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# COMPARATIVE ANALYSIS OF NON-VIRAL GENE DELIVERY SYSTEMS AND THEIR IMPACT ON CELLULAR UPTAKE AND GENE EXPRESSION EFFICIENCY

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## ABSTRACT

Non-viral gene delivery systems have become very popular because they are safer and more universal than viral vectors and have various benefits, including decreased immunogenicity, enhanced scalability, and versatile structure. Their extensive use in clinical practice is, however, restricted by poor efficiency in transfection and numerous intracellular obstacles. This paper provides a comparative study carried out in a systematic manner of 3 of the most well-known non-viral gene delivery platforms, such as lipid-based nanoparticles, polymeric carriers, dendrimers, and hybrid nanomaterials, regarding their ability to influence cellular uptake and cellular efficiency of gene delivery. The systematic analysis framework was used to analyze important parameters of physicochemical qualities in terms of particle size, surface charge, and compositional architecture, and their relation to biological performance. Endocytosis, endosomal escape, cytoplasmic transport, and nuclear import are some of the critical steps in delivery that were studied to find out factors that control the transgene expression rate. The results show that lipid-based and hybrid systems are better uptake and expression systems because of the increased association with membrane and an enhanced endosomal escape system whereas polymeric systems show constraints that is related to cytotoxicity and intracellular degradation. Moreover, profound surface engineering approaches like PEGylation and ligand functionalization are important to increase the stability of delivery and specificity in targets. In general, this research offers mechanical explanations and design principles of the optimization of non-viral gene delivery systems, to obtain a better therapeutic effect.

**KEYWORDS:** Non-viral gene delivery, nanocarriers, cellular uptake, gene expression, lipid nanoparticles, polymeric vectors

## 1. INTRODUCTION

Gene therapy is a breakthrough technology in treating genetic and acquired diseases as it provides a means of targeted delivery of nucleic acids into the cells with therapeutic purposes. Nevertheless, even with the significant advancement, the clinical application of gene therapy is still limited by the issues with the efficiency and safety of delivery. Another emerging focus is non-viral gene delivery systems as the viable alternative to viral vectors, which are less immunogenic, safer, and more flexible than viral vectors in design (Mohammadinejad et al., 2020; Wahane et al., 2020). These platforms comprise lipid-based carriers, polymeric nanoparticles, and hybrid nanomaterials and can provide scalable and customizable nucleic acid delivery platforms (Riley and Vermerris, 2017; Xiang et al., 2017). Recent breakthroughs in nanotechnology have made more complex non-viral carriers, which are highly complex regarding targeting and their physicochemical properties, which can be fine-tuned. Specifically, lipid-based systems have proven to be very promising since they can effectively interact with cellular membranes and can be used to entrap nucleic acids (Ponti et al., 2021). Moreover, PEGylation and other surface engineering methods have become common with the aim of enhancing the stability of nanoparticles, their circulation time, and biocompatibility (Suk et al., 2016). Although there are these advances, non-viral methods of gene delivery still have serious biological drawbacks that restrain their overall output. The process of genetic material delivery is complicated by a rather intricate chain of intracellular events, such as cellular uptake, endosomal escape, cytoplasmic trafficking, and nuclear import (Zhou et al., 2017). Endosomal entrapment is one

of the key bottlenecks among them since a significant amount of internalized cargo is destroyed before entering the cytoplasm (Brock et al., 2019; Pei and Buyanova, 2019). Moreover, the intracellular movement and nuclear penetration of plasmid DNA are also the most serious bottlenecks, especially with non-dividing cells (Bai et al., 2017). The net effect of these barriers is the low transfection efficiency that may be seen in most non virus systems. Though there is a lot of research work done on the development of individual delivery platforms, the research which has been done is usually based on single types of carriers or individual isolated mechanisms without providing a comprehensive comparative analysis of their performance. Specifically, systematic evaluation of the connection between physicochemical characteristics of delivery systems and the efficacy of cellular uptake and gene expression has not been performed. It is a weakness that makes it difficult to determine the best design strategies to enhance delivery outcomes. In this regard, the current research will make a detailed comparative discussion of the key non-viral gene delivery systems with special regard to their effects on cellular uptake and the efficiency of gene expression. The mutual influence of the insights obtained in the delivery mechanisms, material design enables the work to determine critical factors that control performance during transfection and suggest design principles by which to develop an effective and biocompatible non-viral gene delivery platform.

## 2. RELATED WORK

The recent progress in non-viral delivery of genes has seen the creation of various carrier systems that are designed to enhance transfection efficacy and reduce toxicity. Among the most researched platforms, lipid-based vectors, in particular, liposomes and lipid nanoparticles (LNPs), are capable of encapsulating nucleic acids and being transported to cells by means of membrane fusion and endocytic processes (del Pozo-Rodriguez et al., 2016; Zylberberg et al., 2017). Introduction of cationic lipids also increases the interaction with negatively charged cell membranes, which increases the efficiency of internalization and gene delivery (Ponti et al., 2021). Nonetheless, such issues as insufficient stability and the possibility of cytotoxicity are also central concerns in lipid-based systems (Wahane et al., 2020). There is also the potential of polymeric gene delivery system such as polyethyleneimine (PEI) and other cationic polymers which have shown very good potential as they are strongly electrostatically charged and can form stable polyplex with nucleic acids. These systems are capable of efficient condensation and protection of genetic material, but their functionality is usually impaired by cytotoxic effects and inefficient intracellular release (Riley and Vermerris, 2017; Xiang et al., 2017). Moreover, the effective balance between stability and delivery efficiency of polymer-based carriers is a longstanding problem of polymer-based carriers (Wahane et al., 2020). Combined lipid-polymer-based nanocarriers hybrid structures have become a potential solution to address the weakness of both systems. Such systems are an integration of high transfection capabilities of lipid-based carriers and the structural stability of polymeric systems, leading to better cellular intake and controlled release of genes (Gigante et al., 2019). Moreover, the recent advances in systems based on nanomaterials have allowed creating multifunctional delivery systems with a better targeting and tracking (Riley and Vermerris, 2017). In spite of these technological advances, there are some biological obstacles that still restrict the efficiency of non-viral systems of delivery of genes. Endosomal entrapment is still one of the most significant issues because a significant percentage of internalized nucleic acids is destroyed before entering the cytoplasm (Brock et al., 2019; Pei and Buyanova, 2019). Besides, intracellular movement and nuclear importation of plasmid DNA are further bottlenecks that decrease gene expression efficiency drastically (Bai et al., 2017). Recent research focuses on the significance of the knowledge of the gene delivery cascade, in which each of the steps, such as cellular uptake or nuclear entry, should be optimized to ensure an efficient transfection (Zhou et al., 2017). Whereas the available literature has investigated different carrier systems and design strategies, the majority of the literature deals with a single platform or a system in isolation. Comprehensive comparative studies that specifically correlate physicochemical characteristics, including the size of a particle, surface charge, and composition with biological effects (cellular uptake and the effectiveness of gene expression) are still missing (Mohammadinejad et al., 2020; Wahane et al., 2020). This is where this gap lies, as there is a need to design an integrated evaluation framework that will facilitate rational design of next-generation non-viral gene delivery systems.

## 3. METHODOLOGY

This paper is based on the comparative and analytical approach providing systematic analysis of the performance of non-viral gene delivery systems, particularly their effect on the efficiency of cellular uptake and gene expression. The review is grounded in a comprehensive review of peer-reviewed sources and experimentally confirmed research studies, so only high-quality and relevant information is taken into account. The methodology framework will develop clear connections between physicochemical characteristics of delivery systems and biological outcomes of it.

### 3.1 Selection Criteria

The relevant studies were selected through a constant literature search involving publications, which explore non-viral gene delivery systems in both in vitro and in vivo settings. The inclusion criteria were that the chosen articles specify the analysis of cellular uptake mechanisms, transfection effectiveness, or gene expression levels. Studies that used well-established experiment models such as mammalian cell lines and approved animal models were favored to provide consistency and reliability of the reported results. Further, quantitative or semi-quantitative data on the performance of delivery in the studies was given priority to enable easy comparative study.

### 3.2 Classification of Delivery Systems

Systematic categorisation of the chosen gene delivery systems was carried out according to their structural composition and functionality as in Fig. 1. The lipid-based carriers such as liposomes and lipid nanoparticles were classified under a single category as they are membrane-interactive and have been extensively used in the delivery of nucleic acids. Polymeric systems that include cationic polymers (polyethyleneimine and chitosan derivatives) were grouped differently because of their different electrostatic binding processes and polyplex formation. The systems made using dendrimers were regarded as a special category due to the highly branched structures and tunable surfaces. Moreover, hybrid nanocarriers that combine lipid and polymer backbones were recognized as a separate category because of their new importance in the integration of the benefits of several delivery platforms.

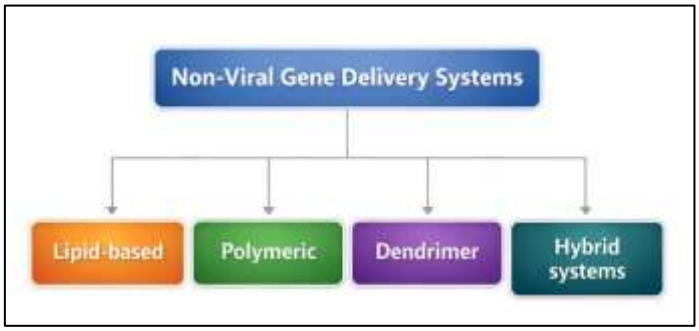


**Fig. 1. Taxonomy of Non-Viral Gene Delivery Platforms Based on Structural Composition**

### 3.3 Evaluation Parameters

Several parameters, both physicochemical and biological, were compared systematically to allow an all-inclusive comparison. Particle morphology and size were deemed as key defining factors of cellular internalization and biodistribution since nanoscale dimension affects endocytic routes. Surface charge which can be commonly characterized by zeta potential was assessed because it has a direct influence on membrane interaction and colloidal stability. The efficiency of cellular uptake was determined using the published cellular rates of internalization and the intensity of uptake using the widely reported measures of gene expression rates, e.g. reporter gene activity or protein to be expressed. In addition, cytotoxicity profiles were evaluated in order to establish the biocompatibility of every delivery system as toxicity is a significant impeding element in non-viral gene delivery.

### 3.4 Analytical Framework

To determine relationships between material traits and biological functions, a comparative analytical model has been created to show correlations as in Fig. 2. In particular, the model connects the physicochemical features, including the size of particles, surface charge, compositional framework, to the cellular uptake efficiency. Simultaneously, endosomal escape, cytoplasmic transport as well as nuclear localization were also measured concerning the results of gene expression. This two-tiered method is applicable to discover the rate-limiting stages in the gene delivery cascade and emphasize how the carrier design can affect overall efficiency of transfection. The approach to methodology has been capable of offering a systematic framework of the comparison of various non-viral delivery systems, offering the best design options through integration of such relationships.

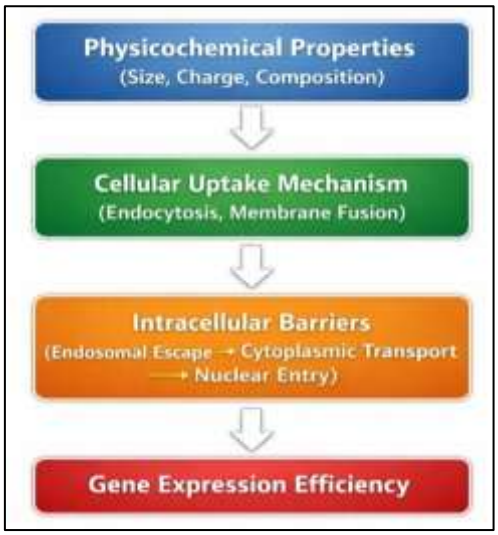


**Fig. 2. Conceptual Framework of Non-Viral Gene Delivery: From Physicochemical Properties to Gene Expression Efficiency**

## 4. RESULTS AND DISCUSSION

### 4.1 Cellular Uptake Efficiency

The key factors controlling cellular uptake efficiency are the size of the nanoparticle, surface charge, and compositional characteristics. Particles with sizes of 50-200 nm exhibit the best internalization by endocytosis, which aligns with the earlier results of nanocarrier action. Carriers with a positive charge (cationic) show an increased binding with negatively charged cell membranes leading to a higher uptake ability (Ponti et al., 2021; Suk et al., 2016). But too much surface charge may cause disruption of membrane integrity, causing greater cytotoxicity, and decreased overall performance. Comparative analysis, as presented in Fig. 3 reveals that lipid systems have a rapid internalization process, which is mediated by membrane fusion, whereas polymeric systems utilize mainly clathrin- or caveolae-mediated endocytosis. Hybrid systems have enhanced uptake efficiency since they have a combination of electrostatic interaction and membrane fusion properties and hence are able to overcome constraints of individual carrier types. These results are in agreement with other literature describing the effectiveness of physicochemical adjustments on the optimization of cellular internalization (Wahane et al., 2020).

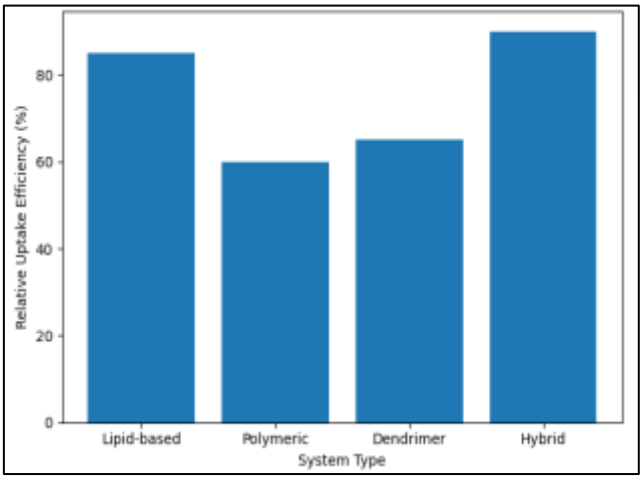


**Fig 3. Comparative Cellular Uptake Efficiency of Non-Viral Gene Delivery Systems**

### 4.2 Endosomal Escape and Intracellular Trafficking

In non-viral gene delivery, endosomal escape continues to be an important rate-limiting process since much of the internalized genetic content is destroyed in endosomal compartments. Polymeric systems have been extensively suggested to employ the proton sponge effect, which allows osmotic swelling and endosomal damage, but its use remains controversial (Brock et al., 2019; Pei and Buyanova, 2019). Lipid-based carriers, on the contrary, favor escape by destabilizing membranes by a process of fusion. Fig. 2 demonstrates that intracellular transport and nuclear localization have an additional impact on efficiency in delivery. Microtubule-mediated processes play a key role in cytoplasmic transport, whereas factors like the size of the plasmid and the dynamics of cell cycles determine nuclear entry (Bai et al., 2017). Hybrid systems can have a better intracellular delivery because of their capacity to combine various escape and transport strategies, and therefore, can improve the overall transfection results.

### 4.3 Gene Expression Efficiency

The successful delivery cascade, involving cellular uptake to nuclear entry, is directly related to gene expression efficiency. Those systems that are effective in overcoming intracellular barriers have a much higher level of transgene expression. Lipid-based and hybrid systems are more efficient in expression of genes than polymeric and dendrimer-based systems according to Fig. 4. Lipid nanoparticles have been demonstrated to show high expression rates because they can mediate uptake as well as endosomal escape resulting in an effective cytoplasmic release. Polymeric systems are efficient in condensing nucleic acids but lower expression levels are observed because of insufficient escape and intracellular degradation. Conversely, hybrid nanocarriers surpass the performance of each system to attain stability, efficiency in delivering therapeutic agents, and low toxicity (Gigante et al., 2019; Wahane et al., 2020). These findings agree with the findings of earlier studies that suggest the delivery cascade model, with the steps in this chain sequentially influencing the efficiency of global gene expression.

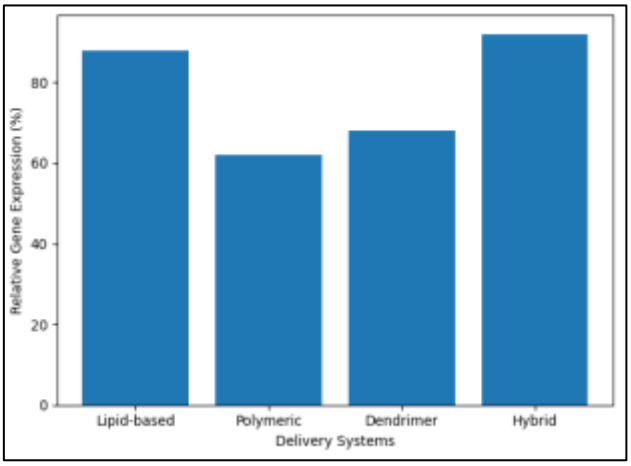


**Fig. 4. Comparative Gene Expression Efficiency of Non-Viral Gene Delivery Systems**

### 4.4 Cytotoxicity and Biocompatibility

A major constraint of non-viral gene delivery systems designs, especially of highly cationic polymeric carriers remains their cytotoxicity. Although the higher the positive charge the more the cellular uptake is increased, membrane disruption and loss of cell viability occur. PEgylation and other surface modification approaches have been demonstrated, to substantially decrease toxicity without affecting delivery efficiency (Suk et al., 2016). Lipid systems tend to be less cytotoxic because of their biocompatibility; but can be problematic with respect to stability at physiological conditions. Hybrid systems show enhanced biocompatibility because it fuses the merits of lipid and polymer constituents, leading to less toxicity without affecting functions. This underscores the necessity of achieving efficiency and safety in the design of carriers.

### 4.5 Comparative Summary

Table 1 gives a comparative analysis of the major non-viral gene delivery systems. The findings show that lipid-based and hybrid systems have a higher cellular uptake and gene expression efficiency, but polymeric systems have limitations in the form of cytotoxicity and intracellular barriers. The systems based on dendrimers demonstrate average efficacy, and the properties can be adjusted to tuning by functionalization of surfaces.

**Table 1. Comparative Performance of Non-Viral Gene Delivery Systems Based on Uptake Efficiency, Gene Expression, and Cytotoxicity**

| System Type | Uptake Efficiency | Expression Efficiency | Toxicity |
|---|---|---|---|
| Lipid-based | High | High | Moderate |
| Polymeric | Moderate | Moderate | High |
| Dendrimer | Moderate | Moderate | Moderate |
| Hybrid | High | High | Low–Moderate |

Comprehensively, the systems comparison shows that no single system is best in every case but rather, it depends upon the interaction between physicochemical properties and biological interactions to determine the performance. The most promising solution to achieve efficient and safe gene delivery lies in the use of hybrid and surface-engineered system.

## CONCLUSION

The study is a comparative analysis of non-viral gene delivery systems in a systematic manner to gain insights on the ways physicochemical properties affect the uptake and expression efficiency of the cells. Combining the lessons of lipid-based, polymeric, dendrimer, and hybrid systems, the work has demonstrated a clear connection between the parameters of carrier design, particle size, surface charge and structure composition, and biological performance. The results indicate that lipid-based and hybrid systems always display higher transfection efficiency as they are more effective in cellular internalization, endosomal escape, and efficient intracellular trafficking. Polymeric systems have been shown to be effective in nucleic acid condensing and protecting, but face cytotoxicity issues and poor cellular processing, showing the necessity of further optimization of structure. One of the main contributions of the given study is the creation of a coherent analytical framework that correlates the properties of the delivery system with every step of the gene delivery cascade allowing to point out the most important rate-limiting steps consisting of endosomal entrapment and nuclear transport. The analysis of the comparative visualization and performance mapping introduction enhances the level of analysis even further, providing a segmented platform on which various delivery strategies will be evaluated. This method takes a step further than descriptive review by giving design-based insights that can inform the rational design of the next generation non-viral vectors. Regardless of these developments, there are still a number of bottlenecks especially regarding how the delivery efficiency and biocompatibility can be efficiently balanced. It is also necessary that future studies concentrate on establishing multifunctional and stimuli-responsive nanocarries which can adapt to

the dynamic intracellular conditions, thus enhancing the targeted delivery and regulated release of genes. Also, the incorporation of artificial intelligence-based design thinking and new gene-editing technologies, including CRISPR and mRNA-based therapeutics, offer remarkable possibilities of developing precision gene therapy. On the whole, this paper indicates the significance of interdisciplinary methods, which integrate material science, nanotechnology, and molecular biology, to get safer and more efficient systems of gene delivery in the clinical practice.

**REFERENCES**


1. Bai, H., Lester, G. M. S., Petishnok, L. C., & Dean, D. A. (2017). Cytoplasmic transport and nuclear import of plasmid DNA. *Bioscience Reports, 37*(6), BSR20160616. https://doi.org/10.1042/BSR20160616
2. Brock, D. J., Kondow-McConaghy, H. M., Hager, E. C., Pellois, J.-P., & Pei, D. (2019). Endosomal escape and cytosolic penetration of macromolecules mediated by synthetic delivery agents. *Bioconjugate Chemistry, 30*(2), 293–304. https://doi.org/10.1021/acs.bioconjchem.8b00743
3. del Pozo-Rodríguez, A., Solinís, M. Á., & Rodríguez-Gascón, A. (2016). Applications of lipid nanoparticles in gene therapy. *European Journal of Pharmaceutics and Biopharmaceutics, 109*, 184–193.
4. https://doi.org/10.1016/j.ejpb.2016.09.027
5. Gigante, A., Li, M., Junghänel, S., Hirschhäuser, C., Knauer, S., & Schmuck, C. (2019). Non-viral transfection vectors: Are hybrid materials the way forward? *MedChemComm, 10*(10), 1692–1718.
6. https://doi.org/10.1039/C9MD00133K
7. Mohammadinejad, R., Dehshahri, A., Madamsetty, V. S., Zahmatkeshan, M., Tavakol, S., Makvandi, P., Khorsandi, D., Pardakhty, A., Ashrafizadeh, M., Ghasemipour Afshar, E., & Zarrabi, A. (2020). In vivo gene delivery mediated by non-viral vectors for cancer therapy. *Journal of Controlled Release, 325*, 249–275.
8. https://doi.org/10.1016/j.jconrel.2020.06.014
9. Pei, D., & Buyanova, M. (2019). Overcoming endosomal entrapment in drug delivery. *Bioconjugate Chemistry, 30*(2), 273–283. https://doi.org/10.1021/acs.bioconjchem.8b00692
10. Ponti, F., Campolungo, M., Melchiori, C., Bono, N., & Candiani, G. (2021). Cationic lipids for gene delivery: Many players, one goal. *Chemistry and Physics of Lipids, 235*, 105032.
11. https://doi.org/10.1016/j.chemphyslip.2020.105032
12. Riley, M. K., & Vermerris, W. (2017). Recent advances in nanomaterials for gene delivery: A review. *Nanomaterials, 7*(5), 94. https://doi.org/10.3390/nano7050094
13. Suk, J. S., Xu, Q., Kim, N., Hanes, J., & Ensign, L. M. (2016). PEGylation as a strategy for improving nanoparticle-based drug and gene delivery. *Advanced Drug Delivery Reviews, 99*, 28–51.
14. https://doi.org/10.1016/j.addr.2015.09.012
15. Wahane, A., Waghmode, A., Kapphahn, A., Dhuri, K., Gupta, A., & Bahal, R. (2020). Role of lipid-based and polymer-based non-viral vectors in nucleic acid delivery for next-generation gene therapy. *Molecules, 25*(12), 2866. https://doi.org/10.3390/molecules25122866
16. Xiang, Y., Oo, N. N. L., Lee, J. P., Li, Z., & Loh, X. J. (2017). Recent development of synthetic nonviral systems for sustained gene delivery. *Drug Discovery Today, 22*(9), 1318–1335.
17. https://doi.org/10.1016/j.drudis.2017.05.001
18. Zhou, Z., Liu, X., Zhu, D., Wang, Y., Zhang, Z., Zhou, X., Qiu, N., Chen, X., & Shen, Y. (2017). Nonviral cancer gene therapy: Delivery cascade and vector nanoproperty integration. *Advanced Drug Delivery Reviews, 115*, 115–154. https://doi.org/10.1016/j.addr.2017.07.021
19. Zylberberg, C., Gaskill, K., Pasley, S., & Matosevic, S. (2017). Engineering liposomal nanoparticles for targeted gene therapy. *Gene Therapy, 24*(8), 441–452. https://doi.org/10.1038/gt.2017.38