



**Figure 1.11 - Non-viral gene therapy studies published over the past decade**

The number of articles published each year returned by pubmed ([www.ncbi.nlm.nih.gov](http://www.ncbi.nlm.nih.gov)) when queried with “non-viral gene therapy”. The total number of articles listed was 567.

### 1.5.2 Non-Viral Gene Delivery

Non-viral gene delivery encompasses the many different methods which do not use a virus as a vector, however, in this dissertation it refers to lipid-based (lipoplex) or polymer-based (polyplex) transfection agents. Electroporation, gene gun, naked DNA and other non-viral gene delivery methods will not be further considered.

Lipoplexes are formed through the interaction of cationic lipids with the negative charge on DNA. Lipofectamine (2,3-dioleoyloxy-N-(2(spermine carbocamido)ethyl)-N,N-dimethyl-1-propanaminium trifluoroacetate (DOSPA) : di-oleoyl phosphatidylethanolamine (DOPE) : cholesterol (3:1:3.36)) (Mahato et al., 2003) and di-oleoyl phosphatidylcholine (DOPC)/DOPE/di-oleoyl trimethylammonium propane (DOTAP) liposomes (Safinya, 2001) are commonly used vectors. Lipoplexes have gained interest in gene therapy and 87 (8.5 %) clinical trials using lipid-based gene therapy have been reported (Edelstein, 2005). Targeted-lipoplexes are discussed further in Section 1.5.3.1.

Polyplexes (Gebhart, 2001) are particles formed through interaction of the positive charge on the polymer with the negative charge on DNA (Gebhart & Kabanov, 2001). When low polymer (+ve) : nucleic acid (-ve) ratios are used polyplexes form aggregates (dependent on the type of polymer). At higher ratios of polymer : nucleic acid the polyplexes are soluble, small and positively charged (Ogris & Wagner, 2002a). Protection of the nucleic acids against DNAses is conferred by the electrostatic interaction with cationic molecules which increases DNA stability in solution and increases the half-life in the circulation (Kircheis et al., 2001b). The electrostatic interaction also condenses the DNA to produce a smaller molecule (Lee et al., 2001). These properties make cationic polymers an interesting delivery vehicle in non-viral gene therapy. The negative charge on DNA, which would hinder entry to the cell due to the negative charges found on the plasma membrane, is balanced or made positive by the polymer and DNA condensation enables easier entry into the cell. A large number of studies have tried to develop non-viral vectors and many cationic polymers have been used; Table 1.5 lists some of the polymeric vectors being investigated. Discussion of the most widely studied cationic polymeric vector, PEI, is made below (Section 1.5.2.1.1) followed by discussion of the more biocompatible vector, chitosan, chosen for this study (Section 1.5.2.1.2).

Table 1.5 – Polymeric non-viral gene delivery vectors

Polymer	Cell lines / <i>in vivo</i>	Molecular weight (kDa)	Reference
PEI (linear)	Primary duckling hepatocytes, Caco2, KB, KBv, COS7, C2C12, MCF7, MCF7 ADR, LLC-PK1, LLC-MDR1, CT26 / ducklings, tree shrews, Wistar rats, human bladder carcinoma (clinical trial)	0.8, 22, 50	(Chemin et al., 1998, Gebhart & Kabanov, 2001, Ohana et al., 2004)
PEI (branched)	Caco2, KB, KBv, COS7, C2C12, MCF7, MCF7 ADR, LLC-PK1, LLC-MDR1, CT26	25	(Gebhart & Kabanov, 2001)
Chitosan	A549, B16, MG63, HEK293, HeLa / Balb/c mice, AKR/J mice	1.2-4.7, 40, 84, 150, 390, 400, 600	(Corsi et al., 2003, Erbacher et al., 1998, Ishii et al., 2001, Koping-Hoggard et al., 2003, Roy et al., 1999)
Poly( $\beta$ -amino) esters	COS-7, NIH 3T3	1-50 (library of polymers)	(Akinc et al., 2003)
Poly(amidoamine)	HepG2	ISA1 = 12.3 ISA4 = 15 ISA22 = 16.5 ISA23 = 14.9	(Richardson et al., 2001)
Poly(amidoamine) dendrimers	CV1 / BALB/c mice	467, 10.4 – 233.3	(Kukowska-Latallo et al., 2000, Tang et al., 1996)
Poly-L-lysine	HuH7 / C57-BL6 mice	9.7, 53.7	(Ziady et al., 1999)
Diethylaminoethyl (DEAE)-Dextran	Primary human macrophages	Not stated	(Mack et al., 1998)
Poly(2-dimethylamino)ethyl methacrylate (pDMAEMA)	COS7	45, 360	(Cherng et al., 1996)
Polyphosphazines	COS7	100, 300	(Luten et al., 2003)
Polybrene/DMSO	J774, HL60	Not stated	(Chisholm & Symonds, 1988)

Non-viral gene delivery systems have several advantages over viral vectors. These include (i) the size of DNA incorporated is largely unlimited, (ii) they can display low toxicity and repeated administration can be made without provoking an immune reaction and (iii) there is greater control over production and characterisation of the vector and the vector/DNA complexes.

### **1.5.2.1 Cationic Polymers in Non-viral Gene Delivery**

#### **1.5.2.1.1 Poly(ethylenimine) as a Non-viral Vector**

The most widely studied cationic polymeric vector is PEI (von Harpe et al., 2000). This vector has a high cationic charge density producing efficient transfection (Boussif et al., 1995). The mechanism of endosomal escape has been widely debated. Studies suggest that the PEI polyplex is taken up into endosomes where the pH is buffered causing osmotic swelling and endosomal membrane rupture. This has been termed the 'proton sponge effect' (Boussif et al., 1995, Cho et al., 2003, Zuber et al., 2001). The buffering of ATP driven H<sup>+</sup> ion influx causes concomitant influx of Cl<sup>-</sup>. This increases the osmotic potential of the endosome and, in turn causes an increase in volume until such point as the membrane bursts (Akinc et al., 2005).

Another hypothesis proposed that the efficient transfection properties of PEI are due to the protonation of the amines causes ionic repulsion leading to extension of the PEI molecule and endosomal membrane disruption. This theory is supported by the lack of difference in the lysosomal pH found between that measured in PEI transfected cells and that measured in non-transfected cells (Godbey et al., 2000). The transfection efficiency of pDNA polyplexes with PEI is 10-fold higher when PEI is added dropwise to the pDNA (Boussif et al., 1995). Polyplexes have been found to form toroid structures of 40-80 nm when condensed with PEI (Kircheis et al., 2001b).

The main problem with PEI is its toxicity (Florea et al., 2002a). Low molecular weight PEI has a lower toxicity and 25 kDa linear PEI is perceived as the best compromise between toxicity and high transfection efficiency (Ahn et al., 2002). Linear PEI (22 kDa) is available commercially, JetPEI<sup>®</sup>, as a reagent for *in vitro* transfection (Fermentas, 2005).

Several PEI derivatives have been made that were found less cytotoxic *in vitro* compared to PEI. These include transferrin-PEG-PEI, galactose-PEG-PEI and N-acylated with alanine of PEI. All these derivatives showed high transfection efficiency (Kursa et al., 2003, Sagara & Kim, 2002, Thomas & Klivanov, 2002). Ahn et al. (2002)