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The Principles, Application and Challenges of Gene therapy in Veterinary Medicine: A Review

Abdela Bulbula[1] and Amanuel Balemi[2]
[1]Animal Health Institute, Sebeta, Oromia Ethiopia
[2] Ambo University, Ambo, Oromia, Ethiopia
Corresponding Author: Abdela Bulbula Ogeto,
Email: abdallahbulbula@gmail.com, phone: +251910398915

Abstract

Gene therapy is the introduction, removal, or change in the content of a genetic code with the goal of treating or curing a disease. The completion of human, mouse, dog, cat, and a growing list of other species genome sequences, mark the start of a new era of knowledge in which it will be possible to delineate the entire genetic program of individual types of cells and, eventually, whole tissues.As a result, the objective of this senior seminar paper is to provide a review of gene therapy, its principles, application and challenges in veterinary medicine.In vivo and ex vivo gene therapy are the two approaches used in gene therapy, by which genes are transferred. The vector can be administered intravenously or injected directly into a specific tissue in the body, where it is taken by individual target cells. Applications of gene therapy in animal health increased immunity of animals with respect to common diseases which these animals are normally exposed to the disease. There are several limitations currently faced in gene therapy which affects its safety and efficacy.Germ-line gene therapy experiments would involve too much scientific uncertainty and clinical risks, and the long term effects of such therapy are unknown. Therefore, the advantages of gene therapy are significantly increased by overcoming biological obstacles, immunological reactions, and potential negative consequences linked to vectors or treatment strategies are recommended.

Keywords: Application, Challenges, Gene, Therapy, Veterinary Medicine

1. Introduction

The large number of disorders that are essentially genetic in origin or involve a genetic predisposition to disease-causing elements in the environment is today's medical sciences' major challenge. These disorders cover a wide range of severe and deadly diseases for which there are few effective treatments or preventative options. Congenital deformities, metabolic problems, and cancer are the most frequent diseases in which genetic anomalies play a role (Wolfe, 2009).

Molecular biology tools have allowed researchers to understand the underlying mechanisms of hereditary disease at the level of DNA, RNA, and protein molecules. In living cells, DNA is the most crucial molecule. It carries genetic information that determines the structures of proteins to be generated within its structure (Bewaji, 2003).Recombinant DNA technology, in which the gene of interest or a healthy gene is inserted into a vector, which can be plasmidial, nanostructured, or viral; the viral vector is the most commonly used due to its efficiency in invading cells and introducing its genetic material, is one of the most widely used techniques (Misra, 2013).

The ability of genetic improvement through the correction of altered (mutated) genes or site-specific alterations that target therapeutic treatment is referred to as gene therapy (Gonçalves and Paiva, 2017). According to the latest definition by the American Society of Gene and Cell Therapy (ASGCT) in 2019, `*Gene therapy (GT) is the introduction, removal, or change in the content of a genetic code with the goal of treating or curing a disease*` (American Society of Gene & Cell Therapy, 2019).

Traditionally, gene therapy was assumed to be a treatment for diseases caused by a specific genetic mutation that could be addressed by restoring the production of the correct version of a gene. Nonetheless, gene therapy has advanced tremendously since its inception. The dogmatic view of gene therapy is no longer sustainable, and the claim that only replacement gene therapy represents the original is nonsensical, as is the idea that one gene encodes one protein. Similarly, simply because gene therapy is concerned with restoring a gene's correct form does not imply that it is limited to that method (Dulak, 2021).

According to the Gene Therapy Clinical Trials Worldwide up to recent year, 3180 clinical trials of gene therapy have been performed, with more than 800 gene therapy trials ongoing in clinical development (High &Roncarolo, 2019).

Although laboratory animals have been extremely useful in gene therapy research, the findings of such studies cannot be directly applied to human patients. As a result, veterinary medicine is becoming a more essential translational link between in vitro and preclinical investigations and human medicine (Pavlin*et al*., 2012).

The use of genetic engineering allows for the development of species-specific gene drugs for animals while avoiding unwanted complications and side effects. Gene therapy also allows for novel modern approaches to treating a variety of animal diseases (Zakirova*et al*., 2022)

Pathogen sequencing investigations, in which scientists analyze the genomes of medical and veterinary pathogens, are producing new, less contentious diagnostic tools. As a result, the objective of paper is to provide a review of gene therapy, its principles, application and challenges in veterinary medicine.

2. Literature review

2.1. Historical background

Humans have known since the beginning that the peculiar characteristics of their parents can be passed down to their descendants. Students in ancient Greece were the first to speculate, and some of these hypotheses persisted for decades. Gregor Mendel, an Austrian monk, characterized the inheritance pattern by studying the traces that were inherited as discrete units, which we now know as genes, in a series of experiments with green peas in the early 1850s. Little was known about the physical nature of genes until 1950, when James Watson, an American biochemist, and Francis Crick, a British biophysicist, created the breakthrough model of double-strand DNA (Gonçalves and Paiva, 2017).

The completion of human, mouse, dog, cat, and a growing list of other species genome sequences, as well as advances in genomics and related sciences, mark the start of a new era of knowledge in which it will be possible to

delineate the entire genetic program of individual types of cells and, eventually, whole tissues(Wolfe, 2009).

The separation of genes at specific locations along the DNA molecule and their repeatable reinsertion were made possible in 1970 by the discovery of a series of enzymes.These genetic advances prepared the scenario for the emergence of genetic engineering with the production of new drugs and antibodies, and as of 1980, gene therapy has been incorporated by scientists (Misra, 2013;Gonçalves&Paiva, 2017).

Diseases brought on by a single gene abnormality are more likely to be successfully treated using gene therapy. It was approved for the treatment of conditions such severe combined immunodeficiency, familial hypercholesterolemia, cystic fibrosis, and Gaucher's disease. Most protocols are intended for the treatment of cancer. Heart disease, Parkinson's, and Alzheimer's diseases, as well as arthritis, are listed as prospective candidates for gene therapy (Scheller&Krebsbach, 2009).

Although several protocols have been successful, the gene therapy process remains complex, and many techniques need new developments. On table 1, a few gene therapy protocols are summarized, which are approved and published for clinical use, exemplifying the disease, the target, and the type of vector used (Gonçalves&Paiva, 2017).

Table 1:Gene therapy protocols

Disease	Objective	Stem cells	Release mode	Countries with the protocol
Adenosine deaminase Deficiency	Substitution of the adenosine deaminase deficiency	Blood	Retrovirus	Italy, Holland, and the United States
~1-antitrypsin deficiency	Substitution of α1-antitrypsin	Respiratory epithelium	Liposome	United States
AIDS	Inactivation of the HIV-presenting antigen	Blood and bone marrow	Retrovirus	United States
Cancer	Improvement of immune function	Blood, bone marrow, and tumor	Retrovirus, liposome, electroporation, and cell-mediated transfer	Austria, China, France, Germany, Italy, Holland (Netherlands), and the United States
Cancer	Tumor removal	Tumor	Retrovirus, non-complexed DNA, cell-mediated transfer	United States
Cancer	Chemoprotection	Blood and bone marrow	Retrovirus	United States
Cancer	Stem cell marking	Blood, bone marrow, and tumor	Retrovirus	Canada, France, Sweden and United States

Cystic fibrosis	Enzymatic substitution	Respiratory epithelium	Adenovirus and liposome	England and the United States
Familial hypercholesterolemia	Substitution of low-density lipoprotein receptors	Liver	Retrovirus	United States
Fanconi anemia	Complement C gene release	Blood and bone marrow	Retrovirus	United States
Gaucher Disease	Glucocerebrosidase substitution	Blood and bone marrow	Retrovirus	United States
Hemophilia B	Factor IX substitution	Skin fibroblasts	Retrovirus	China
Rheumatoid arthritis	Cytokine release	Synovial membrane	Retrovirus	United States

Source: Gonçalves & Paiva, 2017

2.2. Principles of Gene Therapy

Gene therapy is the replacement of faulty genetic material with normal genetic material to treat or cure a disease or abnormal medical condition(US Food and Drug administration) (Xi & Grandis, 2003).The procedure involved in gene therapy includes; identifying the gene of interest, obtaining a normal copy of the gene (therapeutic gene) using a restriction endonuclease enzyme (cutting and splicing), and finally cloning the therapeutic gene into a vector, which serves as a vehicle for delivering the gene of interest (Sunil *et al*., 2012).

Gene transfer efficacy and safety testing system should be done before therapy (Packialakshmi *et al*., 2012). Faulty genes can be corrected by several methods such as, through homologous recombination, a defective gene can be replaced with a normal gene, and normal gene is inserted into nonspecific location within the genome to replace a nonfunctional gene. It is also possible to alter the regulation of a certain gene (the degree to which the gene is switched on or off). A gene that is abnormal is corrected through selected reverse mutation, which restores the gene's normal function(Mammen *et al*., 2007).

2.2.1. Types of gene therapy

Somatic gene therapy

Somatic gene therapy includes inserting a "good" gene into certain cells with the goal of healing the patient, but not the patient's future offspring, because these genes are not passed down to progeny. In other words, even if some of a patient's genes are altered to treat a condition, the patient's offspring are likely to be affected by the same ailment. This is the type of gene therapy used in the majority of genetics laboratories throughout the world (Moss, 2014).

Adult stem cells are already used in the treatment of genetic diseases. Bone marrow transplants and the haematopoietic stem cells contained within are used to treat genetic and acquired blood and immune system diseases. Genetic engineering of haematopoietic stem cells to cure some genetic diseases is one of the most recent developments. Other adult stem cells are being studied in preclinical animal models for transplantation therapy in a variety of genetic diseases, with cellular models being a major potential application (Mackay-Sim&Silburn, 2008).

Furthermore, using an individual's stem cells to generate certain tissues or organs in vitro may be possible in the future. Finally, stem cell research could take a quantum leap forward by merging gene therapy with genetic engineering to create stem cells that can be used to generate healthy tissues and organs(RaziSoofiyani*et al*., 2013).

Germline gene therapy

Injecting foreign genes into fertilized eggs or sperm-producing cells, which will then pass on any genetic modifications to future generations, is known as germline gene therapy (Moss, 2014). The objective of this is to create a favorable or advantageous genetic change that is transmitted to the offspring. When genes are introduced in a reproductive cell, descendant cells can inherit the genes (RaziSoofiyani*et al*., 2013).However, despite the fact that it has the potential to prevent inherited disease, this type of gene therapy is highly contentious, and very little research is currently being done in this field, both for technical and ethical grounds (Moss, 2014).

2.2.2. Gene therapy delivery approaches

In vivo and ex vivo gene therapy are the two approaches used in gene therapy, by which genes are transferred (Grace *et al*., 2022). In the in vivo gene therapy, a new gene with the help of a plasmid or viral vectors is directly introduced into the patient, and now, it is further developed utilizing clustered regularly interspaced short palindromic repeats (CRISPR) strategy (Savić&Schwank, 2016; Gowing*et al*., 2017). Various in vivo gene therapy complications include the viral vector associated with nonspecific gene expression and targeting insertion mutagenesis, gene silencing, and immune responses against the vector gene silencing and immune responses against the vector (Mingozzi& High, 2011). The in vivo gene therapy can also produce strain to CNS cells to work hard making therapeutic molecules. Recent advancements in neural stem cell (NSC) strategies, including the capability to generate autologous induced pluripotent stem cells (iPSC) from the patient’s blood or skin, seem promising in the future for ex vivo gene therapy (Barrett *et al*., 2014).

The ex vivo gene therapy uses in vitro cell modification, and these cells are transplanted for a stable or transient graft based on the patient to serve the purpose of replacement of faculty cells or providing therapeutic proteins (Klein *et al*., 2005; Behrstock*et al*., 2006; Naldini,2011). In ex vivo gene therapy, modified cells’ characterization is done before introducing to the patient, and the patient is not directly exposed to the vector (Liu & Wang, 2015). The cells can undergo differentiation to produce therapeutically relevant tissues, including oligodendrocytes or astrocytes, besides providing the missing or beneficial protein (Grace *et al*., 2022).

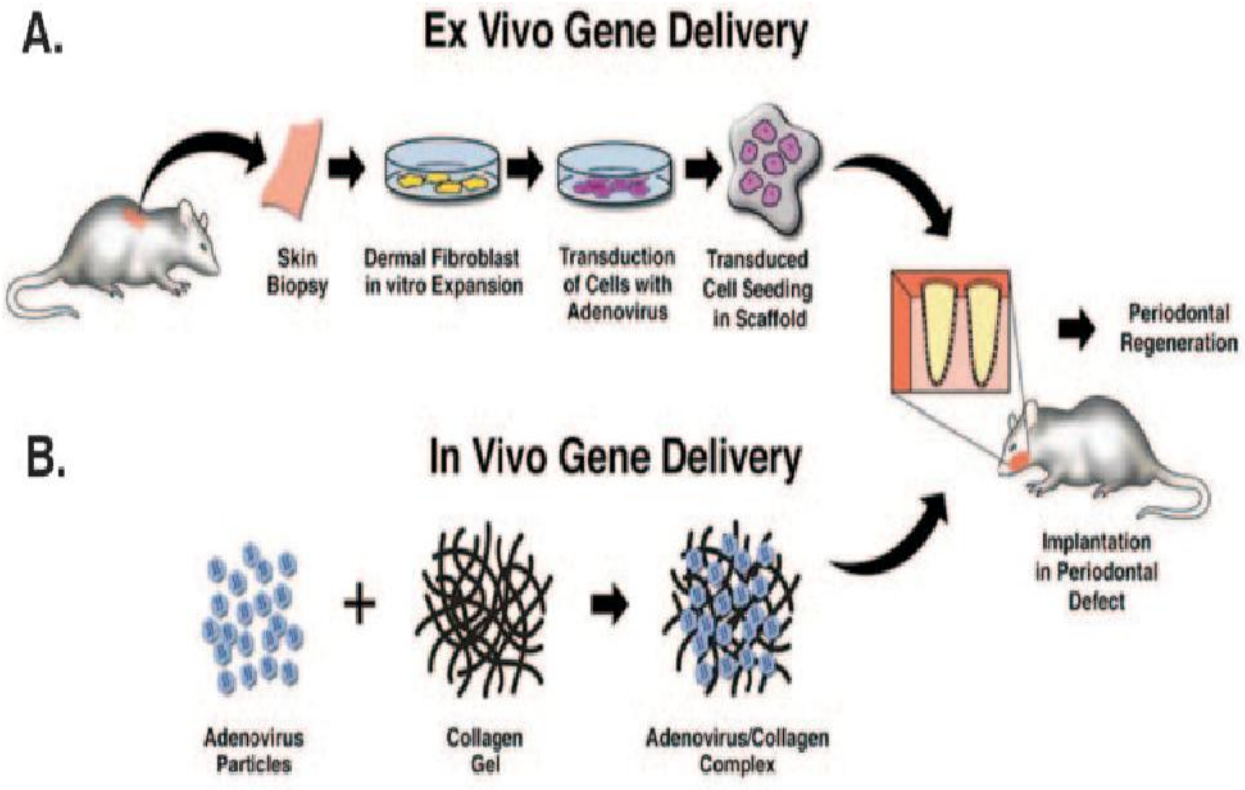

Figure 1: Illustration of gene therapy delivery approaches
Source (Mao *et al*., 2006)

2.3. Vectors Used in Gene Therapy

The vector can be administered intravenously or injected directly into a specific tissue in the body, where it is taken by individual target cells. Alternatively patient‘s representative cells that are removed from the body can be exposed to the vector in laboratory. The cells which now contain the vectors are reintroduced into the patient for therapeutic action. Vector delivers the therapeutic gene into patient‘s target. The target cells become infective with therapeutic gene through vector. Functional proteins are created from the therapeutic gene causing the cell to return to a normal stage (Mammen*et al*., 2007;Prabhakar*et al*., 2011)

The ideal requirements for vectors are due to it should be repeatable and stable, exhibit longevity of expression, and not be immune system detectable (non-immunologic). Must be extremely successful (transfect 100%), High specificity and low toxicity, through the cell membrane, it ought to be able to move and deliver DNA to the nucleus. Vectors ought to be able to control how genes are delivered to certain cells and should be inexpensive and easy to produce in large quantities (Prabhakar*et al*., 2011; Singh *et al*., 2012; Sunil *et al*., 2012,). Currently no single vector type will meet all needs for all tissues, that is different vectors will be needed for different clinical applications (Vagish Kumar, 2014)

Gene therapy uses different kinds of vectors, like viral and non-viral, which may include synthetic macromolecules, cationic polymers carrying specific ligand for cell surface receptors, and lipid carriers, like liposomes. Viral vectors are a fine strategy to pass and express genetic materials to the host cells. The most commonly used and targeted ones are adeno-associated viruses (AAV), herpessimplex virus-1 (HSV1), retrovirus (RV), and lentiviruses (LV). They can invade cells by triggering infection naturally (Teschemacher*et al*., 2005; Bourdenx et al., 2014; .Artusi*et al*., 2018).

The transgene introduction into a vector is a complex procedure, and vectors must possess salient features such as, the vector must allow the easy manipulation for recombinant technology followed by propagation in suitable hosts and possess minimum invasiveness with high cloning capacity. The vector should enable the adaptation of regulatory genes or sequences that ensure the appropriate spatial and temporal regulation of transgene expression and should not have the ability for undesired or uncontrolled alterations of the host genome (Shillitoe, 2009;Inguscie*t al*., 2019).

The vector should be absent from immunogenicity (it should be devoid of genes that bring on immune responses)and transgene selected must have exclusive expression only in the target cells.The vector must allow a prolonged expression of a functional gene that is stable with no alteration in cell progeny (Grace *et al.*, 2022).

2.3.1. Viral vectors

Viral vectors are modified viruses that can infect cells and introduce foreign genes. By changing viral vectors, the genes needed for replication are substituted by therapeutic genes. The important viral vectors utilized in gene therapy include RV; adenoviruses (AV), HSV, AAV, and LV (Grace *et al*., 2022).

1. Retroviruses

Retroviruse is highly effective in transferring genes to the cells that are dividing. The limitation is that they can only infect mitotic cells and have a problem in introducing genes to the post-mitotic neurons(Grace *et al*., 2022). Retroviruses, such as the human immunodeficiency virus (HIV), are a class of viruses that can convert RNA genomes into double-stranded DNA and integrate into host cell chromosomes. One problem with gene therapy using retroviruses is that the integrase enzyme can insert the genome of the virus into any location in the host genome. If the genetic material is inserted into the middle of one of the main genes in the host cell, the function of this gene will be impaired (insertional mutation) (Yazdani *et al*., 2018).

2. Adenoviruses

This class of viruses has a double-stranded DNA genome that causes respiratory, intestinal, and ocular infections in humans. When these viruses infect a cell, they insert their DNA molecules into the host's; however, the genetic material of the adenovirus does not integrate into the genetic material of the host cell. Instead, the DNA molecule remains inside the host cell's nucleus, and this foreign DNA is transcribed like any other gene in the cell. The only difference regarding adenoviruses is that when the host cell is divided, the foreign genes are not replicated; as a result, cells generated by cell division will not have additional genes. Therefore, the treatment of a growing cell population with adenoviruses requires the re-injection of them (Patil*et al*., 2012).

Adenoviruses are popular in gene therapy and can be modified by producing viral gene deletions to make space for the foreign gene insertion, thus generating a replication-deficient virus. They can also be utilized to target the non-dividing cells. Their genes do not merge into host chromosomes and are useful in modulating target gene expression(Grace *et al*., 2022).

3.Adeno-Associated Viruses

Adeno-Associated Viruses comprise a small class of viruses with single-stranded and non-coated DNA. They have the ability to infect both dividing and non-dividing cells with constitutive expression (Patil*et al*., 2012). The viruses are a suitable choice for gene therapy since they can exist in cells in both lysogenic and lytic forms. These viruses lack pathogenicity in the absence of auxiliary viruses like adenoviruses and herpes viruses. In the absence of auxiliary viruses, these viruses can insert their genetic material into a specific location on chromosome 19 (Yazdani *et al*., 2018).

4. Herpes Simplex Viruses

These viruses have double-stranded DNA and attack a particular subset of neural cells. Cold sores and fever blisters are common symptoms of type 1 herpesvirus infection(Verzosa*et al*., 2021). The Herpes Simplex Virus, are primarily exploited in the nervous system for gene transfer. The wild HSV-1 virus has the ability to infect neurons while evading the immune system of the host; yet, this virus may be rendered inactive and result in a lytic cycle of viral reproduction. As a result, a mutant HSV-1 strain that cannot replicate is usually employed(Rosato&Leib, 2015).

2.3.2. Non-viral vectors

Most non-viral vectors are composed of polymeric or lipid particles that package and protect genetic material in their interior to facilitate entry into the cell. The use of non-viral vectors offers several advantages over the use of viral vectors, such as their easily scalable production, a long shelf life, a theoretically unlimited size of the genetic material payload, and a better safety profile. The limitations of non-viral vectors are their poor efficiency at penetrating into the nucleus and their limited ability to achieve long-lasting transgene expression (Maestro *et al*., 2021).

1.Ormasil

Because of the ease of working with silica, ormasil is a viable option for gene delivery. Because of its low toxicity, the most common method of using silica in gene therapy is a combination of nanoparticles and amino silicones. However, because of the reaction between serum proteins as a limiting factor, delivery in the presence of serum reduces the efficiency of this process (Ramamoorth&Narvekar, 2015).

2. Administration of naked genetic material

The most basic method of non-viral vector administration is through naked genetic material. This strategy, however, is hampered by very low efficacy cell entry. Several methods can be used to improve the low efficacy of transfer in the case of hepatocytes. One of the simplest methods is hydrodynamic injection, which involves rapidly injecting a relatively large volume of a solution into the liver, causing very high venous pressure and facilitating the entry of genetic material into the hepatocytes (Ramamoorth&Narvekar, 2015; Song *et al*., 2002).

Because of its inherent simplicity and the ease with which it can be produced in bacteria and manipulated using standard recombinant DNA techniques, naked DNA is an attractive non-viral vector (Wolff &Budker, 2005).Recently, liver electroporation has been used for the expression of a1-antitrypsin (AAT) in AAT-deficient mice, resulting in a reduction of pulmonary emphysema (Sutter *et al*., 2020). Transcutaneous ultrasound has been confirmed to be efficient for the delivery of genetic material to the liver of mice and pigs (Noble-Vranish*et al*., 2018; Endo-Takahashi *et al*., 2020). So far, the use of these approaches in liverdirected GT is still restricted to experimental animal studies (Maestro *et al*., 2021).

3.Polycationic and lipid nanoparticles

Cationic polymers form nanoparticles with nucleic acids through electrostatic interactions allowing the transport of nucleic acids into the cell. One of the most frequently used is polyethylenimine (PEI), which can mediate high transduction efficiencies (Lungwitz*et al*., 2005). Furthermore, the use of galactosylated PEI targeting the ASGPR proved to be very efficient in transducing hepatic cell lines and the livers of mice and rats. PEI nanoparticles and derivatives have been used experimentally for the supply of different drugs, including siRNA and microRNAs (miRNAs), for the treatment of liver malignancies, but no clinical studies have yet been performed (Xue*et al*., 2021).

Lipid nanoparticles (LNPs) have very comparable composition to cell membranes. They are formed by amphiphilic lipids that when dispersed in an aqueous environment spontaneously forms spherical structures with a hydrophilic interior. LNPs are a suitable carrier for nucleic acid

delivery because of their excellent biocompatibility, biodegradability, low toxicity and immunogenicity, structural flexibility, and ease of large-scale preparation(Maestro *et al*., 2021).

2.4. Methods of Gene Delivery

2.4.1. Physical methods

1. Electroporation

Electroporation is a technique for transferring DNA from cell membranes that uses high-voltage short pulses. The principles of electroporation allow the spread of labelled DNA in the cell to be monitored. The use of exogenous plasmid DNA (pDNA) in gene therapy necessitates the expression of a plasmid-cloned gene. Small pores form on the membrane's surface as a result of electrical shock, making it permeable to nucleic acid. Although electroporation can be used on a variety of cell types, the high rate of cell death has limited its use in clinical applications (Patil*et al*., 2012;Sokołowska&Błachnio-Zabielska, 2019).

2. Gene Gun

The gene gun operates on the principle of delivering nucleic acids by bombarding target cells with nucleic acid-coated gold particles at high velocities (pressurized inert gas or high-voltage electronic discharge). In this method, the DNA is coated with gold particles and then placed inside a device which provides the required force to enter the cell (Yazdani *et al*., 2018).

3.Sonoporation

Sonoporation uses an ultrasonic frequency to introduce DNA to a cell. This process is considered an ultrasound cavitation in the cell membrane and leads to DNA movement in the cell (Patil*et al*., 2012).

4.Magnetofection

With magnetofection, DNA is complexed with magnetic particles, and a magnet is placed under the cellular tissue culture container in order to expose the DNA-containing compound to only one cell layer. This method, which is based on the hypothesis of targeted drug delivery, works by the therapeutic gene linking to the magnetic nanoparticles (Yazdani *et al*., 2018).

2.4.2. Chemical methods

1. Oligonucleotides

Synthetic oligonucleotides are used in gene therapy to disable and inactivate genes involved in the disease process. The use of specific antisense for the target gene impairs the transcription of defective genes. Another method is the use of siRNA that leads to the breakdown of a specific sequence of the defective gene mRNA to stop its translation and, thus, its expression (Yazdani *et al*., 2018).

2.Lipoplex and Polyplex

A mixture of DNA and polymers is called polyplex. Most polyplexes include cationic polymers which are based on particle accumulation due to interactions of polyplexes. To improve new DNA delivery to the cell, DNA should be protected from damage and a positive charge. Therefore, anionic and neutral liposomes are used for the formation of lipoplexes as synthetic vectors (Yazdani *et al*., 2018).

3.Dendrimers

The dendrimer is a branched spherical macromolecule. The particle surface can be charged in a variety of ways, and it determines many properties of the particle's final structure. The supplementary charge results in a temporary nucleic acid linkage with the cationic dendrimer in the presence of genetic material such as DNA or RNA. The nucleic acid-dendrimer complex enters the cell via endocytosis (Patil*et al*., 2012; Viswanath&Santhakumar, 2017).

4.Hybrid Methods

Each gene transfer method has its own shortcomings; thus, hybrid methods, which are, in fact, combinations of several techniques, are being developed (Yazdani *et al*., 2018).This hybrid method of gene delivery in respiratory epithelial cells is more effective than the viral or liposome methods alone. In general, this method involves the mixing of different viral vectors with cationic liposomes or hybrid viruses (Patil*et al*., 2012).

2.5. Advantages and Disadvantages of Gene Therapy

2.5.1. Advantages of gene therapy

The key advantages of gene therapy include the fact that it delivers an actual cure rather than just palliative or symptomatic care. For some hereditary illnesses, germ-line gene therapy might be the sole cure. It is possible to avoid the cost and risk of somatic cell treatment for several generations by preventing the transmission of disease genes. The reproductive health requirements of prospective parents who run the danger of passing on major hereditary illnesses should be addressed by medicine. Within the parameters of authorized human study, the scientific community is entitled to unrestricted inquiry. (McCain, 2005;Jafarlou *et al*., 2016).

2.5.2. Disadvantages of gene therapy

Germ-line gene therapy research would involve too much scientific uncertainty and clinical hazards, and the long-term implications of such therapy are unknown. These are the fundamental drawbacks of gene therapy. Such gene therapy would pave the way for efforts to change features unrelated to diseases, which could increase societal and cultural discrimination issues. Germ-line gene therapy research effectively produces generations of unconsulting research subjects because it includes studying early embryos and has an impact on their progeny. Because of its enormous expense, gene therapy will never be given the high social priority it deserves. The right of future generations to inherit an unaltered genetic endowment would be violated by germ-line gene therapy. (McCain, 2005; Jafarlou*et al*., 2016).

2.6. Applications of Gene Therapy in Animal Models

In the sphere of agriculture, and particularly in the health and production of animals, gene therapy has seen some basic applications. This has aided in improving the genetic potential of animals. The use of gene therapy in animal production has resulted in the development of animals with quick maturation and growth rates, improved production of meat and beef, and increased animal immunity to common diseases to which these animals are typically exposed (Ugwu, *et al*., 2019).

Animal models are useful tools in biomedical research to evaluate aspects of viral vector usage such as safety, efficacy, dosage, and transgenic expression localization before proceeding to a potential human trial. When compared to a facility that houses small animals such as mice, rats, and rabbits, housing large animals such as dogs, monkeys, and pigs is more expensive and requires more equipment. Small animal models, as opposed to large animal models, are much easier to handle and maintain, and because they have a limited lifespan, they can be used to quickly evaluate safety and efficacy. Furthermore, in order to advance medicines, it may be advantageous to support research on large models based on key experimental findings from small models(Gopinath, *et al*., 2015;McCarron *et al*., 2021).

Murine models

Murine models are used in various gene therapy studies including those for cancer (Zhang *et al*., 2015, Morrison *et al*., 2006), muscular dystrophies (Dellorusso*et al*., 2002, Larcher*et al*., 2014), hematological (Kuether*et al*., 2012, Negre*et al*., 2015), respiratory (Tuggle*et al*., 2014), liver (Domvri*et al*., 2012) and cardiovascular disorders (Mearini*et al*., 2014). In order to examine safety and enhance treatments, it

is often a good idea to test a novel therapeutic method on an animal model before applying it to human beings. It might be challenging to comprehend and correlate key findings for human applications when mouse models do not always perfectly replicate the phenotypic aspects of human diseases (Shanks*et al*., 2009; Vasireddy*et al*., 2013).

A comparative preclinical study between adult mice and non-human primates (NHPs) showed that intravascular administration of adeno-associated viral vector serotype 9 (AAV9) was able to transduce and actively cross the blood-brain barrier comparatively better in mice than NHPs where poor transduction into brain and peripheral organs was observed. This could be due to the presence of low levels of pre-existing neutralizing antibodies (NAbs) in NHPs that could have blocked transduction into the brain and peripheral organs (Boutin*et al*., 2010).

Canine models

The dog is the most common animal in therapeutic gene transfer in naturally occurring genetic diseases. Dogs' characteristics including size, lifespan and extraordinary medical care level allow a comprehensive longitudinal description of diseases (Casal& Haskins, 2006; Barthélémy et al., 2019).

Foamy virus vector therapy for canine leukocyte adhesion deficiency (CLAD) resulted in complete reversal of the CLAD phenotype in four out of five CLAD dogs that received injections of vector-transduced autologous CD34+ hematopoietic stem cells (HSCs) after a non-myeloablative conditioning regimen (Bauer *et al*., 2008). All treated dogs have been shown to sustain CD18 expression and essentially disease-free for more than 4 years post-treatment. There were no genotoxic effects reported and further the risk of integration near oncogenes was shown to be much lower compared to that of gammaretroviral vectors (Bauer *et al*., 2013). Dogs are considered as suitable animal models for hematopoietic stem cell gene therapy (Bauer *et al*., 2009).

Non-human primate models

Non-human primates (NHPs) such as African green monkeys, baboons, chimpanzees, cynomolgous monkeys, rhesus monkeys and owl monkeys are considered the most suited animal models for preclinical testing as they are evolutionarily and genetically very closely related to humans compared to any other mammals (Gopinath, *et al*., 2015).

Red-green colour blindness, which results from the absence of wavelength sensitive visual photopigments is a common single gene eye disorder. Gene therapy was performed on adult squirrel monkeys that were congenitally colour blind due to the absence of L-opsin. Addition of a third opsin to correct the defect in vision resulted in trichromatic colour vision in the treated monkeys (Mancuso *et al*., 2009).

Porcine models

Cystic fibrosis is one of the diseases where successful gene therapy could be a major concern. Ion transport defect i.e. lack of cAMP-stimulated anion transport in sinus epithelia were corrected in a porcine model using an adenoviral (AdV) vector. Porcine sinus epithelia were transduced using an AV vector expressing CFTR, which lead to restoration of cAMP-mediated anion transport indicating that CFTR expression by a comparatively small number of cells could restore anion transport, a successful step in disease correction which was first demonstrated in a porcine model of cystic fibrosis (Gopinath, *et al*., 2015).In haemophilia A, a bleeding disorder caused by abnormalities in the coagulation factor VIII, gene therapy enabled sustained elevation of factor VIII levels. Currently available mouse models failed to extrapolate the human condition as they could not show similar coagulation ability. Hence, a porcine model was generated from F8 targeted fibroblasts using nuclear transfer(Gopinath, *et al*., 2015). The infusion of human factor VIII using F8 targeting vector, constructed by insertion of two genomic DNA into the plasmid vector pHSv-TK/PGK-Neo resulted in reduced bleeding (Kashiwakura*et al*.,

2012). This would make way for porcine models as efficient animal models for gene therapy of haemophilia A (Gopinath, *et al*., 2015).

Bovine models

The use of cattle for gene therapy is very rare, probably due to its size and also the high cost accrued due to large scale production of recombinant proteins and viral vectors (Casal and, Haskins, 2006). However, using calves could be an alternative as they weigh around 30 kg, which is similar to that of an older child. Bovine model is the only available model for citrullinemia, an inborn error of urea cycle metabolism caused by deficiency of arginosuccinatesynthatase leading to hyperammonemia. Inefficient pharmacological treatments where the oral administration of 15N4 -labelled urea was used to measure the nitrogen flux led to the use of viral vectors. Systemic administration of a first-generation E1-deleted AdV vector expressing human arginosuccinatesynthetase successfully resulted in the transduction of hepatocytes and partial correction of the enzyme defect leading to restoration of urea synthesis (Lee *et al*., 1999).

Equine models

Horses are one of the most commonly used models for osteoarthritis as this condition occurs in them naturally (Frisbie*et al*., 2002). Osteoarthritis is a chronic, debilitating and expensive disease involving the joints (Evans *et al*., 2004).

2.7. Challenges of Gene Therapy in Veterinary Medicine

There are several limitations currently faced in gene therapy which affects its safety and efficacy. There are barriers such as biological barriers as well as immune responses, rate limiting steps in the expression of transgene based on cell type, and possible undesirable effects associated with vectors or treatment strategies. Overcoming these barriers and limitations greatly enhances the benefits of gene therapy (Grace *et al*., 2022).

1. Short-lived nature of gene therapy

Before gene therapy can produce a permanentcure for any condition, the therapeutic DNAintroduced into targeted cells must remainfunctional and the cells containing thetherapeutic DNA must be long-lived and stable.The problem with integrating therapeutic DNAinto the genome and the rapidly dividing natureof many cells prevent gene therapy fromachieving any long-term benefits. Patients willhave to undergo multiple rounds of genetherapy(Ugwu, *et al*., 2019).

2. Immune response

Anytime a foreign object is introduced into human system, the immune system has evolved to attack the invader. The risk of stimulating the immune system in a way that reduces gene therapyeffectiveness is always a possibility. Furthermore, the immune system's enhanced response to invaders that it has seen before makes it much more difficult for gene therapy to be repeated in patients(Ugwu, *et al*., 2019).

3. Problems with viral vectors

Viruses, the carrier of choice in most gene therapy studies, present a variety of potential problems to the patient: toxicity, immune and inflammatory responses, and gene control and targeting issues. In addition, there is always the fear that the viral vector, once inside the patient, may recover its ability to cause disease (Ugwu, *et al*., 2019).

4. Multi-genic disorders

However, diseases caused by multiple genes, such as heart failure, high blood pressure, arthritis, diabetes, Alzheimer's disease, and so on, cannot be treated and chances of inducing tumor (if DNA is inter-placed at wrong place) (Bezabeh et al., 2004; Gonçalves and Paiva, 2017).

The primary challenge of gene therapy in veterinary medicine as a result of most diseases considered candidates for gene therapy are caused by mutations that result in the absence of a specific protein (enzyme deficiencies) or by failure of controlled cell growth (cancer). The most common current gene transfer approach for

providing therapeutic proteins to tissues is to administer normal DNA to express the missing protein, or in the case of cancer, to provide 'suicide genes' or antitumor factors (Casal& Haskins, 2006)

3. Conclusion and Recommendations

The replacement of faulty genetic material with normal genetic material to treat or cure a disease or abnormal medical condition is known as gene therapy. Veterinary medicine is becoming an increasingly important translational link between in vitro and preclinical research in modern medicine.Somatic and germ-line gene therapy offers a true cure, and not simply palliative or symptomatic treatment. This has helped to enhance the genetic capabilities of animals. Applications of gene therapy in animal production have led to the creation of animals with rapid growth and maturity rates, increased meat/beef production and increased immunity of animals with respect to common diseases. However, germ-line gene therapy experiments would involve too much scientific uncertainty and clinical risks, and the long term effects of such therapy are unknown.

Therefore, based on the above conclusions, the following recommendations are given;

- Research on recombinant DNA technology to predict risks and long-term effects for gene therapy should be done,
- The advantages of gene therapy should be increased by overcoming biological obstacles, immunological reactions, and potential negative consequences linked to vectors or treatment strategies.

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Conflict of interest

There is no any conflict of interest among authors.

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