How To Choose the Optimal Gene Delivery Method

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1. Introduction

The facilitated transfer of nucleic acids into eukaryotic cells is one of the most valuable and frequently used techniques of modern biological science. In today's laboratories, this technique is performed for multiple purposes, including gene therapy research, studies of gene regulation, protein structure/function analyses, as well as production of recombinant proteins. There are currently a wide variety of gene transfer methods available, and more are being developed all the time. Choosing the best method for a given application can be a daunting task, particularly when delivering nucleic acids to cells or tissues for which reliable protocols have not yet been established. This article will review the most common gene transfer methods, and help you decide which is best suited to your application.

The numerous gene delivery procedures currently available can be categorized in many different ways. One useful approach is to divide the techniques according to whether a chemical method (termed *transfection*) or a viral method (termed *transduction*) is employed. An additional category of techniques that utilizes physical/mechanical means for gene delivery will also be reviewed.

2. The Early Development of Facilitated Gene Delivery

The efficient transfer of nucleic acids into living cells has been a goal of scientific research for many years. As early as the mid-1950's, it was shown that cells could uptake nucleic acids extracted from viruses and express them as proteins (1, 2). In 1965, Vaheri and Pagano demonstrated that DNA could be delivered into cells when it was mixed with the cationic polymer derivative, DEAE-dextran (3). However, it was not until 1973, when Graham and Van Der Eb published the DNA-calcium phosphate co-precipitation method, that gene delivery became a routine tool for biological research (4). With the birth of recombinant DNA techniques in the 1970's, the existing gene delivery methods helped open a new era in biology that led to the creation of the modern biotechnology industry. Subsequently, transfection methods became further advanced in the late 1980s, when Felgner, *et al.* initiated the use of *cationic* lipids for high efficiency transfection (5). Recent years have seen an explosive growth of commercially available transfection reagents, including cationic lipids, activated dendrimers, and the polymer, polyethylenimine (PEI), as well as viral and mechanical gene delivery methods. We will now review the benefits and drawbacks of each gene transfer method to clarify the applications for which each method is most appropriate.

3. Chemical Methods of Gene Delivery

3.1. DEAE-dextran - Diethylaminoethyl-dextran (DEAE-Dextran) is a polycatonic derivative of the carbohydrate polymer, dextran. Because of its positive charge, DEAE-dextran is able to bind to the anionic phosphodiester backbone of DNA. The resultant complex maintains an overall cationic charge and is able to bind to negatively charged cell membrane surfaces. Subsequently, the complex is internalized, presumably by endocytosis. Among the advantages of the DEAE-dextran transfection method are its simplicity, reproducibility, and low cost. Though less popular today due to the advent of more efficient transfection methodologies, DEAE-dextran is still used by some laboratories, particularly for certain cell types, such as primary cultured human macrophages (6). Major drawbacks of this method are the limited range of cell types with which it works effectively, its lack of efficiency in creating stable cell lines, and its toxicity, especially when DMSO or glycerol is used as a supplemental chemical shock to increase gene transfer efficiency (7). DEAE-dextran is therefore appropriate when transfecting

cell types that have already been proven to efficiently uptake DNA administered via this method, or when higher transfection efficiencies are not required.

3.2. Calcium Phosphate - Calcium phosphate co-precipitation has been one of the most popular and widely used methods for DNA transfection since its introduction by Graham and Van Der Eb in the early 1970's. This technique entails mixing DNA with calcium chloride, and then carefully adding this mixture to a phosphate buffered saline solution followed by incubation at room temperature. This generates a DNA-containing precipitate, which is then dispersed onto cultured cells. The precipitate is then taken into the cells via endocytosis or phagocytosis. The main advantages of the calcium phosphate method are its simplicity, low cost, and its applicability to a wide variety of cell types. Moreover, unlike DEAE-dextran, it can be used to generate stably transfected cell lines, allowing for long-term gene expression studies. Among the disadvantages of the calcium phosphate method are its sensitivity to slight changes in buffer salt concentrations, temperature, and pH, as well as its relatively poor transfection efficiency compared to newer transfection methods, especially in suspension cells such as lymphocytes. Calcium phosphate co-precipitation has a broader range of effectiveness than DEAE-dextran, however it typically does not achieve transfection efficiencies as high as cationic lipids, which will be discussed in the next section.

3.3. Cationic Lipids (Lipofection) - The use of various lipids for mediating gene delivery, known as lipofection, was studied as early as 1980 (8). Researchers found that when they mixed lipids with DNA in water, the lipids formed hollow spheres, called liposomes, with DNA entrapped in the aqueous center. When these liposomes were added to cells growing *in vitro*, some of the liposomes would fuse with cellular plasma membranes and be taken up into the cells via endocytosis. However, the effectiveness of these early liposomes was very poor since they did not bind to target cell membranes efficiently. Also, the endocytotic pathway by which the entrapped DNA entered the cells led to fusion with lysosomes and subsequently to degradation by the digestive enzymes therein. Thus, it was not until the pioneering use of *cationic* lipids by Felgner in 1987, that lipid-mediated transfection became truly feasible. Today, lipofection is probably the most commonly used gene transfer method. Cationic transfection lipids are typically composed of a positively charged head group, such as an amine, a flexible linker group such as an ester or ether, and two or more hydrophobic tail groups. When combined with DNA, cationic lipids spontaneously act to form structures known as lipoplexes, which are much more complex than simple liposomes. When prepared under appropriate conditions, lipoplexes maintain an overall positive charge, enabling them to efficiently bind to negatively charged cell surfaces. Subsequently, the lipoplexes enter cells via the endocytotic pathway. This pathway would normally result in fusion with lysosomes and degradation of the DNA. However, neutral "helper" lipids, such as dioleoylethanolamine (DOPE), are typically included with the cationic lipid, allowing entrapped DNA to escape the endosomes. From there, the DNA can make its way to the nucleus and gain access to the transcriptional machinery of the cell.

The main advantages of cationic lipid transfection reagents are their ability to transfect a wide range of cell types with higher efficiencies than previously developed transfection methods. Also, cationic lipids are valued for their ease of use, their reproducibility, their relatively low cost, and their relatively low toxicity. Additionally, while early cationic lipid formulations could not tolerate the inclusion of serum in the transfection medium, more recent formulations can easily tolerate serum concentrations of 5 to 10%, allowing for less stressful transfection conditions. These combined advantages have made cationic lipids the most widely used commercially available transfection reagents today (9).

Despite these advantages, cationic lipids have some limitations that render them less than optimal for certain gene delivery applications. For example, several types of primary cultured cells, such as primary neurons, primary dendritic cells, and primary endothelial cells remain recalcitrant to non-viral mediated transfection methods, including cationic lipids. Also, the application of cationic lipid to *in vivo* gene delivery remains a difficult proposition, despite advances in lipid-mediated gene transfer to certain tissues such as lung endothelium. Among other reasons, this is due to the hampered ability of cationic lipids to deliver genes efficiently in the presence of high serum concentrations typically found *in vivo* (10).

3.4. Polymers - More recently, a variety of organic polymers have been utilized for transfection. One of the most popular is the polycation, polyethylenimine (PEI). PEI is an organic macromolecule that possesses a high cationic charge density, sometimes known as a “proton sponge”. It condenses DNA into positively charged particles that interact with anionic cell surfaces and enter cells via endocytosis. Because of the close proximity of many linker amino groups in PEI, it retains a substantial buffering capacity at a wide range of pHs. The extensive buffering not only protects DNA inside the lysosome from degradation, but it is also thought to lead to lysosomal swelling and rupture. This in turn provides an escape mechanism for DNA particles to the cytoplasm. Several PEIs have been shown to be effective in various *in vitro* and *in vivo* transfection applications, though they appear to have a narrower range of effectiveness in different cell types compared to cationic lipids.

Dendrimers are another class of polymer made of three-dimensional, bifurcated, branched structures called dendrons. The polyamidoamine (PAMAM) family of dendrimers (also referred to as starburst dendrimers) has shown to be very useful for transfection. Because the spherical polycationic dendrimers are similar to histone clusters in shape and proportion, they can condense DNA to a small size and facilitate entry into cells. Transfection efficiency can also be enhanced by heat-induced degradation of dendrites at their amide linkages. It is also believed that dendrimers have the ability to inhibit lysosomal hydrolases via their pH buffering capacity, thereby stabilizing dendrimer-DNA complexes within the lysosomes. Dendrimers share many of the same advantages and limitations as cationic lipids, and they likely maintain a more consistent particle size once complexed with DNA.

3.5. Targeting Proteins & Peptides - Various protein and peptide sequences have been used to target, mediate, or enhance delivery of nucleic acids in a wide range of applications. Such proteins and peptides are frequently used in combination with cationic lipids. For example, an integrin-targeting peptide has been shown to increase the transfection efficiency of lipid/DNA complexes by two orders of magnitude in a variety of cell types (11). Lipid/DNA complexes containing transferrin show significantly enhanced transfection efficiencies in a variety of primary cultured cells (e.g., lymphocytes, hippocampal neurons, cardiac endothelial cells, and macrophages) compared to DNA/cationic lipid complexes alone (12). In another example, complexation of the polycation peptide, protamine sulfate, with DNA followed by addition of cationic lipids enhances transgene expression in cultured cells compared to delivery with lipid alone (13). Fusogenic peptides, such as GALA (14) and the N-terminal peptide of influenza hemagglutinin HA2 subunit (15) have also been shown to increase transfection efficiencies when added to cationic lipids. Finally, nuclear localization signal (NLS) peptides, such as those derived from SV40 large T antigen, Xenopus nucleoplasmin, HIV Rev and Tat proteins, and the M9 sequence from hnRNP A1, can significantly enhance transfection by facilitating nuclear translocation of transfected DNA (16, 17).

The main benefits of using targeting proteins and peptides are both enhanced transfection efficiencies and targeted delivery. These benefits are particularly attractive for researchers attempting to transfect recalcitrant cells or to apply gene delivery techniques to *in vivo* applications. However, drawbacks of using such proteins and peptides include possible generation of strong immunological responses *in vivo*, as well as their high cost compared to more standard transfection reagents.

4. Viral Vectors for Gene Delivery

Despite the many advances in gene transfer technology, several important cell types have remained, to a greater or lesser degree, resistant to transfection via chemical gene delivery reagents. Since numerous applications require introducing foreign genes into as many cells as possible, many researchers have turned to the use of genetically engineered viruses for high efficiency gene delivery. Viruses have the ability to insert their genetic material into cells, and then hijack the cell's biosynthetic machinery for replication and protein synthesis. Essentially, viruses can be seen as highly evolved gene delivery vehicles. Thus, it was natural that scientists would look to viruses for overcoming difficult transfection obstacles.

4.1. Retroviruses - Retroviruses, particularly those derived from the Moloney murine leukemia virus (MoMLV), are probably the most widely used viral vectors for gene therapy applications (23). Retroviruses carry their genetic material of approximately 8.5 kb in the form of RNA, which is surrounded by a complex protein coat and lipid bilayer. Upon cell entry, the RNA is converted to DNA by the enzyme reverse transcriptase. Retroviruses only infect non-dividing cells because they are unable to pass the nuclear membrane until it breaks down during cell division. This is both an advantage and a disadvantage in terms of transgene delivery. It is an advantage for those applications in which rapidly dividing cancer cells are targeted. However, it is a disadvantage due to the numerous types of non-dividing cells that cannot be targeted. Another key characteristic of retroviruses is the ability to integrate their genetic material into the host cell's chromosome. This presents important advantages in terms of enabling long-term expression of therapeutic transgenes. On the other hand, it is a distinct drawback due to the potential for disrupting important endogenous genes by insertional mutagenesis. Finally, retroviruses have the capacity to accept transgenes inserts of approximately 8 kb or less, which limits their ability to deliver larger genomic sequences.

4.2. Adenoviruses - Adenoviruses (AdV's) are probably the second most commonly used viruses for gene delivery. They carry a double stranded linear DNA chromosome of approximately 36 kb. AdV's can be prepared at high titers and are stable in prolonged storage. They are able to infect a broad range of cells, both dividing and non-dividing. Unlike retroviruses, AdV's do not integrate their DNA into the host cell chromosome; instead, expression occurs from an episome in the cell nucleus. Although this is a definite safety advantage, it also means that AdV's do not support prolonged protein expression. Also, while the expression levels of AdV's are initially very high, they fall off rapidly in a matter of weeks to months. A key drawback of using AdV's is that they tend to elicit a strong immune response. Researchers have identified several key viral genes associated with the immune response and have begun development of new generations of less immunoreactive vectors. Various forms of AdV's have been designed with multiple deletions, and some "gutless" forms are now evolving as well (24). These gutless AdV's possess a transgene insert capacity of nearly the entire length of the viral chromosome.

4.3. Lentiviruses - Lentiviruses are a subclass of retroviruses, which possess some of the characteristics of adenoviruses. Lentiviruses can infect many different cell types, including

dividing and non-dividing cells. They integrate their genetic material into the host chromosomes, allowing for prolonged transgene expression. For instance, animal studies have shown that lentiviral vectors injected into a variety of tissues produced sustained protein expression for more than 1 year (25). They also possess complex envelope proteins, which allows highly specific cellular targeting. For example, the most well known lentivirus, HIV, targets the CD4 surface protein of T helper cells. In addition, they have little immunogenicity *in vivo*.

Although lentivirus vectors have many advantages over other viral vectors, and they are relatively safe to work with, researchers still remain cautious about their use due to the societal stigma attached to them. In response to concerns from government agencies and the general public, scientists have focused on the development of ever-safer versions of lentiviral vectors. For instance, genes encoding viral accessory proteins have been removed in order to ensure viral replication will not occur (26). Such precautions, while important for addressing safety concerns, may result in difficult preparation procedures. Recent generations of lentivirus require up to four separate transcriptional units for preparation of recombinant virus in mammalian cells (27). In addition to these issues, the maximum insert capacity of lentivirus is limited at ~ 8 kb.

4.4. Adeno-Associated Viruses (AAV) - Adeno-associated viruses, a subgroup of the parvovirus family, are the smallest known human viruses. They contain a single DNA strand of approximately 4.7 kb surrounded by a protein coat. At least 85 percent of adults are seropositive for AAV, but no specific disease has been linked to this virus. Thus, AAV can potentially be used for therapeutic gene delivery applications without generating a significant adverse immune response. Moreover, they infect a wide range of dividing and non-dividing cells, and are able to integrate into the host genome. One of the main advantages of AAV is that they only integrate at a specific site on human chromosome 19, which limits the possibility of insertional mutagenesis, while still providing the opportunity for long term expression. However, the key disadvantage with AAV is that its 4.5 kb genome allows for a very limited insert capacity (28). This has proven a formidable barrier to its more prevalent use. Also, the production of AAV vectors is dependent on a helper virus (AdV, HSV, or vaccinia) for replication, and it can be difficult to produce pure stocks of AAV free of contaminating replication-competent helper virus.

4.5. Vaccinia - Vaccinia is a large double stranded DNA virus containing a genome of approximately 200 kb with a complex envelope of proteins and lipids. It can accept large inserts of up to 25 kb, and it can infect nearly any mammalian cell types. Vaccinia is a non-integrating virus, with short-term expression (1-4 weeks). However, the expression levels are quite high during this time. The major disadvantage of vaccinia vectors is that they induce a strong cytotoxic T-cell response, and as a result, they are not used for treating chronic diseases. However, vaccinia does have potential for therapeutic treatment of solid tumors because of its mechanism of action.

4.6. Herpes Simplex Viruses (HSV) - Herpes simplex virus is a linear dsDNA virus with a genome of approximately 150 kb and a large insert capacity of 30 kb. One of the most attractive features of HSV vectors are their ability to infect a wide variety of cells, both dividing and non-dividing, including neurons. Also, they can be conveniently cultured to high titer stocks. HSV is a non-integrating virus, but it can persist in the cytoplasm for years in a latent episomal state. A disadvantage of HSV is the high cytotoxicity of some residual viral proteins. Gutless vectors have been prepared by deletion of Immediate-Early (IE) genes to reduce cytotoxicity, but this has generally resulted in lower titers. Recently however, an HSV genome missing only the cleavage and packaging signals was cloned into a bacterial artificial chromosome (BAC) vector.

This eliminated the need for helper viruses because all the viral genes could be included in one large vector, thus allowing high titer production of replication defective virus (29).

5. Mechanical or Physical Gene Delivery Methods

In addition to chemical and viral gene transfer techniques, several methodologies utilizing physical or mechanical means for translocating nucleic acids into cells have been developed. Such techniques have the advantage of avoiding the introduction of foreign substances, i.e., chemicals or viruses, into the target cells or tissues and therefore offer a “cleaner” alternative approach to gene delivery.

5.1. Microinjection – Conceptually, microinjection is the simplest gene delivery method, but one of the most difficult to apply. It entails the direct injection of DNA into the nuclei of target cells using fine glass needles under microscopy (18). While this method of gene transfer is the nearly 100% efficient, it is painstakingly tedious and time-consuming, typically allowing only a few hundred cells to be transfected per experiment. On the other hand, some recent advances in this technology utilizing automated systems have promised to increase the speed and decrease the labor required. Unfortunately, such technology is very costly. Therefore, the ideal application of microinjection involves any experiment requiring only a small number of cells (e.g.,< 500) to be transfected.

5.2. Electroporation - Electroporation is a method of introducing nucleic acids into cells by exposing the cells to a rapid pulse of high-voltage current, causing pores in the cell membrane to open temporarily. This allows exogenous DNA to pass through the pores and into the cytoplasm of the cells. Typically, the gene transfer efficiency is relatively low, and electroporation frequently results in a high incidence of cell death. Nevertheless, with electroporation there is practically no limit to the size of DNA that can be delivered. In addition, there are few restrictions on the cell types to which this technique can be applied. (19) Recently, advances in electroporation technology have expanded its use to include gene delivery to explant tissues and organs (20), and even *in vivo* gene delivery (21).

5.3. Gene Gun - One method for the direct introduction of DNA into various tissues is the “gene gun”. In this method, plasmid DNA is coated onto metal microparticles and then blasted into cells using either electrostatic force or gas pressure (22). Some of the DNA becomes trapped by a few cells, and may then be expressed to sufficient levels. This technique is fast, simple and safe, and it can transfer genes to a wide variety of tissues. Also, there appears to be no limits to the size or number of genes that can be delivered. However, it can be a challenge to obtain a sufficient number of cells modified by this method to see a biologically significant effect.

5.4. Naked DNA - In the late 1980s, researchers at the University of Wisconsin accidentally discovered that when plain DNA, without any delivery reagent, was introduced to animals in a control experiment, significant expression levels could be detected. The reason why such “naked DNA” is capable of protein expression remains unclear. It is possible that a small amount of tissue damage or increased pressure at the injection site enables the DNA to be assimilated by some cells.

Although direct injection of naked DNA can lead to gene expression *in vivo* and can be used to treat certain genetic diseases, the overall level of gene translocation is much lower than that obtained with either virus or liposome-mediated delivery methods. Also, naked DNA is

susceptible to degradation by serum nucleases. As a result, the use of naked DNA seems to be limited to only a few applications involving easily accessible tissues such as skin, muscles and hard tumors.

6. Factors to Consider When Selecting a Gene Delivery Method

The advancement of transfection and transduction technologies has made gene delivery both more efficient yet more confusing given the formidable array of reagents and methods now available. Determining the best gene delivery approach for any particular experiment requires careful consideration of a number of different factors, which will be reviewed below.

6.1. Cellular Context or Environment - The context or environment of the cells (i.e., in vivo vs. in vitro vs. ex vivo) to which nucleic acids will be delivered is the first factor to consider when deciding upon a gene transfer method. The obstacles and challenges posed by delivery to *in vitro* cell cultures are very different than those posed by *in vivo* or *ex vivo* cells, and require different tools and techniques for overcoming them. For example, targeting nucleic acids to particular cells while avoiding other cells is *usually* a major concern for *in vivo* gene transfer applications, *sometimes* a concern for *ex vivo* applications, but *usually not* a concern for *in vitro* applications. Cell targeting can be addressed either by the use of targeting peptides, such as ligands for cell specific surface receptors, or by the use of viral vectors that are especially efficient at transducing the intended cell type but not other cell types. Another possibility is the use of retroviruses and cationic lipids, which are particularly proficient at delivering genes to dividing cells, such as cancer cells or immortalized cell lines but not to terminally differentiated cells.

Another important issue that must be addressed when planning *in vivo* (but not *in vitro*) gene delivery studies is the immunogenicity of the gene transfer system. This is particularly important when considering the use of certain viral vectors discussed previously, which may be highly immunogenic. In contrast, most of the popular synthetic transfection reagents do not elicit strong immune responses upon injection *in vivo*. Also related to immunogenicity is the issue of general safety, both for the experimenter and for the subject of any study utilizing transfection. Almost always, the use of viral vectors requires more (often very extensive) safety precautions than does the use of synthetic transfection methods.

6.2. Cell or Tissue Type - The origin of the cells or tissue to which nucleic acids will be transferred is typically the second parameter considered when selecting a gene delivery method. For a number of different reasons, the difficulty of cellular nucleic acid delivery varies from cell type to cell type. Because of this, it is always important to review the literature to determine if the cell type of interest has previously been utilized for gene transfer experiments, which exact methods have been used, and what delivery efficiencies were achieved, i.e., what percentages of cells actually transfected or transduced, with each method. (Since delivery efficiencies are frequently *not* noted in published research, it may be necessary to contact the corresponding author to obtain this information. It is also very helpful to contact gene delivery reagent manufacturers for listings of cell types and corresponding efficiencies obtained with their reagents). Certain cell types are often described as “notoriously difficult to transfect”. These include primary cell cultures of many varieties. Successful gene transfer to such cells usually requires a good deal of research before selecting any particular gene delivery method.

6.3. Delivery Efficiency - For most applications, transfecting or transducing as many cells as possible is preferred, especially when it is necessary to detect the presence of rare transgene products. Thus, it is important to determine the level of delivery efficiency required for a given application before deciding on a particular gene transfer method. In most cases, it is found that viral vectors provide the highest delivery efficiencies (though this may not be the case in every circumstance, particularly *in vitro*). For some commonly used and easily transfected cell lines, such as HEK293, COS-1, COS-7, CHO-K1, cationic lipids nearly match viral vectors in their gene transfer efficiency, while providing safer and much quicker procedures.

6.4. Stable vs. Transient Gene Delivery - Gene transfer procedures are frequently categorized by whether the delivered gene remains separate from the host cell chromosome or whether it is integrated into the host cell chromosome. In the first case, known as transient gene delivery, expression of the transgene typically dissipates after a given period of time - usually within several days - because the expression vector is either degraded or expelled from the host cell. In the second case, known as stable gene delivery, expression of the transferred gene is prolonged, or stable, because the vector is integrated into the host cell chromosome. Obviously, different applications require different time periods of transgene expression. Thus, a careful comparison between different gene delivery methodologies will allow a suitable choice for generating the desired transient or stable expression.

In the case of viral vectors, determining whether a particular virus is suited for stable or transient transduction is simply a matter of learning the general characteristics of the vector. In the case of chemical or physical/mechanical gene transfer, determining whether a given method is suitable for transient vs. stable delivery may be as easy as reading sales literature from the manufacturer or checking primary research literature.

6.5. Type of Delivered Molecule - Another factor to consider when selecting a gene delivery method is what type of nucleic acid or other molecule is being transfected or transduced. For example, a reagent that efficiently delivers plasmids may not efficiently deliver DNA oligonucleotides. Similarly, a reagent that efficiently delivers short interfering RNA (siRNA) or proteins may not efficiently deliver plasmids. The factors that determine the transfer efficiency of one reagent over another for a given type of molecule are frequently unknown, and the best reagent is often determined empirically. Because of this, it is frequently easiest to simply review the literature to learn what methods have been used successfully, and to contact other researchers who have experience delivering the molecule of interest. As the saying goes, why reinvent the wheel?

6.6. Cytotoxicity - Another issue that comes up frequently when choosing among different gene delivery methods is the cytotoxicity of the various methods and reagents. Some methods, such as electroporation and gene guns, can be extremely harsh on cells and often result in the death of a majority of cells. Also, the DEAE-dextran can be quite harsh on some cell types, especially when a DMSO or glycerol shock step is used as part of the procedure. In some applications, such as when a small number of transfectants are required from a recalcitrant cell type, this is not a problem. In the majority of applications however, a high percentage of cell death is simply not acceptable. The plethora of available cationic lipids and polymer reagents have varying degrees of toxicity with different cell types, and their concentrations during transfection can be optimized as needed to minimize cell death. The protocol supplied by the manufacturer with a reagent or method will usually provide guidance for optimization. Also, checking the published literature for references before selecting a given method is, once again, a wise approach.

6.7. Suspension vs. Adherent cells - Most commonly transfected cells grow - and are transfected - while attached to some type of support, whether that support is the tissue from which they are derived, some inert membrane or scaffolding, or the surface of a tissue culture plate. Less common are cells that grow while suspended in medium, such as those derived from blood and other bodily fluids. Suspension cells are generally more difficult to transfect; therefore, it may be necessary to rely on harsher gene delivery methods, such as electroporation, gene guns, microinjection, or viral vectors for acceptable transfection levels. Also, some cationic lipids can effectively transfect suspension cells, and should be tried when possible due to their simplicity and cost-effectiveness. Again, it is best to refer to previously published work and manufacturers technical literature for more information.

6.8. Expertise - The degree of difficulty in performing the various transfection techniques can vary significantly, and therefore the expertise and experience of the researcher is usually an important factor to consider. For example, viral transfection methods are invariably more complex and difficult to complete than chemical or physical/mechanical methods. Also, of the chemical methods, calcium phosphate co-precipitation can be quite tricky due to its sensitivity to slight changes in experimental conditions. Generally speaking, cationic lipids and polymer reagents are the easiest to use because they have well developed protocols and the fewest variables subject to experimental error.

6.9. Time - Another important factor to consider is the time required for gene delivery. Viral methods, while highly efficient, can be quite time consuming, typically taking anywhere from two weeks to one month to complete. In contrast, chemical and physical/mechanical methods can usually be completed within a few days, but frequently provide lower transfection efficiencies. Thus, it is not uncommon to have to weigh the benefits of faster results and less labor against the benefit of higher efficiency.

6.10 Cost - Finally, cost can be a major factor when deciding on the best gene delivery method for a given application. The most expensive methods tend to be the physical/mechanical methods, such as electroporation and gene guns, due to the specialized equipment they require. Such methods frequently cost several thousand dollars to complete once the price of the equipment and all accessories are taken into consideration. Viral gene delivery is perhaps the second most expensive method, since it requires the vector reagents, extensive time and labor to complete lengthy vector preparation procedures, as well as proper disposal of viral waste materials. Viral gene delivery can cost anywhere from several hundred to several thousand dollars, depending on the virus used and the degree of difficulty involved in preparing the recombinant viral vector. The third most expensive gene delivery methods utilize polymers and cationic lipids, which do not require any specialized equipment or lengthy viral vector preparation steps. Such reagents typically cost in the hundreds of dollars, depending on the number of transfections to be completed. Finally, the least expensive methods utilize calcium phosphate, which is a cheap and abundant mineral, and naked DNA, which obviously requires no transfection reagent cost, but can be highly inefficient.

Table 1: Gene Delivery Methods Summary and Comparison

Chemical Methods	Recommended Cell Type / Tissue Source	Cellular Context	Length of Expression	Relative Efficiency	Type of Delivered Molecule	Suspension vs. Adherent	Toxicity	Expertise Required	Time Required	Cost
DEAE-Dextran	Easily transfected cell lines, some primary cells	*in vitro*	Transient	Low	Plasmids Oligos MRNA	Adherent	Moderate	Low	1-3 days	< $100
Calcium Phosphate Co-Precipitation	Easily transfected cell lines	*in vitro*	Transient or Stable	Low	Plasmids Oligos MRNA	Adherent	Low	Moderate	1-3 days	< $100
Cationic Lipids	Easy to difficult cell lines, some primary cells	*in vitro* *in vivo* *ex vivo*	Transient or Stable	Moderate	Plasmids Oligos mRNA siRNA Proteins	Adherent and Suspension	Moderate	Low	1-3 days	$100 - $500
Polymers	Easy to difficult cell lines, some primary cells	*in vitro* *in vivo* *ex vivo*	Transient or Stable	Moderate	Plasmids Oligos mRNA	Adherent and Suspension	Low	Moderate	1-3 days	$100 - $500
Proteins & Peptides	Difficult cell lines & primary cells	*in vitro* *in vivo* *ex vivo*	Transient or Stable	Moderate	Plasmids Oligos DNA mRNA	Adherent and Suspension	Low	Moderate	1-3 days	$500 - $1000
Physical / Mechanical Methods	Recommended Cell Type / Tissue Source	Cellular Context	Length of Expression	Relative Efficiency	Nucleic Acids Type	Suspension vs. Adherent	Toxicity	Expertise Required	Time Required	Cost
Microinjection	Difficult cell lines or primary cells	*in vitro* *in vivo* *ex vivo*	Transient or Stable	High	Plasmids Oligos mRNA, siRNA	Adherent	Low	Moderate	1-3 days	>$1000
Electroporation	Difficult cell lines or primary cells	*in vitro* *in vivo* *ex vivo*	Transient or Stable	Low – Moderate	Plasmids Oligos mRNA, siRNA	Adherent and Suspension	High	Moderate	1-3 days	>$1000
Gene Gun	Difficult cell lines or primary cells	*in vitro* *in vivo* *ex vivo*	Transient or Stable	Moderate	Plasmids Oligos mRNA, siRNA	Adherent and Suspension	High	Moderate	1-3 days	>$1000
Naked DNA	*in vivo* and *ex vivo* cells	*in vivo* *ex vivo*	Transient	Low	Plasmids	Adherent and Suspension	Low	Moderate	1-3 days	$100 - $500
Viral Methods	Recommended Cell Type / Tissue Source	Cellular Context	Length of Expression	Relative Efficiency	Nucleic Acids Type	Suspension vs. Adherent	Toxicity	Expertise Required	Time Required	Cost
Retrovirus	Primary cells & difficult cell lines	*in vitro* *in vivo* *ex vivo*	Stable	High	RNA	Adherent and Suspension	Low	High	1-3 weeks	$500-$1000
Adenovirus	Primary cells & difficult cell lines	*in vitro* *in vivo* *ex vivo*	Transient	High	DNA	Adherent and Suspension	Low	High	1-3 weeks	$500 – $1000
Lentivirus	Primary cells & difficult cell lines	*in vitro* *in vivo* *ex vivo*	Stable	High	RNA	Adherent and Suspension	Low	High	1-3 weeks	~$1000
Adeno-Associated Virus	Primary cells & difficult cell lines	*in vitro* *in vivo* *ex vivo*	Stable	High	DNA	Adherent and Suspension	Low	High	1-3 weeks	$500-$1000
Vaccinia	Primary cells & difficult cell lines	*in vitro* *in vivo* *ex vivo*	Transient	High	DNA	Adherent and Suspension	Low	High	1-3 weeks	?
Herpes Simplex Virus	Primary cells & difficult cell lines	*in vitro* *in vivo* *ex vivo*	Transient	High	DNA	Adherent and Suspension	Low	High	1-3 weeks	?

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