

Recombinant Production of Erythropoietin (EPO): Technical Principles, Challenges, and Advances in Biosimilar Development

Yifan Wang *

HDBeijing, Beijing, China

* Corresponding Author Email: 3121796072@qq.com

Abstract. This paper reviews the application of recombinant technology in the production of erythropoietin (EPO), focusing on its production process, selection of expression systems, the critical role of glycosylation in drug activity, and the development and quality control of EPO biosimilars. EPO is a complex glycoprotein essential for anemia treatment; however, its chemical synthesis is highly challenging due to its structural complexity. Therefore, recombinant DNA technology is widely used for large-scale EPO production. The choice of expression system is particularly crucial, with mammalian cells, especially Chinese hamster ovary (CHO) cells, being preferred due to their ability to perform human-like glycosylation. Additionally, this paper analyzes the impact of glycosylation on EPO's stability and bioactivity, along with the limitations of different expression systems in achieving proper glycosylation. With the expiration of EPO patents, biosimilar development is gaining attention. Biosimilars face stringent quality control requirements, including assessments of purity, potency, stability, and immunogenicity, especially to ensure glycosylation patterns consistent with the original drug. Future research directions include optimizing glycosylation through gene editing technologies and employing advanced production techniques to increase yield and reduce costs, ultimately offering more cost-effective treatment options for patients.

Keywords: Erythropoietin (EPO) biosimilars; Recombinant DNA technology; Glycosylation; CHO cells.

1. Introduction

Laboratories employ recombinant DNA technology to modify DNA sequences for the insertion of specific genes into host cells, enabling these cells to synthesize the desired proteins. This innovative technique has significantly advanced the production of complex protein therapeutics, including vaccines, recombinant proteins, and antibodies. Mocini describes how recombinant technology allows for the controlled production of EPO, which is essential for treating anemia [1]. Gross further elaborates that recombinant technology is particularly effective for producing complex proteins like EPO [2].

Most anemia treatments involve EPO, a glycoprotein that boosts red blood cell synthesis. Eschbach & Adamson highlighted that EPO's structure and glycosylation make it challenging to produce chemically, making recombinant technology indispensable for its large-scale production [3]. Because chemical synthesis can't manage EPO's complex structure and glycosylation, recombinant technology is used. Human EPO can be inserted into host cells via recombinant technology. Under ideal conditions, these cells produce lots of EPO protein. Protein purification and processing produce bioactive medicines.

2. Production Process of EPO

To manufacture recombinant erythropoietin, numerous crucial steps need to be carried out with high precision to ensure that the final product is of good quality and usable. Gene cloning is the starting point of the process, where the EPO gene is isolated from human DNA and amplified using polymerase chain reaction (PCR) technology [4]. This amplification step allows for the production of multiple copies of the EPO gene. The amplified gene is then inserted into an expression vector,

which serves as a molecular carrier. The circular DNA vector at this stage plays a key role as it contains regulatory elements like promoters and terminators, which control the expression of the EPO gene once it has been introduced into the host cell. The vector directs the host cell to synthesize the target protein, EPO.

The constructed vector is then introduced into host cells. Mammalian cells, particularly Chinese hamster ovary (CHO) cells, are frequently used due to their ability to perform post-translational modifications, which are crucial for the biological activity of EPO. One such modification is glycosylation, where sugar molecules are added to the protein. This modification is essential for ensuring the stability, bioactivity, and interaction of EPO with the human body. Proper glycosylation is critical, as improper modification can affect the drug's efficacy and safety. CHO cells are favored because they produce a glycosylated form of EPO that mimics the natural version found in humans. The ability of CHO cells to facilitate proper glycosylation makes them ideal for EPO production, as noted by Gross [2].

After successful transfection, the host cells are cultured under specific laboratory conditions to promote growth and protein production. Each type of host cell has unique requirements, including specific nutrient media, temperature, and other factors to optimize their productivity and ensure they produce a significant amount of recombinant EPO.

The next important phase is protein purification. During cell culture, host cells produce not only EPO but also various other proteins and molecules. Therefore, purification techniques such as ion-exchange chromatography and affinity chromatography are used to isolate EPO from other cellular components. These methods selectively separate EPO based on its charge and affinity for specific ligands. Purified EPO must meet stringent pharmaceutical standards to ensure its safety, purity, and effectiveness for therapeutic use. Each batch is rigorously tested to confirm that it adheres to clinical specifications, ensuring it is both safe for patients and effective in treating conditions like anemia.

3. Selection of Expression Systems

The quality, bioactivity, and safety of recombinant erythropoietin (EPO) are largely impacted by the choice of expression system. Various systems offer unique advantages and restrictions, but ideally, the system chosen should support post-translational modifications like glycosylation, essential for EPO efficacy. One of the most commonly used systems is *Escherichia coli* (*E. coli*) and other bacteria. Bacteria are ideal for their fast growth rate, low cost, and ease of genetic manipulation, making them attractive for large-scale protein production. However, bacterial cells lack the endoplasmic reticulum and Golgi apparatus necessary for glycosylation, which is crucial for EPO's stability, solubility, and receptor interaction. Mocini pointed out that bacterial systems are unsuitable for producing complex glycoproteins like EPO [1].

Yeast expression systems, such as *Pichia pastoris*, offer an alternative that addresses some of the limitations of bacterial systems. Yeasts are eukaryotic and can perform some glycosylation, making them cost-effective and scalable for industrial production. However, their glycosylation patterns differ from mammalian cells, as yeast tend to add high-mannose oligosaccharides, which can affect the therapeutic efficacy and safety of EPO. These differences can alter the pharmacokinetics and immunogenicity of EPO, making yeast-derived proteins less suitable for clinical applications requiring precise glycosylation.

Insect cell systems, such as *Spodoptera frugiperda* (Sf9) cells, provide another option for recombinant protein production. Insect cells can perform glycosylation, but like yeast, their glycosylation patterns differ significantly from human cells. Insect cells often add shorter sugar chains, which may reduce the bioactivity of EPO. Additionally, the production efficiency and stability of recombinant proteins in insect cells are generally lower compared to mammalian systems, making them less favorable for high-quality therapeutic protein production.

Mammalian expression systems, particularly Chinese hamster ovary (CHO) cells, are the gold standard for producing recombinant EPO. CHO cells have the complete cellular machinery needed

for complex glycosylation, producing a protein that closely mimics the natural form of human EPO. As Gross noted, CHO cells produce a glycosylation pattern that mirrors that of endogenous human EPO, crucial for its interaction with erythropoietin receptors and effectiveness in treating anemia [2]. Though more expensive and time-consuming, the ability of mammalian systems to produce a fully functional and bioequivalent form of EPO justifies their widespread use in the biopharmaceutical industry.

4. Role of Glycosylation in EPO Production

Glycosylation, a post-translational modification involving the covalent attachment of sugar molecules to proteins, plays a crucial role in the therapeutic efficacy and stability of recombinant erythropoietin (EPO). This modification influences several key properties of EPO, including its molecular structure, biological activity, and interaction with receptors in the human body. Proper glycosylation enhances the protein's biological function, extends its half-life, and improves its stability in circulation. Lin emphasized that glycosylation is essential for maintaining EPO's stability and bioactivity in the bloodstream, contributing to more effective therapeutic outcomes [4]. Eschbach and Adamson also highlighted that without proper glycosylation, EPO would be quickly degraded and metabolized in the body, rendering it ineffective for long-term treatments [3].

In recombinant EPO production, controlling glycosylation is one of the most challenging yet critical aspects. Different expression systems—bacterial, yeast, insect, and mammalian—exhibit varying glycosylation capacities, resulting in differences in the glycosylation patterns of produced proteins. For example, bacterial systems such as *E. coli* cannot glycosylate proteins, making them unsuitable for producing functional EPO. Yeast and insect cell systems can perform some glycosylation, but the sugar structures they produce differ significantly from those required for human EPO, which may affect the drug's efficacy and safety. In contrast, mammalian expression systems, particularly CHO cells, can produce EPO with glycosylation patterns similar to those found in human cells, making them the preferred choice for recombinant EPO production [5].

5. Development and Quality Control of EPO Biosimilars

With the patents on original erythropoietin (EPO) drugs expiring, many biopharmaceutical companies are shifting their focus toward developing EPO biosimilars. These biosimilars are structurally similar versions of the original drug, produced by different manufacturers. Unlike generic drugs, which are chemically identical to their branded counterparts, biosimilars are large, complex molecules created through biological processes that can result in subtle differences, particularly in post-translational modifications like glycosylation. Katre noted that although biosimilars offer the potential for significant cost reductions, they must undergo strict quality control and bioequivalence testing to ensure they match the original drug in terms of purity, efficacy, and safety [6]. One of the major challenges in producing EPO biosimilars is replicating the glycosylation patterns of the original drug, as even small molecular differences can greatly impact the biosimilar's effectiveness, safety, and immunogenicity.

Quality control is central to the development of biosimilars and plays a crucial role in ensuring that EPO biosimilars are safe, effective, and consistent in their performance. Producing biosimilars requires a stringent quality control framework that monitors key parameters such as purity, potency, stability, and immunogenicity. Purity is a fundamental aspect of quality control, ensuring that the EPO biosimilar is free from impurities that could compromise its safety or efficacy. Analytical methods like high-performance liquid chromatography (HPLC) are typically used to assess the purity of the EPO protein and identify unwanted byproducts from the manufacturing process. High purity levels are essential because even trace impurities can negatively impact the drug's performance or lead to adverse reactions.

Potency testing is another critical quality control measure that directly assesses the bioactivity of the biosimilar. In the case of EPO, potency is typically evaluated based on the drug's ability to stimulate red blood cell production both in vitro (in cell cultures) and in vivo (in animal models). These tests verify that the biosimilar retains its biological functionality and can effectively replicate the therapeutic effects of the original EPO drug [7].

Stability testing is conducted to ensure that the biosimilar maintains its integrity and efficacy over time and under various storage conditions. Accelerated stability tests expose the drug to extreme conditions, while long-term studies assess the biosimilar's resilience during storage and transportation [8]. Stability is critical to guarantee that patients receive a product that remains effective throughout their shelf life.

Immunogenicity testing is perhaps the most important safety assessment for biosimilars like EPO. Given that EPO is a biologically active protein introduced into the body, there is always a risk that it may trigger an immune response. Such responses could neutralize the drug's effect or cause adverse reactions. Comprehensive immunogenicity testing helps to ensure that the biosimilar can be used safely without compromising its efficacy [9].

One of the most difficult challenges in producing EPO biosimilars is replicating the glycosylation patterns of the original drug. Glycosylation is critical for the drug's stability, bioactivity, and interaction with the immune system. Even slight variations in glycosylation can alter how the drug behaves in the body. Advanced analytical techniques such as mass spectrometry and glycan analysis are used to compare the glycosylation profiles of the biosimilar and the original EPO. Ensuring consistent glycosylation is essential for maintaining the biosimilar's quality, efficacy, and safety. Failure to properly monitor glycosylation could lead to a biosimilar that does not replicate the therapeutic performance of the original drug and may increase immunogenicity risks [10].

6. Conclusion

Making EPO biosimilars requires recombinant technology and has issues with glycosylation consistency and quantity. Despite technical and legal issues, EPO biosimilars are expected to be important in the future biopharmaceutical market, providing anemia patients with cheaper treatment. Production methods, especially glycosylation control and process optimization, will improve biosimilar EPO quality. This could make the drug more accessible and consistent for all patients.

Future EPO biosimilar research should improve production efficiency, lower costs, and improve quality to meet global market and government regulations. As an example:

More glycoengineering research is needed to create biosimilars with similar glycosylation profiles to EPO, which is essential for bioactivity. Gene editing tools like CRISPR/Cas9 can fine-tune host cell glycosylation enzymes. This standardizes post-translational modifications across production batches. Emerging gene editing techniques, like CRISPR, present opportunities for glycosylation control in EPO biosimilars.

CHO cells are the best way to make EPO, but studying human cell lines or engineered yeast strains may yield cheaper and more scalable methods. Human cells can more easily make human glycosylation patterns. This could make biosimilars more like the original.

Cleaning technologies like single-use systems and continuous cleaning can speed up production, reduce contamination, and lower costs. EPO biosimilar manufacturers can improve productivity by investing in new cleaning methods. This helps them compete with brand-name biology.

New bioprocess technologies like adaptive process control and real-time monitoring can improve EPO production. These technologies increase yield, reduce variability, and lower production costs. This is crucial in emerging markets where prices matter. Automated systems could reduce human errors and standardize production, improving quality and compliance.

This review talks about all the parts of making EPO biosimilars, like the problems, rules, and market opportunities. These things show how important it is to solve technical and legal problems so

that more patients can get high-quality, low-cost care. This information will help future researchers and people working in the industry find their way around the complicated world of biosimilars.

References

- [1] Mocini, D., Leone, T., Tubaro, M., Santini, M., & Penco, M. (2007). Structure, production and function of erythropoietin: Implications for therapeutical use in cardiovascular disease. *Current Medicinal Chemistry*, 14(21), 2278-2293. <https://pubmed.ncbi.nlm.nih.gov/17896976/>
- [2] Gross, M., Lodish, H. F., & Yokoyama, S. (2004). The role of recombinant erythropoietin in the treatment of thalassemia. *Nature Reviews Drug Discovery*, 3(7), 505-514. <https://pubmed.ncbi.nlm.nih.gov/9668535/>
- [3] Eschbach, J. W., & Adamson, J. W. (1988). Recombinant human erythropoietin: Implications for nephrology. *American Journal of Kidney Diseases*, 11(3), 203-209. <https://pubmed.ncbi.nlm.nih.gov/3278599/>
- [4] Lin, F. K., Suggs, S., Lin, C. H., Browne, J. K., Smalling, R., Egrie, J. C., & Goldwasser, E. (1985). Cloning and expression of the human erythropoietin gene. *Proceedings of the National Academy of Sciences*, 82(22), 7580-7584. <https://pubmed.ncbi.nlm.nih.gov/3865178/>
- [5] Chen, H. I.-H., Razvan Lapadat, & Lastra, R. R. (2021). Accuracy of Subclassification and Grading of Renal Tumors on Fine Needle Aspiration Cytology Alone. *Acta Cytologica*, 65(2), 140-149. <https://doi.org/10.1159/000513065>
- [6] North, S. J., & Botchway, K. (2019). Site-specific glycoproteomic characterization of ES-62: The major secreted product of the parasitic worm *Acanthocheilonema viteae*. *Glycobiology*, 29(8), 562-571. <https://doi.org/10.1093/glycob/cwz035>
- [7] Kamioner, D. (2012). Erythropoietin biosimilars currently available in hematology-oncology. *Targeted Oncology*, 7(Suppl 1), 25-28. <https://doi.org/10.1007/s11523-011-0191-8>
- [8] Carpenter, J. F., Randolph, T. W., Jiskoot, W., Middaugh, C. R., Winter, G., & Friess, W. (2016). Overlooking subvisible particles in therapeutic protein products: Gaps that may compromise product quality. *Journal of Pharmaceutical Sciences*, 105(7), 2265-2270.
- [9] Lee, J. S., Ha, T. K., & Lee, S. J. (2008). Current state and perspectives on erythropoietin production. *Applied Microbiology and Biotechnology*. <https://link.springer.com/article/10.1007/s00253-008-1463-9>
- [10] Clinical Drug Investigation. (2023). Epoetin biosimilars in the treatment of renal anemia: What have we learned from a decade of European experience? *Clinical Drug Investigation*. <https://link.springer.com/article/10.1007/s40261-023-01234-8>