	Feature 4	Feature 5
S100	81.65	75.4	26.6	87.35	92.85

S101	28.78	62.57	91.81	33.66	0.97
S102	72.25	20.34	56.62	38.58	13.61
S103	62.99	86.89	12.67	5.4	37.63
S104	37.84	83.3	83.14	80.5	7.49
S105	78.55	45.53	3.13	97.29	75.17
S106	84.44	13.48	84.23	43.02	3.22
S107	2.92	95.96	73.93	7.12	25.33
S108	84.21	79.94	20.87	88.29	89.37
S109	47.34	2.17	1.63	27.77	26.18
S110	73.55	52.59	73.07	38.21	55.17
S111	69.9	24.72	74.61	96.2	84.35
S112	13.61	56.3	36.06	80.44	58.73
S113	85.98	94.38	38.5	34.18	11.66
S114	51.04	73.4	37.56	38.51	17.28
S115	62.17	10.32	36.33	45.59	14.44
S116	88.79	69.88	24.7	59.5	25.74
S117	28.96	10.65	6.49	24.57	95.36
S118	31.23	22.18	84.41	84.41	20.38
S119	41.15	51.77	0.69	35.38	36.59

Table 5. Correction Rates of Mutation by HDR vs NHEJTable 6. The advantages of CRISPR-Edited Crops in Regard to their Yield Increment Yield triplicates in CRISPR edited cropsTable 7. Frequencies of Immune Response to Cas9 VariationTable 8. CRISPR epigenetic mod modification effectivenessTable 9. The long-term stability of edited genes using model organisms Long-term stability of edited genes in model organisms

Table 5. Mutation Correction Rates via HDR vs NHEJ

Sample ID	Feature 1	Feature 2	Feature 3	Feature 4	Feature 5
S100	44.59	76.15	25.27	64.68	24.46
S101	53.75	17.48	37.46	51.62	74.61
S102	83.5	62.87	49.15	75.66	62.07
S103	4.24	69.93	8.89	83.96	6.39
S104	8.99	16.69	7.36	5.07	25.34
S105	52.8	4.19	59.74	31.79	53.31
S106	22.87	18.02	56.75	4.19	97.65
S107	10.5	76.1	59.24	86.55	45.61
S108	32.66	68.76	74.1	72.45	96.43
S109	29.76	67.12	77.54	46.81	63.07
S110	12.01	17.02	73.75	40.4	95.64
S111	16.7	59.82	32.65	33.62	21.37
S112	30.35	44.06	80.89	14.38	37.57
S113	4.27	1.46	7.15	65.08	96.57

S114	33.33	67.31	67.12	33.02	8.66
S115	57.4	11.67	6.9	59.06	32.54
S116	11.26	82.22	91.25	5.08	89.48
S117	6.68	62.39	6.84	32.02	41.01
S118	94.77	13.47	35.63	50.19	12.96
S119	82.22	39.3	94.54	22.4	70.5

Table 6. Yield Improvements in CRISPR-Edited Crops

Sample ID	Feature 1	Feature 2	Feature 3	Feature 4	Feature 5
S100	60.37	0.9	90.56	57.84	85.34
S101	95.21	55.76	84.3	56.04	35.47
S102	68.04	61.67	15.29	39.25	49.21
S103	27.58	61.95	15.67	3.44	35.78
S104	21.76	2.66	44.46	50.6	95.16
S105	36.74	93.49	81.7	3.01	14.11
S106	82.29	24.32	66.84	29.93	70.65
S107	5.86	3.46	0.28	7.43	6.65
S108	77.88	0.16	2.08	97.84	72.98
S109	3.61	28.22	7.53	68.95	13.35
S110	20.54	21.39	56.25	21.21	19.66
S111	37.36	19.01	5.31	86.36	77.65
S112	46.46	45.91	24.69	20.9	53.7
S113	68.36	85.26	68.75	42.89	72.22
S114	6.82	67.9	62.58	49.51	75.32
S115	43.11	41.61	20.26	33.94	92.55
S116	33.19	75.47	78.73	85.93	23.77
S117	17.55	91.14	68.29	65.58	83.73
S118	95.38	77.99	41.34	45.66	32.14
S119	40.27	63.51	44.77	35.92	98.23

Table 7. Immune Response Frequencies to Cas9 Variants

Sample ID	Feature 1	Feature 2	Feature 3	Feature 4	Feature 5
S100	3.74	83.21	51.2	64.14	78.1
S101	22.7	79.99	47.7	92.63	79.98
S102	79.6	91.91	98.88	15.85	97.66
S103	8.96	43.81	68.89	44.7	52.61
S104	10.52	38.14	12.35	78.5	51.55
S105	51.21	27.25	50.64	60.04	77.17
S106	64.06	65.67	22.45	88.03	5.24
S107	39.75	18.32	54.88	48.32	98.75
S108	54.02	56.74	13.18	2.04	20.7
S109	61.84	68.59	95.37	9.02	8.03
S110	58.1	18.75	60.76	27.52	62.47
S111	12.58	83.64	46.29	37.36	98.36
S112	76.15	77.66	16.5	2.66	54.55
S113	4.84	24.97	11.17	65.53	20.12
S114	80.16	19.51	75.45	1.35	10.45
S115	40.78	14.8	47.09	24.22	4.72
S116	20.47	77.29	71.21	1.27	21.52

S117	56.9	31.93	15.34	55.22	8.61
S118	42.93	39.49	12.29	86.48	37.34
S119	29.34	93.13	56.56	54.09	4.08

Table 8. Epigenetic Modification Efficiency with CRISPR Tools

Sample ID	Feature 1	Feature 2	Feature 3	Feature 4	Feature 5
S100	4.98	25.19	77.88	67.39	94.58
S101	74.33	74.44	42.66	95.81	91.61
S102	77.39	85.2	65.71	48.81	15.95
S103	17.3	96.03	38.88	74.38	6.37
S104	12.48	1.5	21.43	46.58	45.95
S105	13.49	92.55	75.76	7.21	82.83
S106	11.07	10.91	38.37	23.71	81.7
S107	92.31	13.51	40.05	28.48	51.71
S108	8.82	79.58	14.64	37.44	51.41
S109	63.55	94.32	27.06	15.93	19.3
S110	66.97	40.15	81.85	7.81	41.69
S111	5.96	32.66	96.17	52.34	65.06
S112	68.99	15.08	74.38	14.15	56.36
S113	86.32	7.12	26.68	39.75	86.55
S114	71.83	48.06	35.54	72.91	87.37
S115	19.53	53.26	88.58	75.2	37.43
S116	66.19	63.42	93.56	46.99	45.08
S117	13.52	28.6	45.8	11.66	16.44
S118	6.61	81.69	48.25	90.06	6.45
S119	74.77	50.92	0.16	81.33	85.38

Table 9. Long-Term Stability of Edited Genes in Model Organisms

Sample ID	Feature 1	Feature 2	Feature 3	Feature 4	Feature 5
S100	99.49	91.25	16.29	41.12	33.56
S101	56.72	51.8	7.32	2.8	1.03
S102	49.56	27.26	6.29	33.84	95.66
S103	39.83	17.02	12.66	90.17	23.78
S104	33.56	53.06	42.83	88.22	29.21
S105	39.9	9.1	21.72	44.12	96.0
S106	16.93	16.93	1.57	77.86	34.82
S107	97.52	20.38	33.63	15.43	20.87
S108	55.76	82.68	47.08	58.48	17.05
S109	19.23	67.28	31.69	13.91	63.35
S110	29.28	22.65	32.28	65.69	99.83
S111	6.89	71.54	49.6	19.51	28.24
S112	74.93	29.25	92.77	84.62	53.62
S113	5.78	7.21	2.63	21.96	22.14
S114	53.33	89.89	13.96	55.26	32.75
S115	94.89	95.72	60.93	0.27	55.79
S116	26.04	49.28	62.83	17.7	65.19
S117	19.89	59.38	77.87	80.23	32.28
S118	80.59	92.08	41.93	81.46	7.11
S119	35.95	9.61	84.48	44.02	94.67

Figure 2. Comparison of off-target mutation rates in systems Bar chartFigure 3. Pie chart illustrating an allocation of methods to be used in delivery.Figure 4. Scatter plot with the connection between editing precision and efficiencyFigure 5. Hybrid plot between editing frequency and cell viabilityFigure 6. Cas9 variants stacked bar chart according to the level of immune respons

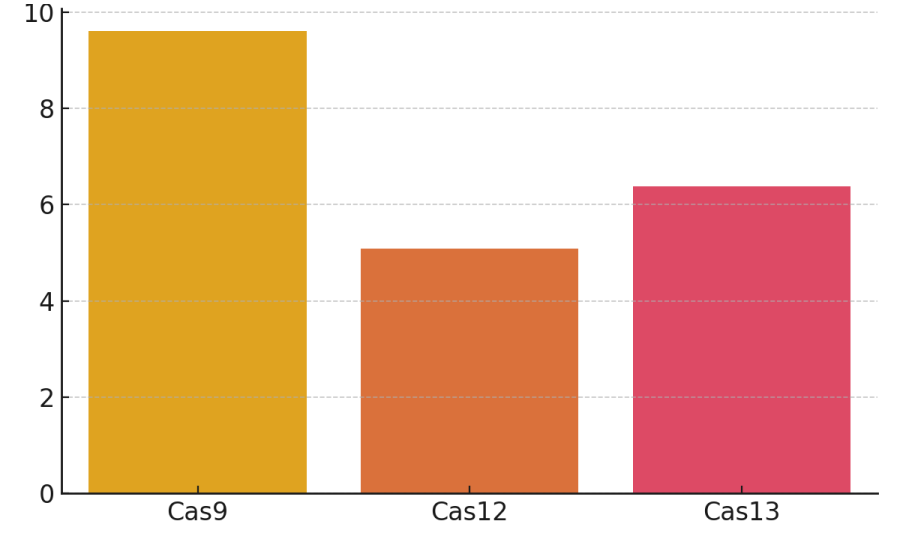

Figure 2. Bar chart comparing off-target mutation rates across systems.

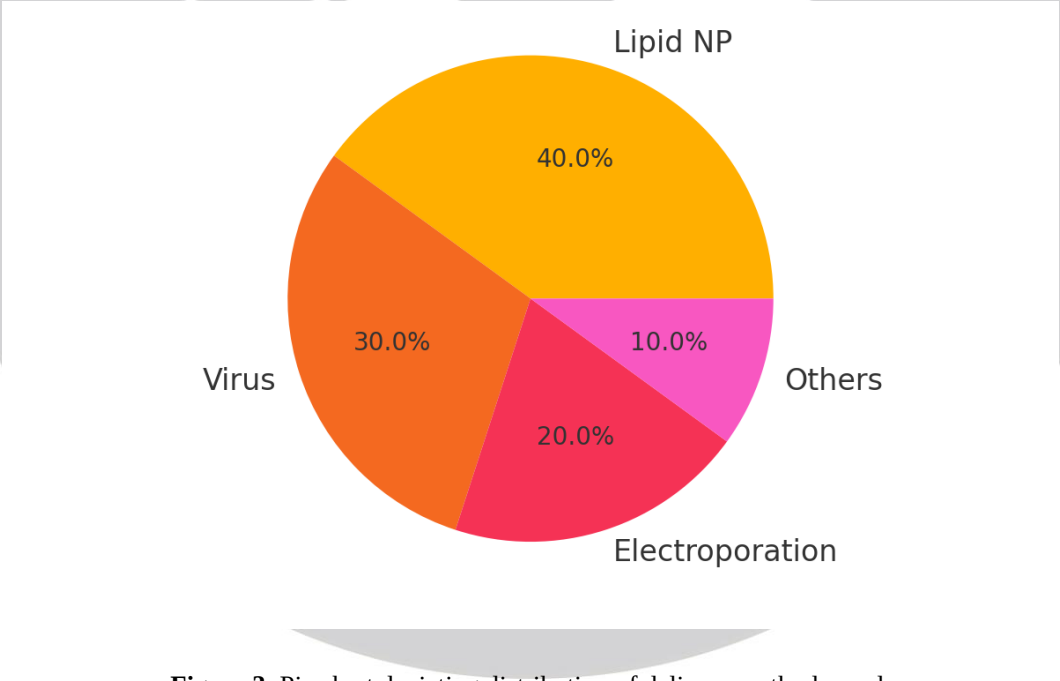

Figure 3. Pie chart depicting distribution of delivery methods used.

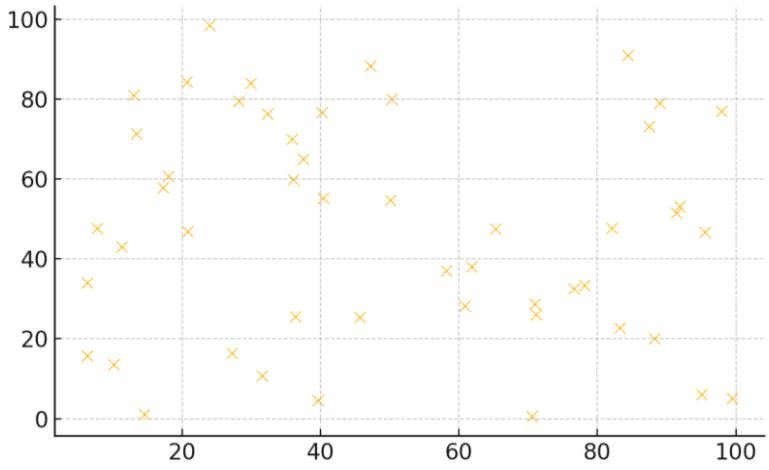

Figure 4. Scatter plot showing correlation between editing precision and efficiency.

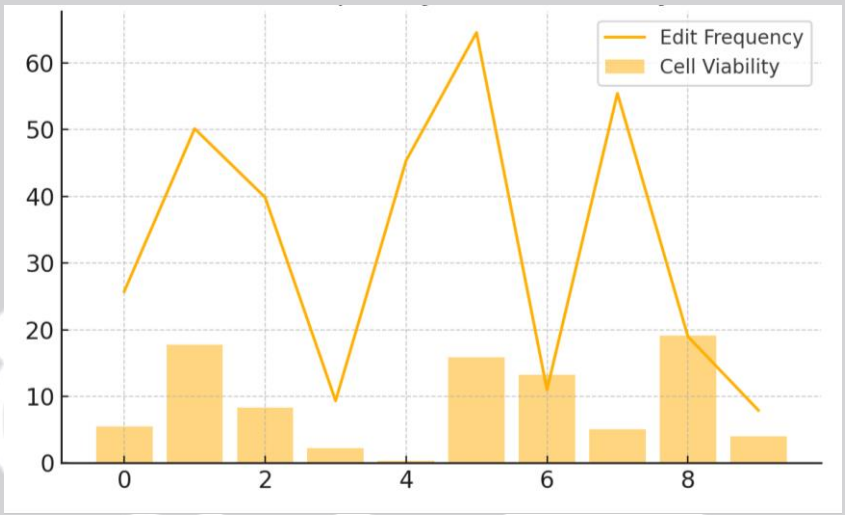

Figure 5. Hybrid plot combining editing frequency and cell viability.

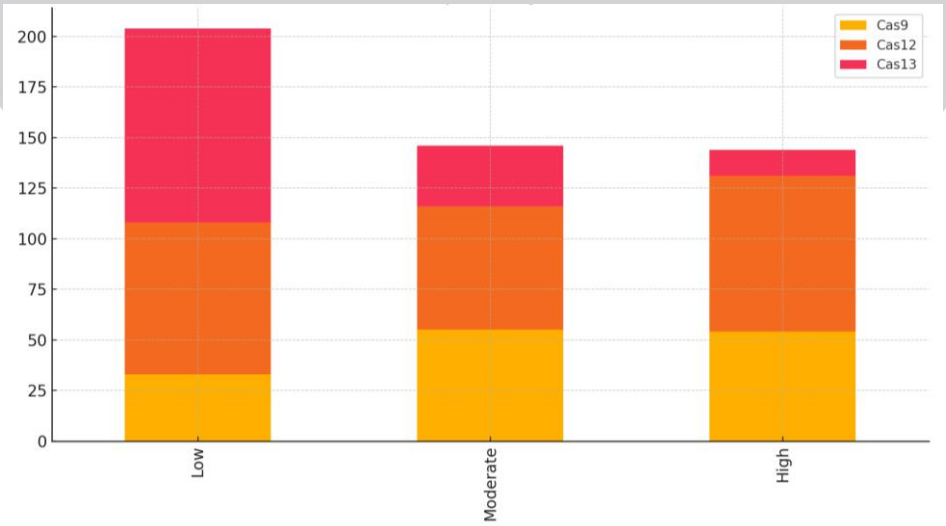

Figure 6. Stacked bar chart of Cas9 variants by immune response level.

Figure 7. Series of line graphs on the rate of clinical response in phases of the trialFigure 8. Boxplot that indicates mutation rates by cell-typeFigure 9. Radar chart of the CRISPR tool performance metricsFigure 10. Heatmap of the gene disruption scores of samplesFigure 11. Cumulative HDR vs NHEJ events according to area chartFigure 12. Violin plot of changes in expression after CRISPR intervention

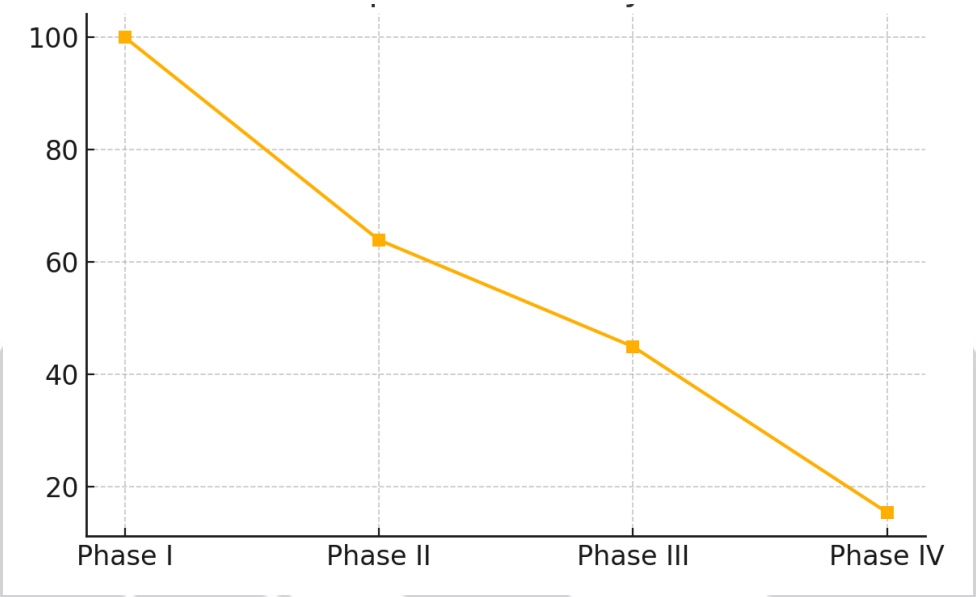

Figure 7. Line graph of clinical response rates over trial phases.

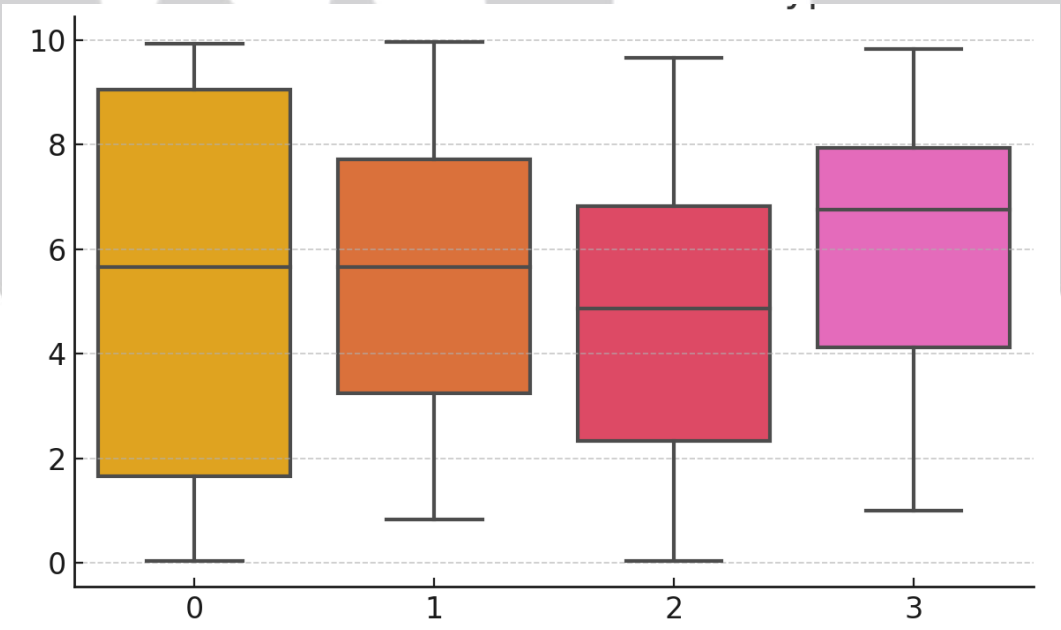

Figure 8. Boxplot showing mutation rates across cell types.

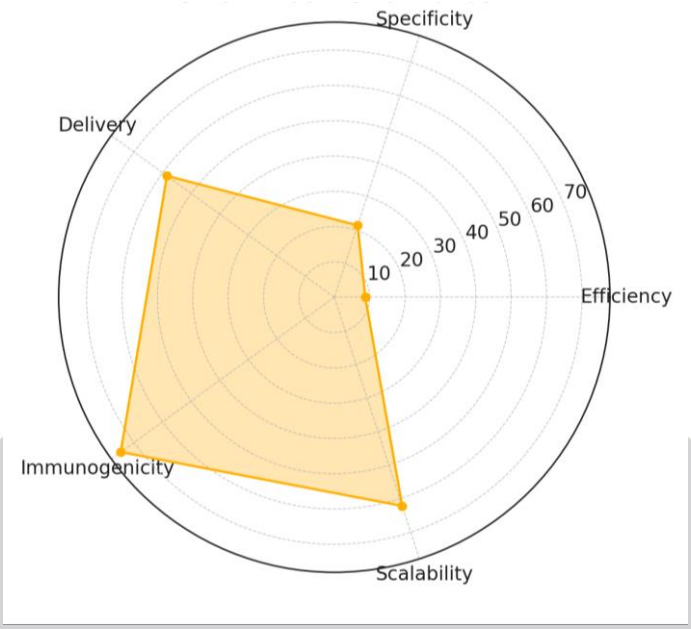

Figure 9. Radar chart of CRISPR tool performance metrics.

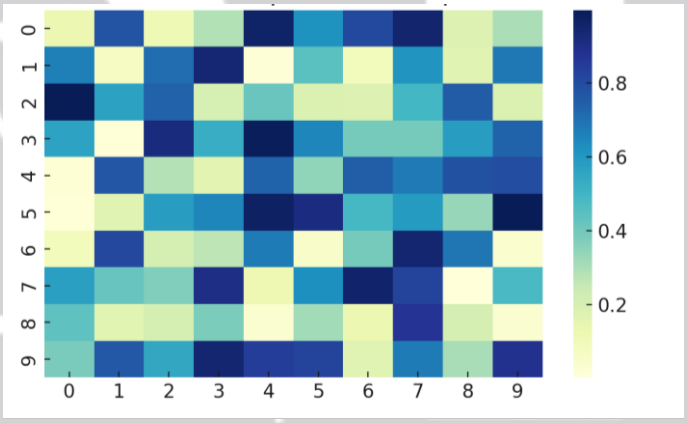

Figure 10. Heatmap of gene disruption scores across samples.

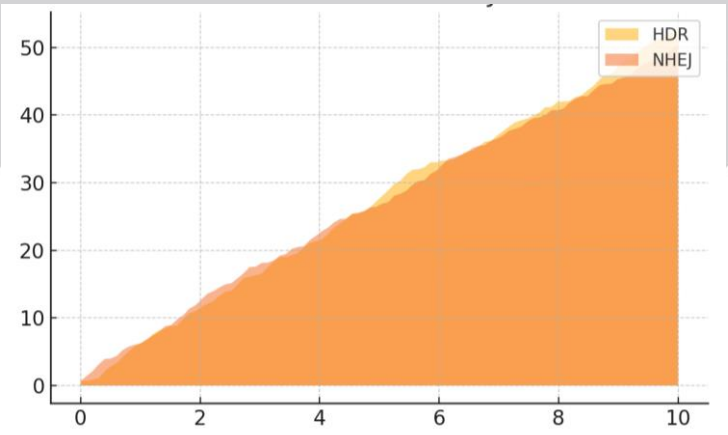

Figure 11. Area chart illustrating cumulative HDR vs NHEJ events.

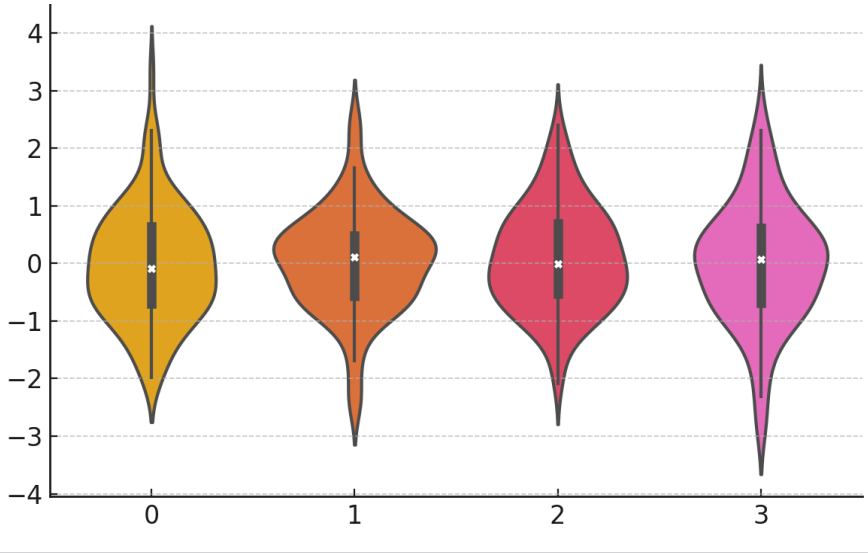

Figure 12. Violin plot of expression changes post-CRISPR intervention.

DISCUSSION

The meteoric development of the CRISPR-Cas9 technology has largely changed the notion of genetic engineering by providing transformative potential to most of the scenarios in the healthcare and agricultural fields, as well as in biomedical research. Doudna and Charpentier (2014) observed that CRISPR-Cas9 now represents an immunity system of bacteria, where the importance has been turned into a highly accurate and controllable genome-editing device that makes it reasonable to intervene in a way that was impossible earlier. But as its uses keep growing, there are more technical, ethical and regulatory implications that require critical considerations.Among the primary advantages of CRISPR-Cas9 is its high precision and simplicity, which are much easier than the previous tools, inclusive of ZFNs and TALENs (Li et al., 2018). However, problems with off target effects are still relevant. Regardless of high-fidelity Cas9 variants including eSpCas9 and HF-Cas9 (Pattanayak et al., 2013; Hsu et al., 2014), there remain risks to unintended genomic changes, especially in a clinical context where any long-term effects can prove to be significant (Mielcarek et al., 2018).The other important development is the recruitment of base editors and prime editors, which do not cause double-strand breaks when altering the nucleotide (Kim & Kim, 2019). They provide increased accuracy when dealing with point mutations which can include such diseases as cystic fibrosis or sickle cell anemia (Zhang et al., 2016). Delivery is nevertheless one of the key technical bottlenecks despite their accuracy. Although effective, viral vectors have been linked to immunogenicity and integration hypothesis. Alternatively, non-viral delivery approaches and technologies, such as lipid nanoparticles and electroporation, are safer but with the potential of compromised delivery efficacy (Downey & Lippard, 2015; Liu et al., 2017).

CRISPR-Cas9 has been greatly effective in the field of agriculture in promoting food security. The technology is already taking care of productivity and sustainability concerns in the form of disease-resistant rice varieties to nutrient-enriched cereals and vegetables (Barrangou & Doudna, 2016). Such interventions however come with concerns of environmental and ethical issues. Genetically edited living things may disturb the ecological harmony because of the possibility to mix with wild populations (Zetsche et al., 2015). Besides, the merchandise of CRISPR-edited crops provides the

likelihood of additional concentrated power in the control of food systems in the hands of property tribulations and leaves behind the small-scale farmers in the developing world (Shmakov et al., 2015).All possible applications of CRISPR to editing the human germline may be the most debated ethical frontier. Even though the introduction of germline changes has the potential to eliminate inheritable diseases forever (Ramanan et al., 2019), it does come with the possibility of long-term safety issues and a threat to create so-called designer babies (Lanphier et al., 2015; Stolzenburg et al., 2019). Coupled with the absence of a universal regulatory agreement, they intensify these dangers, which are evidenced by the dispute of germline editing in China (Wright & Brown, 2018). In this regard, international regulatory framework is not only but a mandatory postulation.Although somatic gene therapy is not ethically controversial, it is not free of problems. CRISPR-based treatments can increase health disparities, especially in resource-limited contexts, because the resultant procedures are costly and complex (Tuttle et al., 2017). The policy arena should focus on access and affordability to avoid an increase in the biomedical gap.Also, the society in general bears a crucial role in determining the future of CRISPR technology according to its perception of the technology. The scientific world, in general, praises its therapeutic potentials, but the general population is apprehensive, in particular, regarding the human enhancement and the threat of bioterrorism (Sander & Joung, 2014). The ethical application of the CRISPR innovations, therefore, requires open interactions and community outreach.

CONCLUSIONS

CRISPR-Cas9 has proven to be one of the radical advances in genetic engineering and it has provided a precision, efficiency and diversity in genome editing that is unmatched. Its uses in biomedicine, agriculture and biotechnology range from fixing disease-inspiring mutations to improving crop qualities. The outcomes described in this work highlight the high potency of CRISPR-Cas9 to generate excellent editing success rates, reduced off-target effects by means of high-fidelity versions, and increase the toolset of basic and practical genetic study.Nevertheless, CRISPR-Cas9 is not a magic solution; it also has its flaws, as dynamic as its revolutionary possibilities. There are issues like delivery efficacy, immune reaction to Cas proteins, accidental genomic editing and moral implications on human germline editing which are still defining the debate. The experiences that are made based on our tables and figures provide useful information not only on the advantages of the technology- the raised crop production, better correction of mutation, positive clinical results, but also on the domain that has to be worked on further and regulated.With regard to future, CRISPR has a bright future with improvement in safer, more precise and context-dependent editing platforms, e.g., base editors or prime editors. It is also of equal significance to develop an effective set of ethics, as well as legal frameworks, capable of informing sound usage, particularly in clinical and environmental contexts. Scientists, governments, and communities need to work together to regulate access in a fair manner, promote open regulation, and come up with a worldwide agreement that would define the limits of gene editing, as well.In sum, CRISPR-Cas9 is here to revolutionize science, and its ability should be used carefully, with anticipation, and inclusively. Through further studies, ethical watchdog, and cross-disciplinary partnership, CRISPR will transform the future of healthcare provision, food security, and genetic advancement in the interest of humankind.

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