Designer Materials for Nucleic Acid Delivery

Theresa M. Reineke and Mark W. Grinstaff, Guest Editors

Abstract

The Human Genome Project continues to reveal the genetic basis for numerous acquired and inherited diseases ranging from cancer, HIV, and heart disease to muscular dystrophy and hemophilia. With this wealth of information, the ability to design patient-specific drugs that alter the cellular machinery at the genetic level in a way that controls or treats a specific disease will increasingly become a reality. Designing nucleic acid drugs as well as engineering novel delivery vehicles that encapsulate and effectively transport genetic materials into cells provides an opportunity to enhance the understanding of disease mechanisms and may help treat or cure these diseases. This issue of *MRS Bulletin* on "Designer Materials for Nucleic Acid Delivery" explores the diverse materials—polymers, lipids, nanoparticles, biocompatible scaffolds, and engineered peptides—that are being evaluated for the intracellular delivery of nucleic acids. These synthetic delivery systems are actively being investigated for many research purposes that range from gene-based therapy, genetic vaccine, and RNA interference to gene function and cellular signaling studies. This area is currently being pursued by a broad group of academic, clinical, and industrial researchers at both the fundamental and applied level, motivated by the widespread implications for human health. In this introductory article, we provide a general tutorial to gene-based therapies and a brief overview of the many areas of materials research that are currently making a tremendous impact on this interdisciplinary field. We conclude with a discussion of the future challenges that materials researchers face in developing viable nucleic acid delivery vehicles.

Keywords: *DNA, gene delivery, lipid, lipoplex, liposome, nonviral vector, nucleic acid delivery, peptide, polyplex, RNA, viral vector.*

Introduction

From the simplicity of a unicellular organism to the complexity of the networked systems in human beings, all living things are made up of an elaborate architecture of biologically synthesized materials. Biological systems are created from a diversity of macromolecules such as lipids, proteins, and nucleic acids that are self-assembled into small entities called cells.[1] Each cell operates in harmony with its surroundings to direct every simple and complex function, from the uptake of nutrients to neuronal communication in the brain. At the very core of these small cellular entities lies the nucleus, which stores and organizes the genetic information responsible for directing all these processes. Although most living organisms are made up of different cell types that perform a variety of functions, each and every cell within a particular organism contains the same set of genomic information, organized into chromosomes within the nucleus. It is the spatial and temporal manner in which this information is deciphered by each cell that controls every unique function that allows life to exist. This process of decoding the specific regions in the genome, or genes,[2] that contain this explicit information is termed gene expression (Figure 1). During this process, a specific gene is transcribed to RNA, which is then translated into functional proteins that differentiate the cells that make up all of our organs and tissues.

It is to the core of these processes, the chromosomal and genomic level, that genetic-based medicines are targeted to manipulate the way in which this raw data is interpreted by the cellular machinery.[3] In more traditional forms of gene therapy, a gene sequence that repairs or replaces the defective gene must be incorporated into the host genome to cure the disease. Antigene and antisense agents alter the expression of a defective gene through binding to either the defective gene or RNA, which prohibits transcription or translation of a specific nucleic acid sequence implicated in disease. Recently, the discovery of RNA interference as a possible therapeutic tool has inspired the study of short interfering RNA (siRNA) fragments that are known to bind with and degrade messenger RNA (mRNA) sequences implicated in synthesizing disease-causing proteins. Lastly, DNA decoys represent another therapeutic route. These specific oligonucleotide sequences can block gene expression by binding up transcription factors, which are proteins that turn on both essential and detrimental gene transcription through binding specific sites in the genome.

Although many strategies exist for altering gene expression, a major obstacle with this form of treatment is the method of delivering the nucleic acids that will aid in regulating disease. Many nucleic acid forms such as DNA, RNA, and PNA (peptide nucleic acid) can now be readily synthesized to yield any size, shape, and

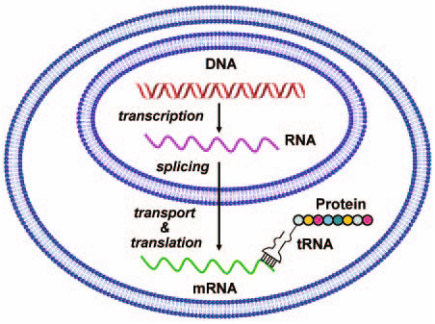

Figure 1. The process of gene expression within a cell. A specific region of DNA (a gene) in the cellular nucleus is transcribed to RNA, which is further processed to messenger RNA (mRNA) and then transported to the cytoplasm of the cell. Transfer RNA (tRNA) decodes the mRNA transcript to synthesize specific protein sequences (translation).

sequence of the drug; however, the major limiting factor to the clinical development of these treatments is actually transporting genetic drugs to the affected cells.[4] Delivery systems are needed to compact nucleic acids into small structures that can be taken up by cells, to protect genetic materials from enzymatic degradation during extra- and intracellular transport, to neutralize the negative surface charge of nucleic acids, and to provide a means of targeting the drug to the specific site of disease and region within the cell.

For many years, genetically modified viruses that incorporate a therapeutic gene instead of the infective sequence have been utilized for the delivery of genetic therapies.[5,6] Viruses have evolved to include many natural mechanisms to facilitate cell surface binding and internalization through a process called endocytosis (Figure 2). In addition, these natural gene carriers have special peptide sequences that allow them to escape degradation in the endosomes and unload their infective payload to the proper area within the cell, either to the cytoplasm or the nucleus.

Unfortunately, several problems have occurred in clinical trials with this viral delivery method. For example, some viruses can randomly integrate genetic materials into the human genome, which disrupts proper gene function, and patients have developed leukemia. Other viral types have revealed toxicity or caused devastating immune and inflammatory responses, resulting in patient death.[7–9]

Because of these problems, alternative methods involving the development of synthetic materials are currently being studied for the delivery of nucleic acids.[10–15] Systems that mimic the positive features of viral delivery vehicles yet circumvent the negative aspects of these systems are highly sought-after. Chemical synthesis offers a powerful tool to tailor-make new materials with the ability to deliver any type of nucleic acid into specific and diverse cell types in a manner similar to viruses (and, as shown in Figure 2, into the proper region within the affected cell). Examples of two synthetic vector types are shown in Chart 1.

This issue of *MRS Bulletin* explores the many diverse materials that are being researched to carry nucleic acid therapies. Polymers, lipids, nanoparticles, and biocompatible scaffolds offer several promising new approaches to gene delivery. Also, conjugating specific peptide sequences to both the synthetic system and/or the nucleic acid itself offers a creative means to including functional viral sequences that may promote cellular uptake, endosomal release, and nuclear delivery of therapeutic genetic materials. This issue is focused on the synthetic design of such materials to overcome the cellular barriers faced during nucleic acid transport. Understanding how to overcome these barriers through creative materials chemistry and engineering is the first step toward developing safe, stable, and successful delivery vehicles for systemic delivery of genetic therapeutics.

Polymers

The broad area of "Polymeric Controlled Nucleic Acid Delivery" is presented in the first article, by Leong, who discusses the various polymer structures being developed for the delivery of nucleic acids. For many years, polymers have demonstrated the ability to bind nucleic acids through electrostatic, hydrophobic, and hydrogen bonding interactions. This area has recently exploded, thanks to the ease of designing and synthetically manipulating the chemical makeup of different polymeric systems to direct certain biological functions, from nucleic acid binding to nontoxic cellular delivery. Cationic polymers have been particularly examined because of their high binding affinity for the polyanionic backbone of DNA, the ease in which they compact DNA into nanoparticles termed "polyplexes," and the protection they offer genetic materials from nuclease degradation. Although these features are very useful for effective delivery *in vitro*, the transport of nucleic acids *in vivo* presents several challenges. In this review, Leong describes the major barriers of nonviral gene transfer and the many diverse polymers being designed to effectively facilitate polymer–nucleic acid binding and compaction, salt and serum stabilization, intracellular transport, and the release of nucleic acid drugs to specific cellular destinations. For example, nanoparticles can be formed through chemically crosslinking both ionic and nonionic polymers, such as polyethylenimine and poly(ethylene oxide), to decrease salt and serum aggregation. Biodegradable amphiphilic polymers [poly(vinyl alcohol) grafted

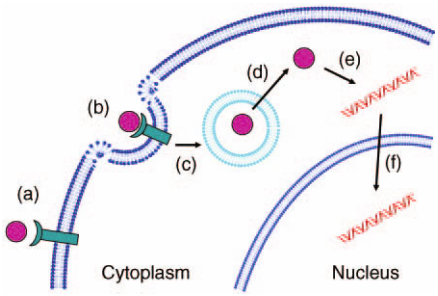

Figure 2. Various materials can bind with and compact nucleic acids into nanoparticles that can be taken up into cells through the process of endocytosis. This process first involves (a) the binding of the nanoparticles to cell-surface glycoproteins. (b) The binding event triggers part of the cellular membrane to envelop the nanoparticle and bring it into the cytoplasm with a membrane-bound vesicle (endosome). (c) Endosomes fuse with other cellular endosomes and mature into degradative lysosomes. (d) The nanoparticles must escape these vesicles to prevent degradation of their therapeutic payload. (e) The material must then release the nucleic acid to perform its therapeutic function in either the cytoplasm, or (f) the genetic material must traverse the nuclear membrane for gene regulation.

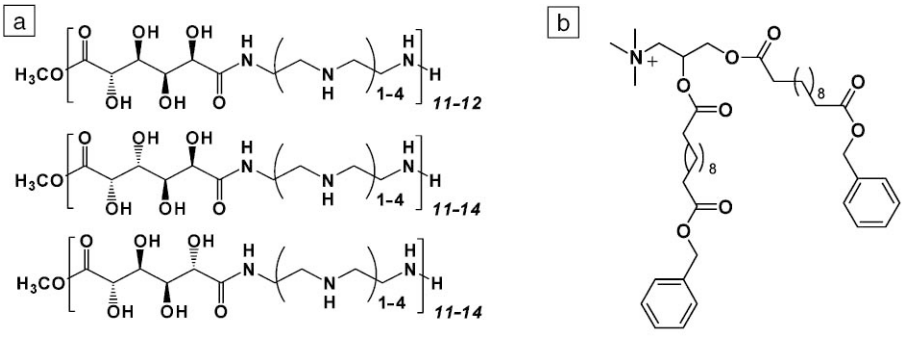

Chart 1. Examples of materials used to deliver nucleic acids. (a) Poly(glycoamidoamine)s synthesized by Reineke et al. exhibit high DNA delivery efficiency and low toxicity in vitro.[16,17] *(b) Functional lipids created by Grinstaff et al. undergo an electrostatic transition to promote the release of DNA from the lipoplex.*[18]

with D,L-lactides and glycolides] have been synthesized to facilitate DNA encapsulation while enhancing nucleic acid release through material degradation. Materials can also be added, such as pluronic block copolymers that serve as an adjuvant. These types of systems do not formally associate with DNA but have been shown to enhance gene expression.

Lipids

The second article in this issue reports on the recent progress with lipids for gene delivery. In their article, "Functional Amphiphiles for Gene Delivery," Barthélémy and Camplo describe amphiphilic lipids that bind DNA and form supramolecular assemblies. Many of these systems are widely used and commercially available for gene delivery *in vitro*, and a few are under clinical evaluation. The proposed pathway for DNA delivery using these synthetic lipid vectors includes DNA–synthetic vector complexation (i.e., lipoplex), endocytosis or plasma membrane fusion, endosomal escape, nuclear entry, and finally transcription (as shown in Figures 1 and 2). These systems are being investigated by a large number of research groups since the chemical structure of the lipid can be easily changed to increase the cationic charge, alter the hydrophobic chain length, or introduce new structures with unique properties (e.g., bio-inspired nucleoside-based amphiphiles). Based on these results, several groups are designing and evaluating synthetic vectors that respond to local environmental effects in an effort to facilitate overall gene transfection. These synthetic vectors are based on mechanistic, rational design principles and represent the next generation of lipids. Barthélémy and Camplo provide examples of lipid vectors that are pH-, redox-, and electrostatic-sensitive, and discuss how this increase in functionality affords enhanced gene transfection efficiencies.

Nanoparticles

The role and use of nanoscale science and nanotechnology in the delivery of DNA is described in the article "Nanotechnology, Nanoparticles, and DNA delivery" by Luo. The advantages of precisely controlled structures and compositions with nanomaterials and DNA provide new opportunities to design delivery systems. The first example described in the article is cationic dendrimers. These well-defined, highly branched macromolecules bind DNA and facilitate the transport of DNA across the cell membrane. This discussion expands to include the use of novel self-assembling DNA-based dendrimers that spontaneously form buckyball-like structures. These structures, when derivatized with peptides and complexed with DNA, mimic natural viruses. Recently, nanotube-, nanorod-, and nanoparticle-based DNA delivery systems have been reported. Luo discusses how these reports begin to show the potential of nanomaterials for gene delivery. Lastly, throughout the article, Luo emphasizes the bottom-up approach to creating new synthetic and synthetic–natural hybrid materials.

Surfaces

In the article titled "Gene Delivery by Immobilization to Cell-Adhesive Substrates," Bengali and Shea report on the recent advances in substrate-mediated gene delivery. Surface immobilization of DNA has several advantages, including minimizing the amount of DNA needed to achieve a desired effect, preventing DNA/vector aggregation, reducing toxicity, and delivering DNA to specific cellular sites. This approach works for both viral and synthetic vectors where these vectors are noncovalently or covalently bound to a biomaterial-coated surface. The authors further discuss the challenges and opportunities of substrate-mediated DNA delivery for basic research, diagnostic, and clinical therapies. For example, gene delivery coupled with a degradable polymer for a tissue engineering application provides both a temporary scaffold for cellular infiltration and molecular signaling to guide tissue repair. As with the other delivery platforms described in this issue, the synthesis, characterization, and knowledge of material properties are the keys to optimizing gene delivery.

Peptides

With all the previously mentioned synthetic delivery systems, the most challenging hurdles to successful and specific nucleic acid transfer are the cellular barriers involved. For example, the delivery vehicle must promote efficient cellular uptake of the genetic drug. Then, the nucleic acid has to survive the uptake event, avoid degradation, and be successfully released into the cellular cytoplasm, which is often the final therapeutic destination within the cell.

However, in many cases, the nucleic acid must be transported into the nucleus to perform its proper therapeutic effect. Bergen and Pun describe several novel strategies to evade these significant difficulties in their article, "Peptide-Enhanced Nucleic Acid Delivery." Inspired by the promising aspects of viruses, scientists may overcome these barriers by incorporating engineered viral sequences into materials-based vectors to enhance the efficacy of nonviral nucleic acid delivery. As discussed in the article, cell-penetrating peptides (CPPs) are highly basic sequences that promote translocation of materials across the cellular membrane. Endosomal release may be enhanced and gene degradation avoided through incorporating fusogenic peptides that undergo pH-dependent structural transitions known to disrupt cellular membranes and thus enhance the cytoplasmic delivery of synthetic vectors. In addition, amino acid chains, termed nuclear localization sequences (NLSs), are being engineered into vector systems to promote nuclear delivery of genetic materials. These proteins recognize and bind to other proteins within the cellular cytoplasm that are known to transport biological materials across the nuclear membrane. Bergen and Pun describe specific peptides being developed to facilitate the targeting of synthetic carriers to specific cell types and particular intracellular organelles.

Conclusion

The field of nucleic acid delivery represents a tremendous and unique opportunity for materials researchers to have an impact on modern medicine. Currently, the ability to transport nucleic-acid-based therapies into cells in a manner that is nontoxic, non-immunogenic, and with high specificity and efficacy is a significant challenge. As highlighted in this issue of *MRS Bulletin*, several different methods involving the use of polymers, lipids, nanoparticles, functionalized surfaces, and engineered peptides to transport genetic drugs are currently being designed and evaluated. Although the number of researchers in this field has increased due to the high potential impact on human health, the area is still in its infancy with regard to creating a safe synthetic system that transfects with viral-like efficiency.

Because much of the work in this area is concentrated on overcoming the cellular barriers, it is clear that further investigations are needed to develop these systems for systemic and clinical applications. New materials that guard polyplex, lipoplex, and nanoparticle-based structures against physiological salt and serum aggregation, prevent nonspecific interactions with blood components and the vasculature, and escape the reticuloendothelial system are needed. In addition, these materials must have the ability to pass through the vascular endothelium and extracellular matrix to deliver their payload in a specific manner to diseased cells while avoid-

ing healthy tissues. These stable complexes must also retain the ability, once inside the cell, to release the therapeutic agent. Consequently, the move from passive to active gene delivery materials that respond to their local environment or external stimuli, or are part of a systems approach, represents a key research pathway for the future. Studies are also needed to understand the biological mechanisms that occur during extra- and intracellular transport, cellular uptake, and release of materials-based carriers. Lastly, understanding how the chemical and structural properties of synthetic materials influence all the biological mechanisms involved in nucleic acid delivery is essential to developing specific delivery vehicles for certain diseases and research purposes. As scientists and engineers, we should not let this particular opportunity go uninvestigated where our knowledge, creativity, and determination can have a profound impact on the ultimate success of nucleic-acid-based therapeutics.

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Barthélémy's areas of research include medicinal, organic, and physical chemistry. Currently, he is working on amphiphilic structures possessing molecular features capable of mimicking natural systems and creating nanometer- to micrometer-scale objects. Included in this research is the systematic study of self-assembly in the presence or absence of nucleic acids. One of his main objectives is to develop novel bio-inspired amphiphiles for multiple applications ranging from molecular building blocks for self-assemblies to drug and biomolecule delivery systems.

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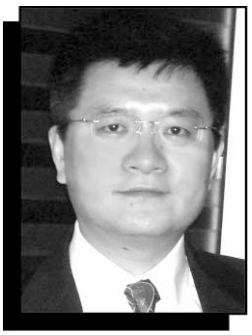

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peptides that enhance nonviral nucleic acid delivery.

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Leong received his PhD degree from the University of Pennsylvania and was a postdoctoral fellow at MIT. He has over 30 patents and more than 150 refereed publications. He serves on the editorial boards of *Biomaterials, Molecular Therapy, Nanomedicine,* the *International Journal of Nanomedicine,* and the *Journal of Controlled Release.*

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