Transferrin and epidermal growth factor (EGF)-containing PEI/DNA complexes demonstrated up to 100-fold enhancement of luciferase gene expression in murine tumour models (Ogris et al., 2003). When transferrin was covalently attached to PEI, and complexes of transferrin-PEI/PEI/pDNA made, a 20-fold increase in gene expression was found in the tumour over other organs after administration to mice bearing neuroblastoma tumours (Kircheis et al., 2001a). Interestingly, in this study a large uptake of the polyplex was observed in the liver but with low expression of the luciferase protein; the explanation made was that uptake was performed by Kupffer cells and RES which degraded the polyplex (Kircheis et al., 2001a). It is interesting to note from Table 1.6 that Kunath et al. (2003b) found decreased transfection using galactose-PEI polyplexes whilst Zanta et al. (1997) found a massive increase in transfection using galactose-PEI polyplexes. The reason for this difference is unclear as similar PEIs were used at similar degrees of galactose substitution and the cell lines transfected were the same.

In a more complicated approach: PEI/DNA polyplexes were first prepared and then coated with streptavidin, EGF-PEG-biotin was then immobilised on the polyplex surface through the high affinity biotin-streptavidin interaction (Lee et al., 2002). The resultant polyplex lead to a 10-fold increase in the transfection efficiency for the most effective polyplex over PEI alone on A431 cells, a human epidermoid carcinoma cell line (Lee et al., 2002). From these studies it can be seen that receptor-mediated targeting of polyplexes to increase the efficiency of gene delivery is feasible but that effective solutions must still be sought.

1.6 Aims of this Research

The overall aim of this research was to develop a peptide-directed, uPAR targeted, non-viral gene delivery system based on chitosan as a non-viral vector. The hypothesis being that a peptide derived from the binding region of uPA when conjugated to 6-O-carboxymethyl trimethyl chitosan would increase the uptake of the derivative by tumour cells. Thus it was envisaged that polyplexes formed from such chitosans containing this targeted derivative would be useful for tumour-targeted cancer gene therapy.

First it was essential to optimise chitosan trimethylation and show that the reaction was reproducible. To enable later studies on the effect of trimethylated chitosan molecular weight, two chitosan molecular weight fractions were used for these studies:

chitosan oligomer (3-6 kDa) and chitosan polymer (~ 100 kDa) (Chapter 3). The chitosan-based products synthesised were then investigated for their toxicity and transfection efficiency (Chapter 4). To design the trimethylated chitosan derivatives as targeted vectors it was necessary to conjugate them to the uPA derived peptides u7 and u11. In addition, fluorescent probes were added (Oregon Green and fluorescein) (Chapter 3) to allow monitoring of uptake and intracellular localisation (Chapter 5).

Before studying the ability of the conjugates to form polyplexes for gene delivery, it was necessary to establish cell models expressing uPAR (Chapter 5). Using MCF-7, COS-7 and DU145 as uPAR-expressing cell lines, the binding and uptake of the two peptide ligands (u7 and u11) and their conjugates was investigated (Chapter 5). Finally, having selected the optimum peptide-ligand construct, the ability of this targeted polyplex to mediate transfection was investigated (Chapter 6).