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A BRIEF REVIEW ON GENE THERAPY

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Abstract:
Now days gene theraphy has interested area for reasearch as a purpose of medical condition or disease. Currently, gene therapy studies a broad range of potential therapeutic interventions, including the body's immune reaction to tumors, new blood vessels in the heart to alleviate heart attacks and to stop HIV-replication in patients with AIDS (Coleman et al., 2003). There is also renewed emphasis on the gene therapy of genetic diseases, such as hemophilia A and B, and cystic fibrosis. Human gene therapy experimentation raises many issues. In this review article, background of gene therapy, introduction, genetic diseases, gene function, germ line gene therapy, hurdles in gene therapy, methods for gene therapy, ex vivo, in vitro and in vivo-gene therapy, risks associated with gene therapy, have been given.

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1. INTRODUCTION[1-2]

A gene is the basic physical and functional unit of heredity. Genes, who are made up of DNA or segment of DNA act as instruction for regulating and synthesis of protein. In humans, genes vary in size from a few hundred DNA bases to more than 2 million bases. The Human Genome Project has estimated that humans have between 20,000 and 25,000 genes.

Every person has two copies of each gene, one inherited from each parent. Most genes are the same in all people, but a small number of genes (less than 1 percent of the total) are slightly different between people. Forms of the same gene with small differences in their sequence of DNA bases OR found at the same place on the chromosomes are called as Allele. These small differences contribute to each person's unique physical features.

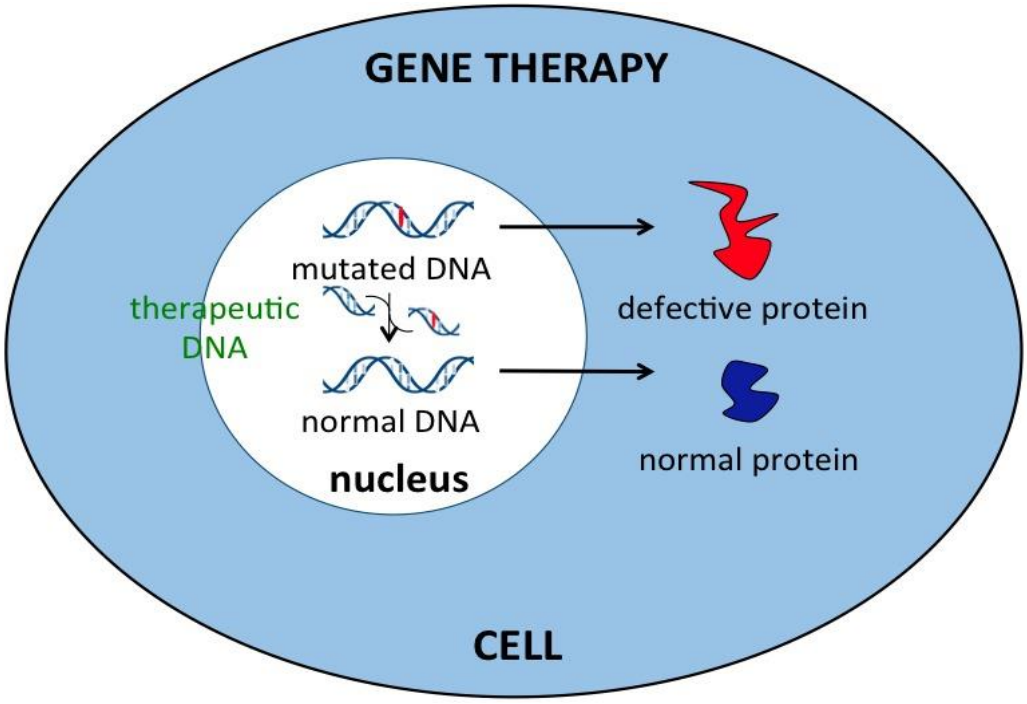

Figure 1:- Gene Therapy

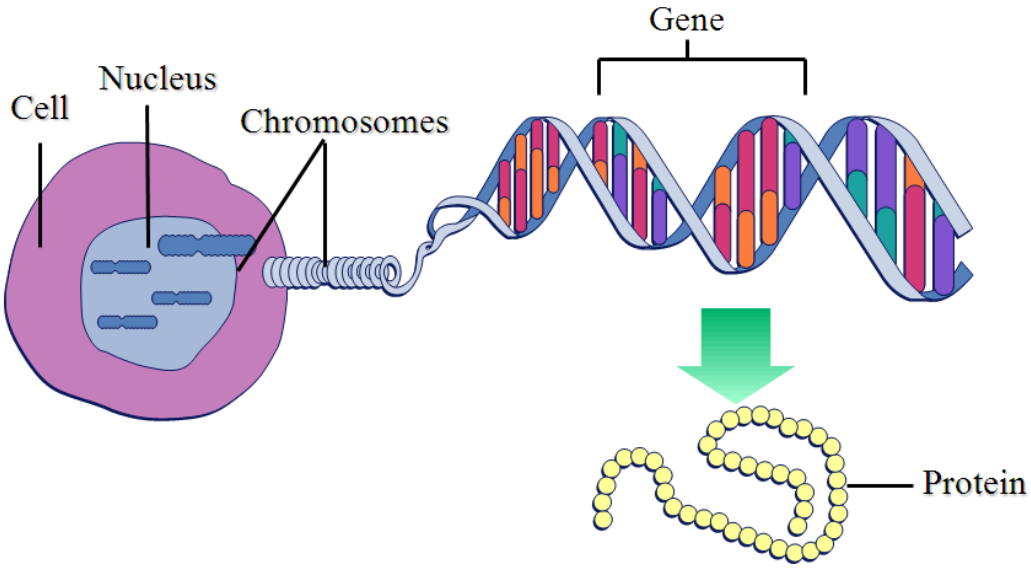

Figure 2:-Protein Synthesis

1.2 GENE THEARAPY:-

The theory behind gene therapy is to treat the disease by repairing the abnormal gene. This is achieved by replacing the disease causing faulty gene with a "normal" copy into an individual's cells. In other words it is an experimental technique for correcting defective gene that is responsible for disease development.

The most common form of gene therapy involves inserting a normal gene to replace an abnormal gene.

1.3 Advantages and Disadvantages:-

- **Advantages-**

1. It has the ability to replace defective cells.
2. It promises a great untapped potential.
3. It can help eradicate diseases.

- **Disadvantages-**

1. It can damage the gene pool
2. It would modify human capabilities.

a. This is one big drawback of gene therapy—it may be used to enhance and modify human capabilities, which means that the standards for normal human life would be altered.

b. By experimenting with the technology, certain countries could create enhanced and unstoppable armies.

3. It has the potential to give rise in other disorders.

There is an exact point in the host genome where the right genes should be brought in, while there is no assurance that the viral enzyme used in the process can bring in the right genes at such point. When things go wrong, it can lead to severe disorders.

1.4 Applications of Gene Therapy:-

(1) Gene therapy used in blood and vascular system.
(2) Gene therapy used in orthopedics.
(3) Gene therapy used in genitourinary system.
(4) Gene therapy used in other diseases,
-Parkinson's disease
-Cancers
-Blindness
-Hemophilia

2. History and Development of Gene Therapy[3]

2.1 Journey of Clinical Trials in Gene Therapy [1960 – 2017]

- **1960:-** The concept of gene therapy was introduced.
- **1970:-** Friedman and Robin cited the first attempt to perform gene therapy "gene therapy for human genetic diseases".
- **1990:- (1)** The first approved gene therapy case at the national institute of health U.K.it was performed on 4 year old girl. It was treated for genetic defect that left her with an immune system deficiency.
  (2) New gene therapy approach i.e. error in messenger RNA derived from the defective gene. This technique has the potential to treat the blood disorder Thalassaemia, cystic fibrosis, and some cancer.
  (3) Sickle cell disease is successfully treated in mice.
- **1992:-** Doctor Claudio work on university Milan Italy performed the first procedure of gene therapy using hematopoietic stem cells as vectors to deliver gene intended to correct hereditary disease.
- **1999:-** Death of Jesses Gelsinger in a gene therapy experiment result in setback to gene therapy research in United state.
- **2006:-** Scientist at the national institute of health have a successfully treated metastatic melanoma in two patient this study constituent that gene therapy can be effective in treating cancer.
- **2007-2011:-** Research is still ongoing and number of diseases that has been treated successfully by gene therapy includes Retinal disease, color blindness, Parkinson's diseases, Cancer
- **2011:-** Medical community accepted that it can cure HIV by using gene therapy.
- **2012:-** The FDA approved Phase 1 clinical trials on thalassemia major patients in the US for 10 participants in July.
- **2014**:- In January researchers reported that six choroideremia patients had been treated with adeno-associated virus.
  By 2016, 32 patients had been treated with positive results and researchers were hopeful the treatment would be long-lasting.
- **2015:-** In February, a gene therapy treatment undergoing clinical trials for treatment of beta thalassemia. In March researchers delivered a recombinant gene encoding a broadly neutralizing antibody into monkeys infected with simian HIV; the monkeys' cells produced the antibody, which cleared them of HIV. The technique is named immunoprophylaxis by gene transfer (IGT). Animal tests for antibodies to Ebola, malaria, influenza, and hepatitis etc.
- **2016:-** In April the Committee for Medicinal Products for Human Use of the European Medicines Agency endorsed a gene therapy treatment called strimvelis (that ex vivo

stem cell gene therapy to treat patients with a very rare disease ADA-SCID[SEVERE COMBINED IMMUNODEFICIENCY due to adenosin deaminase] caused by absence of an essential protein i.e. ADA.

This treats children born with ADA-SCID and who have no functioning immune system — sometimes called the "bubble baby" disease.

- **2017:-** In February Kite Pharma announced results from a clinical trial of CAR-T cells in around a hundred people with advanced Non-Hodgkin lymphoma.

2.2 Ethical and Social Consideration[4]

Gene therapy is a powerful new technology that might have unforeseen risks, scientists first develop a proposed experiments i.e. protocol, that incorporates strict guidelines. After the approval from FDA, the organization continues to monitor the experiment. In the course of a clinical trial, researchers are required to report any harmful side effects. Critics and proponents all agree that risks of gene therapy must not be substantially larger than the potential benefit. Gene therapy poses ethical considerations for people to consider.49 Some people are concerned about whether gene therapy is right and it may be used ethically. Some of the ethical considerations for gene therapy include:

- Deciding what is normal and what is a disability;
- Deciding whether disabilities are diseases and whether they should be cured;
- Deciding whether searching for a cure demeans the live of people who have disabilities;
- Deciding whether somatic gene therapy is more or less ethical than germ line gene therapy Initial experiments using gene therapy have been conducted primarily in patients for whom all other treatments have failed, so that the risks are small. Many people feel that because gene therapies use altered genes and potentially dangerous viruses, those treatments should be tested more extensively.

2.3 STEM CELL THERAPY [4]:-

Stem cell therapy or therapeutic cloning does not involve gene therapy itself. However, in the future it might be used in conjunction with gene therapy for regeneration of tissue and organs after they have been treated with corrective genes. Visually, stem cells are not distinguishable from any other cells of the human body. Under a common microscope (magnification 20 to 40 times), those cells can only be observed using special dyes. Visually there is no significant difference in such cells. The real differences exist at the DNA level, where gene expression is amendable to signals influencing protein expression. The cells can differentiate into any of the 220 cell types of the human body (e.g., kidneys, heart, liver, skin, or retina), a phenomenon called pluripotency. At birth, stem cells can be harvested from an individual's bone marrow, fat tissue, and the umbilical cord. Embryonic stem cells are harvested from embryos up to a few days after fertilization.

Another characteristic of stem cells is their capability to grow indefinitely. Whereas the remaining body cells have a biological programming that limits the number of cell divisions they can go through before dying, stem cells can be maintained indefinitely in a Petri dish with nutritive media. Stem cell therapy provides hope for a cure for patients of incurable afflictions such as Parkinson's disease and Alzheimer's disease, and also for people suffering from paralysis resulting from spinal cord injuries.

At first, some opponents speculated that stem cells would be used in nurseries to produce organs such as livers, hearts, and virtually any other body part. However, most organs possess complex structures with ducts and valves, making it impossible to produce them outside of the organism. Stem cells have opened a new avenue for disease treatment. For example, the injection of stem cells into the liver of a patient with cirrhosis or hepatitis could result in new tissue capable of performing its role. Stem cell therapy also has great potential to cure rheumatoid arthritis and some heart diseases. Recent research has found that spine-injured mice suffering from paralysis were able to move their legs following an injection of stem cells.

Some people believe that if human stem cells are as versatile as those of mice, they might be the long sought after fountain of youth. The combination of stem cells with gene therapy might allow rebuilding of new body parts to substitute for old and defective ones. Right now, different procedures are being tested for curing AD deficiency. Somatic cell gene therapies have the limitation of lasting for only a few months, which in turn requires repeated applications. With the use of stem cells to regenerate healthy bone marrow cells, a permanent cure is expected, as healthy cells have the capability to grow and divide continuously. Embryonic stem cells, from embryos about four days old, have been at the center of a heated debate due to ethical issues. The main disagreement is whether or not a four-day-old embryo is already a human life. When would an embryo or a fetus reach the status of life?

Life begins at conception (i.e., at the moment of the fertilization of the egg by the sperm). For many, the

destruction of embryos for the purpose of treating another human being is wrong. Recently, in the United States, the Bush administration broadened the definition of a child eligible for coverage under the Children's Health Insurance Program by classifying a developing fetus as an "unborn child." Many activists are arguing that the Bush administration's proposal demonstrates its commitment to the strategy of undermining a woman's right to choose abortion by ascribing legal rights to embryos.

3 Types & Basic process of Gene Therapy[4-7]

3.1 **Somatic Gene Therapy**

- Ex vivo gene therapy
- In vivo gene therapy

3.2 **Germ Line Gene Therapy**

3.1 Somatic Gene Therapy

Somatic cells are non reproductive. Somatic cell therapy is viewed as a more conservative, safer approach because it affects only the targeted cells in the patient, and is not passed on to future generations. In other words, the therapeutic effect ends with the individual who receives the therapy. However, this type of therapy presents unique problems of its own. Often the effects of somatic cell therapy are short-lived. Because the cells of most tissues ultimately die and are replaced by new cells, repeated treatments over the course of the individual's life span are required to maintain the therapeutic effect.

Transporting the gene to the target cells or tissue is also problematic. Regardless of these difficulties, however, somatic cell gene therapy is appropriate and acceptable for many disorders, including cystic fibrosis, muscular dystrophy, cancer, and certain infectious diseases.

3.1.1 Ex vivo somatic gene therapy

Which means exterior (where cells are modified outside the body and then transplanted back in again)? In some gene therapy clinical trials, cells from the patient's blood or bone marrow are removed and grown in the laboratory. The cells are exposed to the virus that is carrying the desired gene. The virus enters the cells and inserts the desired gene into the cells. The cells grow in the laboratory and are then returned to the patient by injection into a vein. This type of gene therapy is called ex vivo because the cells are treated outside the body.

3.1.2 In-vivo somatic Gene Therapy

Which means interior (where genes are changed in cells still in the body).This form of gene therapy is called in vivo, because the gene is transferred to cells inside the patient's body. I.e. the vector is administered directly in to the blood stream which may spread to entire body.

3.2 GERM-LINE CELL THERAPY:-

The main advantages of germ-line cell gene therapy are the following:

1. It offers the possibility for a true cure of several diseases and it is not only a temporary solution.
2. It might be the only way to treat some genetic diseases.
3. The benefits would be extended for several generations, because genetic defects are eliminated in the individual's genome and, consequently, the benefits would be passed to his or her offspring.

Some of the arguments presented against germ-line cell gene therapy are the following: 1. it involves many steps that are poorly understood, and the long-term results cannot be estimated.

2. It would open the door for genetic modifications in human traits with profound social and ethical implications.
3. It is very expensive and it would not benefit the common citizen.
4. The extension of the cure to a person's offspring would be possible only if the defective gene was directly modified, but probably not if a new gene was added to another part of the genome.

Table 1:- Difference between in vivo and ex vivo Gene Delivery Systems

In vivo	Ex vivo
Less invasive	More invasive
Technically simple	Technically complex
Vectors introduced directly	No vectors introduced directly
Safety check not possible	Safety check possible
Decreased control over target cells	Close control possible

Table 2:- Difference between Somatic GT and Germ line GT

Somatic gene therapy	Germ line gene therapy
Not Result in permanent change.	Result in permanent change.
Short lived.	Long lived.
Therapeutic gene transfer in to somatic cell. E.g. introduction of gene into bone marrow, blood cell, skin cell.	Therapeutic gene transfer in to germ line cell. e.g. introduction of gene into sperm and egg
Not Inherited to later generation.	Inherited later generation.

3.3 Basic process of Gene Therapy

In general a gene cannot be inserted directly in to the person's cell.it must be inserted using a carrier or Vector. Vector system can be divided in to-

I. **Viral vector**
II. **Non viral vector**

3.3.1 Viral Vectors:-

Both Somatic and Germ line gene therapy need a way to insert DNA into a cell therefore carrier molecule called a vector must be used to deliver the therapeutic gene to the patient's target cells. The most efficient and effective vectors to date are viruses. Viruses can be genetically altered to carry normal human DNA, then passing on the healthy genes to human cells. Much like a chauffeur who picks up and delivers people to certain locations. Some examples of viruses that are used as vectors are: Retroviruses, Adeno-associated viruses, and Herpes simplex viruses.

3.3.2 Non viral vector [CHIMERAPLASTY]:-

A non- viral method that is still being researched for its potential in gene therapy. It is done by changing DNA sequences in a person's genome using a synthetic strand composed of RNA and DNA. This strand of RNA and DNA is known as a chimera last. The chimera last enters a cell and attaches itself to the target gene. The DNA of the chimera last and the cell complement each other except in the middle of the strand, where the chimera last's sequence is different from that of the cell. The DNA repair enzymes then replace the cell's DNA with that of the chimera last. This leaves the chimera last's new sequence in the cell's DNA and the replaced DNA sequence then decays or decomposes. Method of non viral gene delivery have also been explored using **physical** (carrier free gene delivery) **chemical** approaches (synthetic vectors-based gene delivery). (Here, in gene therapy chimera is the molecule that does not exist in nature that is part DNA, part RNA, a molecule of incongruous parts, and the chimeraplast)

Table 3:- Vectors used in Gene Therapy

Viral Vector	Non-viral Vectors
Adenovirus	Lipid complex
Retrovirus	Liposomes
Adeno- Associated Virus	Peptide/protein
Lentivirus	Polymers
Vaccinia virus	
Herpes simplex virus	

3.4 Various Types of Gene transfer:-

The different approaches including-

I. **Physical**
- Microinjection
- Gene gun

II. **Chemical**
- Lipoplexes
- Polyplexes

III. **Electrctrical method**
- Electroporation method

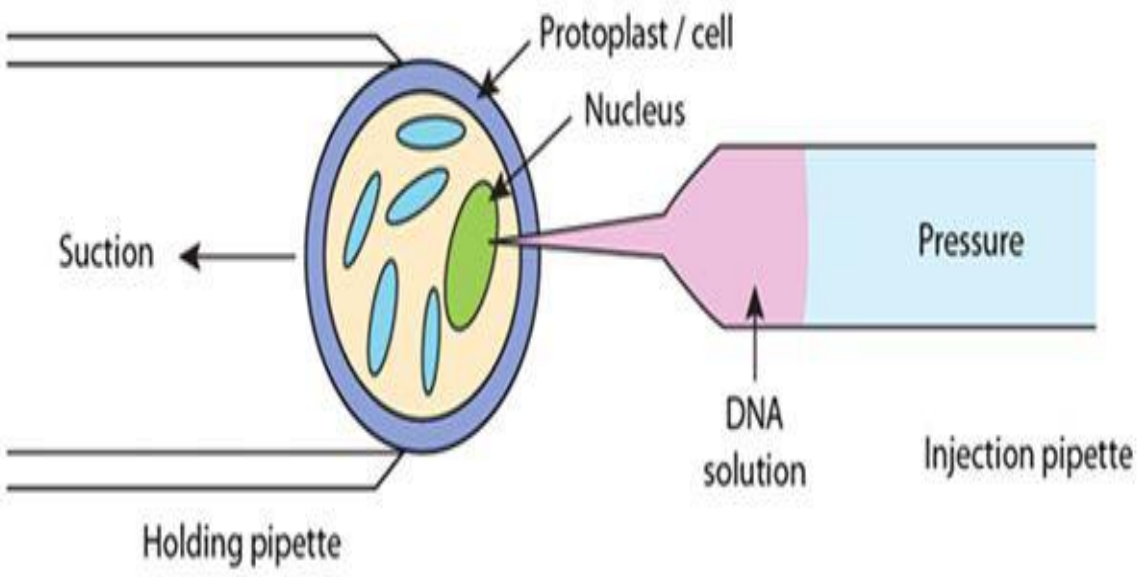

Figure 3:- Microinjection method

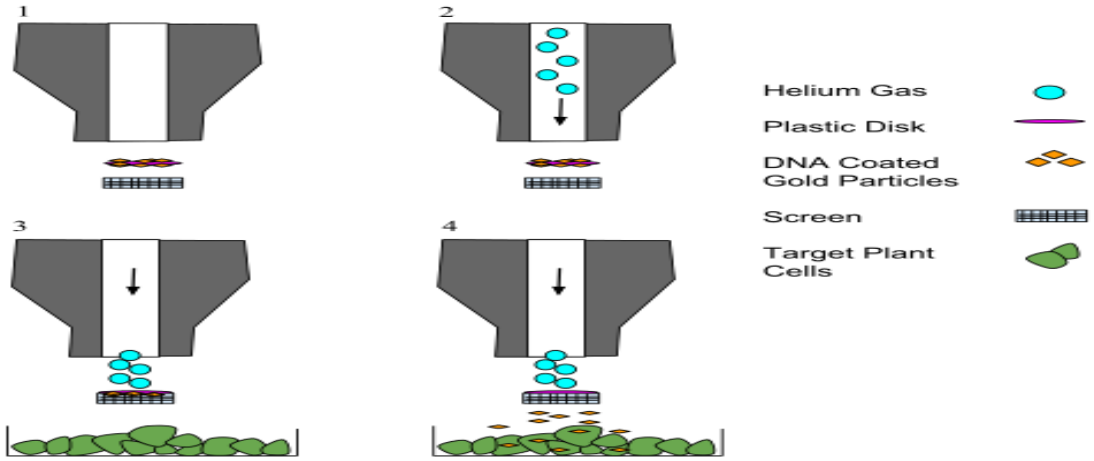

Figure 4:- Gene gun method (Micro projectile)

A gene gun or a biolistic particle delivery system, originally designed for plant transformation, is a device for delivering exogenous DNA (transgenes) to cells. The payload is an elemental particle of a heavy metal coated with DNA (typically plasmid DNA). This technique is often simply referred to as biolistics. This device is able to transform almost any type of cell, including plants, and is not limited to transformation of the nucleus.

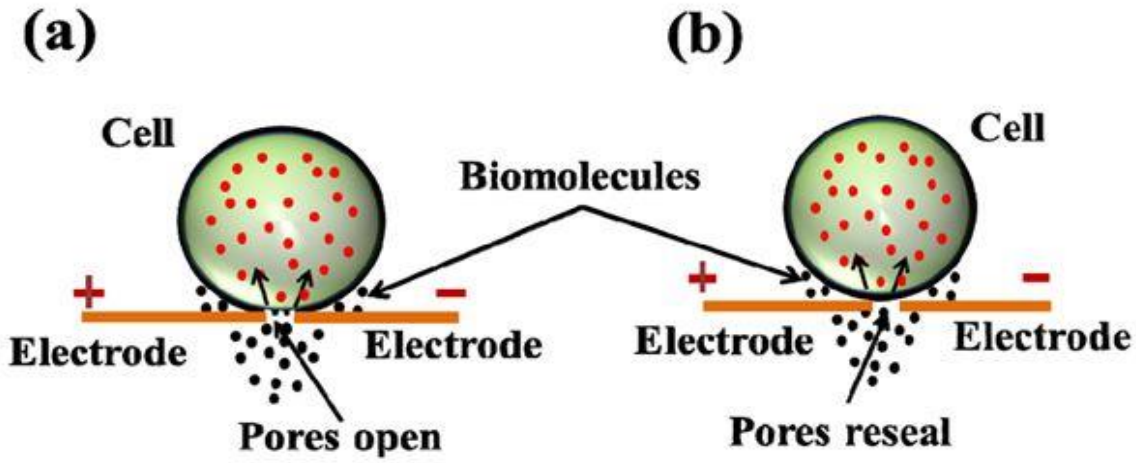

Figure 5:- Electroporation method

4 Success of Gene Therapy [5-9]

4.1 In Blindness [5]-

Gene therapies are being developed to treat several different types of inherited blindness—especially degenerative forms, where patients gradually lose the light-sensing cells in their eyes. Encouraging results from animal models (especially mouse, rat, and dog) show that gene therapy has the potential to slow or even reverse vision loss.

Sight-restoring therapy for the visually impaired and blind is a major unmetmedicalneed.Ocular genetherapyisarationalchoiceforrestoringvisionorpreventingthelossofvisionbecausemostblindingdiseases originates in cellular component so the eye, a compartment that is optimally suited for the delivery of genes, and many of the disease shave a genetic origin or genetic component. In recent years we have witnessed major advances in the field of ocular gene therapy, and proof-of-concept studies are under way to evaluate the safety and efficacy of human gene therapies. Here we discuss the concepts and recent advances in gene therapy in the retina. Our review discusses traditional approaches such as gene replacement and neuro protection and also new avenues such as optogenetic therapies. We conjecture that advances in gene therapy in the retina will pave the way for gene therapies in other parts of the brain

Our capability to perform ocular gene therapy has increased substantially in the past decade owing to the enormous progress made in uncovering novel genetic causes and risks in blinding eye diseases, in developing and analyzing animal models, in developing in vivo imaging. Modalities in human patients, and in refining gene-delivery tools. Despite all this progress, many questions still remain unanswered how to choose promoters and prepare vectors for clinical use; how to decide on the volume of injection and the location of intraocular vector administration whether to use gene-replacement or rather mutation-independent gene therapy; how to choose and standardize patients for a given therapy and how to evaluate visual function before and after gene therapy. To provide the most relevant therapy, we need further improvement in our understanding of geno type phenotype correlations and in the diagnosis of the functional status of retinal cells in vivo in patients. The enthusiasm to provide therapy for such a major unmet medical need propels the field of ocular gene therapy forward to answer these questions. We believe that there cent major advances in gene therapy for the eye will pave the way for gene therapies in other parts of the brain.

4.2 In Parkinson's disease [7]-

Patients with Parkinson's disease gradually lose cells in the brain that produce the signalling molecule dopamine. As the disease advances, patients lose the ability to control their movements. With 0.1–1% prevalence, Parkinson's disease (PD) is one of the most widespread neurodegenerative disorders. The majority of cases are acquired and its biological cause is generally unknown but may be related to oxidative stress, lack of neurotrophic support, or exposure to

toxins. The disease is characterized by a loss of dopamine-producing neurons, specifically, dopaminergic neurons of the substantia nigra that project to the striatum. Tremor, rigidity and movement disorder result from the loss of inhibitory input on the extrapyramidal system. The current treatment, oral L-Dopa therapy, becomes less effective with progression of the disease, and the number of side effects increases.

The effect of oral L-Dopa indicates that the restoration of the neuronal circuitry is not necessary for improvement, but local delivery of L-Dopa is an alternative therapy. The enzyme tyrosine hydroxylase (TH) is responsible for the biosynthesis of L-Dopa from tyrosine. A single gene introducing TH to cells in regions of terminal loss can therefore increase the local supply of L-Dopa. An established animal model in rodents allows testing of the efficiency of gene therapy in Parkinson's disease. The injection of a neurotoxin, 6-hydroxydopamine, destroys nigro-striatal dopaminergic neurons and results in elimination of nigral dopaminergic input and up regulation of dopamine receptors in the lesioned striatum, while the striatal dopamine receptor density in the lesioned side remains unchanged. The asymmetry caused by the resulting differential postsynaptic receptor sensitivities between denervated and intact striatum results in rotational behavior after application of Apo morphine.

Direct gene delivery of the TH gene into the denervated striatum has been achieved with several viral vectors. During and colleagues used defective HSV vector encoding TH and Kaplitt *et al.* showed long term expression *in vivo* in lesioned animals using the AAV vector. Previous reports mostly using adenoviral vectors were not able to retain long-term transgene expression.

Although fetal tissue has been effective in experimental models and partially effective in applications in humans, access to tissue and characterization prior to transplantation are problematic. In addition, transplantation of adrenal chromaffin cells has proven unsuccessful in preclinical and clinical trials. Currently, the use of genetically modified cells that produce TH is one of the major interests in gene therapy. Fibroblasts, retro virally transfected with the TH gene and implanted into the striatum, are able to reduce experimentally induced rotational behaviour in 6-hydroxydopamine lesioned rats. These data have shown that a small number of TH-producing graft cells are capable of inducing behaviour improvements in this model. Despite graft cell survival for at least 2 months after injection, however, the number of TH expressing cells decreases with increasing time. Methods which extend the duration of *in vivo* transgene expression remain to be developed.

4.3 Haemophilia [8]-

People with hemophilia are missing proteins that help their blood form clots. Those with the most-severe forms of the disease can lose large amounts of blood through internal bleeding or even a minor cut.

In a small trial, researchers successfully used an adeno associated viral vector to deliver a gene for Factor IX, the missing clotting protein, to live cells. After treatment, most of the patients made at least some Factor IX, and they had fewer bleeding incidents. Our capability to perform ocular gene therapy has increased substantially in the past decade owing to the enormous progress made in uncovering novel genetic cause sand risks in blinding eye diseases, in developing and analyzing animal models, in developing in vivo imaging modalities in human patients, and in refining gene-delivery tools. Despite all this progress, many questions still remain unanswered: how to choose promoters and prepare vectors for clinical use; how to decide on the volume of injection and the location of intraocular vector administration; whether to use gene-replacement or rather mutation-independent gene therapy; how to choose and standardize patients for a given therapy; and how to evaluate visual function before and after gene therapy. To provide the most relevant therapy, we need further improvement in our understanding of genotype phenotype correlations and in the diagnosis of the functional status of retinal cells in vivo in patients. The enthusiasm to provide therapy for such a major unmet medical need propels the field of ocular gene therapy forward to answer these questions. We believe that the recent major advances in gene therapy for the eye will pave the way for gene therapies in other parts of the brain.

4.4 Cancer [9-10]-

Rogers et al. was one of the first to demonstrate an initial proof-of-concept of virus mediated gene transfer. What he showed was that foreign genetic material can be transferred to cells of interest by utilizing viruses. Motivated by the results he went even further and tested it in humans. With this experiment, Rogers became the first to perform a human gene therapy trial. In that study, Rogers used a wild-type Shape papilloma virus with the intention to introduce the gene for arginase into two girls suffering from a urea cycle disorder (i.e. hyperargininemias). He hypothesized that the Shape

papilloma virus would naturally encode the gene for arginase activity and that this gene could be transferred by introducing the virus to the patients. Unfortunately, the outcome of the trial was negative. There was no change in the arginine levels, nor was there a change in the clinical course of the disease in these patients. Even though Rogers "out of the box" thinking was intriguing, it was doomed to fail as it later turned out that the Shope papilloma virus genome does not encode the arginase gene. The US Food and Drug Administration (FDA) approved the first gene therapy protocol, which was carried out in 1989. Therein, tumor infiltrating lymphocytes collected from advanced melanoma patients were ex vivo transduced with a marker gene (i.e., not a therapeutic gene), expanded in vitro, and re-infused to the patients. The first clinical trial on cancer with an therapeutic intend was started in the following year, wherein patients with advanced melanoma were treated with tumor infiltrating lymphocytes genetically modified ex vivo to express tumor necrosis factor.

Another important milestone in the history of gene therapy was the study conducted by Cline et al. Cline treated thalassaemia patients, wherein he extracted bone marrow cells from these patients and transfected ex vivo with plasmids containing the human globulin gene. After cells were transfected they were administered back to the patients. The reason why this study presents a milestone in the history of gene therapy is not because of the failure of the study itself, but because the study was done without the consent to perform these studies from the University of California, Los Angeles (UCLA) Institutional Review Board. This case demonstrated that knowledge was very limited and that human gene therapy would be technically, as well as ethically much more complex than expected.

HUMAN GENOME PROJECT [11]:-

The human genome is the complete set of nucleic acid sequences for humans, encoded as DNA within the 23 chromosome pairs in cell nuclei and in a small DNA molecule found within individual mitochondria. Human genomes include both protein-coding DNA genes and non coding DNA. Haploid human genomes, which are contained in germ cells (the egg and sperm gamete cells created in the meiosis phase of sexual reproduction before fertilization creates a zygote) consist of three billion DNA base pairs, while diploid genomes (found in somatic cells) have twice the DNA content. While there are significant differences among the genomes of human individuals (on the order of 0.1%), these are considerably smaller than the differences between humans and their closest living relatives, the chimpanzees (approximately 4%) and bonobos.

The Human Genome Project produced the first complete sequences of individual human genomes, with the first draft sequence and initial analysis being published on February 12, 2001. The human genome was the first of all vertebrates to be completely sequenced. As of 2012, thousands of human genomes have been completely sequenced, and many more have been mapped at lower levels of resolution. The resulting data are used worldwide in biomedical science, anthropology, forensics and other branches of science. There is a widely held expectation that genomic studies will lead to advances in the diagnosis and treatment of diseases, and to new insights in many fields of biology, including human evolution.

Although the sequence of the human genome has been (almost) completely determined by DNA sequencing, it is not yet fully understood. Most (though probably not all) genes have been identified by a combination of high throughput experimental and bioinformatics approaches, yet much work still needs to be done to further elucidate the biological functions of their protein and RNA products. Recent results suggest that most of the vast quantities of non coding DNA within the genome have associated biochemical activities, including regulation of gene expression, organization of chromosome architecture, and signals controlling epigenetic inheritance.

There are estimated 19,000-20,000 human protein-coding genes. The estimate of the number of human genes has been repeatedly revised down from initial predictions of 100,000 or more as genome sequence quality and gene finding methods have improved, and could continue to drop further. Protein-coding sequences account for only a very small fraction of the genome (approximately 1.5%), and the rest is associated with non-coding RNA molecules, regulatory DNA sequences, LINEs, SINEs, introns, and sequences for which as yet no function has been determined. In June 2016, scientists formally announced HGP-Write, a plan to synthesize the human genome.

Applications and proposed benefits [12]:-

The sequencing of the human genome holds benefits for many fields, from molecular medicine to human evolution. The Human Genome Project, through its sequencing of the DNA, can help us understand diseases including: genotyping of specific viruses to direct appropriate treatment; identification

of mutations linked to different forms of cancer; the design of medication and more accurate prediction of their effects; advancement in forensic applied sciences; biofuels and other energy applications; agriculture, animal husbandry, bioprocessing; risk assessment; bioarcheology, anthropology and evoluti on. Another proposed benefit is the commercial development of genomics research related to DNA based products, a multibillion-dollar industry.

The sequence of the DNA is stored in databases available to anyone on the Internet. The U.S. National Center for Biotechnology Information (and sister organizations in Europe and Japan) house the gene sequence in a database known as Gen Bank, along with sequences of known and hypothetical genes and proteins. Other organizations, such as the UCSC Genome Browser at the University of California, Santa Cruz, and Ensembl present additional data and annotation and powerful tools for visualizing and searching it. Computer programs have been developed to analyze the data, because the data itself is difficult to interpret without such programs. Generally speaking, advances in genome sequencing technology have followed Moore's Law, a concept from computer science which states that integrated circuits can increase in complexity at an exponential rate. This means that the speeds at which whole genomes can be sequenced can increase at a similar rate, as was seen during the development of the above-mentioned Human Genome Project.

6 Future Prospects [13-14]

Future Prospects Before gene therapy can become the strategy of choice in a wide variety of clinical settings, improvements in the efficiency of gene transfer into target cells and in the maintenance of expression from the relevant transferred gene must occur. The problem of efficient gene transfer will re- quire not only further research to improve delivery systems and vector constructions but also a parallel effort to understand the biology of the target cells. Advances in understanding how target cells divide and differentiate may compensate for deficiencies in currently available delivery systems. For instance, if it could be determined how to cycle hematopoietic stem cells efficiently cx vivo without simultaneously driving these cells to commitment to one or more of the hematolymphoid lineages, retroviral transduction would most likely be- come a more efficient means of introducing relevant genes into these cells for wider therapeutic application.

As the field of gene therapy matures, it is becoming apparent that improvements must also occur in expression of the introduced gene. Research into cellular enhancer and promoter combinations that can stably promote expression of therapeutic genes in the appropriate cell types will need to be identified; viral enhancers and promoters found in many of the current vectors do not provide optimal expression in certain cell types and tissues. An example of using cellular transcription control regions within viral vectors to yield stable expression in the appropriate cell type is the demonstration by Ponnazhagan et al. These investigators showed that an aglobin promoter placed within the context of a recombinant AAV vector yielded more efficient expression in an erythroid cell line than either the HSV-TK or SV4O promoter.

One possible approach to resolving the expression dilemma would be to "harness" the transcriptional activity of a known gene whose expression profile one would want the therapeutic gene to possess. One could harness the expression pattern of a known gene simply by homologously recombining a promoter less therapeutic gene downstream of the promoter of the known gene. Unfortunately, the homologous recombination techniques that are currently being applied to murine embryonic stem cells are not sufficiently robust for use in human gene therapy protocols. However, advances in understanding the mechanism of homologous recombination may eventually put this approach into practice.

A potential approach to utilizing cellular transcription control regions at their native locations would be gene delivery via enhancer-trap retroviruses. These retroviruses contam an enhancer deletion in the U3 region of the 3' long terminal repeat (LTR). Retroviruses with such deletions self-inactivate transcription from the LTR promoter during transduction ofa target cell. Enhancer-trap viruses have been shown to yield stable expression of a reporter gene in lymphoid cell types, whereas similar vectors utilizing the intact Moloney LTR have been shown to yield weak and unstable expression patterns. In some cell types, expression from the Maloney LTR may even be suppressed.

Hence, self-inactivating retroviral vectors provide a means to trap and harness the transcriptional activity of cellular en- hankers and thus could provide stable and sustained expression of a therapeutic gene. The one obvious drawback to this approach is that only retroviral integrations that occur near a cellular enhancer will be transcriptionally active. However, there is ample evidence that retroviruses prefer to

integrate in transcriptionally active regions of the genome, and thus the frequency of active integrations in target cells may not be substantially less with an enhancer less virus than with one with an intact enhancer region.

In addition to improvements in delivery and expression technologies, future efforts will focus on new areas of gene therapy application. These include
(1) Identification and use of "new" resistance genes that will efficiently protect the bone marrow from alkylating agents and radiation;
(2) Intracellular immunization with therapeutic genes for use in adoptive immunotherapies against a variety of life-threatening infectious diseases (e.g., cytomegalovirus); and
(3) Novel adoptive immune therapies employing T cells that have been gene modified to express "new" receptors (e.g., chimeric T cell receptors)

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