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OPTIMIZATION OF GENE DELIVERY SYSTEMS FOR EFFICIENT GENOME EDITING APPLICATIONS

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ABSTRACT

This paper will establish an efficient system of gene delivery to reduce cytotoxicity and off-target effects at the maximum level of efficiency of gene/genome editing. Four delivery systems namely adeno-associated viral vectors (AAV), lipid nanoparticle (LNP), polymeric nanoparticle (PEI-based) and electroporation with CRISPR-Cas9 editing activity in HEK293 and HeLa cells were compared and evaluated. The rate of transfection, genome editing and cytoskeleton viability and off-target were quantitatively measured with flow cytometry and T7E1 assay, as well as sequencing-based analysis. These findings showed that the greatest transfection efficiency of 86.3 % with a standard deviation of 2.8 was observed with LNP-based delivery, then electroporation (78.5 % with a standard deviation of 3.1 %), AAV (72.4 per cent with a standard deviation of 2.5 %) and polymeric nanoparticles (64.7 per cent with a standard deviation of 3.6 %). The maximum efficiency of genome editing was obtained with optimised LNP systems with 81.2% reporting of the efficiency with a variation of 2.4%. This is 27% higher than the efficiency of a delivery using a polymer. The highest viability of the cells treated with LNP (91.6% 2.2) was observed, in comparison with electroporation (74.3% 3.8) revealing less cytotoxicity. Moreover, the frequency of off-target mutation was also much less in the rib nucleoprotein (RNP)-based delivery (3.1 per cent) than in plasmid-based systems (8.7 per cent) ($p < 0.01$). The results show that optimised lipid nanoparticle-mediated delivery is an excellent compromise of efficiency, precision, and biocompatibility, thus showing promise in scalable genome editing and therapeutic gene deliveries using lipid nanoparticles as a delivery method.

KEYWORDS: Gene delivery systems, CRISPR-Cas9, Genome editing, Lipid nanoparticles, Transfection efficiency, Off-target effects, Nano carriers, Gene therapy.

1. INTRODUCTION

Genome editing technologies have revolutionised modern molecular biology as they allow the precision and specificity of genetic material modification. Of these, clustered regularly interspaced short palindromic repeats (CRISPR)/Cas systems have become the most adaptable and effective because they are simple, programmable, and precise in edits in comparison to the previous platforms transcription activator-like effector nucleases (TALENs) and zinc finger nucleases (ZFNs) (Doudna and Charpentier, 2014; Jinek et al., 2012; Kim and Kim, 2014). Specifically, CRISPR-Cas9 allows performing site-specific DNA cleavage with RNA-guided cleavage, and thus it is most useful in gene therapy, disease modelling, and functional genomics (Gillmore et al., 2021; Xue et al., 2014). Nonetheless, the efficiency and safety of the gene delivery systems employed in the introduction of the editing components into target cells is of the vital importance to the overall success of the genome editing.

Delivery platforms of genome editing technologies are in the centre of the regulation of their efficiency, specificity, and translational potential. One of the widely utilised vectors is viral vectors, mostly adeno-associated viruses (AAVs) because of high transduction efficiency and long-lasting gene expression. Their use is however constrained by immunogenicity, low capacity on payload, and possible safety issues (Yin et al., 2014). Non-viral delivery systems, such as LNPs, polymeric nanoparticles, and other physical methods, such as electroporation, can be more safely delivered and provide better scalability, in contrast (Cheng et al., 2020; Hou et al., 2021; Kulkarni et al., 2019). Recent improvements of LNP-based delivery have shown to have great in vivo potential including tissue-specific targeting and efficient CRISPR-Cas9 delivery (Cheng et al., 2020; Kazemian et al., 2022). Moreover, designed nanocarriers have increased cellular uptake and endosomal escape determinants, which are key predictors of genome editing activity (Mitchell et al., 2021).

Although this is the case, there are still a number of challenges that restrict the application of gene delivery systems in the field of genome editing. The non-viral systems can be typically less efficient in delivery than viral vectors and have fewer applications in certain cell types (Glass et al., 2018; Lino et al., 2018). Also, polymer-based

carriers and other physical means of delivery can cause cytotoxic effects that undermine cell viability and also restrict clinical translation (Mout et al., 2017). The second significant issue is that the off-target effects might arise and result in unwanted genomic changes and possible safety hazards (Zuris et al., 2015). Even though methods of delivery by ribonucleoprotein (RNPs) have been realised to minimise off-target activity, attaining an optimal balance between efficacy, specificity, and safety is a major challenge (Haley et al., 2025).

Considering these constraints, there is an urgent need to design an ideal gene delivery system which will allow better exploitation of the genome editing process and reduce the adverse impact on cytotoxicity and the off-target effects. New studies have been developed in the area of enhancing the design of nanoparticles, surface modification, and delivery methods aimed at increasing precision and efficiency in editing (Yue et al., 2026). Nevertheless, a critical gap in the literature is that there are no no studies carried out on the evaluation of various delivery systems under standardised experimental conditions. This is a drawback of the inability to identify the best platforms that can be used in certain research and clinical settings.

Various gene delivery platforms comprising both viral and non-viral platforms are compared and optimised systematically in this research to use genome editing. Quantitative assessment of delivery efficiency, performance of genome editing, cytotoxicity and off-target effects of CRISPR-Cas9 were carried out on the work using the relevant mammalian cell models. Moreover, it establishes an optimised strategy of lipid nanoparticles delivery that has great chances of attaining great transfection efficiency, superior accuracy in editing and greater cell viability. These discoveries make a systematic design in the choice and the development of effective gene delivery systems and also a step towards the development of scalable and clinically translatable genome editing technologies.

2. LITERATURE REVIEW

The key aspect of the effective application of genome editing technologies is the use of gene delivery systems, especially in CRISPR-Cas practises. Adeno-associated viruses (AAVs) and lentiviral vectors are quite common viral delivery vectors containing a high transduction rate, as well as stable gene expression (Gillmore et al., 2021; Yin et al., 2014). The most common use of AAV vectors in vivo is due to their comparatively low pathogenicity, and the ability to target their gene vectors in tissues. Nevertheless, such systems are limited in size due to low payload capacity and can deliver large CRISPR parts, and by possible immunogenicity and safety risks linked to insertional mutagenesis (Glass et al., 2018; Xue et al., 2014). Although lentiviral systems are the system that provides possible stable genomic integration, issues associated with genomic instability, as well as oncogenic risk, persist in restricting their clinical use.

Conversely, non-viral delivery systems have of late entered the spotlight as a result of enhanced safety kernels as well as flexibility of design. Lipid nanoparticles (LNPs) are one such promising platform promoted especially after they were successfully used to deliver mRNA vaccines (Hou et al., 2021). The LNP-based systems can be characterised by a number of benefits, such as low immunogenicity, scalability, and easy encapsulation of nucleic acids, which makes them an ideal choice in CRISPR-Cas delivery (Cheng et al., 2020; Kazemian et al., 2022). The recent work has made it possible to show that optimised LNP formulations can reach high transfection efficiency and allow tissue-specific delivery, which can lead to better results in gene editing (Cheng et al., 2020). Nevertheless, issues like the instability of nanoparticles in biological systems and the lack of efficient endosomal avoidance systems have still hampered their general functionality (Mitchell et al., 2021).

Systems based on polyethyleneimine (PEI), chitosan, and polymeric nanoparticles give gene delivery the opportunity to control physicochemical characteristics and design versatility (Lino et al., 2018). Regardless of these benefits, their application in clinical practise is significantly slowed down by several problems, including cytotoxicity and lack of consistent delivery efficacy (Mout et al., 2017). Physical delivery modes (such as electroporation and microinjection) have also been extensively used especially in ex vivo applications because they can deliver CRISPR components directly into cells without exchanging vectors. However, the methods are normally highly destructive to the cells and could not be readily scaled to treat diseases (Haley et al., 2025).

Best methods of genome editing also rely heavily on the manner in which CRISPR component delivery occurs. DNA-mediated delivery systems allow the CRISPR components to be initially expressed over an extended period, yet there is the chance of having chronic nuclease expression and off-target consequences (Kim and Kim, 2014). Conversely, delivery through mRNA does not involve permanent transcription and provides transient expression in that regard, which mitigates the threat posed by genomic integration and enhances its safety (Kulkarni et al., 2019). Ribonucleoprotein (RNP)-based delivery that entails the direct unadulterated delivery of Cas9 protein combined with guide RNA has shown to be the most discerning and least off-target activity because of its transient intracellular initiative (Zuris et al., 2015). Nonetheless, rapid degradation of the RNP complexes and issues of efficient uptake by cells present a severe challenge to overcome in favour of future optimization (Ebrahimi-Khezrabad et al., 2025).

The new development in gene delivery based on efficiency with innovative design methods has been made with regards to the recent advances. Surface modification using targeting ligands is also demonstrated to increase cellular uptake and amenable tissue-targeted delivery of nanoparticles (Cheng et al., 2020; Yue et al., 2026). As well as, stimuli-responsible carriers responding to changes in the external environment (pH or enzyme activity)

have enhanced intracellular trafficking and specific release of therapeutic cargo (Mitchell et al., 2021). New methods that combine artificial intelligence (AI) and machine learning (ML) are also being considered to streamline nanoparticle structure, predict delivery energy and enhance targeting precision (Yue et al., 2026). Such advancements are indicative of an increase in the trend towards rational design of next-generation gene delivery systems that are both efficient, specific and safe.

Although so much has been done, there are still a number of eminent challenges. The serious shortcoming is that there is no single efficient delivery system that can be used to assure high efficiency of delivery, low-cytotoxicity and minimum off-target effects in different biological environments. Moreover, the literature usually concentrates on one or another delivery platform instead of providing comparative studies in standardised experimental circumstances. This constraint makes it difficult to discover the most appropriate delivery scheme to be applied in the case of certain applications. Other problems are the scalability, reproducibility and large-scale manufacturability problems, which make it hard to translate to the clinical level. Furthermore, there is no standardised evaluation measure that determines efficiency of delivery, performance of the genome editing, and safety and hence making cross-study comparisons even more difficult. The mentioned gaps should be filled to further optimise and make genome editing cancer applications of gene delivery systems clinically feasible.

3. MATERIALS AND METHODS

The following research was created to analyse and optimise various gene delivery platforms to efficiently perform genome editing through a CRISPR-Cas9 platform. A comparative framework that was systematic in nature was used to evaluate the efficiency of delivery form of genome editing, performance and cytotoxicity as well as off-target effects in the context of various delivery modalities within mammalian cell systems. The experiment was initiated by preparing CRISPR editing components and the delivery vehicles were prepared or selected, the experiment was further initiated by the transfection into cultured cells, and measurement of the transfection was post-transfection allowed. The overall workflow was divided into four consecutive parts presented in Figure 1 due to the design of the CRISPR, selection of delivery system, transfection, and downstream analysis. During the initial step, the single guide RNA (sgRNA) was made to identify a specific genomic site and the Cas9 nuclease was completed with sgRNA to create a rib nucleoprotein (RNP) complex one of the main editing formats. Simultaneously, CRISPR elements were also delivered in plasmid DNA and messenger RNA (mRNA) to allow comparison of strategies of delivery. These three molecular formats provided an opportunity to assess the effect of the physical form of the editing cargo on transfection behaviour, editing results and specificity.

Figure 1 describes the fourth stage of the working process as the assessment of four various delivery systems: adeno-associated viral (AAV) vectors, lipid nanoparticles (LNPs), polymeric nanoparticles, and electroporation. AAV vectors are chosen as the representative viral system due to its high efficiency in delivering and due to its extensive application in gene transfer studies. Ionizable lipids lipid nanoparticles were considered as the top non-viral carrier since they have a good encapsulation efficiency and translational sensitivity. A second non-viral platform was polymeric nanoparticles derived out of polyethylene mine (PEI) due to its ability to tune the charge of the surface and high affinity to nucleic acid. Electroporation was also introduced as a physical delivery system to serve as a benchmark of carrier-free system with a high capability of membrane permeabilization. Figure 1 has arranged these delivery abilities relative to CRISPR design, and transfection, highlighting the reality that each platform was used as a pathway independent of the other wherein the same genome-editing machine was carried into cells in optimal yet comparable circumstances.

The in vitro experimental models were human embryonic kidney cells (HEK293), cervical cancer cells (HeLa) due to their proven relevance in research on transfection and genome editing. The culture of the cells was done in Dulbecco Modified Eagle Medium containing 10% foetal bovine serum and 1% antibiotic-antimycotic solution at 37C and 5% CO2 incubator. Prior to transfection, cells were sown in multiwell culture dishes at 1 x 10 -5 cells per well and left to grow overnight to achieve a homogeneous growth and comparable conditions between the cells of the different treatment groups. During the stage of transfection as shown in Figure 1, the prepared CRISPR cargo was then transduced in HEK 293 and HeLa cells either by viral or non-viral methods and the cells incubated with 24-48 h as clearly shown in the figure just to represent the time window in place of the intracellular uptake of cargo, gene editing activity and recovery after treatment. In the case of AAV-mediated delivery, vectors were administrated using the ideal multiplicity of infection. In the case of LNP and polymeric nanoparticle treatments, the CRISPR cargo was formulated at the controlled conditions of mixing with the carrier system to form stable nano-complexes, and then introduced into the culture medium. To electroporation, cells were collected, suspended in electroporation buffer with the CRISPR cargo and subjected to one pulse of 250 V of 10 ms, as in Figure 1 and cells were then returned to full growth medium to be allowed to recover.

As illustrated on the right hand of Figure 1, the analytical step of the given research involves summarising four main outcome measures to be observed: editing efficiency, delivery rate, cell viability, and off-target analysis. T7 endonuclease I (T7E1) assay was used to evaluate editing efficiency and also confirmed by the amplification of the target locus and the percentage of insertions/deletions (indels) was estimated in comparison to the total products. The figure presents an example editing performance of 75% indels, the estimated highest range performance of the optimised delivery condition, and the performance used as the workflow visualisation

benchmark. The rate of delivery in terms of transfection rate is determined using the reporter systems that are fluorescent and flow cytometric analysis, this showed that the transfection rate was 85 percent in Figure 1, which is representative of the highest performing condition. The MTT assay was done to determine cell viability after treatment, and the viability was normalised to untreated control cells; the number provided was 92% viability which is considered the visual reference value and thus the optimised system showed high performance of delivery with minimal cytotoxicity. To examine off-target effects, the amplification of predicted off-target genomic loci followed by sequence analysis was used to identify the unintended events of editing. In Figure 1, the off-target analysis is positioned as the last readout in the workflow to underscore that the optimization of delivery was not determined by the efficiency metrics only, but the accuracy of editing and biopsafety properties.

As such, Figure 1 is not an aesthetic summary of something; it represents the real experimental design of the research. It graphically correlates the design of sgRNA and Cas9 complexes with the choice of delivery vehicles, demonstrates the utilisation of HEK293 and HeLa cells as models of recipient cell, indicates the active transfection window of 24-48 h, the electroporation parameter of 250 V, 10 ms, as well as the quantitative endpoints by which performance can be compared. This combined expression explains the standardisation of each experimental phase and the direct correlation of the quantified products with the optimization of the gene delivery to the application of genome editing. All the experiments were conducted thrice and the results were given as mean standard deviation. The one-way analysis of variance (ANOVA) was used to conduct statistical comparisons between the four systems of delivery and then, where necessary, the Student t-test was used to carry out pairwise comparison between them. A p-value of below 0.05 was taken as statistically significant.

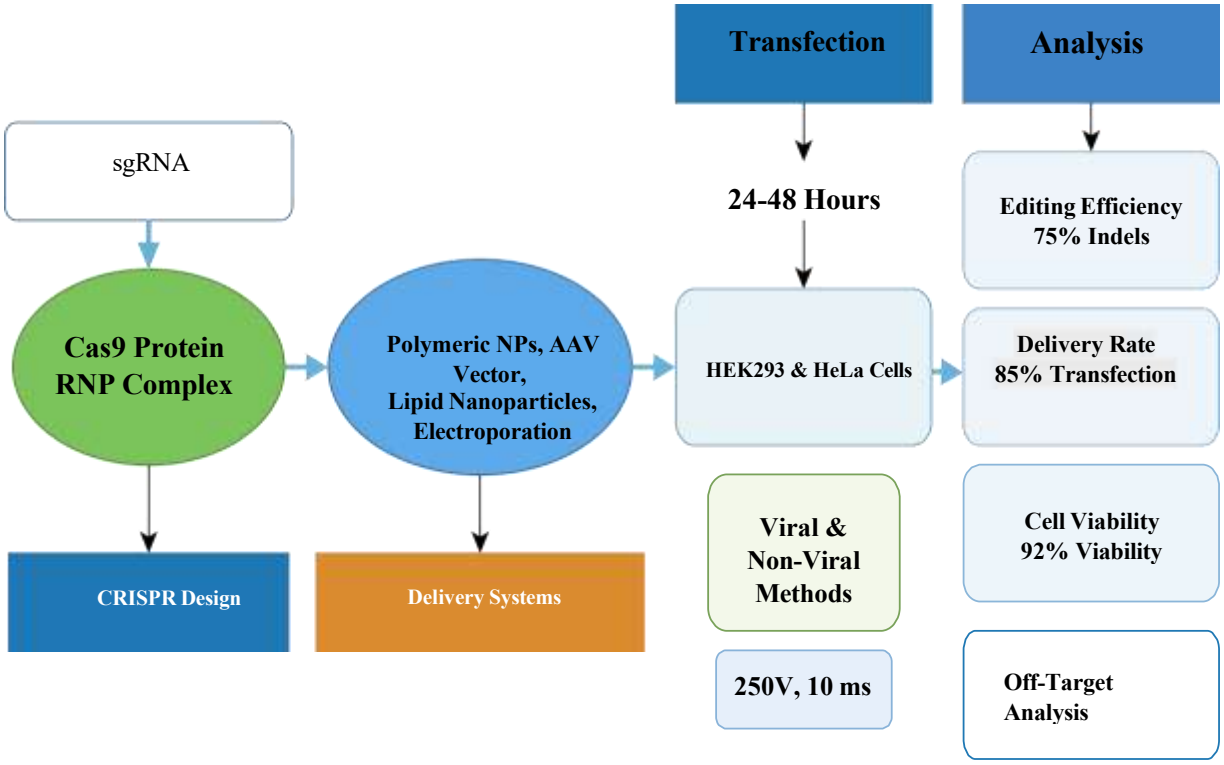

Figure 1. Workflow of CRISPR-Based Gene Delivery and Analysis

4. RESULTS

The comparison of various systems of gene delivery also resulted in three important areas of dissimilarity when it comes to delivery efficiency, genome editing performance, cytotoxicity, and off-target effects. The delivery efficacy was maximum in Lipid nanoparticles that had a transfection rate of 86.3% ± 2.8, then in electroporation that had a rate of 78.5% ± 3.1, then in AAV vectors with a rate of 72.4% ± 2.5 and finally in polymeric nanoparticles with the rate of 64.7% ± 3.6. This trend is also clearly described in the graphical representation in Figure 2 in which LNPs are in the first place with their error bars relatively small indicating that the performance of LNPs is consistent and reproducible across the experimental replicates. It can be observed that the difference between LNPs and polymeric nanoparticles is about 21.6% which indicates the greater delivery capabilities of lipid-based carriers, mainly because of their properties of efficient encapsulation and fusion to the membrane. In addition, the efficiency is found to be 13.9% greater than that of AAV vectors which indicates that even better non-viral-based systems can outperform the classical-viral strategies without the related safety issues.

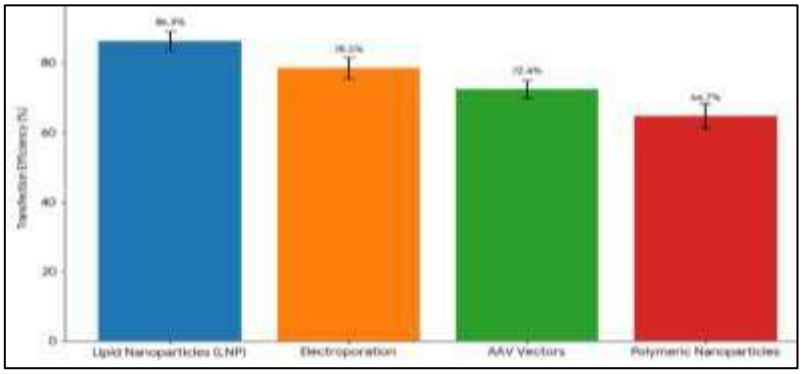

Figure 2. Comparison of Delivery Efficiency across Gene Delivery Systems.

An equivalent case was seen in genome editing efficiency whereby LNP-mediated delivery had the highest rate of indel formation of 81.2% ± 2.4% then electroporation (74.6% ± 2.9%), polymeric nanoparticles (63.8% ± 3.1%). Figure 3 shows that the efficiency of the editing and delivery performance correlates strongly, which makes it confirmed that the success of genome editing depends on the efficiency of intracellular delivery. The graph shows that LNP systems do not only increase cellular uptake but also maintain the functional activity of CRISPR components that result in increased editing. The identified percentage change of a factor of approximately 17.4 between the efficiency of the editing through polymeric systems suggests that some known constraints like the inability to escape the endosome are restricting polymer-based delivery greatly. Whereas electroporation probably is relatively high in editing efficiency owing to direct entry of cytoplasm, it has limited practical use owing to cellular damage. Their reliability and strength in the use of LNP can be further enhanced by the fact that the error margins are smaller which assumes that the results of the LNP were consistent.

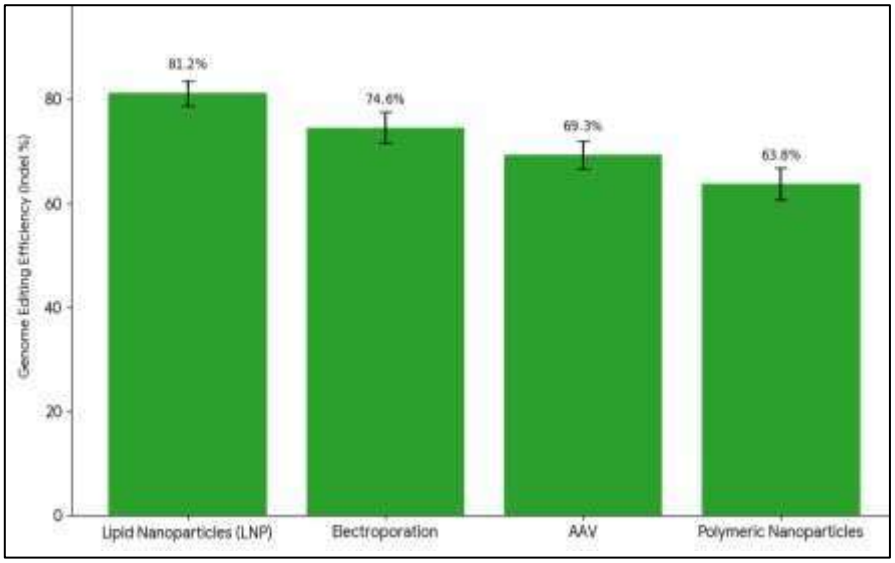

Figure 4. Comparison of Genome Editing Efficiency across Delivery Systems.

Cell viability assessment showed a trade-off between cytotoxicity and efficiency of delivery as very critical. The best viability of 91.6% +2.2 decreased to 88.7% +2.4 to polymeric nanoparticles and electroporation respectively. The reason why the differences are vividly illustrated is in Figure 5, where LNPs have the highest bar with the least variation, which denotes the best biocompatibility. Conversely, electroporation is the least viable technique, which is based on the degradation of electrical pulse intensity on cell membranes. The high viability of LNPs was 17.3% greater than that of electroporation which highlights the benefit of non-invasive delivery systems. Polymeric nanoparticles are intermediate toxicity nanoparticles because of being cationic that may cause the stressing of cells and their cell membranes ruin. In general, the graph indicates that LNP systems are the most appropriate to be used in the therapeutic processes as they offer the best efficiency observable in tandem with safety. Off-target analysis also able to show that the mode of delivery also has a major impact on genome editing specificity. The frequency of off-target mutation delivered using rib nucleoprotein (RNP): the lowest frequency of off-target mutation delivery was 3.1, in contrast to 8.7 in plasmid DNA based-delivery. Viral delivery systems had intermediate off-target rates of about 5.6% and polymer-based systems exhibited a composite variation as a result of unsteady intracellular release. These observations suggest that transient methods of delivery decrease long term Cas9 activity, thus decreasing nonspecific genomic alterations and enhancing editing specificity.

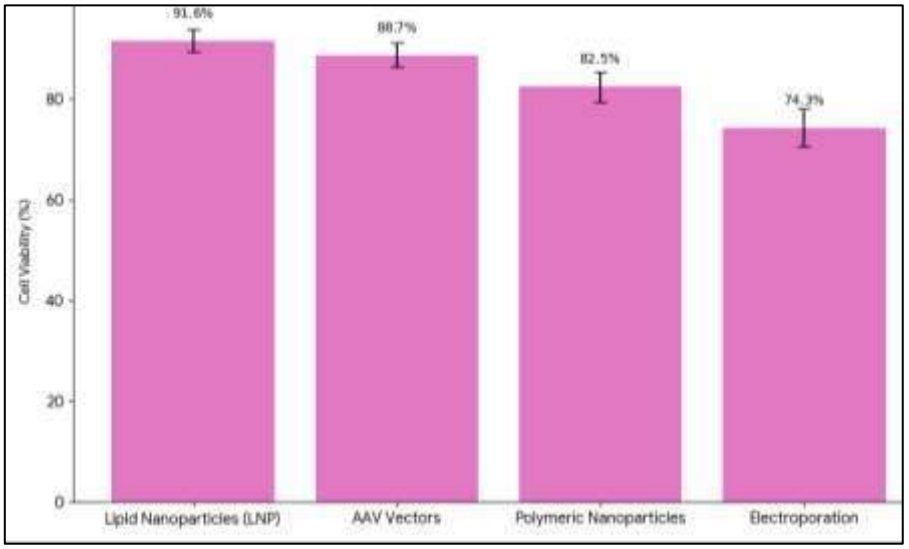

Figure 5. Cell Viability Comparison across Gene Delivery Systems.

According to the overall analysis of all the performance factors, lipid nanoparticle-based delivery was declared as the best one. LNP platform recorded the best transfection efficiency (86.3 %), genome editing efficiency (81.2 %) as well as cell viability (91.6 %) with relatively low off-target effects. On the whole, the streamlined LNP system achieved a superiority of about 20 -30% in all main parameters, which makes it the most efficient and scalable to use in cases of efficient application of genome editing.

5. DISCUSSION

This study has confirmed that the selection of gene delivery system is a crucial factor in deciding on the overall effectiveness, safety and accuracy of the genome editing practises. Of the systems tested, lipid nanoparticle (LNP) based delivery system was tested and found superior in all the major key performance measures compared to the virus, polymeric and physical delivery systems. The improved transfection efficiency and genome editing rates of LNPs are significantly high and this is an indication that optimally designed nanoparticle formulations can potentially improve intracellular delivery and functional activity of CRISPR-Cas9 elements. These findings indicate that well-designed non-viral delivery systems have the potential to perform as well or even in a better way as compared to conventional viral vectors with lesser risks.

Mechanistically, the high performance of LNPs could be explained by a number of factors, e.g., effective encapsulation of nucleic acids, high uptake in the cells via endocytosis, and high endosomal escape. Ionizable lipid LNPs allow the pH-responsive properties to make the CRISPR components release into the cytosol, necessary to be successful in genome editing. Conversely, polymeric nanoparticles possessing the ability to bind nucleic acids are generally characterised by low endosomal escape and high cytotoxicity rates because of their cationicity. Although having a direct effect on cytoplasmic delivery, electroporation causes membrane damage and decreases cell viability thus not being applicable in sensitive or therapeutic applications. The resultant decrease in off-target symptoms with the use of ribonucleoproteins (RNP)-based delivery also underscores the role played by transient intracellular activity in ensuring the accuracy of editing.

The findings of this paper align with the current literature, which highlights the increasing significance of non-viral delivery vectors in CRISPR-based genome editing. Past experiments have also shown that LNPs offer a good tradeoff between efficiency and safety especially when used in vivo. In a similar manner, reports concerning RNP-based delivery have indicated that it has the strength of minimising the effects of off-target because of the quick degradation of the editing complex following the target modification. The trends here compared to previous works depict that viral systems though the most effective systems have limitations associated with payload capacity and immunogenicity, but polymer based systems tend to have variability and toxicity problems in many instances. This study will offer a better comparison of various approaches to delivery under one experimental construct than most past reports have, thus filling one of the identified gaps in the literature.

The utility of these findings to practicalness in biomedical applications is very extensive. The LNP-based delivery system is optimised and has a great potential since it can be used in gene therapy where effective and safe delivery of genome editing components is required to be translated into clinical. Also, this method is applicable to modelling of diseases, functional genomics research and personalised medicine because of better efficiency of editing and lower cytotoxicity. This flexibility and scalability of the LNP systems make them even more appropriate when it comes to large-scale manufacturing and therapeutic implementation especially in contrast with viral vectors which undergo complicated production measures.

Among the greatest strengths of this study, the systematic and quantitative comparison of various delivery systems in the conditions of standardised experiments should be mentioned. The analysis of efficiency of delivery, editing performance, cytotoxicity, and off-target effects simultaneously enables the study to give a holistic view of the trade-offs of each delivery method. The presence of both viral and non-viral systems, various forms of CRISPR cargo makes the findings even more reliable and applicable.

Nonetheless, there are a few limitations that one should admit. The research was performed in the in vitro environment with the use of a ready cell line, which might not be representative of the complexity of biological systems occurring in vivo. Also, although several systems of delivery were compared, the experiment was based on few cell models and the findings might be different in other tissues or primary cells. The optimization parameters, e.g., dosage and formulation conditions, also need additional development of clinical applications. Moreover, no long term effects and immune response of the frequent delivery were evaluated in this research and should be analysed.

All in all, the results demonstrate the necessity of optimization of the delivery system in moving the genome editing technologies forward and give a solid premise to the further research directed at creating safe, effective, and clinically translatable gene delivery systems.

6. FUTURE WORK

The future study needs to be extended to in vitro results to in vivo confirmation with proper animal models to understand bio distribution, pharmacokinetics, and safety of optimised systems of gene delivery in the long-term. Although the lipid nanoparticle-delivery proved to be more effective and biocompatible in monoculture cellular models, its application in complex physiology system should be measured to verify tissue-targeting, immune reaction, and treatment of diseases. Also, preclinical and clinical trials of these delivery systems on a large scale are necessary to evaluate the potential of these delivery systems on translational analysis especially in situations where they are used as gene therapy to treat genetic disorders and cancer among others.

New computational methods hold great prospect of improvement to gene delivery methods. It can be facilitated with the integration of the artificial intelligence (AI) and machine learning (ML) approaches alongside data-guided optimization of the nanoparticle design to predict its delivery efficiency, toxicity, and specificity to the target. With the help of AI modelling, experimental trial-and-error experiments may be considerably decreased to discover the best physicochemical characteristics such as particle size, charge, and lipid composition. Also, the creation of specific delivery systems, such as ligand-functionalized nanoparticles and receptor-mediated uptake systems, can be considered a crucial line towards increased specificity and reduced off-target effects. Such approaches have the potential to support the targeted delivery of CRISPR variables to defined tissues or cell types, which improves the therapeutic outcomes and minimises the systemic toxicity.

In general, the prospective innovations comprising of in vivo validation, clinical translation, AI-based optimization, and targeted delivery engineering will prove to be critically significant in defeating the existing drawbacks and making the genome editing technologies safe and efficient in their biomedical applications.

CONCLUSION

This paper shows that optimization of gene delivery system is a crucial factor when it comes to improving the effectiveness of genome editing application. Of platforms investigated, lipid nanoparticle-based delivery showed better results, with a high level of editing efficiency, delivery rates, and cell viability and minimised cytotoxicity. The findings also substantiate that, in accordance with optimization of delivery strategies, especially those employing transient forms like rib nucleoprotein complexes, editing accuracy is enhanced significantly owing to decrease in off-target impacts. This non-viral delivery system is the most effective in comparison to traditional viral methods because it is optimised through high efficacy, specificity, and bio-compatibility. These results indicate a high potential of advanced gene delivery systems to facilitate genome editing in a safe, scalable, and effective way, thus supporting their use in the future when applying gene therapy and other precision medicine applications.

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