Review Article
Volume 7:3, 2024

Journal of Biomedical and Pharmaceutical Sciences

ISSN: 2952-8100 Open Access

An Overview on the Production of Therapeutics and their Applications

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Abstract

Human cells produce an enormous number of proteins and dysfunction in these may lead to serious diseases and developmental abnormalities. To treat these protein de iciencies the missing or dysfunctional molecules are complemented or substituted with therapeutics provided by different biological systems. However, protein therapeutics must unavoidably adhere to quality constraints that are much stricter than those for chemical industries. Although it is undoubtedly a challenging task to obtain an active protein in a way that is economically feasible, biopharmaceuticals (recombinant proteins, monoclonal antibodies or vaccines) are the largest group of drugs developed in the pharmaceutical industry. Market calculations estimated the recombinant protein drug industry to be around 10% of the entire drug market, predicting an even larger proportion in the future. The global market of biopharmaceuticals is estimated to grow at a Compound Annual Growth Rate (CAGR) of 13.8% from 2018 to 2025, according to the latest report developed by allied market research. The report biopharmaceuticals market by type and application: Global opportunity analysis and industry forecast, presenting an analysis of pro iles of the major player projects that the global biopharmaceuticals market, which reached $186,470 million in 2017, will have reached $526,008 million by 2025. The more detailed characteristics of the expected value growth of particular biologic types in the biopharmaceuticals market (divided into dominating monoclonal antibody, growth and coagulation factor, interferon, vaccine, insulin, erythropoietin and hormone) are available in the report and on the allied market research website.

Keywords: Education • Training • Attainment • Empowers • Job tasks

Introduction

The technologies behind the synthesis of biopharmaceuticals have changed since several protein drugs were approved in the 1980s, although protein molecules have been used as biopharmaceuticals since the 1920s (reports on insulin from pig pancreas) and there were several milestones in the utilization and development of various expression systems. In 1982, the date when humulin (human insulin) was approved by the Food and Drug Administration (FDA) as the first recombinant biopharmaceutical was recognized as a scientific turning point in this review. That was the point at which genetic and biotechnological research finally met medical needs and standards, consequently providing a new generation of therapeutics. Therefore, after more than 35 years, we present a brief overview of the existing expression platforms, together with the main extraction and purification methods [1].

It should be noted here that the terminology connected to biopharmaceuticals varies between scientific communities or industrial units, sometimes referring to different subcategories of therapeutics within the general category. In the context of this review the term recombinant protein biopharmaceuticals includes any pharmaceutical protein drug (e.g., recombinant proteins and peptides, vaccines and monoclonal antibodies) that was obtained *via* engineering of biological sources [2].

Literature Review

Therapeutic proteins and their production

Proteins and peptides are a wide variety of therapeutic molecules ranging from enzymes to cytokines. These therapeutics have several biological functions and interact with various biological pathways. For example, hormones, neurotransmitters and growth factors all represent distinct functions of proteins and peptides. New classes of proteins and peptides continue to be of significant research interest. However, there are several hurdles to cross for companies seeking to develop new protein and peptide therapies. Humans have been using cultured cells since the dawn of civilization. A case can be made that the fermentation of grains by microbes into beer lead to the rise of agriculture and city states [3].

For centuries, microbes were primarily used to produce human consumables including bread, cheese, alcohol and vinegar. It was

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Received: 29 November, 2019, Manuscript No. JBPS-23-5157; **Editor assigned:** 04 December, 2019, Pre QC No. P-5157; **Reviewed:** 18 December 2019, QC No. Q-5157; **Revised:** 30 June, 2023, Manuscript No. R-5157; **Published:** 28 May, 2024, DOI: 10.37421/2952-8100.2024.07.415

not until the 20th century that cultured cells found widespread medicinal and industrial use. Large scale production was initially based on native plant and animal sources. With the advent of recombinant DNA technology and use of cultured cells, a large variety of proteins became available. Today, more than 170 recombinant proteins are used worldwide in medicine. Initially, Pharmaceutical Recombinant Proteins (PRPs) were designed to be as similar as possible to the naturally occurring human protein. More recently, genetic variants of existing proteins and entirely new protein designs have been created for superior therapeutic values and protein stability. These include gene-fusion products to extend the circulating half-life of coagulation proteins or engineered antibodies for cancer treatment. Recombinant proteins for industrial use (IRPs) were developed at the same time as PRPs. Included are enzymes (proteases, lipases, amylases etc.) and structural proteins with wide-ranging applications including the production of food and beverages, conversion of carbohydrates into fuel ethanol or biodiesel, components for clothing and cosmetics, biopolymers, cleaning materials and waste management. IRPs are also genetically engineered for advantageous traits such as improved stability at a different pH, insensitivity towards oxidation and resistance against heat-induced inactivation, misfolding or aggregation (Tables 1 and 2) [4].

Pharmaceutical protein			
Product	**Cell line**	**Application retail**	**Price per kg R**
Rituximab	Hamster	Lymphoma	$9,500,000.00
Eculizumab	Murine myeloma	PNH	$23,000,000.00
rHGH	*E. coli*	GH deficiency	$137,000,000.00
rFVIIa	Hamster	Hemophilia with inhibitor	$2,070,000,000.00

Table 1. Important pharmaceutical proteins with their applications.

Industrial protein			
Product	**Cell line**	**Application retail**	**Price per kg R**
Cellulase	*T. reesei*	Fuel ethanol	$10.00
rβ-Glucosidase	*E. coli*	Fuel ethanol	$37.00

Table 2. Products of industrial proteins.

Basic steps in the manufacturing of recombinant proteins

Whether manufactured for industrial or pharmaceutical use, recombinantly produced proteins follow the same basic manufacturing process. The first step in manufacturing the Protein of Interest (POI) is to isolate the corresponding nucleic acid sequence (GOI). In the early phases of the biotech industry, cloning of DNA sequences from mRNA was a crucial step. Today, this is mostly replaced by direct DNA synthesis aided by the availability of genome sequences from a wide variety of species. GOI expression plasmids are then inserted into an appropriate host system to establish a recombinant cell/organism which can be frozen for later use. From vials of frozen cell banks, cultures can be established to initiate a production run. Typically, harvests are executed when cultures have achieved high cell density. In first step is manufacturing the Protein of Interest (POI) is to isolate the corresponding nucleic acid sequence (GOI). In the early phases of the biotech industry, cloning of DNA sequences from mRNA was a crucial step. Today, this is mostly replaced by direct DNA synthesis aided by the availability of genome sequences from a wide variety of species. GOI expression plasmids are then inserted into an appropriate host system to establish a recombinant cell/organism which can be frozen for later use. From vials of frozen cell banks, cultures can be established to initiate a production run. Typically, harvests are executed when cultures have achieved high cell density in batch or fed-batch cultures. At harvest, the production medium and/or cells that contain the POI are then processed further for purification, packaging and distribution. Although most recombinant proteins follow this basic manufacturing outline there are vast differences in the processes depending on the POI and its application. These are not only between industrial and pharmaceutical manufacturing processes but also amongst different proteins produced for industrial or for pharmaceutical purposes [5].

Discussion

Therapeutic vaccines and their importance

Therapeutic vaccines are able to treat a disease by firming an immune system. Therapeutic vaccines are presently produced against chronic viral infections, for example Human Papilloma Virus (HPV), herpesvirus, Human Immunodeficiency Virus (HIV), Hepatitis B (HBV) and C (HCV) viral infections. It is alternative to antiviral treatment. These therapeutic vaccines activate immune system of patients to kill virus or eliminate viral infection. Targets of therapeutic vaccines are chronic viral infections that cannot completely eliminate with other therapies. Complete understanding of immune response is necessary for the designing of therapeutic vaccines. Ideal therapeutic vaccine can activate both innate and adaptive immune response that provide strong multi-specific T-cell as well as B-cell immunity effective against several viral antigens [6].

Production of therapeutic vaccines from plants

Production of therapeutic vaccines from transgenic plants offers many advantages in terms of storage, transportation, absence of human pathogens and heat stability as well as cost effective in production due to the elimination of fermenters. Among all parts of plant, genetic engineered chloroplast most common used for the production of therapeutic vaccine due to the high level of transgene expression along with multigene expression as well as maternal inheritance. Therapeutic vaccines that are derived from chloroplast have great functionality by several assays like assay used for macrophage lysis, protection of HeLa cells as well as provide immune response against pathogens. Novel transgenic expression vectors developed in plants for the production of therapeutic vaccines that control bioterrorism attacks with increased safety as well as high scalability. Production of vaccines from green biofactories is an attractive platform for therapeutic vaccines due to the huge amount of plastid DNA present in chloroplast that are beneficial for the large amount of vaccine production with high efficacy. Vaccines that are derived from plants save for the health of patient as well as for environment [7].

Recently recombinant influenza VLP virus like particles produced in transgenic plants that expressing hemagglutinin. These virus-like particles are produced only in the absence of neuraminidase or sialidase extraction that insure VLP production in plants. Another recent research carried out for the production of anti-caries DNA vaccine in transgenic tomato plants by the fusion of chlorella toxin B subunit with PAcA gene. Bacterial infections that are chronic in nature, influence on the growth of dental caries within oral cavity. Development of effective vaccine is important that provide protection against caries, by inducing anti-colony immunity against *Streptococcus mutans* infections. Agrobacterium mediated transformation technology is used for the construction of plasmids that were integrated into tomato genomes. PCR, GUS and western blot were used for the confirmed expression of genes at transcription and protein level. So, transgenic tomatoes provide useful system that help for the production of human caries antigen [8].

Production of therapeutic vaccines from microbes

Microbial system also plays an important role for the production of therapeutic vaccines. Variety of secondary metabolites produced in different bacterial and fungal species. These natural products have great therapeutic applications against human diseases. Recently recombinant DNA technology used for the wide array of biopharmaceutical products like recombinant vaccine. Microbial system has been used for the novel production of vaccine products. *Lactococcus lactis* host factory has being utilized for the production of therapeutic vaccine as well as host itself used as live vaccine against infectious diseases by inducing both systemic and mucosal immune responses also have adjuvant properties. Due to the lack of containment strategies, no live vaccine under clinical trial [9].

Recently microbial vehicles are used for the production of molecular allergen vaccine that are based on proteins and peptides related to allergen. These types of vaccine have low immunogenicity, thus appropriate delivery vehicle is needed. Different viruses, bacterial species along with their protein components have been widely used due to their adjuvant capacity. Immunomodulator properties of microbial system used for the production of allergen vaccine. Microbial delivery vehicle has been extensively used in allergy immunotherapy. Phage based vaccines are developed by recombinant fusion of antigens with any surface protein of virions. Antigenic sequence of phage virion present in genome that allows the stable production of vaccine within bacterial construct. Lambda, T7, T4 and filamentous M13, F1 most commonly used for the production of these vaccines [10].

mRNA vaccines

RNA based technology have been extensively used for the production of therapeutic vaccines. mRNA vaccines represent new era in vaccinology as well as promising approach as compared to conventional vaccines in terms of high efficacy, low manufacturing cost and save to use. Multiple mRNA vaccines used for infectious diseases and different types of cancer both for humans and animal models. For example, Zika virus, rabies virus and influenza virus. mRNA vaccines modulate innate and adaptive immunogenicity along with antigen specific T cell response as well as improve delivery. mRNA vaccines quickly produced and provide safe and long-lasting immune response. Different pathways are involved in the production of RNA vaccines. mRNA cannot integrate into host genome but degrade naturally during the antigen expression therefore much safer as compared to other vaccine. Influenza mRNA vaccines is an egg free platform for the production of antigen within mammalian cells with high fidelity [11].

Therapeutic antibodies and their applications

Therapeutic antibodies especially monoclonal antibodies are able to activate, repress or modify endogenous immune responses towards specific cells or molecules. Antibody based drugs revolution in the treatment of cancer, inflammatory and autoimmune disease as well as different types of disease. Immunotherapy is a form of antibody therapy in which monoclonal antibodies are used to bind specifically to cells or proteins. This type of treatment can stimulate the immune system of patients to attack on those cells. Currently therapeutic antibodies are utilized as pharmaceuticals for the treatment of cancer, inflammatory disease, cardiovascular disease, infection, respiratory disease and ophthalmological disease. Antibodies can be largely used as therapeutics due to their high specificity and affinity to target molecule along with high efficacy. They have the ability to target diverse molecules and their modes of action allow them to use as therapeutic target [12].

Production of therapeutic antibodies from plants

Recombinant antibody technology allows the production of improved and novel therapeutic agent that are immunological reagents within plants. It is a cost-effective approach for the production of therapeutic antibodies on large scale. Plant expression system reduced deglycosylation burden *in vitro* and provide therapeutics that are useful for targeted biological activity. Plants have the capacity to producing homogenous N-glycan that involve in the production of novel therapeutics antibodies with high safety and efficacy. Most of plant-based therapeutics are in preclinical and clinical trials and most of them approved by regulatory authority. Plants offers an optimal system for the production of antibodies, ELELYSO and ZMapp both have regulatory approval from FDA thus,

obvious a new era in the field of plant based therapeutic antibodies [13].

Antibodies produced in plant have advantageous as compared to animal cells due to the absence of contamination of other animal proteins. Recently produced therapeutic proteins from plants are used against rotavirus infection, human respiratory virus, rabies, ebola and anthrax etc. Monoclonal antibodies against lymphoma and breast cancer are in clinical trial. Transgenic plants involve in the production of therapeutic proteins and antibodies in which expression of foreign protein within the stable transformed plant cell occurred. Recombinant anti-cancer monoclonal antibodies are produced in transgenic plants. Monoclonal antibodies against anti-chikungunya virus produced in glycoengineered *Nicotiana benthamiana* plants. These antibodies are highly effective both *in vivo* and *in vitro* activity [14,15].

Production of therapeutic antibodies from microbes

Variety of microbial systems are used for the production of therapeutic antibodies. Bacterial cells most commonly used for the production of monoclonal antibodies due to their rapid growth rate. Microbial cell culture system required less time for establishment as compared to mammalian cell culture system. Engineered yeast and *E-coli* cells are also used for the production of therapeutic antibodies. Recently industrialized glycol engineered *P. pastoris* will used for the production of mAbs at lower costs. Next generation therapeutic antibodies have great potential with enhanced efficacy. Hybridoma technology and antibody display technology also plays an important role in the development of effective antibodies. Monoclonal antibody fragment fab is used as therapeutic agent developed in yeast, *E-coli*. Quality of this product largely influence on the success of pharmaceutical industry. Now a day bacterial system used for the production of small monoclonal fragments that does not required post translational modification therefore successfully produced in bacterial system [16].

Production of therapeutic antibodies from mammalian cell culture

Mammalian cell lines including CHO and NS0 used for the production of therapeutic antibodies on large industrial level. PREC6 human cells, Chinese hamster ovary cells also have the ability for high productivity with good quality attributes. Recently engineered cell lines also play an important role for the production of therapeutic antibodies. Production efficacy yield is an important parameter for the production of antibodies. First recombinant biopharmaceutical approved was produced in CHO cell lines in 1986. Human embryonic kidney cells most commonly used for the transient expression of monoclonal antibodies. Therapeutic antibodies are the most dominating technology in biopharmaceutical industry due to their versality in designing and therapeutic applications. They also used as target drug delivery system with increased bioavailability. Nanocarrier technology enhances the potential to control release and stability of mAbs (Table 3) [17].

Products	Indications	Marketing company	Regulatory approval
Bevacizumab (Avastin™)	Colorectal cancer	Genetech	2004
Trastuzumab (Herceptin™)	Brest cancer	Genetech Roche	1998
Panitumumab	Colorectal	Amgen	2006
Omalizumab (Xolair™)	Allergic asthma	Genetech Roche	2003

Table 3. Approved antibodies produced in CHO cells.

Conclusion

Due to the fact that 2017 marked 35 years since the first recombinant medicine was approved by FDA, we provided a brief reminder of the available prokaryotic and eukaryotic expression systems and of variations of the first to try, most established method of purification: Chromatography. Proteins and peptides belong to an increasingly important category of biopharmaceuticals. Taking into consideration the fact that within the next 10 years at most around 50% of all the medicines developed will be biopharmaceuticals; it is understandable that both expression systems and associated technologies of protein recovery arouse interest in scientific and business circles. The production of recombinant therapeutics is a complex, multidisciplinary and expensive process. The time required implementing the initial idea of a therapeutic compound and gain a functional product has been estimated at about 15 years. In spite of this, according to the 15th annual report and survey of biopharmaceutical manufacturing capacity and production released by bioplan associates, Inc. (The organization that has been analyzing the life sciences and biotechnology market for 30 years), the continuously growing world market of biopharmaceuticals has now reached over 250 million. Clearly, compared to drugs, biotherapeutics have appeared to be profitable investments. Although, in general, their manufacturing cost is high, biopharmaceuticals are usually developed mostly for diseases that currently lack a good alternative treatment. Consequently, this creates a ready market for them on one hand and supports high prices on the other.

Acknowledgments

We acknowledge the administration staff of Kinnaird college for women, for providing support.

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How to cite this article: Shafiq, Mehwish, Muqaddas Rana and Shahnaz Chaudhary. "An Overview on the Production of Therapeutics and their Applications." *J Biomed Pharm Sci* 7 (2024): 415.