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Biopharmaceuticals from microorganisms: from production to purification

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ABSTRACT

The use of biopharmaceuticals dates from the 19th century and within 5–10 years, up to 50% of all drugs in development will be biopharmaceuticals. In the 1980s, the biopharmaceutical industry experienced a significant growth in the production and approval of recombinant proteins such as interferons (IFN α, β, and γ) and growth hormones. The production of biopharmaceuticals, known as bioprocess, involves a wide range of techniques. In this review, we discuss the technology involved in the bioprocess and describe the available strategies and main advances in microbial fermentation and purification process to obtain biopharmaceuticals.

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Introduction

Biopharmaceuticals are mostly therapeutic recombinant proteins obtained by biotechnological processes. They are derived from biological sources such as organs and tissues, microorganisms, animal fluids, or genetically modified cells and organisms.[1,2] Although several different expression systems may be employed including mammalian cell lines, insects, and plants, new technological advancements are continuously being made to improve microorganism production of biopharmaceuticals. This investment is justified by the well-characterized genomes, versatility of plasmid vectors, availability of different host strains, cost-effectiveness as compared with other expression systems.[2,3]

Bioprocessing is a crucial part of biotechnology. There is an anticipation that within the next 5 to 10 years, up to 50% of all drugs in development will be biopharmaceuticals. Examples include recombinant proteins obtained through microbial fermentation process.[2,3] Bioprocessing for biopharmaceuticals production involves a wide range of techniques. In this review, we describe the main advances in microbial fermentation and purification process to obtain biopharmaceuticals.

Biopharmaceuticals and the pharmaceutical industry

Drug development is an extremely complex and expensive process. According to the Tufts Center for the Study of Drug Development[4] (http://www.csdd.tufts.edu), it may take approximately 15 years of intense research from the initial idea to the final product and development and costs usually exceed $2 billion. Low-molecular mass molecules are generically named as drugs while high-molecular mass drugs, which are represented by polymers of nucleotides (RNA or DNA) or amino acids (peptides and proteins), are called biopharmaceuticals.[5] Biopharmaceuticals based in nucleic acids, such as small interfering RNA (siRNA), DNA vaccines, and gene therapy, are very promising strategies. However, clinical protocols were approved only very recently[6] and just a few nucleic acids-based drugs have been therapeutically used to date[7] and recent reviews addressed the state of the art of nucleic acids in therapies.[8,9] In this review, we focused on peptides and proteins because they represent the major class of biopharmaceuticals.[10]

The use of proteins as drugs has been highlighted mainly by the high versatility of these biomolecules, which have different physiological roles in the human body including as catalysts, receptors, membrane channels, macromolecule carriers, and cellular defense agents.[10,11] Some protein therapies provide high specificity, such as replacement of a patient's defective protein or even fulfill its absence due to genetic defects or immunological complications.[10]

Biopharmaceuticals: reference, biosimilars, and biobetters

It is worth emphasizing that the same gene product, which encodes the identical amino acid sequence, could be obtained by extraction from an animal tissue or by recombinant DNA techniques. However, the same protein produced by different manufacturers present different characteristics. In order to differentiate the products, the first biopharmaceutical version of the same therapeutic protein is set as the reference medicine, whereas the following ones are denominated biosimilars. Biosimilars may present differences because of post-translational modifications (phosphorylation, glycosylation) and different manufacturing processes. The term biobetter, also named biosuperiors, was recently used to refer to therapeutic macromolecules of the next generation, which present more effective drug delivery system, are modified by chemical methods (e.g., PEGylation) and/or engineered by means of molecular biology techniques to present better pharmacologic properties such as higher activity, enhanced stability, fewer side effects, and lower immunogenicity.[12,13] Therefore, while a biosimilar represents a generic version of the original biopharmaceutical, biobetters need original research and development and the costs are significantly higher.[14]

Additionally, while the first biopharmaceuticals were predominantly delivered by injections, biobetters adopt different approaches to drug delivery administration as oral, dermatological and inhaled formulations which are related with different encapsulation approaches aiming to minimize the biologic instability caused by protein aggregation and denaturation as consequence of physicochemical modifications processes of the biodrug as deamination, hydrolysis, oxidation, among others.[15] Protein engineering and rational modification is also a very promising area in new biopharmaceuticals and some aspects will be discussed later.

The use of biopharmaceuticals has grown worldwide in the last few years. In 2016, the total number of products approved by the Foods and Drugs Administration (FDA) and European Medicines Agency (EMA) for use in humans reached 1357, of which >130 have different formulations (reference products), 737 are biosimilars, and the remaining 482 are classified as biobetters[16] (http://www.biopharma.com). From 2013 to 2016, 73 biopharmaceuticals were approved for use in humans. Among them, high prominence was given to monoclonal antibodies (23 approvals) widely used in several diagnostic procedures, treatment of inflammatory diseases, and neoplastic tumors[16] (http://www.biopharma.com).

In addition, the European Medicine Agency (EMA) licensed two new products based on gene therapy (insertion of a corrective gene able to produce a normal protein in the patient's genome to cure a genetic disease) for use in human therapeutic protocols. These products were Glybera, developed by the German company UniQure for the treatment of lipoprotein lipase deficiency, and Strimvelis, developed by GlaxoSmithKline (GSK) for the treatment of adenosine deaminase deficiency.[17] Although biopharmaceuticals can be very effective for disease control or cure, treatment costs can reach up to $1 million per patient.[18]

Biobetters based in protein structure engineering

One of the most promising areas of the biobetters relies in protein structure engineering aiming the development of biodrugs with better pharmacological properties including higher activity, fewer side effects, and lower immunogenicity. The breakthrough in the determination of protein structures and their use as medicines dates from 1980s as a consequence