Preface

Gene delivery is the science of transferring genetic material, either DNA or RNA, into cells to alter specific cellular function or structure at a molecular level [1]. Cloned genes can be delivered to cells for biochemical characterization, mutational analyses, investigation of the effects of gene expression on cell growth, understanding of gene regulatory elements, and producing specific proteins. Inversely, the delivery of RNA can be used either to induce protein expression or to repress it using antisense or RNA interference (RNAi) [2].

As the delivery of "naked" nucleic acids is the safest but the least efficient way to transfect mammalian cells and tissues [3], a variety of different vectors, which can be roughly categorized into viral and nonviral systems, have been extensively investigated over the last three decades. Unfortunately, none of them can be applied to any different kind of cells type with no limitation and side effects [4]. That is mainly because gene delivery pathways are complex combinations of multiple, potentially rate-limiting, biological processes and approaches to the design of delivery vehicles focusing on any single barrier individually will likely be suboptimal [5]. Furthermore, many current approaches are frustrated both by mislocalization and by sequestration in nontarget sites [6]. No matter what their origin, strain, and family, viruses have naturally evolved exquisite strategies to reach and penetrate specific target cells where they hijack the cellular machinery to express genes [7]. Even though engineered replication-defective viral vectors outperform nonviral systems and their inherent cell-specific tropism reduces off-target transduction, this, together with other shortcomings, has hitherto precluded the delivery of nucleic acids to alternative cell types and tissues [8]. Recent years have thus witnessed a surge of interest in nonviral delivery systems [9]. Cationic lipids and polymers are nowadays relatively safe, with tunable chemistries and cell targeting moieties, and potential for large-scale production with high reproducibility and at acceptable costs [10]. Nevertheless, despite transfectants are becoming increasingly optimized (at least) for benchtop laboratory research, nonviral gene delivery is still arguably in its infancy. First binary complexes between cationic lipids and polymers with the DNA date back to the mid-70s [11, 12]. Since then several scientists have made substantial contributions to this domain by developing more and more sophisticated, still conventional or classical, chemicals and formulations [13, 14]. Additionally, drawing inspiration from processes naturally occurring in vivo, major strides forward have been made in the development of more effective transfectants. Specifically, smart vectors sensitive to a variety of physiological stimuli such as cell enzymes, redox status, and pH are substantially changing the landscape of gene delivery by helping to overcome some of the systemic and intracellular barriers that viral vectors naturally evade [9]. Stimuli-responsive transfectants are now at the forefront of gene delivery vectors technology [15]. Clearly, existing vectors need to be streamlined further [16]. The promises are still great, and the problems have been identified (and they are surmountable) [17].

Some other fellows and I trust that a key pitfall that plagues science, among other worthy causes, is the difficulty in reproducing results because of the huge amount of variability existing not only between labs but also from time to time in the same lab and never reported in peer-reviewed papers [18, 19]. Furthermore, members of our thriving scientific community

come from different backgrounds and are not historically accustomed to talking a common scientific language with each other. It therefore follows that a handbook of best laboratory practices and detailed experimental procedures including organic synthesis going through chemical-physical characterization to biological testing is badly needed.

The volume of the *Methods in Molecular Biology* series provides the readers with a wide collection of the latest and foremost, readily reproducible technical protocols available in the field of nonviral gene delivery vectors, written such that a competent scientist unfamiliar with these methods can carry out the technique(s) successfully at the first attempt by simply following the detailed practical procedure(s) being described.

Such a collection of chapters is organized into three major parts: (1) Part I on conventional bolus gene delivery vectors (*see* Chapters 1–13) introduces typical transfection approaches relying on the addition of transfectants to the cell culture medium where the cells grown in; (2) another one on stimuli-responsive bolus transfectants (*see* Chapters 14–17) covers advanced topics on gene delivery complexes delivered by dripping onto cells, made of smart polymers or stimuli-responsive polymers that undergo changes depending on the environment they are in; (3) an example of substrate-mediated gene delivery (*see* Chapter 18), also termed reverse transfection, or solid-phase delivery concerns the immobilization of a gene delivery vector onto a surface as opposed to more typical bolus delivery from the medium.

Each chapter covers the development and/or characterization and/or testing of a typical transfectant, as apparent by the very specialized title, representative of a wide class of nonviral gene delivery systems. It is worthy of note that it is the running title that will provide readers with general information about the specialty subset of nonviral gene delivery vectors they are going to read and learn about. In this respect, the complete set of information comprises pure cationic lipid-based lipoplexes (*see* Chapter 1), cationic and zwitterionic lipid-based lipoplexes (*see* Chapters 2 and 3), anionic and zwitterionic lipid-based lipoplexes (*see* Chapter 4), non-ionic surfactant-based lipoplexes (*see* Chapter 5), stealth lipoplexes (*see* Chapter 6), targeted lipoplexes (*see* Chapter 7), anionic polymer-strengthened lipoplexes (*see* Chapter 8), pure cationic polymer-based polyplexes (*see* Chapters 9 and 10), stealth polyplexes (*see* Chapter 11), cationic lipid-coated polyplexes (*see* Chapter 12), anionic lipid-coated polyplexes (*see* Chapter 13), redox-responsive lipoplexes (*see* Chapter 14), pH-responsive polyplexes (*see* Chapter 15), photo-responsive polyplexes (*see* Chapter 16), thermo-responsive polyplexes (*see* Chapter 17), and surface-tethered polyplexes (*see* Chapter 18). New and future gene delivery vectors will be dealt with on the basis of this body of knowledge.

The structure of each single chapter, organized in four consecutive and interrelated sections each encompassing different types of information, is the real hallmark typifying the *Methods in Molecular Biology* series. Each chapter opens with a coherent and authoritative account of the very idea underlying the method(s) being described. The Materials section lists all the raw materials, buffers, disposables, and equipment necessary for carrying out every protocol claimed. As the overall aim of this volume is to provide researchers with a full account of the practical steps necessary for carrying out each protocol successfully, the Methods section contains detailed and lucid step-by-step descriptions of every protocol for the successful completion of each method undertaken. The Notes are intended to complement Materials and Methods sections, highlighting critical experimental details and how best to troubleshoot issues that might arise when executing the protocol(s). Finally, a comprehensive list of all the References contains a great deal of useful material in addition to the main text, best provide readers with entry to the literature.

As this volume has been written for experimentalists, it is my most sincere hope that *Nonviral Gene Delivery Vectors: Methods and Protocols* will be an essential part of many laboratory bookshelves and would help novice and professionals alike succeed in their research in this field.

Mere words cannot express my sincere appreciation and gratitude to all and each of the authoritative contributors for providing this volume with such high-quality manuscripts and to Prof. John Walker, the Editor-in-Chief of the *Methods in Molecular Biology* series, for his timely help and guidance.

Milan, Italy ***Gabriele Candiani***

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