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Review Paper

Gene transfer in eukaryotic cells: current applications and implications

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Abstract

Gene transfer is indispensable to modify the genome of organisms and enhance their overall performance as compared to the average performance of their ancestors. Gene transfer in eukaryotic cells gives way to decipher the genotypes responsible for different traits, agricultural enhancement and therapeutic strategy. The three commonly used gene transfer methods are physical, chemical and biological based ones. It is reported that the application of transgenenesis in livestock agriculture comprises of increment of growth rate, milk production and composition, feed usage and carcass composition, disease resistance, enhanced reproductive and prolificacy enhancement. Now days, liposome mediated gene transfer is believed to be the most promising widely used gene transfer method in eukaryotic cells. In principle, the DNA fragments encapsulated with liposomes adheres to the cell membranes which in turn will fuse with them to transfer genes or DNA fragments of interest. Thus, the DNA fragment of interest enters into the nucleus of a cell that will later undergo reproduction. In order to achieve maximum transfection efficiency in eukaryotic cells, it is essential to optimize several parameters including lipid to DNA charge ratio and DNA amount concentration. Liposome mediated gene transfer is relatively easy, direct, inexpensive, reproducible and efficient.

Keywords: Eukaryotes, Cells, Liposome, Gene.

Introduction

Gene delivery is interesting issue in bioscience and medicine[1]. Methods of gene transfer can be classified into Physical, Chemical and Biological mediated ones. Moreover, gene transfer methods can also be categorized into stable and transient. Transient transfection is a temporary expression of foreign gene which lasts only for several days. It may rapidly lost as DNA never integrates into DNA of the host Cell. However, Stable gene transfer/ transfection refers to gene expression maintained for the long term because of the foreign DNA integrates to the host genome[2]. It has been reported that there are conditions when and where one method of gene transfer works efficient as compared to the rest and their similarities and differences have been illustrated in the table below[2]. Chemical transfection was used to transfer genes into mammalian genes and is still among the most widely used methods to know the function of genes or gene products[3].

Among the non-biological methods, cationic liposomes and polycations are widely investigated[4,5]. In order to gethighestgene transfer efficiency, optimizing several parameters in liposome mediated transfection is necessary and the two most critical parameters are the lipid to DNA charge ratio and DNA amount concentration[6,7]. Successfulness of gene transfer requires proliferating and in good condition cells in stage of divisions. Hence, it was reported that DNA/RNA to be transfected might be of possibly with highest purity in a stage of high growth rate. Generally, liposome mediated gene transfer is advantageous in that it protects DNA/ RNA from nuclease digestion, has low cell toxicity, it is stable media as it stores nucleic acids due to encapsulation in liposomes and is highly reproducible and wildly applicable[7,41-43].

Gene transfer in Agriculture

Transgenesis in agriculture refers to genetic composition alteration of animals and crops with the aim of increasing their overall performance[8,9]. However, Releasing of genetically modified animals on farms seems to delay as compared to products from plants and the reasons include the cost required and the generation interval in livestock which is longer as compared to plants. Some studies also indicate that there is an opportunity for correcting genetic diseases and providing animals that fulfill the requirement set by customers[10,11]. There are different methods of gene transfer experienced in eukaryotic cells such sperm mediated and somatic cell cloning[12].

Retroviral vectors based and liposome mediated methods are relatively better biological and chemical gene expression classes respectively[13]. Different vectors like lentiviruses have been reported to be efficient in generation of transgenic cattle[13].

Gene transfer has been becoming applicable to select crops that have excellent disease resistance and better productivity.

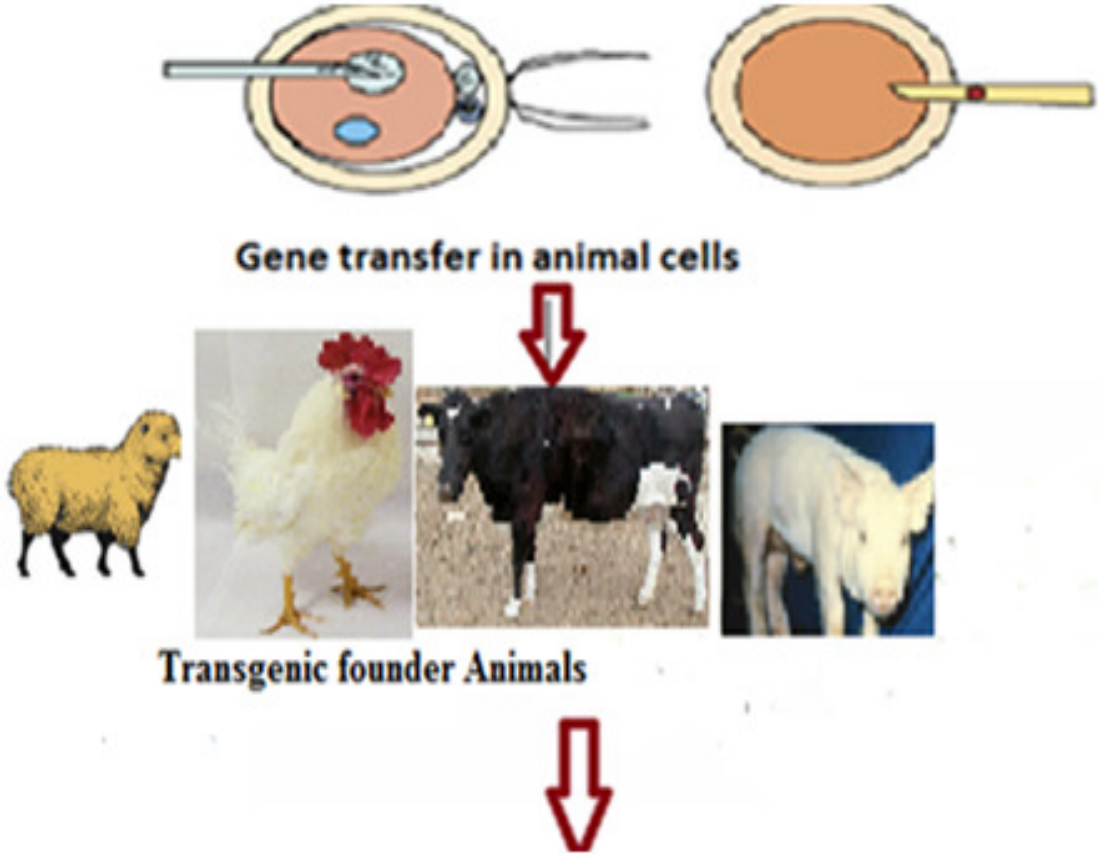
Moreover, gene transfer has also enabled to produce different transgenic livestock with increased production, reproduction and disease resistance[14-16]. Liposome based gene transfer aiming at facilitating foreign DNA uptake by Sperm in swine showed relatively in gene transfer efficiency[13].

Gene transfer and diseases therapeutics

There are different promising innovative researches that are in progress to find vaccines for different diseases like malaria and Ebola. It is also reported that clinical trials for AIDS vaccine programs have already begun in some countries. Gene therapy still requires several years before it will make a noticeable impact on different diseases treatments. The most noticeable limitations in gene transfer aiming at therapeutics include poorgene delivery and post delivery expression conditions. This is due to lack of understanding on the process through which vectors are constructed, availability of regulatory sequences and lack of knowledge on how to overcome in vivo immune defenses[1]. Gene therapy advancement is promising for cancer treatment and primarily in the area of melanoma followed by ovarian carcinoma, brain tumor and lung cancer[19-24]. Currently, there seems strong interest to embark on gene therapy trials with anticipation of curing people suffering from cardiopathies, neurologic diseases and AIDS in USA[25,26]. Gene transfer is potentially regarded as a means for vaccine development. Liposome nanoparticles can detour the efflux pumps of drug-resistant strains and decrease the drug efflux which has been reported to be potentially applicable against carcinoma.

Gene therapy entails adding a normal copy of a gene to the genome of an individual whose gene has got defective copies. Hence; this indicates the potential to treat genetic diseases in human beings. Gene therapeutics involves in vivo repair of a mutant and human genome disorder[26,29].

Gene transfer can be target fully conducted in order to treat individuals against diseases in clinical trials comprised: familial hypercholesterolemia, gene therapy for infectious disorders, ADA deficiency, melanoma, hemophilia B, cystic fibrosis, Fanconi's anemia, alpha-1-antitrypsin deficiency, Gaucher's disease, Hunter syndrome, ovarian carcinoma 22, sarcoma, brain tumor, and lung cancer, and LDL-receptor deficiency[30-33].



Improved milk production and composition
Improved growth rate, feed usage and carcass composition
Increased disease resistance, enhanced productive performance and prolificacy

Figure-1: Modified from (Wheeler, 2007), Agricultural applications for transgenic livestock[9].

Gene transfer and the biology of altered genotypes

There is recently developed gene targeting method that uses chimeric RNA/DNA oligonucleotides. This technique is believed to be relatively efficient way of in vivo induced site-directed mutagenesis and thereby knowing phenotype of the altered genotype [26].

Methods of gene transfer in Eukaryotic cells

The principle in gene transfer is that a gene of interest is transferred to the nucleus of the intended cell. Hence, that gene will be used as a template for mRNA synthesis and production of specific protein which is absent or mutated in a receipt's cell. There are vectors with a wide range of molecular sizes that are used to deliver genes of interest into mammalian cells[6]. Generally, there are various methods of genes transfer categorized into physical, Chemical and biological classes. These methods of gene transfer do have different characteristics as explained below[6,27,34].

Liposome mediated gene transfer

Liposomes are spherical particles consisting of lipid bilayers that impersonate biological membranes structures. The genes of interest aimed to transfer are packaged with liposomes in-vitro where in liposome will serve in transferring it to a target tissue in vivo and cationic liposomes are popular vesicles believed to play significant role intheraptic purpose[35,36]. In contrary to viral vectors, the DNA/liposome complexes are easy to prepare and are not size limited.

Liposomes are microscopically small sized particles. They contain phospholipid bilayers which are concentric in nature. Liposomes enclose aqueous chamber and can entrap water soluble molecules called lipid bags where many plasmids are enclosed in them. Liposomes are synthetic vectors becoming popular for gene transfer in Eukaryotic cells. Liposome mediated gene transfer is reproducible, uses simple and shortcut step and does not have the real risks implicit in virus vectors such as insertional mutagenesis, oncogene activation or virus-mediated pathology including encephalitis and other inflammations[37]. Although there are factors that affect their efficiencies, liposomes are believed to be highly effective in transfecting cultured cells and are able to carry varying amounts of DNA[34]. In contrary, liposomes are not thoroughly guaranteed from being degraded by cellular enzymes and nuclear transport with long-term high level transgene expression which attributed to the specific mode of action of lipid vesicles. Cationic liposomes probably fuse with cell membranes and later on release DNA into the cellcytoplasm[37], but the precise structures formed by lipids and DNA still remain somewhat controversial[34]. Transfection of vascular tissues in multiple animal models has been variably successful, but most previous attempts have used viral vectors[38]. Recent findings indicate that surface modifications of liposomes with polyethylene glycol (PEG) resulted in lipofection efficiency improvement[39-41].

Table-1: Summary of different gene delivery methods.

Category	Methods	Advantages	Disadvantages
Biological	Virus-mediated	High efficiency	Potential hazard to laboratory personnel
		Easy to use	Insertional mutagenesis - Immunogenicity
		Effective on dissociated cells, slices and in vivo	DNA package size limit
Chemical	Cationic polymer	No viral vector	Chemical toxicity to some
	Calcium phosphate	High-efficiency	Variable transfection efficiency by cell type or condition
	Cationic lipid	Easy to use	Hard to target specific cells
		Effective on dissociated cells and slices	
		Plenty of commercially available products	
		No package size limit	
Physical	Direct injection	Simple principle and straight forward	Needs special instruments
	Biolistic particle delivery	Physical relocation of nucleic acids into cell	Vulnerable nucleic acids
	Electroporation	No need for vector	Demands experimenter skill, laborious procedure.
	Leser-irridiation	Less dependent on cell type and condition	
	Sonoporation	Single-cell transfection	
	Magnetic nanoparticle		

Modified from; Tae etal, 2010[2]. Whitelaw, 2008[13]

Conclusion

Gene transfer in eukaryotic cells gives way to decipher the genotypes responsible for different traits, agricultural enhancement and therapeutic strategy. The potential benefits of gene transfer in eukaryotic range from the production of large quantities of pharmaceutically relevant proteins to agricultural improvement, therapeutics against different diseases and to decipher the functional biology although it is time-consuming and expensive because of the inefficiency of the existing techniques. Different studies in different countries are currently undergoing to find vaccines for different diseases. However, gene therapy has to address the development of efficient in-vivo gene delivery systems. Other factors being there, types of cells are fundamental for an efficient way of capturing and transfer of gene of interest. Generally, there are different types of gene transfer methods under the three; Physical, Chemical and Biological categories. Of the different methods, liposome mediated gene transfer is a very nice and promising non viral gene transfer method in Eukaryotic cells. It is simple, direct, and relatively cheap and easy to apply. In contrast to biological vectors, it does not require ex-vivo manipulation. Generally, there will be the need to explore fast track and effective gene delivery and transfer methods for their wide range employment in the Biological and developmental sciences, agriculture and manufacturing as well as Biomedical therapeutics.

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