

Modern Biotechnology Plays a Key Role in Medicine

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Abstract

Modern biotechnology refers to using sophisticated scientific methods to alter genes and cells, mainly through genetic engineering. This field makes it possible to transfer genetic traits between different species and is crucial in medicine, agriculture, and ecology. In contemporary medicine, biotechnology has become a driving force, introducing numerous breakthroughs that are transforming how we treat various illnesses. A significant advancement brought about by these innovations is the rise of targeted therapy, which offers a customized way to treat patients. Rather than using a one-size-fits-all method, healthcare providers can now analyze a patient's genetic makeup to pinpoint specific mutations, allowing them to adjust treatments to fit individual requirements.

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Introduction

Molecular biotechnology employs various methods to isolate and transfer genes between organisms [1]. Central to these techniques is the ability to attach a specific DNA sequence to a vector that can be introduced into a suitable host. This technique, known as recombinant DNA technology or molecular cloning, has many variations based on a solid grasp of fundamental molecular biology, bacterial genetics, and enzymes related to nucleic acids.

Bioreactors and fermenters are regarded as the backbone of any bioprocess, playing a vital role in producing antibiotics, vaccines, single-cell proteins, fermented foods, chemicals, wastewater treatment, and large-scale bioenergy production [2]. Historically, alcoholic drinks like wine and beer were made with yeast in traditional wooden bioreactors. Across the globe, various fermented foods, including Kefir, Kombucha, Sauerkraut, Tempeh, Miso, and numerous fermented vegetables are created using controlled microorganisms and enzymes that transform raw materials into these products. In 1857,

Louis Pasteur revealed that microorganisms could spoil food but could also create lactic acid and ethanol under controlled conditions. His introduction of pasteurization demonstrated that heating the medium could kill all present microorganisms, leading to longer shelf lives for products. Pasteur also formulated the germ theory, highlighting the significance of microorganisms in industrial bioprocesses. In 1928, Alexander Fleming accidentally discovered penicillin, which effectively combats gram-positive pathogens, and it became widely used during World War II. Penicillin was the first antibiotic, and industrial production began within a decade. By 1942, the mass production of penicillin launched modern industrial biotechnology in healthcare.

Biotechnology

Medical biotechnology focuses on applying molecular technologies to identify and treat human diseases [1]. At the heart of these innovations are methods for pinpointing and isolating specific genes and multiplying them in host organisms. These tools emerged from a thorough understanding of cell biochemistry,

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genetics, and molecular biology. Molecular cloning involves taking a gene or another DNA sequence from one organism, inserting it into a vector, and then introducing that vector into a host cell. The key breakthrough for this process came with the discovery of restriction endonucleases, which allow for precise and repeatable cutting of the target (insert) and vector DNAs, preparing them for joining. A restriction endonuclease is a protein that attaches to DNA at a specific sequence and cuts the phosphodiester bond in both strands of the DNA within that sequence. By using the same restriction endonuclease on the target and vector DNA, compatible single-stranded ends are created that can link together through complementary base pairing, aided by the enzyme DNA ligase, which helps form phosphodiester bonds. Another method called recombinational cloning does not use restriction endonucleases or DNA ligase for inserting target DNA into a vector; instead, it takes advantage of a mechanism used by certain viruses to integrate into the host genome through recombination at designated attachment sites.

Cloned DNA is transferred into bacterial host cells that can take up external DNA, a process called transformation. The vectors that carry target DNA into these host cells typically come from natural bacterial plasmids, which have been genetically modified to include multiple restriction sites for easier cloning. A vector can multiply within a host cell if it has a DNA sequence that allows replication (origin of replication). Although transformation is often not very efficient, the transformed cells can be identified by checking for gene activity present on the vector, such as antibiotic resistance or the ability to produce colored substances.

To effectively clone and express genes coding for eukaryotic proteins in a bacterial host, it's necessary to remove the introns first. Purified mRNA serves as a template for making cDNA, using the enzyme reverse transcriptase. Oligonucleotide primers can be crafted to specifically target certain mRNAs for cDNA synthesis or to attach to the poly(A) tails found on most eukaryotic mRNAs, enabling the creation of a cDNA library that encompasses all coding sequences from the eukaryotic source. On the other hand, constructing a genomic DNA library from a prokaryote is simpler and involves cutting the DNA into overlapping fragments for cloning. Various methods are used to screen these libraries to find clones that have a specific sequence or that produce a desired protein.

Amplifying and sequencing DNA are essential techniques in medical biotechnology. A powerful approach called PCR allows for the creation of millions of DNA copies from tiny samples. This amplification occurs through a series of 30 or more cycles, including the denaturation of template DNA, the annealing of two oligonucleotide primers to match complementary sequences near a target gene in single-stranded DNA, and the synthesis of DNA extended from the primer using a heat-resistant DNA polymerase. PCR can detect specific nucleotide sequences in complex biological samples or yield large quantities of a particular DNA sequence for cloning or sequencing purposes.

Biotechnology has seen remarkable transformations over the last fifty years [3]. Current advancements heavily rely on

breakthroughs in molecular biology, particularly in genetic engineering. Organisms ranging from bacteria to cows are being genetically altered to produce drugs and food supplies. Furthermore, innovative techniques for isolating, analyzing, and detecting disease-related genes, as well as gene therapy, are set to change the face of medicine.

In principle, the genetic engineering process is relatively straightforward. First, researchers determine the changes to make in a specific DNA molecule. In some situations, it may be beneficial to adjust a human DNA strand to fix errors linked to diseases like diabetes. Alternatively, scientists may introduce new instructions into a DNA molecule that it does not naturally contain, such as providing bacteria with the ability to create insulin. Additionally, existing DNA can be modified to correct mistakes or insert new information. These techniques are now well-established. Finally, researchers seek methods to introduce the recombinant DNA into the target organisms where it will act. Once integrated, the new DNA can instruct cells in humans to rectify genetic disorders, enable bacteria to produce new substances, or serve various other functions in different cell types.

Genetic engineering has led to numerous impressive achievements. Many products that were formerly available only from limited natural sources can now be produced abundantly by genetically modified microorganisms at a low cost. Examples include insulin, human growth hormone, tissue plasminogen activator, and alpha interferon.

Genes

The gene that carries the information for a specific protein can be extracted and incorporated into another DNA segment, allowing for the protein's production through cell growth [4]. With these techniques available, researchers began to explore how they could leverage this technology to create safer vaccines. Since antibodies and T cells focus on particular sections of viral proteins, it made sense to use recombinant DNA technology to generate a small number of viral proteins in large amounts for use as vaccines. These subunit vaccines would be inherently safe since they do not contain any virus, only some of its proteins. Moreover, producing these vaccines would be much less complicated compared to the challenging methods required for growing viruses or ensuring they are properly inactivated.

Before we understood the receptors of the innate immune system, creating adjuvants relied largely on trial and error. Early examples of adjuvants included killed bacteria. At that time, immunologists were unaware that these dead bacteria could activate certain innate immune receptors. Aluminum salt is another adjuvant frequently found in various vaccines. While the exact mechanism of its immune-stimulating action is unclear, it may act as an irritant that triggers tissue damage and inflammation, thereby activating innate immunity. Today, thanks to our understanding of innate immune receptors, we can use drugs that specifically target these receptors as adjuvants. However, even with this insight, the process of formulating adjuvants is still largely empirical.

There is another method for creating subunit vaccines. Researchers select a virus that infects human cells, such as an adenovirus, known for causing mild colds and diarrhea. The virus's genes are altered to prevent replication once it infects a human cell, ensuring it doesn't cause illness. Using recombinant DNA technology, the genes that provide information for essential proteins from the virus needing a vaccine are inserted into the DNA of the modified adenovirus. This modified adenovirus, referred to as the vector, contains the virus's insert. When the vaccine is administered, the vector enters human cells, and the inserted genes lead to the production of the corresponding proteins. This prompts the immune response towards those proteins. Vaccines created using this method do not require adjuvants since the vector itself is a live virus capable of triggering innate immune responses.

Drug Discovery

Patients depend on researchers in drug discovery to push for new ideas, make progress, and provide new treatments that enhance their well-being [5]. The process of discovering new drugs is both complicated and expensive, involving various stages necessary to bring a drug to the market. As competition in drug discovery intensifies, it becomes increasingly vital for researchers to adopt innovative strategies to maintain a steady flow of new products.

Progress in developing new drugs heavily depends on basic biological research conducted in pharmaceutical and biotechnology firms, as well as in academic institutions. This research focuses on finding new biological targets, validating those targets, and establishing their significance in disease conditions. At the start of drug discovery, researchers choose a target that can be influenced by a modulator, which could be a protein or a small molecule. Once a target is selected, scientists need to prove its relevance to a disease using both live cells and animal models. The ability to sequence entire genomes, recent insights into the molecular causes of diseases, advancements in validation technologies, and high-throughput screening (HTS) techniques all open up new possibilities for target validation in drug discovery. Following this validation, the hunt begins for a 'hit' molecule that can interact with the chosen target. These 'hits' can come from nature, be designed from scratch, or identified through HTS; however, they often need to be refined into 'leads' through cycles of modifying the molecule's structure and properties paired with further screening. Throughout this journey, lead compounds are fine-tuned for desired absorption, distribution, metabolism, excretion, and toxicological (ADMET) characteristics. This ADMET optimization results in the 'lead compound' that progresses to later phases of drug development.

A case study on phosphodiesterase (PDE) inhibitors demonstrates the effective use of modern drug discovery and development principles. After discovering endothelium-derived relaxing factors and understanding the roles of nitric oxide, cyclic guanosine monophosphate (cGMP), and PDEs in vasodilation, researchers at Pfizer theorized that a PDE inhibitor could be useful in treating angina. Their analysis of cGMP's structure, particularly how it interacts with PDEs, alongside

Zaprinast, a weak vasodilator and another PDE inhibitor, provided further backing for their theory. By screening existing compound libraries and rationally designing analogues, they discovered active molecules aimed at PDE-5, which is a cGMP-specific PDE found in coronary smooth muscle. Refining these compounds for better potency and ADMET properties ultimately led to a clinical candidate for angina; however, the compound was found ineffective, resulting in the cessation of its development as a cardiovascular drug.

The outlook for industrial biotechnology is bright, with many exciting technological innovations and applications on the way. Several key trends are influencing the future of industrial biotechnology:

1. Microbiome engineering focuses on the human microbiome, a complex collection of trillions of microorganisms living in and on our bodies, which significantly impacts our health and wellness. This area of research aims to manipulate these microbial communities to enhance human health and address various diseases. It is a rapidly expanding field with numerous potential applications in personalized medicine and disease prevention.
2. CRISPR-Cas genome editing: The CRISPR-Cas technology serves as an incredible tool for editing genomes, allowing for accurate and effective changes to genetic information. This system has numerous possible uses in industrial biotechnology, such as creating new microbial strains with enhanced characteristics and modifying crops for better yield and sustainability.
3. Advanced fermentation techniques: Recent progress in fermentation technology is leading to the creation of innovative and more effective fermentation methods for use in industrial biotechnology. Techniques like continuous fermentation, fed-batch fermentation, and other advanced options help increase productivity, minimize waste, and reduce costs of production.
4. Circular economy: In a circular economy, waste is kept to a minimum and resources are utilized more sustainably and efficiently. Industrial biotechnology plays a significant role in fostering the circular economy, with numerous applications focused on cutting waste, promoting recycling, and creating renewable feedstocks.
5. Artificial intelligence and machine learning: The role of artificial intelligence and machine learning is growing more critical in industrial biotechnology. These technologies can help optimize process conditions, forecast product quality and output, and discover new microbial strains suited for industrial uses.

Overall, the outlook for industrial biotechnology is highly optimistic, with many exciting advancements on the way. This field blends aspects from biology, chemistry, engineering, and other areas to generate products and processes that are sustainable and environmentally friendly, ultimately benefiting society.

Vaccines

Vaccines rank among the most significant achievements in public

health within modern medicine, having saved millions of lives [6]. Immunizations serve as a reliable method for preventing and controlling infectious diseases. Traditionally, bacterial and viral vaccines were made by cultivating the pathogens and then killing them using chemicals or heat. Another method involves creating a less harmful version of the pathogen (an attenuated strain) through genetic changes, which allows for the production and administration of these strains, with or without additional inactivation. Thanks to progress in vaccinology and immunology, scientists can now identify the protective components of a pathogen. When these protective antigens are produced in adequate amounts, they can be extracted from the pathogen itself (a process known as homologous expression). Vaccine antigens sourced from natural microbial hosts include toxoids, like those derived from *Corynebacterium diphtheriae* and *Clostridium tetanus*, as well as capsular polysaccharides from *Salmonella Typhi* and *Streptococcus pneumoniae*. The purified antigens are then modified through inactivation or by linking them to a carrier, and they may or may not be combined with an adjuvant. Recently, advancements in molecular biology and biotechnology have led to the creation of acellular vaccines, including therapeutic proteins, using expression systems that are not the natural hosts (heterologous expression).

Heterologous expression systems come with several benefits over homologous ones:

The antigen expression levels can be heightened by choosing the right cell lines, utilizing strong promoters that can be activated at specific times, resulting in higher antigen production.

The production process in yeast or bacteria, like *Escherichia coli*, is well understood. These organisms are grown in carefully controlled media, allowing the antigen to be purified to a high degree. Effective purification methods can be developed to eliminate unwanted substances such as bacterial endotoxins or residual DNA, with their absence confirmed through established tests. This increases vaccine safety, as no pathogens are included in the final product.

Using recombinant antigen expression enables the creation of proteins with desired structures, including specific posttranslational modifications such as glycosylation.

Certain antigens can't be produced in homologous systems because the pathogenic organism does not have scalable cell culture options, so heterologous systems provide a way to produce these antigens in large amounts.

Vaccination

Vaccination is one of the most effective ways to prevent infectious diseases and has arguably had a significant impact on human health compared to any other medical procedure [7]. The creation of vaccines has come about due to the consistent advancement of new technologies. Key developments include the discovery of toxoids, the purification of polysaccharides, the ability to culture viruses using cell culture methods, regulated virus attenuation techniques, the conjugation of polysaccharides

with proteins, the use of genetically-engineered cells for producing protein vaccines, and the reassortment of viruses. By applying modern biotechnology tools, we have seen a range of vaccine candidates emerge from various sources, opening up possibilities for the prevention or treatment of many infectious and chronic diseases. This has transformed our ability to engineer and manufacture vaccines that can be safer, more effective, easier to produce, and more affordable. The new technologies being developed continue the innovative spirit that has always been part of vaccine development. Presently, several new technologies are gaining significant attention, such as genetically-engineered vaccines, live vectors, nucleic acid vaccines, innovative delivery systems, and novel adjuvants.

This revolution in biotechnology poses significant challenges for traditional vaccine development to ensure that assessments are both adequate and timely, so that we can maximize the benefits of these innovations. Developing vaccines successfully requires a lengthy commitment from a network of scientists and involves a continuous process with regulatory and manufacturing organizations.

The pathway to vaccine licensure is both pyramidal and selective. It follows a step-by-step process that connects basic research, development, large-scale production in recognized facilities, and clinical evaluations to confirm safety and protective effects. While the overall process is similar to that of conventional vaccines, clinical input is particularly crucial at many points during the discovery process, which can be outlined as follows:

1. identifying medical needs,
2. selecting the rationale and developing the strategy based on preclinical findings,
3. proving concepts in early clinical trials,
4. crafting and executing an integrated clinical development plan, and
5. constantly evaluating safety and effectiveness.

Conclusion

Modern biotechnology involves applying scientific principles and techniques to alter and manipulate living organisms, cells, and molecules for various purposes. It utilizes sophisticated tools and technologies to explore, analyze, and modify biological systems at the molecular level. This field covers a wide array of disciplines such as genetic engineering, genomics, proteomics, bioinformatics, synthetic biology, and tissue engineering, with applications across numerous sectors.

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