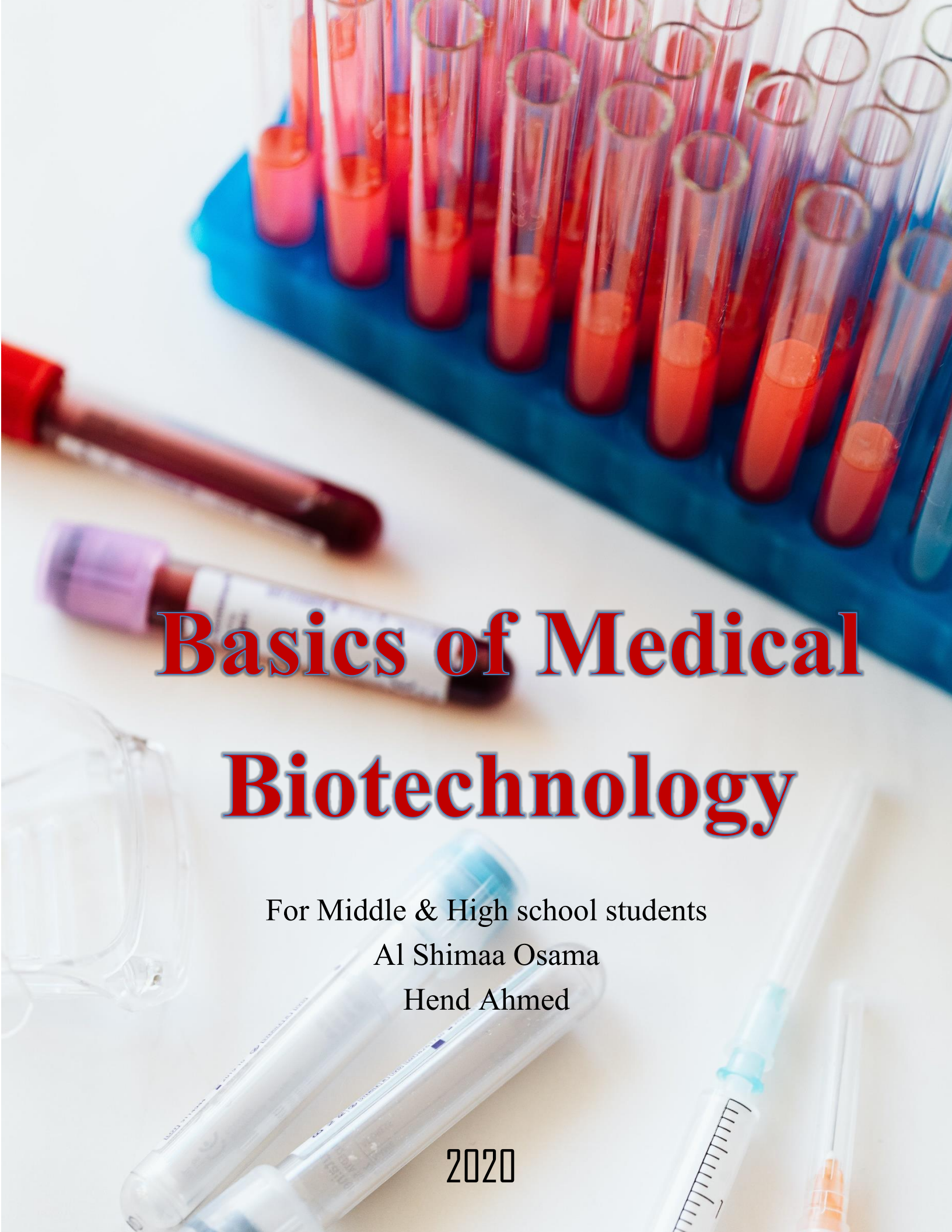

Basics of Medical Biotechnology

For Middle & High school students

Al Shimaa Osama

Hend Ahmed

2020

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Introduction

The field of medical biotechnology is experiencing rapid growth in recent years, leading to the development of several innovative techniques for preventing, diagnosing, and treating diseases. Novel methodologies, including polymerase chain reaction, gene sequencing, fluorescence in situ hybridization, microarrays, cell culture, gene silencing using interference RNA, and genome editing, have significantly contributed towards improving health science, such as the sequencing of the human genome, use of stem cells for regenerative medicine, tissue engineering, development of antibiotics, and the generation of monoclonal antibodies for therapy. This book will summarize and update important techniques used and the products generated using these tools in the field of medical biotechnology. If the current growth rate continues, medical biotechnology will soon become a major pillar of health science.

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Chapter 1: Background of Medical Biotechnology

Section 1.1 Medical Biotechnology

What is Medical Biotechnology?

Medical biotechnology is a branch of drugs which utilizes living cells and cell materials to analysis, and then generate pharmaceutical and diagnosing products. These assist cure and impede diseases. From the Ebola vaccine to mapping human DNA to agricultural influences, medial biotechnology is creating huge progressions and assisting lots of folks.

Some of the newest usages of biological technology is work in genetic testing, drug treatments, and artificial tissue growth. With myriad advancements in medical biotechnology, there are novel concerns that arise. From funding to ethics, there are many things to identify and regulate

when it comes to this fast-paced industry. Learn about the many technical biology advancements, and the concerns surrounding them here.

Major medical biotechnology advancements. From cancer analysis to agriculture advancements, medical biotechnology has lot of promising avenues of technological growth that has the potential to assist many people.

- *CRISPR.*

CRISPR technology or CRISPR-Cas9, uses a protein known as Cas9, that acts as a pair of molecular scissors and can cut DNA. CRISPRs are specialized stretches of DNA and are utilized in medical biotechnology as a tool to edit genomes. It permits scientists to change DNA and modify gene functions, typically known as genetic engineering. There are several applications, such as correcting genetic defects, treating diseases, impeding the spread of diseases, improving crops, and more. But the science of altering genomes has many ethical concerns surrounding it. From the capability to mutate genes, and the unknowns surrounding gene mutation, CRISPR is a controversial area of biomedical science. Some new studies even demonstrate that perhaps CRISPR technology can make tumors and cancer, with DNA deletions that aren't controlled or precise. Of course, pharmaceutical firms and other scientific organizations that improve and utilize CRISPR technology are attempting to downplay the concerns and problems, so the reality of the benefits and damage of the technology is somewhat unknown.

- *Tissue Nano transfection.*

New science could have the flexibility to heal folks with one bit. Sound too sensible to be true? It's not. Tissue Nano transfection works by injecting genetic code into skin cells, that turns those skin cells into the other kinds of cells needed for curing diseases. In some laboratory tests, one touch of TNT completely repaired the injured legs of mice over a period of a few weeks by turning skin cells into vascular cells. And reportedly, this biotech can work on other types of tissue besides skin. The potential for this type of gene therapy is huge, from assisting car crash victims to active-duty soldiers. Medical

biotechnology has made this advancement possible, and the continued research and testing will only help develop this tech and adopt it across hospitals and medical centers.

- *Recombinant DNA technology.*

Recombinant DNA technology is combining DNA molecules from 2 completely different species, and so inserting that new DNA into a bunch organism. That host organism can turn out new genetic mixtures for drugs, agriculture, and business. There are myriad instances of recombinant DNA technology being used, from biopharmaceuticals and medicine, to energy applications like biofuel, to agricultural biotechnology with changed fruits and veggies. The genetically changed products can perform in a much better manner than the regular medicine or produce. Recombinant agriculture is able to be more pest resistant or weather resistant, recombinant medicine as insulin is able to better work with bodies, etc. Due to lots of benefits that recombinant DNA holds for a different product, scientists are optimistic about the future it has within biosciences, and in other industries similarly.

- *Genetic testing from 23andMe.*

Genetic and ancestry kits are popular nowadays, and they are beneficial for more than only assisting human beings perceive their genetics and heritage. Novel studies are demonstrating that saliva kits can test for things like breast cancer by noticing gene mutations. Specific races are conjointly more likely to inherit some mutations or human diseases, and knowing what races make up your genetic material able you to assist in being ready. Whereas 23andMe test results shouldn't be a cause to make decisions about treatments, interpreting your heritage and how that could influence your health is valuable. 23andMe is also approved to analyze for a variety of diseases including Parkinson's and Alzheimer's.

Medical and ethical problems of biotechnology.

While there are beneficial progress and positives to medical biotechnology, anything this fast-growing and powerful is certain to go together with some concerns and problems. Medical biotechnology is a controversial medical topic, with medical ethical problems associated.

- *Risk to human life in clinical trials.*

A huge risk of medical biotechnology is its effect throughout clinical trials. As a result of it's such new technology, Human beings can and have gotten hurt and even died during trials of the technology. According to these risks, Intensive analysis should be performed before even thinking of introducing tech to human subjects, and those who are participating in a trial should be extremely aware of any and all probabilities. Unfortunately, the paradox is that myriad times individuals who are ill are willing to attempt novel things for the chance to get cured. This means researchers and doctors have a huge ethical responsibility to outline for a patient what the prices may be, and respect their final call.

- *High price might exclude the poor.*

While medical biotechnology has vast potential to form drugs more efficient and simpler, what's the cost? This technology is commonly hugely expensive compared to traditional treatments. There is an ongoing give and take about finding new medical advancements, and the cost it takes to do research and then market the findings for purchase. There is also the concern that high costs of tech treatments can exclude a complete class of people from being able to use them. This is also a huge give and take, with science and medicine having a responsibility to assist almost all patients, not only those who are rich enough to buy the best care.

- *There are privacy concerns.*

Privacy is a current problem in our technology world, but reading someone's DNA seems to be a large privacy breach. Imagine a doctor looks at a young child's DNA and discover they are likely to develop a heart disease or terminal issue. Does their employer have the proper to grasp that? Should this information influence their capability to get a house, or insurance? HIPPAA offers some protection, but as medical biotechnology continues to progress the ability to read genes, insurance firms, doctors, and governments will have to come up with new programs and privacy tactics to match all the new desires that will arise.

- *Some teams oppose stem cell analysis.*

Medical biotechnology is type of a hot-button political issue, with presidential candidates even being asked about their stance. The idea of working with fetal tissue, or other tissue, to learn about regrowth conjures images of Frankenstein's monster. Scientists and researchers have been cautioned multiple times to be ethical and moral when doing this research. For instance, alyze for different diseases including Parkinson's and Alzheimer's. using human tissue for research can be noticed as ethical, while using an embryo's tissue can be seen as unethical because it can damage the embryo. It is still early in the stem-cell research process, but as technology and research continues to advance in that area, scientists will have to take into account moral and ethical lines even more.

- *Bioterrorism is a national concern.*

Medical biotechnology has been used for security measures to assist in impeding a large number of people from possible bioterrorism. However, the development of these projects takes away funding and time from curing known diseases. It becomes a real question of how to divide resources among projects, and recognizing where the resources are most required. It's difficult because we don't know if people will die from bioterrorism, but with so myriad ones being concerned, it seems like a worthwhile place to spend time and money.

Any way you look at it, there are a number of concerns when it comes to medical biotechnology, and as we continue to make advancements, these ethical considerations will have to be made.

Medical biotechnology is a field that is exploding, and along with its potential for saving lives, it generates some ethical questions. As the field continues to grow, people from all types of industries are going to be needed to create decisions to assist in regulating this field.

Role of nurses in the biotechnology industry.

Nurses have an ongoing role in medical biotechnology according to their direct experience with patient care. Nurses can use their knowledge and experience in hospitals and clinics to interpret and show how medicines and drugs would influence large populations. Beyond knowing the science, they have the human element that occasionally scientists lack. They are able to understand how a patient would respond to a potential treatment, and can help researchers take into account new approaches to technology and adoption practices.

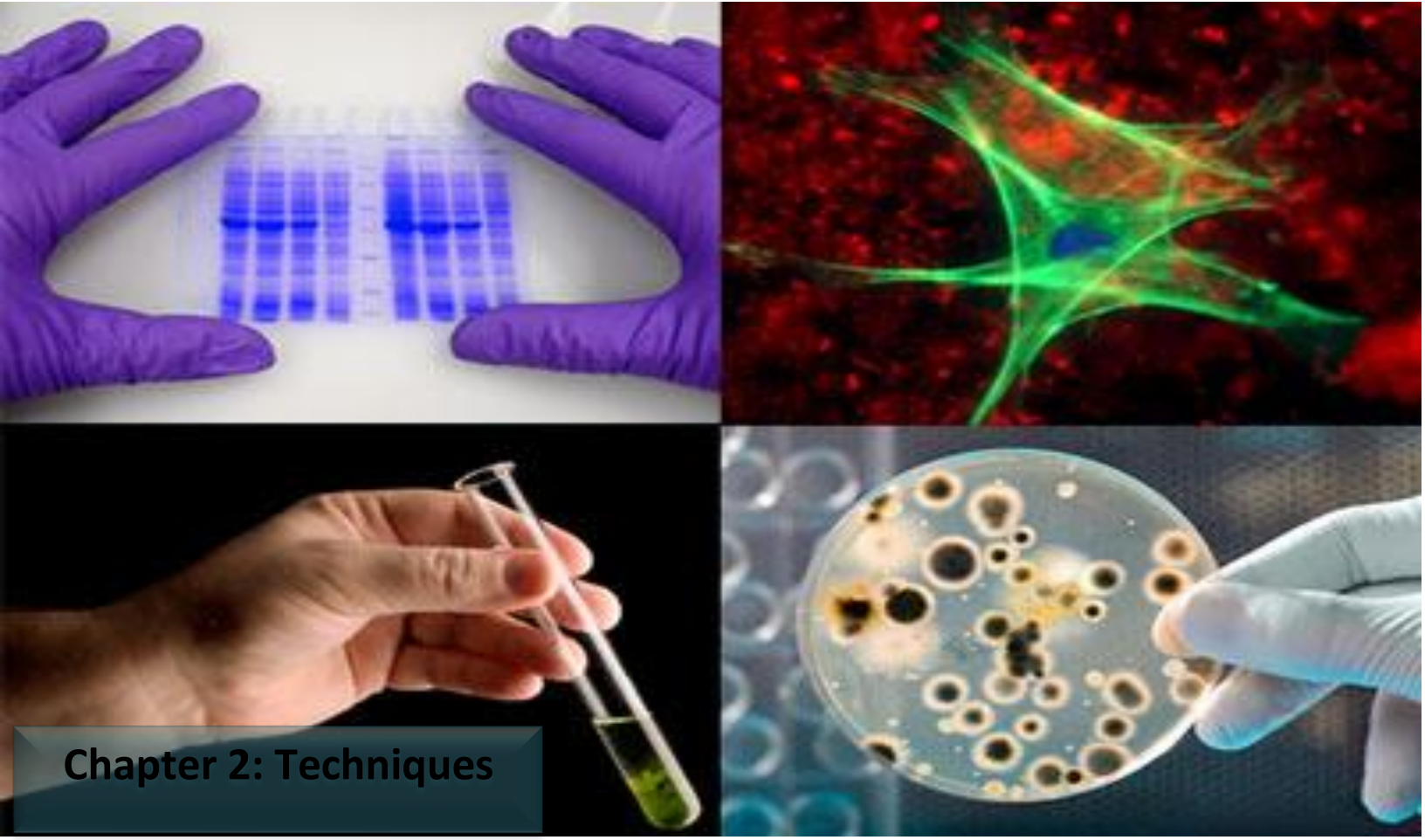
Medical biotechnology is a field that is exploding, and along with its potential for saving lives, it generates some ethical questions. As the field continues to grow, people from all types of industries are going to be needed to create decisions to assist in regulating this field.

Section 1.2: History of Medical Biotechnology

During the twentieth century there have been the greatest gains in health in most parts of the world due to dramatic reductions in infant mortality, eradication of life-threatening diseases, such as smallpox, and considerable improvements in life expectancy in developing and industrialized countries. In the past, life for most people was coarse, lacking in adequate nutrition, poor housing and, above all, short in years. With the advent of improved sanitation and better living conditions, together with the availability of vaccinations and antibiotics, there has been, for many, a vast improvement in health status. However, health status still differs widely among nations and by geographical region. For instance, life expectancy is less than 50 years in some sub-Saharan African countries but over 75 in established industrialized countries. The wealthiest economies appear to be the healthiest. A crucial factor related to life expectancy is access to safe water! In much of the developing world, simply drinking water is a high-risk exposure.

Undoubtedly, the real gains in health over the last century can be attributed mainly to the impact of public health and disease prevention rather than to medical interventions. Public health can be primarily distinguished from clinical medicine by placing emphasis on the prevention of disease rather than the curing, and having a main focus on populations and communities rather than the individual patient. It is essential to continue to develop a public health approach that will protect populations and create prevention strategies for groups and not just for individuals. Biotechnology has, and continues to play, a major part in establishing programs for achieving clean drinking water and waste treatment technology.

Nowadays in industrialized societies, infectious diseases are no longer the main threat to life but rather it is the chronic diseases (cancer, cardiovascular disease, Alzheimer's disease, etc.) that plague our increasingly ageing population. Much of the increased life span achieved in the last 50 years has not prolonged youth but extended dotage. The late John F. Kennedy said in the 1960s: 'It is not enough for a great nation to have added



Section: 2.1 polymerase chain reaction (PCR)

Polymerase chain reaction (PCR) is a laboratory technique used to make multiple copies of a portion of DNA. The polymerase chain reaction allows scientists to make a large amount of DNA from just a small fraction. This DNA region can be anything the experimenter is interested in. For example, it may be a genetic marker used by forensic scientists to match crime scene DNA with suspects. Polymerase chain reaction (PCR) is used in many areas of biology and medicine, such as molecular biology research and medical diagnostics.

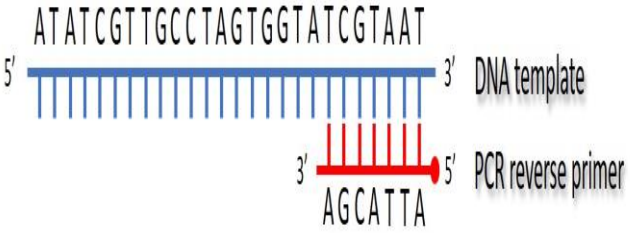
There are many steps for making PCR, these steps will be discussed later in this section. First, we need to know what is Taq polymerase?

PCR requires the DNA polymerase enzyme that makes new strands of DNA, using the existing strands as templates. The DNA polymerase normally used in the polymerase

chain reaction is called **Taq polymerase**, after the refractory bacteria from which it was isolated (Thermus aquaticus).

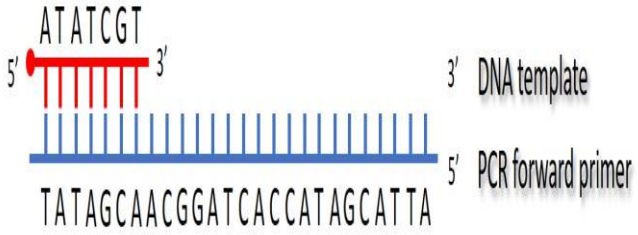
Thermus aquaticus lives in hot springs and hydrothermal vents. Its DNA polymerase is very heat stable and is more active at about 70 ° C / 70 ° C, 70 ° C, and this thermal stability makes Taq polymerase ideal for PCR.

But this Taq polymerase will not reduce a polymerase without primer- Short pieces of single-stranded DNA- typically around 20 nucleotides in length. Two primers are used in each PCR reaction, and they are designed to surround the target region. (as shown in figure)



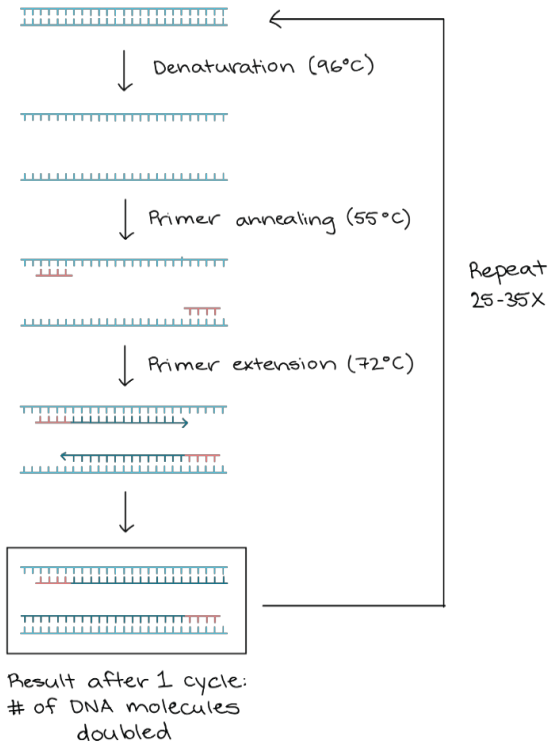
Steps of PCR:

1- Denaturation: this step must occur at (96 °C), Denaturation means separation, it uses this heat to separate the DNA into two strands to be able to follow the next step.



2- Annealing the DNA strand must be at temperature in the range (55°C - 65° C): to Cool the reaction so the primers can bind to their sequences on the single strand of DNA.

3- Extension the reaction temperature increases again to reach (72°C), so that Taq polymerase extend the primer making new strand of DNA.



These steps are shown in the figure.

There are many ways to visualize the results of PCR, like gel electrolysis, that we will discuss in this section.

Gel electrophoresis: is a technique in which fragments of DNA are pulled across a gel matrix by an electric current, and the DNA fragments are separated according to their size.

DNA fragments of the same length (band) form on the gel. A DNA strand consists of several copies of the main DNA region. Because DNA is microscopic, there must be a lot of copies of it before we can see it with the eye. Therefore, PCR is an important tool: it produces enough copies of DNA sequences that we can see or manipulate.

PCR is used in research labs, forensics, genetic testing, and diagnostics. PCR can also be used to test for a bacterium or DNA virus in a patient's body.

Section: 2.2 Fluorescent in situ hybridization (FISH)

Fluorescent in situ hybridization (FISH):

is a macromolecule recognition technology that relies on the integral nature of DNA or DNA / RNA double strands.

Situ hybridization is used to locate DNA sequence locations on chromosomes.

This technique was initially developed as a physical mapping tool for identifying genes within chromosomes. The high analytical accuracy of single gene level, sensitivity and high specificity enabled immediate application of the genetic diagnosis of common structural aneuploidy and microdeletion / replication syndromes and rearrangements without telomere.

as we know, Adenine in one strand of DNA binds to thymine on a complementary DNA strand, and this cytosine also binds to guanine. Due to the numerous hydrogen bonds formed between these bases, the double helix is an uncommonly stable structure. so that,

if the hydrogen bonds that bind the helix together are broken by heat or chemicals, the helix can reconstitute when conditions are suitable. when DNA helix can remodel this is the basis of molecular hybridization.

In molecular hybridization, an assorted DNA or RNA sequence is used as a probe to determine the natural match of the sequence in a sample. It was realized that molecular hybridization could be used to locate a DNA sequence in situ. The radioactive transcription for ribosomal DNA sequencing can be used to detect the complementary DNA sequences in the nucleus of a frog egg. Since those original observations, several improvements have increased the inconstancy and sensitivity of the procedure to such an extent that it is on situ. Fluorescent labels have replaced the radioactive labels in hybridization probes due to their safety, stability, and ease of detection. Indeed, most of the current hybridizations are performed in situ using FISH procedures.

Steps of FISH:

1- The first step is to make a fluorescent copy of the probe sequence or a modified version of the probe sequence that can be converted to a fluorescent image later in the procedure.

2- The second step: staining the target and the sensor sequences using heat or chemicals prior to hybridization. This step is necessary for the new hydrogen bonds (as discussed in the PCR 2.1 technique).

3- The third step is to mix the probe and target sequences. The probe is hybridized with its complementary sequence on the chromosome. If the probe is indeed illuminated, it will be possible to directly detect the hybridization site.

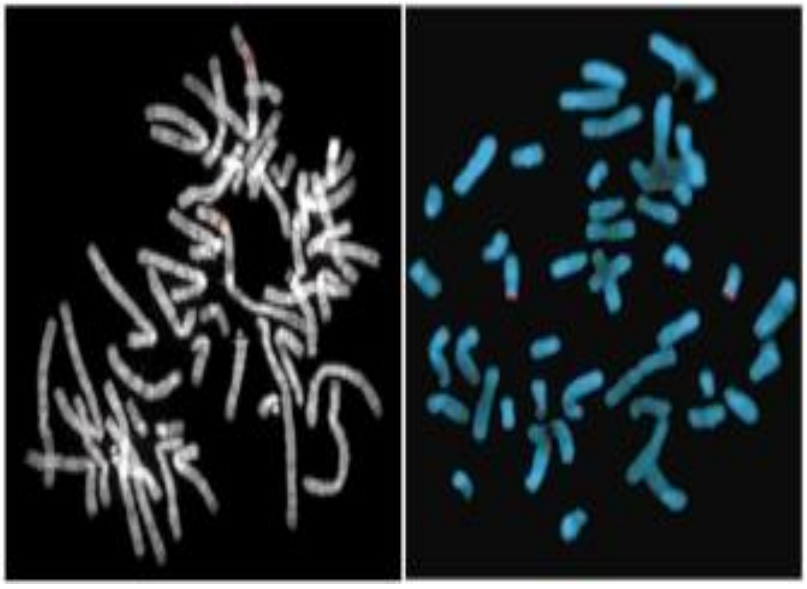
There are some other cases that require a further step to visualize the hybrid probe. Hybrid bodies formed between the probes and their chromosome targets can be detected using a fluorescent microscope.

there are two factors that must be taken in consideration t if they are needed or not. these factors are sensitivity and resolution. We must know whether the sensitivity and resolution required for the experiment lie within the technical limits of fluorescence microscopy. Sensitivity depends on the ability of the microscope to collect light, so that small target sequences, which are more difficult to see than large target sequences, can be detected. Resolution is the ability to distinguish two points along a chromosome. Ultimately, light microscopy cannot resolve objects separated by less than 200-250 nm, which is the lower limit of the visible light spectrum. putting these limits in mind, researchers also need to look at the formation of the DNA within the chromosome. Metaphase chromosomes are thousands of times more compressed than interphase chromosomes, which in turn are compressed at least ten times that of naked DNA.

When all these factors are considered together, investigators typically expect to have accuracy within the massive bases range for positioning on metaphase chromosomes and accuracy in the tens of thousands of kilobases for interphase chromosomes.

Using FISH to Identify the Positions of Genes:

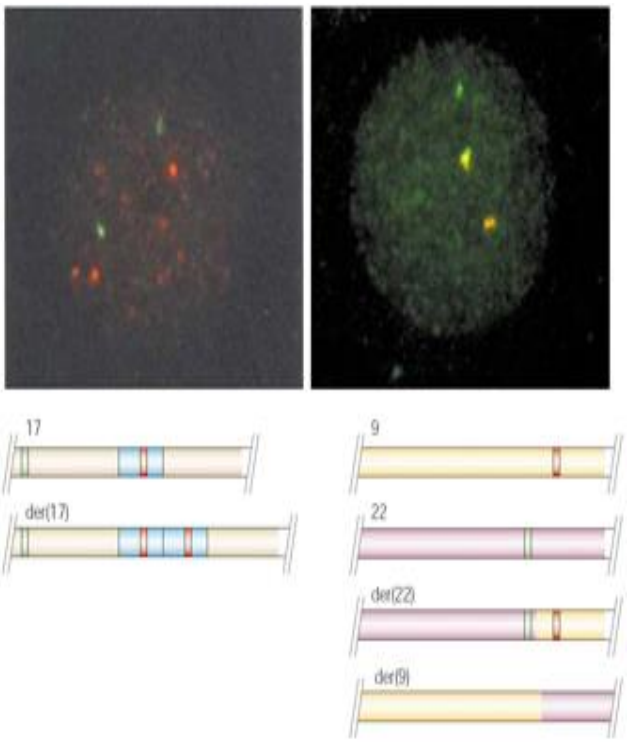
FISH can locate cloned DNA sequences on metaphase chromosomes. The cloned DNA sequences are hybridized to normal metaphase chromosomes. Red bands are detected at the hybridization sites on two homologous chromosomes, which can be identified by the distinct banding patterns. The examination shows that each red stripe is composed of two points, corresponding to the sister chromatids of the mitotic chromosome. (As shown in the figure):



The advantages of FISH:

The cells must not be cultured several times before they are prepared for analysis. They can be used to analyze chromosomes from samples such as solid cancer, which are very important, however they cannot be divided repeatedly, and researchers can observe the multiplicity of sites together, using different fluorescent compounds.

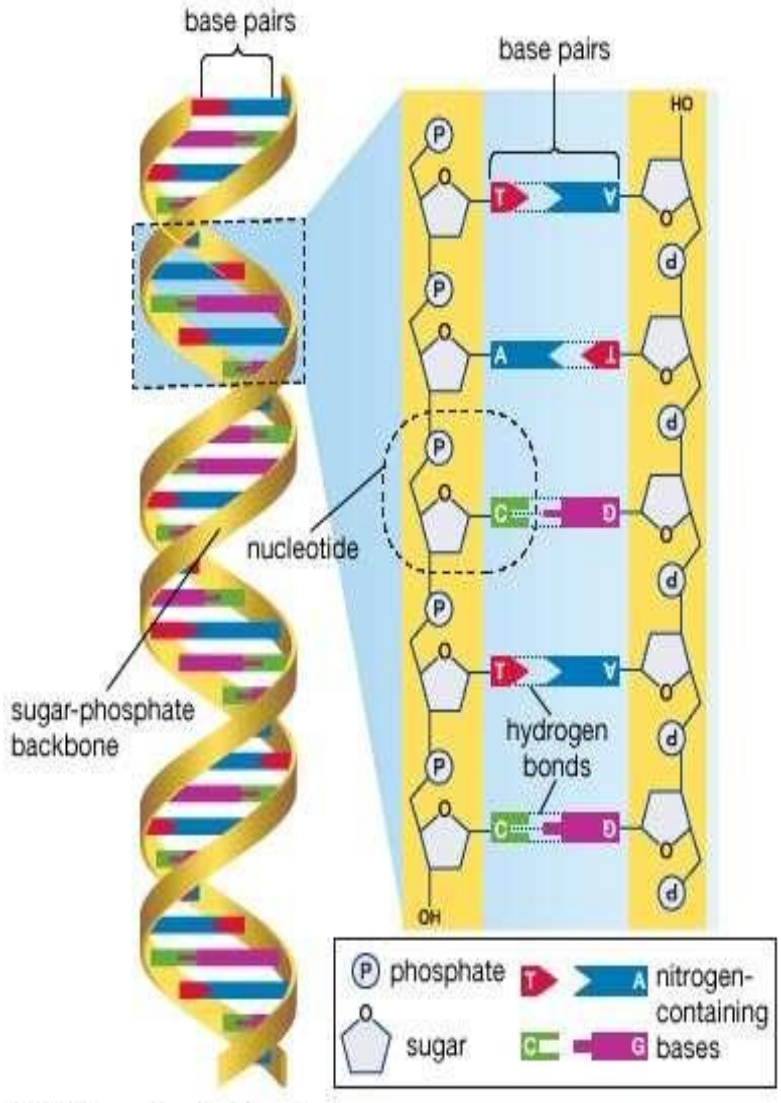
An example of FISH analysis:



(as in the figure) FISH analysis used to detect the presence of chromosomal translocations in a patient with chronic myelogenous leukemia. In case of this disease, the portion of chromosome 9 that contains the primary oncogene (ABL) fuses with the breakpoint group region (BCR) on chromosome 22 during cross-transmission. Demonstrates that BCR-ABL fusion can be readily recognized by FISH when a green-labeled hybridization probe surrounding the BCR is applied with a red-labeled probe surrounding the ABL. In this image, normal copies of chromosomes 9 and 22 are revealed as red and green spots, respectively. On the other hand, the Philadelphia chromosome be a fused complex macula, which appears to have a central yellow region with red and green subregions on either side. It can be resolved in FISH analysis like metaphase chromosomes.

Section: 2.3 Sequencing

Sequencing Is a technique that is used to determine the sequence of the nucleotides of DNA. The nucleotide is used to find out a gene or genome. It is the shame that contains the orders for building an organism, and it is the base of understanding genetic functions and evolution.



Recently, sequencing was made in many steps, so it takes effort and costs a lot of money, but now because of the developed technology, it become easier and faster.

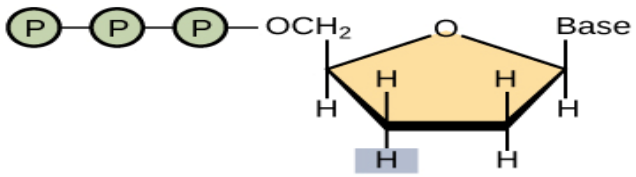
There are two techniques that are recently used in sequencing.

1- Sanger Sequence: chain termination method.

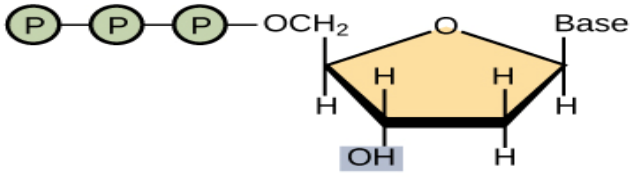
DNA sequence containing about 900 base pairs are arranged using a method called Sanger sequencing or the chain termination method. This method was developed by the British biochemist Fred Sanger and his colleagues in 1977. In the Human Genome Project, Sanger sequencing has been used to determine the sequence of small parts of human DNA. Segments were aligned based on overlapping fragments to assemble sequences of larger regions of DNA and, ultimately, whole chromosomes.

There are some needs for Sanger sequencing:

Sanger sequencing involves making multiple copies of a target DNA region. To complete the Sanger sequence, you need:



Dideoxynucleotide (ddNTP)



Deoxynucleotide (dNTP)

DNA polymerase enzyme

Primer, a short piece of single-stranded DNA, to bind to the DNA template and act as a (starter) for the polymerase.

Nucleotides: (dATP, dTTP, dCTP, dGTP)

DNA template, to be sequenced

Also, the Sanger sequence contains unique needs:

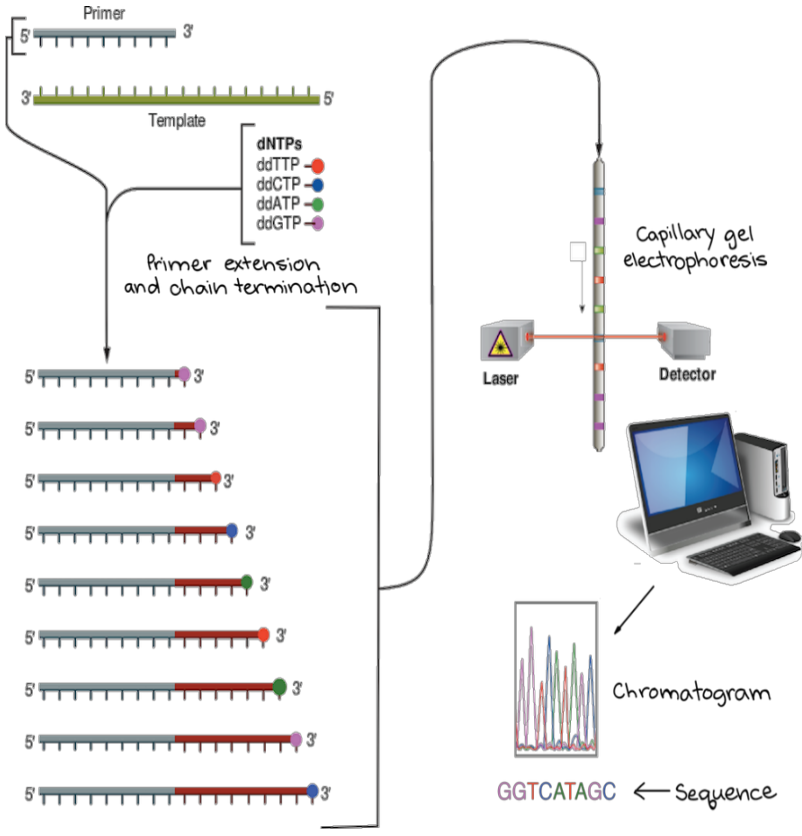
Dideoxy, or termination chain, versions of all four nucleotides (ddATP, ddTTP, ddCTP, ddGTP), each labeled with a different color of the dye (as shown in the figure)

The technique of Sanger sequencing method:

The DNA is combined in a tube with a primer, DNA polymerase, and DNA nucleotides (dATP, dTTP, dGTP, and dCTP). The dyed four-nucleotides, which terminate in chain, are added, but in smaller amounts, normal amounts.

This mixture is first heated to separate the DNA strands, and then cooled to make the primer bind to the single-chain DNA template. When the primer binds, the temperature will rise, allowing the DNA polymerase to synthesize new DNA starting with the primer. The DNA polymerase will continue adding nucleotides to the chain until it adds a dioxide nucleotide in place of the original. At this point, no nucleotides will be added, so the dioxide nucleotide strand will end up. This process will be repeated several times until it is

complete. It is almost guaranteed that a dioxide nucleotide is incorporated into every single locus of target DNA in at least one reaction. Therefore, the tube will contain fragments of different lengths, terminating at each of the nucleotide positions in the original DNA (**as in the figure**). The ends of the fragments will be labeled with pigments indicating their final nucleotides.



After the reaction ends, the fragments pass through a long, thin tube containing a gel matrix, this process called capillary gel electrophoresis. The short fragments move rapidly through the gel pores, the long fragments move slowly, and when each piece reaches (finish line) at the end of the tube, they will be illuminated by the laser, allowing the attached dye to be detected.

The smallest chunk reaches the finish line first, followed by the next smaller chunk and so on. Therefore, from the colors of the dyes arranged one after the other on the detector, the sequence of the original piece of DNA can be constructed from one nucleotide at a time. The data recorded by the detector consists of a series of peaks in the intensity of fluorescence as shown above **in the figure**.

Advantages and disadvantages of the Sanger sequence:

Sanger sequencing gives high-quality sequences over relatively long distances of DNA. It is usually used to sequence individual pieces of DNA, such as bacterial plasmids or DNA transcribed in PCR.

However, Sanger sequencing is expensive and ineffective for large projects, so sequencing technology has been widely developed and is now the most widely used as it is faster and less expensive.

2- Next-generation sequencing

There are several technologies that make DNA sequencing using next generation technology. However, most of them share a common set of features, namely:

Highly parallel: Several sequence reactions occur simultaneously

Micro Scale: Interactions are small and many of them can be performed all at once on a slide Fast: Because the reactions take place in parallel, the results are ready faster, Low cost: Genome sequencing is cheaper than Sanger sequencing

Shorter length: readings typically range from 50-700 nucleotides.

Next-generation sequencing is a very large number of small-chain Sanger reactions operating in a parallel fashion. With this parallelism and small scale, large quantities of DNA can be sequenced more quickly and at a lower cost using next-generation methods compared to Sanger sequencing.

Section: 2.4 Microarrays

Microarray is one of the most recent developments used in cancer research. It helps in pharmacological approaches to treat various diseases including oral lesions. Microarray technology helps in analyzing many pre-recorded samples or new samples; It even helps test for the occurrence of a particular marker in tumors. Until recently, the use of microarray in dentistry was very limited, but in the future, as the technology becomes more affordable, there may be an increase in its use.

Here, we discuss the different techniques and applications of microarrays or DNA segments.

For microarray analysis, messenger RNA particles are usually collected from both an experimental sample and a reference sample. For example, the reference sample can be collected from a healthy individual, and the experimental sample may be collected from an individual with a disease such as cancer.

The mRNA samples are then converted into complementary DNA (cDNA), and each sample is marked with a fluorescent probe of a different color. For example, an experimental cDNA can be labeled with a red

fluorescent dye, while a reference cDNA can be called a green, fluorescent dye. Then the two samples are mixed and allowed to bind to a microarray slide. The process in

which cDNA molecules bind to the DNA probes on the slide is called hybridization. After hybridization, the micro-matrix is examined to measure the expression of each gene printed on the slide. If the expression of a

gene is higher in the experimental sample than in the reference sample, then the corresponding spot on the micro-matrix is shown in red.

In contrast if the expression in the experimental sample is less than in

the reference sample, the stain will appear green.

Finally, if there is equal expression in the two samples, the stain will appear yellow. The data collected through the microarrays can be used to create gene expression profiles, which show simultaneous changes in the expression of several genes in response to a specific condition or treatment.

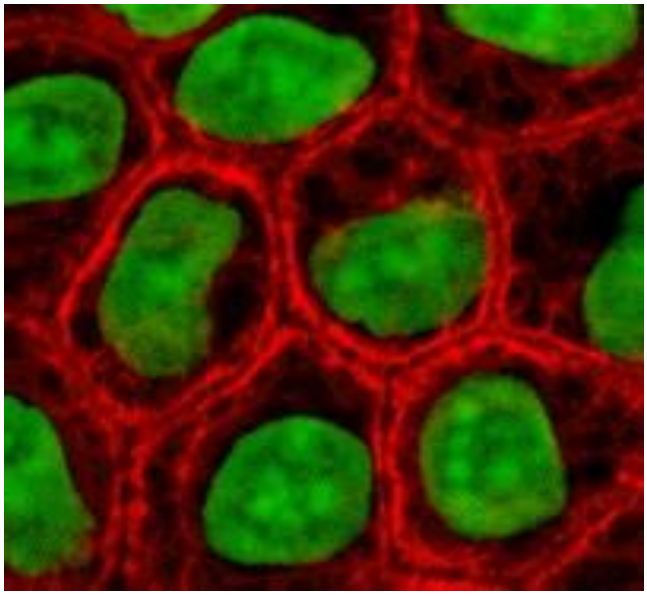
Section: 2.5 Cell culture

Cell culture is the process by which cells grow under controlled conditions outside of their natural environment. After cells are isolated from living tissue, they can be subsequently preserved under carefully controlled conditions. These conditions differ for each type of cell, but they generally consist of a suitable container with a substrate or medium that provides essential nutrients (amino acids, carbohydrates, vitamins, and minerals), growth factors, hormones, and gases (CO2, O2), and regulates the physical and chemical environment (buffer pH, pressure, Osmotic, temperature). The term cell culture now refers to cell culture derived from multicellular eukaryotes, especially animal cells. The laboratory technique of maintaining live cell lines, a group of cells descended

from a single cell and containing the same genetic makeup, separated from the original tissue source, became more powerful in the mid-twentieth century.

Cell isolation:

Epithelial cells in culture



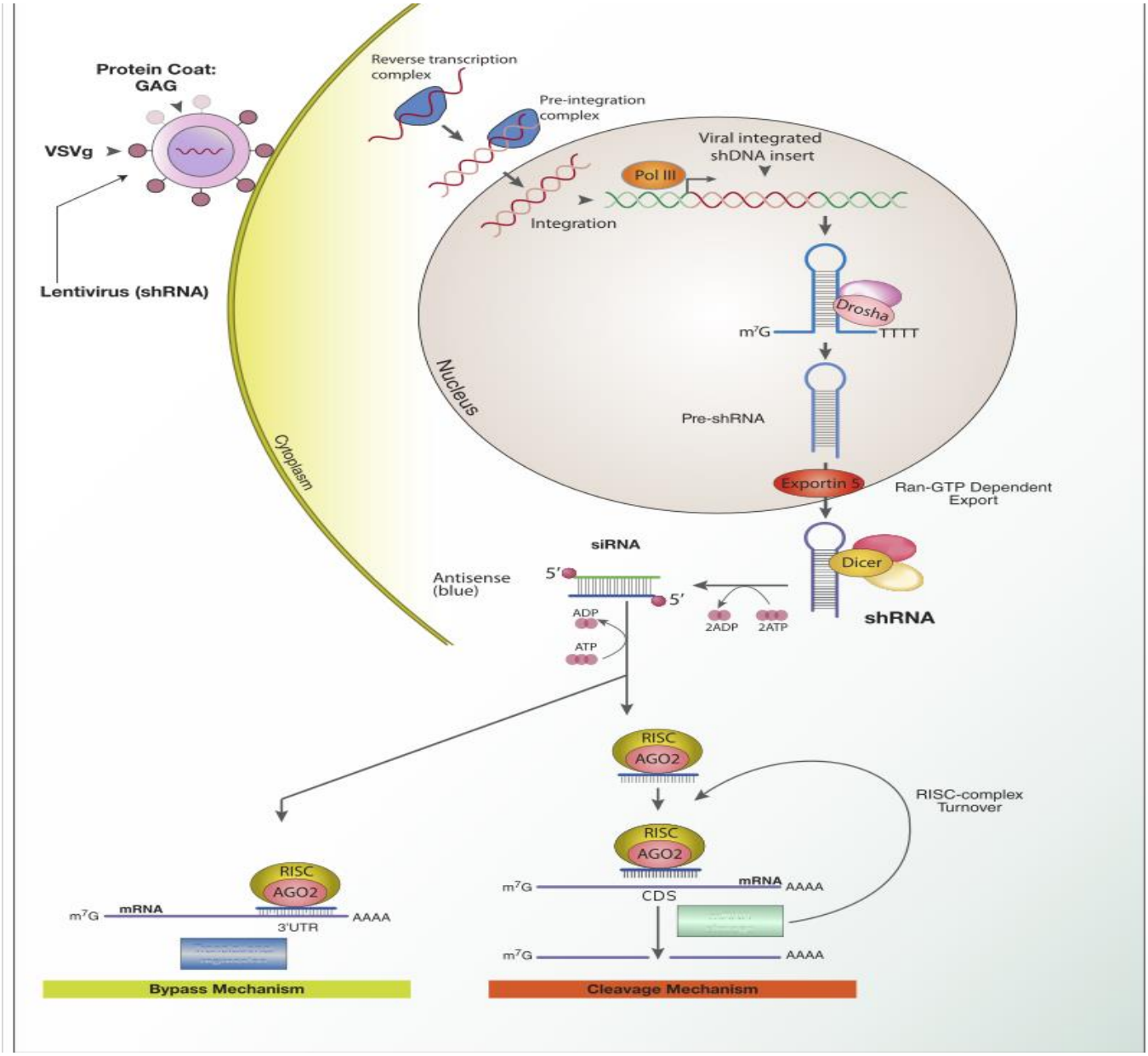
Cells can be isolated from tissues for cultivation ex vivo in several ways. Cells can be removed from the blood easily. However, only white cells can grow in the culture. Cells can be isolated from solid tissues by digesting the extracellular matrix with enzymes such as collagenase, trypsin, or pranas, before moving the tissue to release the suspended cells. Alternatively, pieces of tissue may be placed in the growth medium, and the cells that are growing are available for transplantation.This method is known as plant culture. Cells cultured directly from a subject are known as primary cells.

Section: 2.6 RNA interference

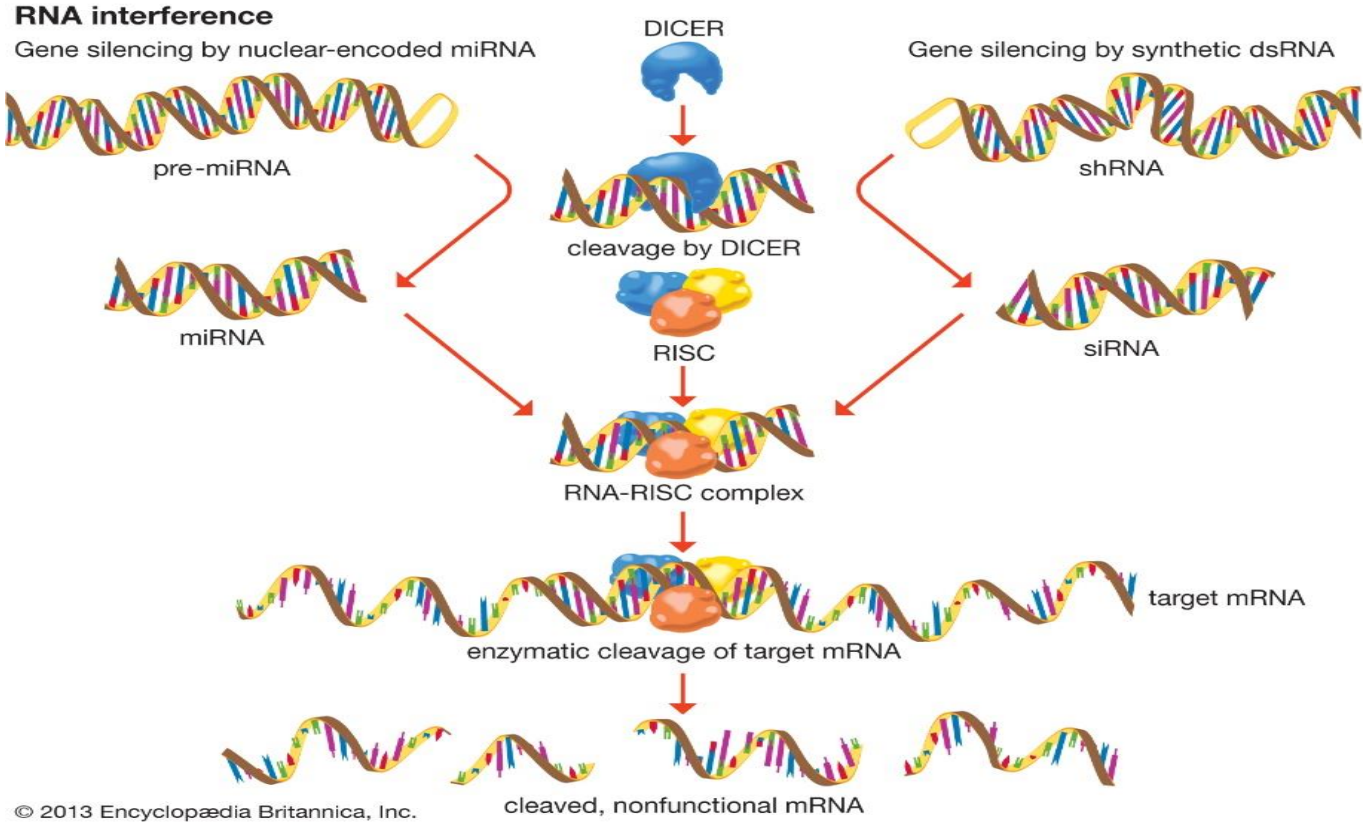
RNA interference (RNAi) is a biological process in which RNA molecules prevent gene expression, or translation, by neutralizing the target mRNA molecules. Since the discovery of RNAi and its regulatory potentials, it has become evident that RNAi has enormous potential in suppressing desired genes. RNAi is now known to be accurate, effective, stable, and better than anti-allergy treatment for gene suppression.There are two types of small RNA molecules that are the focal points of RNA interference. RNAs are the direct product of genes, and these small RNAs can direct enzyme complexes to break down RNA (mRNA) molecules and thus reduce their activity by blocking translation, by silencing the genes after transcription. Moreover, transcription can be inhibited by the pre-transcriptional silencing mechanism of RNA interference, by which the enzyme

complex induces DNA methylation at genomic loci complementary to the complex siRNA or miRNA. RNA interference plays an important role in the defense of cells against the parasitic nucleotide sequence - viruses and transposons. It also affects development.

The RNAi pathway is found in many eukaryotes, including animals, and is initiated by the Dicer enzyme, which splits long dsRNA molecules into short double-stranded fragments.



(ssRNAs), stirrup strand and guide strand. The stirrup strands Each siRNA is decoded into two single-stranded RNA is degraded and the guide strand is incorporated into the RNA-induced silencing complex (RISC). The most studied result is post-transcriptional gene silencing, which occurs when the guide strand is paired with a complementary sequence in the messenger RNA molecule and induces cleavage by Argonaut 2 (Ago2), the catalytic component of RISC. In some organisms, this process is systematically pervasive, despite initially limited molar concentrations of siRNA. The technique of RNA interference (RNAi) It is an RNA-dependent gene silencing process that is controlled by an RNA-induced silencing complex (RISC) and initiated by short double-



stranded RNA molecules in the cytoplasm of the cell, where they interact with the catalytic Argonaut component RISC. When dsRNA is exogenous, the RNA is imported directly into the cytoplasm and broken down into short parts by Dicer. The initiator dsRNA can also be originating in the cell, as in pre-microRNAs expressed from RNA-coding genes in the genome. The primary copies of these genes are first processed to form the characteristic pre-miRNA stem-loop structure in the nucleus, and then exported to the cytoplasm. Thus, two dsRNA pathways, external and internal, converge in RISC.

External dsRNA initiates RNAi by activating the Dicer RNA, which binds and cleaves double-stranded RNAs (dsRNAs) in plants, or short-haired RNAs (shRNAs) in humans, to produce fragments Double-chain 20-25 base pairs. These short, double-stranded fragments are called small interlocking RNAs (siRNAs). These siRNAs are then separated into single strands and combined into an active RISC, by the RISC-Loading complex (RLC). TATA-bound protein-binding factor 11 (TAF11) aggregates RLC by facilitating Dcr-2-R2D2 tetramerization, increasing siRNA binding affinity by 10-fold. Linking with TAF11 will convert the R2-D2-Initator (RDI) complex to RLC. R2D2 carries double-stranded RNA-binding domains side by side to recognize the thermodynamically stable end of siRNA double impellers, whereas Dicer-2 is the other less stable end. Loading is asymmetric: the MID domain of Ago2 recognizes the thermodynamically stable end of siRNA. Therefore, the (stirrup) thread whose five ends are discarded by MID, while the retained (guide) thread cooperates with the AGO to form the RISC.

After fusion into RISC, the siRNAs base pair into its own target mRNA and fix it, thus preventing its use as a translation template. Unlike siRNA, the miRNA-loaded RISC complex scans cytoplasmic mRNAs for possible integration. Instead of destructive cleavage (by Ago2), miRNAs instead target untranslated 3 regions (UTR) of mRNAs where they normally associate with incomplete integration, thus preventing ribosomes from reaching translation. An exogenous dsRNA is detected and linked to a responsive protein, known as RDE-4 in C. elegans and R2D2 in Drosophila, which induces gambler

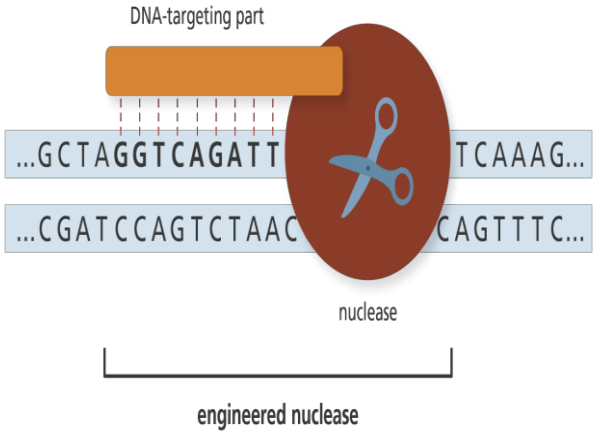
activity. The mechanism that produces this specific length is unknown and this protein binds only to long dsRNAs.

Section: 2.7 Genome editing

Genome editing is a technique used to modify DNA accurately and efficiently inside a cell. It involves making cuts in specific DNA sequences using enzymes called (engineered nucleases). Genome editing can be used to add, remove, or alter the DNA in the genome. By editing the genome, the properties of a cell or an organism can be altered. Genome editing can be used to edit the genome of any organism. It is against the law to use genome editing in human embryos that would be allowed to develop after 14 days.

Genome editing can be used:

- For research: Genome editing can be used to alter the DNA in cells or organisms to understand their biology and how they work.
- To treat disease: Genome editing has been used to modify human blood cells that are then returned to the body to treat conditions such as leukemia and AIDS.
- It may also be used to treat other infections (such as MRSA) and minor genetic conditions (such as muscular dystrophy and hemophilia).
- For biotechnology: Genome editing has been used in agriculture to genetically modify crops to improve their yields and resist disease and drought, as well as to genetically modify cattle that have no horns.

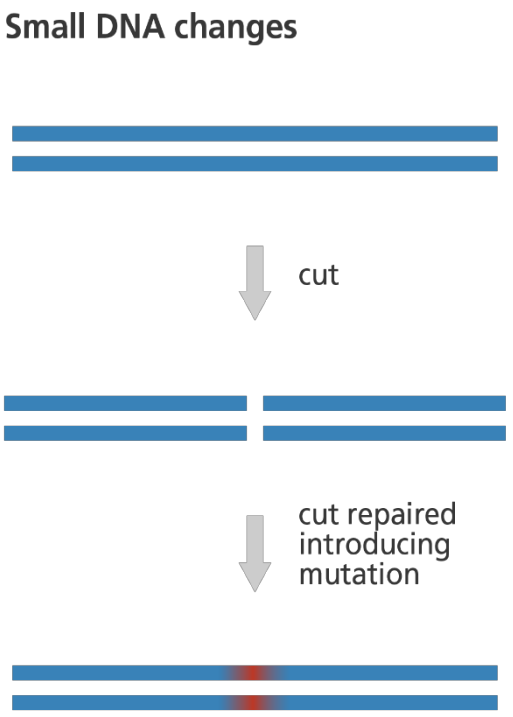


how the process occurs?

Genome editing uses a type of enzyme called an (engineering nuclease) that cuts the genome at a specific location.

Engineering nucleases consist of two parts:

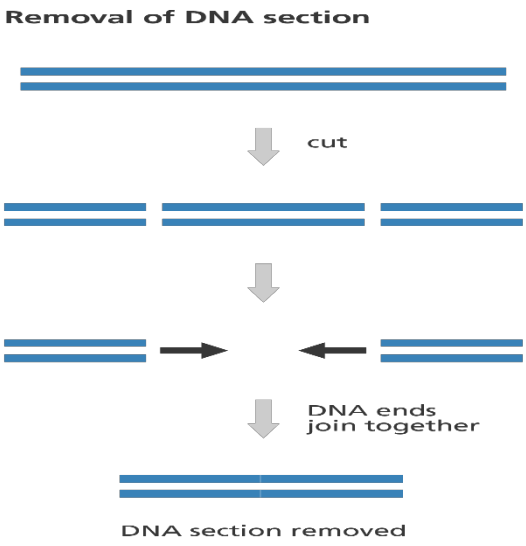
The nuclease fraction cuts DNA:



DNA targeting fragment designed to direct the nuclease to a specific DNA sequence. After cutting DNA at a specific location, the cell will naturally repair the wound. We can manipulate this repair process to make changes to the DNA at that site in the genome.

A Small change in DNA, the nuclease enzyme is designed to cut at a specific location in DNA.

After cutting DNA using an engineered nuclease, does the normal DNA repair of a cell, the machines will recognize the damage and tie the two cut ends of DNA together again. This simple fix is not 100 percent perfect and is often a few, They are lost or added around the cut site when repaired. This small change In the DNA will affect the function of this part of the DNA, which could mean a gene? Not working properly or not working at all.

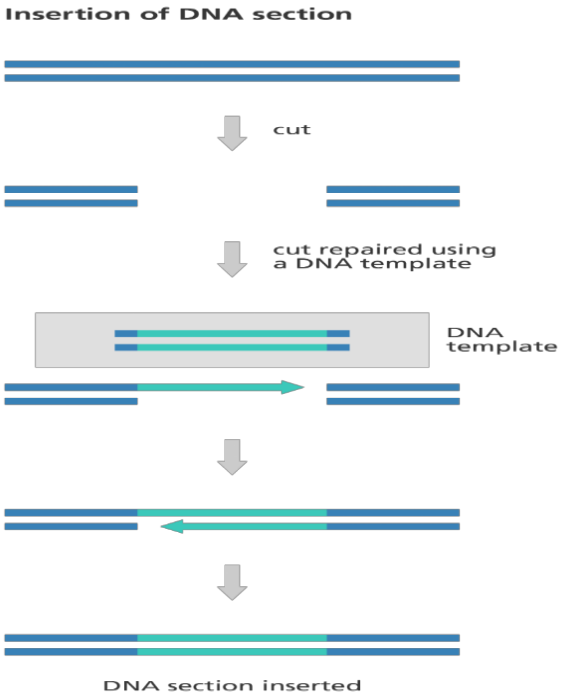


Removing part of the DNA

To remove a portion of DNA, nucleases are designed to make cuts in the DNA on either side of the section we want to remove. After a nuclease breaks the DNA, the cell's natural DNA repair mechanism will recognize the damage but may mistakenly bind the wrong ends of the DNA together, removing the DNA between the two pieces.

Inserting a section of DNA

The normal DNA repair system can be hijacked to introduce a portion of DNA into the genome-by-genome editing. Usually before the cell divides, is all the DNA replicated so that the two newborn cells are replicated? A complete copy of the genome can be obtained.



If there is a break in one copy of the DNA, the cell repairs the break using the other copy as a template. This process ensures that two copies of the DNA are matched again and is called (homology directed repair.)

With genome editing, it is possible to leverage this DNA repair system to trick a cell into inserting a piece of DNA. The nuclease enzyme is designed to cut at a specific location in DNA. After cutting the DNA, a modified piece of DNA like the sequence is introduced to the cut site. The cell uses the modified piece of DNA as a template to repair the fracture, filling the separator with a copy of the new DNA.

Can this approach be used to insert a new section of DNA, or to replace an existing section of DNA with an altered copy, Genome Editing Systems There are several different types of engineered nuclease used in genome editing. They all contain a nuclear part to cut DNA and a part that targets the DNA to identify the DNA sequence they cut.

They differ mainly in how the DNA to be cut is identified:

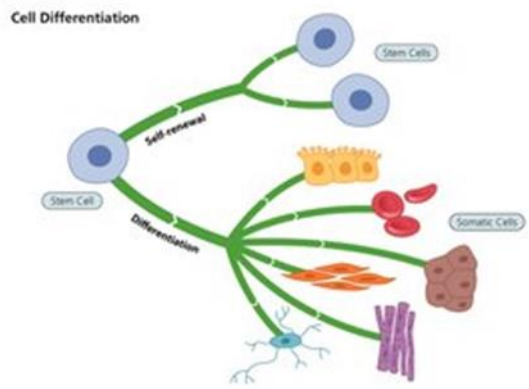
- RNA-based: It contains a short sequence of RNA that binds to the target DNA to be cut.
- Protein: It contains a protein that recognizes and binds to the target DNA to be cut for example, to correct a point mutation, Within a gene.

Chapter 3: Emerging Trends

Section 3.1: Stem Cells

What are stem cells?

Stem cells are cells that are able to produce a copy of themselves by dividing. However, division does not create two new stem cells, it is an asymmetric cell division that creates two different cells. One of the newly created cells is a duplicate of the mother cell and has the same characteristics. a new stem cell is created. The other cell, which was created by asymmetric cell division, develops into a specialized cell type, it differentiates. The stem cells are then able to mature by differentiation into further cell types and represent an essential basis for the development



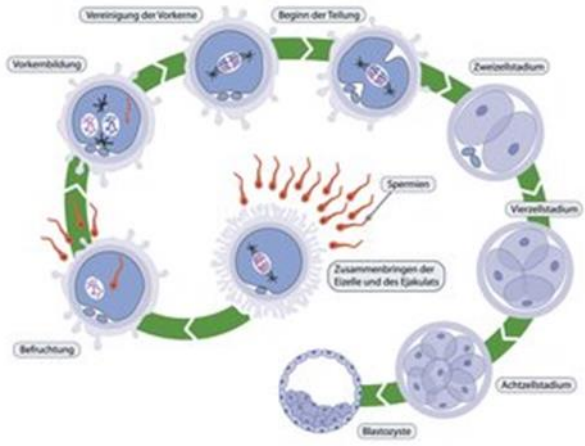
Asymmetric cell division enables to maintain the pool of stem cells (self-renewal) and to develop new somatic cells (differentiation

of complex tissues and organs, such as the heart and kidneys. Through the mechanism of asymmetric cell division, it is possible both to maintain the pool of stem cells and to form new cells for differentiation. When we talk about stem cells, we have to distinguish between different stem cell types. They differ in their potential to form different cell types by division.

Types of the Stem cells

Stem cells can be classified according to their origin into four broad types

- from embryos
- from the infants
- from the adult.



the developmental stages from the fertilized egg to the blastocyst

Embryonic Stem Cells

Embryonic stem cells are pluripotent, self-renewing cells that can be derived from both mouse or human blastocysts, they are taken from the very early stages of embryo development after 4-5 days after fertilization. They can be stored in culture as undifferentiated cell lines and can be stimulated to differentiate into any cell line. They can differentiate into endoderm, mesoderm, and ectoderm embryonic germ layers, and also any type of somatic cells. They, therefore, hold a great capacity in tissue regeneration therapy. Figure ()

Embryonic Germ Stem Cells: Embryonic Germ (EG) cells are taken from the later stages of the embryo development cells. They are derived from Primordial Germline Cells (PGCs) in the early development. They are mainly isolated from the fetal tissue in narrow-window timing. The PGC-derived cells were pluripotent, although, it was not possible to demonstrate pluripotency by generating the formation of teratomas in mice.

Fetal stem cells: Fetal stem cells are primal cell types found in the organs of the fetuses. They are able to differentiate into two types of stem cells: pluripotent stem cells and hematopoietic stem cells. Neural crest stem cells, fetal hematopoietic stem cells and pancreatic islet cells have been isolated in the fetuses. Human fetal stem cells have been used by many people, children and adults that are suffering from many of mankind's most devastating diseases.

Infant stem cell

Umbilical cord stem cells: Umbilical cord blood contains prevalent stem cells which differ from those of bone marrow and adult peripheral blood. Cord blood stem cells have shown to be multipotent as it being able to differentiate into neurons and liver cells.

Wharton's jelly: Wharton's jelly, which is the umbilical cord matrix, is considered to be a source of mesenchymal stem cells. These cells express typical stem cell markers, can be propagated for long times and can be induced to differentiate in vitro into neurons.

Adult stem cell

Adult stem cells are any stem cells taken from mature tissue; they are found in the tissues of a fully developed child (whole embryo) or adult and can only produce a limited number of cell types. They have limited potential as compared to the stem cells that derived from embryos and fetuses because of the stage of development of these cells. They play a vital role in tissue repair, regeneration; and they are referred to their tissue origin. Bone marrow is an abundant source of adult stem cells.

Mesenchymal stem cells: Mesenchymal Stem Cells (MSCs) are a different population of cells with the potential to differentiate into various somatic lineages. They were at first described as adherent cells with a fibroblast-like appearance that can differentiate into osteocytes, chondrocytes, adipocytes, tenocytes and myocytes.

MSCs can be isolated from the bone marrow and readily discreted from the hematopoietic stem cells due to their plastic adherence. They are used in tissue

engineering and regenerative medicine. They are character by long-storage without major loss of their potency.

Hematopoietic stem cells: Hematopoietic stem cells are cells having the self-renewing potential and the capacity to give rise to differentiated cells of all hematopoietic lineages. Therefore, they transplanted for complete healing of hematologic disorders and after high-dose chemotherapy against malignant diseases.

Neural Stem Cells: Neural stem cells are multipotent and self-replication cells; they are established in specialized molecular microenvironments in the adult mammalian brain. They can display the potential role in cellular therapy of the brain.

Gastrointestinal stem cells: The stem cells of the gastrointestinal tract reside in a "niche" in the intestinal crypts and gastric glands. The mechanism and the direction of the diffusion of this converted clone in the gastrointestinal mucosa are hotly disputed, and the central to this case is the position and nature of the gastrointestinal stem cells.

Types of stem cells according to their differentiation

Researchers categorize stem cells, according to their potential to differentiate into other types of cells.

- Totipotent: These stem cells can differentiate into all possible cell types. The first few cells that appear as the zygote starts to divide are totipotent.

- Pluripotent: These cells can turn into almost any cell. Cells from the early embryo are pluripotent.

- Multipotent: These cells can differentiate into a closely related family of cells. Adult hematopoietic stem cells, for example, can become red and white blood cells or platelets.

- Oligopotent: These can differentiate into a few different cell types. Adult lymphoid or myeloid stem cells can do this.

- Unipotent: These can only produce cells of one kind, which is their own type. However, they are still stem cells because they can renew themselves. Examples include adult muscle stem cells.

- Embryonic stem cells are considered pluripotent instead of totipotent because they cannot become part of the extra-embryonic membranes or the placenta.

What are the uses of stem cells?

- Transplants with stem cells are already helping people with diseases such as lymphoma.

Stem cells themselves do not serve any single purpose but are important for several reasons.

First, with the right stimulation, many stem cells can take on the role of any type of cell, and they can regenerate damaged tissue, under the right conditions.

This potential could save lives or repair wounds and tissue damage in people after an illness or injury. Scientists see many possible uses for stem cells.

- Tissue regeneration

Tissue regeneration is probably the most important use of stem cells. Until now, a person who needed a new kidney, for example, had to wait for a donor and then undergo a transplant. There is a shortage of donor organs but, by instructing stem cells to differentiate in a certain way, scientists could use them to grow a specific tissue type or organ.As an example, doctors have already used stem cells from just beneath the skin's surface to make new skin tissue. They can then repair a severe burn or another injury by grafting this tissue onto the damaged skin, and new skin will grow back.

- Cardiovascular disease treatment

In 2013, a team of researchers from Massachusetts General Hospital reported in PNAS Early Edition that they had created blood vessels in laboratory mice, using human stem

cells. Within 2 weeks of implanting the stem cells, networks of blood-perfused vessels had formed. The quality of these new blood vessels was as good as the nearby natural ones. The authors hoped that this type of technique could eventually help to treat people with cardiovascular and vascular diseases.

- Brain disease treatment

Doctors may one day be able to use replacement cells and tissues to treat brain diseases, such as Parkinson's and Alzheimer's. In Parkinson's, for example, damage to brain cells leads to uncontrolled muscle movements. Scientists could use stem cells to replenish the damaged brain tissue. This could bring back the specialized brain cells that stop the uncontrolled muscle movements. Researchers have already tried differentiating embryonic stem cells into these types of cells, so treatments are promising.

- Cell deficiency therapy

Scientists hope one day to be able to develop healthy heart cells in a laboratory that they can transplant into people with heart disease. These new cells could repair heart damage by repopulating the heart with healthy tissue. Similarly, people with type I diabetes could receive pancreatic cells to replace the insulin-producing cells that their own immune systems have lost or destroyed. The only current therapy is a pancreatic transplant, and very few pancreases are available for transplant.

- Blood disease treatments

Doctors now routinely use adult hematopoietic stem cells to treat diseases, such as leukemia, sickle cell anemia, and other immunodeficiency problems. Hematopoietic stem cells occur in blood and bone marrow and can produce all blood cell types, including red blood cells that carry oxygen and white blood cells that fight disease.

Researchers and doctors hope that studies of stem cells will help:

- Increase knowledge about how diseases emerge.

By watching stem cells mature into cells in bones, heart muscle, nerves, and other organs and tissue, researchers and doctors may better understand how diseases and conditions develop.

- Generate healthy cells to replace diseased cells (regenerative medicine)

Stem cells can be guided into becoming specific cells that can be used to regenerate and repair diseased or damaged tissues in people.

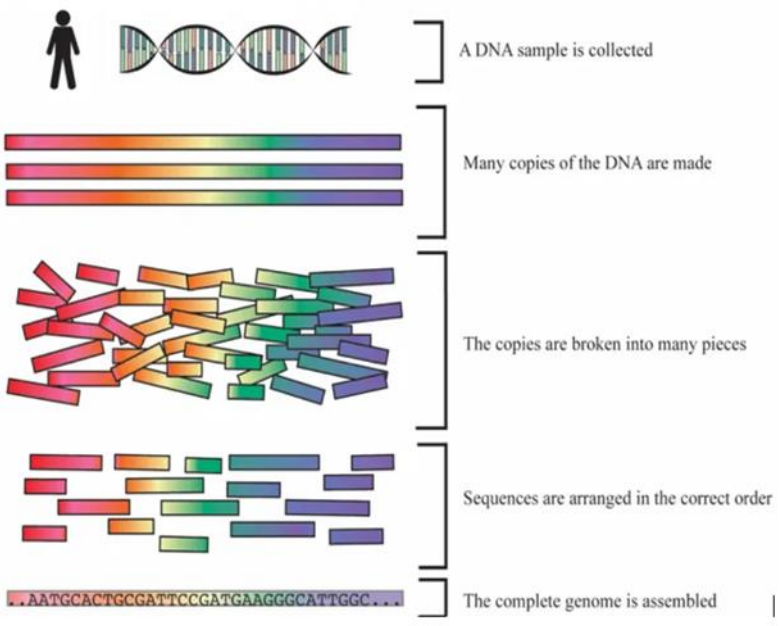
Stem cells may have the potential to be grown to become new tissue for use in transplant and regenerative medicine. Researchers continue to advance the knowledge on stem cells and their applications in transplant and regenerative medicine.

- Test new drugs for safety and effectiveness

New areas of study include the effectiveness of using human stem cells that have been programmed into tissue-specific cells to test new drugs

For instance, nerve cells could be generated to test a new drug for a nerve disease. Tests could show whether the new drug had any effect on the cells and whether the cells were harmed.

Section 3.2: The Human Genome Project



The Human Genome Project was an international research project that sequenced all of the genes found in humans. This ambitious project began in 1990 and concluded in 2003. One goal of the project was to accurately sequence the 3 billion nucleotide base pairs in the human genome. A second goal was to map and identify all of the human genes present in the DNA

sequence. (The number of genes is currently estimated to be between 20,000 and 25,000.) An additional aim of the Human Genome Project was to publicly store all of the sequence information collected in Internet databases.

In order to sequence the human genome, the first portion of the Human Genome Project involved fragmenting chromosomes into large, overlapping segments. The fragments were then sequenced and assembled in order. Finally, any remaining gaps were sequenced. The success of the Human Genome Project provided scientists with a vast amount of information, which continues to be used to study the functions of unknown genes, understand human health, and identify genes associated with disease.

Along with sequencing the human genome, the Human Genome Project also involved sequencing the genomes of a number of other organisms, including yeast, E. coli, fruit flies, roundworms, and mice.

Human genome project goals

- Genetic Mapping

Complete the 2- to 5-cM map by 1995. (Goals for map resolution remain unchanged.)

Develop technology for rapid genotyping.

Develop markers that are easier to use.

Develop new mapping technologies.

- Physical Mapping

Complete a sequence tagged site (STS) map of the human genome at a resolution of 100 kb. (Goals for map resolution remain unchanged.)

- DNA Sequencing

Develop efficient approaches to sequencing one- to several mega base regions of DNA of high biological interest.

Develop technology for high-throughput sequencing, focusing on systems integration of all steps from template preparation to data analysis.

Build up a sequencing capacity to allow sequencing at a collective rate of 50 Mb per year by the end of the period. This rate should result in an aggregate of 80 Mb of DNA sequence completed by the end of FY 1998.

- Gene Identification

Develop efficient methods for identifying genes and for placement of known genes on physical maps or sequenced DNA.

- Technology Development

Substantially expand support of innovative technological developments as well as improvements in current technology for DNA sequencing and for meeting the needs of the Human Genome Project as a whole.

- Model Organisms

Finish an STS map of the mouse genome at a 300-kb resolution.

Finish the sequence of the Escherichia coli and Saccharomyces cerevisiae genomes by 1998 or earlier.

Continue sequencing Caenorhabditis elegans and Drosophila melanogaster genomes with the aim of bringing C. elegans to near completion by 1998.

Sequence selected segments of mouse DNA side by side with corresponding human DNA in areas of high biological interest.

- Training

Continue to encourage training of scientists in interdisciplinary sciences related to genome research.

- Technology Transfer

Encourage and enhance technology transfer both into and out of centers of genome research.

- Outreach

Cooperate with those who would establish distribution centers for genome materials. Share all information and materials within 6 months of their development. This should be accomplished by submission of information to public databases or repositories, or both, where appropriate.

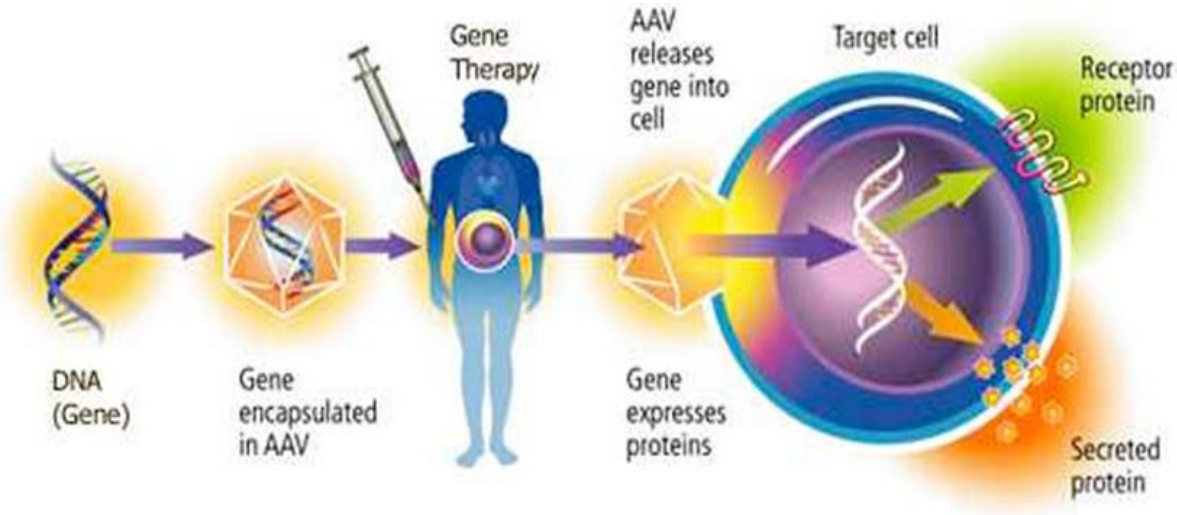
Section 3.3: Gene Therapy

What is gene therapy?

Gene therapy is an experimental technique that uses genes to treat or prevent disease. In the future, this technique may allow doctors to treat a disorder by inserting a gene into a patient's cells instead of using drugs or surgery

How the gene therapy work?

Gene therapy is designed to introduce genetic material into cells to compensate for



abnormal genes or to make a beneficial protein. If a mutated gene causes a necessary protein to be faulty or missing, gene therapy may be able to introduce a normal copy of the gene to restore the function of the protein. A gene that is inserted directly into a cell usually does not function. Instead, a carrier called a vector is genetically engineered to

deliver the gene. Certain viruses are often used as vectors because they can deliver the new gene by infecting the cell. The viruses are modified so they can't cause disease when used in people. Some types of virus, such as retroviruses, integrate their genetic material (including the new gene) into a chromosome in the human cell. Other viruses, such as adenoviruses, introduce their DNA into the nucleus of the cell, but the DNA is not integrated into a chromosome.

The vector can be injected or given intravenously (by IV) directly into a specific tissue in the body, where it is taken up by individual cells. Alternately, a sample of the patient's cells can be removed and exposed to the vector in a laboratory setting. The cells containing the vector are then returned to the patient. If the treatment is successful, the new gene delivered by the vector will make a functioning protein.

Is Gene Therapy safe?

Gene therapy is under study to determine whether it could be used to treat disease. Current research is evaluating the safety of gene therapy; future studies will test whether it is an effective treatment option. Several studies have already shown that this approach can have very serious health risks, such as toxicity, inflammation, and cancer. Because the techniques are relatively new, some of the risks may be unpredictable; however, medical researchers, institutions, and regulatory agencies are working to ensure that gene therapy research is as safe as possible.

Comprehensive federal laws, regulations, and guidelines help protect people who participate in research studies (called clinical trials). The U.S. Food and Drug Administration (FDA) regulates all gene therapy products in the United States and overseas research in this area. Researchers who wish to test an approach in a clinical trial must first obtain permission from the FDA. The FDA has the authority to reject or suspend clinical trials that are suspected of being unsafe for participants.

The National Institutes of Health (NIH) also plays an important role in ensuring the safety of gene therapy research. NIH provides guidelines for investigators and

institutions (such as universities and hospitals) to follow when conducting clinical trials with gene therapy. These guidelines state that clinical trials at institutions receiving NIH funding for this type of research must be registered with the NIH Office of Biotechnology Activities. The protocol, or plan, for each clinical trial is then reviewed by the NIH Recombinant DNA Advisory Committee (RAC) to determine whether it raises medical, ethical, or safety issues that warrant further discussion at one of the RAC's public meetings.

An Institutional Review Board (IRB) and an Institutional Biosafety Committee (IBC) must approve each gene therapy clinical trial before it can be carried out. An IRB is a committee of scientific and medical advisors and consumers that reviews all research within an institution. An IBC is a group that reviews and approves an institution's potentially hazardous research studies. Multiple levels of evaluation and oversight ensure that safety concerns are a top priority in the planning and carrying out of gene therapy research.

What are the ethical issues surrounding gene therapy?

Because gene therapy involves making changes to the body's set of basic instructions, it raises many unique ethical concerns. The ethical questions surrounding gene therapy include:

- How can "good" and "bad" uses of gene therapy be distinguished?

-Who decides which traits are normal and which constitute a disability or disorder?

- Will the high costs of gene therapy make it available only to the wealthy?

- Could the widespread use of gene therapy make society less accepting of people who are different?

- Should people be allowed to use gene therapy to enhance basic human traits such as height, intelligence, or athletic ability?

Current gene therapy research has focused on treating individuals by targeting the therapy to body cells such as bone marrow or blood cells. This type of gene therapy cannot be passed to a person's children. Gene therapy could be targeted to egg and sperm cells (germ cells), however, which would allow the inserted gene to be passed to future generations. This approach is known as germline gene therapy.

The idea of germline gene therapy is controversial. While it could spare future generations in a family from having a particular genetic disorder, it might affect the development of a fetus in unexpected ways or have long-term side effects that are not yet known. Because people who would be affected by germline gene therapy are not yet born, they can't choose whether to have the treatment. Because of these ethical concerns, the U.S. Government does not allow federal funds to be used for research on germline gene therapy in people.

Section 3.4: Recombinant DNA

What is the Recombinant DNA?

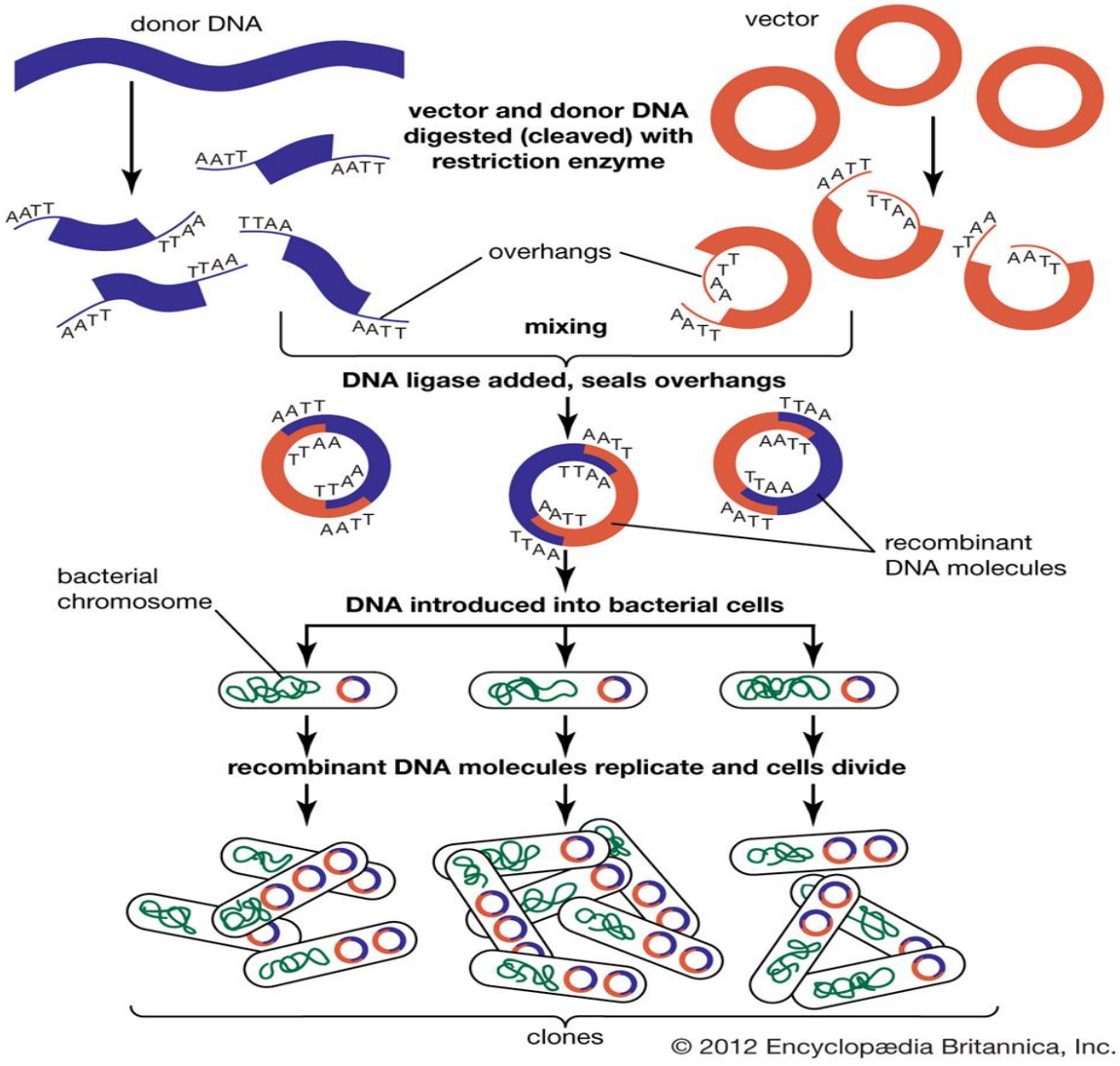
Recombinant DNA technology is the joining together of DNA molecules from two different species. The recombined DNA molecule is inserted into a host organism to produce new genetic combinations that are of value to science, medicine, agriculture, and industry. Since the focus of all genetics is the gene, the fundamental goal of laboratory geneticists is to isolate, characterize, and manipulate genes. Recombinant DNA technology is based primarily on two other technologies, cloning and DNA sequencing. Cloning is undertaken in order to obtain the clone of one particular gene or DNA sequence of interest. The next step after cloning is to find and isolate that clone among other members of the library (a large collection of clones). Once a segment of DNA has been cloned, its nucleotide sequence can be determined. Knowledge of the sequence of a DNA segment has many uses.

When the recombinant DNA is invented?

The possibility for recombinant DNA technology emerged with the discovery of restriction enzymes in 1968 by Swiss microbiologist Werner Arber. The following year American microbiologist Hamilton O. Smith purified so-called type II restriction enzymes, which were found to be essential to genetic engineering for their ability to cleave at a specific site within the DNA (as opposed to type I restriction enzymes, which cleave DNA at random sites). Drawing on Smith's work, American molecular biologist Daniel Nathans helped advance the technique of DNA recombination in 1970–71 and demonstrated that type II enzymes could be useful in genetic studies. About the same time, American biochemist Paul Berg developed methods for splitting DNA molecules at selected sites and attaching segments of the molecule to the DNA of a virus or plasmid, which could then enter bacterial or animal cells. In 1973 American biochemists Stanley N. Cohen and Herbert W. Boyer became the first to insert recombined genes into bacterial cells, which then reproduced.

How the recombinant DNA is useful?

Through recombinant DNA techniques, bacteria have been created that are capable of synthesizing human insulin, human growth hormone, alpha interferon, hepatitis B vaccine, and other medically useful substances. Recombinant DNA technology also can be used for gene therapy, in which a normal gene is introduced into an individual's genome in order to repair a mutation that causes a genetic disease. The ability to obtain specific DNA clones using recombinant DNA technology has also made it possible to add the DNA of one organism to the genome of another. The added gene is called a transgene, which can be passed to progeny as a new component of the genome. The resulting organism carrying the transgene is called a transgenic organism or a genetically modified organism (GMO). In this way a "designer organism" is made that contains some specific change required for an experiment in basic genetics or for improvement of some commercial strain.



Tools of Recombinant DNA Technology

- The enzymes which include the restriction enzymes help to cut, the polymerases- help to synthesize and the ligases- help to bind. The restriction enzymes used in recombinant DNA technology play a major role in determining the location at which the desired gene is inserted into the vector genome. They are two types, namely Endonucleases and Exonucleases.

- The Endonucleases cut within the DNA strand whereas the Exonucleases remove the nucleotides from the ends of the strands. The restriction endonucleases are sequence-specific which are usually palindrome sequences and cut the DNA at specific points.

They scrutinize the length of DNA and make the cut at the specific site called the restriction site. This gives rise to sticky ends in the sequence. The desired genes and the vectors are cut by the same restriction enzymes to obtain the complementary sticky notes, thus making the work of the ligases easy to bind the desired gene to the vector.

- The vectors – help in carrying and integrating the desired gene. These form a very important part of the tools of recombinant DNA technology as they are the ultimate vehicles that carry forward the desired gene into the host organism. Plasmids and bacteriophages are the most common vectors in recombinant DNA technology that are used as they have a very high copy number. The vectors are made up of an origin of replication- This is a sequence of nucleotide from where the replication starts, a selectable marker – constitute genes which show resistance to certain antibiotics like ampicillin; and cloning sites – the sites recognized by the restriction enzymes where desired DNAs are inserted.

- Host organism – into which the recombinant DNA is introduced. The host is the ultimate tool of recombinant DNA technology which takes in the vector engineered with the desired DNA with the help of the enzymes.

There are a number of ways in which these recombinant DNAs are inserted into the host, namely – microinjection, biolistics or gene gun, alternate cooling and heating, use of calcium ions, etc.

Process of Recombinant DNA Technology

The complete process of recombinant DNA technology includes multiple steps, maintained in a specific sequence to generate the desired product.

Step-1. Isolation of Genetic Material.

The first and the initial step in Recombinant DNA technology is to isolate the desired DNA in its pure form. free from other macromolecules.

Step-2. Cutting the gene at the recognition sites.

The restriction enzymes play a major role in determining the location at which the desired gene is inserted into the vector genome. These reactions are called 'restriction enzyme digestions.

Step-3. Amplifying the gene copies through Polymerase chain reaction (PCR).

It is a process to amplify a single copy of DNA into thousands to millions of copies once the proper gene of interest has been cut using the restriction enzymes.

Step-4. Ligation of DNA Molecules.

In this step of Ligation, joining of the two pieces – a cut fragment of DNA and the vector together with the help of the enzyme DNA ligase.

Step-5. Insertion of Recombinant DNA Into Host.

In this step, the recombinant DNA is introduced into a recipient host cell. This process is termed as Transformation. Once after the insertion of the recombinant DNA into the host cell, it gets multiplied and is expressed in the form of the manufactured protein under optimal conditions.

As mentioned in Tools of recombinant DNA technology, there are various ways in which this can be achieved. The effectively transformed cells/organisms carry forward the recombinant gene to the offspring.

Application of Recombinant DNA Technology

- DNA technology is also used to detect the presence of HIV in a person.
- Gene Therapy – It is used as an attempt to correct the gene defects which give rise to heredity diseases.
- Clinical diagnosis – ELISA is an example where the application of recombinant
- Recombinant DNA technology is widely used in Agriculture to produce genetically-modified organisms such as Flavr Savr tomatoes, golden rice rich in proteins, Bt-cotton to protect the plant against ball worms and lot more.

- In the field of medicines, Recombinant DNA technology is used for the production of Insulin.

- In Medicine

Drug delivery systems in medicine that are based on bacterial or viral hosts could prove hazardous if either the organism is genetically unstable and converts to a pathogenic type or if purification is incomplete. In an analogous proof of concept from the agricultural sphere, use of the soil bacterium Agrobacterium tumefaciens as a vehicle for gene transfer is very effective and has become widely adopted despite its tumorigenicity, causing crown gall disease of dicotyledonous plants. Genetic reversion is also a major concern regarding the experimental technique of gene therapy to treat or prevent otherwise incurable genetic disorders and acquired diseases, research into which was slowed in the early 2000s due to cases of viral vector instability. Consequently, identification of a preferred system to safely and efficiently deliver an altered gene of choice has become a priority as the technology advances from development and laboratory research to clinical translational trials.

- In Biotechnology

An appreciable biotechnological success and novel commercial application is the production of genetically modified fluorescent zebrafish, Danio rerio, and similar species using genes encoding glowing characteristics. This is marketed under the Goldfish patent in the US where fish colored bright red, green, orange-yellow, blue and purple are sold as pets to be kept in the controlled environment of an indoor aquarium. In the event of release, inadvertent or deliberate, into the environment the survival capacity of these constantly fluorescent fish is markedly reduced due to increased vulnerability to predation compared to wild type fish; thus, the risk of sustained ecological impact is considered to be marginal.18 However, in-depth research to confirm or refute this notion is currently not possible because of insufficient understanding and

a lack of technology to study the nexus of evolutionary biology and ecology with specific reference to the introduction of a novel species into, and its subsequent migration from, an ecosystem.

Section 3.5: Biochips

A biochip is a set of diminished microarrays that are placed on a strong substrate that allows many experiments to be executed at the same time to obtain a high throughput in less time. This device contains millions of sensor elements or biosensors. Not like microchips, these are not electronic devices. Each and every biochip can be considered as a microreactor that can detect a particular analyte like an enzyme, protein, DNA, biological molecule or antibody. The main function of this chip is to perform hundreds of biological reactions in a few seconds like decoding genes (a sequence of DNA).

Working Principle of a Biochip:

The working of Biochip mainly includes the following steps.

Step1: The operator generates a low-power electromagnetic field through radio signals

Step2: The fixed biochip gets turn on

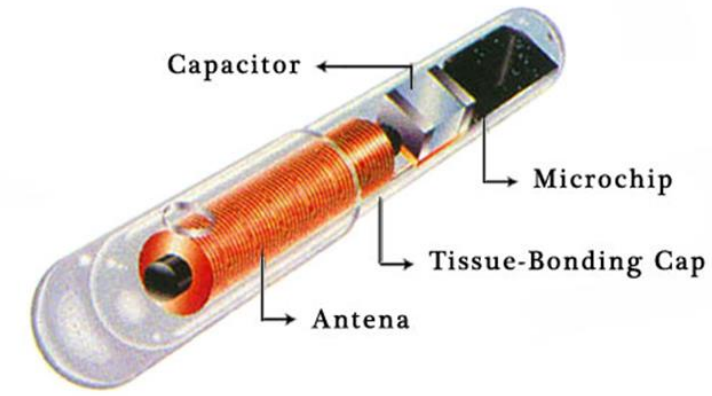
Step3: The activated chip transmits the identification code reverse to the operator through radio signals

Step4: Reader strengthens the received code to change it into digital form and finally exhibits it on LCD.

Components of Biochip:

A Biochip comprises of two components, a transponder, and a reader.

(a) Transponder



Transponders are two types' namely active transponder and passive transponder. This is a passive transponder which means that it doesn't contain any of its own energy or battery whereas in passive, it is not active until the operator activates it by giving it a low electrical charge. This transponder consists of four parts such as antenna coil, computer microchip, glass capsule, and a tuning capacitor.

The computer microchip stores a unique identification (UID) number that ranges from 10 digits to 15 digits long.

The antenna coil is very small, primitive and this type of antenna is used to send and receive the signals from the scanner or reader.

The charging of the tuning capacitor can be done with the small signal i.e, 1/1000 of a watt which is sent by the operator.

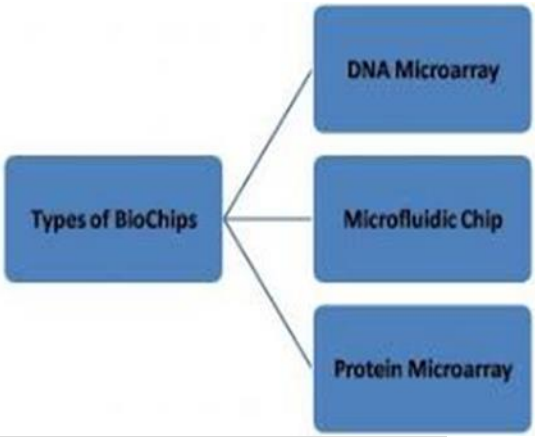
The glass capsule holds the antenna coil, capacitor, and microchip, and it is made with a biocompatible material namely soda lime glass.

(b). Reader:

It consists of a coil called exciter which is used for creating an electromagnetic field(emf) with the help of radio signals. It provides the required energy to trigger the chip. A receiving coil is present for receiving the transmitted code directed back from the excited implanted chip.

Types of Biochip:

The following three types of Biochips are available: –



DNA Microarray –

It comprises of a large number of tiny DNA spots which are fixed to a strong surface. It is used to calculate the expression levels for a large number of genes. Each and every DNA mark comprises of probes (the picomoles of

a particular gene). Generally, probe-target hybridization is observed and calculated by recognition of fluorophore (a fluorescent chemical compound which can re-emit light upon light excitation) labeled targets to decide the relative quantity of nucleic acid series in the target. Innovative arrays of macromolecule were macro arrays concerning nine cm X twelve cm and also the at the start machine-driven icon-based analysis was revealed within the year 1981.

Microfluidic Chip –

They are a replacement for a biochemical laboratory. They are used for a large number of reactions such as DNA analysis, molecular biology procedures and many more biochemical reactions. These chips are really complex because they contain thousands of components. These parts are designed physically known as a bottom-up full-custom arrange, which may be a terribly giant workforce.

Protein Microarray –

These chips are used to track the activities as well as connections of proteins, and to find out their function on a large scale. Its main advantage is that it can be used for tracking a large number of proteins in parallel. This protein chip comprises a surface for supporting like microtiter plate or bead, nitrocellulose membrane, the glass slide. These are automated, fast, economical, very sensitive, consume less quantity of samples. The first methodology of protein chips was introduced in antibody microarrays of scientific publications in the year 1983. The technology behind this chip was quite easy to develop for DNA microarrays, which have become the foremost generally used microarrays.

Advantages:

Biochips has the following advantages –

- They are very small in size and are powerful and faster.
- It can perform thousands of biological reactions in a few seconds.
- Biochip and help in various diseases.

Disadvantages:

Biochips has the following disadvantages –

- They are expensive.
- They can be fixed inside a human body even without their consent.
- They can raise serious problems of individual privacy.

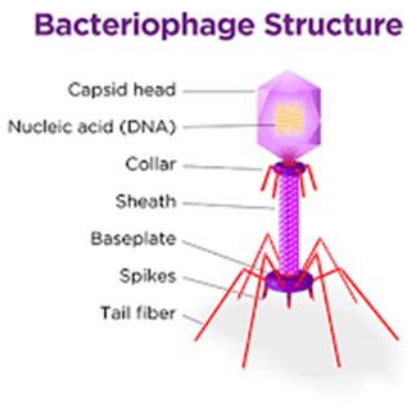
Biochips Applications

The applications of biochip include the following:

- By using this chip, we can trace a person or animal anywhere in the world.
- This chip is used to store and update the information of a person like medical financial and demographics.
- A biochip leads to safe E-commerce systems
- These chips are effective in restoring the records of medical, cash, passport, etc.
- The biochip can be applicable in the medical field as a BP sensor, glucose detector, and oxygen sensor.

Section 3.6 Phage Therapy

Is the therapeutic use of lytic bacteriophages to treat pathogenic bacterial infections especially multiple antibiotic-resistant bacteria infections.



Introduction of Phage Therapy

Phage therapy (PT) is not a new science but was first developed more than a century ago during World War 1, when French-Canadian microbiologist Félix d'Hérelle, working at the Pasteur Institute

in Paris, observed the case of a dysentery patient cured by administering a 'phage cocktail' to eliminate the bacterial infection.

Essentially, phage therapy is the use of viruses to attack bacteria. Application of bacteriophages in this way carries a key advantage in that they only attack bacteria (the name means "eater of bacteria") and cause no harm to flora and fauna. While bacteriophages might look like alien invaders, they are an organic part of life on Earth, prevalent in soil, sewage and water and are nature's way of keeping control of bacterial growth.

Bacteriophages kill bacteria by lysis, first binding themselves to the target bacterium and injecting their DNA or RNA into the cell. This causes copies of the phage to reproduce inside the cell, making as many as a thousand copies in each bacterium, which eventually bursts, releasing the new bacteriophages. Once bacteria are lysed, they are dead and cannot multiply. As with viruses in general, phages can remain inactive until suitable bacterial host 'targets' appear.

Approaches of Phage Therapy

Monophage Therapy

Monophage therapy refers to the use of a single phage type as therapeutic agent. It is primarily used for development of phage therapy experimental models, as proof of concept during the design and testing of phage preparations. The disadvantages of monophage therapy are that it requires precise matching between the pathogen and the phage.

Polyphage Therapy (Phage Cocktails)

Polyphage therapy, also known as phage cocktails, utilizes a combination of phages as therapeutic agent. Compared to monophage therapy, polyphage therapy targets multiple strains of a single bacterial species or multiple species. In Russia and Georgia, phage

cocktails are used as over-the-counter medication for the treatment of bacterial infections of broad etiology.

Phage-Derived Proteins

Virion-associated peptidoglycan hydrolases (VAPGH) and polysaccharide depolymerases are two groups of proteins that are required during phage adsorption to the host and ejection of its genome. VAPGH on the phage base plate can locally degrade bacterial peptidoglycan layer, accelerating the ejection of phage genome into the host. Polysaccharide depolymerases can specifically degrade the polysaccharide components of bacterial cell envelope, even the biofilms. These enzymes help to phage adsorption, invasion, and disintegrating to the host bacteria. In view of the special functions of these phage-derived proteins, they can be explored and developed into novel antibiotics, adjuvants for antibiotics, and bacterial biofilm disrupt ants.

Bioengineered Therapeutic Phages

Phages can be genetically engineered into therapeutic agents by incorporating dominant sensitive genes of reversing antibiotic resistance or antibiotic genes into phage genome. And engineered phages can deliver therapeutic agents into target bacterial cells. Besides, phages can be also genetically modified to have a broader therapeutic potential, including expanding its host range, changing host tropism, and modification of phage capsids.

Phage therapy advantages

A 2011 research review, Pros and cons of phage therapy, by Catherine Loc-Carrillo and Stephen T Abedon, listed principal advantages and disadvantages of phage therapy.

On the plus side, Loc-Carillo and Abedon found that:

- Phages can be effective against antibiotic-resistant bacteria.
- Phages can work as stand-alone therapies or in combination with drugs.

- Phages multiply organically, reducing dosage requirements.
- They have minimal side-effects against benign bacteria in humans.
- Phages are not toxic to humans, animals, plants, or environment.

Phage therapy disadvantages

On the minus side, the review suggested that phage therapy needs more research to find out how well it works. For example, it remains to be established that phages cannot harm people or animals in ways unrelated to direct toxicity.

Other identified disadvantages of phage therapy included:

- Difficulty in preparing phage-based treatments;
- Uncertainty over dosages or timescales;
- Challenges in identifying and isolating the most effective phage type;
- Uncertainty over phage effects on human immune system;
- Limited pool of identified phage types;
- Potential for bacteria to become resistant to specific phage therapies.

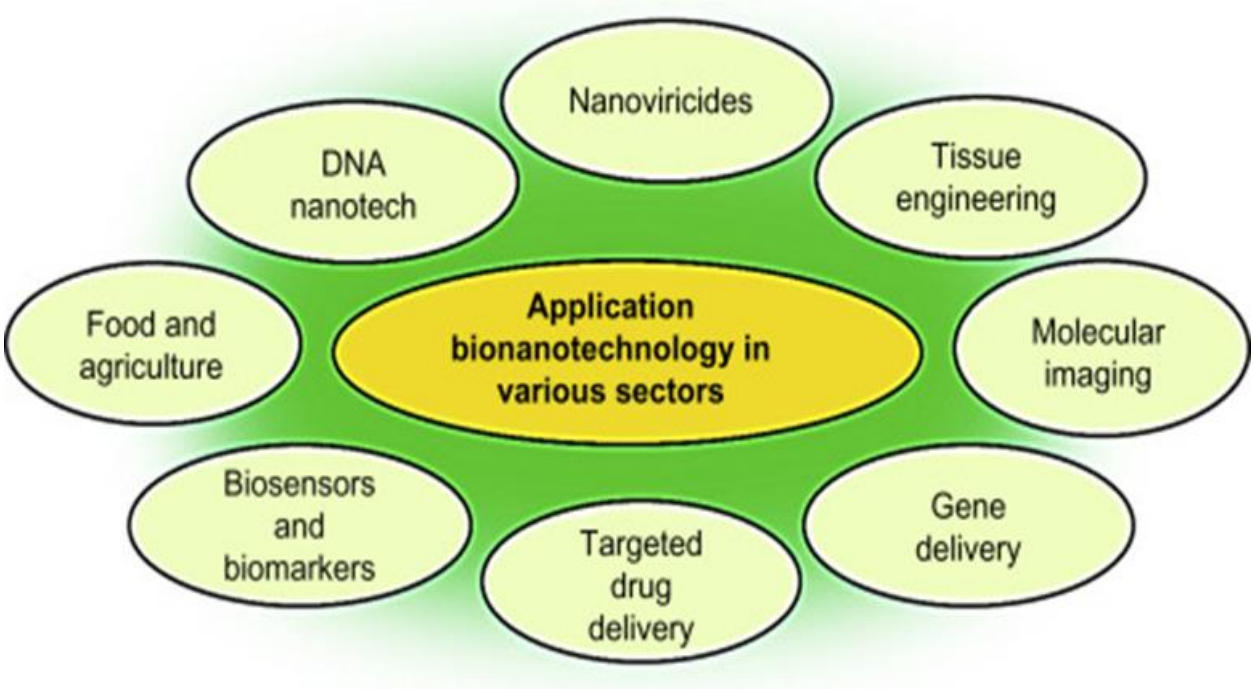
Current therapeutic use of phages

The prime focus of this research is in addressing infections that don't respond to antibiotics, such as the 'hospital killer', Staphylococcus MRSA. There have also been anecdotal 'freelance' successes, such as the well-documented case from San Diego, California, of doctors using bacteriophages to cure a 68-year-old man, whose Acinetobacter baumannii infection had resisted all antibiotics.

Section 3.7: Bionanotechnology

Bionanotechnology is the study of biology, in particular biological machines, and the application of biological building blocks to solve engineering challenges and create new

areas of technological development. Learning about the structure and function of the inner workings of biological systems such as cells, bacteria and viruses has been used to improve existing applications of nanotechnology and to develop entirely new applications.



Nanotechnology is usually defined as the manipulation of materials that range from the nanometer (nm) to the micrometer (um) scale. For comparison, the cells of living organisms are typically around 10 um across. That puts the machinery and components of living cells within the nanoscale size range, making them ideal for interactions with functional nanoparticles and nanomachines.

Nanoscale materials have unusual properties distinct from bulk materials. Some of these enhanced properties include surface area, cation exchange capacity, ion adsorption, and complexation. A high proportion of the atoms in a nanoparticle are present on its surface, meaning that compared to macro-scale materials, nanoparticles have different surface compositions, different reactivity, and different types of surface interaction sites.

In Biotechnology

The field of biotechnology is focused on basic research into the mechanisms of disease toward the development of new therapeutic and diagnostic devices. Bionanotechnology applications within biotechnology include the development of microfluidic devices for high throughput drug discovery assays, nanotechnology-based drug delivery devices, genome sequencing, proteomics, and imaging.

One example is the use of nanoparticles for drug delivery. A therapeutic is chemically attached to the nanoparticle. Radio or magnetic signals are then used to guide it to its target in the body. Precisely targeted drug delivery enhances efficacy and side effects due to off-target activity.

Gene delivery is another area of active research in bionanotechnology. Nanoparticle-based non-viral vectors of about 50 to 500 nm have been studied for delivery of plasmid-based DNA for gene therapy.

In Pharmaceuticals

Nanotechnology is also being developed for use in the pharmaceutical industry to enhance pharmacokinetics, biopharmaceutical properties of drugs, and drug delivery. Nanomaterials can alter properties of the drug or other components of a drug formulation, and overcome difficulties in absorption of the drug or how it functions in the body.

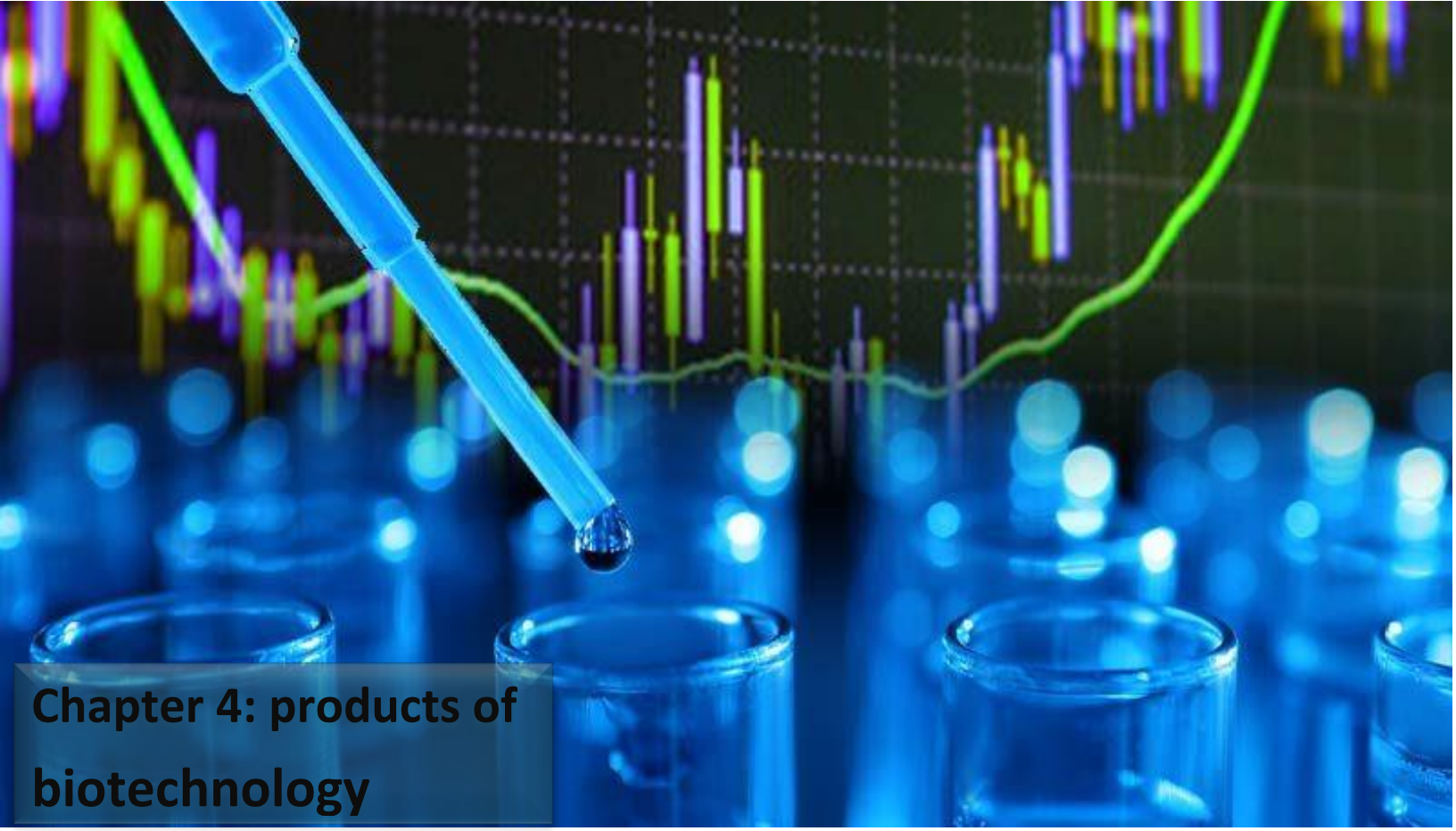
In Medicine

Nanotechnology has applications in clinical medicine and diagnostics. As an example, nanotechnology-based diagnostic devices can be used to accurately test blood for disease markers.

Nanorobotics is a developing field wherein machines are engineered from nanoscale components. In the field of nanomedicine, nanorobots have been used to carry out some interesting operations. Harvard and MIT scientists attached RNA strands to nanoparticles loaded with a chemotherapy drug, creating nanomachine that could target and kill cancer cells. In another example, nanorobots were used to work with white blood cells to repair tissue.

In Agriculture

Nanotechnology is being developed in agriculture to overcome the limitations of conventional farming. For example, nanotechnologies have the potential to increase the use of soil nutrients by plants. Nanofabricated materials containing plant nutrients in aqueous suspension and hydrogels are being studied for use in growing crops. Zerovalent iron nanoparticles or nanoparticles from iron rust could be used to remediate soils contaminated with pesticides, heavy metals, and radionuclides. Nanotechnology is also being used for genetic modification of plants through delivery of genes and drug molecules at the cellular level.



Chapter 4: products of biotechnology

Section: 4.1 Antibiotics

Antibiotics are a group of medicines that are used to treat infections by destroying or slow down the growth of bacteria. Antibiotics are called antibacterial or antimicrobial.

Antibiotics are taken as liquids, tablets, or capsules, or by injection. Antibiotics can be in the form of creams, ointments, or lotions to apply to the skin to treat some types of skin infections.

Antibiotics only work in cases of infections caused by bacteria and some parasites. They are a type of germs that need to live on or in another organism. Antibiotics do not work against infections caused by viruses, fungi, and fungal infections. skin. Sometimes the bacteria transform into other forms, so antibiotics will be needed in this case.

There are many antibiotics available and they come by different names. Antibiotics are usually grouped together based on how they work. Each type of antibiotic only works against certain types of bacteria or parasites. This is the reason why different antibiotics are used to treat different types of infections.

The main types of antibiotics include:

- Penicillin - for example, phenoxymethylpenicillin, flucloxacillin and amoxicillin.
- Cephalosporins - for example, cefaclor, cefadroxil, and cephalexin.
- Tetracyclines - for example, tetracycline, doxycycline, and lymecycline.
- Aminoglycosides, gentamicin, and tobramycin.
- Macrolides - for example erythromycin, azithromycin, and clarithromycin.
- Clindamycin.
- Sulfonamides and trimethoprim, co-trimoxazole.
- Metronidazole and Tinidazole.
- Quinolones - for example, ciprofloxacin,
- levofloxacin, and norfloxacin.
- Nitrofurantoin - used for urinary tract infections.

There are other types of antibiotics that are used for uncommon infections.

We knew about types of antibiotics, but how does

they work?

Some antibiotics work by killing germs (bacteria or parasites). This is often done by interfering with the cell wall structure of a bacterium or parasite. Some of them work by preventing the bacteria or parasite from reproducing. Antibiotics are usually only prescribed for more serious bacterial infections. Most common infections are caused by viruses when an antibiotic is not helpful. Even if you have a mild bacterial infection, most bacterial infections can be eliminated by your immune system.

For example, antibiotics do little to speed up healing of most ear, nose, and throat infections caused by bacteria. However, you may need antibiotics if you have a certain serious infection caused by bacteria, such as meningitis or pneumonia. In these cases, antibiotics are often lifesaving. Antibiotics may also be prescribed to treat a less serious condition. For acne, antibiotics can be taken orally or applied directly to the skin. The choice of antibiotic depends mainly on the type of

infection you have and the germs (bacteria or parasites) that your doctor thinks are causing the infection. This is because each antibiotic is only effective against certain bacteria and parasites. For example, if you have pneumonia, your doctor knows which types of bacteria usually cause most cases of pneumonia. But what are the side effects of antibiotics and when will these side effects be harmful? As with all medicines, there are several side effects that have been reported with each of the different antibiotics. Most antibiotic side effects are not dangerous. Common side effects include soft stools, diarrhea, or a mild stomach upset such as feeling sick (nausea). Less commonly, some people have had an allergic reaction to an antibiotic, and some have died from a severe allergic reaction, which is very rare. Antibiotics can kill the natural defense bacteria that live in the intestine and vagina. This may then allow thrush or other bad bacteria to grow.

You should tell your doctor if you have any of the following side effects:

Severe watery diarrhea and abdominal cramps: signs of a serious bacterial infection in the intestine (Clostridium difficile infection). Shortness of breath, hives, rash, swelling (of the lips, face, or tongue), fainting: signs of an allergic reaction. Vaginal itching or discharge: signs of vaginal thrush. White spots on the tongue: signs of oral thrush. Being sick (vomiting). Some antibiotics may interact with other medications you may be taking. This may cause reactions or reduce the effectiveness of one or the other treatments. Therefore, when you are prescribed an antibiotic, you should tell the doctor if you are taking other medicines.

Section: 4.2 Recombinant proteins

Recombinant proteins are a new combination of genes that make up DNA. Recombinant DNA technology allows wild type, human and mammalian proteins to be produced in large quantities. Recombinant proteins are made from cloned DNA sequences that normally encode an enzyme or protein with a known function. Recombinant protein is made through genetic engineering, also called gene linkage or recombinant DNA technology. By placing human, animal, or plant genes into the genetic material of bacteria, mammals, or yeast cells, these microorganisms can be used as factories or producers to make proteins for medical, academic and research uses. A vector is simply a tool for manipulating

DNA and can be considered a (transport vehicle) for producing proteins from specific DNA sequences that are cloned into them.

Protein purification and expression can sometimes be very complex and time consuming, so an additional marker is used in addition to the specific DNA sequence that will facilitate purification and removal of the recombinant protein. Recombinant proteins are proteins whose DNA has been artificially formed. DNA from two or more sources fuses into a single recombinant molecule. The DNA is first treated with a nuclease restricted enzyme in which the ends of the pieces contain a dangling piece of ingle stranded DNA. These are called (adhesive ends) because they in pair with any DNA molecule containing the complementary sticky end. DNA ligase covalently binds the two strands into a single recombinant DNA molecule. The recombinant DNA molecule must be repeated multiple times to provide material for analysis and sequencing. The production of many identical copies of the same recombinant DNA molecule is called cloning. Cloning takes place in the laboratory, through a process called polymerase chain reaction (PCR) as discussed in chapter 2.1. In vivo cloning can take place in single-celled microbes such as Escherichia coli, single-celled eukaryotes such as yeast and in mammalian cells that grow in tissue culture. The cell must take the recombinant DNA in a form in which it can be replicated and expressed. This is accomplished by incorporating DNA into a vector. Several viruses (both bacterial and mammalian cells) can act as vectors. Recombinant DNA is also sometimes referred to as a delusion. When two or more different strands of DNA are combined, there are three different ways in which recombinant DNA is made. Firstly, Transformation, secondly, Phage infection thirdly, Transformation of yeast, plants, and mammals. When using the transformation method, one needs to select a piece of DNA to be inserted into a vector, cut a piece of DNA with a restriction enzyme, and bind the DNA insert into the vector using the DNA Ligase. The appendix contains an optional marker that allows identification of recombinant molecules. The antibiotic marker is used to cause the death of a host cell that does not contain the vector when exposed to a specific antibiotic. Transformation information is the vector entry into the host cells. The host cells are ready to take in the foreign DNA. Identifiable markers are used for antibiotic resistance, color changes, or any other characteristic that can distinguish transformed hosts from non-transformed hosts. The transformation of yeast, plants

and mammals is done by precise injection of DNA into the nucleus of the cell being transformed. The phage

transfection process is equivalent to the transformation except for the fact that lambda phage or MI3 is used in place of bacteria. These bacteriophages produce plaques containing

recombinant proteins that are easily distinguished from nonrecombinant proteins through various selection methods. The host produces large amounts of the recombinant protein only when expression genes are added. Protein expression depends on the genes that surround the DNA of interest, and this set of genes act as signals that provide instructions for transcribing and translating the DNA of interest to the cell.

These signals include:

the promoter, ribosome binding site, and terminator. Recombinant DNA is inserted into the expression vectors containing the promoter, ribosome binding site and terminator.

In prokaryotic systems, the promoter, ribosome binding site and terminator must be from the same host since bacteria are unlikely to understand the signals of human and terminator stimuli. The gene must not contain human introns because the bacteria do not recognize it and this leads to premature termination, the recombinant protein may not be processed properly, folded properly, or it may degrade. The peptide sequence can be added as an extension in the N-terminal. Researchers can choose the specific purification system they wish to use. The available unique vectors have many of the features needed to produce large amounts of a target protein. The peptide sequence is usually placed in the vector such that it is designed to be the point of attack for a specific protease. Thus, after the recombinant protein is expressed and extracted from bacteria, a specific peptide

extension can be used to purify the protein and subsequently remove it from the target protein to generate an almost normal sequence on the final product. 6 or more of the consistent histidine residues serve as a mineral binding site for the purification and expression of the recombinant protein. The hexa-His sequence is called the His-Tag sequence that can be placed on the N-terminal of a target

protein using vectors from various commercial molecular biology companies. The His-Tag contains the cleavage site for a specific protease. The His-Tag recombinant proteins are purified by metal affinity chromatography such as nickel ion

columns that are used as the heavy metal ion, and the His-Tag protein is extracted from the metal chelate column with histidine or imidazole. Then the purified His-Tag protein is treated with the specific protease for cleavage of His-Tag tag or not if the tag does not affect the active site of the protein.

Proteins contain mineral binding sites that can be used to purify recombinant and natural proteins. This type of purification is rather simple when using a gel bead that is covalently modified so that it exposes a chelating group to bind to a heavy metal ion such as Ni2 + or Zn2 +. The chelating set on the gel bead contains a small amount of bonds needed to stabilize the metal ion. So, when the protein metallic binding site finds the heavy metal, it will combine by providing the bonds from its metallic binding site to contact with the metallic ion displayed on the chelating site of the gel granule. This purification method is completely identical to affinity chromatography when purifying the class of metal-bound proteins.

Section: 4.3 Hybridoma and Mab

Hybridomas

Hybridomas are cells that form through the fusion between a short-lived antibody-producing B cell and an immortal myeloma cell. Each hybridoma essentially expresses a large amount of a single specific mAb, and the cell lines of the preferred hybridoma can be cryopreserved to produce long acting mAb. As a result, researchers typically prefer generating hybridomas over other mAb production methods to maintain an adequate, never-ending supply of important mAb. Hybridoma generation is a five-step process that takes advantage of the host animal's natural ability to generate high-resolution, high affinity functional mAbs. In short, the first stage involves development and improvement of immunogenic antigen (Ag). Next, a host animal is immunized with Ag to elicit an immune response and initiate the B-cell maturation process. The third stage involves the isolation of these B cells from the spleen of the host animal and their fusion with

the myeloma cells to form a hybridoma. During the fourth stage, the generative hybridomas undergo multiple rounds of screening and selection to determine which hybridomas produce the best mAbs for their intended application. The fifth and final stage is amplification of these specific hybrids and subsequent mAb purification.

MAb treatments

Compared to other biosimilars, mAbs can maintain a very high affinity towards their target. Given this high affinity and specificity, researchers have begun to investigate the therapeutic potential of mAbs as metabolic stimulants, inhibitors, and immunomodulators. While the first few of the FDA-approved mAb therapies, such as muromonab-CD3,

were only produced in mice, it has become evident that to avoid immune rejection, future mAb-based therapies must undergo humanization. Since muromonab-CD3 was approved in 1986, the FDA has approved nearly 80 additional mAb treatments for diseases ranging from autoimmune disorders

to inflammatory diseases, HIV, and cancer. Interestingly, although harmonic display libraries were discovered in 1984 as an alternative mAb discovery platform, the majority of mAb therapies were originally discovered using hybridoma technique in human or rat compatible mice. The reason for this preference is likely attributable to the natural ability of the mouse immune system to generate highly specific mAbs that lead to strong stable field functions with limited post humanized immune activity.

Section: 4.4 Vaccines

A vaccine is a biological preparation that provides active acquired immunity to a specific disease. The vaccine typically contains a disease-like microorganism medium and is often made from weakened or killed forms of the bacterium, its toxins, or one of its surface's proteins.

Giving vaccines is called a vaccination. Vaccination is the most effective way to prevent infectious diseases. The World Health Organization (WHO) reports that licensed

vaccines are currently available for twenty-five different types of preventable infections. The terms vaccine and vaccination are derived from Variole (cowpox) vaccine, a term coined by Edward Jenner (who developed the concept of vaccines and created the first vaccine) to refer to cowpox. He used the phrase in 1798 to get the long title of his book Investigating the oriole Vaccine Known as Cowpox, in which he escribed the protective effect of cowpox against smallpox. In 1881, to honor Jenner, Louis Pasteur suggested that the terms be extended to include new preventive vaccinations that are then developed. Vaccinations given to children, adolescents, or adults are generally safe, and harmful effects, if any, are generally mild, and the rate of side effects depend on the vaccine in question. Some common side effects include fever, pain around the injection site, and muscle aches.

Additionally, some individuals may be allergic to the components of the vaccine. The MMR vaccine is rarely associated with febrile seizures. Severe side effects are extremely rare. Varicella vaccine is rarely associated with complications in immunocompromised individuals, and rotavirus vaccines are moderately associated with intussusception.

There are several different types of vaccines. Each type is designed to teach your immune system how to fight specific types of germs and the dangerous diseases they cause. When scientists create vaccines, they consider:

How does your immune system respond to the germ? Who needs vaccination against germs? The best technique for making a vaccine Based on a number of these factors, scientists decide what kind of vaccine to manufacture?

There are 4 main types of vaccines:

(Live attenuated vaccines - inactivated vaccines - for subunit, recombinant vaccines, polysaccharide, and conjugate vaccines - toxoid vaccines)

Live attenuated vaccines:

Live vaccines use a weak form of the germs that cause disease. Since these vaccines are very similar to the natural infection they help prevent, they create a strong and long-lasting immune response. Just one or two doses of most live vaccines can give you life-long protection against the germs and the disease they

cause. But there are also some restrictions on live vaccines, because they contain a small amount of live attenuated virus, some people must speak to a health care provider before receiving them, such as people with weak immune systems, long-term health problems, or people who You have undergone an organ transplant. They must remain calm, so that they do not travel well. This means that they cannot be used in countries with limited access to refrigerators.

Live vaccines are used to protect against:

(Measles, mumps, rubella, and rotavirus).

Inactivated vaccines:

Inactivated vaccines use the killed version of the pathogen. Inactivated vaccines usually do not provide as strong immune protection as live vaccines. So, you may need booster shots over time to have continuous immunity against diseases.

Inactivated vaccines are used to protect against:

(Hepatitis A - influenza (injection only) - polio (injection only)- Rabies).

Subunit, recombinant, polysaccharide, and conjugate vaccines:

Subunit, recombinant, polysaccharide, and conjugate vaccines use specific pieces of germs - such as a protein, sugar, or capsid (envelope around germs).

Because these vaccines use only specific pieces of germs, they give a very strong immune response that targets the main parts of the germs. It can also be used on nearly everyone who needs it, including people with

weak immune systems and long-term health problems.

One limitation of these vaccines is that you may need booster shots to get ongoing protection against disease.

These vaccines are used to protect against:

(Hib (Haemophiles influenzae type b) disease – hepatitis B-HPV (human papillomavirus) – whooping cough (part of the combined DTaP vaccine) - pneumococcal disease – pneumococcal disease Herpes zoster).

Toxin vaccines:

Toxoid vaccines use a toxin produced by disease-causing germs. They create immunity to the parts of the germ that cause disease rather than the germs themselves. This means that the immune response targets the toxin rather than the entire bacterium. Like some other types of vaccines, you may need booster shots to get ongoing protection against disease.

Toxoid vaccines are used to protect against:

(Diphtheria - tetanus).

Section: 4.5 Stem cell therapy

Stem cell therapy is the use of stem cells to treat or prevent disease. As of 2016, the only treatment that uses stem cells is a hematopoietic stem cell transplant, and this usually takes the form of a bone marrow transplant, but the cells can also be derived from umbilical cord blood. Stem cell therapy has become controversial following developments such as the ability of scientists to isolate and grow embryonic stem cells, to create stem cells using somatic cell nucleus transfer and their use of techniques to create induced pluripotent stem cells. This controversy is often linked to abortion and human cloning policies. Additionally, efforts to commercialize transplantation-based stored cord blood treatments have been controversial.

Stem cell expansion

For use in research or treatment applications, large numbers of eligible stem cells are needed. It is imperative to develop a culture system to produce pure groups of tissue-specific stem cells in the laboratory without losing stem cell potential. Two main approaches are taken for this purpose of two dimensional and three-dimensional cell culture. In 2D platforms, cells are usually exposed to a flat, solid surface on

the basal side and to liquid on the apical surface. The establishment of such a solid 2D substrate requires significant adaptation of the surviving cells as it lacks the unique extracellular matrix for each cell type, which may alter the cell's

metabolism and reduce its functions.3D cell culture systems may create a biomimetic stem cell microenvironment, like the original 3D extracellular matrix (ECM). Advanced biomaterials have contributed significantly to 3D cell culture systems in recent decades, and more complex and unique biomaterials have been proposed to improve stem cell proliferation and controlled differentiation. Among them, nanostructured biomaterials are of particular interest because they have the advantage of a high surface-to-volume ratio, and they also simulate the physical and biological features of normal stem cells at the nanoscale level.

Life applications on stem cell therapy:

Neurodegeneration

Research has been conducted on the effects of stem cells on animal models of brain degeneration, such as Parkinson's disease, amyotrophic lateral sclerosis and Alzheimer's disease, and preliminary studies related to multiple sclerosis have been conducted. Healthy adult brains contain neural stem cells, which divide to maintain general stem cell numbers, or become progenitor cells. In healthy adult laboratory animals, progenitor cells migrate within the brain and function primarily to maintain neuronal populations for smell.

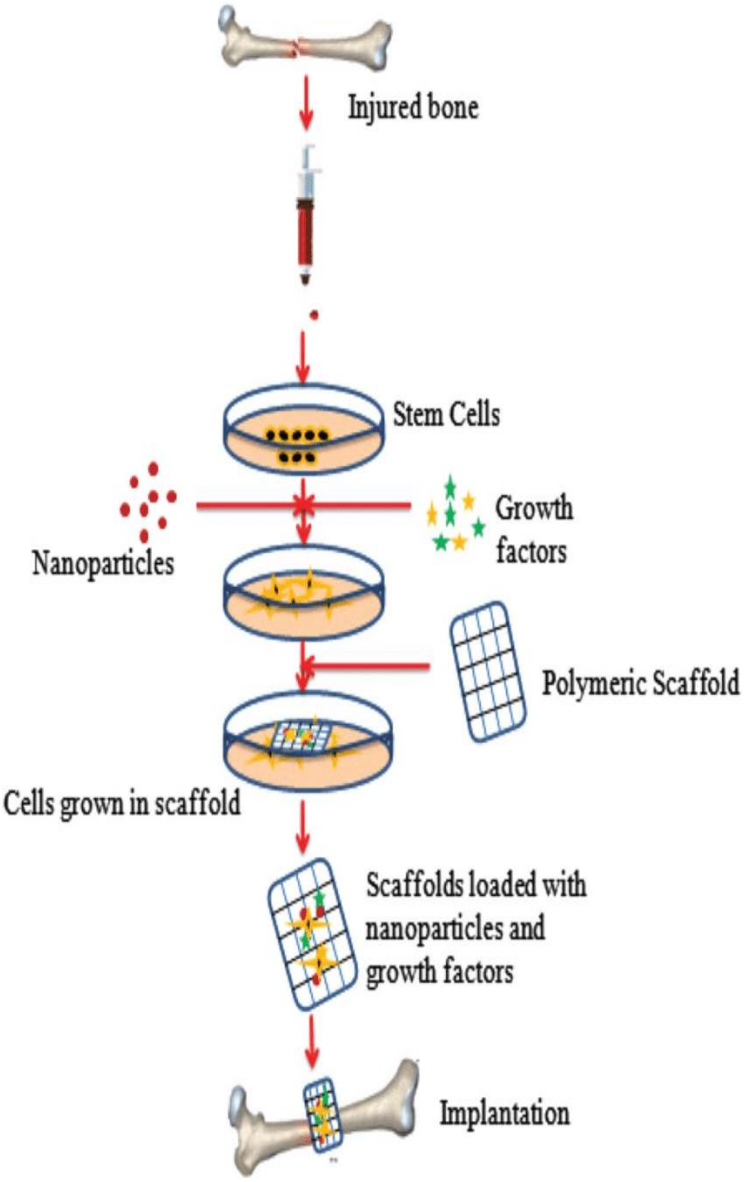
Pharmacological activation of autonomic neural stem cells has been reported to induce neuroprotection and behavioral recovery in adult rat models of neurological disorder. Injury to the brain and spinal cord Stroke and traumatic brain injury lead to cell death, which is characterized by the loss of neurons and oligodendrocytes within the brain.

Clinical and animal studies have been conducted on the use of stem cells in SCI cases.

Frailty syndrome

A small-scale study of individuals 60 years of age or older impaired aging, following intravenous therapy with MSCs from young, healthy donors, showed significant improvement in physical performance measures.

Section4.6;Tissue engineering



Tissue engineering has evolved out of the field of biomaterial development and refers to the assembly of scaffolds, cells, and bioactive molecules in functional tissues. The goal of tissue engineering is to bring together functional structures that restore, maintain, or improve damaged tissues or entire organs. The terms (tissue engineering) and (regenerative medicine) have become largely interchangeable, as the field hopes to focus on therapies rather than treatments for complex diseases. This field continues to evolve. In addition to medical applications, non-therapeutic applications include the use of tissues as biosensors to detect biological or chemical threat agents, and tissue chips that can be used to test the toxicity of an experimental drug. How does the two systems work? Cell clusters make and secrete their own supporting structures, which are called the extracellular matrix. This Matrix does more than just support cells, it also acts as a relay station for various signal particles. Thus, cells receive messages from many sources that become available from the local environment. Each signal can initiate a chain of responses that determine what happens to the cell. By understanding how individual cells respond to signals, interact with their environment, and organize in tissues and organisms, researchers have been able to manipulate these processes to repair damaged tissues or even create new tissues. The process often begins with building a scaffold from a wide range of potential sources, from proteins to plastics. Once scaffolds are established, cells with or without (a mixture) of growth factors can be introduced. If the environment is appropriate, the

fabric will evolve. In some cases, all cells, scaffolds, and growth factors are mixed at once, allowing the tissues to self-assemble. Another way to create a new fabric is using an existing scaffold. The donor organ cells are stripped, and the remaining collagen scaffold is used to grow new tissue.

This process has been used in the bioengineering of heart, liver, lung, and kidney tissues. This approach holds great promise for using scaffolds from human tissue that are thrown away during surgery and integrating them with the patient's own cells to make customized organs that the immune system will not reject. Currently, tissue engineering plays a relatively small role in treating patients. Complementary bladder, small arteries, skin grafts, cartilage, and even complete trachea has been implanted in patients, but these procedures are still experimental and very expensive. While more complex organic tissues such as heart, lung, and liver tissues have been successfully reproduced in the laboratory, they are still very far from being fully reproducible and ready for transplantation into a patient. However, these tissues can be very useful in research, and especially in drug development. Using human tissue to aid in screening of candidate drugs can speed up the development process, provide key tools to facilitate personalized medicine while saving money and reducing the number of animals used in research.

Chapter 5 Modelling Human Diseases

Section 5.1: Molecular Biology and Human Disease

Since the beginning of the Human Genome Project and subsequent developments in proteomics and metabolomics there has been a remarkable upsurge in deciphering the molecular basis of complex human diseases. New in vitro molecular diagnostic tests (nucleic acid probes, microarrays, etc.) are now quantitatively measuring response to therapy, and can monitor disease progress and redict recurrences. Central to the success of these diagnostic procedures is the correct identification of suitable human biomarkers.

Human biomarkers have long been utilized in medicine and have evolved over time from simple, single physiological (heart rate, blood pressure) or laboratory (cholesterol, white blood cells) parameters to highly complex imaging modalities or multimarkers in genome/proteome panels. Any measurement that can predict a person's disease state or response to a drug treatment can be called a biomarker. DNA-based biomarkers are already being incorporated into routine patient management. In many ways this molecular diagnostic approach supports a future market for

personalized medicine. Revenue from molecular biomarker-related products and services is expected to exceed US$2 billion by 2009.

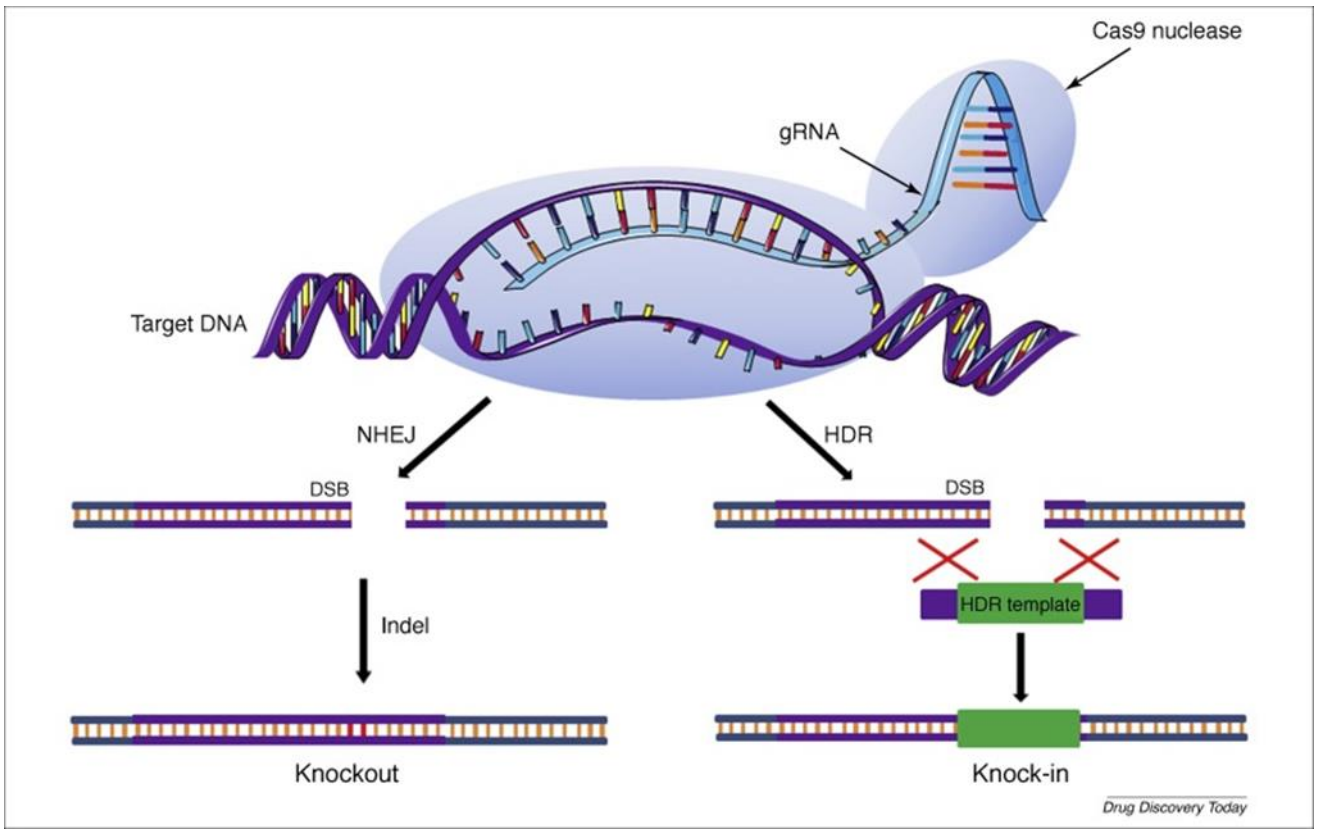
The overall field of medical diagnostics generated c. US$29 billion of which over 80% was related to identification of infectious diseases and the rest made up of assays related to genetic diseases, predictive testing, cancer and paternity testing. A vast range of such testing kits is now available over the counter. Over 500 companies now have molecular diagnostics as a part or all of their business. Molecular diagnostics have brought technical advances with improvements in sensitivity, speed and selectivity. While in vitro diagnostic tests dominate the medical market, in vivo systems are being developed. However, unlike in vitro systems, in vivo products will have to undergo extensive, time-consuming clinical trials to prove their safety in use.

Section 5.2: CRISPR Cas 9

What Is CRISPR Cas 9?

The functions of CRISPR (Clustered Regularly Interspaced Short Palindromic Repeats) and CRISPR-associated (Cas) genes are essential in adaptive immunity in select bacteria and archaea, enabling the organisms to respond to and eliminate invading genetic material. These repeats were initially discovered in the 1980s in E. coli, but their function wasn't confirmed until 2007 by Barrangou and colleagues, who demonstrated that S. thermophilus can acquire resistance against a bacteriophage by

integrating a genome fragment of an infectious virus into its CRISPR locus. Three types of CRISPR mechanisms have been identified, of which type II is the most studied. In this case, invading DNA from viruses or plasmids is cut into small fragments and incorporated into a CRISPR locus amidst a series of short repeats (around 20 bps). The loci are transcribed, and transcripts are then processed to generate small RNAs (crRNA – CRISPR RNA), which are used to guide effector endonucleases



that target invading DNA based on sequence complementarity.

How the CRISPR work?

- The CRISPR-Cas9 system consists of two key molecules that introduce a change (mutation?) into the DNA. These are:

- An enzyme called Cas9. This acts as a pair of 'molecular scissors' that can cut the two strands of DNA at a specific location in the genome so that bits of DNA can then be added or removed.

- A piece of RNA called guide RNA (gRNA). This consists of a small piece of pre-designed RNA sequence (about 20 bases long) located within a longer RNA scaffold.

The scaffold part binds to DNA and the pre-designed sequence ‘guides’ Cas9 to the right part of the genome. This makes sure that the Cas9 enzyme cuts at the right point in the genome.

- The guide RNA is designed to find and bind to a specific sequence in the DNA. The guide RNA has RNA bases that are complementary to those of the target DNA sequence in the genome. This means that, at least in theory, the guide RNA will only bind to the target sequence and no other regions of the genome.

- The Cas9 follows the guide RNA to the same location in the DNA sequence and makes a cut across both strands of the DNA.

- At this stage the cell recognizes that the DNA is damaged and tries to repair it.

- Scientists can use the DNA repair machinery to introduce changes to one or more genes? in the genome of a cell of interest.

How was it developed?

- Some bacteria have a similar, built-in, gene editing system to the CRISPR-Cas9 system that they use to respond to invading pathogens like viruses much like an immune system.

- Using CRISPR the bacteria snip out parts of the virus DNA and keep a bit of it behind to help them recognize and defend against the virus next time it attacks.

- Scientists adapted this system so that it could be used in other cells from animals, including mice and humans.

What are the applications and implications?

- CRISPR-Cas9 has a lot of potential as a tool for treating a range of medical conditions that have a genetic component, including cancer hepatitis B or even high cholesterol.

- Many of the proposed applications involve editing the genomes of somatic? (non-reproductive) cells but there has been a lot of interest in and debate about the potential to edit germline? (reproductive) cells.

- Because any changes made in germline cells will be passed on from generation to generation it has important ethical implications.

- Carrying out gene editing in germline cells is currently illegal in the UK and most other countries.

- By contrast, the use of CRISPR-Cas9 and other gene editing technologies in somatic cells is uncontroversial. Indeed, they have already been used to treat human disease on a small number of exceptional and/or life-threatening cases.

What's the future of CRISPR-Cas9?

- It is likely to be many years before CRISPR-Cas9 is used routinely in humans.

- Much research is still focusing on its use in animal models or isolated human cells, with the aim to eventually use the technology to routinely treat diseases in humans.

- There is a lot of work focusing on eliminating 'off-target' effects, where the CRISPR-Cas9 system cuts at a different gene to the one that was intended to be edited.

Better targeting of CRISPR-Cas9

- In most cases the guide RNA consists of a specific sequence of 20 bases. These are complementary to the target sequence in the gene to be edited. However, not all 20 bases need to match for the guide RNA to be able to bind.

- The problem with this is that a sequence with, for example, 19 of the 20 complementary bases may exist somewhere completely different in the genome. This means there is potential for the guide RNA to bind there instead of or as well as at the target sequence.

- The Cas9 enzyme will then cut at the wrong site and end up introducing a mutation in the wrong location. While this mutation may not matter at all to the individual, it could affect a crucial gene or another important part of the genome.

- Scientists are keen to find a way to ensure that the CRISPR-Cas9 binds and cuts accurately. Two ways this may be achieved are through:

1- The design of better, more specific guide RNAs using our knowledge of the DNA sequence of the genome and the 'off-target' behavior of different versions of the Cas9-gRNA complex.

2- The use of a Cas9 enzyme that will only cut a single strand of the target DNA rather than the double strand. This means that two Cas9 enzymes and two guide RNAs have to be in the same place for the cut to be made. This reduces the probability of the cut being made in the wrong place.

Section 5.3: Treat Diseases

▪ Blindness

What causes blindness?

The following eye diseases and conditions can cause blindness:

- Glaucoma refers to different eye conditions that can damage your optic nerve, which carries visual information from your eyes to your brain.

- Macular degeneration destroys the part of your eye that enables you to see details. It usually affects older adults.

- Cataracts cause cloudy vision. They’re more common in older people.

- A lazy eye can make it difficult to see details. It may lead to vision loss.

- Optic neuritis is inflammation that can cause temporary or permanent vision loss.

- Retinitis pigmentosa refers to damage of the retina. It leads to blindness only in rare cases.

- Tumors that affect the retina or optic nerve can also cause blindness.

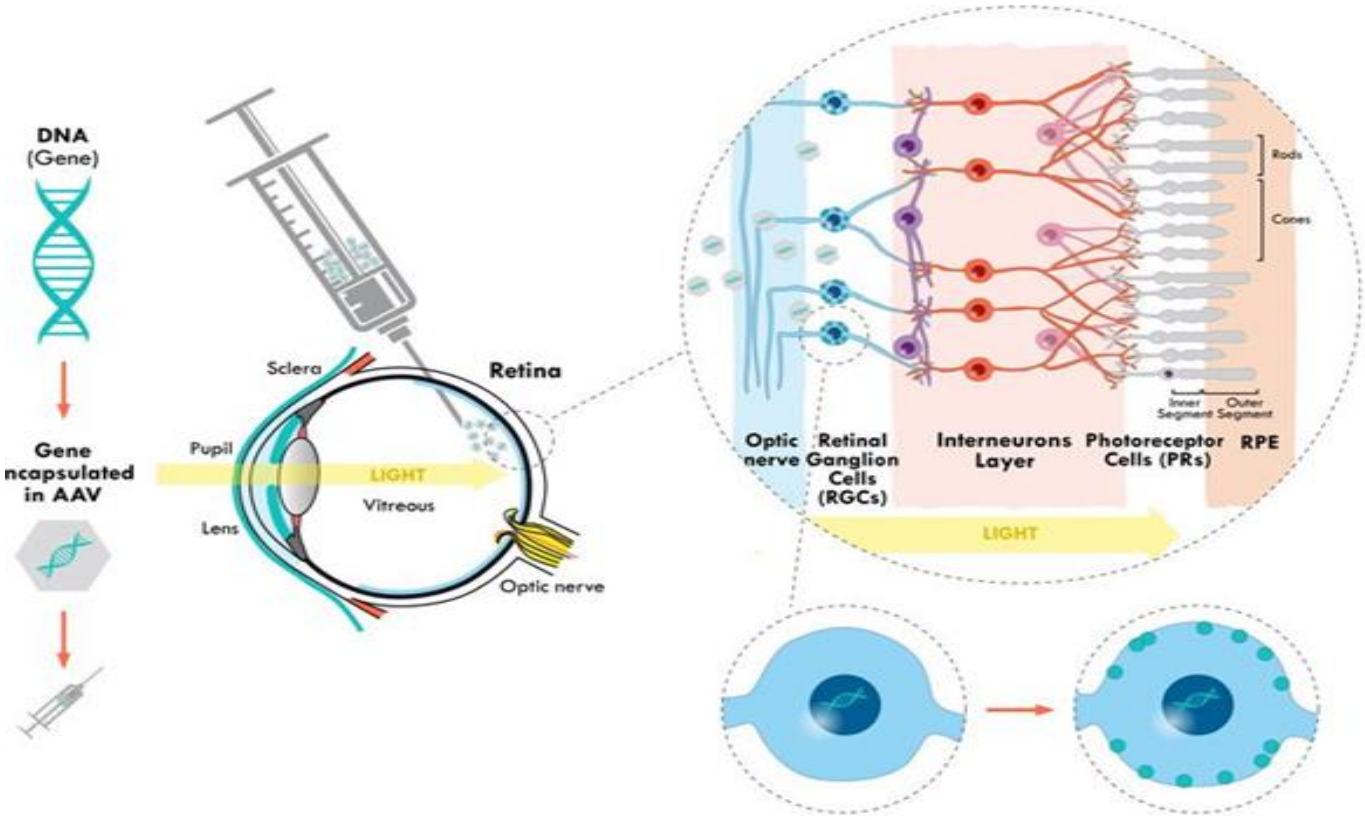
Gene Therapy Tools for Curing Blindness

Leber congenital amaurosis

Leber congenital amaurosis is significant example of an eye disorders that primarily affects the retina, which is the specialized tissue at the back of the eye that detects light and color. People with this disorder typically have severe visual impairment beginning in infancy. The visual impairment tends to be stable, although it may worsen very slowly over time. Leber congenital amaurosis is also associated with other vision problems, including increased sensitivity to light (photophobia), involuntary movements of the eyes (nystagmus), and extreme farsightedness (hyperopia). The pupils, which usually expand and contract in response to the amount of light entering the eye, do not react normally to light. Instead, they expand and contract more slowly than normal, or they may not respond to light at all. Additionally, the clear front covering of the eye (the cornea) may be cone-shaped and abnormally thin, a condition known as keratoconus.

Leber congenital amaurosis can result from mutations in at least 14 genes, all of which are necessary for normal vision. These genes play a variety of roles in the development and function of the retina. Mutations in any of the genes associated with Leber congenital amaurosis disrupt the development and function of the retina, resulting in early vision loss. Mutations in the CEP290, CRB1, GUCY2D, and RPE65 genes are the most common causes of the disorder, while mutations in the other genes generally account for a smaller percentage of cases. In about 30 percent of all people with Leber congenital amaurosis, the cause of the disorder is unknown.

Leber congenital amaurosis usually has an autosomal recessive pattern of inheritance. Autosomal recessive inheritance means both copies of the gene in each cell have mutations. The parents of an individual with an autosomal recessive condition each carry



one copy of the mutated gene, but they typically do not show signs and symptoms of the condition.

When Leber congenital amaurosis is caused by mutations in the CRX or IMPDH1 genes, the disorder has an autosomal dominant pattern of inheritance. Autosomal dominant inheritance means one copy of the altered gene in each cell is sufficient to cause the disorder. In most of these cases, an affected person inherits a gene mutation from one affected parent. Other cases result from new mutations and occur in people with no history of the disorder in their family.

Gene therapy contributions

Gene therapy involves inserting the correct copy of a gene into cells that have a mistake in the genetic sequence of that gene, recovering the normal function of the protein in the cell. The eye is an ideal organ for testing new therapeutic approaches, including CRISPR. That is because the eye is the most exposed part of our brain and thus is easily accessible.

In recent years, breakthrough gene therapy studies paved the way to the first-ever Food and Drug Administration-approved gene therapy drug, Luxturna TM, for a devastating childhood blindness disease, Leber congenital amaurosis Type 2.

This form of Leber congenital amaurosis is caused by mutations in a gene that codes for a protein called RPE65. The protein participates in chemical reactions that are needed to detect light. The mutations lessen or eliminate the function of RPE65, which leads to our inability to detect light – blindness.

The treatment method developed simultaneously by groups at the University of Pennsylvania and University College London and Moorefield's Eye Hospital involved inserting a healthy copy of the mutated gene directly into the space between the retina and the retinal pigmented epithelium, the tissue located behind the retina where the chemical reactions take place. This gene helped the retinal pigmented epithelium cell produce the missing protein that is dysfunctional in patients.

Other tools of gene therapy

- *CRISPER*

Lately, scientists have been developing a powerful new tool that is shifting biology and genetic engineering into the next phase. This breakthrough gene-editing technology, Gene therapy that involves CRISPR promises a permanent fix and a significantly reduced recovery period. A downside of the CRISPR approach is the possibility of an off-target effect in which another region of the cell's DNA is edited, which could cause undesirable side effects, such as cancer. However, new and improved strategies have made such a likelihood very low. which is called CRISPR, enables researchers to directly edit the genetic code of cells in the eye and correct the mutation causing the disease.

- *Delivery vehicles for genes*

Children suffering from the disease Leber congenital amaurosis Type 10 endure progressive vision loss beginning as early as one year old. This specific form of Leber

congenital amaurosis is caused by a change to the DNA that affects the ability of the gene – called CEP290 – to make the complete protein. The loss of the CEP290 protein affects the survival and function of our light-sensing cells, called photoreceptors.

One treatment strategy is to deliver the full form of the CEP290 gene using a virus as the delivery vehicle. But the CEP290 gene is too big to be cargo for viruses. So, another approach was needed. One strategy was to fix the mutation by using CRISPR

These studies led to the formulation of the first-ever in human CRISPR gene therapy clinical trial. This Phase 1 and Phase 2 trial will eventually assess the safety and efficacy of the CRISPR therapy in 18 Leber congenital amaurosis Type 10 patients. The patients receive a dose of the therapy while under anesthesia when the retina surgeon uses a scope, needle, and syringe to inject the CRISPR enzyme and nucleic acids into the back of the eye near the photoreceptors.

An ongoing project in the laboratory focuses on designing a gene therapy approach for the same gene CEP290. Contrary to the CRISPR approach, which can target only a specific mutation at one time, my team is developing an approach that would work for all CEP290 mutations in Leber congenital amaurosis Type 10. This approach involves using shorter yet functional forms of the CEP290 protein that can be delivered to the photoreceptors using the viruses approved for clinical use

- *Engineered gold particle*

scientists have developed a revolutionary technology, which enables mice and human retinas to detect infrared radiation. This ability could be useful for patients suffering from the loss of photoreceptors and sight. The researchers demonstrated this approach, inspired by the ability of snakes and bats to see heat, by endowing mice and postmortem human retinas with a protein that becomes active in response to heat. Infrared light is light emitted by warm objects that is beyond the visible spectrum. The heat warms a specially engineered gold particle that the researchers introduced into the retina. This

particle binds to the protein and helps it convert the heat signal into electrical signals that are then sent to the brain.

- **Cancer**

Throughout our lives, healthy cells in our bodies divide and replace themselves in a controlled fashion. Cancer starts when a cell is somehow altered so that it multiplies out of control. A tumor is a mass composed of a cluster of such abnormal cells. Most cancers form tumors, but not all tumors are cancerous. Benign, or noncancerous, tumors do not spread to other parts of the body, and do not create new tumors. Malignant, or cancerous, tumors crowd out healthy cells, interfere with body functions, and draw nutrients from body tissues. Cancers continue to grow and spread by direct extension or through a process called metastasis, whereby the malignant cells travel through the lymphatic or blood vessels -- eventually forming new tumors in other parts of the body.

The term "cancer" encompasses more than 100 diseases affecting nearly every part of the body, and all are potentially life-threatening. The major types of cancer are carcinoma, sarcoma, melanoma, lymphoma, and leukemia. Carcinomas -- the most commonly diagnosed cancers -- originate in the skin, lungs, breasts, pancreas, and other organs and glands. Lymphomas are cancers of lymphocytes. Leukemia is cancer of the blood. It does not usually form solid tumors. Sarcomas arise in bone, muscle, fat, blood vessels, cartilage, or other soft or connective tissues of the body. They are relatively uncommon. Melanomas are cancers that arise in the cells that make the pigment in skin.

Cancer has been recognized for thousands of years as a human ailment, yet only in the past century has medical science understood what cancer really is and how it progresses. Cancer specialists, called oncologists, have made remarkable advances in cancer diagnosis, prevention, and treatment. Today, more people diagnosed with cancer are living longer. However, some forms of the disease remain frustratingly

difficult to treat. Modern treatment can significantly improve quality of life and may extend survival.

Can CRISPR-Cas9 Be Utilized in Cancer Treatment?

The CRISPR-Cas9 system is being explored as a way to improve cancer treatment. In a keynote address delivered at the AACR Virtual Special Conference: Tumor Heterogeneity, immunologist, oncologist, and Fellow of the AACR Academy Carl June, MD, described the potential utility of CRISPR-Cas9 in adoptive cell therapy, a form of cancer therapy that uses a patient's own immune cells to destroy cancer cells.

In adoptive cell therapy, T cells (a type of immune cell found in blood) are extracted from the patient, engineered to express a T-cell receptor that will recognize the patient's cancer, multiplied, and reintroduced into the patient. Adoptive cell therapy has been successful for the treatment of many blood cancers in both adult and pediatric patients; however, several challenges remain to using this therapy widely, including cancer-mediated activation of immune checkpoints.

Immune checkpoints constitute a normal regulatory process that prevents overactivation of the immune response and subsequent damage to normal tissue. Immune checkpoints are activated by the binding of certain receptors (PD-1 or CTLA-4) on the surface of T cells to their ligands (PD-L1 or B7, respectively) on target cells.

Some cancer cells express high levels of the PD-L1 or B7 ligands even in the absence of immune activation, allowing cancer cells to activate immune checkpoints, turn off the T-cell response, and circumvent the immune system. This presents a problem for adoptive cell therapy: While the engineered T cells can recognize the patient's cancer, they are inactivated before they can mount an antitumor immune response.

One approach to addressing this challenge is to utilize CRISPR-Cas9 to delete the gene expressing the PD-1 receptor in the patient's T cells. Researchers hypothesize that the absence of the immune checkpoint would allow the T cells to remain active. Various studies have demonstrated that this strategy improves responses in preclinical models, but the feasibility of this approach in patients remains unclear.

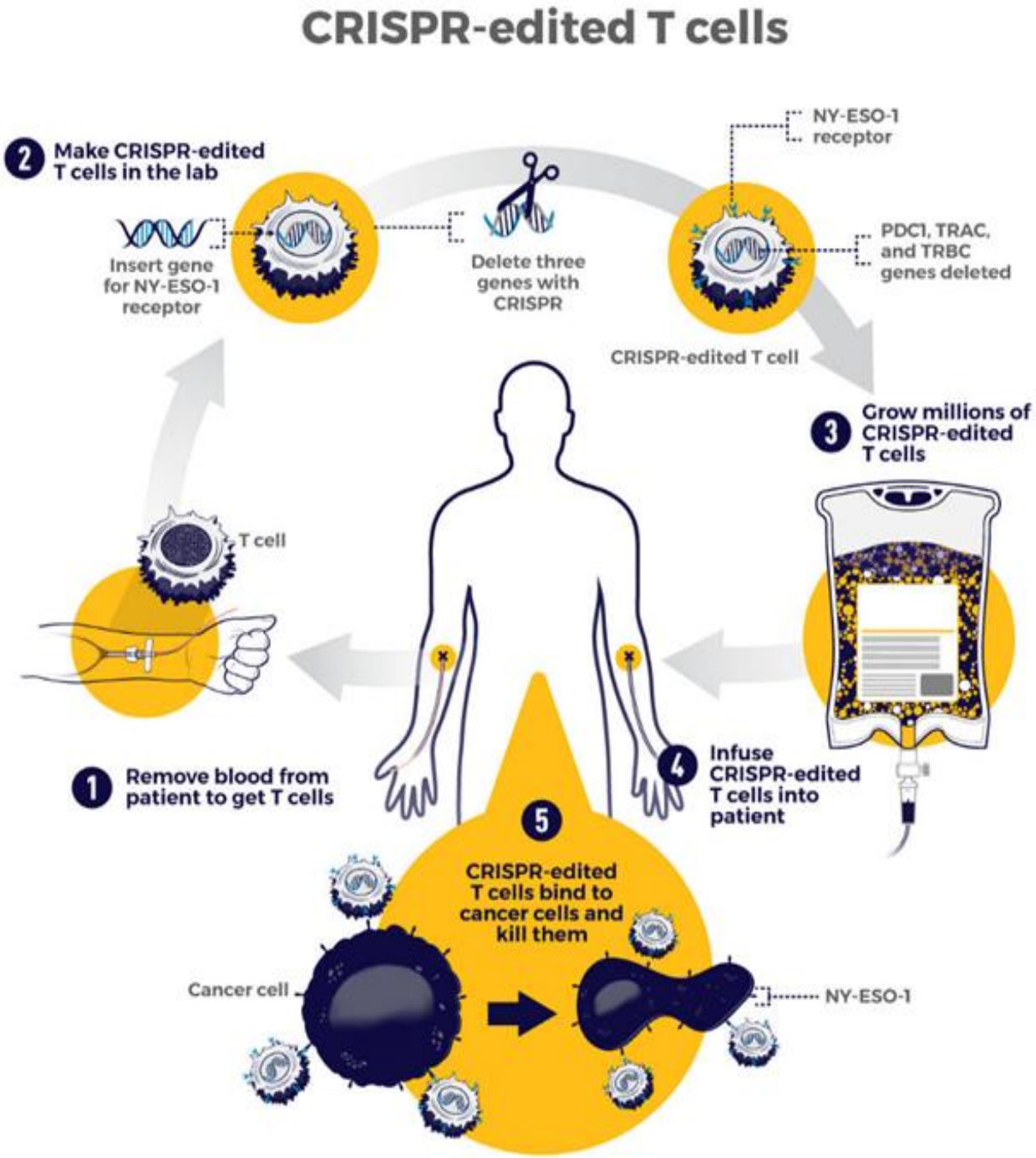
In his presentation, June discussed results from the first-in-human trial of CRISPR-Cas9-modified T-cell therapy, which were published earlier this year. In this study, June and colleagues used CRISPR-Cas9 to delete from T cells the gene expressing PD-1, as well as genes expressing endogenous T-cell receptors to prevent competition with the engineered T-cell receptor. The CRISPR-Cas9-modified T cells were administered to three patients with advanced cancer, including two patients with refractory advanced melanoma and one patient with refractory metastatic sarcoma. Stable levels of the T cells were detected in the blood of all three patients several months after infusion.

None of the patients experienced any serious adverse effects from the treatment, and there was no evidence of autoimmunity or cytokine release syndrome, a potentially lethal side effect that can be associated with adoptive cell therapy. The engineered cells were able to recognize and destroy cancer cells from all three patients, and the treatment led to stable disease in two of the patients. The third patient had a mixed response, which included a 50 percent reduction of one of their tumors but progression of other tumors.

Gene expression analysis of the infused cells in one patient showed that the engineered T cells had increased expression of genes associated with T-cell memory, but not T-cell exhaustion, suggesting that deletion of PD-1 may have prevented the activation of immune checkpoints.

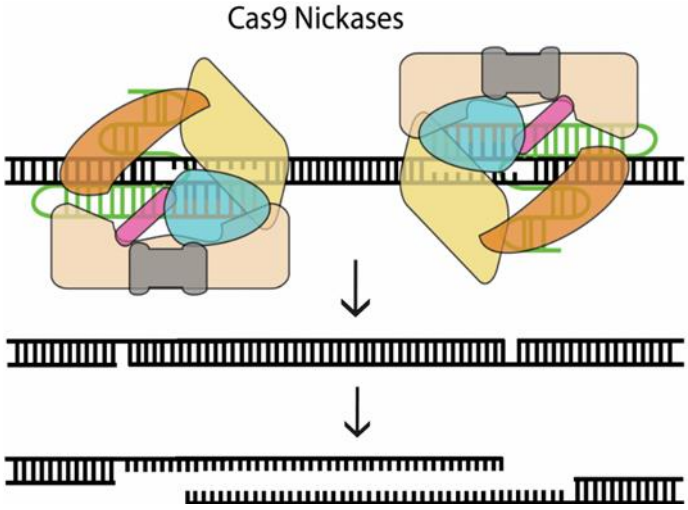
It was concluded that editing of T cells using CRISPR-Cas9 technology was safe and feasible for cancer treatment. Further research will be required to determine the

potential of this approach to prevent immune checkpoint activation and improve clinical responses.



Huntington's disease

Huntington's disease is an illness caused by a faulty gene in your DNA (the biological 'instructions' you inherit which tell your cells what to do). If you have Huntington's, it affects your body's nervous system – the network of nerve tissues in the brain and spinal cord that co-ordinate your body's activities.



Cas9 Nickases cut single-strand breaks into DNA. When used in pairs, this allows for more precise targeting of specific DNA sequences.

Huntington's can cause changes with movement, learning, thinking and emotions. Once symptoms begin, the disease gradually progresses, so living with it means having to adapt to change, taking one day at a time.

Living with Huntington's disease can be very challenging. Getting the right information and support is vital and we're here to help. Treating Huntington's could be tricky, as any off-target effects of CRISPR in the brain could have very dangerous consequences. To reduce the risk, scientists are looking at ways to tweak the gene editing tool to make it safer.

While previous technologies, such as TALEN and zinc-finger nucleases (ZFN), have made gene-editing possible, CRISPR/Cas9 has the advantage of acting faster and being easier to use than its predecessors. Now, the use of a Cas9 nickase pair provides a safe and efficient means of targeting the huntingtin gene in Huntington's patients.

Blood Disorders

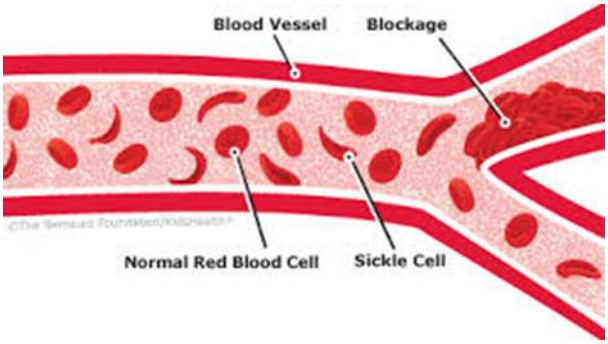
Blood disorders can affect any of the three main components of blood:

- Red blood cells, which carry oxygen to the body's tissues
- White blood cells, which fight infections
- Platelets, which help blood to clot
- Blood disorders can also affect the liquid portion of blood, called plasma.

Treatments and prognosis for blood diseases vary, depending on the blood condition and its severity.

Blood Disorders Affecting Red Blood Cells

Blood disorders that affect red blood cells include:



Anemia: People with anemia have a low number of red blood cells. Mild anemia often causes no symptoms. More severe anemia can cause fatigue, pale skin, and shortness of breath with exertion.

Iron-deficiency anemia: Iron is necessary for the body to make red blood cells. Low iron intake and loss of blood due to menstruation are the most common causes of iron-deficiency anemia. It may also be caused by blood loss from the GI tract because of ulcers or cancer. Treatment includes iron pills, or rarely, blood transfusion.

Anemia of chronic disease: People with chronic kidney disease or other chronic diseases tend to develop anemia. Anemia of chronic disease does not usually require treatment. Injections of a synthetic hormone, epoetin alfa (Epogen or Procrit), to stimulate the production of blood cells or blood transfusions may be necessary in some people with this form of anemia.

Pernicious anemia (B12 deficiency): A condition that prevents the body from absorbing enough B12 in the diet. This can be caused by a weakened stomach lining or an autoimmune condition. Besides anemia, nerve damage (neuropathy) can eventually result. High doses of B12 prevent long-term problems.

Aplastic anemia: In people with aplastic anemia, the bone marrow does not produce enough blood cells, including red blood cells. This can be caused by a host of conditions, including hepatitis, Epstein-Barr, or HIV -- to the side effect of a drug, to chemotherapy medications, to pregnancy. Medications, blood transfusions, and even a bone marrow transplant may be required to treat aplastic anemia.

Autoimmune hemolytic anemia: In people with this condition, an overactive immune system destroys the body's own red blood cells, causing anemia. Medicines that suppress the immune system, such as prednisone, may be required to stop the process.

Thalassemia: This is a genetic form of anemia that mostly affects people of Mediterranean heritage. Most people have no symptoms and require no treatment. Others may need regular blood transfusions to relieve anemia symptoms.

Sickle cell anemia: A genetic condition that affects mostly people whose families have come from Africa, South or Central America, the Caribbean islands, India, Saudi Arabia, and Mediterranean countries that include Turkey, Greece, and Italy. In sickle cell anemia, the red blood cells are sticky and stiff. They can block blood flow. Severe pain and organ damage can occur.

Polycythemia vera: The body produces too many blood cells, from an unknown cause. The excess red blood cells usually create no problems but may cause blood clots in some people.

Malaria: A mosquito's bite transmits a parasite into a person's blood, where it infects red blood cells. Periodically, the red blood cells rupture, causing fever, chills, and organ damage. This blood infection is most common in parts of Africa but can also be found

in other tropical and subtropical areas around the world; those traveling to affected areas should take preventive measures.

Blood Disorders Affecting White Blood Cells

Blood disorders that affect white blood cells include:

Lymphoma : A form of blood cancer that develops in the lymph system. In lymphoma, a white blood cell becomes malignant, multiplying and spreading abnormally. Hodgkin's lymphoma and non-Hodgkin's lymphoma are the two major groups of lymphoma. Treatment with chemotherapy and/or radiation can often extend life with lymphoma, and sometimes cure it.

Leukemia : A form of blood cancer in which a white blood cell becomes malignant and multiplies inside bone marrow. Leukemia may be acute (rapid and severe) or chronic (slowly progressing). Chemotherapy and/or stem cell transplantation (bone marrow transplant) can be used to treat leukemia, and may result in a cure.

Multiple myeloma: A blood cancer in which a white blood cell called a plasma cell becomes malignant. The plasma cells multiply and release damaging substances that eventually cause organ damage. Multiple myeloma has no cure, but stem cell transplant and/or chemotherapy can allow many people to live for years with the condition.

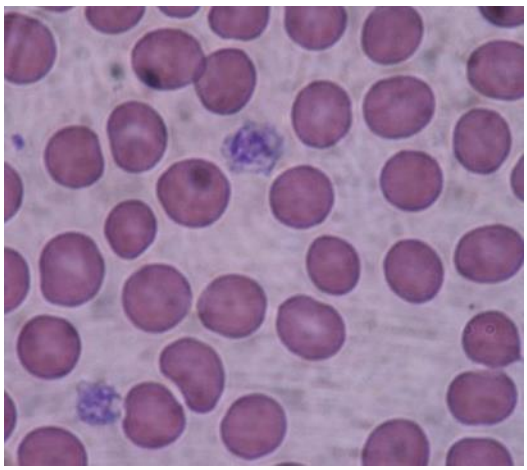
Myelodysplastic syndrome: A family of blood cancers that affect the bone marrow. Myelodysplastic syndrome often progresses very slowly, but may suddenly transform into a severe leukemia. Treatments may include blood transfusions, chemotherapy and stem cell transplant.

Blood Disorders Affecting Platelets

Blood disorders that affect the platelets include:

Thrombocytopenia: A low number of platelets in the blood; numerous conditions cause thrombocytopenia, but most do not result in abnormal bleeding.

Idiopathic thrombocytopenic purpura: A condition causing a persistently low number of platelets in the blood, due to an unknown cause; usually, there are no symptoms, yet abnormal bruising, small red spots on the skin (petechiae), or abnormal bleeding can result.



Two giant platelets (stained purple) are visible in this image from a light microscope (40×) from a peripheral blood smear surrounded by red blood cells. One normal platelet can be seen in the upper left side of the image (purple) and is significantly smaller in size than the red blood cells (stained pink).

Heparin -induced thrombocytopenia: A low platelet count caused by a reaction against heparin, a blood thinner given to many hospitalized people to prevent blood clots

Thrombotic thrombocytopenic purpura: A rare blood disorder causing small blood clots to form in blood vessels throughout the body; platelets are used up in the process, causing a low platelet count.

Essential thrombocytosis (primary thrombocythemia): The body produces too many platelets, due to an unknown cause; the platelets do not work properly, resulting in excessive clotting, bleeding, or both.

Blood Disorders Affecting Blood Plasma

Blood disorders that affect blood plasma include:

Hemophilia: A genetic deficiency of certain proteins that help blood to clot; there are multiple forms of hemophilia, ranging in severity from mild to life-threatening.

von Willebrand disease: von Willebrand factor is a protein in blood that helps blood to clot. In von Willebrand disease, the body either produces too little of the protein, or produces a protein that doesn't work well. The condition is inherited, but most people with von Willebrand disease have no symptoms and don't know they have it. Some people with von Willebrand disease will have excessive bleeding after an injury or during surgery.

Hypercoagulable state (hypercoagulable state): A tendency for the blood to clot too easily; most affected people have only a mild excess tendency to clot, and may never be diagnosed. Some people develop repeated episodes of blood clotting throughout life, requiring them to take a daily blood thinning medicine.

Deep venous thrombosis: A blood clot in a deep vein, usually in the leg; a deep venous thrombosis can dislodge and travel through the heart to the lungs, causing a pulmonary embolism.

Disseminated intravascular coagulation (DIC): A condition that causes tiny blood clots and areas of bleeding throughout the body simultaneously; severe infections, surgery, or complications of pregnancy are conditions that can lead to DIC.

Blood disorders can be cured by CRISPR Cas 9

The first CRISPR trial in Europe and the US, which enrolled its first patient in February this year, aims to treat beta-thalassemia and sickle cell disease, two blood disorders that affect oxygen transport in the blood. The therapy, developed by CRISPR Therapeutics and Vertex Pharmaceuticals, consists in harvesting bone marrow stem cells from the patient and using CRISPR technology to make them produce fetal hemoglobin, a natural form of the oxygen-carrying protein that binds oxygen much better than the adult form.

Before the trial started, the FDA put it on hold in the US to clear out some safety questions. A few months later, the hold was lifted and the treatment was given fast track designation for both conditions.

Hemophilia is another blood disorder that CRISPR technology could tackle. CRISPR Therapeutics is working with Casebia on an in vivo CRISPR therapy where the gene editing tool is delivered directly to the liver.

Treating Sickle Cell Disease with Genetic Editing Tools

As sickle cell disease is a well-known genetic disorder, it is considered a leading candidate for gene editing therapies. Studies published in 2016 described a successful

proof-of-concept in treating sickle cell disease in mice using the CRISPR-Cas9 gene editing tool.

CRISPR-Cas9 is a programmable RNA-guided DNA endonuclease, which has been gaining significant attention over the last decade due to its ability to treat genetic disorders such as sickle cell disease. Guided by a single RNA strand, the Cas9 nuclease—originally isolated from bacteria—can be programmed to cut a target DNA sequence and modified by inserting, deleting, or replacing it with a genetic sequence.

In the 2016 studies, blood-forming stem cells were removed from the bone marrow and edited to remove the disease-causing mutation. They were then re-introduced into the bone marrow in the hopes that "normal" hemoglobin might proliferate. Although only a small percentage (~5%) of transplanted, edited cells were found to produce normal, functioning hemoglobin, researchers and clinicians speculated that this might lie at the threshold of what would be required to alleviate patient distress.

Cystic Fibrosis

Cystic fibrosis is a hereditary disease that affects the lungs and digestive system. Cystic fibrosis is caused by mutations in the cystic fibrosis transmembrane conductance regulator (CFTR) gene that affect the production of the CFTR protein. This protein forms ion channels in cell membranes throughout the body that regulate the flow of ions and water molecules in and out of cells. When the CFTR protein is not made correctly its ability to transport ions is compromised, affecting the balance of salt ions and water inside and outside of the cell. This imbalance leads to thick, sticky mucus in the lungs, pancreas and other organs. The mucus coughed up from the respiratory tract is called sputum.

Mucus that doesn't clear is a breeding ground for bacteria and leads to these chronic lung infections in CF patients. It's also hard for therapeutic agents to penetrate the mucus and eradicate the bacteria.

The first step in understanding bacterial infections is to quantify what's going on. The NIH Common Fund Human Microbiome Project (HMP) was established in 2008 to characterize healthy human microbiota and investigate their role in disease. The first phase of the project characterized oral, gut and skin microbiomes, but didn't investigate the lungs. Culturing lung samples on a petri dish – the traditional way of identifying bacteria – didn't show anything, so scientists concluded healthy lungs were sterile. Subsequent research with more advanced sequencing techniques showed that in fact multiple bacteria species inhabit healthy lungs.

However, the differences between a healthy and unhealthy lung microbiome is 'a pretty controversial area right now', explains Ann Field, senior director of drug discovery at the Cystic Fibrosis Foundation, a US non-profit. Some studies identify lists of organisms that appear to be present in lungs where there isn't an infection, but other studies identified considerable overlap in the types and quantities of organisms in healthy and infected lungs. 'The jury is still out on whether there is a "healthy lung microbiome",' Field says.

Three images showing different kinds of bacteria

The presence of Pseudomonas, Staphylococcus and Achromobacter bacteria generally means reduced lung function in CF patients

The most common method of quantifying bacteria is with 16S ribosomal RNA (rRNA) gene sequencing, where PCR amplifies the 16S rRNA gene present in DNA collected from sputum samples. The 16S rRNA gene is present in all bacteria, and acts as a barcode for different species.

When they conducted 16S rRNA sequencing on sputum from 77 cystic fibrosis patients, researchers at Emory University and Georgia Institute of Technology, both in Atlanta, US, found the presence of Pseudomonas, Staphylococcus and Achromobacter in significant quantities correlates to reduced lung function in CF patients, while increased

microbiome diversity correlates to improved lung function. However, they also found exceptions to these trends.

There are some drawbacks to using DNA-based methods to quantify bacteria in the lungs. In CF patients a lot of host and bacterial DNA is present in samples, but the quantities of DNA may not represent the actual composition of bacteria present. 'Bacterial DNA accumulates at different rates with different stabilities; a lot of bugs in the airways make pretty good DNases, so they tend to degrade the DNA around them. Whereas with pathogens like Pseudomonas the DNA accumulates continually,' says Michael Surrette, a professor of microbiology at McMaster University in Ontario, Canada.

Surrette argues a more accurate way to quantify bacteria is to look for bacterial RNA. The drawback of RNA-based sequencing methods is although it gives a more accurate snapshot of species present, RNA isn't stable, and has half-life of minutes. Extracting RNA from sputum is also difficult. 'Sputum is the worst possible sample to do this with,' Surette says. His group has developed a protocol that breaks down the saliva matrices, stabilizes the RNA before amplification and sequencing, and takes less than five minutes to perform.

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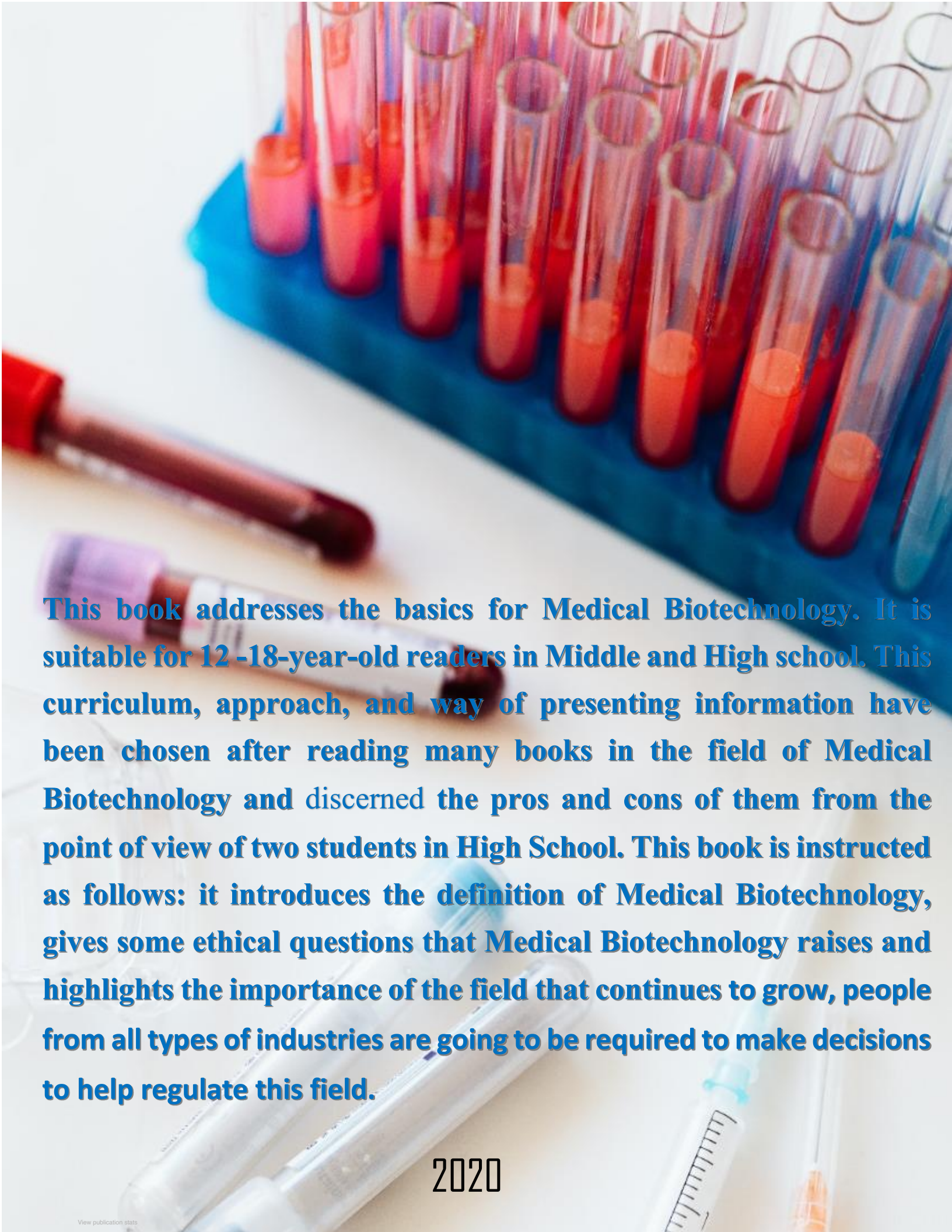
Conclusion

As the field of medical biotechnology grows with new products and discoveries, so does the need for a holistic view of biotechnology in medicine. Biotechnology in Medical Sciences fulfills that need by delivering a detailed overview of medical biotechnology as it relates to human diseases and epidemiology, bacteriology and antibiotics, virology and vaccines, immunology and monoclonal antibodies, recombinant DNA technology and therapeutic proteins, stem cell technology, tissue engineering, molecular diagnostics and forensic science, gene therapy, synthetic biology and nanomedicine, pharmacogenomics, bioethics, bio business and intellectual property rights, and career opportunities.



This book addresses the basics for Medical Biotechnology. It is suitable for 12 -18-year-old readers in Middle and High school. This curriculum, approach, and way of presenting information have been chosen after reading many books in the field of Medical Biotechnology and discerned the pros and cons of them from the point of view of two students in High School. This book is instructed as follows: it introduces the definition of Medical Biotechnology, gives some ethical questions that Medical Biotechnology raises and highlights the importance of the field that continues to grow, people from all types of industries are going to be required to make decisions to help regulate this field.

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