# Generation of recombinant CHO cells towards bioengineered heparin and heparan sulfate production

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論 文 名　：Generation of recombinant CHO cells towards bioengineered heparin and heparan sulfate production
(ヘパリンおよびヘパラン硫酸のバイオ生産に向けた組換え CHO 細胞の作製)

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## 論　文　内　容　の　要　旨

Heparin, a well-established sulfated glycosaminoglycan, has been essential in treating and preventing thrombotic disorders since its discovery in 1916. While its anticoagulant properties are well-known, recent research has highlighted its potential in various therapeutic areas, including anti-inflammatory, antiviral, antibacterial, anti-thrombotic, anti-metastatic, anti-hypolipidemic, and anti-angiogenesis applications. This versatility is especially significant during the COVID-19 pandemic, where heparin has shown promise in reducing mortality through its multifaceted effects.

The complex architecture of heparin, typically sourced from animal tissues, enables its interaction with a broad spectrum of biological molecules, a captivating characteristic that has intrigued researchers for decades. Nevertheless, reliance on animal sources presents challenges such as supply vulnerabilities and contamination risks, as evidenced by past shortages and quality control issues.

To address these challenges, significant progress has been made in bioengineering and nanotechnology. Advancements in microbial engineering and enzyme-assisted alteration have provided a new horizon for the biosynthesis of heparosan, the precursor to heparin and heparan sulfate, through microbial fermentation. Heparosan offers advantages in safety, yield, and cost-effectiveness, making it a strong candidate for bioengineered heparin synthesis through subsequent enzymatic sulfation. Furthermore, advancements in both prokaryotic and eukaryotic systems have opened new avenues for producing high-purity heparin. Notably, the use of recombinant Chinese hamster ovary (CHO) cells has been particularly promising. These CHO cells can be genetically engineered to produce heparin with high purity and consistency, offering a reliable and scalable alternative to traditional animal-sourced heparin. This approach minimizes contamination risks and supply vulnerabilities associated with animal sources, thus enhancing the overall safety and availability of heparin.

In our study, we utilized several strategies by applying advanced technologies such as CRISPR-Cas9 technology to knocking out many targets (including chondroitin sulfate biosynthesis pathways and desulfation enzymes) to enhance heparin production. Moreover, temperature shift strategies were applied to optimize conditions for protein expression and cellular metabolism. These tactics attempt to increase the production of heparin with desired quality, on which further investigation is in progress. Another key area of interest is in the development of scalable bioreactor protocols for maintaining process stability and economic feasibility, particularly when scaled for large production.

To sum up, the continually evolving therapy potential and new bioengineered production methods of heparin have opened an exciting opportunity to enhance our abilities to improve patient recovery and redefine therapeutic interventions across a wide range of medical fields. There is still a lot of research work to be done and its full potential as a therapeutic molecule will only be realized with continued work on its better structuring.