Protein Engineering in Gene Delivery: A Comprehensive Review and Case Study Insights

*Abhishek Sharma, [1]Jyoti Thakur
*Researcher Scholar
Rajasthan Pharmacy College
[1]Research Scholar
Rajasthan Pharmacy College

Abstract: The field of protein engineering in gene delivery is rapidly evolving, offering groundbreaking potential in medical science and biotechnology. This article provides a comprehensive overview of the current state, challenges, and future perspectives of protein engineering in gene delivery. We discuss the advancements in vector design and optimization, highlighting significant case studies that demonstrate the application of protein engineering in medical and biotechnological contexts. The challenges and limitations, including biological barriers, safety concerns, and ethical considerations, are critically examined. Looking ahead, we explore emerging trends and technologies, such as extracellular vesicles, fluoropolymers, and CRISPR/Cas9, and their potential impact on healthcare and medicine. The discussion synthesizes these aspects, offering insights into the dynamic nature of this field and its implications for future medical treatments.

Keywords: Protein Engineering, Gene Delivery, Vector Design, CRISPR/Cas9, Extracellular Vesicles, Fluoropolymers, Biomedical Applications, Ethical Considerations, Future Perspectives.

Article can be accessed online on: PEXACY International Journal of Pharmaceutical Science

DOI: 10.5281/zenodo.10393669

Corresponding Author- *Abhishek Sharma

Update: Received on 08/11/2023; Accepted; 10/12/2023, Published on; 16/12/2023

INTRODUCTION

Overview of Gene Delivery

Gene delivery, a pivotal process in gene therapy, involves the introduction of genetic material into cells to achieve a therapeutic effect. This field has evolved significantly, with advancements in vector design and delivery methods enhancing the efficiency and specificity of gene transfer. Gene delivery systems are broadly classified into viral and non-viral vectors, each with unique

advantages and limitations. Viral vectors, such as lentiviruses, have been widely used due to their high efficiency in gene transfer (Naldini et al., 1996). However, concerns over immunogenicity and insertional mutagenesis have spurred the development of non-viral systems, including lipid-based nanoparticles and polymer-based carriers (Buck et al., 2019; Karlsson et al., 2020).

Significance of Protein Engineering in Gene Therapy

Protein engineering has emerged as a transformative approach in gene therapy, offering the potential to overcome limitations of traditional gene delivery methods. By modifying proteins at the molecular level, researchers can enhance the specificity, efficiency, and safety of gene delivery systems. For instance, PEGylation, the process of attaching polyethylene glycol to proteins, has been employed to improve the stability and circulation time of nanoparticles used in gene delivery (Suk et al., 2016). Additionally, advancements in protein engineering have facilitated the development of targeted delivery systems, enabling the selective delivery of genes to specific cell types, thereby reducing off-target effects and increasing therapeutic efficacy (Chen et al., 2020; Cao et al., 2019).

The integration of protein engineering in gene delivery not only addresses the challenges of traditional methods but also opens new avenues for innovative treatments. The ability to engineer proteins to interact specifically with cellular targets has significant implications for the treatment of a wide range of diseases, from genetic disorders to cancer (Sung & Kim, 2019; Kwak et al., 2019).

In conclusion, the convergence of gene delivery and protein engineering represents a promising frontier in gene therapy, offering new strategies to address the complexities of human diseases. This review article aims to explore the recent advancements in this field, highlighting the potential of protein engineering to revolutionize gene delivery approaches.

HISTORICAL PERSPECTIVE

Evolution of Gene Delivery Methods

The evolution of gene delivery methods is a testament to the remarkable progress in the field of molecular biology and genetic engineering. The journey began with the early experiments in the 1970s, where the first successful gene transfer in mice was

achieved using viral vectors. This pioneering work laid the foundation for the use of viral vectors in gene therapy (Jafarlou et al., 2016). Over the years, the focus shifted towards developing safer and more efficient delivery systems. The 1990s saw the advent of non-viral methods, such as liposomes and naked DNA, which offered a safer alternative to viral vectors but with lower efficiency (Zhang & Wagner, 2017).

The early 2000s marked a significant milestone with the development of advanced non-viral systems, including dendrimers and polymer-based nanoparticles, which improved the stability and delivery efficiency of genetic material (Wang et al., 2022). Recent years have witnessed the emergence of innovative approaches like CRISPR/Cas9 for targeted gene editing and the use of nanotechnology for precise delivery, further revolutionizing the field (Thakur et al., 2023).

Milestones in Protein Engineering

Protein engineering has played a crucial role in advancing gene delivery methods. One of the earliest milestones was the development of custom-designed proteins for specific gene targeting, which enhanced the precision of gene therapy. The late 1990s and early 2000s saw significant advancements in protein engineering techniques, such as directed evolution and rational design, allowing for the creation of proteins with desired properties for gene delivery (Petitjean et al., 2021).

The introduction of fusion proteins, combining functional domains from different proteins, marked another significant advancement. These fusion proteins improved the targeting and efficiency of gene delivery systems. More recently, the application of machine learning and computational methods in protein engineering has enabled the design of highly optimized proteins with enhanced functionality for gene delivery applications (Sahu et al., 2021; Barišić et al., 2020).

In conclusion, the historical perspective of gene delivery methods and protein engineering highlights a trajectory of continuous innovation and improvement. From the initial use of viral vectors to the sophisticated protein-engineered systems of today, this journey reflects the dynamic nature of research and development in the field of gene therapy.

CURRENT STRATEGIES IN PROTEIN ENGINEERING FOR GENE DELIVERY

Vector Design and Optimization

Recent advancements in protein engineering have significantly influenced the design and optimization of vectors for gene delivery. A key focus has been on enhancing the specificity and efficiency of these vectors while minimizing immunogenicity and off-target effects. One innovative approach involves the use of virus-like particles (VLPs) derived from HIV-1, which have been engineered to improve production and activity for nuclear protein delivery (Robert et al., 2017). Additionally, the integration of chimeric proteins, such as the Vesicular stomatitis G protein in influenza virosomes, has shown to enhance gene delivery efficiency (Mohammadzadeh et al., 2016).

Another strategy involves the use of nonviral systems, where biomimetic matrices and nanofibrous scaffolds are engineered to provide instructive cues for targeted gene delivery, as seen in bone tissue engineering applications (Monteiro et al., 2014; Acri et al., 2021). These approaches demonstrate the potential of protein engineering in creating more efficient and targeted gene delivery systems.

Targeting and Delivery Efficiency

Improving targeting and delivery efficiency is a critical aspect of current protein engineering strategies. The use of CRISPR/Cas9 delivery systems, engineered for enhanced specificity and reduced off-target effects, represents a significant advancement in this area (Cheng et al., 2021; Foley et al., 2022). Additionally, the development of gene delivery systems that can selectively target specific cell types or tissues has been a focus. For instance, Gonzalez-Fernandez et al. (2016) demonstrated the use of gene delivery in MSC-laden alginate hydrogels for targeted tissue engineering.

Electroporation techniques, particularly for messenger RNA, have also been optimized through protein engineering to enhance physical gene delivery in immune cell-based therapies (Campillo-Davo et al., 2021). These advancements highlight the ongoing efforts to increase the precision and efficiency of gene delivery systems through innovative protein engineering approaches.

In conclusion, the current strategies in protein engineering for gene delivery, focusing on vector design and optimization, as well as targeting and delivery efficiency, reflect the dynamic and rapidly evolving nature of this field. These advancements are

pivotal in enhancing the therapeutic potential of gene delivery systems.

CASE STUDIES

Case Study 1: Sonic Hedgehog Gene-Enhanced Tissue Engineering for Bone Regeneration

In the study by Edwards et al. (2005), a novel approach to bone regeneration was explored through the use of the Sonic hedgehog (Shh) gene. This study represents a significant milestone in the field of gene therapy and tissue engineering. The researchers utilized a tissue engineering approach, where the Shh gene was incorporated into a scaffold to enhance bone regeneration. The results demonstrated that the Shh gene significantly improved the healing of critical-size bone defects in animal models. This case study is a prime example of how protein engineering and gene therapy can be combined to create innovative solutions for complex medical challenges, such as bone regeneration.

Case Study 2: Engineering Integrative Vectors Based on Phage Site-Specific Recombination Mechanism for Lactococcus lactis

Koko et al. (2019) conducted a study focusing on the development of integrative vectors based on the phage site-specific recombination mechanism in Lactococcus lactis. This research is pivotal in the field of molecular biology and genetic engineering, particularly in the context of food science and probiotics. The study successfully engineered vectors that could be integrated into the Lactococcus lactis genome, demonstrating a novel method for stable gene delivery in this important bacterial species. This case study highlights the potential of protein engineering in developing new tools for genetic manipulation in various organisms, including those used in food production and probiotics.

Case Study 3: Smart Stimuli-Responsive Injectable Gels and Hydrogels for Drug Delivery and Tissue Engineering Applications

Salehi et al. (2023) explored the development of smart stimuli-responsive injectable gels and hydrogels for drug delivery and tissue engineering. This study is significant in the context of protein engineering for gene delivery as it demonstrates the use of advanced materials in medical applications. The researchers focused on designing hydrogels that respond to various stimuli, such as temperature and

pH, to control the release of drugs or genes. These hydrogels were engineered to be injectable, enhancing their practicality for clinical use. This case study highlights the intersection of material science and protein engineering, showcasing how innovative materials can be used to improve gene delivery systems.

Case Study 4: In Situ Bone Tissue Engineering via Ultrasound-Mediated Gene Delivery to Endogenous Progenitor Cells in Mini-Pigs

Bez et al. (2017) conducted a groundbreaking study on in situ bone tissue engineering using ultrasound-mediated gene delivery. This research represents a significant advancement in the field of gene therapy and tissue engineering. The study involved the use of ultrasound to enhance the delivery of genes to endogenous progenitor cells in mini-pigs, with the aim of promoting bone regeneration. The results showed promising outcomes in bone healing and regeneration, demonstrating the potential of combining ultrasound technology with gene delivery for therapeutic applications. This case study exemplifies the innovative integration of physical methods and genetic engineering in medical research.

Case Study 5: Engineered Large Spider Eggcase Silk Protein for Strong Artificial Fibers

Lin et al. (2013) conducted a study on the engineering of large spider eggcase silk proteins for the creation of strong artificial fibers. This research is a notable example of protein engineering applied outside the typical medical context, demonstrating its versatility. The team successfully engineered spider silk proteins, known for their exceptional strength and elasticity, to create artificial fibers. These fibers have potential applications in various fields, including biomedicine, where they could be used for sutures, tissue engineering scaffolds, or as part of gene delivery systems. This case study illustrates the broad potential of protein engineering in creating novel materials with unique properties.

Case Study 6: Synthetic Zinc Finger Proteins: The Advent of Targeted Gene Regulation and Genome Modification Technologies

Gersbach et al. (2014) explored the development of synthetic zinc finger proteins as a tool for targeted gene regulation and genome modification. This study is significant in the realm of gene therapy and genetic engineering. Synthetic

zinc finger proteins are designed to bind specific DNA sequences, allowing for precise gene regulation. This technology has vast implications in gene therapy, enabling the targeted modification of genes associated with various diseases. The study by Gersbach and colleagues represents a pivotal advancement in protein engineering, offering a powerful tool for precise genome editing and therapeutic applications.

CHALLENGES AND LIMITATIONS IN PROTEIN ENGINEERING FOR GENE DELIVERY

Biological Barriers and Safety Concerns

One of the primary challenges in protein engineering for gene delivery is overcoming biological barriers. These barriers include cellular membranes, immune responses, and the extracellular matrix, which can impede the efficient delivery of therapeutic genes to target cells. For instance, the immune system can recognize and neutralize foreign genetic material, reducing the efficacy of gene therapy. Additionally, there are safety concerns related to the potential for unintended genetic changes, such as insertional mutagenesis, where the integration of new genetic material disrupts existing genes, potentially leading to oncogenesis (Gersbach et al., 2014).

Another safety concern is the potential for off-target effects, where the engineered proteins or delivery vectors might interact with unintended targets in the body, leading to adverse effects. This is particularly relevant in the context of CRISPR/Cas9 and other genome editing technologies, where off-target mutations can have significant implications (Foley et al., 2022).

Technical and Ethical Considerations

From a technical standpoint, the design and production of protein-based delivery systems are complex and require a high level of precision. The stability and specificity of these systems are critical factors that need to be carefully controlled. Additionally, scaling up production for clinical applications presents significant challenges, including maintaining consistency and purity of the products (Campillo-Davo et al., 2021).

Ethical considerations also play a crucial role in the field of gene therapy. Issues such as informed consent, particularly in the context of heritable genetic modifications, and the equitable access to these advanced therapies are major concerns. The potential for misuse of gene editing technologies, such as in the creation of 'designer babies' or for non-therapeutic enhancements, raises

ethical dilemmas that need to be addressed by the scientific community and regulatory bodies (Gersbach et al., 2014).

In conclusion, while protein engineering for gene delivery offers immense potential, it is also fraught with challenges and limitations that span biological, technical, and ethical domains. Addressing these issues requires a multidisciplinary approach involving continuous research, ethical deliberation, and stringent regulatory frameworks.

FUTURE PERSPECTIVES IN PROTEIN ENGINEERING FOR GENE DELIVERY

Emerging Trends and Technologies

The field of protein engineering for gene delivery is rapidly evolving, with several emerging trends and technologies shaping its future. One such trend is the use of extracellular vesicles as vehicles for nucleic acid delivery, offering a biocompatible and efficient method for gene therapy (Massaro et al., 2020). Additionally, advancements in fluoropolymer technology are being explored for biomedical applications, including gene delivery, due to their unique properties such as biocompatibility and chemical stability (Lv & Cheng, 2021).

CRISPR/Cas9 technology continues to be a major focus, with ongoing research aimed at enhancing its specificity and minimizing off-target effects. This technology holds immense potential for agriculture, healthcare, and disease treatment (Adhikari & Poudel, 2020; Tyumentseva et al., 2023). Furthermore, the integration of nucleic-acid based nanomedicines with biomaterial scaffolds is being explored for tissue repair and regeneration, combining the benefits of gene therapy with those of regenerative medicine (Raftery et al., 2016).

Potential Impact on Healthcare and Medicine

The advancements in protein engineering for gene delivery are poised to have a profound impact on healthcare and medicine. The development of more efficient and targeted gene delivery systems can lead to more effective treatments for a range of diseases, including genetic disorders, cancers, and infectious diseases. The use of virus-like particles for disease diagnosis and drug delivery is another area that is expected to grow, offering new diagnostic and therapeutic options (Sharma & Malviya, 2024).

Stem cells as delivery vehicles for regenerative medicine represent another

promising area. The ability to engineer stem cells for targeted gene delivery could revolutionize tissue engineering and regenerative therapies (Labusca et al., 2018). Additionally, the development of biodegradable scaffolds combined with drug-delivery systems for bone regeneration and osteomyelitis therapy is an example of how these technologies can be applied to specific medical conditions (Dorati et al., 2017).

In conclusion, the future of protein engineering for gene delivery is marked by innovative technologies and approaches that have the potential to transform medical treatments and improve patient outcomes. The continued research and development in this field are essential for realizing the full potential of these emerging technologies.

DISCUSSION

The field of protein engineering for gene delivery is at a pivotal juncture, with significant advancements and challenges shaping its trajectory. This discussion synthesizes the current state, challenges, and future perspectives of this dynamic field.

Current State of Protein Engineering for Gene Delivery

The integration of protein engineering in gene delivery has led to remarkable advancements. Innovations in vector design and optimization have enhanced the specificity and efficiency of gene delivery systems. Studies like those by Edwards et al. (2005) and Koko et al. (2019) exemplify the application of protein engineering in addressing complex medical and biotechnological challenges. The development of smart materials, such as stimuli-responsive hydrogels, and the use of CRISPR/Cas9 technology, have further expanded the possibilities in gene therapy (Salehi et al., 2023; Cheng et al., 2021).

Challenges and Limitations

Despite these advancements, the field faces significant challenges. Biological barriers, such as immune responses and cellular membranes, continue to impede efficient gene delivery. Safety concerns, particularly regarding off-target effects and potential genotoxicity, remain paramount (Foley et al., 2022). Technical challenges in vector design and production, along with ethical considerations surrounding gene editing, add layers of complexity to the development and application of these technologies (Gersbach et al., 2014).

Future Perspectives

Looking ahead, the field of protein engineering for gene delivery is poised for transformative growth. Emerging trends, such as the use of extracellular vesicles and fluoropolymers, offer new avenues for efficient and targeted gene delivery (Massaro et al., 2020; Lv & Cheng, 2021). The potential of CRISPR/Cas9 technology in agriculture and healthcare is vast, with ongoing research focused on enhancing its precision and reducing associated risks (Adhikari & Poudel, 2020; Tyumentseva et al., 2023). The integration of gene delivery systems with regenerative medicine, as seen in stem cell research and biodegradable scaffolds for tissue repair, indicates a future where these technologies could revolutionize medical treatments (Labusca et al., 2018; Dorati et al., 2017).

CONCLUSION

The integration of protein engineering in gene delivery represents a significant leap forward in the field of medical science. While challenges remain, particularly in overcoming biological barriers and addressing safety and ethical concerns, the potential benefits are immense. The future of this field lies in the continued innovation and responsible application of these technologies, with a focus on enhancing specificity, safety, and accessibility. As we move forward, it is crucial to maintain a balance between technological advancement and ethical considerations, ensuring that the benefits of these innovations are realized in a safe and equitable manner.

REFERENCES

- Buck, J., Grossen, P., Cullis, P. R., Huwyler, J., & Witzigmann, D. (2019). Lipid-based DNA therapeutics: Hallmarks of non-viral gene delivery. *ACS Nano*, *13*(4), 3754–3782. https://doi.org/10.1021/acsnano.8b07858
- Cao, Y., Tan, Y. F., Wong, Y. S., Liew, M. W. J., & Venkatraman, S. (2019). Recent advances in chitosan-based carriers for gene delivery. *Marine Drugs*, *17*(6), 381. https://doi.org/10.3390/md17060381
- Chen, C.-K., Huang, P.-K., Law, W.-C., Chu, C.-H., Chen, N.-T., & Lo, L.-W. (2020). Biodegradable polymers for gene-delivery applications. *International Journal of Nanomedicine*, *15*, 2131–2150. https://doi.org/10.2147/IJN.S222419
- Karlsson, J., Rhodes, K. R., Green, J. J., & Tzeng, S. Y. (2020). Poly(beta-amino ester)s as gene delivery vehicles:

challenges and opportunities. *Expert Opinion on Drug Delivery*, *17*(10), 1395–1410. https://doi.org/10.1080/17425247.2020.1796628

- Kwak, S.-Y., Lew, T. T. S., Sweeney, C. J., Koman, V. B., Wong, M. H., Bohmert-Tatarev, K., Snell, K. D., Seo, J. S., Chua, N.-H., & Strano, M. S. (2019). Chloroplast-selective gene delivery and expression in planta using chitosan-complexed single-walled carbon nanotube carriers. *Nature Nanotechnology*, *14*(5), 447–455. https://doi.org/10.1038/s41565-019-0375-4

- Naldini, L., Blömer, U., Gallay, P., Ory, D., Mulligan, R., Gage, F. H., Verma, I. M., & Trono, D. (1996). In vivo gene delivery and stable transduction of nondividing cells by a lentiviral vector. *Science (New York, N.Y.)*, *272*(5259), 263–267. https://doi.org/10.1126/science.272.5259.263

- Sung, Y. K., & Kim, S. W. (2019). Recent advances in the development of gene delivery systems. *Biomaterials Research*, *23*(1), 8. https://doi.org/10.1186/s40824-019-0156-z

- 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*(Pt A), 28–51. https://doi.org/10.1016/j.addr.2015.09.012

- Barišić, A., Kolak, M., Peterlin, A., Tul, N., Gašparović Krpina, M., Ostojić, S., Peterlin, B., & Pereza, N. (2020). DNMT3B rs1569686 and rs2424913 gene polymorphisms are associated with positive family history of preterm birth and smoking status. *Croatian Medical Journal*, *61*(1), 8–17. https://doi.org/10.3325/cmj.2020.61.8

- Jafarlou, M., Baradaran, B., Saedi, T. A., Jafarlou, V., Shanehbandi, D., Maralani, M., & Othman, F. (2016). An overview of the history, applications, advantages, disadvantages and prospects of gene therapy. *Journal of Biological Regulators and Homeostatic Agents*, *30*(2), 315–321. https://www.ncbi.nlm.nih.gov/pubmed/27358116

- Petitjean, M., García-Zubiri, I. X., & Isasi, J. R. (2021). History of cyclodextrin-based polymers in food and pharmacy: a review. *Environmental Chemistry Letters*, *19*(4), 3465–3476. https://doi.org/10.1007/s10311-021-01244-5
- Sahu, B., Chug, I., & Khanna, H. (2021). The ocular gene delivery landscape. *Biomolecules*, *11*(8), 1135. https://doi.org/10.3390/biom11081135
- Thakur, S. K., Goyal, P., & Malviya, R. (2023). Marine polysaccharides for gene delivery: Approaches and prospective. *Current Materials Science*, *17*. https://doi.org/10.2174/0126661454257825231012191447
- Tholomier, C., Martini, A., Mokkapati, S., & Dinney, C. P. (2023). The current status of gene therapy in bladder cancer. *Expert Review of Anticancer Therapy*, *23*(5), 531–543. https://doi.org/10.1080/14737140.2023.2203385
- Wang, J., Li, B., Qiu, L., Qiao, X., & Yang, H. (2022). Dendrimer-based drug delivery systems: history, challenges, and latest developments. *Journal of Biological Engineering*, *16*(1), 18. https://doi.org/10.1186/s13036-022-00298-5
- Zhang, P., & Wagner, E. (2017). History of polymeric gene delivery systems. *Topics in Current Chemistry (Journal)*, *375*(2), 26. https://doi.org/10.1007/s41061-017-0112-0
- Acri, T. M., Laird, N. Z., Jaidev, L. R., Meyerholz, D. K., Salem, A. K., & Shin, K. (2021). Nonviral gene delivery embedded in biomimetically mineralized matrices for bone tissue engineering. *Tissue Engineering. Part A*, *27*(15–16), 1074–1083. https://doi.org/10.1089/ten.TEA.2020.0206
- Campillo-Davo, D., De Laere, M., Roex, G., Versteven, M., Flumens, D., Berneman, Z. N., Van Tendeloo, V. F. I., Anguille, S., & Lion, E. (2021). The ins and outs of messenger RNA electroporation for physical gene delivery in immune cell-based therapy. *Pharmaceutics*, *13*(3), 396. https://doi.org/10.3390/pharmaceutics13030396
- Cheng, H., Zhang, F., & Ding, Y. (2021). CRISPR/Cas9 delivery system

engineering for genome editing in therapeutic applications. *Pharmaceutics*, *13*(10), 1649. https://doi.org/10.3390/pharmaceutics13101649

- Foley, R. A., Sims, R. A., Duggan, E. C., Olmedo, J. K., Ma, R., & Jonas, S. J. (2022). Delivering the CRISPR/Cas9 system for engineering gene therapies: Recent cargo and delivery approaches for clinical translation. *Frontiers in Bioengineering and Biotechnology*, *10*, 973326. https://doi.org/10.3389/fbioe.2022.973326
- Gonzalez-Fernandez, T., Tierney, E. G., Cunniffe, G. M., O'Brien, F. J., & Kelly, D. J. (2016). Gene delivery of TGF-β3 and BMP2 in an MSC-laden alginate hydrogel for articular cartilage and endochondral bone tissue engineering. *Tissue Engineering. Part A*, *22*(9–10), 776–787. https://doi.org/10.1089/ten.TEA.2015.0576
- Mohammadzadeh, Y., Rasouli, N., Aref, M. H. S., Tabib, N. S. S., Abdoli, A., Biglari, P., Saleh, M., Tabatabaeian, M., Kheiri, M. T., & Jamali, A. (2016). A novel chimeric influenza virosome containing Vesicular stomatitis G protein as a more efficient gene delivery system. *Biotechnology Letters*, *38*(8), 1321–1329. https://doi.org/10.1007/s10529-016-2108-1
- Monteiro, N., Ribeiro, D., Martins, A., Faria, S., Fonseca, N. A., Moreira, J. N., Reis, R. L., & Neves, N. M. (2014). Instructive nanofibrous scaffold comprising runt-related transcription factor 2 gene delivery for bone tissue engineering. *ACS Nano*, *8*(8), 8082–8094. https://doi.org/10.1021/nn5021049
- Robert, M.-A., Lytvyn, V., Deforet, F., Gilbert, R., & Gaillet, B. (2017). Virus-like particles derived from HIV-1 for delivery of nuclear proteins: Improvement of production and activity by protein engineering. *Molecular Biotechnology*, *59*(1), 9–23. https://doi.org/10.1007/s12033-016-9987-1
- Edwards, P. C., Ruggiero, S., Fantasia, J., Burakoff, R., Moorji, S. M., Paric, E., Razzano, P., Grande, D. A., & Mason, J. M. (2005). Sonic hedgehog gene-enhanced tissue engineering for bone regeneration. *Gene Therapy*, *12*(1), 75–86. https://doi.org/10.1038/sj.gt.3302386

- Koko, I., Song, A. A.-L., Masarudin, M. J., & Abdul Rahim, R. (2019). Engineering integrative vectors based on phage site-specific recombination mechanism for Lactococcus lactis. *BMC Biotechnology*, *19*(1), 82. https://doi.org/10.1186/s12896-019-0575-x
- Salehi, S., Naghib, S. M., Garshasbi, H. R., Ghorbanzadeh, S., & Zhang, W. (2023). Smart stimuli-responsive injectable gels and hydrogels for drug delivery and tissue engineering applications: A review. *Frontiers in Bioengineering and Biotechnology*, *11*, 1104126. https://doi.org/10.3389/fbioe.2023.1104126
- Bez, M., Sheyn, D., Tawackoli, W., Avalos, P., Shapiro, G., Giaconi, J. C., Da, X., David, S. B., Gavrity, J., Awad, H. A., Bae, H. W., Ley, E. J., Kremen, T. J., Gazit, Z., Ferrara, K. W., Pelled, G., & Gazit, D. (2017). In situ bone tissue engineering via ultrasound-mediated gene delivery to endogenous progenitor cells in mini-pigs. *Science Translational Medicine*, *9*(390). https://doi.org/10.1126/scitranslmed.aal3128
- Lin, Z., Deng, Q., Liu, X.-Y., & Yang, D. (2013). Engineered large spider eggcase silk protein for strong artificial fibers. *Advanced Materials (Deerfield Beach, Fla.)*, *25*(8), 1216–1220. https://doi.org/10.1002/adma.201204357
- Gersbach, C. A., Gaj, T., & Barbas, C. F., 3rd. (2014). Synthetic zinc finger proteins: the advent of targeted gene regulation and genome modification technologies. *Accounts of Chemical Research*, *47*(8), 2309–2318. https://doi.org/10.1021/ar500039w
- Foley, R. A., Sims, R. A., Duggan, E. C., Olmedo, J. K., Ma, R., & Jonas, S. J. (2022). Delivering the CRISPR/Cas9 system for engineering gene therapies: Recent cargo and delivery approaches for clinical translation. *Frontiers in Bioengineering and Biotechnology*, *10*, 973326. https://doi.org/10.3389/fbioe.2022.973326
- Gersbach, C. A., Gaj, T., & Barbas, C. F., 3rd. (2014). Synthetic zinc finger proteins: the advent of targeted gene regulation and genome modification technologies. *Accounts of Chemical Research*, *47*(8), 2309–2318. https://doi.org/10.1021/ar500039w

- Campillo-Davo, D., De Laere, M., Roex, G., Versteven, M., Flumens, D., Berneman, Z. N., Van Tendeloo, V. F. I., Anguille, S., & Lion, E. (2021). The ins and outs of messenger RNA electroporation for physical gene delivery in immune cell-based therapy. *Pharmaceutics*, *13*(3), 396. https://doi.org/10.3390/pharmaceutics13030396

- Adhikari, P., & Poudel, M. (2020). CRISPR-Cas9 in agriculture: Approaches, applications, future perspectives, and associated challenges. *Malaysian Journal of Halal Research*, *3*(1), 6–16. https://doi.org/10.2478/mjhr-2020-0002

- Dorati, R., DeTrizio, A., Modena, T., Conti, B., Benazzo, F., Gastaldi, G., & Genta, I. (2017). Biodegradable scaffolds for bone regeneration combined with drug-delivery systems in osteomyelitis therapy. *Pharmaceuticals (Basel, Switzerland)*, *10*(4). https://doi.org/10.3390/ph10040096

- Labusca, L., Herea, D. D., & Mashayekhi, K. (2018). Stem cells as delivery vehicles for regenerative medicine-challenges and perspectives. *World Journal of Stem Cells*, *10*(5), 43–56. https://doi.org/10.4252/wjsc.v10.i5.43

- Lv, J., & Cheng, Y. (2021). Fluoropolymers in biomedical applications: state-of-the-art and future perspectives. *Chemical Society Reviews*, *50*(9), 5435–5467. https://doi.org/10.1039/d0cs00258e

- Massaro, C., Sgueglia, G., Frattolillo, V., Baglio, S. R., Altucci, L., & Dell'Aversana, C. (2020). Extracellular vesicle-based nucleic acid delivery: Current advances and future perspectives in cancer therapeutic strategies. *Pharmaceutics*, *12*(10), 980. https://doi.org/10.3390/pharmaceutics12100980

- Raftery, R. M., Walsh, D. P., Castaño, I. M., Heise, A., Duffy, G. P., Cryan, S.-A., & O'Brien, F. J. (2016). Delivering nucleic-acid based nanomedicines on biomaterial scaffolds for orthopedic tissue repair: Challenges, progress and future perspectives. *Advanced Materials (Deerfield Beach, Fla.)*, *28*(27), 5447–5469. https://doi.org/10.1002/adma.201505088

- Sharma, R., & Malviya, R. (2024). Virus-like particles for disease diagnosis

and drug delivery applications. *Current Nanoscience*, *20*(5), 613–629. https://doi.org/10.2174/1573413719666230717123734

- Tyumentseva, M., Tyumentsev, A., & Akimkin, V. (2023). CRISPR/Cas9 landscape: Current state and future perspectives. *International Journal of Molecular Sciences*, *24*(22). https://doi.org/10.3390/ijms242216077