Bioorganic & Medicinal Chemistry Letters 25 (2015) 1171–1176

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

BMCL Digest

Conceptual and technical aspects of transfection and gene delivery

Lars Kaestner *, Anke Scholz, Peter Lipp

Institute for Molecular Cell Biology, Research Centre for Molecular Imaging and Screening, Saarland University, 66421 Homburg/Saar, Germany

ARTICLE INFO

Article history:
Received 13 October 2014
Revised 30 December 2014
Accepted 9 January 2015
Available online 28 January 2015

Keywords:
Gene transfer
Gene therapy
Transduction
Lipofection
Electroporation

ABSTRACT

Genetically modified animals are state of the art in biomedical research as gene therapy is a promising perspective in the attempt to cure hereditary diseases. Both approaches have in common that modified or corrected genetic information must be transferred into cells in general or into particular cell types of an organism. Here we give an overview of established and emerging methods of transfection and gene delivery and provide conceptual and technical advantages and drawbacks of their particular use. Additionally, based on a flow chart, we compiled a rough guideline to choose a gene transfer method for a particular field of application.

Genetically modified animals are state of the art routine in biomedical research as gene therapy is a promising perspective in the attempt to cure hereditary diseases. Both approaches have in common that modified or corrected genetic information must be inserted into cells in general or into particular cells of an organism.

The DNA needs to be available as a plasmid to be transfected. However, the topic of this Digest is not about cloning strategies but focuses on different methods of transfection and gene transfer and their conceptual application. Transfection can be categorized into physical, chemical and biological methods. In the history of gene delivery into cells, initial successful transfection was achieved with chemical methods.[1,2] The calcium phosphate co-precipitation method was examined by Graham and van der Eb[3] in the early 1970s and became a very popular method, which is still used (see below). An overview of selected transfection and gene-transfer methods is given in Table 1.

Physical transfection approaches include microinjection,[4] optical transfection,[5,6] particle guns (ballistic gene delivery),[7,8] electroporation,[9] sonoporation,[10] magnetofection[11] and electric field-induced molecular vibration.[12] The first two examples are methods where usually only one cell is transfected at a time. The disadvantage is an ultra low throughput, but the certainty that the cell of interest is indeed transfected. Therefore methods like microinjection are popular for gene transfer when only a limited number of cells are available, like a variant in the creation of transgenic animals when the DNA is directly injected into the male pronucleus. After the fusion of the pro-nuclei the diploid zygote nucleus is formed and the zygote is cultivated to the state of the two-cell embryo.

A particular interesting concept of physical transfection is laser-mediated transfection. This can be a direct membrane perforation,[13] methods taking advantage of nanoparticles[14] or techniques using wave-guided optical wave guides (WOW).[15] The idea of the latter is to use μTools that can be moved and navigated by the use of laser beams that are manipulated by programmable diffraction patterns resulting in so called holographic optical tweezers (HOT).[16] The μTool are designed such that their tip can be brought in contact with the cell of interest. The energy of laser-generated photons, which are fed into the tail of the μTool, and that travel through the intrinsic optical waveguides result in a thermoporation at the tip of the μTool (compare Table 1).

A further advantage is that physical transfection does not depend on particular chemical or biological cell properties[17] and therefore cells that are difficult to be transfected by other methods (see below), like cells of the immune system, for example, T-cells, can be successfully manipulated by physical transfection approaches. Methods that can be applied to cell suspensions, for example, electroporation, are particular popular.[17]

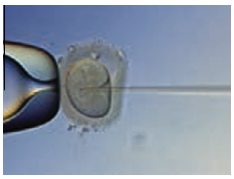
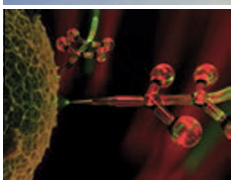
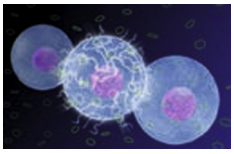
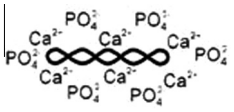
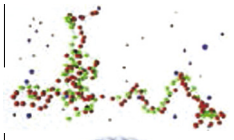
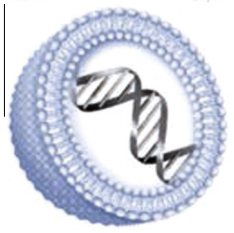
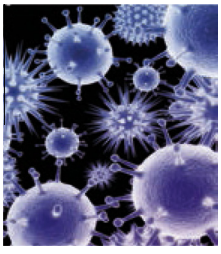
Chemical transfection methods are techniques that catalyze DNA cross-membrane transport through the use of Ca^{2+} phosphate,[18] polycations[19] or dendrimers.[20]

Transfection with Ca^{2+} phosphate is one of the least expensive methods and is therefore still applied, whenever large amounts of cells need to be transfected simultaneously, for example, for the production (and later purification) of particular proteins or virus (see below). The method is effective with many different cultured cell types.

* Corresponding author. Tel.: +49 6841 16 26103; fax: +49 6841 16 26104.
E-mail address: lars_kaestner@me.com (L. Kaestner).

http://dx.doi.org/10.1016/j.bmcl.2015.01.018
0960-894X/

Table 1
Overview of selected popular transfection and transduction methods

Method	Principle	Scheme	Advantages	Disadvantages	Field of application
Microinjection[4]	Direct injection of the genetic information into the nucleus of the target cell under visual (microscopic) control		Most direct transfection; after initial training high yield of success	Tedious, very limited number of transfected cells, more difficult on small cells	Making transgenic animals, studying RNA trafficking, immuno-cytochemistry
µTool based thermoporation[15]	µTools can be manipulated by laser beams and thermoperforate the cell by laser energy delivered to the tail of the µTool and wave-guided to its tip		Versatile tool with high potential for special requirements	Technical demanding; currently under development and only available in specialized labs	To be determined
Electroporation[9,10,17]	Application of changing electrical potentials to induce the formation of pores in the cell membrane		Established and effective method for otherwise hard to transfect cells in suspension and to some extend in tissue; often used for primary isolated cells	Needs specific adaptation and optimization of parameters for particular cell types; tendency for high level of damaged cells	Wide range, whenever a limited cell viability is tolerable; local transfection in vivo with specialized electrodes (needles)
Calcium phosphate[3,18]	After adsorption of the DNA, calcium phosphate coprecipitates to the cell surface and is taken up by phagocytosis		Cheap and easy to perform; large amounts of cells can be transfected	Relatively low transfection rate; primary cells and cells in suspension can hardly be transfected	Transfection for protein purification and similar approaches
Polycations[19]	The polycations form complexes with the polyanionic DNA molecules and these complexes are taken up by phagocytosis		Large size of DNA can be transfected and it should be useful in gene therapy when viral gene delivery is not useful because of the immune response	Sometimes poor efficiency; cytotoxicity for sensitive cells and high mutation rate of the DNA	Gene therapy together with drug delivery
Lipofection[21–24]	Vesicles of cationic lipids bind to DNA and positively charged complexes bind to the cell surface (negatively charged silica acid residues) followed by uptake into the cells		Simple and fast procedure with high reproducibility	Not suitable for most primary isolated cells	Most popular method in cell biology and related research fields
Dendrimers[20]	Positively charged dendrimers bind with the negatively charged phosphates of the DNA molecule (electrostatic) and the DNA–dendrimer complexes with a positive net charge are taken up by the cell		No or low cytotoxicity; high efficiency in numerous cell lines	Not suitable for most primary isolated cells	Often applied in combination with lipofection, therefore similar field of application
Receptor mediation[7]	Utilises endocytosis for uptake of proteins, DNA is bound to the ligand of the target receptor via a DNA binding moiety (like poly-L-lysin)		Cell specific transfection, very low cytotoxicity and reapplication possible	Effective transfection only possible with cells carrying a high density of the receptor	Cell therapy
Virus[30]	Genetic information is incorporated into a virus and when the virus infects the cell, the protein of interest is transduced in the infected cell		Broad selection of different virus types; high specificity by tissue-specific promoters and tissue tropism (only AAV); fast expression (Semliki-Forrest virus); long constant expression levels (especially Lentivirus); genome integration (only AAV and Lentivirus)	Limited size of DNA; may induce cytopathic effects	Can be used for almost all cell types, except cells of the immune system; preferentially used for terminally differentiated cells like neurons and cardiomyocytes; broad application in vivo