MEDICAL BIOTECHNOLOGY

OXFORD
UNIVERSITY PRESS

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Biotechnology and Healthcare

After reading this chapter, the readers will be able to

- Understand the role of biotechnology in healthcare
- Know the advancement of diagnosis, therapy, and intervention
- Learn about different methodologies in diagnosis and treatment of various diseases

1.1 BIOTECHNOLOGY IN HEALTHCARE

Molecular biotechnology is globally emerging as the cutting-edge technology of medicine. The biotechnological innovations are directly applicable to the welfare of human health and society at large. The core of molecular biotechnology is the advancement of diagnosis, therapy, and intervention, which involves harnessing the natural process microbes, plants, and animal cells for the benefit of industrial processes and/or products like vaccines, antibiotics, and therapeutic proteins. Hence, molecular biotechnology is recognized as a discipline with basic and applied sciences being merged.

The rapid advances made in recombinant DNA technology has revolutionized into a new branch of medical biotechnology which has given an insight into the control of biological processes. This technology has made it possible to manipulate the heritable material of the biological systems and generate the desired end result.

Thus, more than 60% of the biotechnological innovations are directly pertinent to the field of medicine. One such innovation is the introduction of a monoclonal antibody based diagnostic test developed in 1981. This was followed by the generation of *humulin*, a recombinant human insulin produced commercially by Genetech, USA.

For investigations in bioprocess optimization, expression and/or scaling up off recombinant proteins, another field *bioprospecting* has emerged. With the dawn of the millennium, emerged another medical field called *nanomedicine* with the genetically engineered products being fabricated or designed *in silico* at the nano level.

1.2 ADVANCEMENT OF DIAGNOSIS, THERAPY, AND INTERVENTION

The field of biotechnology has led to the revolutionization of human health and medicine with its potential applications. The involvement of biotechnology in healthcare can be discussed on the aspects of

- Diagnosis of disorders
- Prevention, treatment, and management of diseases
- Counselling for health problems

A health problem can arise due to an infection by microorganism, a nutritional deficiency, or a hereditary cause.

1.2.1 Diagnosis

Diagnosis of the disease is very important, specifically when it is caused by an infectious agent or a gene. Diagnosis can be accurate only when a specific organism causing a symptom or a group of symptoms is properly identified. Accurate diagnosis is critical for effective management and cure of a disease.

Immunological Assays

Disease is usually diagnosed by specimen cultures to identify a cause, immunological assays for specific antigens present on the surface of pathogens, and antibodies produced in response to these antigens. The immunological assays also help in diagnosis of a disease or identification of a pathogen.

Monoclonal antibodies Another area of interest in diagnosis of diseases is monoclonal antibodies. A monoclonal antibody (MAb) is specific to a single antigenic determinant (epitope) of a single antigen. They are produced from *hybridoma clones*. Each hybridoma clone is derived from the fusion of a single *myeloma cell* with a single antibody producing *lymphocyte*. The various applications of MAbs include clear detection of pathogens, early and accurate detection of cancers, and blood typing (ABO, Rh).

MAbs play an important role in treatment and management of certain infectious diseases and cancers. They provide passive immunity against infectious diseases, for example leprosy. They deliver toxic molecular agents of immunotoxins to cancer cells in a specific manner.

Enzyme-linked immunosorbent assay Enzyme-linked immunosorbent assay (ELISA) (Fig. 1.1) is a highly sensitive technique for detecting and measuring antigens or antibodies. It is generally used in AIDS detection and other infectious diseases.

It is a heterogeneous, solid phase assay. Performing an ELISA involves at least one antibody with specificity for a particular antigen. The sample with an unknown amount of antigen is immobilized on a solid support (usually a *polystyrene* microtiter

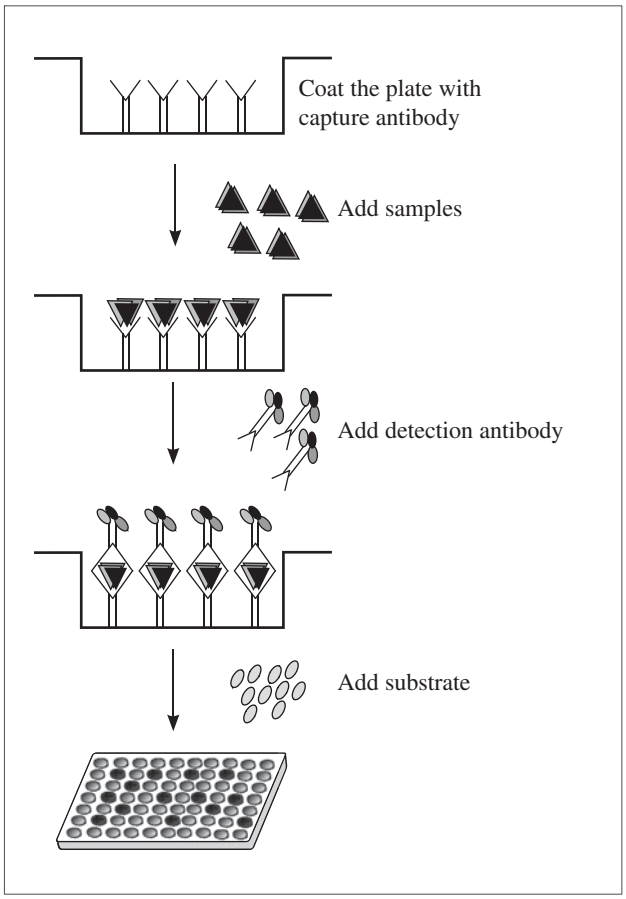

FIGURE 1.1 ELISA technique

plate), either non-specifically (via *adsorption* to the surface) or specifically (via capture by another antibody specific to the same antigen, in a *sandwich* ELISA).

Once the antigen is immobilized, the detection antibody is added, forming a complex with the antigen. The detection antibody can be covalently linked to an *enzyme*, or can itself be detected by a *secondary antibody* which is linked to an enzyme through *bioconjugation*. Between each step, the plate is typically washed with a mild *detergent* solution to remove any proteins or antibodies that are not specifically bound.

After the final wash step, the plate is developed by adding an enzymatic *substrate* to produce a visible signal, which indicates the quantity of antigen in the sample. Older ELISAs utilize *chromogenic* substrates, while newer assays employ *fluorogenic* substrates enabling much higher sensitivity.

Diagnostic test based on immunological protocols are lengthy and strenuous and may yield ambiguous results because of polymorphic nature of a disease, which are sometimes unreliable. New development in biotechnology leads to novel diagnostic

approaches which are precise and rapid. Some of the important protocols are based on probes and MAbs.

Diagnosis Using Probes

Probes are oligonucleotides of about 30 bases of DNA or RNA, employed to detect the presence of complementary sequences in nucleic acid samples of pathogens from the diseased individuals. Probes are usually labelled with radioactive/non-radioactive substances for easy identification of unknown sequences.

Use of probes for diagnosis is advantageous over conventional procedures because of its specificity, reliability and safety, as they are non-infectious agents. Probes can be used in clinical diagnosis for the detection of pathogens in various samples, for example, plasmodium, schistosomes, mycobacterium tuberculosis, and herpes virus.

Probes can also be used in the southern blot, dot blot, *in situ* hybridization, and ligase chain reaction (LCR) for the detection of a variety of pathogenic microorganisms.

Polymerase Chain Reaction

Polymerase Chain Reaction (PCR) and the immuno PCR for detection of DNA by amplification and for detection of antigen-antibody complex linked to a marker DNA, respectively, help in detection of pathogens and their antigens.

PCR (Fig. 1.2) is a technique widely used in molecular biology, microbiology, genetics, diagnostics, clinical laboratories, forensic science, environmental science, hereditary studies, paternity testing, and many other applications. The name, polymerase chain reaction, comes from the DNA polymerase used to amplify (replicate many times) a piece of DNA by *in vitro* enzymatic replication.

The original molecule or molecules of DNA are replicated by the DNA polymerase enzyme, thus doubling the number of DNA molecules. Then each of these molecules is replicated in a second cycle of replication, resulting in four times the number of the original molecules. Again, each of these molecules is replicated in a third cycle of replication. This process is known as the *chain reaction* in which the original DNA template is exponentially amplified.

With PCR, it is possible to amplify fragments of DNA over many cycles, generating millions of copies of the original DNA molecule. PCR has been extensively modified to perform a wide array of genetic manipulations, diagnostic tests, etc.

Ligase Chain Reaction

Ligase chain reaction (LCR) is the technique on similar lines to PCR. The ligase chain reaction is an offshoot of PCR (Fig. 1.3) utilizes two pairs of oligonucleotides—one pair complementary to the upper template strand, and the other pair complementary to the lower template strand. Each member of a pair hybridizes to adjacent positions on the template such that the 5′-phosphate of one oligonucleotide abuts the 3′-hydroxyl of the other. The resulting nick is sealed by DNA ligase.

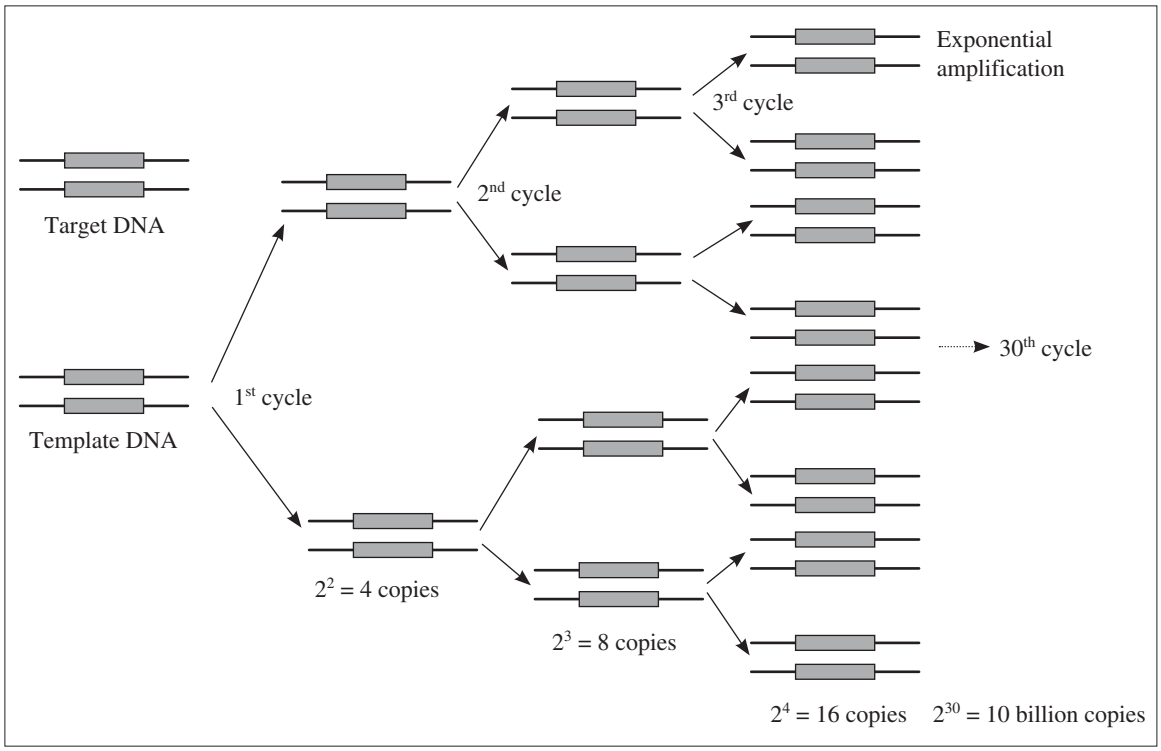

FIGURE 1.2 PCR technique

The product of one round of ligation can serve as a template for a subsequent cycle; thus, using a thermostable ligase and performing sequential cycles of denaturation and ligation results in an exponential accumulation of the product. Because a mismatch in the ligation junction inhibits the ligation reaction, LCR is ideal for the diagnosis of known mutations.

However, there are different protocols of diagnosis for inherited conditions in addition to the methods described above. They are amniocentesis, chorionic villus sampling where foetal cells are cultured and analysed for biochemical (α-fetoproteins), cytological (karyotyping), and molecular markers (RFLP, SNPs) associated with such inherited disorders.

RFLP/SNPs (restrictions fragment length polymorphism/single nucleotide polymorphisms) help us to delineate heterogeneity of the condition with varied genotype (for example, *Retinitis pigmentosa*), whereas karyotyping and *in situ* study helps to know chromosomal aberrations associated and molecular markers specific to chromosomal loci affected in inherited conditions.

'Prevention is better than cure' an epitome makes one to probe into the possibility of preventing various diseases rather than their cure, especially when treatment is difficult and impossible. One of the important products for prevention is the generation of vaccines. Vaccines are biological preparations which effectively

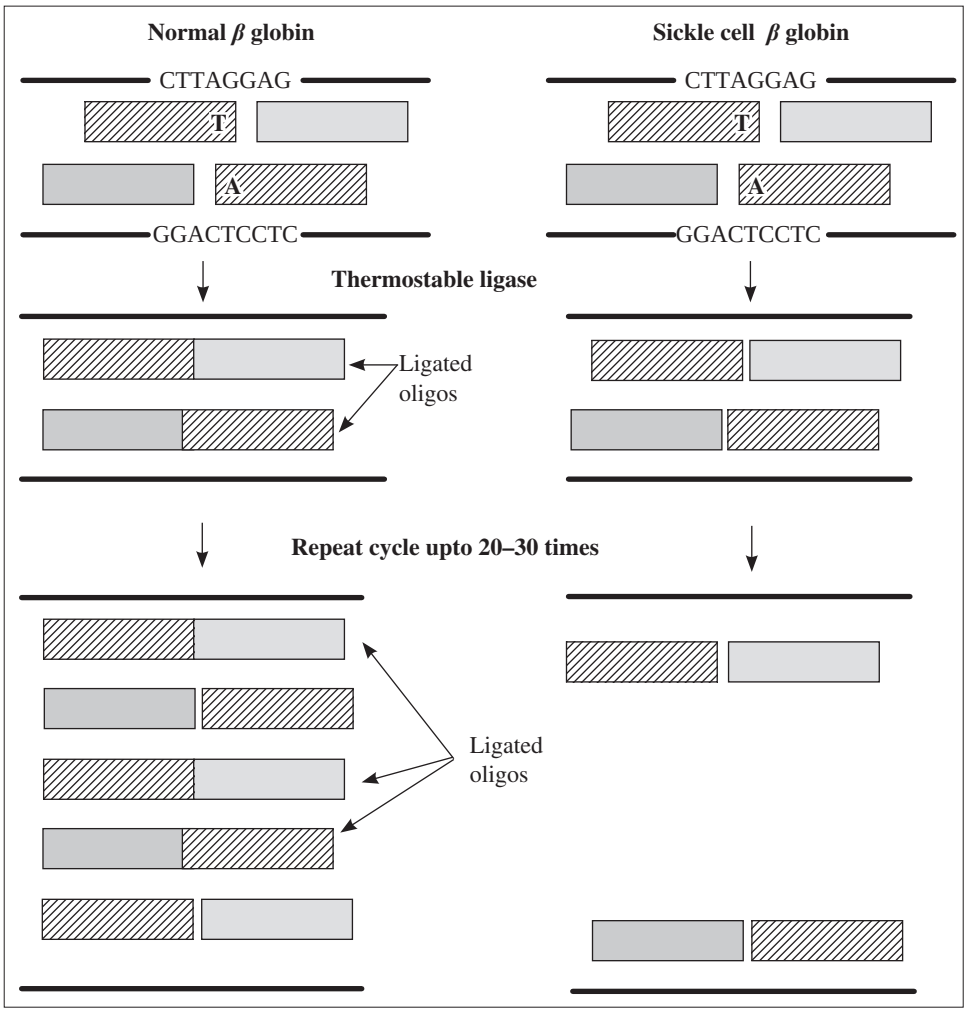

FIGURE 1.3 LCR technique for detection of sickle cell anemia

prevent the onset of the disease for a particular period depending on the type of vaccine and the pathogen against which it acts.

An ideal vaccine should be safe and produce long lasting humoral and cellular immunity. Recombinant vaccines are in use now either in the form of protein/polypeptide subunit vaccine or DNA vaccines which are always a better choice over conventional vaccines.

A large number of genes encoding antigenic proteins have been integrated into vaccinia virus which was then used for vaccination. Some of the important examples are:

- Rabies virus glycoprotein
- Herpes simplex virus glycoprotein D
- Hepatitis B surface antigen

1.2.2 Treatment

Treatment of diseases utilizes a variety of preparations of compounds from plants, microorganism, cultured cells, and recombinant organisms. The various products include whole organisms like lactobacillus, biomass-based products like single cell proteins, antibiotics, vitamins, enzymes, organic acids. Such compounds can also be produced through plant and animal cell cultures, transgenic plants, and animals.

The advantages of such production over conventional methods are their cost-effectiveness, no risk of contamination, and large production within a short time. Proteins of human origin were made available like humulin in place of insulin usually extracted from an animal. *Interferons* used in the treatment of cancers and as antiviral agents and growth factors involved in correcting some metabolic and immune disorders are some of the examples cited. *Erythropoietin* is one of the growth factors used in the treatment of anemia.

Antisense RNA of 25 to 35 bases pairs with 5′ end mRNA preventing its translation, killing many of the parasites including *trypanosomes*.

Drug designing is one of the active areas of research in biotechnology. This approach is aimed at designing drugs which fit into the functional domain of target molecules, thereby inactivating them. The molecule which is targeted by this method can be a protein/enzyme in the metabolism or replication of DNA, a hormone receptor, or some other molecule of interest in causing the disease.

Once a drug is designed, it has to be properly delivered to the tissue or organ affected. The innovative methods are administration of drugs via parenteral administration. Alternatively, drugs can be encapsulated in liposomes which are tagged with ligands (monoclonal antibodies) to reach specific targets, thus delivering the DNA very effectively to the tissue/organ.

Gene therapy is aimed at correcting errors due to single gene mutations. Gene therapy may be affected at gametic level or zygotic level (germ line gene therapy), somatic level (somatic cells) which has been tried for cancer and blood disorders.

The two approaches of gene therapy, viz. addition or augmentation therapy, targeted gene transfer (homologous recombination) which was successful in the case of severe combined immunodeficiency (SCID) and adenosine deaminase (ADA) deficiency is well documented.

An advancement made in the field of regenerative medicine is tissue engineering based on the principles of cell transplantation and engineering towards the development of biological substitutes that can restore and maintain normal function.

Stem cell biology has come of age with emphasis on the self-renewal property of stem cells, which is also similarly identified in cancer cells via the signalling pathways. Hence, stem cell therapy is the latest development to treat cancers and other genetic disorders and to create artificial skin in case of burns and skin injuries. Last but not the least, bioinformatics is the bridge course which integrates information at DNA and mRNA level to protein, with the prediction of the structure, function, and model.

EXERCISES

Review Questions

1. Discuss the role of biotechnology in healthcare.
2. What are the different methodologies to be adopted in the diagnosis and treatment of various diseases?
3. Write short notes on
   (a) Labelled probes
   (b) LCR
   (c) PCR
   (d) Immuno PCR
   (e) ELISA
   (f) MAb
4. What is RFLP? Explain how it is performed.
5. What is ELISA? Explain its application with one example.
6. Differentiate between PCR and immuno PCR.
7. What are the applications of PCR?
8. What are the applications of immuno PCR?
9. Explain the utility of MAb in the treatment of cancers and other diseases.
10. What is hybridoma? How is it helpful in production of MAb?
11. What is LCR? Explain with one example.
12. Discuss the protocol for the development of a labelled probe.

Laboratory Exercise

1. Perform PCR for a given sample.
2. Perform ELISA to detect antigen in a given sample.