POLYPHOSPHAZENES FOR BIOMEDICAL APPLICATIONS

Edited by

Alexander K. Andrianov

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PREFACE

Polymers play a key role in the development of drug delivery systems, medical devices, and biosensors. More than ever they face challenging requirements, as clinical science dictates increasingly sophisticated sets of properties and design parameters. Interactions with specific biological targets, biocompatibility, environmental responsiveness, modulated degradation, and formation of supramolecular assemblies are among some of the desired features that have to be integrated in biomedical polymers of the next generation. Yet most synthetic macromolecules used in the biomedical area were not designed originally for these applications and lack the desired chemical flexibility. It has become increasingly evident that the creation of novel macromolecules for life sciences applications can only be realized through the successful merger of a biological rationale with a highly versatile synthetic platform.

Polyphosphazenes, macromolecules with a phosphorus and nitrogen backbone, provide an ideal background for the realization of this objective. A unique synthetic approach, the key feature of polyphosphazene chemistry, allows easy introduction of multiple functionalities and biological modules in a polymer while supporting it with high-throughput discovery methods, which are still largely uncommon in macromolecular chemistry. Unlike many other classes of synthetic polymers, polyphosphazenes offer a hydrolytically degradable backbone with side groups providing reliable "dial-in" controls for rate modulation. Flexibility of the backbone and two side groups at every monomeric unit, which potentially render high functional density, create further opportunities for fine tuning of biologically relevant properties.

These advantages were sufficiently important to trigger a genuine interest in polyphosphazenes as a unique template for constructing biomedical polymers. Still, the attention shows signs of caution as the field advances to provide more robust synthetic approaches, allowing adequate control of macromolecular parameters. The concern is especially valid to industrial scientists, who have to look for efficient and practical solutions to their immediate challenges. The genuine excitement about the opportunities is frequently mixed with reservations about the novel and somewhat unknown behavior of these polymers in living systems. In this book we organize the most recent developments in various areas of biomedical polyphosphazenes to give the reader the opportunity to review the current status of knowledge on the interface of biological sciences and polyphosphazene chemistry.

The book begins, with an introductory section in which general aspects, the most critical advances, and future directions of the technology are discussed. It includes a brief overview of the main synthetic approaches, rational design in polyphosphazene chemistry as it relates to biological applications, and the main representatives of biomedical polyphosphazenes. Further sections are organized based on specific areas of potential clinical applications of polyphosphazenes. A substantial part of the book is dedicated to the most advanced class of biomedical polyphosphazenes, polyelectrolytes, which have been studied extensively in both preclinical and clinical research as vaccine adjuvants. Part II contains an overview of polyphosphazene adjuvants, an extensive collection of in vivo data in various animal models using a variety of antigens, analyzes the importance of the delivery routes, and provides condensed information on their production, control, and potential mechanism of action. It also showcases a critical role of unique structural features possessed by a polyphosphazene family in their interactions with biological targets, including proteins and cells.

Chapters in Part III are focused on the advantages that polyphosphazenes can provide as potential biomaterials. Discussed are applications of polyphosphazenes as scaffolds for tissue engineering, use for surface modification, and in composite and nanofabricated materials. Polymers prepared by condensation polymerization and their evaluation in cytotoxicity studies are also reviewed here. Various aspects of the technology, such as biocompatibility, biodegradability, surface properties of polyphosphazenes, and their buffering capacity in blends are covered in this section.

Drug delivery remains one of the most important and promising areas of biomedical polyphosphazenes. Chapters in Part IV review a broad range of topics, starting with supramolecular polyphosphazene assemblies such as environmentally responsive liposomes and micelles for intracellular targeting and the use of polyphosphazenes for the production of nanostructured materials, and continuing to prodrugs and potential cancer therapies. Although to date computational chemistry and molecular simulations have rarely been applied to biomedical polyphosphazenes, the existing knowledge base on phosphazene membranes and polyelectrolytes is expected to be of interest to the reader. In fact, it can easily be extended to such biologically relevant systems as ionically cross-linked polyphosphazene microspheres and coatings. Thus a review of the relationship between polyphosphazene structure and molecular transport is also included. Detection and monitoring in biological systems and the role that polyphosphazenes can play in such devices are discussed in Part V.

Part VI of the book deals with one of the most fundamental subjects of biomedical polymers: well-defined macromolecular structures and synthetic approaches to their synthesis. A review of chemical regularity in polyphosphazene copolymers can provide an important starting point for scientists interested in the development of polyphosphazenes with multiple functionalities. Finally, a chapter on cyclic phosphazenes is also included, since an

indispensible database of biologically relevant properties established for these compounds can open new opportunities for the rational synthesis of new polyphosphazenes for biomedical applications.

The book is intended to reach a broad audience interested in pharmaceutical sciences and biomaterials and to assist researchers and clinicians in enhancing their understanding of polyphosphazene technology. It can also be useful for both graduate and undergraduate students, as it can extend their knowledge base to an important but somewhat less publicized class of biomedical polymers. I hope that the book will help motivate the readers to take a closer look at the fascinating class of polyphosphazene compounds and their potential role in the development of future biomedical polymers.

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PART I
Introduction

1 Polyphosphazenes for Biology and Medicine: Current Status and Future Prospects

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Synthetic polymers have played a fascinating role in the successful development of biomedical devices and drug delivery systems. However, until recently, polymers for health care applications were commonly adopted from other industries without their substantial redesign for medical use. Although this strategy helped to resolve many pressing needs and even resulted in successful medical treatments, it is no longer acceptable for modern-day systems, many of which demand combinations of unique biological characteristics. In many life sciences applications, researchers are facing major challenges in creating materials with specific patterns of degradation profiles, biological interactions, release characteristics, and physicochemical and mechanical properties [1]. Today's medical treatments demand macromolecular systems with the ability to participate in cellular signaling processes, modulated interactivity with biomacromolecules, varied environmental responsiveness, and the ability to self-assemble into supramolecular structures. The choice of polymers for life sciences applications, especially those that combine modulated biodegradability and the ease of chemical derivatization, remains scarce. In this regard, synthetic polymers with phosphazene backbones offer unique opportunities in the life sciences arena.

Polyphosphazenes, macromolecules with a phosphorus-nitrogen backbone and organic side groups (Fig. 1), possess a number of properties that make

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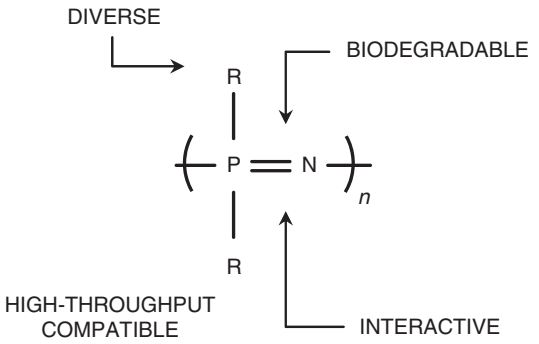

Figure 1 Polyphosphazenes.

them highly attractive for life sciences applications. The following features distinguish them from other classes of biomedical polymers.

1. The inorganic backbone is capable of hydrolytical degradation, which can be modulated through selection of the appropriate side group.
2. The unique synthetic pathway to these polymers, *macromolecular substitution*, allows a huge selection of substituents to be introduced by common organic chemistry methods, free of many ambiguities and restrictions associated with polymerization processes.
3. Such methods lend themselves to high-throughput synthesis, which accelerates the discovery process.
4. The unique flexibility of the backbone and its ability to participate in noncovalent bonding and formation of supramolecular assemblies create new opportunities for an interface with biological systems.

Polyphosphazene chemistry, largely due to pioneering contributions of Professor Harry Allcock, has become an important area of polymer research. Although the features of polyphosphazenes should at least induce curiosity in these compounds and stimulate their testing in challenging applications, these polymers are still infrequent guests on the benches of biomaterials and drug delivery scientists. Limited commercial availability, inadequate information on material quality, and a scarce database on the structure–property relationship make them inaccessible to most application researchers. Despite this, a steady flow of publications, patents, and even clinical trial reports indicate advancement in the field. Multifunctionality and unique biological features are among the key factors in the selection of these macromolecules for biological applications.

Recent discovery of extraordinarily high immunostimulating activity of ionic polyphosphazenes has inspired extensive research in the area and aroused interest in their commercial development. The fact that polyphosphazene

polyelectrolytes were overwhelmingly more active than their conventional counterparts, as well as the ability of polyphosphazene backbones to undergo degradation, triggered extensive preclinical and clinical research in the area [2–11]. Recent developments in the field with regard to in vivo activity and chemical control of such systems with emphasis on the unique behavior of these compounds are summarized in several subsequent chapters.

Interestingly, intense development of water-soluble polyphosphazene immunostimulants revealed other important advantages of this class of polymers. Initially, a lead compound, poly[di(carboxylatophenoxy)phosphazene] (PCPP), was introduced in the vaccine industry as a potent immunostimulating excipient for water-soluble formulations (Fig. 2). It was not long before simple, ''protein-friendly'' aqueous coacervation methods were developed to prepare slow-release microspheres in which a potent immunostimulant, PCPP, also serves as a wall-forming material [12,13]. Slow-release microspheric systems are considered to be important in achieving persistent memory immune responses or as carriers for mucosal immunization. The use of polyphosphazene microspheres not only eliminates the need for an additional microencapsulating agent but allows sustained release of the immunostimulant along with the antigen, a new concept for vaccine delivery which is difficult to achieve with other immunostimulants, such as emulsions or alum.

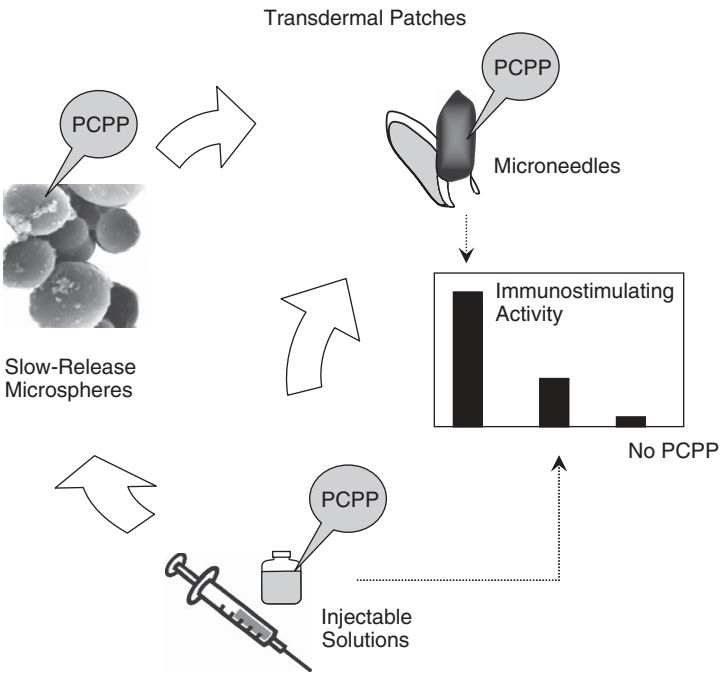

Figure 2 Multifunctionality of polyphosphazene immunostimulants: transdermal, slow release, and solution formulations of PCPP.

Recently, polyphosphazenes have also emerged as a potent class of immunostimulants for intradermal vaccines. Transdermal immunization is one of the promising areas of vaccine development, due to the strength of the skin's defense mechanisms against many infections. Intradermal vaccines have the potentiall to provide improved immune response and antigen sparing. However, topical administration requires the use of special devices, such as arrays of microneedles, due to poor penetration of antigens through the upper layer of the skin: the stratum corneum. Microneedle technology is based on the use of submillimeter structures and often relies on coating such needles with solid-state vaccine formulations. Such systems are designed to be painless, can be self-administered, and in many cases also improve the shelf life of a vaccine over that of its solution formulation. One serious obstacle for the successful development of intradermal vaccines is difficulty in combining the technology with immunostimulants, due either to reactogenicity concerns (alum) or to phase compatibility issues (emulsions). PCPP was shown to be an excellent agent for binding the vaccine formulations to the surface of microneedles (Fig. 2), eliminating the need to use other polymeric excipients, such as carboxymethyl cellulose (CMC). Recent in vivo experiments showed synergy between microneedle and polyphosphazene technologies, with such formulations unexpectedly and dramatically outperforming both CMC microneedle and parenteral polyphosphazene vaccine formulations. These results are discussed thoroughly in Chapter 7. A combination of microspheric and intradermal delivery approach can establish yet another modality of the technology (Fig. 2).

A diverse set of features contributed to the advancement of polyphosphazene polyelectrolytes as immunostimulants. Physicochemical properties linked to immunostimulating activity, such as excellent complex-forming ability, flexibility of the backbone, and high ionic density, were combined successfully with hydrolytically degradable backbone, ionic sensitivity, hydrogel-forming properties, and traditional polymer characteristics such as film-forming properties. This example emphasizes both the unique and multifunctional behavior demonstrated by ionic polyphosphazenes, which led to the introduction of an unparalleled delivery system in this area of the life sciences.

It can be anticipated that polyphosphazenes will also be capable of expanding horizons in other areas of biomedical applications. These expectations are especially high in applications where multicriteria requirements have created considerable obstacles for the successful development of established materials. For example, due to their tailorable biodegradability, polyphosphazenes can provide an important resource for regenerative medicine, a thriving area of research aimed at the treatment of deceased and damaged tissues. The multifaceted strategy employed in the field is directed toward the development of new materials that can interface with tissues structurally, mechanically, and biofunctionally. New materials must provide space, support, and instructive cues while avoiding hostile immune response, and must also degrade slowly to allow for tissue in-growth and removal of the synthetic scaffold.

One of the challenges of the field is that many existing biodegradable synthetic polymers provide minimal biological cues to guide tissue regeneration and have limited bioactivity [14,15]. Structural diversity and synthetic flexibility of biodegradable polyphosphazenes seem to put them in a favorable position since they can provide straightforward routes for the incorporation of peptide sequences with highly specific biofunctionality. Alternatively, sites for protein adsorption can be introduced easily to transform an implant surface into a biological landscape that supports cellular interaction. Recent advances achieved in polyphosphazene use in biomaterials are described in several chapters.

Although most of the research on biodegradable materials is devoted to hydrophobic polyphosphazenes, hydrogels can be as important in such applications. Although their initial mechanical properties cannot compete with those of hydrophobic polymers, hydrogels can better facilitate accelerated tissue formation, due to their aqueous environment. This leads to the rapid development of a natural matrix, which can soon supplement and assume much of the scaffold's mechanical responsibility. A number of preliminary reports indicate that polyphosphazene hydrogels are efficient in cell encapsulation and can provide simple ways for in situ hydrogel formation [16]. Due to the excellent ion-complexing properties of some polyphosphazenes, they can also play important roles in biomineralization processes for bone and cartilage regeneration [17], since they could be a source for the slow supply of calcium and phosphate ions. More research on the use of polyphosphazenes in this field can be expected in upcoming years.

Challenges in drug delivery are another important source of inspiration in polyphosphazene synthesis. Research has been focused primarily on microparticulate and prodrug systems, and some of the insights in the field are reviewed in the present book. Although these developments are vital, new structures and architectures that encompass features currently unachievable in the framework of conventional chemistries pave the road to the recognition of polyphosphazenes in the field. Polyphosphazene alternatives to well-known pharmaceutical carriers currently utilized in drug delivery can potentially introduce superior biological characteristics. For example, originally, PCPP was synthesized to mimic the ionic sensitivity and hydrogel-forming properties of alginic acid. It was introduced successfully as an alternative to alginates in ionic complexation technologies, also bringing the benefits of well-defined structure and high synthetic reproducibility [16,18]. Another important example is a polyphosphazene mimic of poly(vinylpyrrolidone). The latter is used widely in research as a drug carrier. However, its practical use for parenteral administration is severely restricted by its inability to degrade. Recently, polyphosphazene containing pyrrolidone side groups was synthesized [19]. This water-soluble macromolecular system combines functional properties introduced by pyrrolidone groups with hydrolytical degradability and ease of chemical modification.

Delivery of protein drugs is one of the areas where such systems can be of significant interest. The development of many promising protein therapeutics is

obstructed by problems associated with their rapid clearance from the body or undesirable side effects, such as immunogenic reactions. Their chemical modification with a synthetic polymer, poly(ethylene glycol) (PEG), proved to be a powerful approach in improving protein's pharmacokinetics, formulation stability, and safety [20–23]. A number of proteins modified with PEG have been approved by regulatory authorities. Advancement of biodegradable polyphosphazene carriers as an alternative to nonbiodegradable PEGs can potentially bring new architectures and functions to these systems. This can also reduce production costs by eliminating the need for careful fractionation, which is imperative for nonbiodegradable PEG. Such approaches may become increasingly important, as new, high-dose treatments such as antibody therapies are becoming more widespread.

Conceptually, biodegradable polyphosphazene with the appropriate protein-complexing properties can even eliminate the need for the sophisticated site-specific attachment of polymer to the protein. Currently, covalent attachment of PEG molecules to proteins is one of the most challenging stages in commercial manufacturing. Synthesis of monofunctional PEG with controlled molecular weight, chemical activation of PEG, a reaction of covalent conjugation, and purification of the synthesized product requires sophisticated technologies and equipment in multiple-step processes and so dictates high development and manufacturing costs. Covalent attachment methods can also result in a loss of biological activity, due to the nonspecific and random linkage of multiple PEG molecules.

Complexation of proteins to a biologically inert polymer through noncovalent links, provided that the resulting assembly does not show high immunogenicity, can potentially reduce sophisticated chemical manufacturing to a routine formulation step. Efforts to develop of polyphosphazene systems that combine PEG-like features with an ability to form complexes with proteins have recently been initiated [24].

Another interesting area where polyphosphazenes can potentially take advantage of their multifunctionality is in the field of shape-memory polymers, where a combination of modulated flexibility with functional diversity can be important. If biodegradability can be built into such materials, they also have potential for use in minimally invasive surgery. Two examples are biodegradable shape-memory polymer as an intelligent suture for wound closure, and insertion of bulky implants into the human body in compressed form through a small incision, with the implants turning into their application-relevant shape within the body [25,26]. Other areas are biodegradable slow-release coating for stents, where biological parameters have to be blended with intrinsic polymer characteristics [27–29], or biocompatible fluorinated coatings, which can be based on multilayer fluorinated systems [30].

There is little doubt that polyphosphazenes represent an important class of biologically relevant polymers which can provide solutions for various challenging life sciences applications. The realization of this potential is profoundly dependent on the synthetic methods of polyphosphazene chemistry, especially

on their ability to facilitate rapid synthesis of new compounds and to provide adequate control of molecular characteristics.

The polyphosphazene technology platform seems to be ideally positioned for the development of high-throughput synthesis and combinatorial approaches, due to the structural diversity of the class and the dominance of organic chemistry methods. As soon as the starting point of polyphosphazene synthesis—the macromolecular precursor, poly(dichlorophosphazene) (PDCP)—is synthesized by polymerization, it can be reacted with a multitude of nucleophiles to yield a variety of organic polyphosphazene derivatives [31]. The main challenge to development of the parallel synthesis approach is the inherent hydrolytic instability of PDCP, which dictates the need for frequent time-consuming and labor-intensive polymerization runs. Recent breakthroughs in stabilization chemistry for this hydrolytically sensitive compound made possible new opportunities in this area [32]. In fact, semiautomated synthesis of new polyphosphazene derivatives was conducted successfully for the assembly of an immunoadjuvant library [33,34], a library of protein modification polymers [33,35], and process discovery for the synthesis of sulfonated polymers [36]. Hundreds of new polymers and copolymers were synthesized, and thousands of synthetic runs were conducted using the same lot of PDCP. The downstream processes were sufficiently simplified to complete the synthesis and purification, generally in one or two days. A general representation of biomedical polyphosphazene library construction, which in some cases can be extended to the assembly of the corresponding microspheres, is shown in Figure 3.

The perspectives of high-throughput methods in polyphosphazene chemistry may not be limited to discovery of new molecules and materials. Unprecedented diversity of polyphosphazenes combined with a potential for rapid synthesis can facilitate the construction of extraordinarily large databases, which may be difficult to build for other macromolecular classes. This obviously can be an enormous resource in predicting the properties and functional behavior of other polymers, boosting the role of polyphosphazenes as a unique scientific "toolbox."

For years the development of polyphosphazenes for life sciences applications has been somewhat impeded by challenges in regulating macromolecular characteristics and production consistency. Reproducibility of functional properties, control of structural irregularities, and synthetic by-products are the most critical issues that need to be addressed for the materials to be advanced further into the biomedical arena. There are clear indications that the lack of control in the substitution process can lead to residual moieties on polyphosphazenes, thus significantly affecting degradation profile, shelf life, and eventually, biological characteristics [5]. Although it can still be challenging to achieve adequate control of biologically relevant properties of polyphosphazenes in the research environment, much progress have been made in this area. In this regard, the establishment of the first GMP (good manufacturing practices) process for the manufacture of biomedical polyphosphazenes [5,32] and the following clinical trials [10,11] manifest a critical stage in their commercial development.

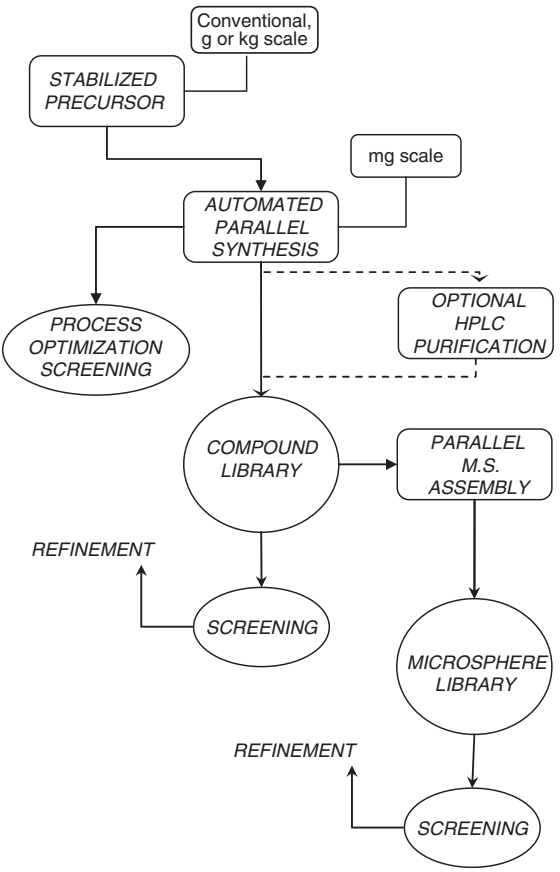

Figure 3 General scheme of high-throughput synthesis and polyphosphazene library construction.

In designing new biomedical polyphosphazenes, it may be important to critically review some of the most traditional approaches presently utilized in the field. The starting point in the blueprinting of any new polyphosphazene is almost always a review of the physicochemical properties of the potential side group. This is obviously important since the material properties of the future polymer will be defined primarily by the characteristics of the substituent. However, the effect of the future side group on the backbone and capability of the letter to interact with other molecules is still almost always neglected. From the chemistry of cyclic compounds it has been known for years that the phosphazene backbone is highly capable of noncovalent bonding and is actively engaged in supramolecular interactions. Obviously, this can be an indispensable resource for macromolecular chemistry as well, since such interactions are proven to be critical for immunostimulating and other biomedical applications of polyphosphazenes.

We look forward to further advancement of biomedical polyphosphazenes and anticipate that the collection of up-to-date articles on their life sciences applications and synthesis in this book will facilitate the process.

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