

Delivery Techniques in Gene Therapy: A Brief Overview

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Abstract

The use of gene therapy is rapidly gaining ground in the field of immunotherapy and the correction of monogenetic disorders. An ongoing challenge to the successful implementation of gene therapy to human subjects remains safe and efficient transfer of the plasmid to the host. Strategies for gene transfer include the use of viral vectors or synesthetic transfection systems incorporating physical, chemical, or electrical methods. While viral vectors remain the standard for transduction efficiency, synthetic systems offer potential safety advantages from an immunological perspective.

Keywords: Gene therapy; Synthetic; Transfection; Transduction; Electrotransfer

Introduction

Gene therapy holds promise for correcting host pathology through manipulation of DNA expression. Gene therapy can involve *transfer*, *repair* or *silencing*. Transfer of genetic material enables the expression of a particular functional protein within the host. Genetic repair attempts to correct a flaw in host DNA, while silencing switches off production of a pathogenic protein. There has been particular interest in gene transfer for correcting monogenetic disorders such as severe combined immunodeficiency and Haemophilia B, with a number of human trials already performed [1-4]. There is also much interest in gene transfer therapy in solid organ transplantation for the induction of transplant tolerance through modulation of co-stimulatory pathways, manipulation of cytokine expression and apoptosis pathways, immunomodulation via enzyme expression, leukocyte migration, and transfer of transduced antigen presenting cells and/or lymphocytes [5].

Gene Transfer Techniques

The transfer of genetic material can be accomplished *in vivo* through local or systemic inoculation or *ex vivo* where the target of interest is collected and modified outside of the organism before return to the host. Transfer of synthetic DNA can be accomplished by transduction or transfection. Such methods of transfer include either direct injection of DNA into the recipient cells, or utilising methods to induce membranes permeabilisation, receptor-mediated uptake or endocytosis. Transduction utilises recombinant virus as a vector for gene transfer. Entry of these vectors is mediated by cell-surface receptors. Concerns regarding the immunogenicity of viral vector systems due to activation of memory responses against constituent viral proteins or a primary response to neoantigens has spawned the evolution of synthetic gene delivery systems which exploit transfection, the transfer of DNA via physical, chemical or electrical methods [6,7]. Benefits of non-viral methods for DNA transfer include a reduction of risks associated with viruses (immune response, insertional mutagenesis) and limitations to gene delivery (such as length of the transgene cassette) [8].

Physical approaches range from microinjection into individual cells or the direct injection of DNA into tissues (i.e. muscle). Generally such techniques result in low level, variable expression. Others include the use of a 'gene gun' which pushes a stream of plasmid-coated gold microparticles through the skin or the use of ultrasound to permeate cells (sonoporation) [9,10].

Chemical methods (i.e. calcium phosphate) have also been employed, and are commonly used in the laboratory setting. Isolated DNA combined with calcium chloride and potassium phosphate can

produce a calcium phosphate DNA precipitate, which will be taken up by a fraction of cells after incubation [11]. The main drawbacks are low frequency of transduction, reduced cell viability and random integration into the host genome.

Electrical methods (electrotransfer) are more well-established. Applying an electrical field to cells alters the resting transmembrane potential, which can induce permeability though the formation of reversible structural membrane changes (electropores) [8]. A large number of animal studies have been performed across on a range of tissues, with the main application being immunotherapy (summarised in [8]). Therapeutic levels of gene expression have been achieved, as well the cotransfer of multiple plasmids [12]. Although more efficient than chemical or physical methods, the efficiency of electrotransfer is still less than that seen with viral vectors.

The choice between transfection strategies compared to transduction with a virus will largely depend on the therapeutic goal. For transient gene expression or repeat dosing scenarios, synthetic delivery systems herald obvious advantages. Conversely, correction of missing protein disorders which require long-term, stable gene expression may be better served by viral vectors which can lead to integration of the transgene with host DNA and more stable constitutive protein expression. Synthetic delivery holds potential benefits in terms of safety, low frequency of gene integration, ability to introduce larger portion of genes and ease of production [13,14]. Another consideration is the efficacy of expression: in general, viral vectors achieve higher efficiency of expression than synthetic systems [15,16]. The development of artificial viral systems (synthetic viruses) remains a future strategy to harness the advantages of viral and synthetic systems.

Synthetic Delivery Systems

The plasmid DNA of interest is susceptible to rapid degradation by biological enzymes necessitating that it be packaged for protection [17]. Synthetic delivery systems accomplish this using polycationic

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polymers and/or cationic lipids which may be complexed with nucleic acid (polyplex, lipoplex or lipopolyplex systems) or lipid encapsidation (liposomes) [18]. The use of cationic packaging takes advantage of the anionic properties of DNA to form an electrostatic bond between the plasmid of interest and the protective coating. The final DNA-packaging construct usually measures between 100 nanometers to 1 micrometer [14]. By using an excess of cationic charge in the packaging molecules, the chance of leaving uncoated anionic DNA is reduced and the final complexes then tend to repulse from one another, minimising the change of precipitation [14]. A consequence of using positively charged particles as a delivery system is their tendency to interact with negatively charged particles. This effect has been demonstrated with synthetic vectors binding to extracellular glycosaminoglycans and heparin sulphate receptors, altering transfection efficiency and distribution [19-21].

Targeting Gene Delivery

An important consideration in gene therapy is ensuring that the pharmacophore is delivered to an area that maximises its therapeutic benefit. This can be especially complex in the living organism due to shared receptors between tissues, circulatory anomalies (such as the blood-brain barrier) and ability of serum proteins to destabilise synthetic vector complexes [14]. In some cases, direct application of the vector to the dysfunctional tissue may be required to maximise effect. In the case of a cationic liposome complexed to plasmid DNA encoding chloramphenicol acetyltransferase, direct injection into murine hepatic tumours resulted in higher levels of gene expression than were achieved with systemic or portal vein inoculation [22]. Lipoplexes complexed to the bcl-2 gene have demonstrated reduced neural apoptosis after transient cerebral ischaemia in an animal model, circumventing the blood-brain barrier by utilising direct intra-thecal injection [23]. Furthermore, the cystic fibrosis transmembrane regulator gene has been successfully packaged with both cationic liposomes and polymers and safely delivered intranasally to cystic fibrosis directly targeting airway mucosa [24-26].

Other targeting techniques include altering the charge of the synthetic vector-DNA plasmid particle: cationic liposomes have been shown to preferentially distribute to the lung after systemic administration, an effect which is lost which decreasing positivity [27]. Size also plays a role as large molecules may be unable to extravasate from the circulation to reach target cells within organ parenchyma. Additionally, constitutive expression of specific ligands on target cells can be manipulated to design advantage, for example the use of dextran-spermine polycation complexing with DNA to target the liver by preferential binding to galactose receptors on hepatic parenchyma [28].

Conclusion

The ideal synthetic vector can safely and reliably target the tissue of interest without systemic distribution or premature degradation, be taken up into the cell, and the synthetic components separated allowing the DNA plasmid to be transported to the nucleus and transfect the target cell. Efficiency of transfection using non-viral strategies remains a challenge which may be addressed by enhancing biodistribution biases and a more comprehensive understanding of endogenous ligand targets.

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