INTRODUCTION

The aim of this thesis was the physicochemical and biological characterization of polyethylenimine and PEGylated polyethylenimine derivatives as non viral delivery systems for oligonucleotides and ribozymes. In this chapter, the technology concerning oligonucleotide and ribozyme delivery will be briefly introduced. The transdermal transport of oligonucleotides will be discussed as a potential method for their local or systemic administration.

OLIGONUCLEOTIDE TECHNOLOGY

In contrast to conventional gene therapy, which attempts the replacement of a defective or missing gene, oligonucleotide (ON) strategies suppress or inhibit unwanted gene expression in neoplastic, infectious, as well as certain inherited diseases [1]. ONs are generally defined as short sequences of single stranded nucleic acid or nucleic acid analogs able to interfere with gene transcription and translation in a sequence specific manner [2]. Different mechanisms of ON activity are described, all of which ultimately prevent the production of a specific protein (Fig. 1).

Antisense DNA-ONs hybridize via complementary Watson-Crick base pairing with specific sequences of mRNA and consequently hinder ribosomal reading sterically [3]. It is further assumed that the RNA strand of the original hybrid duplex between mRNA and DNA-ON is cleaved by the endogenous enzyme, RNase H [4]. Ribozymes are defined as catalytic RNA molecules which can undergo complementary binding and subsequently cleave a target mRNA into two shorter RNA fragments, no longer capable of being translated into a specific protein [5]. Triple-helix-forming ONs bind to the major groove of the DNA double-helix leading to the arrest of the transcription machinery [6]. More recently, small interfering RNAs (siRNA) have emerged. These 21 nucleotide-long siRNA doublexes guide a nuclease for degradation of the target RNA after incorporated into the RNA-induced silencing complex [7].

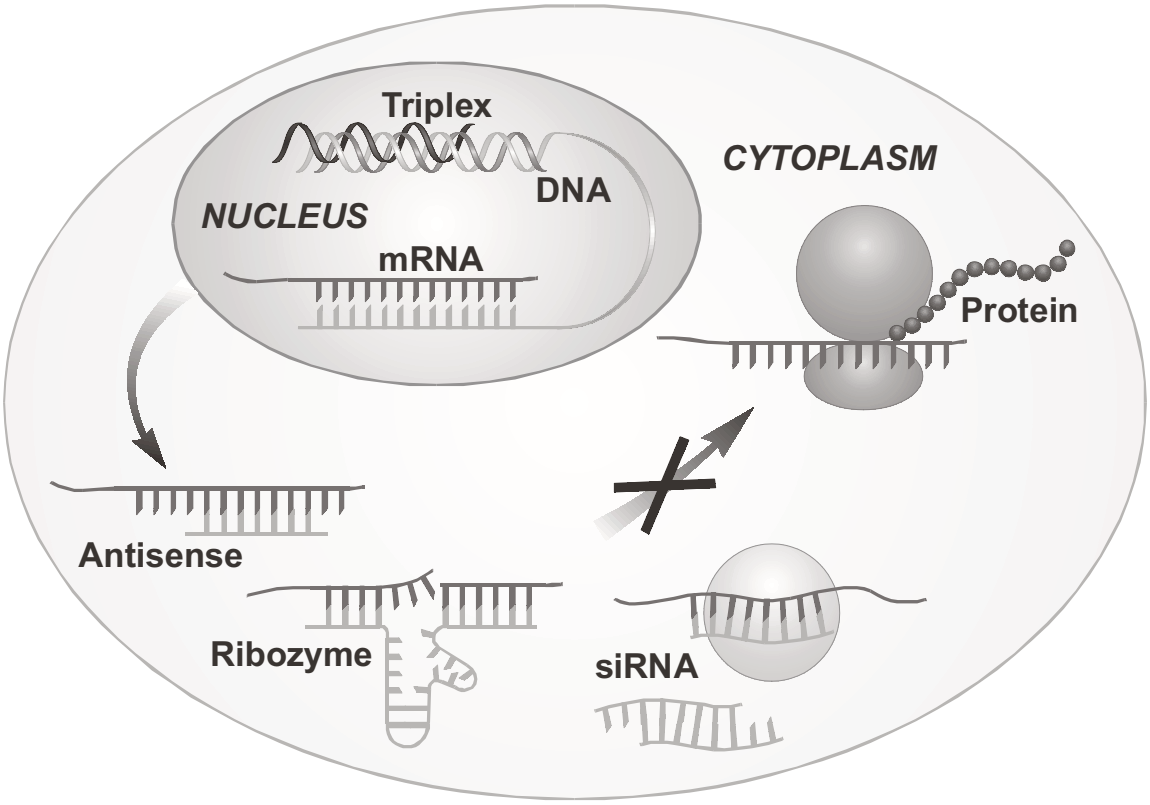

Figure 1: Oligonucleotide based strategies for inhibition of gene expression.

OLIGONUCLEOTIDE STABILITY

A major barrier for efficient ON delivery is their marked instability under in vivo conditions [8]. The rapid degradation of the naturally occurring phosphodiester backbone by endo- and exonucleases limits the physiological half-life of DNA ONs in human serum to approximately one hour [9]. All RNA ribozymes are even more sensitive to enzymatic degradation with half-lifes below one minute [10,11]. A large number of chemically modified ONs showing enhanced nuclease resistance are currently under investigation. The most widely studied derivatives are phosphorothioate ONs (PS), in which the non-bridging oxygen atom in the backbone is replaced by sulfur [12]. Other modifications have been introduced more recently such as 2´-O-methyl-ON, peptide nucleic acids and morpholino-based ONs (for review see [13]). Generally, similar modifications can be incorporated into ribozyme molecules, although the modification of ribozymes is more complicated, due to a potential

loss of catalytic activity [14-16]. A major disadvantage of chemically modified ONs are the resulting nonspecific interactions with proteins, so called non-antisense effects [17]. These effects have led to the observation of serious acute toxicity after phosphorothioate ON application in primates, which was probably the result of a transient activation of the complement cascade [18].

OLIGONUCLEOTIDE DELIVERY

A second major hurdle of the therapeutic application of ONs is their poor cellular uptake [9]. The barriers for delivery of ONs and plasmid DNA on a cellular levels are very similar: (a) the lack of binding to the cell surface, (b) low extent of cell internalization of nucleic acids by endocytosis, (c) poor release from the endosomal or lysosomal compartment, and (d) the low rate of accumulation within the nucleus [19]. However, some substantial differences between plasmids and ON may provide advantages for the delivery of ONs. The significantly larger molecular weight of plasmid DNA renders cellular and nuclear uptake much more difficult and poses additional challenges for gene delivery systems [20]. Furthermore ONs have shown a free diffusion from the cytosolic compartment to the nucleus whereas, lager DNA fragments were immobilized in the cytosol, probably due to protein binding [21]. Although, the display of antisense activity even after the administration of naked ONs has cast doubt on the need for delivery systems [8,19,22], the use of physical methods or carrier systems was shown to enhance ON delivery [20]. While, most of the physical methods in use, such as ultrasound, electroporation and iontophoresis, enhanced ON delivery in vitro or ex vivo, their potential under in vivo conditions has to be investigated [2]. On the other hand, a huge number of different non viral vectors have been reported to both stabilize ONs against enzymatic degradation and increase initial membrane interaction with subsequent cellular uptake [19]. Generally, ribozymes can be delivered using the same carriers [8]. Moreover, ribozymes and siRNAs can be endogenously

delivered via ribozyme encoding plasmids or viral vectors [5]. However, viral delivery systems are associated with serious safety problems and have not yet been investigated extensively for ON delivery [20]. Table 1 provides an overview of commonly used delivery approaches, each of them exhibiting its own advantages and drawbacks. In conclusion, the improvement of these carrier systems with regard to their efficiency, biocompatibility, in vivo performance and stability remains a challenge.

Table 1: Delivery systems for oligonucleotide delivery

Delivery approach	Examples	Review articles
Liposomes	Lipofectine™, Lipofectamine™	[2,8,19,23,24]
Polymers	PEI, dendrimers,	[8,19,20,25,26]
Peptides	pLL, polyornithine	[2,8,19,20,27]
Polysaccharides	Cyclodextrins	[2,9,25,28]
Nanoparticles	Polycyanoacrylates	[2,25]
Microspheres	PLA, PLGA	[2,25,29]
Hydrogels	Poloxamer	[20]
Physical Methods	Electroporation, ultrasound	[2,30]
Viral delivery of Ribozyme encoding plasmid	Retroviral, adeno-associate viral vectors	[5,31,32]

POLYETHYLENIMINE

One of the most widely used polycations for gene delivery today is polyethylenimine (PEI) [26]. In several cell culture models and under in vivo conditions, PEI has demonstrated high transfection efficiencies [33-35]. PEI is a water-soluble, highly cationic polymer able to complex and condense DNA and RNA via electrostatic interactions [36]. The resulting complexes used in transfection are usually produced with an excess of polymer and hence, carry a net positive surface charge [37]. As result, the interaction with negatively charged cell membranes is increased and complexes are taken up by cells possibly via adsorptive endocytosis [38].

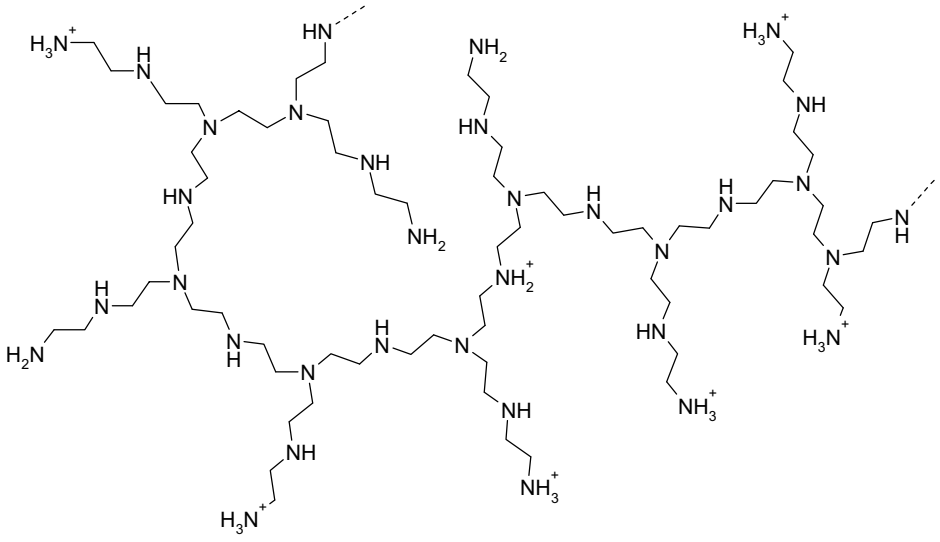

Figure 2: Chemical structure of polyethylenimine.

As advantage of PEI versus other polycations such as pLL, the intrinsic endosomolytic escape mechanism has attracted much interest and is probably responsible for the rapid release of the DNA into the cytosol [33]. Due to its buffer capacity under physiological conditions, PEI is thought to act like a proton sponge, capturing the protons being pumped into the early endosome [39].

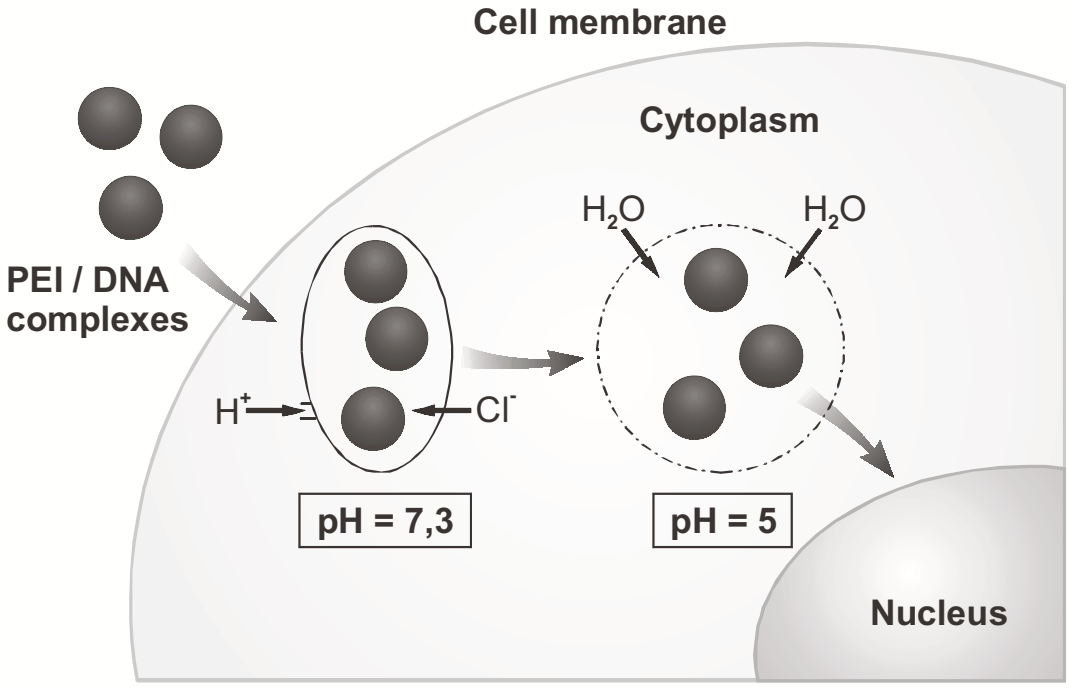

Figure 3: Scheme presenting the proton sponge hypothesis. (adapted from [40]).

The resulting passive influx of chloride and water is assumed to cause osmotic swelling and endosomal rupture [40]. Several PEI derivatives of different molecular weight, different degree of branching and of linear form are available. Recently, the influence of polymer structure on the transfection efficiency, biocompatibility and DNA condensation were investigated for plasmid DNA [37,41,42]. Some guidance might be drawn from these studies for ON delivery using PEI, however, so far scant experimental evidence has been reported in this field [20] (see Table 2).

Table 2: Studies using PEI based systems for oligonucleotide delivery

Mechanistic studies			
PEI type	Topic	ON type	Ref.
PEG-PEI	Physicochemical characterization	ODN & PS	[43,44]
PEI 25 kDa	Subcellular processing	RZ	[45]
PEI/PLGA	Physicochemical characterization	ODN	[46-48]

Cell culture studies			
PEI type	Target	ON type	Ref.
PEI 800 kDa	Thyroid hormone receptor	ODN	[33]
PEI 50 kDa	Ca^{2+} channel βsubunit mRNA	PS	[49]
Lactosylated	Factor IX genomic DNA	chimeric ON	[50,51]
PEI	Ha-ras mRNA	ODN & PS	[52]
Transferrin	Human mdr 1 mRNA	PS	[53]
PEGylated	Luciferase test system	morpholino	[54]
PEI/virus	Aquaporin 5 (rAQP5) cDNA	triplex	[55]
PEI	Mutant codon EGF protein	chimeric ON	[56]
Linear PEI	Genomic DNA repair via chimeraplasty	chimeric ON	[57]
PEI/liposome	p53 protein antisense	PS	[58]
PEI/PLGA	Anti $TGF\beta_1$ or model ODN	ODN	[46-48]

In vivo studies				
PEI type	Target	Application	ON type	Ref.
Lactosylated	Factor IX	liver (rat)	chimeric ON	[50]
Linear PEI	Hepatitis B virus	i.v. (duck)	ODN & PS	[59,60]
PEI-pluronic	Random	i.v. (mice)	PS	[61]
PEI-PEG	NF-κB	i.v. (mice)	DNA decoy	[62]
PEI 2.7 kDa	Pleiotrophin	intratumoral (mice)	RZ	[63]
PEI 20 kDa	Platelet-DGF	carotid-catheter (rat)	chimeric RZ	[64]