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Physical methods for gene transfer: Improving the kinetics of gene delivery into cells

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Abstract

One factor critical to successful gene therapy is the development of efficient delivery systems. Although advances in gene transfer technology, including viral and non-viral vectors, have been made, an ideal vector system has not yet been constructed. This review describes the basic principles behind various physical methods for gene transfer and assesses the advantages and performance of such approaches, compared to other transfection systems. In particular, the kinetics and efficiency of gene delivery, the toxicity, in vivo feasibility, and targeting ability of different physical methodologies are discussed and evaluated.

Keywords: Gene transfer; Electroporation; Sonoporation; Gene gun; Magnetofection; Ultrasound

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1. Introduction

With recent advances in molecular biology and the sequencing of the human genome, gene therapy is expected to assume a pivotal role in the treatment of genetic diseases. This innovative therapy involves the introduction of healthy copies of mutated or absent genes into target cells so as to promote the expression of normal protein and to restore correct cellular function. The development of gene therapy vectors with sufficient targeting ability, transfection efficiency, and safety must be achieved before gene therapy can be routinely used in man.

The ideal vector for gene delivery would have at least the following characteristics: (i) specificity for the targeted cells; (ii) resistance to metabolic degradation and/or attack by the immune system; (iii) safety, i.e., minimal side effects; and (iv) an ability to express, in an appropriately regulated fashion, the therapeutic gene for as long as required. In general terms, gene delivery methods can be sub-divided into two categories: (a) the use of biological vectors and (b) techniques employing either chemical or physical approaches. The first implicates viral-mediated processes referred to as infection. Retroviruses and adenoviruses are the most commonly used vectors and have already been tested in clinical trials. They offer several advantages, but also many undesired side effects, such as viral toxicity, host immune rejection, as well as being difficult to prepare [1,2]. Non-viral gene transfer, or transfection, involves treatment of cells by chemical or physical means. Chemical methods cover an array of complexes between DNA and diverse polycations ("polyplexes") or cationic lipids ("lipoplexes"). Technically, the approach is relatively straightforward and easily scaled-up, and it does not provoke specific immune responses. However, efficiency and targeting remain extremely poor.

A naked DNA injection, without any carrier, into local tissues or into the systemic circulation is probably the simplest and safest 'physical/mechanical' approach. However, due to rapid degradation by nucleases and fast clearance by the mononuclear phagocyte system, the expression level, and the area of tissue treated, after a naked DNA injection are severely limited [3,4]. Consequently, attention has turned to a number of other so-called 'physical' manipulations to improve the efficiency (rate and extent) of gene delivery. These methods have also attracted interest for their potential ability to circumvent various "barriers," which significantly compromise the efficiency of gene delivery, including massive dilution of DNA upon injection, accessibility of the target site, and entry into the cell and the nucleus.

In this review, the following physical methods for gene delivery are discussed: microinjection and particle bombardment (gene gun); electroporation, sonoporation, and laser irradiation; and magnetofection. After a brief description of each technique, their applicability to the enhancement of gene transfer, particularly with respect to the rate and extent of delivery, will be compared to other, especially non-viral, transfection techniques. Finally, we will discuss the advantages and limitations of these physical methods, in terms of the kinetics and efficiency of gene delivery, the toxicity, in vivo feasibility, and targeting ability.

2. Mechanical methods

2.1. Microinjection

The most direct method to introduce DNA into cells is microinjection, either into the cytoplasm or into the nucleus. This is a microsurgical procedure that is conducted on a single cell, using a glass needle