INTRODUCTION

In this work, modified polyethylenimines were investigated as non-viral vectors for gene transfer. This chapter serves as a general introduction defining the terms gene therapy, viral and non-viral vectors as well as specifically polyethylenimines and the concept of targeting and charge shielding. A more detailed introduction dealing with the specific objectives of each research topic is presented in each chapter. Furthermore, the objectives of this dissertation are outlined.

GENE THERAPY

Gene therapy is generally defined as the transfer of nucleic acids into cells for the purpose of altering the course of a medical condition or disease [1]. In many cases the nucleic acids such as DNA coding for proteins or RNA interfering with m-RNA need to be transferred into the cell nucleus or cytoplasm, respectively. By the transfer of genes or fragments of genes into human cells, not only monogenetic diseases but also infectious diseases, cancer and other diseases might be treated [2]. Gene therapy strategies can be classified into viral and non-viral approaches.

VIRAL GENE TRANSFER

To efficiently deliver genes into cells, whether *ex* or *in vivo*, a transport vehicle, designated as vector, is necessary. Viruses, which have evolved to become efficient vectors for gene transfer over millions of years, have already been exploited in a number of clinical trials [3]. Currently, four main groups of viral vectors are used in human trials, each having its own advantages and disadvantages. Table 1 gives an overview on the properties of those four groups.

Features	Adenoviral	AAV	Retroviral	Lentiviral
Maximum insert size	~30 kb	3.5-4.0 kb	7-7.5 kb	7-7.5 kb
Integration into host genome	No	Yes/No	Yes	Yes
Duration of expression in vivo	Short	Long	Short	Long
Immunological problems	Extensive	Not known	Few	Few
Pre-existing host immunity	Yes	Yes	Unlikely	Unlikely, exception: AIDS patients
Safety problems	Inflammatory response, toxicity	Inflammatory response, toxicity	Insertional mutagenesis	Insertional mutagenesis

Table 1: Comparison of properties of different viral vector systems (modified according to [4]).

As can be seen from this table, different viral vectors vary in the maximum size of DNA that can be delivered and in the duration of gene expression, and may or may not integrate into host genome. Immunological responses against adenoviral and adeno-associated viruses (AAV) are very likely. They can even occur at the first treatment due to previous infection with naturally occurring viruses [4]. Further treatments will be ineffective and hazardous due to the formation of specific antibodies leading to an inflammatory response [4]. It should be mentioned that the first fatal incidence in gene therapy trials was

caused by adenovirus [5]. By contrast, the most prominent risk of retroviral and lentiviral vectors is insertional mutagenesis. Random integration into the host chromosome could activate oncogenes or inactivate tumor-suppressor genes [4]. Furthermore, the possibility of recombination with wild type virus has to be taken into account as it can result in replication competent and/or pathogenic viruses. Even extremely rare recombination events present a serious risk due to the high number of vectors ($> 10^9$) necessary for gene therapy [2].

NON-VIRAL VECTORS

Due to the above mentioned risks of viral vectors, alternative gene delivery systems are highly desirable. Vector systems with acceptable safety profiles are a prerequisite for widespread clinical application and success of gene therapy.

Non-viral vectors, therefore, are an extremely interesting alternative. Although strategies such as ultrasound transfection [6], gene gun, electroporation or naked DNA injection [7] lead to efficient gene expression under certain circumstances, in most cases vectors will be needed. Cationic polymers and cationic lipids or mixtures thereof are being widely investigated and are promising candidates for non-viral gene therapy [8]. In general, these artificial vectors are considered to be less immunogenic than viruses against which the human body has developed defense mechanisms during evolution. Furthermore, there is almost no size limit for the DNA to be included in these systems [9].

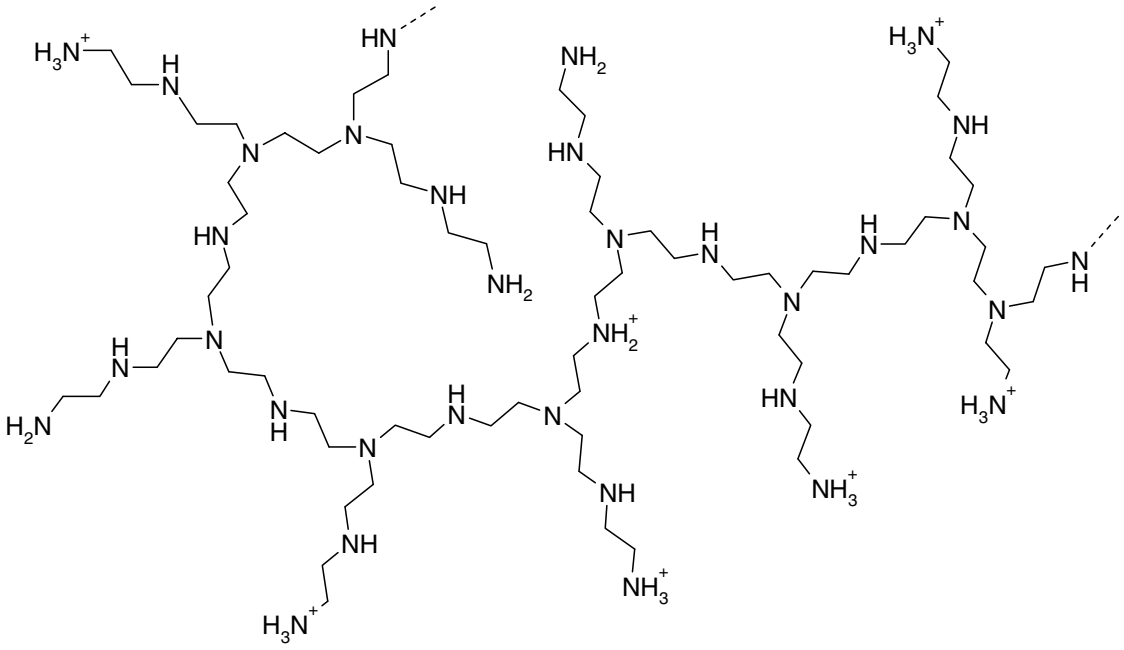
Since naturally occurring neutral lipids are not capable of forming complexes with anionic DNA, cationic lipids such as N-[1-(2,3-dioleyloxy)propyl]-N,N,N-trimethylammonium chloride (DOTMA) have been synthesized and utilized for gene transfer [10]. These lipids form heterogeneous complexes with DNA with a structure different from conventional liposomes [11] and are therefore called lipoplexes [12]. An excellent review may be found in [13].

Also, cationic polymers are known to form complexes with DNA by electrostatic interactions – so-called polyplexes. Polymers seem to be advantageous over cationic lipids as it is easier to specifically tailor them for

particular applications, i.e. by choice of molecular weight or coupling of targeting moieties for specific cells or tissues [8]. A variety of cationic polymers have been used for gene transfer so far, for example poly-L-lysine (pLL), chitosans, polyamidoamine dendrimers and polyethylenimine (PEI), and an excellent review can be found in [14]. Amongst them, polyethylenimine is the most effective transfection reagent.

POLYETHYLENIMINE

The first report about the use of polyethylenimine for gene transfer has been presented in 1995 [15]. Since then, a large number of studies have been conducted with this polymer and it seems to be the most extensively studied and most widely applied cationic polymer for gene transfer today. PEI has a very high density of cationic charges as every third atom in the polymeric backbone is nitrogen and about every fifth to sixth nitrogen group is protonated at physiological pH [16] (Scheme 1).



Scheme 1: Chemical structure of PEI.

PEI can effectively complex even large nucleic acid molecules [9] into colloidal particles of ca. 50 – 100 nm size which can transfect cells with a high efficacy

both under *in vitro* as well as *in vivo* conditions [15,17]. A variety of PEIs differing in molecular weight and branching have been investigated [18-20] and those factors were found to have great impact on gene transfer efficiency, but contradictory results have been reported. The mechanism by which the different PEIs transfect cells is most probably the same. It is generally accepted that PEI mediates transfection by the so-called proton sponge mechanism (Fig. 1) [21].

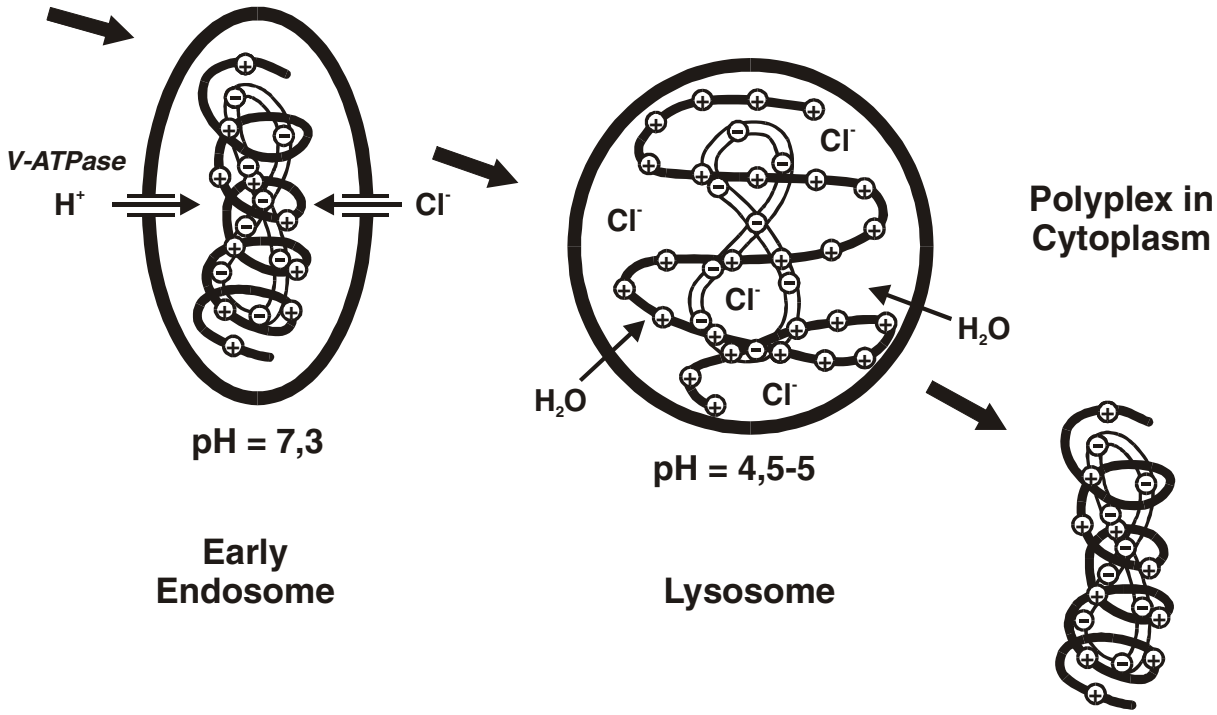


Fig. 1: Schematic presentation of the proton sponge hypothesis (adapted from [22]*).*

PEI/DNA complexes are taken up into cells by adsorptive endocytosis caused by interaction between cationic polyplexes and anionic cell surfaces and then trafficked into lysosomes. The decrease in pH occurring in lysosomes is prevented due to the high buffering capacity of PEI. Consequently, ATPase driven proton accumulation and subsequent passive chloride influx result in water entry caused by the osmotic gradient [15]. Two possible mechanisms are made responsible for the final disruption of lysosomes [23]: Expansion of PEI

molecules due to internal charge repulsion or osmotic swelling leading to a high pressure.

CHARGE SHIELDING

Polyplexes, especially with an excess of positive charge, are rapidly cleared from circulation due to opsonization and complement activation [24]. To avoid opsonization and consequent removal from the circulation by the mononuclear phagocytic system, uncharged, hydrophilic polyplex surfaces are beneficial. A variety of hydrophilic polymers are known to reduce clearance of drugs or particles from blood [25]. Some of them have been tested in combination with cationic polymers, and especially PEG has shown some promising results. Fig. 2 schematically shows polyplex formation from such copolymers.

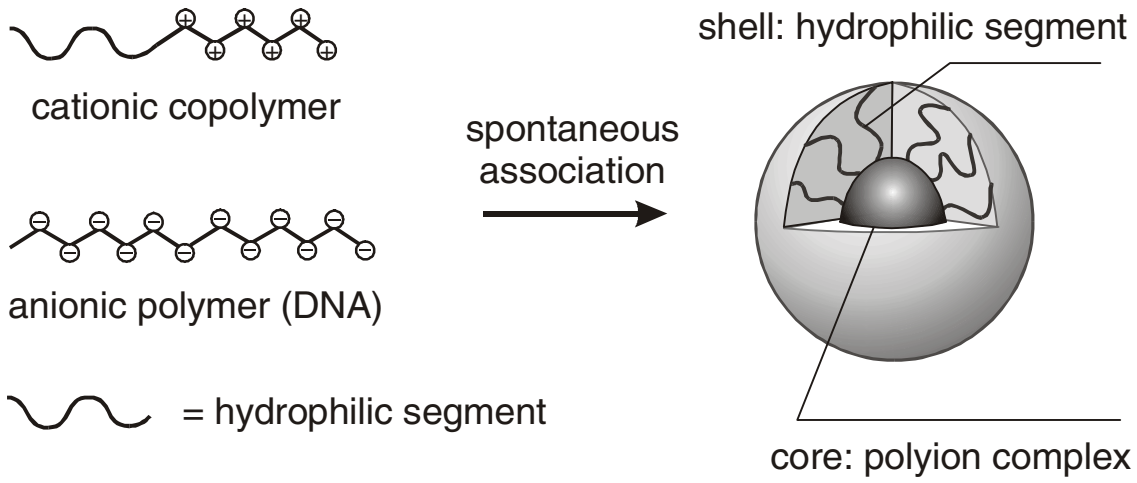


Fig. 2: Polyplex formation from cationic copolymers with hydrophilic segments (adapted from [26]*).*

More information is necessary on the importance of PEG molecular weight, the density of PEG on polyplex surface, and especially on *in vivo* behavior of PEGylated PEI. Also, very little is known about the influence of PEGylation on polyplex targeting.