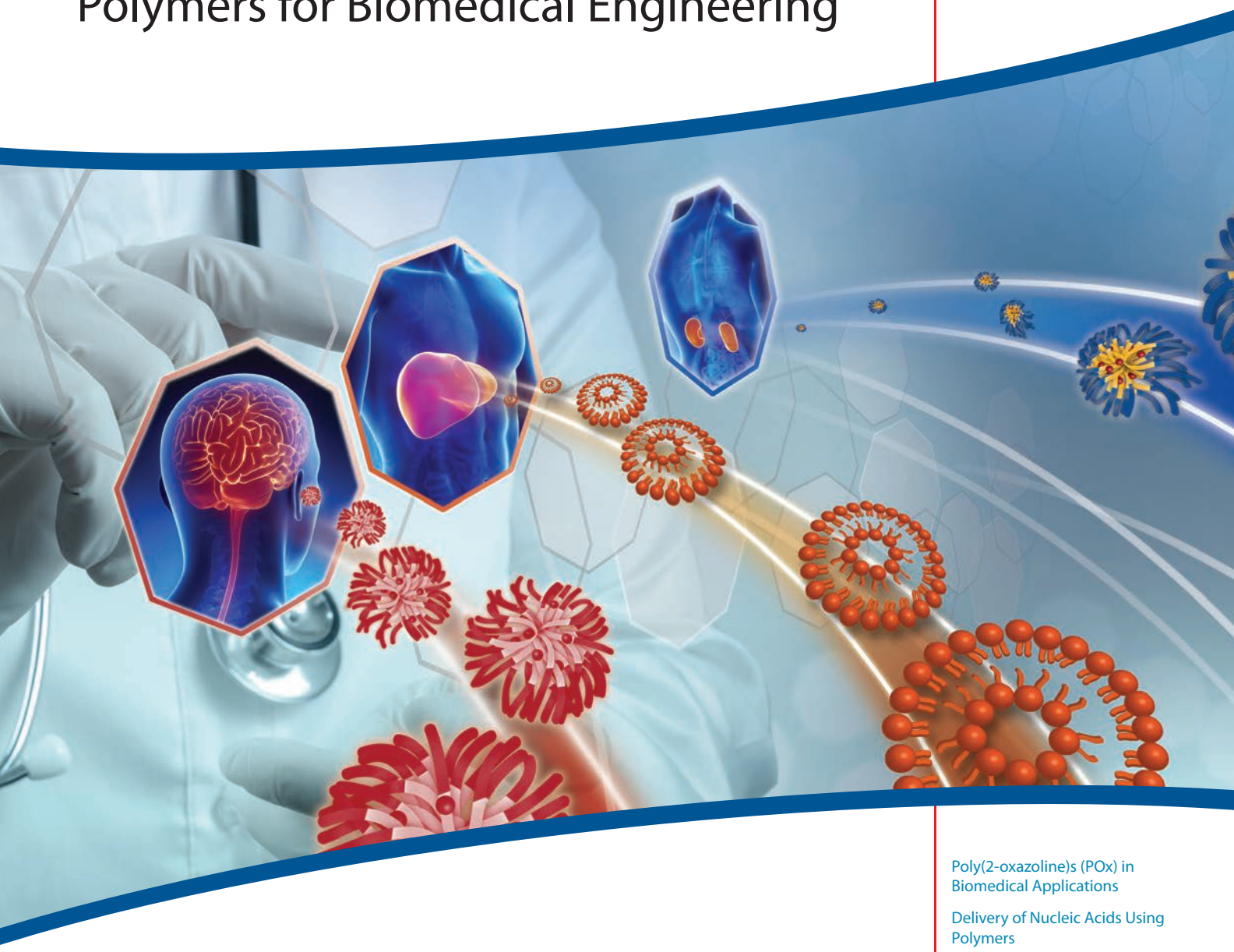
Material Matters™

Volume 8, Number 3

ALDRICH Materials Science

Polymers for Biomedical Engineering



Pushing the Boundaries in Medicine

Poly(2-oxazoline)s (POx) in Biomedical Applications

Delivery of Nucleic Acids Using Polymers

Synthesis and Biomedical Applications of Polyamino Acids

Fabrication of Drug-loaded Microparticles Using Hydrogel Technology and Recent Innovation in Automation

Functional RAFT Polymers for Biomedical Applications

Introduction

Sebastian Grajales, Ph.D.
Aldrich Materials Science

Welcome to the third 2013 issue of ***Material Matters™***, focused on polymers for biomedical engineering. Given the vast nature of this topic, ranging from delivery of small organic molecules, biological polymer materials, to hybrid biomaterial scaffolds, this issue highlights a few key research areas with significant advances focused on novel materials and techniques designed for specific targeted delivery abilities and tissue engineering needs.

The first article, by Rainer Jordan and Robert Luxenhofer (Germany) reviews poly(2-oxazolines) (POx). This family of biocompatible polymers has a range of potential functionalities to cover a variety of physical properties. These properties, such as tunable hydrophilicity, functionalities for conjugation, and temperature-sensitive capabilities, are particularly valuable for biomedical applications. POx materials are capable of forming micelles for drug delivery and hydrogels for tissue engineering, as well as POx-drug and POx-protein conjugates.

In our second article, Olivia Merkel (USA) reviews the delivery of nucleic acids using polyethyleneimine-based polymers (PEI). PEI is used in the non-viral delivery of DNA and RNA and can also be functionalized for cell-specific, targeted delivery. The formation of stable RNA polyplexes for siRNA-mediated gene silencing is also discussed.

Ettigounder Ponnusamy and Jianjun Cheng's research group (USA) review the synthesis and biomedical applications of polyamino acids in the third article. The process to polymerize amino acids enables a range of architectures, solubilities, charge densities, and conformations. These properties are useful in the biomedical applications of gene delivery.

The fourth article, by Jinhyun Hannah Lee, John Garner, and Sarah Skidmore (USA), reviews the formation of drug-loaded microparticles using hydrogels. The hydrogel template approach enables homogeneous particles with a narrow distribution of sizes. Recent developments enable automated formation with enhanced reproducibility.

In the final article in this issue, M. Shahinur Rahman and I (USA) review Reversible Addition–Fragmentation Chain Transfer (RAFT) polymerization and RAFT polymers for biomedical applications. A variety of functionalities, morphologies, and architectures enable bioconjugation, as well as micelle and vesicle formation.

Each article is accompanied by a list of Aldrich® polymers. Contact **matsci@sial.com** if you need materials you cannot locate in the Aldrich Materials Science catalog, or need a custom grade for development work. We welcome new product requests and suggestions as we continue to expand our polymer offering.

About Our Cover

Advances in drug delivery and tissue engineering continue to push the boundaries in medicine. Innovation often hinges on polymeric-based biomaterials research which will enable a tailored environment or tunable properties for the needs of a given application. The cover art of this issue illustrates the ability to form a delivery vehicle that expands the types of drugs used, as well as the release profile and specificity, in modern medicine.

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Your Materials Matter

Bryce P. Nelson, Ph.D.
Aldrich Materials Science Initiative Lead

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Emi Tokuda and Katherine Lewis from Professor Kristi Anseth's group at the University of Colorado (USA) kindly suggested we offer more thiol-functionalized polyethylene glycols (PEGs). Thiol functionality is versatile and commonly used in PEGylated drug delivery,[1,2] hydrogel formation,[3,4] and thiol-ene "click" chemistry.[3-6] Aldrich has introduced several thiol-functionalized PEGs, such as Tetra(ethylene glycol) dithiol (Aldrich Product No. 717142), as well as thiol-containing heterobifunctional PEGs. In addition to lower molecular weight PEGs, we are introducing other biocompatible polymers with thiol functionality such as poly(2-oxazoline)s, polylactides, and diblock copolymers to enable more experimental options in these fields.

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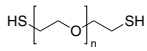
Poly(ethylene glycol) dithiol

PEG dithiol
$HSCH_2CH_2(OCH_2CH_2)_nSH$



▶ **average M_n 1,000**

Molecular weight: M_n 900-1,100

mp . . . . . 29 to 33 °C

store at: 2-8°C

717142-1G 1 g

Poly(2-oxazoline)s (POx) in Biomedical Applications


Robert Luxenhofer[1] and Rainer Jordan[2]
[1]Functional Polymer Materials, Chair of Chemical Technology of Materials Synthesis, University Würzburg, Röntgenring 11, 97070 Würzburg, Germany
[2]Professur für Makromolekulare Chemie, Department Chemie, TU Dresden, Mommsenstr. 4, 01069 Dresden, Germany
Email: Robert.Luxenhofer@uni-wuerzburg.de; Rainer.Jordan@tu-dresden.de

Introduction

Poly(2-oxazoline)s (POx) can be viewed as conformational isomers of polypeptides. They are synthesized via living cationic ring-opening polymerization (LCROP) of 2-oxazolines and were almost simultaneously discovered in 1966 by the groups of Litt, Tomalia, Fukui and Seeliger.[1-3] Extensive studies by Saegusa, Kobayashi, R.C. Schulz, Nuyken, Ringsdorf and many others demonstrated the highly living character of the LCROP, and numerous POx homopolymers, block, random, gradient copolymers and functionalized POx were reported.[2,4] A recent special issue by Wiley-VCH (*Macromol. Rapid Commun.* **2012**, *33* (19), 1593-1719) addresses most aspects of the chemistry, properties and applications of POx and related pseudo-polypeptides in several reviews. Here, we give a brief summary of the synthetic possibilities related to POx and potential applications of POx-based materials with a focus on biomaterials and nanomedicine.

Synthetic Possibilities and Physical Properties

The synthetic possibilities of the POx system are outlined in **Figure 1**. The structural as well as functional variation of POx polymers can be varied by the use of mono- to multi-functional initiators along with the incorporation of functional 2-oxazoline monomers.

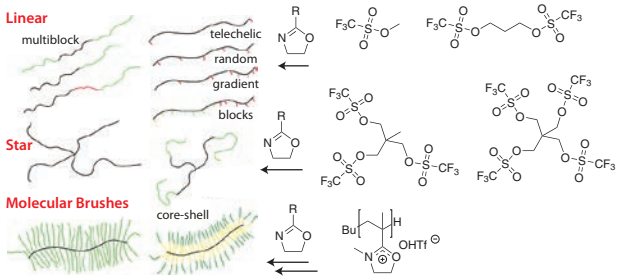


Figure 1. The structural and chemical variability of POx can be realized by the initiator and choice of additional functional monomers. Mono- or difunctional initiators result in linear POx, multifunctional initiators into POx stars, and macroinitiators in well-defined molecular brushes. For the latter, 2-isopropenyl-2-oxazoline is first polymerized by radical or anionic polymerization. Reaction with methyltriflate gives the macroinitiator salts, which react with most 2-oxazolines by LCROP to molecular brushes.

Although promising POx-based systems presented in the early 1990s resulted in initial commercialization, the development of POx lay dormant for almost two decades. Only recently, issues with and limitations of poly(ethylene glycol) (PEG)-related biomaterials triggered an intensive research on POx materials.[5,6]

The main physical properties of POx are defined by the nature of the side chain residue. In general, POx are amorphous or semi-crystalline polymers. While the melting point is found around 150 °C for most semi-crystalline POx, the glass transition temperature lies between +100 °C and –80 °C, depending on the specific nature of the side chain.[7-8]

Interestingly, most POx are miscible with a broad variety of other polymers and soluble in various organic solvents. As the POx main chain features a polar tertiary amide group, the entire range from highly hydrophilic to hydrophobic POx can be realized.

Short (< C4) pendant chains result in water soluble polymers while longer aliphatic or aromatic side chains result in hydrophobic polymers (**Figure 2**). Similar to other water-soluble polymers, aqueous POx solutions exhibit reversible phase-transitions (C2 and C3 side chains) which renders POx as an ideal candidate for the design of "smart polymer materials."[9,10] In contrast to some other prominent temperature-sensitive polymers, such as poly(N-isopropylacrylamide) (PNIPAM, Aldrich Product No. 724459), the phase transition of POx solutions occurs with only a minor hysteresis and within a very narrow temperature interval. Variation of the pendant group, as well as copolymerization of hydrophilic- and hydrophobic-substituted 2-oxazolines with iso- or n-propyl-substituents (PiPOx, PnPOx), allows a broad adjustment of the cloud point (T_{cp}) over the entire temperature range (0 to 100 °C) as well as fine-tuning the soluble-to-insoluble transition temperature around human body temperature. As outlined in **Figure 2**, the introduction of n-alkyl substituents in the pendant chain results in so-called "polysoaps," meaning that each monomer unit has an amphiphilic motif. Block copolymerization of hydrophilic and hydrophobic 2-oxazolines thus yields polymers of an amphiphilic contrast in the monomer unit as well as in the polymer main chain.

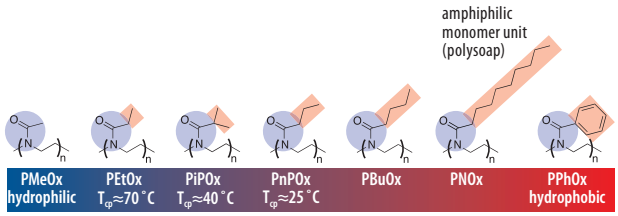


Figure 2. Water solubility of a series of poly(2-oxazoline)s as a function of the polymer pendant group. While poly(2-methyl-2-oxazoline) (PMeOx, Aldrich Product No. 774189) is highly hydrophilic, poly(2-ethyl-2-oxazoline) (PEtOx, Aldrich Product No. 773379) exhibits a so-called "cloud point temperature" (T_{cp}), which varies with architecture, concentration, and molar mass. This is the critical temperature above which the polymer becomes instantaneously water insoluble. The T_{cp} decreases further to 40 °C for poly(2-iso-propyl-2-oxazoline) (PiPOx) and PnPOx until poly(2-butyl-2-oxazoline) (PBuOx) is the first insoluble POx. Longer alkyl-substituents or aromatic moieties render the polymer hydrophobic but with an amphiphilic motif in the monomer unit (polysoap).

The narrow transition temperature window, as well as the tunability for different target temperatures, creates an interesting comparison to other common polymer amphiphiles including PEG systems (Pluronics). This capability to generate material with tailored properties is also applicable to POx of more complex architectures[3,2] and end groups.[11]

Lipopolymers

Functionalization by the initiating and/or terminating step can also be performed with lipids as the hydrophobic moiety resulting in so-called "lipopolymers" (**Figure 3**). In early studies, POx-based lipopolymers were synthesized and studied during the discovery

of the well-known "Stealth Effect" of hydrophilic polymers that are conjugated to biomolecules.[12,13] The stealth effect refers to a decreased immune response and increased residence time. However, only PEG-based systems became widely known for this property. Later, POx-lipopolymers were employed successfully for the design of stable biomimetic model membranes in the form of liposomes, monolayers at the air-water interface, and as solid supported (polymer-tethered) membranes with a favorable performance of POx in comparison to the PEG-based systems. This was due to the broader chemical variability of POx and its hydrophilic nature, as well as the different interaction potential of POx with incorporated transmembrane proteins.[14] Some examples of lipopolymers realized with the POx system are summarized in **Figure 3.**

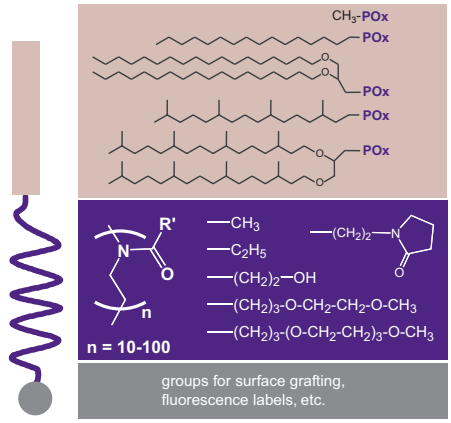


Figure 3. Selection of POx-based lipopolymers with a modulation of the hydrophilic–lipophilic balance through different combinations of n-alkyl or lipid moieties and POx. The variation of the POx pendant groups also allows a modulation of the polymer–water interaction (hydrogen bonding) including oligo(ethylene glycol) (OEG) moieties. The terminal end of the lipopolymers can be further functionalized by surface coupling groups such as silanes (directly using α,ω-aminoalkylsilanes) or by polymer analog reactions after termination with (protected) piperazine (e.g., fluorescence labeling).

Biomedical Applications

Biocompatibility of POx and Issues with PEG

While the early findings on the Stealth Effect triggered the extensive research and clinical application of PEG-based lipopolymers and countless PEGylated conjugates, "POxylated" analogs were forgotten for about 10 years. One reason might be found in the early commercial availability of a broad range of defined PEGs. Recently, further studies on hydrophilic POx (poly(2-methyl-2-oxazoline) (PMeOx) and (poly(2-ethyl-2-oxazoline) (PEtOx, Aldrich Product No. 773379) in a biomedical context underlined the high potential of this material. Analogous to PEG, PMeOx or PEtOx exhibited very little to no organ uptake (including liver) in mice[15] and are essentially not cytotoxic even at rather high doses of administation.[16,17] This also includes amphiphilic POx which are premier candidates for micellar drug delivery.[18] Moreover, safe administration of POx-based polymer therapeutics was recently demonstrated in non-human primates. Non-specific protein binding is also very low on POx-modified surfaces. The performance is, again, similar to PEG-based systems; however, as PEG is a polyether and, thus, prone to oxidative degradation,[19] POx was found to be stable under physiological conditions for a long time and thus probably more suitable for long-term use in animals and humans.[5] Considering that PEG has already been extensively investigated, the POx-based biomaterials must prove their suitability as an equal or better alternative. An important factor will be the immunogenicity of POx-based materials. Recently, it was found that PEGylated compounds activate the immune system to a larger extent than originally expected (activation of the complementary system and formation of antibodies).[20,21] Consequently, PEGylated compounds are rapidly cleared from the blood if administered repeatedly (anti-PEG immunity).[22-24]

Polymer Therapeutics: POx in Nanomedicine and Cancer Research

Because of the highly structural and chemical variability, POx are ideally suited for the design of polymer therapeutics of all main types: polymeric micelles for drug-delivery, POx-drug and POx-protein conjugates, and polyplexes.[3,6,25] In this contribution we can only spotlight recently developed systems to address the advantages and specific potential of POx. In comparison to the currently used "gold standard" PEG and the related poloxamers, all preclinical data indicate the suitability of the polymer for medical use. Until now, however, POx has not entered clinical trials and are not approved by the U.S. Food and Drug Administration (FDA) as part of a formulation or drug that can be administered to humans. PEtOx, however, are approved as an indirect food additive by FDA.

POxylation: POx-drug and POx-protein Conjugates

Modification of proteins with POx (POxylation), analogous to PEGylation, was pioneered by Saegusa.[26] In this early report and since, POx were modified post-polymerization to react with amine moieties of proteins. Proteins such as horseradish-peroxidase, superoxide dismutase (SOD1), albumin, trypsin, granulocyte colony stimulating factor, and others were POxylated.[27-29] The majority of this work was dedicated to finding alternatives to PEGylated proteins. Accordingly, hydrophilic POx such as PMeOx and PEtOx were utilized. However, one major limitation of PEGylation of proteins is the decreased mobility of PEGylated proteins across biological barriers. Since we previously showed that the control over POx composition allows for excellent control over polymer endocytosis,[16] it was hypothesized that amphiphilic POx will facilitate transport of covalently attached proteins via cellular membranes, similar to some members of the poloxamer family.[30-32] Indeed, it was observed that SOD1 modified with amphiphilic POx did exhibit increased neuronal uptake *in vitro*.[28] Decreased concentration of reactive oxygen species (ROS) showed protein delivery in active form. More importantly, it was shown that amphiphilic POx enabled the transport across the blood-brain barrier *in vivo*.

POx-based Polymeric Micelles for Drug Delivery

Solubilization of hydrophobic drugs with amphiphilic small molecules and polymers is a direct approach to prepare a formulation suitable for parenteral administration. However, low drug concentration in the formulation and poor formulation stability are significant drawbacks typically encountered. The LCROP of POx allows excellent and straightforward tailoring of amphiphilic block copolymers to result in non-ionic polysoaps, and amphiphilic POx block copolymers self-assemble into highly defined and relatively small polymeric micelles as shown in **Figure 4**. Surprisingly, no drug delivery platform has been developed using this material for a long time. Jeong and co-workers have utilized a block copolymer comprising PEtOx as the hydrophilic block and poly(ε-caprolactone)[33] for the solubilization of paclitaxel (PTX). Although the formulation was relatively effective and safe, it appears that this approach has not been developed further. One reason may be that the amount of paclitaxel that could be incorporated was rather low by this approach.

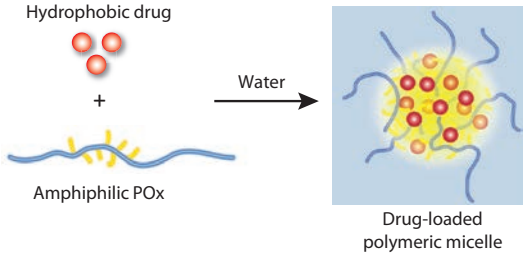


Figure 4. Simple but highly effective solubilization of hydrophobic drugs by amphiphilic POx. After addition of water to polymer–drug films, stable micellar solutions of nearly water-insoluble drugs are obtained.

We recently discovered that block copolymers with poly(2-butyl-2-oxazoline)s as a hydrophobic block allow the solubilization of very large amounts of hydrophobic drugs, including amphotericin B, paclitaxel, cyclosporine A, 17-AAG and others.[18] Not only is the relative amount of the drug in the formulation as high as 50 wt.%, the overall solubility remains very high and stable micellar solutions were observed. Moreover, it is possible to dissolve several drugs simultaneously with the same high drug loading capacity due to excellent solubility and outstanding formulation stability.[34] In the case of the anti-cancer drug, paclitaxel (PTX), the solubility increase is approximately 50,000 fold. No additional formulation stabilizers or cryoprotectants are required. The weight ratio of the POx to the solubilized drug is close to 1:1.

Gene Delivery

POx have been studied in some detail as a platform for complexation of DNA. For example, Hsiue and co-workers used the well-known hydrolysis of POx to PEI to prepare block copolymers of PEtOx and linear PEI.[35,36] The cationic PEI is well-known to condense DNA and help transfection of cells. However, PEI is also well-known for its significant cytotoxicity. A slightly different approach is the use of cationic POx, which can be prepared from monomers with amine-bearing side chains. These amines must be protected during polymerization or loss of control over the polymerization will occur.[37] Schubert, et al. recently utilized this approach to prepare cationic POx based hydrogels for reversible DNA binding.[38]

POx-based Hydrogels

POx have been used to prepare hydrogels for many years.[39,40] Also stimuli-responsive hydrogels of POx are well-known.[41,42] Considering the low protein binding[35] and the excellent synthetic versatility of POx, it is not surprising that POx-based hydrogels have been studied for drug delivery and for tissue engineering applications.[43]

Conclusions and Future Outlook

Polyoxazolines are synthesized via a controlled living cationic ring-opening polymerization. This enables a variety of architectures including linear copolymers, star-shaped or branched structures, and bottle brushes. In addition to structural control, the different substituents on oxazoline-based monomers enable tunability for several properties, including not only functional groups, but also stimuli responsive behavior. POx has been shown to be biocompatible and is being investigated as an equal or superior alternative to polyethylene glycol. It has been used to form micelles in drug delivery applications as well as to form POx-drug and POx-protein conjugates. The current research trends indicate that applications using POx material will continue to expand throughout the drug delivery and tissue engineering fields.

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Poly(2-oxazoline)s

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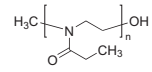
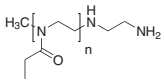
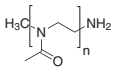
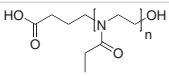
Well-defined Poly(2-ethyl-2-oxazoline)s

Name	Structure	Avg. Molecular Weight (M_n)	PDI	Prod. No.
Poly(2-ethyl-2-oxazoline)		5,000	≤ 1.2	740713-5G
		10,000	< 1.3	741906-5G
		25,000	≤ 1.4	741884-5G

End-group Functionalized Poly(2-oxazoline)s

Name	Structure	Avg. Molecular Weight (M_n)	PDI	Prod. No.
Poly(2-ethyl-2-oxazoline), amine terminated		2,000	< 1.2	773360-1G
		5,000	< 1.2	773379-1G
		10,000	< 1.2	773387-1G
Poly(2-methyl-2-oxazoline), mono amine terminated		10,000	< 1.2	774170-1G
		5,000	< 1.2	774189-1G
		2,000	< 1.2	774197-1G
Poly(2-ethyl-2-oxazoline), propionic acid, hydroxy terminated		5,000	< 1.2	774235-1G

Delivery of Nucleic Acids Using Polymers

Olivia Merkel
Wayne State University, Detroit, Michigan
Email: olivia.merkel@wayne.edu

Introduction

The medical application of molecular nanotechnology, referred to as "nanomedicine," is believed to lead to progress in human therapeutics in terms of improving health at the molecular scale.[1] This has great potential for accessing the currently "undruggable" targets[2] with new and smart medicines equipped with high bioavailability and few side effects. Nanomedicines are expected to have a variety of implications in treatment[3] and diagnosis,[4] which is expressed in the neologism as "theragnostics."[5] While nanotechnology enables diagnosis at the single-cell and molecular level, nanomedical therapeutics are expected to be specifically or even personally tailored. The field of nanomedicine has been developed strongly in the last decade. In particular, advanced drug delivery systems (DDS) have seen attention from multiple disciplines[6] to control the pharmacokinetics, toxicity, immunogenicity, biorecognition, and efficacy of the drug.[7] Drug carriers can be soluble or insoluble polymers which are formulated as nanoparticles using techniques such as the solvent displacement[8] or solvent evaporation/emulsion technique,[9] biopolymers,[10] or dendrimers.[11] Other formulations include polymer complexes,[12] "dendriplexes",[13] liposomes,[14] micelles,[15] and nanogels.[16] This review will focus on the sub-sector of drug delivery concerning the delivery of nucleic acids, with a particular focus on poly(ethyleneimine).

Non-viral Delivery of Nucleic Acids

In contrast to highly efficient viral delivery vectors, non-viral counterparts bear less immunostimulatory, mutagenetic, and oncogenic complications; some can approach transfection efficiencies comparable to viruses.[17] Therapeutic nucleic acids of interest for nanomedicine can be DNA in plasmids (pDNA), antisense oligonucleotides (AONs), ribozymes, DNAzymes, and more recently, siRNA and shRNA. While pDNA is used in gene therapy to deliver missing genes or replace dysfunctioning genes, all of the other therapeutic nucleic acids down-regulate gene expression by post-transcriptional gene silencing. This also explains their different target compartments: pDNA and shRNA expression vectors need to be delivered into the nucleus for transcription, whereas the cytosol is the site of action of AONs, ribozymes, DNAzymes, siRNA, and shRNA. Unfortunately, cells lack an efficient uptake mechanism for nucleic acids. Spontaneous intracellular translocation that retains bioactivity of the macromolecules is unlikely since nucleic acids are labile, negatively charged biomacromolecules. This has generated interest in research focused on protective formulation of nucleic acids into smart nanodevices that have high transfection efficiencies.

Delivery vehicles for nucleic acids that will make it from bench to bedside need to possess biocompatibility and robust processes of assembly, conjugation, and purification.6 A broad variety of lipid-based vectors, polymers, biopolymers, dendrimers, polypeptides, and inorganic nanoparticles have been investigated by groups all around the world.[18] The most prominent polymeric vector is certainly poly(ethyleneimine) (PEI) (Aldrich Product No. 764582), which is commercially available or can be polymerized as low or high molecular weight PEI.[19] PEI was first introduced as non-viral gene delivery vector by Bousif, et al. in 1995,[20] who described its outstanding property the "proton-sponge-effect." While liposomes escape the endo/lysosomal compartment after endocytosis due to fusogenic properties, PEI is believed to attract an influx of chloride and, subsequently, an osmotic influx of water into the lysosome as it is protonated. This leads to swelling and bursting of the lysosomes which release the polymer and nucleic acid into the cytosol.

Unfortunately, almost all polymeric vectors have one thing in common: they either form positively charged or amphiphilic complexes with nucleic acids that cause toxicity by interaction with negatively charged cell membranes,[21] or cellular components and pathways after successful intracellular entry.[22] There seems to be a correlation between transfection efficiency and toxicity up to the point where cells no longer survive. Since it has been reported that low molecular weight (LMW) PEI (Aldrich Product No. 764604) is significantly less toxic than high molecular weight counterparts,[23] a recent study investigated reversible disulfide-based crosslinking of LMW PEI[24] to achieve macromolecular vectors. A common principle for decreasing the surface charge of polycation–nucleic acids composites and their non-specific charge-dependent interactions was adopted from "stealth® liposomes" that are surface-modified with poly(ethylene glycol) (PEG) or other hydrophilic compounds, such as carbohydrates.[14] This steric stabilization decreased self-aggregation and interactions with proteins in biological fluids, and it increased salt and serum stability. Also, recognition and phagocytotic capture by cells of the reticulo-endothelial system (RES) or aggregation within pulmonary capillary beds *in vivo* was prevented, thereby enhancing their circulation half-lives. Derivatives of PEI with PEG, saccharides, and a monoclonal antibody (mAb) have been reported to yield stable complexes that partly retained their transfection efficiency. In a systematic study, different densities of grafted PEG chains as well as varied PEG chain lengths were investigated, suggesting that surface charge and toxicity decreased as a function of PEGylation. Unfortunately, transfection efficiency also decreased at comparable polymer-to-DNA ratios. Fortunately the low toxicity affords an opportunity to increase the polymer concentration to increase transfection. An even smarter system was recently described where PEG chains are connected via a peptide sequence which is cleaved in the presence of matrix metalloproteinases (MPPs). Thus, the emerging multifunctional envelope-type nanodevices (MENDs) are PEGylated extracellularly and lose the PEG block upon contact with an MPP that cleaves the peptide spacer. As non-specific endocytosis is triggered by interaction of cationic particles with heparin sulfate proteoglycans on the cell surface, a certain amount of positive surface charge of non-viral vectors is favorable. There are extensive publications which report a diversity of PEI modifications, including full deacylation and succinylation of commercially available PEIs, crosslinking of branched HMW PEI, conjugation of

melittin, grafting of chitosan, and immobilization on poly-L-lactide (PLLA) films for layer-by-layer assembly of polyelectrolytes. There are also more synthetic approaches to modify PEI, including the synthesis of PEI-alginate composites, amphiphilic and cyclodextrin-threaded triblock copolymers, PEI-cholesterol composites, and alkyl-oligoamine LMW-PEI derivatives. These modifications target enhanced endosomal release, transfection efficiency, pharmacokinetic parameters, and biocompatibility. One particularly interesting modification is the functionalization of PEI with cell-specific ligands to enable conjugation of targeting ligands, which is described below.

Targeted Gene Delivery Using Cell-specific Ligands

Targeted delivery systems are expected to selectively interact with internalizing receptors on certain cell types in a "lock and key" model that eventually triggers receptor-mediated uptake of the delivery system. Therefore, in this approach, non-specific, charge-related interactions that can also lead to non-specific toxicity are not necessary for efficient delivery. The dilemma that non-toxic, non-viral vectors which display a neutral surface charge are often less efficient than their non-shielded counterparts can be overcome by attaching targeting ligands. As another advantage, selective delivery systems require much lower amounts of siRNA or DNA for the same effect as a result of specific transfection. Targeting approaches exploit the fact that certain receptors are overexpressed on a variety of tissues and especially malignant abnormal cells due to their active proliferation and their demand for nourishment. Therefore, many targeted delivery systems are specific for growth factor receptors and are, therefore, suitable for tumor therapy. A variety of ligands have been used for modification of polymers to increase specificity, reduce the dose, and increase transfection efficiency. Conjugates of PEI are listed in **Table 1**.

Even though successful targeting of liposomes was described in the literature almost 29 years ago,[35] until today cell- or tissue-specific delivery has not been clinically exploited.

There are various further possible ligands and a myriad of further multifunctional PEI-based vectors for known and to-be-determined targets. We previously described specificity and activity of a differently synthesized PEG–PEI-based gene delivery system coupled with a novel peptidomimetic small molecule targeting the integrin receptor alpha *v* beta 3.[36]

Concepts of siRNA Mediated Post-transcriptional Gene Silencing

In 2006, Andrew Fire and Greg Mello were awarded the Nobel Prize in Physiology for their discovery of gene silencing by introduction of double-stranded RNA (dsRNA).[37] Their work led to the identification of a catalytic mechanism of a multi-protein complex which incorporates short RNAs that, on their part, are complementary in sequence to mRNA which is subsequently degraded.[37] This mechanism is an evolutionary conserved-defense process for inactivation of foreign, e.g., transposable, viral, or bacterial genetic information, and it can also be exploited biotechnologically, as shown in **Figure 1**. Long dsRNA, which naturally or directedly reach the cytoplasm, are degraded by "Dicer" (an RNase III-like enzyme) into small interfering RNAs (siRNAs) of 21–25 nucleotides in length.[38] While long dsRNA can interact with Toll-like receptor 3 (TLR3), synthetic short interfering RNA (siRNA) no longer than 19–21 base pairs with 2 nt 3′ overhangs is efficient and lacks interferon response. After being transferred into the cytosol where it is incorporated into the RNA-induced silencing complex (RISC), double-stranded siRNA is cleaved upon activation of RISC, and complementary mRNA can bind to the antisense strand. Argonaute (Ago2), an endonuclease in the RISC, subsequently cleaves the mRNA and leads to down-regulation of target gene expression.

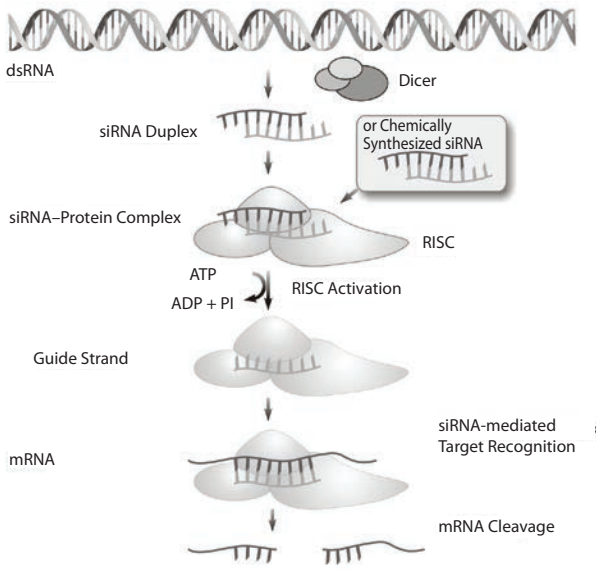


Figure 1. The mechanism of endogenous and induced RNA interference (RNAi). siRNA is eventually incorporated into the RISC where complementary mRNA binds before it is cleaved.

Table 1. Conjugates of poly(ethyleneimine) (PEI) for specific receptor interaction.

Target Receptor	Ligand Used	Type of Cell or Tumor	Ref.
Transferrin Receptor	Transferrin	Erythrocytes, Actively Proliferating Cells	25
Lactoferrin Receptor	Lactoferrin	Bronchial Epithelial Cells	26
Epidermal Growth Factor Receptor (EGFR)	Epidermal Growth Factor (EGF)	Variety of Cancer Cells	27
Human Epidermal Growth Factor Receptor 2 (HER2/neu)	Trastuzumab	Mamma Carcinoma	28
Fibroblast Growth Factor Receptor	Fibroblast Growth Factor (FGF)	Fibroblasts	29
Nerve Growth Factor Receptor (NGFR)	Recombinant Peptide	Neuronal Cells	30
Folate Receptor	Folic Acid	Actively Proliferating Cells, Especially Nasopharyngial KB Carcinoma Cells	31
Integrin Receptor ($\alpha_v\beta_3$)	RGD Peptides	Umbilical Cord Cells, Tumor Endothelial Cells	32
Hyaluronic Acid Receptor	Hyaluronic Acid	Liver and Kidney Cancer Cells	33
Platelet Endothelial Cell Adhesion Molecule (PECAM)	Anti-PECAM Antibody	Airway Endothelial Cells	34

Since the discovery of an RNA interference (RNAi) mechanism in mammalian cells, RNAi is routinely used in functional genomics and drug development. RNA-based therapeutics, on the other hand, are rather sparse. Due to their susceptibility to degradation by ubiquitous nucleases and their strongly negative surface charge, siRNA molecules require effective formulation and cannot easily be compared with small molecule drugs.[39] Besides interaction with plasma proteins and degradation by serum nucleases and fast renal clearance *in vivo,*[39] the bottlenecks in efficient RNAi include both translocation of siRNA across the plasma membrane and its subsequent escape from the endosomal/lysosomal compartment. Despite these drawbacks, successful reports on the silencing efficacy of naked siRNA can be found in the literature where siRNA is instilled into the lung or the vagina, or delivered to the liver by high-pressure tail vein injection.[40]Most clinical trials involving siRNA-based drugs rely on local administration to the eyes, targeting age-related macular degeneration (AMD) and diabetic retinopathy (Acuity Pharmaceuticals, Alnylam Pharmaceuticals, Inc., and Sirna Therapeutics, Inc.) or direct delivery to the brain or the lung (Alnylam Pharmaceuticals, Inc.). While SIRNA-Merck used chemically optimized siRNA in their clinical trial for the treatment of AMD, Alnylam applied cholesterol-attached siRNA targeting the nucleocapsid N gene of the respiratory syncytial virus (RSV), which was successful in their Phase II GEMINI study. Chemical modification of siRNA is not only a means to improve *in vivo* stability, which has been reported to be a result of modifications of the backbone and/or ribose. The additional benefit of methylation of the ribose 2′ hydroxyl group (2′-OMe) is the diminishing of immunostimulatory effects which have been described after liposomal delivery of siRNA. The knockdown kinetics of unmodified and nuclease-stabilized siRNA were shown to be essentially the same. A promising systemic *in vivo* application of siRNA is gene silencing in the liver, which can successfully be achieved with so-called "SNALP" (stable nucleic acid lipid particles) formulations.[42] Naked siRNA that is systemically applied is known to be excreted via the kidneys faster than it is degraded. Other formulations that have been investigated concerning their *in vivo* pharmacokinetics are post-PEGylated protamine-lipoplexes, adamantane-PEG-transferrin-bearing cyclodextrin-containing polyplexes, RNA–gold nanoparticle conjugates, chitosan, liposome and PEI-formulated locked nucleic acids (LNAs), and PEG–PEI polyplexes, as shown in **Figure 2**. While post-PEGylation approaches and cholesterol conjugation both extended the circulatory half-life, polyplexes made of pre-PEGylated PEI often have been presumed to disassemble in tissue or circulation.

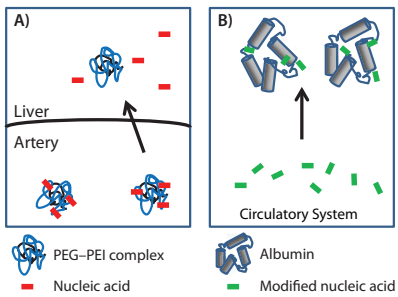


Figure 2. Two delivery methods of nucleic acids are **A)** intravenously applied complexes of nucleic acids and PEG-PEI, which are suspected to disassemble either in the circulation or upon liver passage, and **B)** amphiphilic modifications of nucleic acids which bind to albumin. Adapted from Reference 41.

Even though RNAi-based knockdown of target mRNA or protein was frequently reported in the literature, none of the pharmaceutical companies had robust delivery systems that would lead to broad clinical translation into RNAi-based therapeutics.

A highly interesting case is CALAA-01, the first experimental therapeutic that provided targeted delivery of siRNA in humans. This case just completed phase I tolerability evaluation of intravenous application in adults with solid tumors refractory to standard-of-care therapies by Calando Pharmaceuticals. CALAA-01 referred to siRNA against the M2 subunit of ribonucleotide reductase (RRM2) formulated within the RONDEL™ delivery system, a transferrin-targeted cyclodextrin-containing polymer RNAi-nanotherapeutic.

While DNA polyplexes have been extensively studied, there is not as much research published regarding circulatory half-life and biodistribution of siRNA. We previously described the optimization of a method for non-invasive determination of pharmacokinetics and biodistribution of siRNA polyplexes.[43] Making use of this optimized method, we then correlated *in vivo* pharmacokinetics of various (PEG-)PEI/siRNA polyplexes with their *in vivo* stability, another parameter that is crucially important in the development of polymeric siRNA delivery systems.[44]

Conclusions and Future Outlook

The field of nanomedicine research developed into several sectors focused on many areas. Poly(ethyleneimine) (PEI) has been a prominent polymer used in the development of three areas in particular: non-viral transfection, targeted delivery, and post-transcriptional gene silencing. In non-viral transfection, research is focused on increasing the efficiency while avoiding toxicity. In targeted delivery, there is a need to develop polymers with a high degree of specificity capable of maintaining a high level of drug activity. The further development of siRNA-mediated gene silencing is dependent on forming stable RNA polyplexes. PEI-based polymers will continue to be an integral part of the further development of these fields.

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Polymer Gene Delivery Vehicles

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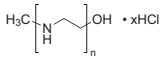
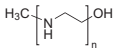
Linear PEI in the HCl Salt Form

Name	Structure	Avg. Molecular Weight (M_n)	PDI	Prod. No.
Polyethylenimine hydrochloride		4,000	≤ 1.1	764892-1G 764892-5G
		8,000	≤ 1.1	764647-1G
		20,000	≤ 1.2	764965-1G

Linear PEI in the Free Amine Form

Name	Structure	Avg. Molecular Weight (M_n)	PDI	Prod. No.
Polyethylenimine, linear		2,500	< 1.2	764604-1G
		5,000	< 1.2	764582-1G
		10,000	≤ 1.2	765090-1G

Gene Delivery Peptides

Name	Peptide Basis, TLC (%)	Peptide Content (%)	Prod. No.
Lys-Lys-Lys	≥97	~ 60	L8901-50MG
Lys-Lys-Lys-Lys	≥95	~ 60	L9026-10MG L9026-50MG
Lys-Lys-Lys-Lys-Lys	≥55	~ 60	L9151-10MG L9151-50MG

Synthesis and Biomedical Applications of Polyamino Acids

Ziyuan Song,[1] Rujing Zhang,[1] Hua Lu,[1] Nathan P. Gabrielson,[1] Lichen Yin,[1] Ettigounder Ponnusamy,[2] and Jianjun Cheng[1]*
[1]Department of Materials Science and Engineering, University of Illinois at Urbana–Champaign, 1304 West Green Street, Urbana, IL 61801, USA
[2]Sigma-Aldrich, 3500 DeKalb Street, St. Louis, MO 63118, USA
*Email: jianjunc@illinois.edu

Introduction

Polyamino acids are able to adopt ordered conformations, such as α-helices and β-sheets, through cooperative hydrogen bonding. These conformations impart polyamino acids with various unique properties and functions in biological environments. The development of controlled ring-opening polymerization (ROP) of α-amino acid *N*-carboxyanhydrides (NCAs) in the past two decades has enabled the synthesis of a large quantity of polyamino acid materials with predictable molecular weight (M_w) and narrow molecular-weight distribution (polydispersity or PDI).[1, 2] The innate ability of polyamino acids to adopt functionally ordered conformations in conjunction with the capability of highly controlled synthesis in large scale has expedited the widespread use of this class of materials, especially in the fields of drug delivery, tissue engineering, catalysis, and self-assembly.[3, 4]

Polyamino acids are the first class of biomaterials used as non-viral gene delivery vectors,[5] among which cationic poly-L-lysine (PLL) is the most widely studied one. While capable of binding and condensing anionic DNA, PLL and its derivatives generally display low transfection efficiencies and, therefore, are largely abandoned in favor of polymers such as polyethyleneimine (PEI).[6-9] Despite the inability of PLL to function as a stand-alone vector, polyamino acids have been adopted as components to increase the delivery efficiency of other gene vectors. In particular, cell penetrating peptides (CPPs) such as Penetration[10] and Tat (HIV Tat-derived peptide with the sequence of RKKRRQRRR)[11] have found use as membrane-active ligands incorporated into existing delivery vectors to promote cell internalization, endosomal escape, and accordingly increase the transfection efficiency.[12] Helical conformation is often observed in CPPs or formed in CPPs during membrane transduction, and has been closely tied to their membrane activity.[13] Due to their short length and insufficient cationic charge density, CPPs lack the capability to mediate gene delivery on their own. Therefore, it is of great interest in the design and synthesis of polyamino acid vectors that possess the structural characteristics of CPPs (i.e., helical secondary structure) with adequate length and cationic charge density to function as stand-alone vectors.

Herein, we report our efforts on the development of a new polymerization method of NCAs, design and synthesis of water-soluble α-helical ionic polyamino acids, and the application of these new polyamino acids in gene delivery wherein the secondary structure plays a crucial role.

Synthesis of Polyamino Acids Using Organo–Silicon Initiators

In 2007, we developed a controlled ROP system for various NCA monomers using hexamethyldisilazane (HMDS) as an initiator.[14] Compared with traditional amine initiators, HMDS allows better control over M_W and MWD, and enables the preparation of well-defined block co-polyamino acids. A unique trimethylsilyl carbamate (TMS-CBM) moiety was identified as the chain propagating group to achieve the controlled polymerization of NCA (**Scheme 1A**). The mechanism of the polymerization, differing from the traditional amine-initiated "amine mechanism" and "activated monomer mechanism," resembles the group transfer polymerization (GTP) for methyl methacrylate.[15] In addition, this metal-free living polymerization can be expanded to various *N*-trimethylsilyl (*N*-TMS) amines and allows the facile functionalization of C-termini of polyamino acids (**Scheme 1B**).[16] A variety of functional groups including alkene, alkyne, and norbornene were easily introduced into polyamino acid chains, which served as reactive sites for further chemical modification. For example, this system can be integrated with ring-opening metathesis polymerization (ROMP) to produce well-defined polyamino acids containing brush[17] or block polymers[18] by using either N-TMS amine-functionalized ROMP monomers or chain-transfer agents (CTAs). These novel hybrid copolymers bearing intrinsic secondary structures have shown interesting patterns and supramolecular architectures in self-assembly.[19]

In addition, we also developed novel NCA monomers to enable side-chain manipulation of polyamino acids. NCA monomers bearing vinyl groups [e.g., γ-(4-vinylbenzyl)-L-glutamate *N*-carboxyanhydride (VB-L-Glu-NCA),[20] γ-(4-allyloxylbenzyl)-L-glutamate *N*-carboxyanhydride (AOB-L-Glu-NCA),[21] and O-pentenyl-L-serine *N*-carboxylanhydride (PE-L-Ser-NCA)[22]) and photo-labile groups (e.g., γ-(4,5-dimethoxy-2-nitrobenzyl)-L-glutamate *N*-carboxylanhydride (DMNB-L-Glu-NCA)[23]] were synthesized and polymerized. The functional groups on the side chain of the resulting polyamino acids allow various chemical reactions to modulate the structures and properties of the materials. For instance, vinyl groups on poly-γ-(4-vinylbenzyl)-L-glutamate (PVBLG) side chains were converted to various functional groups including alcohol, aldehyde, carboxylic acid, vicinal diol, chloride, and aromatic ring via highly efficient post-polymerization modifications. The versatility of this chemistry shows great potential in generating varieties of polyamino acids with diverse side-chain functionalities and properties to broaden the applications of polyamino acid materials.

Water-soluble, Helical, Ionic Polyamino Acids

Helical conformation is one of the most common motifs of polyamino acids and is often associated with their biological activities. However, the usefulness of some natural α-helical polyamino acids (e.g., poly-L-leucine) toward biomedical applications is often limited by their poor aqueous solubility. On the other hand, natural polyamino acids with charged groups on their side chains have excellent water solubility but adopt random coil conformation due to the electrostatic repulsions between side-charged groups.[24] Thus, substantial efforts have been directed toward the preparation of water-soluble polyamino acids with stable helical conformation. By introducing neutral hydrophilic groups (e.g., hydroxy group, sugar moieties, or oligo ethylene glycol) onto the side chains, polyamino acids with distinct helicity and aqueous solubility can be obtained.[25-27] Unfortunately, these neutral polyamino acids are generally not useful for gene delivery due to their weak affinity for DNA.

To address this problem, we recently developed a class of ionic α-helical polyamino acids.[28] When the side-chain ionic groups were placed distally from the polyamino acid backbone, the charge repulsion was minimized and the helical conformation was stabilized by the enhanced hydrophobic interaction due to the elongated hydrophobic side-chain (**Figure 1**). We discovered that when the charged residue was placed 11 σ-bonds away from the backbone, α-helical ionic polyamino acids with good water solubility were obtained (**Figures 2A–2B**). Using the conjugation strategy described above for PVBLG, a series of water-soluble polyamino acids (PVBLG-X) were prepared via ozonolysis and subsequent reductive amination of PVBLG (**Scheme 1C**). For all developed PVBLG-X, the positive-charged amino groups were 11 σ-bonds away from the backbone and showed up to 90% helicity. In addition, when the side chain charges were further situated 17 σ-bonds away from the backbone, polyamino acids with very high helicity (81%) can be obtained even at a low degree of polymerization (DP) of 10.[21]

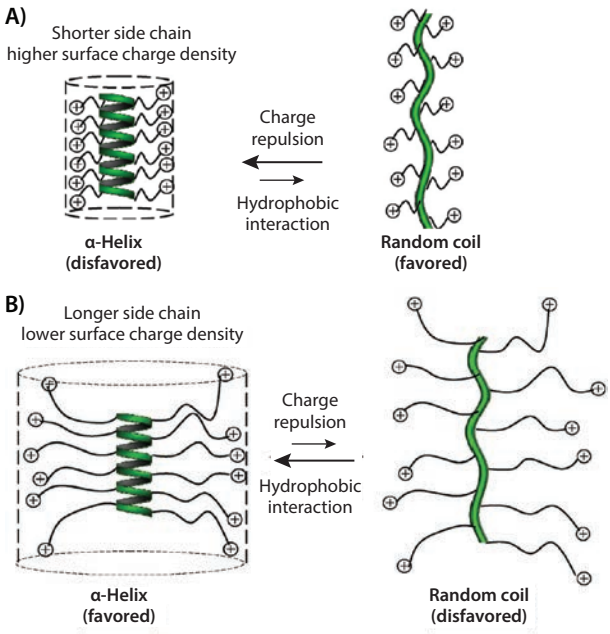
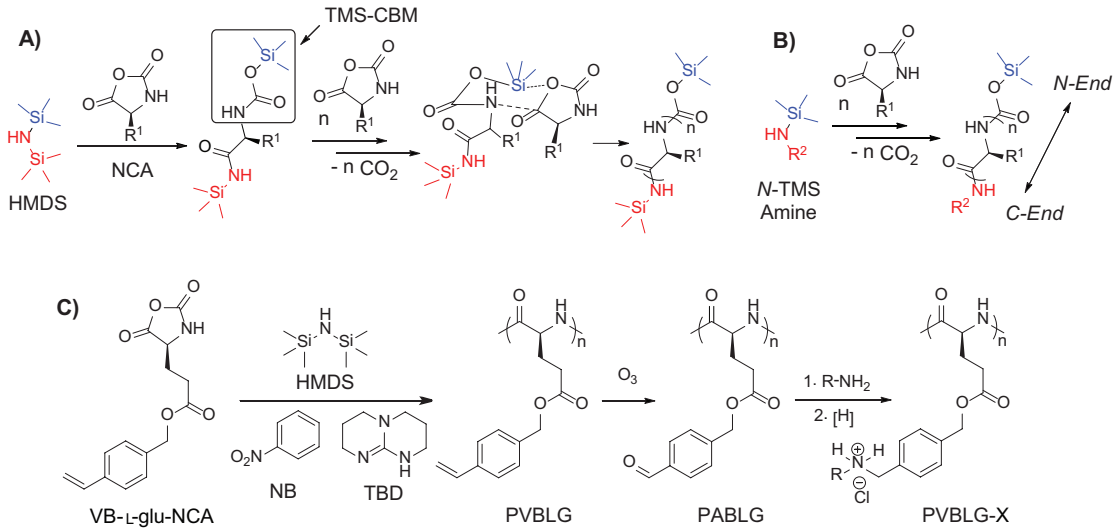


Figure 1. A) Illustration of polyamino acids with short, charged side chains and the postulated helix-to-random coil transition due to side-chain charge repulsion. **B)** Illustration of polyamino acids with long, charged side chains and postulated random coil-to-helix transition due to reduced side-chain charge repulsion and increased hydrophobic interaction.



Scheme 1. A) Proposed mechanism of hexamethyldisilazane (HMDS) initiated controlled NCA polymerization. Trimethylsilyl carbamate (TMS-CBM) moiety was identified as the chain propagating group. **B)** Synthesis of C-termini functionalized polyamino acids initiated by *N*-TMS amines. **C)** Synthesis of water-soluble α-helical polyamino acids PVBLG-X.

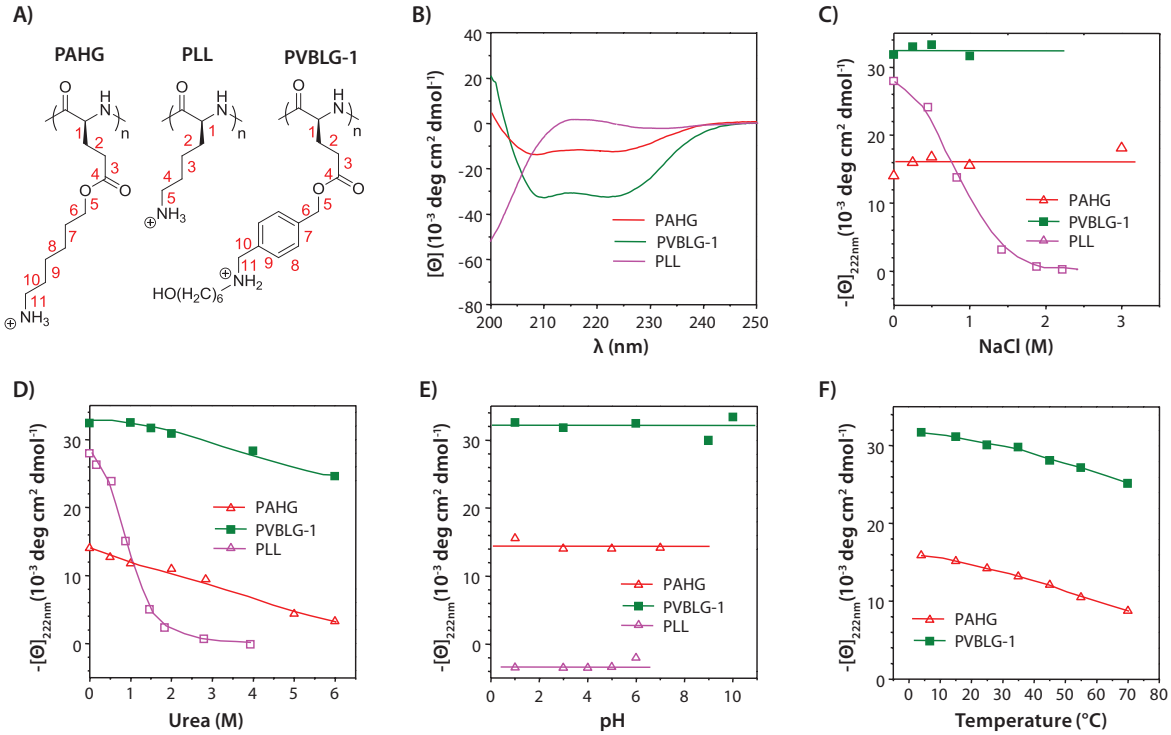


Figure 2. A) Chemical structure of PAHG, PLL, and PVBLG-1. **B)** Circular dichroism (CD) spectra of PAHG, PVBLG-1, and PLL in aqueous solution at pH 3. **C)** Salt concentration dependence of residue molar ellipticity at 222 nm for PAHG and PVBLG-1 at pH 3 and PLL at pH 10. **D)** The helical stabilities of PAHG and PVBLG-1 at pH 3 and PLL at pH 10 in the presence of urea. **E)** The pH dependence of the residue molar ellipticity at 222 nm for PAHG, PVBLG-1, and PLL. **F)** Temperature dependence of residue molar ellipticity at 222 nm for PAHG and PVBLG-1 at pH 3.

The helical stability of the α-helical ionic polyamino acids under various physiological conditions was evaluated with respect to biomedical applications. Particularly, unlike PLL, the helicity of PAHG and PVBLG-1 was stable against high ionic strength and in the presence of urea as the denaturing reagent (**Figures 2C–2D**). The helicity was also stable within a wide range of pH and temperature (**Figures 2E–2F**), both of which are important parameters for consideration in biomedical applications (such as the neutral pH of extracellular compartment and the acidic pH of the endosome). Such unique helical stability thus allows the polyamino acids to maintain their secondary structures and helicity-dependent membrane activities under a variety of complex biological conditions.

Role of Helicity in Cell Penetration and Gene Delivery

We also explored the potential of α-helical ionic polyamino acids toward non-viral gene delivery, wherein their sufficiently positively charged side chains enable effective DNA condensation, and the stable helical conformation potentially enables membrane transduction as noted in many CPPs.[29] As part of our initial work, a library of cationic α-helical polyamino acids with different amine side groups was synthesized and screened in an attempt to identify particular candidates with proper balance between hydrophilicity (i.e., DNA binding strength) and hydrophobicity (i.e., membrane disruption potential). The top-performing material, PVBLG-8 (**Figure 3A**), showed stable helical conformation (**Figure 3B**) and yielded 12-fold higher transfection efficiency compared to the commonly used transfection reagent PEI (**Figure 3C**).

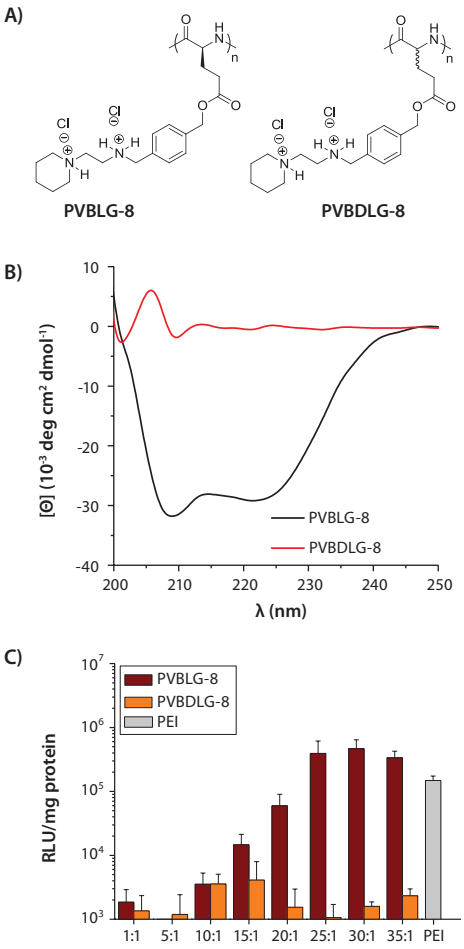


Figure 3. A) Chemical structure of helical PVBLG-8 and random-coiled PVBDLG-8. **B)** CD spectra of PVBLG-8 and PVBDLG-8 in aqueous solution at pH 7.4. **C)** *In vitro* transfection of PVBLG-8/pCMV-Luc complexes and PVBDLG-8/pCMV-Luc complexes in COS-7 cells at various molar ratios of amine to phosphate (N/P ratios). PEI (25 kDa) was included as a control.

Mechanistic studies were conducted to elucidate the function of the α-helical conformation in gene delivery. We showed that PVBLG-8 induced concentration-dependent destabilization of cellular or endosomal membranes (**Figure 4A**). To probe the endosomal escape mechanism, transfection studies were further performed in the presence of inhibitors such as bafilomycin that disrupts the proton sponge effect [30] or nocodazole that leads to the accumulation of endocytosed material in early endosomes via the disruption of active cellular transport processes.[31] Bafilomycin dramatically reduced the transfection efficiency of PEI, known as proton sponges, but had no effect on PVBLG-8 (**Figure 4B**). Comparatively, nocodazole induced a two-fold increase in the transfection efficiency of PVBLG-8, which indicated that the membrane disruption capabilities of PVBLG-8 increased with increasing polymer concentration (**Figure 4B**). These results collectively suggested that the incorporation of helical conformation, a trait shared by many CPPs, into our gene delivery vector yielded polyamino acids which possess the ability to destabilize endosomes by membrane disruption rather than the proton sponge effect. PVBDLG-8, a random-coiled racemic analogue of PVBLG-8 (**Figures 3A–3B**), was shown to have negligible transfection efficiency (**Figure 3C**), which highlights that polymer secondary structure can impact its overall performance. In order to test the breadth of applicability of this bioactive polyamino acid template, PVBLG-8 was also used to transfect cell lines that are traditionally considered to be hard to transfect, such as H9 human embryonic stem cells (hESC).[32] The results revealed that, with the addition of nocodazole, PVBLG-8 exhibited a three-fold enhancement over the transfection efficiency of the commercially available transfection reagent, Lipofectamine® 2000 (LPF2000) (**Figure 4C**).

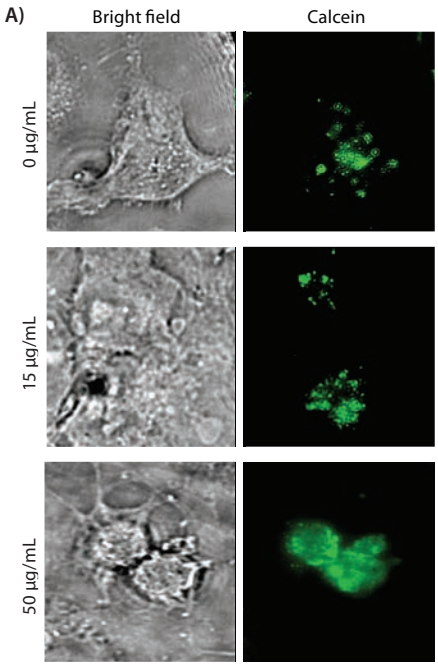


Figure 4A. Calcein uptake in COS-7 cells treated with various concentrations of PVBLG-8. Calcein is unable to cross intact membranes. As the concentration of PVBLG-8 in the extracellular medium is increased, the intracellular fluorescent signal becomes more diffuse, which indicates membrane permeation and non-endosomal uptake.

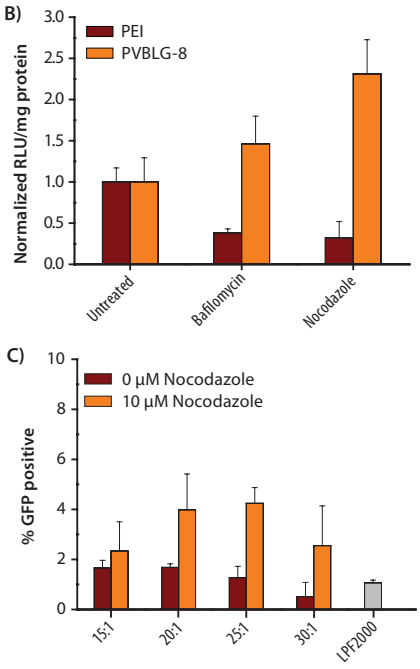


Figure 4. B) *In vitro* transfection of COS-7 cells treated with complexes of 25 kDa PEI or PVBLG-8 in the presence of intracellular inhibitors. The polymer concentration was fixed at 10 µg/mL. **C)** *In vitro* transfection of H9 human embryonic stem cells (hESC) with PVBLG-8 in the presence and absence of 10 µM nocodozole. Commercial transfection agent LPF2000 was included as a positive control.

Further Applications Based on PVBLG-8

In addition to DNA-based therapies, small interfering RNA (siRNA) has recently attracted much interest for the treatment of cancer and a number of other diseases. The RNA interference machinery features the site-specific mRNA cleavage and degradation in a highly efficient and specific manner, and thus enables gene regulation at the post-translation state. However, the successful clinical application of RNA interference has been hampered by the lack of efficient carriers or methods to deliver siRNA to target cells.[33] CPPs have previously been utilized to mediate intracellular siRNA delivery[34] and promote the endosomal escape of internalized siRNA-delivery vehicles. One major limitation of this approach, however, is that CPP activity depends on the formation of an acidic pH environment.[35] Thus, if cargo is internalized via a process that avoids rapid acidification such as caveolae-mediated uptake (a pathway that has been identified as perhaps more efficient than traditional clathrin-mediated uptake), CPPs will be ineffective. To this end, the super-stable helical conformation of PVBLG-8 within a wide range of pH makes it an effective siRNA delivery vehicle which not only facilitates cellular internalization but also aids endosomal escape (**Figures 5A–5B**).[36] Our results suggest that, unlike many other siRNA delivery vectors, PVBLG-8 operates by causing pore formation on cell membranes to allow direct diffusion of the siRNA cargo into the cell cytosol (**Figure 5C**). To further take advantage of such a unique property, PVBLG-8 was also incorporated into self-assembled nanoparticles in an attempt to mediate oral siRNA delivery.[37] The stable helical structure of PVBLG-8 allows it to maintain helicity-dependent membrane permeabilities when passing through the acidic gastric tract and neutral intestinal lumen. The incorporation of PVBLG-8 could also alter the overall intracellular kinetics of complexes. For instance, upon the inclusion of PVBLG-8, supra-molecular self-assembled nano-complexes could enter the cells mainly through energy-independent permeation instead of conventional endocytosis.[38]

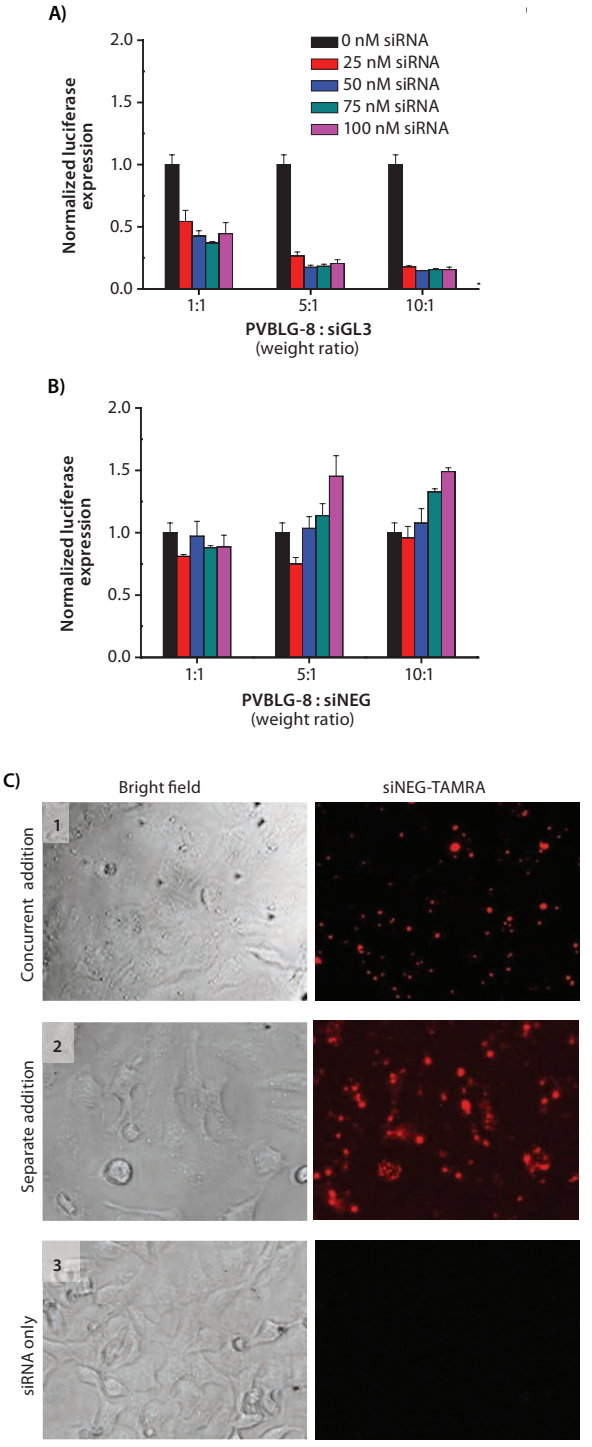


Figure 5. A) *In vitro* transfection of HeLa-Luc cells with luciferase siGL3 at various polymer:siRNA weight ratios and siRNA concentrations. **B)** *In vitro* transfection of HeLa-Luc cells with negative control siNEG. **C)** Fluorescence images of HeLa-Luc cells incubated with complexes formed with TAMRA-labeled siRNA and PVBLG-8. Free polymer and free siRNA were added sequentially in *Pane 1*. Free polymer was added, incubated, and removed prior to addition of free siRNA in *Pane 2*. Free siRNA was added without any polymer in Pane 3. The fluorescence signals observed in *Panes 1 and 2* suggested that PVBLG-8 could cause pore formation on cell membranes to allow direct diffusion of siRNA into the cytoplasm.

Motivated by the desire to further understand the impact of polymer physicochemical properties (M_W, charge density, three-dimensional structure, chain flexibility, and hydrophilicity/hydrophobicity balance, etc.) on the efficiency of non-viral gene vectors,[39] we designed and synthesized various compositionally equivalent yet topologically different PEG-PVBLG-8 copolymers (**Figure 6A**). By using different initiators or grafting materials, di-block, ABA tri-block, graft, and star (8-arms) PEG-PVBLG-8 copolymers were obtained with desired molecular structures.[40] Di-block and tri-block copolymers exhibited lower membrane activities and cytotoxicities due to the incorporation of PEG segments at the end of the polyamino acids. Nonetheless, these block copolymers showed uncompromised gene transfection efficiency compared to the non-PEGylated homopolymers. The graft copolymer, obtained by randomly grafting short PEG chains onto PVBLG-8, displayed lower DNA binding affinity and membrane activity, resulting in subsequently reduced transfection efficiency that is attributed to the steric effect induced by the PEG segments.

The star copolymer, initiated by an 8-arm PEG-NH2, demonstrated the highest membrane activity yet also relatively low cytotoxicity, thus resulting in a transfection efficiency that outperformed the non-PEGylated PVBLG-8 homopolymer and LPF2000 by 3–5 and 3–134 folds, respectively (**Figures 6B–6C**). The potency of the star copolymer was believed to result from its densely charged architecture as a "multivalent cationic sphere," which substantially favored interactions with negatively charged cell membranes. Meanwhile, the lower cytotoxicity of the star copolymer as compared to the homopolymer is attributed to the reduced amount of individual PVBLG-8 moiety in direct contact with the cell membranes. Such studies on the structure–property relationship provide insight into the rational design of future synthetic gene vectors.

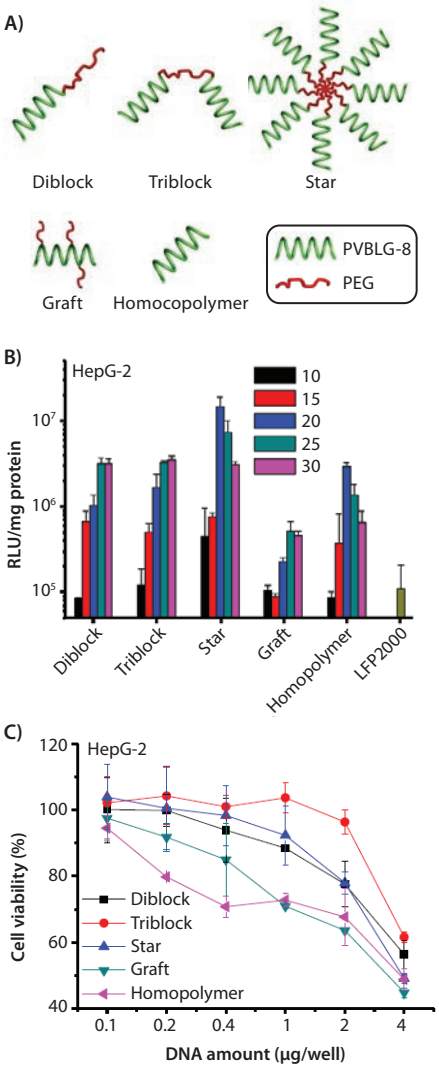


Figure 6. A) Schematic representation of PEG-PVBLG-8 copolymers with different architectures. **B)** Transfection efficiencies of polyplexes in HepG-2 cells at various molar ratios of amine to phosphate (N/P ratios). **C)** Cytotoxicity of polyplexes (N/P ratio of 20) toward HepG-2 cells as determined by the MTT assay.

Conclusions and Future Perspectives

We summarized our work on both the synthesis of water-soluble α-helical cationic polyamino acids and their biomedical applications in non-viral gene delivery. Using amine-TMS initiated NCA polymerization and post-modification of functional side chains, we were able to prepare a series of polyamino acid materials with different end-groups, architectures, and side-chain functionalities. In particular, by elongating the charged side chains to separate the charges from the polyamino acid backbone with a distance of 11 σ-bonds or longer, we were able to develop a series of cationic polyamino acids (PVBLG-X) with excellent water solubility and helical stability. Upon a screening process on PVBLG-X, PVBLG-8 was identified as the top-performing membrane-penetrating and gene delivery material. Due to its secondary structure, PVBLG-8 facilitated cellular internalization and endosomal escape which improved its gene delivery efficiency either independently or in combination with other delivery systems. More specifically, our studies suggest that PVBLG-8 is able to penetrate cellular or endosomal membranes by creating pores on biological membranes, an energy-independent approach distinctly different from traditional endocytosis pathway.

In view of this reactive polyamino acid template, our current studies are focused on modifying the polyamino acids with trigger-responsive, degradable, targeting, or other functional moieties in order to further improve their efficacy for both *in vitro* and *in vivo* gene delivery. On the other hand, given the importance of secondary structure in biomedical applications and the ultra-stability of helicity in this template, it presents great potential for therapeutics under extreme biological conditions, such as the very acidic environment of the gastric tract.

Acknowledgments

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Synthetic Poly(amino acid)s

For a complete list of available poly(amino acid)s, visit aldrich.com/polyaminoacid

Homopolymers

Name	Molecular Weight	Prod. No.
Poly-DL-alanine	1,000-5,000	P9003-25MG P9003-100MG P9003-1G
Poly-L-arginine hydrochloride	5,000-15,000	P4663-10MG P4663-50MG P4663-100MG
	15,000-70,000	P7762-10MG P7762-50MG P7762-100MG
	>70,000	P3892-10MG P3892-50MG P3892-100MG
Poly-L-asparagine	5,000-15,000	P8137-25MG P8137-100MG
Poly-(α,β)-DL-aspartic acid sodium salt	2,000-11,000	P3418-100MG P3418-1G
Poly-γ-benzyl-L-glutamate	30,000-70,000	P5011-500MG P5011-1G
	70,000-150,000	P5386-100MG P5386-1G
	150,000-350,000	P5136-1G
Poly-ε-Cbz-L-lysine	500-4,000	P4510-1G
Poly(γ-ethyl-L-glutamate)	>100,000	P8035-1G
Poly-D-glutamic acid sodium salt	2,000-15,000	P9917-100MG
	15,000-50,000	P4033-10MG P4033-100MG P4033-1G
Poly-L-glutamic acid sodium salt	750-5,000	P1943-100MG
	1,500-5,500 by MALLS	P1818-25MG P1818-100MG
	3,000-15,000	P4636-25MG P4636-100MG P4636-500MG P4636-1G
	15,000-50,000	P4761-25MG P4761-100MG P4761-500MG P4761-1G
	50,000-100,000	P4886-25MG P4886-100MG P4886-500MG P4886-1G
	>50,000	G0421-25MG G0421-100MG G0421-1G
Polyglycine	500-5,000	P8791-100MG P8791-500MG
Poly-L-histidine	5,000-25,000	P9386-10MG P9386-50MG P9386-100MG
Poly-L-histidine hydrochloride	≥5000	P2534-10MG P2534-25MG P2534-100MG P2534-500MG
Poly (α,β-[N-(3-hydroxypropyl)-DL-aspartamide])	5,000-20,000	P0937-100MG

Homopolymers *cont'd*

Name	Molecular Weight	Prod. No.
Poly-D-lysine hydrobromide	1,000-5,000	P0296-10MG P0296-50MG P0296-100MG P0296-500MG P0296-1G
	4,000-15,000	P6403-10MG P6403-50MG P6403-100MG
	30,000-70,000	P7886-10MG P7886-50MG P7886-100MG P7886-500MG P7886-1G
	70,000-150,000	P0899-10MG P0899-50MG P0899-100MG P0899-500MG P0899-1G
	150,000-300,000	P1149-10MG P1149-100MG P1149-500MG
	≥300,000	P1024-10MG P1024-50MG P1024-100MG P1024-500MG P1024-1G
Poly-L-lysine hydrobromide	500-2000	P8954-25MG P8954-100MG P8954-500MG
	1,000-5,000	P0879-25MG P0879-100MG P0879-500MG P0879-1G
	15,000-30,000 by viscosity	P7890-25MG P7890-100MG P7890-500MG P7890-1G
	30,000-70,000	P2636-25MG P2636-100MG P2636-500MG P2636-1G
	40,000-60,000	P3995-100MG P3995-500MG P3995-1G
	70,000-150,000 by viscosity	P1274-25MG P1274-100MG P1274-500MG P1274-1G
	150,000-300,000	P1399-25MG P1399-100MG P1399-500MG P1399-1G
	≥300,000	P1524-25MG P1524-100MG P1524-500MG P1524-1G
	4,000-15,000	81331-50MG 81331-250MG
Poly-L-lysine hydrochloride	15,000-30,000	P2658-25MG P2658-100MG P2658-500MG P2658-1G
	>30,000	P9404-25MG P9404-100MG P9404-500MG P9404-1G
Poly-DL-lysine hydrobromide	25,000-40,000	P9011-25MG P9011-100MG
	>40,000	P4158-25MG P4158-100MG P4158-500MG
Poly-L-lysine–FITC Labeled	precursor poly-L-lysine • HBr15,000-30,000 15,000-30,000	P3543-10MG P3543-25MG
	precursor poly-L-lysine • HBr30,000-70,000 30,000-70,000	P3069-10MG P3069-50MG
Poly-L-lysine, succinylated	>50,000	P3513-100MG P3513-1G

Homopolymers *cont'd*

Name	Molecular Weight	Prod. No.
Poly-L-ornithine hydrobromide	5,000-15,000	P4538-10MG P4538-50MG P4538-500MG
	30,000-70,000	P3655-10MG P3655-50MG P3655-100MG P3655-500MG P3655-1G
	>100,000	P4638-10MG P4638-50MG P4638-100MG P4638-500MG P4638-1G
Poly-L-ornithine hydrochloride	15,000-30,000	P2533-10MG P2533-50MG P2533-100MG P2533-500MG
Poly-DL-ornithine hydrobromide	3,000-15,000	P8638-25MG P8638-100MG P8638-250MG P8638-500MG
	15,000-30,000	P0421-100MG P0421-250MG
	>30,000	P0671-25MG P0671-100MG P0671-500MG
Poly-L-proline	1,000-10,000	P2254-50MG P2254-100MG P2254-500MG P2254-1G
	>30,000	P3886-500MG P3886-1G
Poly-L-threonine	5,000-15,000	P8077-25MG P8077-100MG P8077-250MG
Poly-L-tryptophan	15,000-50,000	P4772-100MG
Poly-L-tyrosine	10,000-40,000	P1800-100MG

Random Copolymers

Name	Molecular Weight	Feed Ratio	Prod. No.
Poly(Ala, Glu, Lys, Tyr) 6:2:5:1 hydrobromide	20,000-30,000	Ala:Glu:Lys:Tyr (6:2:5:1)	P1152-10MG P1152-25MG
Poly(Arg, Pro, Thr) hydrochloride	10,000-30,000	Arg:Pro:Thr (6:3:1)	P9431-25MG
	5,000-20,000	Arg:Pro:Thr (1:1:1)	P9306-25MG
Poly(Glu, Ala, Tyr) sodium salt	20,000-50,000	Glu:Ala:Tyr (6:3:1)	P3899-25MG P3899-100MG
	20,000-50,000	Glu:Ala:Tyr (1:1:1)	P4149-10MG P4149-25MG
Poly(Glu, Lys) hydrobromide	75,000-125,000	Glu:Lys (1:4)	P8619-100MG
Poly(D-Glu, D-Lys) hydrobromide	20,000-50,000	D-Glu:D-Lys (6:4)	P7658-25MG P7658-100MG
Poly(Glu, Lys, Tyr) sodium salt	20,000-50,000	Glu:Lys:Tyr (6:3:1)	P4409-10MG P4409-25MG P4409-500MG
Poly(Glu, Tyr) sodium salt	5,000-20,000	Glu:Tyr (4:1)	P7244-25MG P7244-250MG
	20,000-50,000	Glu:Tyr (4:1)	P0275-10MG P0275-25MG P0275-100MG P0275-250MG P0275-500MG
	20,000-50,000	Glu:Tyr (1:1)	P0151-25MG
Poly(Glu, Tyr)–Agarose	-	Glu:Tyr (4:1)	P6835-5ML
Poly(D,L-lactide-*co*-glycolide)	66,000-107,000	lactide:glycolide (75:25)	P1941-1G P1941-5G
Poly(Lys, Phe) 1:1 hydrobromide	20,000-50,000	-	P3150-10MG P3150-25MG P3150-100MG P3150-500MG
Poly(Lys, Tyr) hydrobromide	20,000-50,000	Lys:Tyr (4:1)	P4659-10MG P4659-25MG P4659-250MG
	50,000-150,000	Lys:Tyr (1:1)	P4274-100MG

Fabrication of Drug-loaded Microparticles Using Hydrogel Technology and Recent Innovation in Automation



Dr. Jinhyun Hannah Lee,[1]* John Garner,[2] Sarah Skidmore[2]
[1]Departments of Biomedical Engineering
Purdue University, West Lafayette, IN, USA
[2]Akina Inc., West Lafayette, IN, USA
*Email: jhannahlee@purdue.edu

Introduction

Microparticle drug delivery systems have been extensively researched and applied to a wide variety of pharmaceutical and medical applications due to a number of advantages including injectability, local applicability to target tissues and sites, and controlled drug delivery over a given time period. Drug-loaded polymer particles are conventionally prepared by emulsion methods. As an oil-in-water emulsion, polymers typically dissolve in a water-immiscible organic solvent along with either the organic soluble drug or dispersion of drug particles and form colloidal droplets in aqueous phase. This mixture is subsequently emulsified into a large volume of water with surfactant and subjected to solvent removal by evaporation/extraction to solidify the droplets into particles. Such methods are quite straightforward; however, there are several drawbacks to this technique: 1) Heterogeneous size distribution; 2) limited shape (sphere) of drugs; 3) difficulty in predicting and controlling their properties; and 4) limited capacity for loading of hydrophilic drugs. A drug-polymer emulsion is prepared as described below and a resultant image is shown in **Figure 1A**. A volume of 100 µL of a dichloromethane solution, consisting of 5% (w/v) poly(L-lactic acid) (M_w: 232 kDa) chemically conjugated to Flamma Fluor FPI749 dye, was added to a Poly(vinyl alcohol) (PVA) 0.5% (w/v) solution emulsified via high-speed homogenization using a traditional homogenizer (IKA, ULTRA-TURRAX®, 10,000 rpm). The emulsification continued for 2 minutes, followed by drying under room temperature. As can be seen, the resultant particles (blue in color due to conjugated dye) exhibit a wide dispersion of sizes and are non-uniform. The disadvantages of emulsion include low drug loading capacity caused by high diffusion of drugs to aqueous continuous phase from solution-dispersed droplets, pore formation during solvent removal, and poor control over microparticle formation. For the smaller particles, the larger surface area-to-volume ratio of the particles results in even lower drug loading in the particles. Also, drug loading would be lowered for the case of non-specific adsorption between polymer particles and drugs during particle formation by emulsion. The reproducibility of particle fabrication is another difficulty because emulsion formation of microparticles is extremely sensitive to conditions and depends on several factors, such as the drug being encapsulated (solubility), the polymer (composition, concentration, molecular weight), the ratio of drug to polymer, the emulsifier being used, the organic solvent (evaporation rate, solubility, viscosity), the viscosity/volume ratio of organic to aqueous phase, and the temperature/speed of the emulsification process.[1] Particles formed by this method also suffer from a high initial and non-controlled burst release which is a result of poor control over microparticle formation and is extremely condition-sensitive. Thus, it is significantly crucial to develop a technology which allows for the generation of controlled (size, shape, and load) microparticles with high drug loading capacity and loading efficiency.

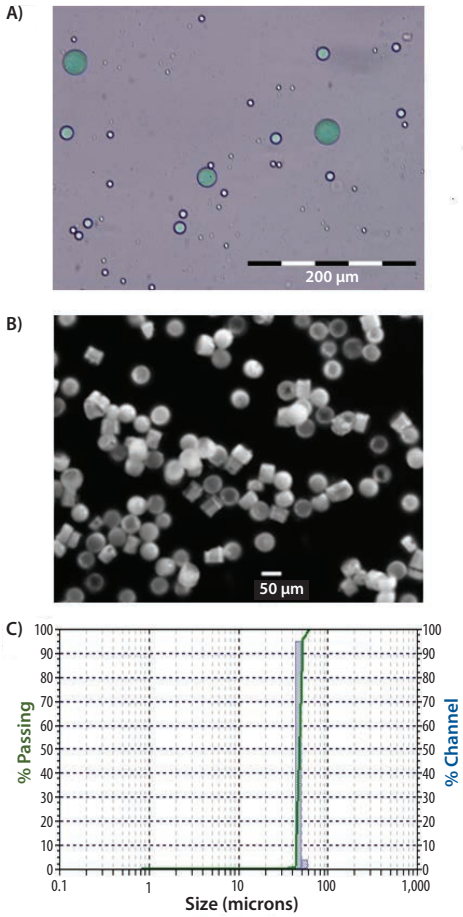

Figure 1. A) Bright field illumination of the emulsion droplets of 5% (w/v) poly(L-lactic) acid (PLA, M_w: 232 kDa) solution dispersed in 1% Poly(vinyl alcohol) (PVA) solution (PLA is chemically conjugated to Flamma Fluor FPI749 dye); **B)** dark field illumination of drug-loaded PLGA microparticles prepared by using a PVA template and SpinSwiper (see **Figure 3**) (adapted from http://SpinSwiper.com with permission); and **C)** size distribution profile of the drug-loaded PLGA microparticles.

These controlled microparticles with homogenous size distribution have been developed for drug delivery by using photolithography-based micro-fluidic techniques,[2,3] soft lithographic techniques,[4] and Particle Replication in Nonwetting Templates (PRINT) approach.[5] However, harvesting the particles prepared by these methods is not simply due to the use of a permanent template. The monodisperse particles of these methods still show high initial burst similar to the particles obtained by conventional methods such as emulsion. In order to overcome this problem, we have developed a hydrogel template approach.[6,7] This technique enables not only the capacity to produce microparticles with homogeneous size distribution and predefined shape and size, but also easily collect particles by dissolving the hydrogel template in an aqueous medium. **Figure 1B** shows an optical photograph of

Poly(D,L-lactide-*co*-glycolide) (PLGA) particles prepared by hydrogel template using an automated device, "SpinSwiper." Compared to the image of emulsion droplets shown in **Figure 1B**, the size of particles prepared by the hydrogel template method are very homogenous and uniform, which is confirmed by the size distribution profile shown in **Figure 1C**.

Fabrication of Microparticles by Hydrogel Template Approach

Hydrogels are materials which have a three-dimensional network structure and can hold or absorb water in the mesh made by crosslink junctions. They are widely used in tissue engineering, implantable devices, drug delivery systems, and even sensors due to their biocompatibility, controllable physical and mechanical properties, and response to external conditions.[8,9] Hydrogels can be classified into physically or chemically crosslinked hydrogels depending on one of the criteria used to classify hydrogels, the reversibility of the network structure to external environments. For feasibly harvesting microparticles from the template, physically crosslinked hydrogels, such as gelatin and PVA with appropriate molecular weight, are used as templates since they lose their integrity upon wetting and can simply dissolve in aqueous medium. In addition, large quantity production of the particles is possible using this method. The steps to prepare a hydrogel template are shown in **Figures 2A–2D**. First, a silicon wafer with defined geometry, producing the desirable shape and size of particles is prepared as a master template by UV irradiation through a mask (**Figure 2A**). Next, an intermediate template made of silicone rubber is also prepared using the master template (**Figure 2B**). Then PVA (or gelatin) solution is poured onto the silicone rubber template and allowed to solidify (**Figure 2C**), and the solidified PVA template is peeled off and is ready to use as a template for fabricating microparticles (**Figure 2D**). To this template 200–300 µL of biodegradable polymer (mostly poly(lactic acid) or poly(glycolic acid) based polymers such as PLGA or PLA are used) in organic solution with the organic soluble or dispersed drug is placed on top of PVA template and uniformly spread by a swiper (**Figure 2E**). After the drug-polymer solution is well solidified, the template is dissolved in an aqueous medium (**Figure 2F**). The particles are then collected by filtration and centrifugation (**Figure 2G**).

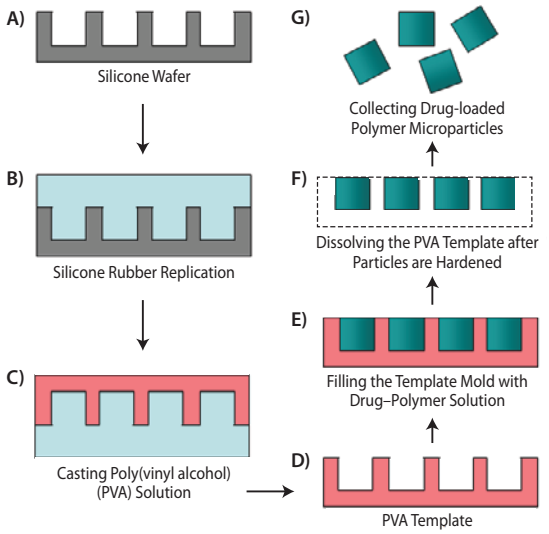

Figure 2. Schematic illustrations of **A)–D)** PVA template preparation process and **D)–G)** of fabrication process drug-loaded polymer microparticles using the PVA template.

The size of particles can be varied depending on the template mold size from 1.5 µm to around 50 µm using the hydrogel template method. Previous research regarding four different drugs (paclitaxel (PTX), progesterone (PRG), felodipine (FDP), and risperidone (RSP)) loaded into PLGA microparticles relative to the particle size reported that the drug release from larger particles is mostly slower and longer, regardless of the type of drug.[6] **Figure 3** shows the drug release profiles of PTX, PRG, FDP, and RSP from cylindrical PLGA microparticles for the sizes of 20 µm and 50 µm.[6] The drug release from the 20 µm particles is faster than from the 50 µm particles. Initial burst of RSP is relatively lower, which was explained due to relatively lower hydrophobicity compared to the others. This is because less hydrophobic drugs tend not to diffuse to the surface of particles when drug solvent evaporates. In addition, the hydrogel template approach is capable of producing microparticles with a variety of geometries and microstructures.

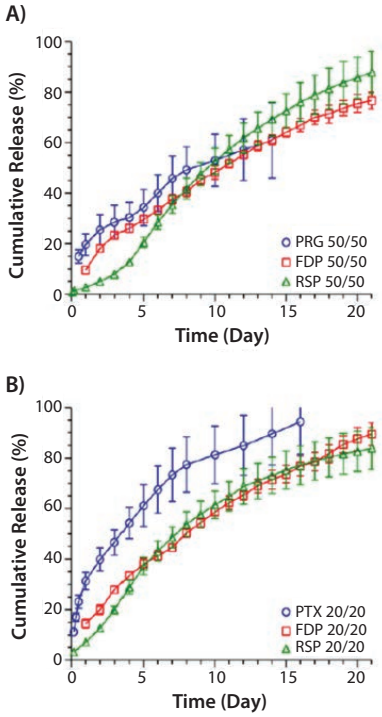

Figure 3. Cumulative release profiles of different drugs (felodipine (FDP), risperidone (RSP), and progesterone (PRG) from drug–PLGA microcylinders. **A)** with 50 µm height and 50 µm diameter (50/50) and **B)** with 20 µm height and 20 µm diameter (20/20). Adapted and reprinted from Reference 6.

Figure 4[7] shows fluorescence images of PLGA microparticles with several different geometries fabricated using the dissolvable hydrogels template and PLGA-Nile Red solution. The geometry of drug particles is one of the crucial factors for development of drug delivery systems. The degradation kinetics of drug particles and their flow behavior in the body system will also be affected by the geometry. Also, the particle geometry plays an important role for tissue engineering materials since cellular responses such as encapsulation in intracellular vesicles is affected by the geometries of the particles.[10] Moreover, the particles prepared by the hydrogel template method can have a multilayered structure by simply filling the mold in the template multiple times.[7] Each layer can have its own unique function or allow for the loading of different drugs. In this manner, particles with multilayered structure can be expected to possess a wide variety of functionalities.

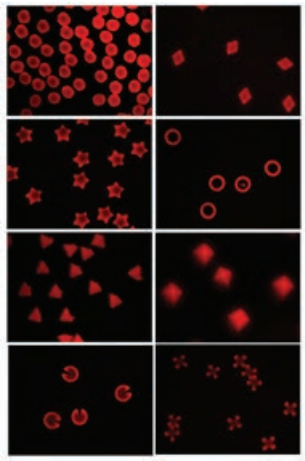

Figure 4. Microparticles with different geometries fabricated by the hydrogel template approach. The particle size was 50 µm. Adapted and reprinted from Reference 7.

Recent Innovation in Automation

Recently, we generated an automated version of the process of filling the template mold with polymer (or polymer-drug) solution by developing the "SpinSwiper" as shown in **Figure 5.** This automated device improves the quantity and speed of microparticles production. To be more specific, the filling step shown in **Figure 3E** is automated. There are more advantages of this device. SpinSwiper reduces the amount of wasted polymer solution, as a specified amount is dispensed and then the solution is contained to prevent evaporation. Another advantage is the reduction of variation that is inherently present when templates are swiped by hand. The whole process setup (**Figure 5A**) includes a syringe pump, syringe, and a SpinSwiper consisting of a rubber tube, a hollow blade, an aluminum plate with rubber backing where a template is placed, and a crossbar where the blade is secured. The drug-polymer (or polymer) solution is loaded into a syringe and placed on a syringe pump. One end of the rubber tubing is inserted over a needle attached to the syringe, and the other end of the rubber tubing is placed in the hollow blade of the SpinSwiper as shown in **Figure 5B**. A template is secured to an aluminum plate with rubber backing, and the plate is placed on the base of the SpinSwiper. The blade is installed on the crossbar of the SpinSwiper (**Figure 5B**) and the crossbar is locked onto the base. The blade is lowered onto the template without any gap. The syringe pump is set to a pre-determined flow rate (adjustable during the run) and the blade is filled with solution. Once the blade is filled, the SpinSwiper is started and the drug-polymer solution fills the mold of the hydrogel template as shown in **Figure 5C**. After the pre-set volume has been dispensed, the SipinSwiper is stopped and the template filled with the drug-polymer solution is peeled away from the rubber backing. The template is dissolved and the particles are harvested as depicted in **Figure 3F–3G.**

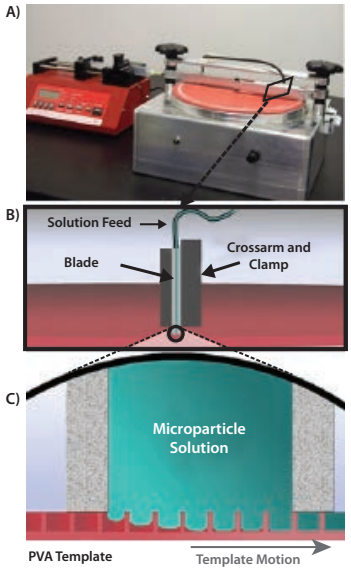

Figure 5. **A)** SpinSwiper setup for filling drug-polymer solution into template molds (see **Figure 2E**); **B)** close up of the filling part of the SpinSwiper; and **C)** close up of the filling process.

Conclusions

The hydrogel template approach makes it possible to easily fabricate homogeneous and high drug-loaded microparticles with predefined shapes and sizes. Thus, the properties and behaviors of these particles can be predicted and controlled. Furthermore, recent development of the automated SpinSwiper device brings out a technological innovation enabling the creation of simple and large-scale production of microparticles with a narrow distribution of sizes and, therefore, enhanced reproducibility. The combination of both the hydrogel template approach and automation will generate significant advances, not only in drug delivery and tissue engineering fields, but also in other material fields dealing with microparticles.

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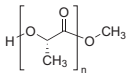
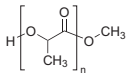
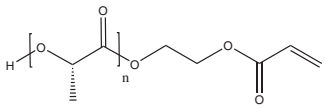
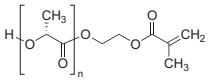
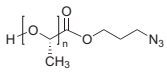
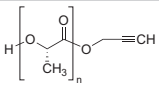
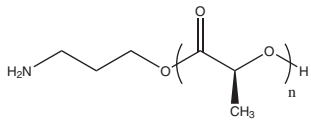
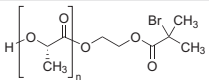
Polylactides

Name	Structure	Molecular Weight	PDI	Degradation Time	Prod. No.
Poly(L-lactide)		average M_n 5,000	≤ 1.2	>3 years	764590-5G
		average M_n 10,000	≤ 1.1	>3 years	765112-5G
		average M_n 20,000	≤ 1.1	>3 years	764698-5G
Poly(D,L-lactide)		average M_n 5,000	≤ 1.1	<6 months	764612-5G
		average M_n 10,000	≤ 1.1	<6 months	764620-5G
		average M_n 20,000	≤ 1.3	<6 months	767344-5G

End-functionalized Poly(L-lactide)s

Name	Structure	Molecular Weight	PDI	Prod. No.
Poly(L-lactide), acrylate terminated		average M_n 2,500	≤ 1.2	775991-1G
		average M_n 5,500	≤ 1.2	775983-1G
Poly(L-lactide) 2-hydroxyethyl, methacrylate terminated		average M_n 2,000	≤ 1.1	771473-1G 771473-5G
		average M_n 5,500	≤ 1.2	766577-1G 766577-5G
Poly(L-lactide) azide terminated		average M_n 2,000	≤ 1.2	774871-1G
		average M_n 5,000	< 1.2	774146-1G
Poly(L-lactide), propargyl terminated		average M_n 2,000	≤ 1.1	774162-1G
		average M_n 5,000	≤ 1.1	774154-1G
Poly(L-lactide) amine terminated		average M_n 2,000	≤ 1.2	776378-1G 776378-5G
		average M_n 5,000	≤ 1.2	776386-1G 776386-5G
Poly(L-lactide), 2-bromoisobutyryl terminated		average M_n 5,500	≤ 1.1	773395-1G
		average M_n 10,000	≤ 1.1	773409-1G
		average M_n 3,000	≤ 1.1	773247-1G

Biodegradable Block Copolymers

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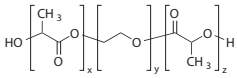
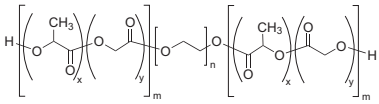
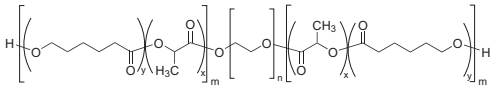
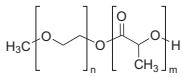
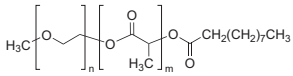
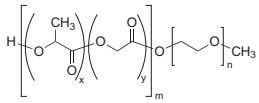
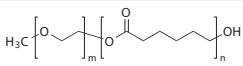
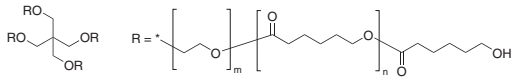
ABA Tribolock Copolymers

Name	Structure	Molecular Weight	PDI	Degradation Time	Prod. No.
Polylactide-*block*-poly(ethylene glycol)-*block*-polylactide		PEG average M_n 900 PLA average M_n 1,500 average M_n (1,500-900-1,500)	< 1.2	<12 months	659630-1G
		PEG average M_n 10,000 PLA average M_n 1,000 average M_n (1,000-10,000-1,000)	< 1.2	<12 months	659649-1G
Poly(lactide-*co*-glycolide)-*block*-poly(ethylene glycol)-*block*-poly(lactide-*co*-glycolide)		PEG average M_n 1,000 PLGA average M_n 2,200 average M_n (1100-1000-1100)	< 2.0	2-3 weeks	764817-1G
		PEG average M_n 1,000 PLGA average M_n 2000 average M_n (1,000-1,000-1,000)	< 1.2	1-2 weeks	764787-1G
Poly(lactide-*co*--caprolactone)-*block*-poly(ethylene glycol)-*block*-poly(lactide-*co*-caprolactone)		PEG average M_n 5,000 PLCL average M_n 6,000 average M_n (1,000-10,000-1,000)	< 1.2	1-2 months	764833-1G

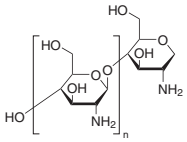
AB Diblock Copolymers

Name	Structure	Molecular Weight	PDI	Degradation Time	Prod. No.
Poly(ethylene glycol) methyl ether-*block*-poly(D,L lactide)		PEG average M_n 2,000 PLA average M_n 2,200 average M_n 4,000 (total)	≤ 1.4	2-4 weeks	764779-1G
Poly(ethylene glycol) methyl ether-*block*-poly(D,L lactide)-*block*-decane		PEG average M_n 2,000 PLA average M_n 2,000 average M_n 4,000 (total)	< 1.2	2-5 weeks	764736-1G
Poly(ethylene glycol) methyl ether-*block*-poly(lactide-*co*-glycolide)		PEG M_n 2,000 PLGA M_n 4,000 average M_n 6,000 (total)	< 1.1 (typical PEG) < 1.5 (overall)	1-4 weeks	764825-1G
		PEG average M_n 5,000 PLGA M_n 10,000 average M_n 15,000 (total)	< 1.6	1-4 weeks	765139-1G
		PEG average M_n 2,000 PLGA average M_n 15,000 average M_n 17,000 (total)	< 1.1 (typical PEG) < 1.8 (overall)	1-4 weeks	764760-1G
		PEG average M_n 5,000 PLGA M_n 55,000 average M_n 60,000 (total)	< 1.2	1-4 weeks	764752-1G
Poly(ethylene glycol)-*block*-poly(ε–caprolactone) methyl ether		PCL average M_n ~5,000 PEG average M_n ~5,000 average M_n ~10,000 (total)	< 1.4 PI	>12 months	570303-250MG 570303-1G
		PCL average M_n ~13,000 PEG average M_n ~5,000 average M_n ~18,000 (total)	≤ 1.4	>12 months	570311-250MG 570311-1G
		PCL average M_n ~32,000 PEG average M_n ~5,000 average M_n ~37,000 (total)	< 1.4	>12 months	570338-250MG 570338-1G
Poly(ethylene oxide)-*block*-polycaprolactone, 4-arm		PCL average M_n ~2,500 PEG average M_n ~2,500 average M_n ~5,000 total)	< 1.2	>12 months	570346-1G

Natural Biodegradable Polymers

For a complete list of available natural polymers, visit aldrich.com/natural

Chitosans (White Mushroom Origin)

Name	Structure	Degree of Acetylation	Avg. Molecular Weight (M_v)	Prod. No.
Chitosan		Degree of acetylation: ≤40 mol. %	60,000-120,000	740063-1G 740063-5G
		Degree of acetylation: ≤40 mol. %	110,000-150,000	740500-1G 740500-5G
		Degree of acetylation: ≤40 mol. %	140,000-220,000	740179-1G 740179-5G

Collagen

Name	Form	Prod. No.
Collagen from bovine achilles tendon	powder, suitable for substrate for collagenase	C9879-1G C9879-5G C9879-10G C9879-25G
	powder, suitable for substrate for collagenase	C4387-10G
	lyophilized	27662-1G
Collagen from bovine nasal septum	Bornstein and Traub Type II, powder	C7806-5MG C7806-10MG
Collagen from bovine tracheal cartilage	Bornstein and Traub Type II, powder	C1188-5MG C1188-10MG
Collagen from calf skin	Bornstein and Traub Type I, (0.1% solution in 0.1 M acetic acid), aseptically processed, BioReagent, suitable for cell culture	C8919-20ML
	Bornstein and Traub Type I, solid, BioReagent, suitable for cell culture	C9791-10MG C9791-50MG C9791-100MG C9791-250MG
	Bornstein and Traub Type I (Sigma Type III), solid	C3511-10MG C3511-50MG C3511-100MG C3511-250MG C3511-1G
Collagen from chicken sternal cartilage	Type II (Miller), powder, BioReagent, suitable for cell culture	C9301-5MG C9301-25MG C9301-100MG
Collagen from Engelbreth-Holm-Swarm murine sarcoma basement membrane	Type IV (Miller), lyophilized powder (from sterile-filtered solution), BioReagent, suitable for cell culture	C0543-1VL
Collagen human	Bornstein and Traub Type I, acid soluble, powder, ~95% (SDS-PAGE)	C5483-1MG
	Bornstein and Traub Type I, recombinant, expressed in *Nicotiana*	C7624-5ML C7624-30ML
	Bornstein and Traub Type I, recombinant, expressed in *Nicotiana*	C7999-30MG
Collagen from human lung	Bornstein and Traub Type I, powder	C5983-1MG
Collagen from human placenta	Bornstein and Traub Type IV, powder	C7521-5MG C7521-10MG C7521-50MG
	Bornstein and Traub Type III (Sigma Type X), powder	C4407-1MG C4407-5MG C4407-25MG
	Bornstein and Traub Type IV, powder, BioReagent, suitable for cell culture	C5533-5MG
	Bornstein and Traub Type I (Sigma Type VIII), powder	C7774-5MG C7774-25MG C7774-100MG
	Bornstein and Traub Type V (Sigma Type IX), powder	C3657-1MG C3657-5MG
	Bornstein and Traub Type IV, solution, ≥95% (SDS-PAGE)	H4417-100UG
	Bornstein and Traub Type IV, powder	C8374-5MG
Collagen from mouse sternum	Bornstein and Traub Type II, acid soluble, powder	C5733-1MG
Collagen from rabbit skin	Bornstein and Traub Type I, powder	C5608-100MG
Collagen from rat tail	Bornstein and Traub Type I, powder, BioReagent, suitable for cell culture	C7661-5MG C7661-10MG C7661-25MG C7661-50MG C7661-100MG
	Bornstein and Traub Type I (Sigma Type VII), powder	C8897-5MG C8897-10MG C8897-25MG C8897-50MG C8897-100MG

Fibronectin

Name	Form	Prod. No.
Fibronectin from bovine plasma	solution, sterile-filtered, BioReagent, suitable for cell culture	F1141-1MG F1141-2MG F1141-5MG
	powder, BioReagent, suitable for cell culture	F4759-1MG F4759-2MG F4759-5MG
Fibronectin from human foreskin fibroblasts	lyophilized powder, BioReagent, suitable for cell culture	F2518-.5MG
Fibronectin from human plasma	lyophilized powder, BioReagent, suitable for cell culture	F2006-1MG F2006-2MG F2006-5MG F2006-5X5MG
	liquid, 0.1% (Solution), BioReagent, suitable for cell culture	F0895-1MG F0895-2MG F0895-5MG
Superfibronectin from human plasma	solution, sterile-filtered, BioReagent, suitable for cell culture	S5171-.5MG
Fibronectin from rat plasma	powder, BioReagent, suitable for cell culture	F0635-.5MG F0635-1MG F0635-2MG
Fibronectin Fragment III_1-C human	recombinant, expressed in *E. coli*, lyophilized powder, ≥90% (SDS-PAGE)	F3542-.5MG
Fibronectin Type III Connecting Segment Fragment 1-25	≥90% (HPLC)	F5007-1MG
Fibronectin Proteolytic Fragment from human plasma	lyophilized powder, 45 kDa	F0162-.5MG
	lyophilized powder, 70 kDa	F0287-.5MG
	lyophilized powder, 30 kDa	F9911-.5MG
Fibronectin-like Protein Polymer genetically engineered	lyophilized powder, autoclaved, BioReagent, suitable for cell culture	F5022-1MG F5022-5MG
Fibronectin-like Engineered Protein Polymer-plus genetically engineered	powder, sterile; autoclaved, BioReagent, suitable for cell culture	F8141-1MG
Fibronectin Adhesion-promoting Peptide	≥95% (HPLC)	F3667-1MG F3667-5MG

Synthetic Peptide Scaffolds

Synthetic Peptide Hydrogels

Name	Form	Prod. No.
HydroMatrix™ Peptide Cell Culture Scaffold	mixture, powder	A6982-1ML A6982-5ML A6982-10ML
	24 well plate	H4040-1EA
	6 well plate	H3915-1EA

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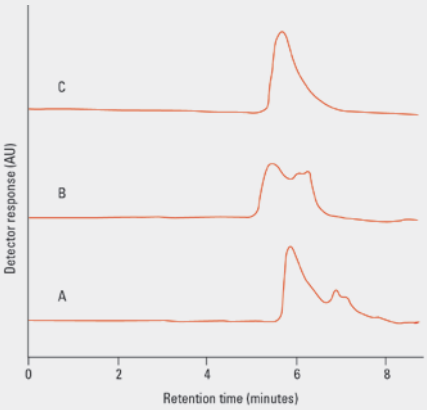
TSKgel Series	Particle Composition	Application	Examples
SW type (SW, SWXL, SuperSW)	Silica with hydrophilic bonded phase	Proteins, Biopolymers	Monoclonal Antibodies, Antibody-drug Conjugates
PW type (PW, PWXL, SuperMultiporePW)	Methacrylate[1]	Water-soluble Polymers	Celluloses, Polysaccharides, Polyacrylamide, Cationic Polymers
Alpha and SuperAW	Methacrylate[1,2]	Polar Organic-soluble Polymers	Sodium Chondroitin Sulfate, Polyvinyl Alcohol
H type (HXL, HHR, SuperMultiporeHZ)	Polystyrene/DVB copolymer	Organic-soluble Polymers	Acrylic and Epoxy Resins, Polyethylene, Polyester

[1]Hydrophilic polymethacrylate resin
[2]High degree of crosslinking

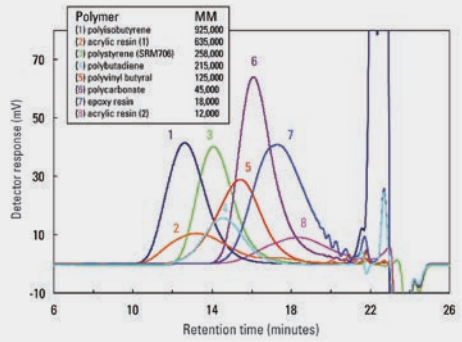
Figure 1



Columns: A: TSKgel GMHHR-H, 5 µm, 7.8 mm ID × 30 cm
B: TSKgel GMHXL, 9 µm, 7.8 mm ID × 30 cm
C: TSKgel GMHHR-H(S), 13 µm, 7.8 mm ID × 30 cm
Mobile phase: THF
Flow rate: 1.0 mL/min
Detection: UV @ 245 nm
Temperature: 25 °C
Sample: polystyrene standard F2000 (2.06 × 10^7 Da)
20 µL (0.025%)

Figure 1 shows how shear degradation was reduced for a 20 million Dalton molecular mass polystyrene polymer by using a larger particle size TSKgel GMHHRH(S) column.

Figure 2



Columns: TSKgel SuperMultiporeHZ-M, 4 µm, 4.6 mm ID × 15 cm × 4
Mobile phase: THF
Flow rate: 0.35 mL/min
Detection: RI
Temperature: 25 °C
Injection: 10 µL
Sample Conc.: 0.3%

Figure 2 shows data that demonstrates the performance of four 15 cm × 4.6 mm ID TSKgel SuperMultiporeHZ-M columns for a wide variety of organic-soluble polymers. Multipore columns are packed with particles of a uniform size, with each particle having a very broad pore size distribution. This innovative multi-pore approach, pioneered by Tosoh Corporation, essentially creates a linear calibration curve within each particle.

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*TSKgel columns are not available from Sigma-Aldrich in the Asian region.

Functional RAFT Polymers for Biomedical Applications

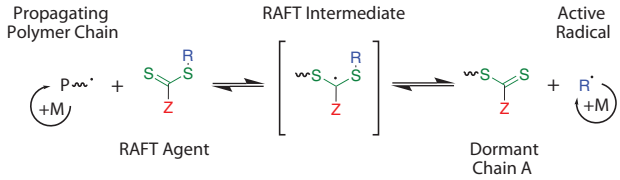

M. Shahinur Rahman,* Sebastian Grajales
Aldrich Materials Science, Sigma-Aldrich Co. LLC
*Email: Shahinur.Rahman@sial.com

Introduction

Reversible addition–fragmentation chain transfer (RAFT) polymerization is rapidly moving to the forefront in construction of drug and gene delivery vehicles. The RAFT technique allows unprecedented latitude in the synthesis of water soluble or amphiphilic architectures with precise dimensions and appropriate functionality for attachment and targeted delivery of diagnostic and therapeutic agents. To date, efforts have focused on the use of RAFT polymerization for generating block copolymer micelles, vesicles, star polymers, nanoparticles, and capsules as potential advanced drug carriers and also polymer-drug conjugates as prodrugs (**Figure 1**).[1] This review focuses on the overview of the RAFT process, selection of appropriate RAFT agents, and its potential for building tailor-made (block) copolymers, functional polymers, and polymers with a wide range of biological recognition.

RAFT Polymerization

Controlled radical polymerization (CRP) techniques have been previously reviewed in articles,[2,3] books,[4] and a guide.[5] These techniques rely upon quickly establishing an equilibrium between an active, propagating polymer chain and a dormant moiety. The RAFT process generates polymers with precisely controlled structural parameters such as random, block, gradient, grafted, and star copolymers.[6-9] The advantages of RAFT polymerizations include the ability to operate in a wide variety of reaction conditions and solvents. In RAFT polymerization, chain transfer agents (commonly referred to as CTAs or RAFT agents) create a reversible addition/fragmentation equilibrium (**Scheme 1**). In this process, the initial generation of a radical produces a propagating polymer chain. This polymer chain will (ideally) quickly react with a RAFT agent, which will functionalize the end of the chain with a RAFT moiety, therefore causing dormancy. The remaining moiety of the original RAFT agent will then initiate a new second polymer chain. This second chain will eventually react with a RAFT-functionalized polymer chain, creating an environment in which polymer chains are alternating between being dormant and active and therefore growing at similar rates. This ideally yields polymer chains that are all comparable in chain length.



Scheme 1. Simplified mechanism of a RAFT polymerization. Essentially, a radical (in the form of a propagating polymer chain) reacts with a RAFT agent, which can reversibly fragment to create a new active radical (in blue). As the radical reacts with nearby monomer units, "R" will represent a second propagating polymer chain. This results in the thiocarbonylthio group (in green) transferring from chain to chain acting as a "protecting group." The Z group (in red) dictates which types of monomers can be effectively controlled.

While RAFT is versatile and there are RAFT agents available for a wide variety of monomers, the limitation of RAFT is that there is not any one RAFT agent capable of controlling all types of monomers. RAFT agents must be selected based upon the corresponding monomer type used in order to obtain the desired level of control of the polymerization. Previously published compatibility tables aid in this selection,[5] and many varieties of functionalized RAFT agents are commercially available from Sigma-Aldrich. In general, there are four types of RAFT agents, all of which contain a thiocarbonylthio moiety (as seen in **Scheme 1**). RAFT agents such as dithioesters[10] and trithiocarbonates[11] work well with controlling the polymerization of "more-activated" monomers (MAMs), such as styrene (Sty), methyl acrylate (MA), and methyl methacrylate (MMA), meanwhile dithiocarbamates[12,13] and xanthates[14] must be used to control the polymerization of "less-activated" monomers (LAMs), such as vinyl acetate (VAc), *N*-vinylpyrrolidone (NVP), and *N*-vinylcarbazole (NVC). Improper pairing (e.g., dithiocarbamate RAFT agent with MMA monomer) will inhibit or significantly limit the polymerization.[11] In an attempt to address this, there are pH-switchable RAFT agents (such as Aldrich Product Nos. 751227 and 736236) capable of generating poly(MAM)-block-poly(LAM).[15]

RAFT agents of particular interest for drug and gene delivery allow for facile pre- or post-polymerization conjugation to biological compounds. In particular, RAFT agents containing carboxylic acid (Aldrich Product Nos. 723010 and 722995), azide (Aldrich Product No. 741698), NHS-ester (Aldrich Product Nos. 741035 and 751227), pentafluorophenyl ester (Aldrich Product No. 740810), alkyne (Aldrich Product No. 765147), and thalimido end groups (Aldrich Product No. 777072) and photocleavable groups (Aldrich Product No. 765147) (see **Figure 1**) are now commercially available for synthesis of well-defined homopolymers and copolymers for specific biomedical application.

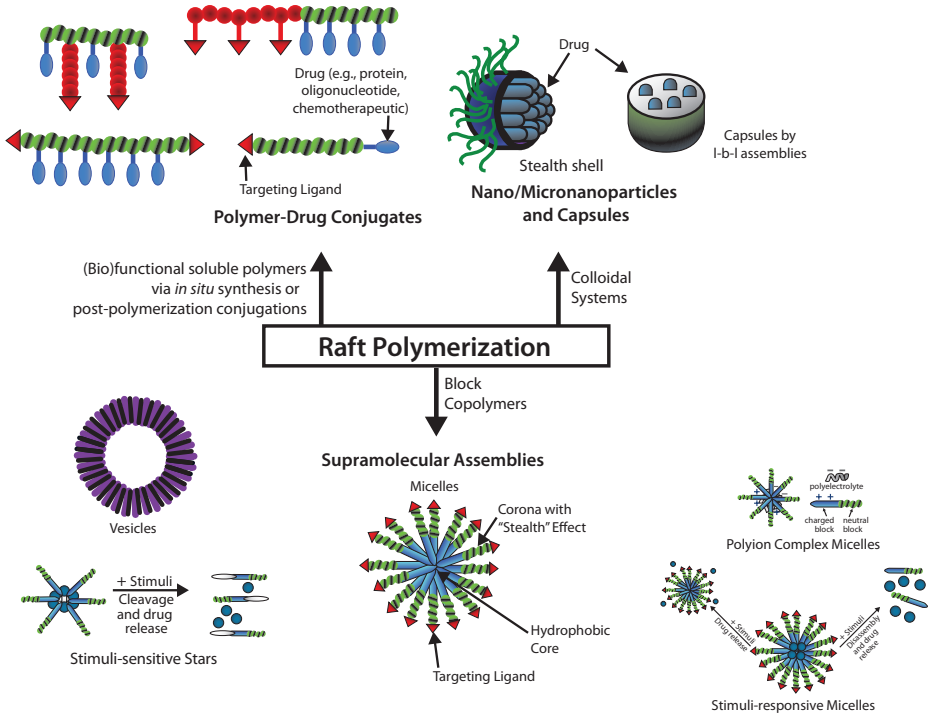


Figure 1. Examples of Controlled Drug Release Systems Generated by RAFT-Polymers: supramolecular assemblies including micelles, vesicles, and stars; Nanoparticles, microparticles, and capsules; and polymer-drug conjugates. Adapted from Liu, et al, Reference 1.

Functional RAFT Polymers for Bioconjugation

Bioconjugates of vinyl polymers have been increasingly used in biomedicine, biotechnology, and nanotechnology.[16] In general, they have been prepared using semitelechelic polymers having one functional end group which could be used for conjugation directly or after chemical modifications.[1] While the entry of controlled radical polymerization (CRP) techniques to the bioconjugation field has enabled the *in situ* synthesis of bioconjugates of polymers[17] as well as the direct synthesis of well-defined semitelechelic polymers suitable for conjugation to biomolecules without the need for post polymerization modifications,[18,19] they have also brought the possibility of one-step synthesis of well-defined telechelic polymers. This is accomplished because the functionalized RAFT agent remains on the chain end after polymerization. Capitalizing on this inherent end group functionality, RAFT enables synthesis of various well-defined polymers for chemoselective conjugation to biomolecules. End functional polymers having carboxylic acid, azide, amine, and thiol groups have been proven to be suitable for selective bioconjugation.[1] RAFT synthesized polymers with carboxylic acid end groups have been successfully bioconjugated with biotin and PEGylated biotin.[20] Similarly, the amine functionalized polymer was successfully conjugated to an activated fluorescent compound, 6-[Fluorescein-5(6)-carboxamido] hexanoic acid N-hydroxysuccinimide ester (5-SFX) (Sigma Product No. 46940). It is easily imaginable that such techniques could be used to produce bioconjugates such as peptides, proteins, or targeting moieties for drug or gene delivery.

RAFT polymers containing azide and thiol were suitable for conjugation with biomolecules using "Click Chemistry."[21] Li and coworkers[21]adopted copper-catalyzed azide-alkyne click chemisty to synthesize responsive protein-polymer conjugates, as shown in **Figure 2**. In their study, BSA was functionalized with an alkyne moiety via reaction of its free cysteine residue with propargyl maleimide. Azido terminated poly(NIPAAm) was prepared via RAFT, and the protein-polymer coupling was accomplished by copper-catalyzed azide-alkyne cycloaddition (**Figure 2**).

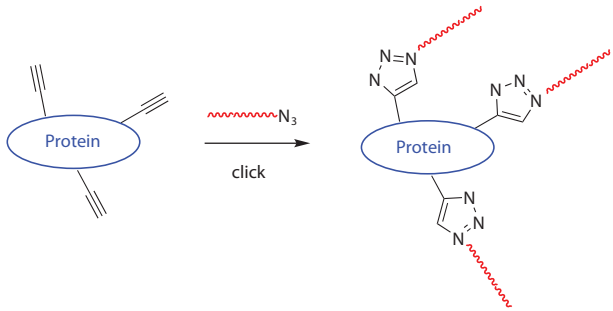


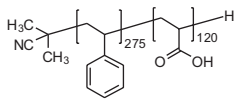
Figure 2. Polymers generated by RAFT and functionalized with a clickable azide can be used to form polymer-protein bioconjugates.

In recent years, many thiol-based reactions have been recognized and used as highly efficient processes for polymer synthesis and functionalization.[22] The increasing number of routes available to transform the thiocarbonylthio end-group to a thiol provides an avenue to explore and exploit chemistry on the thiol functional 'handle.' Examples of reactions that can be performed at the terminal thiol group in RAFT-prepared (co)polymers include thiol–ene, thiol–yne, thiol–isocyanate, thiol–halo and thiol–oxirane reactions, many of which possess the key characteristics of click reactions.[22]

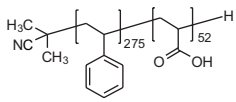
Aldrich Materials Science developed a wide range of functional polymers with a precise molecular weight and narrow polydispersity (PDI <1.1), many of which are functionalized with carboxylic acid, azide, amine and thiol groups. These are commonly used as is or as precursors for synthesizing desired block copolymers for potential biomedical applications.

Amphiphilic Copolymers and Self-assembly

The self-assembly of amphiphilic di- and triblock copolymers into micelles and vesicles (polymersomes) has been investigated widely in pharmaceutical applications ranging from sustained-release technologies to gene delivery.[23-26] Therapeutic molecules can be incorporated into micelles and vesicles via hydrophobic interactions, electrostatic attractions, hydrogen, and/or covalent bonds. Biodistribution, stability, solubility, immunogenicity, and nonspecific bioactivity of therapeutics can be altered using micelles/vesicles rationally designed for a particular application.[20,26-27] Micellar structures can be programmed to release the therapeutics upon an environmental trigger such as temperature and pH or by passive diffusion, depending on the application.[28] Immense attention in the RAFT polymerization field has been given to the generation of amphiphilic block copolymers as building blocks of micelles/vesicles for potential drug delivery applications. RAFT polymerization provides a versatile route to the generation of block copolymer micelles with controllable features, such as block lengths affecting the critical micelle concentration (thus stability), hydrodynamic size, and morphology, and chemical functionalities in the micelle corona and core offering possibilities to stabilize the supramolecular structure via covalent bonds (i.e., shell or core cross-linking), conjugating with biologically active molecules such as cell specific targeting molecules and therapeutics. Sigma-Aldrich developed a series of well-defined amphiphilic polystyrene-*block*-poly(acrylic acid) (PS-*block*-PAA) copolymers using RAFT polymerization (**Figure 3**). The resulting copolymers show controlled molecular weight as well as narrow polydispersity indices (PDI <1.2) (**Table 1**). The thioester, RAFT agent end group was removed by radical induced reduction to get colorless, non-toxic polymers.



Aldrich Prod. No. 746983



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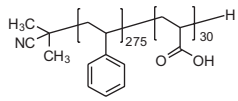

Aldrich Prod. No. 747009

Figure 3. Structures of PS-*block*-PAA with PAA content 30%, 15%, and 10%, respectively.

Micellization of Polystyrene-*block*-poly(acrylic acid)

Micellization of block copolymers, especially those comprised of PS-*block*-PAA, have been investigated extensively.[29] It has been found that the morphologies are influenced by many variables, including the composition of the block copolymers, molecular weight, copolymer concentration, and type of the common solvent.

Morphological overview and the molecular characteristics of PS-*block*-PAA are shown in **Table 1**. In general, micelles of ~30 nm in diameter and vesicles of 75–300 nm in diameter can be achieved by simply changing the block composition and molecular weight of the polymers.

Table 1. The molecular characteristics, composition, morphology and micellar sizes of polystyrene-*block*-poly(acrylic acid) (PS-*block*-PAA) copolymers.[29]

PS-*block*-PAA	Mole % PAA	M_n (kDa)	Morphology	Diameter (nm)
197-*b*-47	19	24	Micelles	20
132-*b*-61	32	18	Micelles	26
130-*b*-120	48	22	Micelles	28
120-*b*-120	50	21	Micelles	37 (crosslinked)
248-*b*-47	16	30	Vesicle	75
40-*b*-40	50	7	Micelles	80 (crosslinked)
188-*b*-34	15	22	Vesicle	86
310-*b*-28	8	34	Vesicle	90
300-*b*-44	13	35	Vesicle	90
275-*b*-47	15	32	Vesicle	100
310-*b*-52	14	36	Vesicle	100
132-*b*-26	17	16	Vesicles	100
310-*b*-36	10	35	Vesicle	125
434-*b*-47	10	49	Vesicle	292

We studied the micellization of three PS-*block*-PAA copolymers with PAA content of 30%, 15%, and 10% (**Table 2**) by dynamic light scattering (DLS).

Table 2. Molecular characteristics and aggregation behaviors of polystyrene-*block*-polyacrylic acid (PS-*block*-PAA) block copolymers. M_n is number average molecular weight, M_w is weight average molecular weight, Rh is hydrodynamic radius.

PS-*block*-PAA	Mole % PAA	M_n	M_w/M_n (PDI)	Morphology	Rh (nm)
275-*b*-120	30	37,000	1.15	Micelles	30–50
275-*b*-52	15	32,000	1.17	Vesicles	~100
275-*b*-30	10	31,000	1.14	Vesicles	~300

Micelles/vesicles were prepared in 1 wt% polymer solution in 1,4-dioxane with 20% water (***v/v***). The DLS data show the size of the micelles/vesicles increase with the decrease of PAA content (**Table 2**). This has attributed due to the increase of hydrophobic content and the strong hydrophobic interaction of PS block in water. The ability to control the size of micelle/vesicle formed by using the correct block copolymer enables further research in interesting applications such as active drug loading and controlled release for biomedical application.

Conclusion

The RAFT polymers have been increasingly used in potential drug delivery applications. The potential toxicity of thiocarbonylthio groups can be eliminated easily by post polymerization treatments of the RAFT polymers.[30] However, toxicity assay results determined in a few published studies[31] suggest that the removal of the active thiocarbonylthio functionality from the RAFT-synthesized polymers may not always be necessary for *in vitro* experiments depending on the type of the RAFT agent (substituent groups), the type of the polymer and cells, and the concentration of the polymer used. While the systematic investigations on the pharmacological profile, such as metabolic cytotoxicity, of polymeric RAFT agents are yet to be performed, the increased popularity in the literature indicates that more RAFT polymers have been applied in the controlled drug delivery field by polymer chemists, material scientists, and biomedical researchers. Commercial availability of the RAFT agents and RAFT polymers will continue to enhance industrial adoption.

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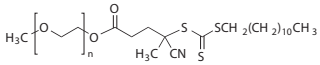
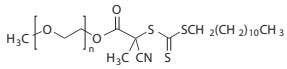
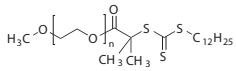
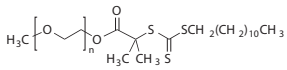
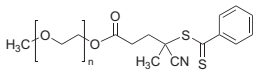
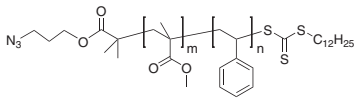
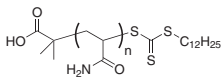
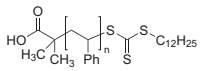
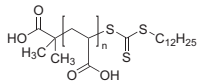
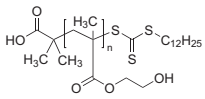
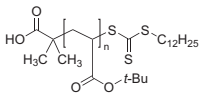
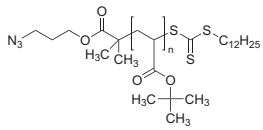
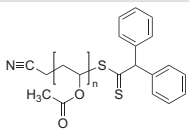
Macro Chain Transfer Agents (macroCTAs)

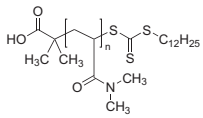
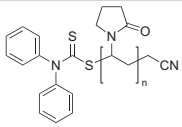
For a complete list of available macroCTAs, visit aldrich.com/raftagent

PEG macroCTAs

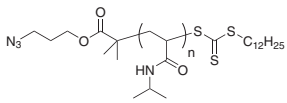
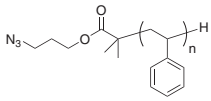
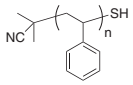
Name	Structure	Avg. Molecular Weight (M_n)	PDI	Prod. No.
Poly(ethylene glycol) methyl ether (4-cyano-4-pentanoate dodecyl trithiocarbonate)		1,400	≤ 1.1	752487-1G 752487-5G
		2,400	≤ 1.1	751634-1G 751634-5G
		5,400	≤ 1.1	751626-1G 751626-5G
Poly(ethylene glycol) methyl ether 4-cyano-4-[(dodecylsulfanylthiocarbonyl)sulfanyl]pentanoate		10,000	≤ 1.1	753033-1G
Poly(ethylene glycol) methyl ether 2-(dodecylthiocarbonothioylthio)-2-methyl-propionate		1,100	≤ 1.1	740705-1G
		5,000	≤ 1.1	736325-1G
Poly(ethylene glycol) methyl ether (2-methyl-2-propionic acid dodecyl trithiocarbonate)		10,000	≤ 1.1	752495-1G
Poly(ethylene glycol) 4-cyano-4-(phenylcarbonothioylthio) pentanoate		2,000	≤ 1.1	764914-1G
		10,000	≤ 1.1	764930-1G

Functionalized macroCTAs

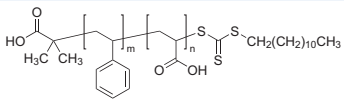
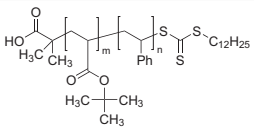
Name	Structure	Avg. Molecular Weight (M_n)	PDI	Prod. No.
Poly(styrene)-*block*-poly(methyl methacrylate) DDMAT, azide terminated		10,000	< 1.1	777919-1G
Polyacrylamide, DDMAT terminated		10,000	< 1.1	773611-1G
Polystyrene, DDMAT terminated		5,000	< 1.1	772577-1G
		10,000	≤ 1.1	772569-1G
Poly(acrylic acid), DDMAT terminated		10,000	≤ 1.1	775843-1G
Poly(hydroxyethyl methacrylate), DDMAT terminated		7,000	< 1.2	772542-1G
Poly(*tert*-butyl acrylate), DDMAT terminated		7,000	< 1.2	772550-1G
Poly(*tert*-butyl acrylate), DDMAT terminated, azide terminated		7,000	≤ 1.2	776424-1G
Poly(vinyl acetate), cyanomethyl diphenylcarbamodithioate		5,000	< 1.2	773328-1G

Name	Structure	Avg. Molecular Weight (M_n)	PDI	Prod. No.
Poly(*N*,*N*-dimethylacrylamide), DDMAT terminated		10,000	< 1.1	773638-1G
Poly(vinyl pyrrolidone), cyanomethyl diphenylcarbamodithioate		6,000	≤ 1.2	773417-1G

Functionalized Polymers

Name	Structure	Avg. Molecular Weight (M_n)	PDI	Prod. No.
Poly(*N*-isopropylacrylamide), azide terminated		15,000	< 1.2	747068-1G
Polystyrene, azide terminated		11,000	< 1.2	746916-1G
Polystyrene, thiol terminated		5,000	< 1.2	746924-1G
		11,000	< 1.2	746932-1G

Diblock Copolymers

Name	Structure	Avg. Molecular Weight (M_n)	PDI	Description	Prod. No.
Polystyrene-*block*-poly(acrylic acid)		37,000	< 1.2	30 wt. % PAA	746983-500MG
		32,000	< 1.2	15 wt. % PAA	746991-1G
		31,000	< 1.2	10 wt. % PAA	747009-500MG
		-	≤ 1.1	PS:PAA 3,000:5,000	776351-500MG
Polystyrene-*block*-poly(*tert*-butyl acrylate), DDMAT terminated		12,000	≤ 1.2	acid terminated block ratio 50:50	776432-1G

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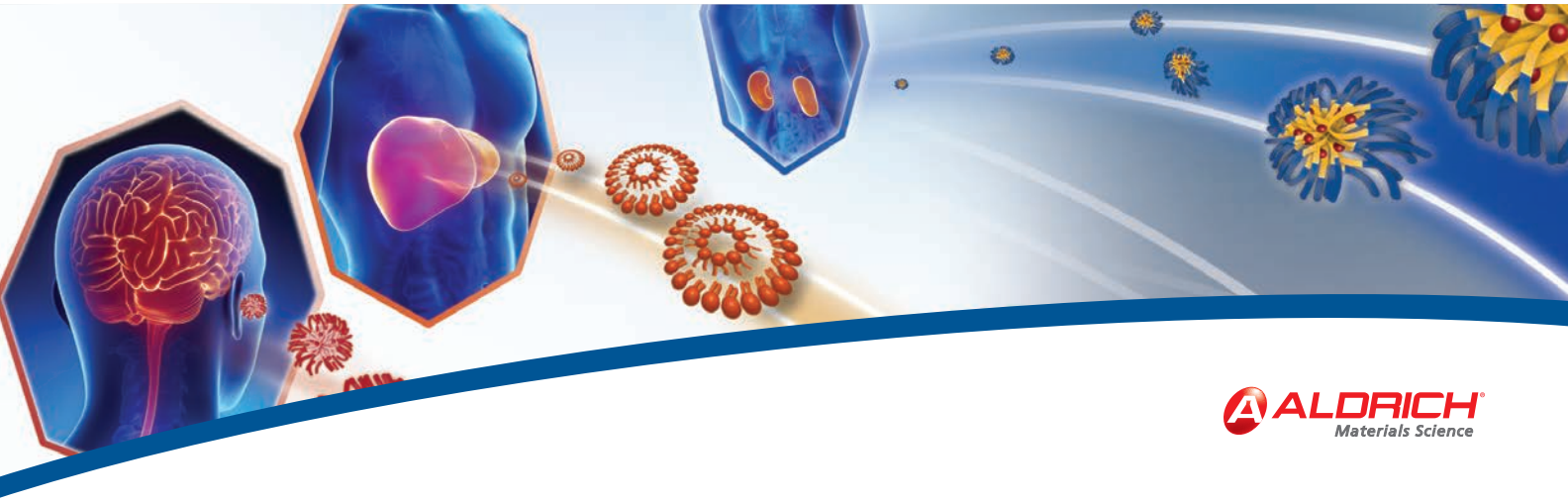

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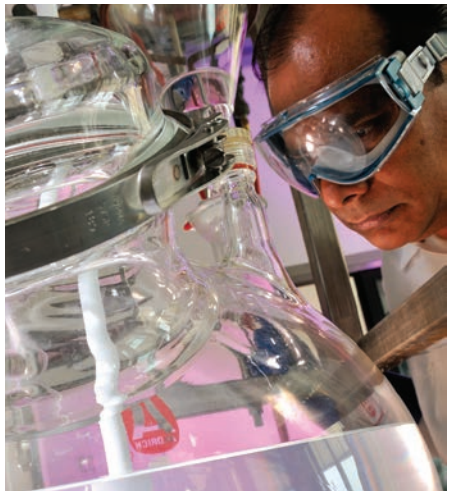

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