

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)

produced a PEG-PEI with the aim of reducing toxicity whilst retaining transfection efficiency of higher molecular weight PEI (Ahn et al., 2002). Toxicity was reduced (80 % viability of control) compared to 25 kDa PEI (40 % viability of control) and transfection efficiency higher than that of the starting Mw PEI (1.8 kDa) (Ahn et al., 2002). However, no direct comparison to 25 kDa transfection efficiency was made but PEG-PEI co-polymers were acknowledged to be less efficient (Ahn et al., 2002).

The first clinical trial using a polymeric vector, PEI, was published in 2005 (Ohana et al., 2004). Bladder cancer (two human subjects) was treated through bladder installation of a transurethral catheter and a > 75 % reduction in tumour size with no adverse side effects was reported (Ohana et al., 2004).

In the studies reported in this thesis PEI has been used as a positive control. Its cytotoxicity and transfection efficiency compared with that of the chitosan derivatives prepared. PEI was also used in some studies as an additional component in the development of an improved non-viral gene delivery system.

#### ***1.5.2.1.2 Chitosans: Natural Origin Cationic Polymers as Non-viral Vectors***

Chitosan is a naturally occurring polysaccharide of  $\beta$ 1-4 linked *N*-acetyl-D(+)-glucosamine and D(+)-glucosamine. It is produced through the deacetylation of chitin, the extent of which is usually 40-100 % (reviewed in Thanou & Junginger, 2004). Chitin is found in the cell walls of fungi; this was where it was first discovered in 1811 by H. Braconnot who named it fungine (Domard & Domard, 2002). It is also found in the exoskeleton of insects, and crustacea (crab, shrimps) and was termed chitin, after the Greek word for coat, chitos, by C. Odier in 1823 after the discovery in the elytrum of the cock chafer beetle (Domard & Domard, 2002).

The main commercial source of chitin is shell waste from the food industry. Chitin is processed by treatment with 3-5 % (w/v) aqueous alkali (NaOH) at 80-90 °C to remove protein, this is followed by treatment with 3-5 % (w/v) aqueous acid (HCl) to remove inorganic materials (i.e. calcium carbonate). The purified chitin is deacetylated, producing primary amines, using 40 % sodium hydroxide at 120 °C for 1-3 h (Kumar, 2000).

Chitosan is widely regarded as being a non-toxic biologically compatible polymer (Corsi et al., 2003). It is approved for dietary applications in Japan, Italy and Finland (Illum, 1998) and it has been approved by the FDA for use in wound dressings (McCue, 2003). Chitosans (<5 kDa, 5-10 kDa and >10 kDa) were found to display little