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REVIEW

Bibliographic update on cell and protein engineering

Actualización bibliográfica sobre ingeniería celular y de proteínas

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ABSTRACT

Introduction: bioprocessing is undergoing a revolution driven by innovations such as single-use systems and continuous bioprocessing. The direct approach to cell engineering is to introduce to or omit from the cell a gene or genes by genetic engineering methods to endow a particular phenotype in order to improve the cellular processes. Over three-quarters of therapeutic proteins are produced using Chinese hamster ovary cells, that raises as the most common cell line used. The aim was to characterize cell and protein engineering.
Method: a literature review was conducted, where 18 articles in English and Spanish were selected, published in the last five years on the subject, in databases such as: Scopus, PubMed, Springer.
Results: deleting nonessential genes may increase the productivity by directing cellular resources toward product biosynthesis. Cell culture system provide controlled environments to study cellular process, mammalian cell cultures are valuable for virology, vaccine production, tissue regeneration, genetic engineering. The use of these modified cells is essential for producing recombinant proteins, antibodies and vaccines.
Conclusions: cell and protein engineering raised as alternative tools for the development of specific therapies in cancer and immunologic diseases in the last few years. Recombinant protein production lay on areas as cell culture, mammalian cells for specific antibody expression, cell culture technologies and bioreactors as the corner stone for bioprocess.

Keywords: Bioprocessing; Bioengineering; Monoclonal antibodies; Recombinant protein production; Mammalian cells; Bioreactors.

RESUMEN

Introducción: el bioprocesamiento está experimentando una revolución impulsada por innovaciones como los sistemas de un solo uso y el bioprocesamiento continuo. El enfoque directo de la ingeniería celular consiste en introducir u omitir uno o más genes en la célula mediante métodos de ingeniería genética para dotarla de un fenotipo específico y mejorar los procesos celulares. Más de tres cuartas partes de las proteínas terapéuticas se producen utilizando células de ovario de hámster chino, que se cría como la línea celular más utilizada. El objetivo era caracterizar la ingeniería celular y proteica.
Método: se realizó una revisión de literatura, donde se seleccionaron 18 artículos en inglés y español, publicados en los últimos cinco años sobre el tema, en bases de datos como: Scopus, PubMed, Springer.
Resultados: la eliminación de genes no esenciales puede aumentar la productividad al destinar los recursos celulares a la biosíntesis de productos. Los sistemas de cultivo celular proporcionan entornos controlados para el estudio de procesos celulares. Los cultivos de células de mamíferos son valiosos para la virología, la

producción de vacunas, la regeneración tisular y la ingeniería genética. El uso de estas células modificadas es esencial para la producción de proteínas recombinantes, anticuerpos y vacunas.
Conclusiones: la ingeniería celular y proteica se ha convertido en una herramienta alternativa para el desarrollo de terapias específicas contra el cáncer y las enfermedades inmunológicas. La producción de proteínas recombinantes se basa en áreas como el cultivo celular, las células de mamíferos para la expresión de anticuerpos específicos, las tecnologías de cultivo celular y los biorreactores como piedra angular de los bioprocesos.

Palabras clave: Bioprocesamiento; Bioingeniería; Anticuerpos Monoclonales; Producción De Proteínas Recombinantes; Células De Mamíferos; Biorreactores.

INTRODUCTION

Early efforts to develop cell culture were driven by the need to find a replacement for animals and animal tissues.[1,2,3,4] The emergence of antibody as a class of therapeutic agent changed drug discovery to a hypothesis-driven and design-based process.[1]

By the middle of the twentieth century, the first human cell line, HeLa, was successfully isolated from a cervical tumor and cultured in vitro.[3] Cohen and Boyer, created the recombinant DNA technology in 1973.[1] On the other side, cell culture is a century-old science, pioneered by Ross Harrison in 1907 who grew frog nerve fiber on lymph fluid in vitro for weeks on coverslips.[5]

Cell engineering and protein engineering are tools that can facilitate the production of some proteins, that are difficult to express due factors like toxicity to the cells, complex post-translational modifications or quaternary structure, or instability after secretion, to name a few.[6] The direct approach to cell engineering is to introduce to or omit from the cell a gene or genes by genetic engineering methods to endow a particular phenotype in order to improve the cellular processes.[2] Bioprocessing is undergoing a revolution driven by innovations such as single-use systems and continuous bioprocessing.

Advancements in the area of recombinant protein production have changed the previous trend, making the yield much higher and the cost much lower, thus allowing the production of such proteins on an industrial scale and opening the door for the treatment of multiple diseases and disorders.[7] The use of licensed compounds for the induction/repression of gene transcription, such as antibiotic resistance operons including macrolide inducible resistance operons and pharmacological agents that can be used to regulate gene expression, have been exploited for biopharmaceutical production.[2]

The first generation of recombinant DNA therapeutic proteins, including human growth hormone and insulin, were produced in Escherichia coli. The second wave of human therapeutic proteins, including tissue plasminogen activation (tPA), erythropoietin (EPO), and Factor VIII, required extensive posttranslational modifications (such as multiple disulfide bonds and complex glycosylation) not attainable in microbial hosts.[3]

Over three-quarters of therapeutic proteins are produced using Chinese hamster ovary (CHO) cells,[1,2] that raises as the most common cell line used. The other prominent host cells are derived from mouse myeloma (SP2/0, NS0), Syrian hamster kidney cells (BHK), human embryonic kidney (HEK293) cells and most recently added the human retina derived (PerC6).[1,5] Other examples: bacteria, yeast, insect cells, transgenic animals, and transgenic plants.[7]

The relevant value-added products in biopharmaceutical industry include monoclonal antibodies, interferons, hormones, growth and coagulation factors, vaccines, and others.[8] Around 570 monoclonal antibodies (mAbs) have been tested, of those tested, 79 mAbs have been approved by the Food and Drug Administration (FDA) for commercial use. A substantial majority of the approved mAbs are used in treatments of cancer and autoimmune disorders.[8]

Bioengineering is expanding rapidly with broad societal impacts, driven by technological convergence and decreasing costs. It spans applications from personalized medicine to environmental solutions, but also raises ethical, regulatory, and equity concerns. With a bibliographic point of view this investigation was conducted, the aim was to characterize cell and protein engineering.

METHOD

A bibliographic investigation was carried out. To this end, a search was conducted in databases such as PubMed, Scopus, and Springer using the following keywords: Bioprocessing; Bioengineering; Monoclonal antibodies; Recombinant protein production; Mammalian cells; Bioreactors. These terms were used in combination with the connector “and.” A total of 18 articles were selected, more than 60 % of which were published in the last five years, in both Spanish and English. Relevant information was extracted, and after organizing and synthesizing it, a well-structured development was achieved.

RESULTS

Cell lines for therapeutic protein production were traditionally constructed by random integration of linearized plasmid DNA containing a gene of interest (GOI) into the host cell genome, followed by selection and amplification of GOI copy number to ensure a high transcript level. Host cell engineering will likely become more prevalent, especially with multi-gene manipulation to engineer pathways or traits to create a cell line with ideal metabolic behavior, production capabilities, and product quality characteristics.[6]

Targeted genetic alterations are increasingly being pursued to increase the productivity of natural products. Deleting nonessential genes may increase the productivity by directing cellular resources toward product biosynthesis.[1] However, selection of high production clones, minimizing the cost of media preparation, bioreactor design and optimization, and streamlining the product recovery,[9] are all important research areas for cost effective production that need to be promoted.

On the other side, autologous cell therapy focuses on bioprocesses that allow a patient's own cells to be manufactured into an advanced therapy medicinal product (ATMP). The major advantage of autologous cell therapies is that the implanted cells will not trigger a graft-vs-host immune response thus reducing the need for immunosuppression.[10] However, some factors like host viability, the economic costs and the accessibility to this therapy limit its world spreading.

Funding sources and political economy strongly influence bioengineering research directions and accountability. There is also a critical need for robust ethical frameworks and inclusive policymaking to manage risks and ensure fair distribution of benefits.

Specifications

The vast majority of recombinant therapeutic proteins are glycoproteins. The extent of glycosylation and the structure of the glycans on those glycoproteins may have a profound effect on their activities and circulatory half-life.[3]

Protein glycosylation is an important characteristic and plays a crucial role in the efficacy, serum half-life, and antigenicity of a recombinant biopharmaceutical.[7] Cell engineering of glycosylation patterns and in vitro glycosylation has provided new possibilities for biopharmaceutical design and optimization thus lowering immunogenicity effects, increasing stability and enhancing functional activity.[2] The level of host cell proteins (HCP) in the product can also be controlled to an acceptable regulatory level, due its con centration may alter product characteristics over long-term storage.[6]

With recombinant DNA technology, virus surface antigens may be produced using engineered host cells. The protein antigen is then used to vaccinate, without using the actual virus particle itself. It is also possible to employ a recombinant virus by inserting the gene coding for the surface antigen of the target virus into the genome of the carrier virus.[3]

Cell engineering research has also started to focus on other regulatory elements of the cell such as mRNA processing pathways and microRNA (miRNA). Recently, it has been discovered that if X-Box binding protein 1 spliced (S) (XBP 1(S)) was obtained from the transcript XBP-1 by mRNA processing, it had the possibility to lead to increased secretion, cell size, membrane synthesis, and energy production.[2]

Cell culture

Cultured cells are the standard vehicle for the production of biologics, both proteins and viruses. The quantity of products produced each year varies widely, from hundreds of kilograms of proteins produced in stirred-tank reactors of tens of cubic meters to merely a thousand liters of a virus-infected culture medium.[3] Their ability to generate homogenous data sets is crucial for understanding cellular mechanisms without the confounding factors present in vivo.

Cell culture processes are used to produce the vast majority of protein therapeutics, valued at over US$ 180 billion per annum worldwide. For more than a decade now, these processes have become highly productive.[6]

A typical cell culture process starts with growing and expanding cells in suspension through a series of reactors of increasing size (also known as the seed train), until a sufficient number of cells have been produced to seed the production reactor.[6] Cell culture fermentation is used for the production of several important biologicals including interferons, growth factors, vaccines, hormones, and monoclonal antibodies.[9,11]

In designing a process and selecting a medium for a particular cell line, one has to keep stoichiometric balance in mind. Depending on the amount of cells to be reached, the medium must supply sufficient amounts of all components, including inorganic materials (phosphate, potassium, magnesium, etc.), in the medium.[3]

Three-dimensional culture systems and organoids represent a significant advancement, allowing cells to self-organize into functional clusters that better mimic physiological conditions.[12]

For cell therapy, vaccine, and viral vector applications, the cell culture process is largely similar to that for the manufacturing of biologics, with emphasis on deploying disposable apparatus, achieving high cell concentration, and for cell therapy applications, keeping the product cells at a high viability and potency state.[6]

Mammalian cells

Therapeutic antibodies are characterized by complex post translational modifications to ensure bioactivity and low toxicity of the proteins. Therefore, mammalian cell lines like Chinese hamster ovary (CHO) cells are utilized for the expression of these complex biomolecules instead of microbial hosts.[8,13] They have the capacity to express large and complex recombinant proteins.[7]

A number of mammalian host cell lines are commonly used for genetic modification to produce a product, including Chinese Hamster Ovary (CHO) cells, Human embryonic kidney cells (HEK-293), mouse myeloma (NS0), and baby hamster kidney (BHK) cells.[6,14]

Mammalian cells have the unique ability to produce therapeutic proteins with oligosaccharides attached to their serine/threonine (O-linked glycosylation) or asparagines (N-linked glycosylation) residues. This unique advantage outweighs the costs associated with mammalian cell culture, which are far greater in terms of development time and manufacturing when compared to bacterial culture.[2] These cells are highly specialized, preforming varied physiological functions, which must be considered when selecting cell types for specific purposes.

The functional biological activity of the glycoprotein is directly affected by glycosylation, including its trafficking and folding within the host cell. Once secreted, solubility, aggregation, stability and immunogenicity of the protein may be affected by its glycosylation pattern.[2]

Over the past 15 years, the use of mammalian cells has contributed to the leverage of methods in diagnosis, therapy and processes that improve the quality of life, as can be seen in the industrial environment cell culture, along with bioprocess engineers (BE) knowledge, enables large scale production of pharmaceuticals, vaccines, cosmetics, in addition to in vitro cell tissue production, which helps the regeneration of tissues and organs.[15] The use of mammalian cells is crucial for producing recombinants proteins, antibodies, and vaccines that require proper folding and post-traslational modifications unique to higher organisms.

Bioreactor

Bioreactors are the corner stone of the bioprocessing industry, although each bioreactor design has its advantages and disadvantages. Between 2003 and 2023, studies on bioreactor technology and the de sign of these devices increased by approximately 1200 % in an effort to develop versatile and sophisticated equipment that meets the needs of the current market in the various industrial sectors in which fermentation processes are used.[16]

A bioreactor plays a dominant role in the production of biologicals, and it has been regarded as the central production wheel for the biopharmaceutical industry.[17] Bioreactors can be defined as equipment used to cultivate animal, plant, or microbial cells on a small or large scale.[5,16] Compared to antibiotic manufacturing, cell culture bio reactors are smaller in size.[1]

Bioreactors have emerged as indispensable tools in the field of bioprocessing, playing a pivotal role in the production of various biopharmaceuticals, biofuels, and industrial enzymes. These versatile devices facilitate the cultivation of cells or microorganisms in a controlled environment, allowing for the efficient production of desired products.[10,16,18]

Omic technologies are transforming bioengineering and bioprocessing by providing comprehensive molecular insights. The integrations of omics with bioinformatics and artificial intelligence accelerates discovery and enhances predictive capabilities, improving process control and product quality.

Recent developments in metabolic engineering also include the use of gene-editing tools for successful clone and product development. Innovations in cell engineering, including the use of RNA information, ribozyme engineering, and CRISPR-Cas-based techniques, have been applied in pursuit of better strategies for antibody production.[7]

Nevertheless, ethical considerations around genomic data privacy and equitable access to omic-driven therapies also require attention.

Besides CHO cell-based production of protein therapeutics, other cell culture processes, including virus production for vaccine and gene therapy applications, may benefit from - omic tools.[6] Some approaches utilized for the modification of the glycan pattern of recombinant proteins include the selection of a proper expression host, glycoengineering, and upstream process optimization to control protein glycosylation.[7]

Several new technologies have been introduced to overcome positional dependent inactivation. For example, using chemicals such as sodium butyrate and sodium propionate, which inhibit histone deacteylases since histone actylation is generally related to enhanced expression, but these compounds tend to have cytotoxic effects as well.[2]

CONCLUSIONS

Cell and protein engineering raised as alternative tools for the development of specific therapies in cancer and immunologic diseases in the last few years. Recombinant protein production lay on areas as cell culture,

mammalian cells for specific antibody expression, cell culture technologies and bioreactors as the corner stone for bioprocess.

BIBLIOGRAPHIC REFERENCES

1. Bandyopadhyay AA, Khetan A, Malmberg L-H, Zhou W, Hu W-S. Advancement in bioprocess technology: parallels between microbial natural products and cell culture biologics. Journal of Industrial Microbiology and Biotechnology 2017;44:785-97. https://doi.org/10.1007/s10295-017-1913-4.

2. Kuystermans D, Krampe B, Swiderek H, Al-Rubeai M. Using cell engineering and omic tools for the improvement of cell culture processes. Cytotechnology 2007;53:3-22. https://doi.org/10.1007/s10616-007-9055-6.

3. Hu W. Cell Culture Bioprocesses and Biomanufacturing, 2017, p. 289-318. https://doi.org/10.1002/9781119159056.ch10.

4. Malathi H, Singh SK, Narayan J. Investigating the Role of Decellularization in Liver Regeneration and Scaffold Development for Transplantation. Seminars in Medical Writing and Education 2023;2:143. https://doi.org/10.56294/mw2023143.

5. Yodsanga S, Poeaim S, Chantarangsu S, Swasdison S. Investigation of Biodegradation and Biocompatibility of Chitosan–Bacterial Cellulose Composite Scaffold for Bone Tissue Engineering Applications. Cells 2025;14. https://doi.org/10.3390/cells14100723.

6. O'Brien SA, Hu W-S. Cell culture bioprocessing — the road taken and the path forward. Current Opinion in Chemical Engineering 2020;30:128-34. https://doi.org/10.1016/j.coche.2020.100663.

7. Tripathi NK, Shrivastava A. Recent Developments in Bioprocessing of Recombinant Proteins: Expression Hosts and Process Development. Front Bioeng Biotechnol 2019;7:420. https://doi.org/10.3389/fbioe.2019.00420.

8. Reyes SJ, Durocher Y, Pham PL, Henry O. Modern Sensor Tools and Techniques for Monitoring, Controlling, and Improving Cell Culture Processes. Processes 2022;10. https://doi.org/10.3390/pr10020189.

9. Ozturk SS. Engineering challenges in high density cell culture systems. Cytotechnology 1996;22:3-16. https://doi.org/10.1007/BF00353919.

10. Papantoniou I, Lambrechts T, Aerts MJ. Bioprocess engineering strategies for autologous human MSC-based therapies: one size does not fit all. Cell Gene Therapy Insights 2017;3:469-82. https://doi.org/10.18609/cgti.2017.040.

11. Das C, S RJ, Rohini. Adaptive Control Strategies for Bioprinting Tissues and Organs in Biomedical Engineering Applications. Seminars in Medical Writing and Education 2023;2:100. https://doi.org/10.56294/mw2023100.

12. Horta-Martínez LE. 3D printing in the medical field. Seminars in Medical Writing and Education 2022;1:8. https://doi.org/10.56294/mw20228.

13. Reger LN, Saballus M, Matuszczyk J, Kampmann M, Wijffels RH, Martens DE, et al. Boosting Productivity for Advanced Biomanufacturing by Re-Using Viable Cells. Frontiers in Bioengineering and Biotechnology 2023; 11-2023.

14. Bhardwaj U, Samal P, Malathi H. Stem Cell-Based Cartilage Engineering Approaches for Osteochondral Defect Regeneration and Functional Tissue Repair. Seminars in Medical Writing and Education 2023;2:148. https://doi.org/10.56294/mw2023148.

15. Pereira S, Coutinho D, Matos A, Silva W, Fabrino D. Three-dimensional cell culture, opportunities and challenges for bioprocess engineers. Brazilian Journal of Biological Sciences 2016;3:263-77. https://doi.org/10.21472/bjbs.030603.

16. Palladino F, Marcelino PR, Schlogl AE, José ÁH, Rodrigues RD, Fabrino DL, et al. Bioreactors: Applications

and Innovations for a Sustainable and Healthy Future—A Critical Review. Applied Sciences 2024;14. https://doi.org/10.3390/app14209346.

17. Sharma R, Harrison S, Tai S. Advances in Bioreactor Systems for the Production of Biologicals in Mammalian Cells. ChemBioEng Reviews 2021;9. https://doi.org/10.1002/cben.202100022.

18. Kaya U. Advancements in Bioreactors: Revolutionizing bioprocessing Technologies. Pharmaceutical Bioprocessing 2023; 11:120-1. https://doi.org/10.37532/20489145.2023.11.

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