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Optimizing Minicircle DNA Vaccine Production for SARS-CoV-2 RBD Expression

Vlasova Hill*

Department of Chemistry, University of Potsdam, Potsdam, Germany

Introduction

Minicircle DNA vaccines have emerged as a promising platform for the rapid and efficient production of immunogenic antigens, particularly in response to emerging infectious diseases such as SARS-CoV-2. Unlike conventional plasmid DNA vaccines, minicircle DNA vaccines lack bacterial sequences, thereby enhancing transgene expression, improving immunogenicity, and reducing potential inflammatory responses. The optimization of minicircle DNA vaccine production expressing the Receptor-Binding Domain (RBD) of SARS-CoV-2 is crucial for developing effective and scalable immunization strategies against COVID-19. The production of minicircle DNA vaccines involves a two-stage process that includes the initial propagation of parental plasmids in bacterial cultures followed by an in vivo recombination step to remove bacterial backbone sequences. This process results in a purified minicircle DNA construct that contains only the gene of interest and necessary regulatory elements, enhancing the efficiency of gene expression in host cells. To maximize the production yield and quality of minicircle DNA vaccines expressing SARS-CoV-2 RBD, various parameters must be optimized, including bacterial strain selection, culture conditions, recombination efficiency, purification methods, and transfection efficiency.

Description

One of the critical factors influencing minicircle DNA vaccine production is the choice of bacterial strains used for propagation. Escherichia coli strains engineered for minicircle production, such as ZYCY10P3S2T, have been optimized for high-yield plasmid replication and efficient recombination. These strains contain inducible recombinase systems that enable the precise excision of bacterial backbone sequences upon induction, yielding high-purity minicircle DNA. The optimization of growth conditions, including temperature, aeration, and nutrient composition, plays a crucial role in maximizing plasmid replication before the induction of recombination. The induction of recombination is a pivotal step in minicircle DNA production and must be carefully controlled to achieve high conversion efficiency while minimizing unwanted byproducts. The use of chemical inducers such as L-arabinose can trigger site-specific recombination, leading to the excision of bacterial sequences and the generation of minicircle DNA. The timing and concentration of inducers must be optimized to balance cell viability and recombination efficiency, ensuring maximum minicircle yield. Additionally, temperature shifts during induction can enhance the activity of recombinase enzymes, further improving the efficiency of minicircle formation [1].

Following recombination, the purification of minicircle DNA is essential to remove bacterial contaminants, endotoxins, and residual parental plasmids. High-Performance Liquid Chromatography (HPLC), anion-exchange chromatography, and ultracentrifugation techniques can be employed to achieve high-purity minicircle DNA preparations suitable for downstream applications. The choice of purification method significantly impacts the overall vaccine quality, as residual contaminants can affect transfection efficiency and immunogenicity. Scalable purification strategies are particularly important for large-scale vaccine production, ensuring batch-to-batch consistency and compliance with regulatory standards. The expression of SARS-CoV-2 RBD from minicircle DNA vaccines relies on efficient delivery into host cells, where the antigen is produced and presented to the immune system. Various non-viral delivery methods, including electroporation and lipid nanoparticle (LNP)-mediated transfection, have been explored to enhance cellular uptake and gene expression. Electroporation facilitates the direct entry of minicircle DNA into cells by temporarily permeabilizing the membrane using electrical pulses. While effective, this method requires specialized equipment and can induce cellular stress. LNP-based delivery systems, on the other hand, encapsulate minicircle DNA within lipid vesicles, promoting efficient cellular entry and endosomal escape. The optimization of LNP formulations, including lipid composition and particle size, is crucial for maximizing transfection efficiency and antigen expression [2].

The immunogenicity of minicircle DNA vaccines expressing SARS-CoV-2 RBD is influenced by various factors, including promoter selection, codon optimization, and adjuvant incorporation. Strong eukaryotic promoters such as the Cytomegalovirus (CMV) promoter drive high levels of transgene expression, enhancing the immune response. Codon optimization ensures efficient translation in human cells, further improving antigen production. Additionally, the inclusion of immune-stimulatory sequences or co-administration with adjuvants such as CpG oligodeoxynucleotides can enhance the magnitude and durability of the immune response. Studies have demonstrated that minicircle DNA vaccines elicit robust humoral and cellular immunity, with higher antigen-specific antibody titers compared to conventional plasmid DNA vaccines. Scaling up minicircle DNA vaccine production for mass immunization programs presents logistical and technical challenges that require further optimization. Bioreactor-based fermentation systems offer a scalable approach for high-density bacterial culture and minicircle production. Fed-batch fermentation strategies can enhance plasmid yield by providing a controlled supply of nutrients, minimizing metabolic stress, and extending the bacterial growth phase. Furthermore, advancements in automated purification systems enable large-scale processing of minicircle DNA with high reproducibility and efficiency [3].

Regulatory considerations play a crucial role in the clinical translation of minicircle DNA vaccines. The safety and efficacy of these vaccines must be thoroughly evaluated in preclinical and clinical studies to ensure compliance with regulatory guidelines. The absence of antibiotic resistance genes and bacterial sequences in minicircle DNA constructs enhances their safety profile, reducing concerns related to horizontal gene transfer and genomic integration. However, long-term immunogenicity, stability, and storage conditions must be assessed to determine the viability of minicircle DNA vaccines as a widespread immunization platform. The emergence of SARS-CoV-2 variants has further highlighted the need for adaptable vaccine technologies capable of responding to viral mutations. Minicircle DNA vaccines offer a flexible and rapid platform for antigen design, allowing for the swift modification of genetic sequences to match emerging variants. The modular nature of minicircle constructs enables the incorporation of updated RBD sequences, ensuring continued vaccine efficacy against evolving strains. Moreover, the combination of minicircle DNA vaccines with other immunization strategies, such as mRNA or protein subunit vaccines, could provide broader and more durable protection against COVID-19 [4,5].

***Address for Correspondence:** Vlasova Hill, Department of Chemistry, University of Potsdam, Potsdam, Germany, E-mail: hillvlasov@gmail.com*

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Conclusion

In conclusion, the optimization of minicircle DNA vaccine production expressing SARS-CoV-2 RBD represents a significant advancement in genetic immunization technologies. By refining bacterial culture conditions, recombination efficiency, purification strategies, and delivery methods, researchers can maximize vaccine yield, purity, and immunogenicity. The scalability and adaptability of minicircle DNA vaccines make them a promising candidate for addressing current and future infectious disease threats. Continued research and innovation in this field will pave the way for the development of next-generation vaccines that offer enhanced efficacy, safety, and accessibility on a global scale.

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Conflict of Interest

None.

References

1. Antony, Priya and Ranjit Vijayan. "Role of SARS-CoV-2 and ACE2 variations in COVID-19." *Biomed. J* 44 (2021): 235-244.
2. Kaneko, Syuzo, Ken Takasawa, Ken Asada and Norio Shinkai, et al. "Epigenetic mechanisms underlying COVID-19 pathogenesis." *Biomed* 9 (2021): 1142.
3. Baden, Lindsey R., Hana M. El Sahly, Brandon Essink and Karen Kotloff, et al. "Efficacy and safety of the mRNA-1273 SARS-CoV-2 vaccine." *N Engl J Med* 384 (2021): 403-416.
4. Hadj Hassine, Ikbel. "Covid-19 vaccines and variants of concern: A review." *Rev Med Virol* 32 (2022): e2313.
5. Lee, Jihui, Shreedevi Arun Kumar, Yong Yu Jhan and Corey J. Bishop. "Engineering DNA vaccines against infectious diseases." *Acta Biomater* 80 (2018): 31-47.

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