Gene therapy has gained significant attention over the past two decades as a potential method for treating genetic disorders such as severe combined immunodeficiency, cystic fibrosis, and Parkinson’s disease, as well as an alternative method to traditional chemotherapy used in treating cancer. Research efforts are currently focused on designing effective carrier vectors that compact and protect oligonucleotides for gene therapy as free oligonucleotides and DNA are rapidly degraded by serum nucleases in the blood when injected intravenously. Initial research has concentrated on using viral carriers, including both retroviruses and adenoviruses, as these vectors exhibit high efficiency at delivering both DNA and RNA to numerous cell lines. However, fundamental problems associated with viral vector systems, including toxicity, immunogenicity, and limitations with respect to scale-up procedures, have encouraged the investigation of other potential scaffolds to carry exogenous DNA into the targeted tissues/cells. Non-viral vector systems including cationic lipids, polymers, dendrimers, and peptides offer potential routes for compacting DNA for systemic delivery. However, unlike viral analogues that have evolved means to overcome cellular barriers and immune defence mechanisms, non-viral gene carriers consistently exhibit significantly reduced transfection efficiency as they are hindered by numerous extra- and intracellular obstacles. However, biocompatibility and potential for large-scale production make these compounds increasingly attractive for gene therapy. As a result, a significant amount of research in the past decade has focused on designing cationic compounds that can form complexes with DNA and can avoid both in vitro and in vivo barriers for gene delivery.

One of the most successful and widely studied gene delivery polymers reported to date is polyethylenimine (PEI), an off-the-shelf polycation containing a high density of primary, secondary, and tertiary amines, which offer very good buffering capacity and very high transfection efficiency. Due to its relatively high gene delivery efficiency and ready availability, branched PEI (25 kDa) has become a gold standard to which other polymers, especially newly designed and synthesized materials, are often compared. PEI offers significant protection to the complexed nucleic acids against enzymatic degradation, possibly due to their higher charge density and more efficient complexation. It mainly exists in either linear or branched form. Several groups have reported that transfection efficiency of PEI also depends upon molecular weight of the polymer and its toxicity appears to decrease with decreasing polymer size. It has been shown that degree of branching also plays an important role in determining the transfection efficiency of the PEI polymer.

Modifications to the surface of PEI are necessary as in spite of having good transfection efficiency it is cytotoxic in nature which limits its use in in vivo studies.
Incorporation of modifications converts the bad charge of primary amines to the good charge, thereby making it non-toxic to the cells. In this study, the main focus was to design a suitable carrier with improved transfection efficiency and reduced cytotoxicity. Both linear and branched PEIs with different molecular weights were modified and optimized for gene delivery efficacy.

**Chapter I** discusses about gene therapy in general, various barriers and outlines the types of vectors used in gene delivery. The mechanism of cellular uptake of DNA complexes through endocytosis is discussed. A brief discussion of viral vectors and a detailed discussion of non-viral vectors are given with the main focus on cationic polymers. The advantages of non-viral vectors over viral ones and the parameters that are to be taken into consideration while designing a gene delivery vector have been discussed in detail. A brief overview on the applications of nanotechnology in gene delivery has also been included. At the end, it outlines the scopes and objectives of the present study.

**Chapter II** summarizes the details of all the experiments carried out in the present study. It deals with the documentation of source of all the chemicals, reagents and instruments used in the present investigation. The details of all the experiments and techniques involved in the characterization of particles, viz., particle size, zeta potential and various spectroscopic techniques, have been discussed. The protocols for the preparation and purification of plasmid DNA used in the present study, for in vitro as well as in vivo, have been given. The experimental details of in vitro transfection efficiency and cytotoxicity in mammalian cell lines, intracellular trafficking of nanoparticles or DNA complexes and in vivo gene expression in Balb/c mice have been described.

**Chapter III** describes the formation of a series of polyethylenimine (PEI)-g-polyglutamic acid (PGA) nanocomposites (PPGA) and their evaluation in terms of cell viability and transfection efficiency in vitro and in vivo. On complexion with pDNA, the positively charged PPGA/DNA nanocomposites resulted in a higher level of in vitro reporter gene transfection (2.7-7.9 fold) as compared to native PEI, and selected commercial reagents with >95% cell viability in HEK293, HeLa and HepG2 cell lines. Further, PPGA-5 nanocomposite (the best working system in terms of transfection efficiency among the series) was found to efficiently transfect primary mouse keratinocytes upto 22% above the control level. PPGA-5, when tested for in vivo cytotoxicity in Drosophila, did not induce any stress in the exposed larvae in comparison to control. In vivo gene expression using PPGA-5/DNA complex showed the highest transfection efficiency in spleen of mouse closely followed by heart tissues after intravenous injection through tail vein. Besides, these nanocomposites also delivered siRNA...
efficiently into mammalian cells, resulting in ~80% suppression of EGFP expression. These results together demonstrated the potential of the projected nanocomposites for in vivo gene delivery.

In Chapter IV, formation of Dextran–PEI grafted nanoparticles as transfection agents have been reported. To address the concerns associated with PEI, dextran was first crosslinked with 1,4-butanediol diglycidyl ether (BDE), a commercially available homobifunctional crosslinker, to form its nanoparticles (DN), which were then partially oxidized with sodium periodate to generate aldehyde functionalities on them. The resultant nanoparticles were grafted with branched polyethylenimine (bPEI, 25 kDa) to obtain a series of DN–PEI nanocomposites (DP), having comparatively smaller size, enhanced buffering capacity and competent binding ability with DNA. Subsequent to biophysical characterization, these nanocomposites were evaluated for their transfection efficiency in serum and serum-free environments and toxicity in various mammalian cell lines. A significantly (p < 0.05) improved transfection efficiency of these nanocomposites concurrent with their minimal cytotoxicity as compared to the selected commercial transfection agents and native PEI was observed. These results were further validated by intracellular trafficking, wherein DP4 (the best working system in the series) was able to carry the DNA inside the nucleus after 1 h of the addition of the complex. The in vivo gene expression profile of the DP4/DNA complex in male Balb/c mice exhibited maximum expression in their spleen tissue raising promise of using these nanocomposites as improved non-viral transfection agents.

Chapter V discusses the formation of chitosan chlorohydrin and its subsequent reaction with varying amounts of IPEI(2.5kDa) to obtain a series of chitosan-IPEI(2.5kDa) copolymers (CP). These copolymers were then characterized and evaluated in terms of transfection efficiency (in vitro and in vivo), cell viability, DNA release and buffering capacity. The CP-4 copolymer (the best among the CP series) showed enhanced transfection (2-24 folds) in comparison to chitosan, IPEI(2.5kDa), bPEI(25kDa) and commercial transfection reagent in HEK293, HeLa and CHO cells. The buffering capacity (in the pH range of 3-7.5), as shown by confocal microscopy, and DNA release capability of the CP copolymers were found to be significantly enhanced over chitosan. Intravenous administration of CP-4 polyplexes in mice followed by the reporter gene analysis showed the highest gene expression in spleen. Collectively, these results demonstrate the potential of CP-4 copolymer as a safe and efficient non-viral vector.

Chapter VI deals with the development of efficient and safe nucleic acid carriers for the success of gene therapy. The transfection efficiency of depolymerized chitosans (7 and 10
kDa) conjugated via their terminal endo groups with branched low molecular weight polyethylenimine, bPEI (1.8 kDa) (CP) were evaluated in vitro and in vivo. The resulting CP conjugates interacted efficiently with DNA and siRNA also showed higher cellular uptake. The CP conjugates on complexation with DNA yielded nanoparticles in the size range of 100-130 nm (in case of C7P) and 115-160 nm (in case of C10P). They exhibited significantly higher transfection efficiency (~2 - 42 folds) in various cell lines as compared to native chitosan (high and low mol. wt.) and selected commercially available transfection reagents retaining the cell viability almost comparable to native chitosan. Of the two CP conjugates, chitosan 7 kDa-PEI (C7P) performed slightly better than C10P conjugates in terms of transfection efficiency in the presence and absence of serum. DNase I assay showed that C7P3 had good binding affinity with DNA that resulted in only ~17% degradation of DNA after 2 h of treatment. The reporter gene analysis in male Balb/c mice receiving intravenous administration of C7P3/DNA polyplex shows the maximum expression in spleen cells. Further, tuftsin reported as targeting macrophage, was tethered to C7P3 and the resulting complex, i.e. C7P3-T/DNA, exhibiting significantly higher gene expression in cultured mouse peritoneal macrophages as compared to unmodified C7P3/DNA complex without any cytotoxicity, demonstrates the suitability of the conjugate for targeted applications. Taken together, the study demonstrates the potential of the projected conjugates for gene delivery for wider biomedical applications.

Chapter VII discusses the formation of a series of electrostatically crosslinked nanoparticles, N-(2-hydroxyethyl)- polyethylenimine-PEG_{600} (HePP), prepared by allowing N-(2-hydroxyethyl)- polyethylenimine (HeP) to interact with polyethylene glycol (600) dicarboxylic acid (HOOC-PEG_{600}-COOH, PEG_{600}dc) and their subsequent evaluation to transfect cells in vitro and in vivo. Dynamic light scattering (DLS) studies revealed the size of the HePP nanoparticles in the range of 106-170 nm, which efficiently condensed nucleic acids and provided sufficient protection against nuclease degradation. These nanoparticles exhibited considerably higher cell viability than branched polyethylenimine (bPEI, 25 kDa) and other widely used commercial transfection reagents. Transfection studies in mammalian cells showed the capability of the entire series of nanoparticles to deliver genetic material to the insides of the cells with HePP-3, being the best, acted as an efficient gene delivery vector in HEK293, CHO and HeLa cells outcompeting the native polymer and commercially available transfection reagents. Also, HePP-3 mediated sequential delivery of GFP specific siRNA resulted in ~76% suppression of the target gene. Confocal laser scanning microscopic (CLSM) examination showed a significant presence of HePP-3/DNA complex in the nucleus
after 60 min. Intravenous administration of HePP-3/DNA complex in Balb/c mice followed by monitoring of the reporter gene analysis post 7d revealed the highest gene expression in spleen, which implies the suitability of the formulation for in vivo applications. Together, these results advocate the potential of HePP nanoparticles as efficient vectors for gene delivery in vitro and in vivo.