Preface

Nobel Laureate Richard P. Feynman gave a talk at the annual meeting of the American Physical Society on Dec. 29, 1959, at the California Institute of Technology, that has become one of the twentieth century's classic science lectures, titled "There's Plenty of Room at the Bottom". He emphasized on a technological vision to manipulate and control things on miniature scale. Feynman envisaged a technology of building nano-objects atom by atom or molecule by molecule. Since the 1980s, several inventions and discoveries in the fabrication of nano-objects have realized his vision. The term "nanotechnology" was coined by Tokyo Science University Prof. Norio Taniguchi in 1974 and defined as: Nano-technology mainly consists of the processing of separation, consolidation, and deformation of materials by one atom or one molecule. Nanotechnology enables the production and application of physical, chemical, and biological systems at sizes ranging from individual atoms or molecules to submicron dimensions. Innovation in nanotechnology promises breakthroughs in areas such as materials and manufacturing, nanoelectronics, medicine and healthcare, energy, biotechnology, information technology, and national security. A unique parameter of nanotechnology is the highly increased ratio of surface area to volume present in many nanoscale materials which opens new avenues in surface-based science, such as catalysis. A number of physical properties become noticeably visible as the size of the system decreases. These aspects include statistical mechanical effects, as well as quantum mechanical effects, for example the "quantum size effect" where the electronic properties of solids are altered with great reductions in particle size.

This book on "Theory, Techniques and Applications of Nanotechnology in Gene Silencing" highlights the recent past advances at the interface between

the science and technology of nanotechnology and their biological applications. As highlighted in this book these applications include using nanoparticles for *in vitro* and *in vivo* siRNA delivery for gene silencing. The potential implications of nanotechnology have been demonstrated recently by a broad variety of applications in the study of sub-cellular processes of fundamental importance in biological systems. Examples include but not limited to study of mechanism, diagnosis, treatment of deadly diseases such as cancer. Chapter 1 provides an introduction to nanotechnology detailing the evolution of nanoparticles as distinguished drug and gene delivery systems. It also described the potential importance and application of nanotechnology in drug, gene and siRNA delivery. Chapters 2, 3 details about the various tools and techniques employed to study nanoparticles physicochemical properties and the role therein in investigation for delivery applications. It provides an overall aspect of various strategies adapted for tracing the movement of nanoparticles while in various *in vitro* and *in vivo* applications.

The success of nanoparticles mediated siRNA delivery and henceforth gene silencing is hampered by various *in vitro* and *in vivo* obstacles. The mechanism of RNAi takes place in the cytoplasm of the cells and is a multi-step process; Chapter 4 describes the mechanism of siRNA mediated gene silencing i.e., RNA interference. It also gives an account of the barriers encountered and the strategies adapted to successfully combat them. The barriers have been sub divided into two classes i.e., the *in vitro* and *in vivo* barriers. Although modifications such as PEGylation have improved the circulation time of nanoparticles, the RES still manages to capture a significant amount of the injected dose. Henceforth, a subtle balance between stability and cellular uptake is desirable for siRNA to meet its potential as a therapeutic modality. The potential of nanoparticles can further be manipulated by making them target specific by decoration of surface with various targeting ligands. Chapter 5 mentions the cell surface modifications that occurs in the diseased cells and the strategies designed to exploit that situation by employing different targeting ligands to nanoparticles such as antibodies, transferrin etc. Nanoparticles, being compact, are well suited to traverse cellular membranes to mediate gene delivery. It is also expected that due to smaller size, nanoparticles would be less susceptible to reticuloendothelial system clearance and will have better penetration into

tissues and cells, when used in *in vivo* therapy. Chapter 6 highlights the pros and cons of using cationic polymeric nanoparticles for siRNA delivery.

Chitosan, an aminoglucopyran, which is biocompatible and biodegradable along with low toxicity, has been largely explored as siRNA delivery candidate. It has been employed in numerous *in vitro* and *in vivo* studies for gene silencing applications in cell and animal models. Chapter 7 provides account of properties of chitosan and preparation methods in addition to preparation of nanoparticles for siRNA delivery. Polyethylenimine (PEI) often considered as the gold standard of gene transfection have recently been investigated as a siRNA delivery vector. PEI exists in different molecular weights and polymer isoforms. Chapter 8 provides brief summary of the studies undertaken to investigate the siRNA delivery potential of PEI. Poly-L-lysine (PLL) is one of the first polymers investigated for non-viral gene delivery, but found limited applications for siRNA. Chapter 9 explores the literature citing implications of PLL for siRNA delivery. Atelocollagen was the first biomaterial with potential application as a gene delivery vector and obtained by pepsin treatment from type I collagen of calf dermis. siRNA complexed with atelocollagen is resistant to nucleases and is transduced efficiently into cells, thereby allowing long-term gene silencing. Chapter 10 details the studies done to tap the hidden potential of atelocollagen for gene delivery in several mice models.

Use of protamine for siRNA delivery was first reported by Song *et al.* which further followed by several studies. Chapter 11 deals with application of protamine in different formulations to provide safe and effective siRNA delivery. Dendrimers, the branched, multivalent molecules with a well defined structure found ample of applications in gene delivery and also explored for siRNA delivery. Chapter 12 summarizes different types of dendrimers, structural arrangements and their siRNA delivery potential. Cyclodextrin containing cationic polymer (CDP) for gene delivery can be further modified by inclusion-complex-formation due to presence of large amounts of CD moieties. Chapter 13 reports the use cationic polymers such as linear and branched PEI, and PAMAM dentrimer, modified by grafting CDs on the polymers, and studied for siRNA delivery. Although, cationic polymers (i.e., nucleic acids condensing agents) seem to be an excellent substitute for viral vectors, limitations including toxicity due to excess positive charge of the polymer, limits the application for systemic delivery. Chapter 14 explores the promising

PLGA-based nanoparticles with the encapsulation of siRNA, for delivery to plethora of applications. Nanomaterials such as gold nanoparticles, carbon nanotubes, and silica nanoparticles attracted considerable attention in their pharmaceutical applications due to their biocompatibility and ability to facilitate the delivery of therapeutic cargos. Chapter 15 discusses the properties and use of these nanomaterials for siRNA delivery.

Collectively, this book should prove a useful resource not only to those who are using or wish to use nanoparticles for siRNA delivery in cell lines and animal models, but also to those interested in applications of nanotechnology more generally. We hope that this book will also serve as a catalyst spurring others to use nanomaterials not yet being widely studied for siRNA delivery and to develop new methodologies that would contribute to both the fundamental understanding of nanomaterials mechanism of action and their potential utility.