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Combinatorial approaches in post-polymerization modification for rational development of therapeutic delivery systems

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Abstract

The combinatorial polymer library approach has been proven to be effective for the optimization of therapeutic delivery systems. The library of polymers with chemical diversity has been synthesized by (i) polymerization of functionalized monomers or (ii) post-polymerization modification of reactive polymers. Most scientists have followed the first approach so far, and the second method has emerged as a versatile approach for combinatorial biomaterials discovery. This review focuses on the second approach, especially discussing the post-modifications that employ reactive polymers as templates for combinatorial synthesis of a library of functional polymers with distinct structural diversity or a combination of different functionalities. In this way, the functional polymers have a consistent chain length and distribution, which allows for systematic optimization of therapeutic delivery polymers for the efficient delivery of genes, small-molecule drugs, and protein therapeutics. In this review, the modification of representative reactive polymers for the delivery of different therapeutic payloads are summarized. The recent advances in rational design and optimization of therapeutic delivery systems based on reactive polymers are highlighted. This review ends with a summary of the current achievements and the prospect on future directions in applying the approach of post-polymerization modification of polymers to accelerate the development of therapeutic delivery systems.

Graphical Abstract

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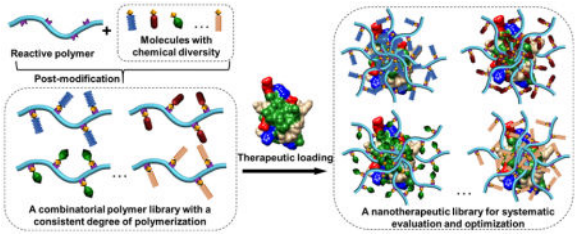
Disclosure

The authors have no conflicts to disclose.



Keywords

reactive polymers; post-modification; combinatorial biomaterials discovery; therapeutic delivery; high-throughput screening

1. Introduction

Polymeric delivery systems have been considered to have controllable chemical structures and compositions, relatively low cytotoxicity, and manageable surface chemistry, making them widely useful in the delivery of various kinds of therapeutics, including genes, small-molecule drugs, proteins, and peptides, to treat human diseases.[1–13] The structures of polymeric delivery vehicles, which include micelles, vesicles and dendrimers, have been demonstrated to affect the therapeutic delivery behaviors.[3, 14, 15] For polymeric delivery vehicles having similar structures, the chemical compositions of polymers usually decide the therapeutic binding affinity, release profile, and targeted delivery efficiency.[3, 16, 17] However, a strategy to rationally design and systematically optimize polymers for the efficient delivery of specific therapeutics is lacking. The combinatorial polymer library approach, which was first suggested by Langer and coworkers at the beginning of the 21th century, could be an efficient approach to this end.[18, 19] Based on the Michael addition reaction between monomers containing amines and diacrylates, respectively, an initial library of 3 structurally unique, degradable, cationic poly(β-amino ester)s was synthesized by polymerization.[18] The library was amplified to 140, and then 2350, unique polymers by using a semi-automated synthesis method (the polymer synthesis routes are shown in Fig. 1).[19, 20] Through a high-throughput and cell-based screening, more than 30 polymers were finally identified from the library that transfected the monkey kidney fibroblast COS-7 cells with a higher efficiency than the gold standard poly(ethyleneimine) (PEI) *in vitro*.[20] This combinatorial approach was further demonstrated to be effective for the optimization of therapeutic delivery polymers in suicide gene therapy for localized cancer treatment *in vivo*.[21] An account by Green *et al.* published in 2007 summarized the results of employing structurally diverse, biodegradable poly(β-amino ester)s synthesized by the combinatorial library approach for gene delivery and brought deep insight into the structure-property-function relationships in these gene delivery systems.[22] Combinatorial libraries that consisted of other types of polymers were synthesized by different chemistries, such as epoxide-amine reaction,[23] ring-opening polymerization,[24, 25] and divinylsulfonamide-amine Michael addition reaction[26]. These were also proven to be efficient for polymer optimization for gene delivery, showing improved performance, including higher transfection efficiency and lower cytotoxicity, than PEI and other commercial transfection reagents.[19, 20, 23] It was noticed that the monomer composition often affects the outcome

of the polymerization, resulting in a broad range distribution of chain length in the libraries (sometimes more than 20 times in molecular weight difference) (Fig. 2a).[19, 27, 28] The heterogeneous polymeric backbones in molecular weights and polydispersities may underestimate the efficacy of certain functionalities in optimizing the polymers for therapeutic delivery.

Another approach for combinatorial polymer library synthesis is post-polymerization modification of reactive polymers with diverse functionalities (noted as post-modification).[17, 27–33] The chemistries applied in modifying polymers, including thiol exchange, atom transfer radical addition, and the Huisgen 1,3-dipolar cycloaddition, have been comprehensively reviewed in 2009 by Klok and coworkers.[30] Emerging methodological studies in combinatorial polymer library synthesis via post-modification of reactive polymers for the optimization of therapeutic delivery systems have yet to be systematically summarized and discussed. Reactive polymers are able to be decorated with a variety of molecules in a quantitative manner through post-modification and could be ideal templates for the synthesis of polymers with controlled chemical diversity and molecular weights.[27–29] The key of this approach (of employing reactive polymer precursors for combinatorial polymer library synthesis to optimize therapeutic delivery systems) is to rationally select a series of small molecules with structural comparability and analogous physicochemical properties that can be conjugated to a reactive polymer backbone to yield a combinatorial polymer library for therapeutic encapsulation and delivery. This usually leads to a library of nanocarriers having similar nanostructures/sizes but significantly different therapeutic loading and delivery behaviors.[29, 31] In this way, the relationships between therapeutic-binding moieties and therapeutic loading/delivery behaviors can be simply studied without considering the physical aggregation properties of the self-assembled polymer nanostructures. It is worth noting that this approach is effective for the precise control and systematic optimization of the polymer compositions, however, relatively weak in controlling the self-assembled polymer nanostructures. This approach usually neglects the influence of polymer compositions on the self-assembled nanostructures/sizes. Therefore, it is particularly applicable in the systems having similar self-assembled polymer nanostructures/sizes. The prominent merits of this approach include: (i) Functionalization of reactive polymer precursors by a series of small molecules to produce a library of functional polymers with significant structural diversity and a consistent polymer backbone (Fig. 2b). This allows for parallel comparison of different therapeutic delivery polymers. (ii) The integration of multiple types of functionalities in the therapeutic delivery polymers, which provide opportunities to tailor the encapsulation of therapeutics by multivalent and hybrid interactions. The synergistic combination of hybrid interactions is particularly important for protein encapsulation and delivery,[7, 31] (see Section 3.3). (iii) Screening of small molecules for focused polymer synthesis and evaluation. As the structural diversity of the therapeutic delivery polymers generally originates from the functional small molecules that are used for the post-modification, a simplified screening approach can save time when evaluating and optimizing experimental delivery vehicles, especially when combined with virtual screening.[17, 31] We will further discuss this simplified approach in Section 4.2. These advantages make the post-modification approach greatly useful in combinatorial polymer synthesis for the optimization of therapeutic delivery polymers.

In this review, we discuss the perspectives of the approach of post-modification of reactive polymers in combinatorial polymer synthesis for the optimization of therapeutic delivery systems. The studies on combinatorial polymer synthesis by post-modification approach without therapeutic delivery application have been comprehensively reviewed by Klok and coworkers,[30, 34] and, therefore, are not included or only briefly discussed herein. We summarize representative reactive polymers and their applications in the delivery of different cargoes, e.g., nucleic acids, small-molecule drugs, and protein therapeutics. The principles guiding the selection of functional small molecules for post-modification and the methods to build the combinatorial polymer library are discussed. We also highlight recent advances towards the structure-based approach for polymer design, combinatorial synthesis, and screening using a well-defined reactive polymer template, i.e., telodendrimer. Lastly, we summarize current achievements and give our prospective on the future application of the approach of post-polymerization modification of polymers for the rational development of therapeutic delivery systems.

2. Reactive polymers for combinatorial synthesis of therapeutic delivery polymers

Reactive polymer precursors are the basis for the post-modification-based optimization of therapeutic delivery systems.[27–29] The reactive polymers are isolated from natural sources[35, 36] or synthesized by various methods, including chemical modification,[37] free radical polymerization,[27] atom transfer radical polymerization (ATRP),[38–40] reversible addition-fragmentation chain transfer (RAFT) polymerization,[28, 33, 41, 42] and peptide chemistry[17, 31, 43]. By using controllable synthesis methods, the resulting reactive polymers are able to possess controlled molecular weights and narrow polydispersity.[17, 28] Different kinds of reactive polymers usually have diverse properties, including solubility and reactivity. It is key to rationally utilize the reactivity of these polymers for post-modification. In this section, five categories of typical reactive polymers (Table 1) that have been used as templates for the post-modification-based optimization of therapeutic delivery polymers are detailed. Other potentially useful reactive polymers are also discussed.

2.1. N-Hydroxysuccinimide-functionalized polyacrylates

The early application of the approach of post-polymerization modification of polymers for the optimization of therapeutic delivery systems is based on the polymers constructed by polyacrylate backbones and reactive side chains, such as poly(*N*-methacryloxysuccinimide) (Fig. 3a and Table 1).[29, 44–47] These reactive polymers were first synthesized by Ferruti *et al.* in the early 1970s, which were proved to be good polymethacrylamide precursors, yielding derivatives by reaction with primary or secondary aliphatic or cycloaliphatic amines.[48] In 2009, Wong *et al.* reported the screening of combinatorial libraries of functional polymers synthesized by post-modification of poly(*N*-methacryloxysuccinimide) in order to investigate how the polymer parameters, such as the molecular weights, cationic pendant groups (Fig. 3b), and hydrophobic pendant groups (Fig. 3c) of the functional polymers, affect the gene delivery efficiency.[29] They found the functional polymers with a large molecular weight (30 or 50 kDa) containing primary amino (compound 1 in Fig. 3b)

and imidazole (compound 4 in Fig. 3b) groups showed the highest levels of transfection among the combinatorial polymeric delivery vehicle library, comparable to the gold standard PEI. Further, this optimized polymer vector had superb toxicity profiles. The combination of primary amino and imidazole groups was demonstrated to be important for efficient gene delivery: the primary amino groups offered superior charge-neutralizing and size-condensing capacities while the imidazole groups appeared to bind to DNA molecules through non-electrostatically mediated interactions. Thus, stable polyplexes were produced that were resistant to premature dissociation. The hydrophobic pendant groups were found to affect polyplex formation and stability in addition to the interacting with membrane components. This study demonstrated the use of the post-modification method to build combinatorial polymer libraries for the optimization of polymeric delivery vehicles. At the same time, it also provided a versatile and robust platform for the investigation on structure-property-function relationships. However, a limitation of this poly(*N*-methacryloxysuccinimide) precursor approach is that purification steps are needed to remove reaction byproducts (e.g., 1-hydroxypyrrolidine-2,5-dione, Fig. 3a) and excess reactants, which restricts its application in high-throughput polymer synthesis and screening.

2.2. Azlactone-functionalized polymers

Poly(2-vinyl-4,4-dimethylazlactone) (PVDMA) is the one of the most representative azlactone-functionalized polymer, which is an important reactive polymer and has attracted considerable attention since its chemical modification was pioneered by Heilmann and coworkers in 1984.[49–52] PVDMA can be functionalized by primary amines under facile reaction conditions without added catalysts producing a stable amide linkage without formation of byproducts (Fig. 4a and Table 1). Several reports have detailed the use of PVMDA-containing polymers as reactive matrices. For example, Lynn and coworkers reported the layer-by-layer assembly of PVDMA-based multilayers for DNA delivery.[53] Fontaine and coworkers developed a class of stable azlactone-functionalized thermoresponsive nanoparticles which have potential applications in theranostics.[54] By utilizing the reactivity of PVDMA, Kilbey and coworkers created surface scaffolds for immobilization of biomolecules, manipulated the solution/interface properties, and constructed reactive polymer brushes based on reactive PVDMA homopolymers and dually reactive block copolymers.[55–59] A comprehensive review of azlactone-functionalized polymers as reactive platforms for the design of advanced materials was published in 2012 by Lynn and coworkers.[60]

The successful use of PVDMA-based polymers as delivery vectors for efficient gene delivery was presented by Lynn and coworkers in 2010.[27] In their study, PVDMA with a number-average molecular weight of 74000 was used as a reactive polymer precursor to interact with compounds having both primary and tertiary amine functionalities (Fig. 4b) to synthesize a library of 12 cationic polymers. The primary amine groups on each compound could rapidly react with PVDMA while the tertiary amine groups did not react with the azlactone functionality.[27] Exhaustive functionalization of PVDMA in 12 individual reactions resulted in a small library of polymers with a consistent number of repeating units and a consistent polymer chain length. The differences between these 12 polymers are the species of cationic groups and the numbers of positioned carbons from the cationic groups to

the polymer backbone. By conducting cell-based screening experiments, two important structural motifs enhancing DNA delivery efficiency were identified: sterically hindered tertiary amine groups and shorter carbon chains leading to the cationic groups (e.g., compounds 3, 7, and 8 in Fig. 4b). In addition, the polymer molecular weights were found to significantly affect the DNA delivery efficiency: A shorter PVDMA with a number-average molecular weight of 5800 was attempted to incorporate compounds 3, 7, and 8, but the resulting polycations showed low levels of transfection. Because the molecular weights of polymers significantly affect the delivery efficiency, the parallel comparison of different polymers with an identical degree of polymerization becomes extremely important, which is, however, challenging to be realized in the polymer systems that synthesized by the approach of polymerization of functionalized monomers (Fig. 2a). In comparison, the reactive polymers are ideal templates for this parallel comparison (Fig. 2b). The study demonstrated the great promise to use azlactone-functionalized polymers as templates in combinatorial polymer synthesis for the optimization of therapeutic delivery systems. Unfortunately, to the best of our knowledge, this is the only example to use azlactone-functionalized polymers in combinatorial polymer synthesis for rational design and optimization of therapeutic delivery vehicles. The use of azlactone-functionalized polymers for combinatorial polymer synthesis and delivery system optimization is neglected probably because the post-modification is often conducted in organic solvents such as tetrahydrofuran (THF) due to poor aqueous solubility of the azlactone-functionalized polymers and additional purification steps are sometimes needed to remove the excess, unreacted small molecules, which may limit the throughput of polymer preparation and screening. However, the post-modification conditions and purification steps can be further optimized to fulfill the requirement of high-throughput polymer screening (e.g., introducing polyethylene glycol (PEG) to prepared PEG-PVDMA block copolymers to improve the aqueous solubility, using equivalent units of small-molecule reactants for post-modification to avoid purification steps). Another concern is the biocompatibility and degradability of PVDMA, which call for further studies on it. Overall, given the easy, controllable, and rapid synthesis procedure, we believe that azlactone-functionalized polymers are promising and convincing candidates for use in the fabrication of novel polymeric biomaterials and as potentially useful templates for the development of screening-based methodologies.

2.3. Hydrazide-functionalized polymers

Hydrazide-functionalized polymers can react readily with various functional aldehydes by post-modification to form acyl hydrazones that are sufficiently stable under physiological conditions.[28, 61–66] Different from PVDMA, the hydrazide-functionalized polymers, e.g., poly(acryloyl hydrazide) (Fig. 5a and Table 1), are usually water soluble, which enables the post-modification reaction(s) to occur in aqueous solutions or dimethyl sulfoxide (DMSO)/water mixtures.[28, 67, 68] In 2016, Montenegro and coworkers reported the *in situ* functionalization of poly(acryloyl hydrazide) with positively charged and hydrophobic aldehydes (Fig. 5b) and the application for the efficient delivery of small interfering RNA (siRNA) screened in cell culture.[28] This advanced *in situ* functionalization strategy will be discussed in detail in Section 4.1. It should be mentioned that the small-molecule aldehydes can be consumed completely during the post-modification and no catalyst or co-reagent was used, therefore, no further purification was required for the removal of small molecules. In

this work, the cationic aldehyde that contains a guanidinium group was kept constant, and different hydrophobic aldehydes were used for polymer synthesis and screening. It was found in this study that the species and the content of the hydrophobic functionalities significantly affected the membrane transport ability and siRNA transfection efficiency. Similar to PVDMA, the biocompatibility and degradability of poly(acryloyl hydrazide) need to be studied to assess its potential application for therapeutic delivery *in vivo*.

The idea to use hydrazide-functionalized polymers for the optimization of therapeutic delivery polymers could be applied equally to a peptide system (linear oligo(amino acid)s). Arginine and hydrazides were introduced into a peptide system by Montenegro and coworkers, wherein the position and the number of the positively charged arginine still kept constant, and the position of hydrazides also kept constant for the combination of 28 different hydrophobic aldehydes to yield amphiphilic peptides with structural diversity that could form polyplexes for cell-based delivery vehicle screening towards efficient gene delivery.[43] By using this approach, 3 hydrazone-modulated amphiphilic peptides were identified that delivered plasmid DNA into HeLa cells with better efficiency and less cytotoxicity than the standard commercial reagents.

The hydrazide-functionalized macromolecule precursors (polymers and peptides) usually have fine aqueous solubility and the post-modification reactions readily occur in aqueous solution or DMSO/water mixtures. In addition to the aqueous solubility, isolation and purification of the synthesized hydrazone-modulated macromolecules are not required. Given the consistent degree of polymerization, aqueous solubility, and dispensable purification, we believe that hydrazide-functionalized macromolecules are potentially ideal templates for high-throughput screening of polymeric delivery vehicles through an informed choice of aldehydes.

2.4. Natural polysaccharides and derivatives

The natural polysaccharides such as chitosan, dextran, hyaluronic acid, alginic acid have been widely used in the field of biomedical engineering as they are inexpensive, readily available (usually isolated from natural sources), and have excellent biocompatibility and biodegradability.[69–72] In terms of using a combinatorial polymer library approach for delivery vehicle optimization, Amiji and coworkers demonstrated that hyaluronic acid (Table 1) could act as a reactive polymer backbone for post-modification with polyamines and lipids of varying carbon chain lengths and nitrogen content through an amidation reaction for siRNA encapsulation and tumor-targeted delivery.[35] Some natural polysaccharides can be converted into highly reactive derivatives through single-step chemical reactions that would be useful as templates for combinatorial polymer synthesis. For example, Abeylath *et al.* reported the conjugation of 5-chloro-1-pentyne to a dextran backbone for the synthesis of *O*-pentynyl dextran that enabled post-modification with varying lengths of lipid chains, thiol groups, and PEG via click chemistry for the construction of a library of functional polymers.[36] This library demonstrated efficacy in self-assembling into nanoparticles for the encapsulation of various small-molecule anticancer drugs with different hydrophobicities. However, the relatively broad molecular weight distribution of the natural polysaccharides and their derivatives, together with the use of coupling reagents or copper in the amidation

and click reactions, may limit the application of these materials and chemistries in high-throughput polymer synthesis for the optimization of therapeutic delivery systems. To their benefit, copper-free methods for compound synthesis by click chemistry have been developed,[73] which may circumvent residual copper-associated toxicity that could potentially occur with this click chemistry-based approach.

2.5. Oligo(amino acid)s-based platforms

Linear poly(amino acid)s such as polylysine and polyaspartic acid contain reactive primary amine or carboxyl groups, and they have the potential to be useful as templates in combinatorial polymer synthesis. Such poly(amino acid)s have been rarely used in combinatorial polymer synthesis studies, mainly due to their high synthesis costs. However, linear or dendritic oligo(amino acid)s with easy synthesis procedures and low synthesis costs have been well used in combinatorial macromolecule synthesis for the optimization of therapeutic delivery systems.[17, 31, 43] Compared to natural polysaccharides and traditional reactive polymers synthesized by polymerization, the chemical structures of the oligo(amino acid)s-based platforms synthesized via peptide chemistry are well-defined and can be characterized clearly.[7, 17, 31, 43] In addition to the well-defined structures, biocompatible oligo(amino acid)s-based platforms are constructed via peptide bonds, which can be hydrolyzed by peptidases *in vivo*.[7] The use of linear oligo(amino acid)s with hydrazide-functionalized peptides as reactive templates for combinatorial delivery vehicle synthesis[43] was discussed in Section 2.3. Compared to linear macromolecules that mostly have random coil structures, the more globular shapes in the dendritic segments of dendrimers and linear-dendritic copolymers are generally considered to potentially affect their physiochemical and biological properties. This has led to the discovery of interesting effects related to macromolecular architecture.[74, 75] For instance, Gitsov and coworkers reported that the PEG-*b*-dendritic poly(benzyl ether) could selectively interact with glycan moieties on a laccase to enhance its enzymatic activity by providing hydrophobic depots for substrates, while the linear PEG-*b*-polystyrene polymer consistently inhibited the catalytic activity.[76, 77] In terms of dendritic oligo(amino acid)-based systems, we reported the use of a chemically well-defined telodendrimer[78–86] composed of linear PEG-blocking-dendritic oligolysine and capping peripheral building blocks (Fig. 6) for structure-based design of polymers for therapeutic delivery.[17] Efficient peptide chemistry was used in telodendrimer synthesis that allowed for free and precise control over the architecture and the functionality of the telodendrimer. The peripheral groups on the dendritic oligolysine have good flexibility in interacting with drug molecules. Three generations of telodendrimers with distinct architectures were developed: (i) first generation (G1) telodendrimers with 8 drug-binding molecules (DBMs) (Fig. 6a), (ii) second generation (G2) telodendrimers with hybrid peripheral groups of 4 DBMs and 4 amphiphilic cholic acids (CAs) for better stabilization and aqueous solubility of the telodendrimer micelles (Fig. 6b), and (iii) third generation (G3) telodendrimers with functionally segregated peripheral groups of 4 DBMs together for drug binding and 4 CAs together for stabilizing the payload-containing telodendrimer micelles (Fig. 6c). By using these linear-dendritic architectures, various DBMs can be conjugated in a well-defined manner to produce telodendrimers with different species but the same number of DBMs, which is critical for parallel evaluation of different polymeric delivery vehicles. However, compared to the readily available natural

polysaccharides and the conventional reactive polymers synthesized by single-step polymerization, the oligo(amino acid)s-based platforms usually have tedious synthetic procedures (Table 1). Employing peptide chemistry for functionalization involves conjugation reactions using coupling reagents, which may necessitate purification steps. This may limit the applications for high-throughput screening of large polymer libraries.

2.6. Other reactive polymers potentially useful in combinatorial polymer synthesis for therapeutic delivery

Along with the five types of classic reactive polymers mentioned above are other reactive polymers that have been used, or have the potential to be used, as templates for combinatorial polymer synthesis. For instance, Bradley and coworkers have demonstrated that poly(allyl glycidyl ethers) can be functionalized through a tetrazine-mediated post-modification, and this modification can be used for triggered release of doxorubicin from the polymer nanoparticles.[87, 88] The inverse electron demand Diels-Alder reaction used in these studies can be conducted in either organic or aqueous solvent, without the need for additives or catalysts,[87] which shows great promise for post-modifications in polymers aimed at biological applications. Poly(pentafluorophenyl methacrylate) (PPFMA) that can react with primary amines has been used for the preparation of a diverse water-soluble polymer library by post-modification without inducing any additional toxicity,[33] indicating the potential to use PPFMA as a reactive polymer precursor in parallel polymer synthesis for the optimization of therapeutic delivery systems. A drawback of using PPFMA as a reactive polymer precursor is that purification steps are needed to remove the functionalization reaction byproducts of 2,3,4,5,6-pentafluorophenol, which may limit the application of PPFMA in high-throughput polymer synthesis. Recently, Li *et al.* reported the synthesis of reactive polymers with both pentafluorophenyl ester and azlactone functionalities, that can be selectively modified by different amines in a controlled manner to yield polymers with bespoke functionalities.[89] This is realized by fully exploiting the difference in reactivity of these two activated esters toward different amines. Epoxide group-containing polymers, such as poly(glycidyl methacrylate) (PGMA),[58, 59] that can react with functional molecules containing amine, hydroxyl, or carboxyl groups, are promising reactive polymer templates for combinatorial polymer synthesis although the undesired chain-branching reactions may occur when improper nucleophilic reagents (e.g., primary amines) are used. The post-modification via epoxide ring opening can be catalyzed by either tertiary amine small molecules or tertiary amine group-containing polymers, such as poly(2-(dimethylamino)ethyl methacrylate) (PDMAEMA).[34, 90] Moreover, the pendant tertiary amine groups in the PGMA-*co*-PDMAEMA brushes were demonstrated to accelerate the post-modification rate via epoxide ring opening with primary amines in aqueous solution at room temperature.[91] A successful case to use epoxide as a “modification handle” for therapeutic delivery polymer optimization was presented by Anderson and coworkers in 2015.[92] In their study, 1536 structurally distinct nanoparticles with cationic cores and variable shells were synthesized based on the ring-opening reactions between epoxide-functionalized block polymers and amines in a combinatorial fashion for high-throughput screening of polymers toward the efficient delivery of siRNA and DNA.[92] Some other amine- or carboxyl-containing synthetic polymers, such as polyallylamine hydrochloride and poly(acrylic acid), could be useful as templates for combinatorial polymer synthesis.

However, similar with the oligo(amino acid)-based platforms, coupling reagents are necessary for the post-modification of these polymers and additional purification is required. Notably, branched PEI with reactive primary amine groups is not a good candidate template for combinatorial polymer synthesis as the chemical structure of PEI is complex and not well-defined. Moreover, the secondary amine groups in PEI may compromise functionalization if small molecules with strong reactivity (e.g., organic acid anhydrides) are used. The design and synthesis of new reactive polymers with high reactivity and good aqueous solubility/stability, in which case the post-modification can be conducted at room temperature in an aqueous solution without catalysts and further purification, will greatly promote the development of reactive polymer-based combinatorial synthesis and screening methods for optimization of therapeutic delivery vehicles.

3. Therapeutic delivery

This section discusses three types of therapeutics used as model payloads in combinatorial polymer synthesis by the post-modification approach for the rational fabrication of therapeutic delivery systems: genes, small-molecule anticancer drugs, and proteins (Table 2). It should be pointed out that the therapeutic cargoes are not limited to these three types. The selection of functional small molecules used for post-modification of reactive polymers relies on the properties of the therapeutic cargoes. Therefore, in this section, we discuss the principles guiding functional small molecule selection along with an introduction on the therapeutic cargoes.

3.1. Genes

Gene therapy is an attractive therapeutic option that holds promise to treat diseases; especially for cancer treatments by targeting vital genes for cancer survival.[21, 44, 93] Nonviral gene delivery systems are under intense investigation due to many advantages over viral systems, such as simple preparation procedures, better safety, higher stability, low immunogenicity, and the possibility for further modification to realize advanced functions (e.g., molecular imaging guided gene delivery).[21, 94, 95] Combinatorial polymer synthesis and screening has been widely used for the optimization of polycation-based gene delivery vehicles.[19–22] In fact, most examples of using a combinatorial polymer synthesis approach to optimize functional polymers synthesized by either polymerization of functionalized monomers or post-modification of reactive polymers focused on the delivery of genes.[19, 20, 23, 27, 44]

While gene therapy is promising to treat diseases, there are other underlying reasons for scientists to focus on the development of efficient gene delivery vectors by combinatorial polymer synthesis and screening. Firstly, unlike small-molecule drugs that can diffuse into diseased cells, the negatively charged, hydrophilic DNA molecules are generally cell-impermeable by themselves, requiring delivery vehicles to condense them and mediate their membrane transport, endosomal escape, and nuclear import. In an account describing the combinatorial polymer library approach for gene delivery, Anderson and coworkers quoted Verma as saying, “…there are only three problems in gene therapy - delivery, delivery and delivery.”, to expound their opinion that the development of efficient delivery vehicles is key

for progressing the field of gene therapy.[22, 96] Secondly, compared to small-molecule drugs and proteins with great structural diversity, the structures of genes are relatively simple and fixed. Optimized gene delivery polymers screened using a model gene may have universality for other genes. Electrostatic interactions are the main driving forces for gene loading in the polycationic delivery vehicles, while the hydrophobic functionalities in polymeric delivery vehicles were demonstrated to influence the gene delivery efficiency by affecting membrane transport ability.[43] Lastly, cell level evaluation for the optimization of gene delivery vehicles is reliable, relatively simple, and feasible. Unlike delivery vehicles for small-molecule drug or protein delivery that usually need *in vivo* treatment efficacy characterizations, high-throughput, cell-based screening has been demonstrated to be reliable and efficient for the identification of optimal polymers for DNA delivery.[20] Incidentally, this cell-based screening method is also suitable for the high-throughput optimization of siRNA delivery vehicles.[24, 28] These three factors make genes the most popular therapeutic cargoes used in combinatorial polymeric delivery vehicle screening studies. We have shown several examples (Tables 1 and 2) of using the reactive polymer-based combinatorial polymer synthesis and screening method to optimize vehicles for efficient gene delivery[27–29, 35, 43] in Sections 2.1–2.4. Here we provide one more example showing how to rationally synthesize and optimize the gene delivery polymers.

Both amino and hydrophobic functionalities in polymeric nonviral gene vectors seriously affect the gene delivery efficiency.[97–99] On the one hand, the optimization of amino functionalities can efficiently improve the DNA condensation and facilitate the cellular uptake and endosomal escape.[97] On the other hand, the hydrophobic functionalities usually influence the stability of DNA-polymer complexes and the interactions with cell membranes.[98, 99] Ge and coworkers recently presented a one-pot synthetic route for simultaneous optimization of both amino and hydrophobic functionalities in polymeric nonviral gene vectors by employing thiolactone chemistry (Fig. 7).[37] This one-pot combinatorial synthesis of multifunctional polymers was achieved through the aminolysis of the thiolactone unit, followed by the release of a mercapto group for Michael addition reaction with poly(2-(acryloyloxy)-ethyl methacrylate). In this way, both amino and hydrophobic functionalities could be conjugated to the polymer backbones in a facile manner, yielding 15 multifunctional polymers for DNA condensation and cell-based screening. A polymer (R_1 = D, R_2 = 3, see Fig. 7) based on tetraethylenepentamine and heptafluorobutyric acid-functionalized thiolactone was finally identified to have the highest gene transfection efficiency and low cytotoxicity, which performed better than the gold standard PEI and Lipofectamine 2000 in both HeLa and HepG2 cell lines. In their study, the thiolactone chemistry was used for the first time for combinatorial synthesis of polymers having both amino and hydrophobic functionalities, which presented an effective way for the optimization of gene delivery polymers.

Compared to the more popular research on gene delivery polymers using the combinatorial library approach, the utilization of combinatorial polymer synthesis to optimize polymers for the delivery of small-molecule drugs and protein therapeutics is relatively backward. This is mainly attributed to the largely varied structures and properties of small-molecule drugs and protein therapeutics, which add significant difficulty to the development of universal polymers for the delivery of such cargoes. Structural precision is critical in the combinatorial

fabrication of polymers for drug/protein delivery, as slight structural uncertainty may lead to unforeseen difficulties when conducting systematic delivery vehicle evaluation and optimization. Therefore, the development of reactive polymers and functionalized polymers with chemically well-defined structures is essential for combinatorial polymer synthesis and optimization for drug/protein delivery. We will show two examples of employing a well-defined telodendrimer platform (Fig. 6) for polymer optimization by the combinatorial library approach towards the efficient delivery of small-molecule anticancer drugs and protein therapeutics in Sections 3.2 and 3.3, respectively.

3.2. Small-molecule anticancer drugs

As a major public health problem worldwide, many small-molecule drugs have been developed for the treatment of various cancers.[100, 101] A majority of anticancer drugs have undesirable aqueous solubility, poor pharmacokinetics, and may cause severe toxic side effects.[101–107] Polymeric nanoparticle-based drug encapsulation increases drug solubility and stability, minimizes toxic side effects, and, most importantly, delivers drugs specifically to tumor sites by the enhanced permeability and retention (EPR) effects.[104, 108–111] Many factors, including the physicochemical properties of polymer nanoparticles (such as size, shape, charge density, hardness, and surface composition) and the tumor environments, seriously affect (or limit) the extent of the EPR effects.[78, 112] For example, we previously demonstrated that the size of the drug-loaded polymer micelles greatly influenced their *in vivo* biodistribution, and the smaller ones (hydrodynamic diameter, D_h: 17 and 64 nm) were more readily and efficiently able to carry the loaded anticancer drugs to the tumor sites, when compared to the larger one (D_h: 154 nm).[78] Moreover, the modification of ligands on polymer nanoparticles can further improve the tumor uptake by active targeting effects.[113] Recently, Kobayashi and coworkers showed that the EPR effects could be increased by manipulating either local tumor or systemic conditions, and they suggested three important modifiable parameters that improve the resistance of the tumor capillary wall: (i) modulating tumor blood flow, (ii) modulating tumor vasculature and stroma, and (iii) killing cancer cells to reduce the barrier function.[112]

Anticancer drugs have diverse chemical structures and properties, which require rational and customized polymer design for efficient drug encapsulation and delivery. The significance of combinatorial design of polymeric nano-delivery systems and formulation customization for cancer therapy has been reviewed by Amiji and coworkers.[36, 114, 115] Reactive polymer-based combinatorial synthesis and screening can effectively optimize polymer synthesis for targeted drug delivery. By using doxorubicin as a model anticancer drug and telodendrimers (Fig. 6 and Table 2) as a model reactive platform, we presented a study on drug-specific polymer design for efficient anticancer therapy.[17] A number of lipophilic vitamins and natural molecules (Fig. 8a) were collected into a model library for virtual screening, followed by combinatorial polymer synthesis and systematic evaluation/optimization. This computer-aided strategy to facilitate the experimental screening will be discussed in Section 4.2. Rhein (Rh), an aromatic-rich molecule, was identified to be the best DBM for doxorubicin with high binding affinity, as well as to have biocompatibility and bioactivity of a building block. The telodendrimers were synthesized using amphiphilic CAs as co-core forming building blocks with Rh, which efficiently stabilized nanocarriers into small-sized

particles and prevented further aggregation. Rh- and CA-containing telodendrimer nanocarriers were demonstrated to have sustained doxorubicin release (Fig. 8b), improved circulation (Fig. 8c), increased maximum tolerated dose, reduced toxic side effects (Fig. 8d), effective tumor targeting (Fig. 8e). The proper stability and drug release profiles of these nanoformulations yielded the significantly improved anticancer effects (Fig. 8f) when compared to both free doxorubicin and Doxil (a stealth liposomal nanoformulation of doxorubicin).

We show this example to illustrate a method for functional small molecule selection when constructing reactive polymer-based systems for the delivery of an anticancer drug. We can study the structure-property relationship of DBMs and drug loading properties in these systems: Firstly, hydrogen bonding and hydrophobic interactions may play important roles for binding the anticancer drugs. For example, aromatic-rich molecules generally had high binding affinity with aromatic-rich doxorubicin. Steric hindrance is another important factor to determine binding affinity, as doxorubicin was not the most favorable binder to itself. Therefore, serious consideration of the drug structure and drug-DBM interactions may help us to narrow the selection of functional small molecules before the screening of polymers. Secondly, the strongest drug binding and encapsulation may not be the best option. For correct function, the payload needs to be released after the nanoparticles reach the tumor site(s). If the nanoformulation (e.g., Doxil) is too stable to release a drug, the anticancer efficiency may be suboptimal (Fig. 8f). Therefore, the goal is to use reactive polymer-based combinatorial polymer synthesis and screening methods to identify the optimal DBM and fabricate drug delivery vehicles to improve the drug release profile, the *in vivo* pharmacokinetics, and the targeted drug delivery ability. Many amphiphilic polymers have been prepared to optimize the particle size, stability and drug loading capacity via tailoring the balance of hydrophobicity and hydrophilicity of the system.[108, 116–118] We demonstrated an inside-out approach in polymer design through precise control of binding affinity between drugs and polymers, which not only improved drug loading and release profiles, but also optimized the size and stability of nanoformulations, therefore optimizing the *in vivo* efficacy for disease treatment. Furthermore, we want to emphasize the importance of rational design of polymers based on the structure of a small-molecule anticancer drug. The polymers designed for the delivery of one drug may not be suitable for another drug, therefore an approach for drug-specific polymer design is warranted.

3.3. Proteins

Protein therapy has been considered a safe and direct approach to treat human disease since insulin (Fig. 9a) was used as the first human recombinant protein therapeutic.[119–121] Currently, more than 130 bioactive proteins have been approved to treat human diseases.[122, 123] Delivery systems are able to enhance the therapeutic efficacy of proteins through the modification of their pharmacokinetics. Protein PEGylation has a long-standing history of efficiently prolonging circulation time, increasing stability, and reducing the immunogenicity of protein therapeutics, especially for recombinant proteins.[124, 125] Physical encapsulation of proteins into nano- or micro-particles has been intensively studied for systemic or local administration.[126–128] It is a critical issue to maintain protein structure and activity in such encapsulation/modification processes, especially for processes

involving lyophilization or organic solvents. For example, the use of organic solvents in the oil/water emulsion technique[129] for the encapsulation of proteins into biodegradable polymeric microparticles (e.g., polylactic acid and polycaprolactone) usually causes denaturation of proteins and at least partial loss of activity.[130] Encapsulation of proteins in aqueous environments, such as in hydrogels[131, 132] and nanogels[133–135], represent an efficient way to preserve protein structures and activities. However, these processes mostly rely on polymerization or chemical reactions to crosslink bulk or granular hydrogels or within the dispersed nanoaggregates. The chemical process may lead to unexpected complication in control of the physical properties, and the chemicals used in these reactions may remain as toxic impurities that hinder *in vivo* and clinical applications. Efficient encapsulation of proteins *in situ* in biologically relevant environments without extra chemicals or procedures required, is ideal for clinical development of protein nanotherapeutics. The interplay of hydrogen bonding, electrostatic interactions, and hydrophobic interactions stabilizes and maintains protein three-dimensional structures. Polar amino acids, including both positively and negatively charged ones, mostly display on the surface of proteins for protein dispersion in aqueous solutions (Fig. 9a). In addition, hydrophobic residues aggregate into hydrophobic grooves to minimize aqueous exposure, as well as for binding of other hydrophobic materials and for the reinforcement of electrostatic interactions by decreasing the static dielectric constants of surroundings.[136–138] The design of a scaffold to target both polar groups and hydrophobic grooves on protein surfaces represents a promising solution for protein coating driven by both enthalpy (electrostatic)- and entropy (hydrophobic)-favored processes.

Inspired by the cooperative and/or multivalent effects[139–141] in biological systems, we have developed a hybrid telodendrimer composed of a linear PEG and a dendritic polyelectrolyte decorated with different hydrophobic biocompatible protein binding moieties tethered by a flexible linker (Fig. 9b and Table 2), that could encapsulate proteins in aqueous solution without the addition of co-reagents.[7] The decoration of telodendrimers with multiple charged and hydrophobic moieties on the dendritic periphery was demonstrated to be able to match protein surface chemistries by both electrostatic and hydrophobic interactions to facilitate protein binding and stabilize the generated nanoconstructs. The freedom in engineering the telodendrimers allows for generating well-defined structures with modularly tailored functionalities to optimize protein loading efficiency and to fine-tune the physical properties of the nanocomplex. Both charged and hydrophobic groups in the telodendrimers were found to affect the protein encapsulation and delivery. The selection of charged functionalities in telodendrimers should account for the net charges on protein surfaces. However, the species of the charged groups need to be carefully considered based on the protein properties and functions. For example, guanidine or tertiary amine groups may be used to improve the intracellular delivery efficiency of telodendrimers for cell-impermeable proteins, such as cytochrome C and truncated diphtheria toxin, that require delivery into the cytosol to impart their functions.[7, 142] In comparison, primary amino groups in the telodendrimer may be a better choice for the delivery of proteins, such as insulin, that can bind to extracellular receptors.[31] Moreover, the lysine-containing telodendrimer was found to bind to insulin stronger than the equivalent arginine-containing analog in our previous study.[31] This is presumably because the amine groups on lysine

have more flexibility in orientation to interact with the carboxylic groups on insulin to form efficient salt bridges, while arginine can form strong bivalent hydrogen bonds only with the planar guanidinium,[143] which may reduce the chance to form stable salt bridges between the telodendrimer and insulin during the assembly process. The hydrophobic moieties in the telodendrimers are largely variable, which have been known to be important for protein encapsulation and delivery.[7] We have presented a computer-aided approach for rational and customized design of telodendrimers for therapeutic protein delivery.[31] Representative hydrophobic protein binding molecules (Fig. 9c) were collected for virtual screening against a model protein of human insulin, followed by precise conjugation on the reactive telodendrimer backbone preinstalled with charged moieties in a combinatorial manner. Protein-loading stability, binding affinity between proteins and telodendrimers, and blood glucose levels controlled by sustained insulin release were studied for systematic evaluation and optimization. D-α-tocopherol (vitamin E)-containing telodendrimers were identified to be optimal by this combinatorial polymer library approach, showing strong binding affinity for insulin loading and leading to improved blood glucose control. This study not only demonstrated the importance of synergetic combinations of rationally selected charged and hydrophobic groups for stable protein loading and efficient protein delivery, but also affirmed the concept of utilizing a combinatorial polymer library for the optimization of therapeutic delivery polymers and validated the approach of structure-based polymer design for protein delivery.

4. Advances to facilitate the reactive polymer-based combinatorial polymer synthesis and screening

The last decade have witnessed the initial development of the post-modification approach in combinatorial polymer synthesis for the optimization of therapeutic delivery systems, and a number of representative therapeutic delivery polymers with desirable properties have been identified by this approach.[17, 27, 28, 31] Despite these achievements, there are still large demands to improve this approach by developing advanced reactive polymer-based systems useful for high-throughput screening of therapeutic delivery polymers and efficient ways to facilitate the rational selection of functional small molecules used for post-modification of reactive polymers. In this section, we introduce recent advances towards the optimization of the post-modification approach for the identification of therapeutic delivery polymers.

4.1. In situ functionalization of reactive polymers towards high-throughput polymer synthesis for therapeutic delivery

Beyond the delivery vehicle evaluation methods, the lack of efficient reactive polymers also limits the use of the post-modification approach for the high-throughput screening of therapeutic delivery polymers. Although the exploration of facile high-throughput evaluation methods is needed for polymer optimization when delivering small-molecule drugs and proteins, we mainly focus on the development of novel strategies to facilitate high-throughput polymer synthesis in this section. Polymer microarray approach is an efficient way to afford a polymer library containing large numbers of biomaterials with diverse structures, which allows for rapid, microscale screening of polymer-cell interactions.[144, 145] Besides, we have showed an example of *in situ* functionalization of a reactive polymer,

poly(acryloyl hydrazide), for combinatorial polymer synthesis and screening[28] in Section 2.3. This *in situ* functionalization occurred under aqueous conditions and without further purification, which may allow for high-throughput polymer synthesis. Learning from this study, we know that the reaction solvent and further purification steps are the primary barriers that may restrict the high-throughput polymer synthesis by post-modification approach.

4.1.1. Reaction medium—Unlike lipidoids[120, 142, 146, 147] (cationic lipid-like materials frequently used in combinatorial delivery vehicle library studies) that can be synthesized in solvent-free conditions at elevated temperatures, polymers are usually highly viscous and, therefore, require solvents for dissolution and efficient functionalization.[20] Selection of the solvent is critical and directly links to the throughput of functional polymer synthesis.[28] If an unsuitable solvent is used, complicated isolation steps become unavoidable for solvent removal, which significantly limits the functionalization throughput. Indeed, the optimal solvent for post-modification should be water as further delivery efficiency evaluations are usually carried out in aqueous solutions.[28] However, some reactive polymers are not water-soluble or have poor stability in water.[27] A small amount of DMSO or alcohol can be used for functionalization since it has been proved that <5 % (volume percentage) of DMSO or alcohol in aqueous solutions is generally safe in both cell cultures and *in vivo* studies.[142] Normally, 5% organic solvents are not sufficient for reactive polymer dissolution. We may use a high concentration, but small volume, of organic solvents (e.g., DMSO and alcohol) for the functionalization, followed by dilution with water to reach an organic solvent content of <5%. Alternatively, organic solvents with low boiling points (e.g., methanol and THF) can be used for the functionalization reactions and, after dilution with water, be simply removed by evaporation. These dilution and evaporation steps won't dramatically limit the throughput of combinatorial polymer functionalization since they can be performed in the same vessel. For example, the functionalization reaction, dilution, and evaporation steps can be completed in sequence in one well of a multi-well plate to yield an aqueous solution of functional polymers ready to be used for further therapeutic loading or delivery efficiency evaluation (Fig. 10).

4.1.2. Polymer purification—Purification and isolation steps increase the time required and the cost of the discovery process.[28] Therefore, these steps have to be avoided for high-throughput polymer synthesis. Generally, the reactivity of polymers dictates the need to use catalysts or co-reagents and indirectly affects the need for isolation/purification to remove the catalysts or co-reagents. For example, in the reactive polymer systems that employ peptide chemistry for post-modification, the use of coupling reagents usually cannot be avoided.[78] Therefore, isolation/purification steps of the candidate polymers have to be performed to remove the small-molecule coupling reagents. Further, the formation of reaction byproducts also make purification steps necessary, such as in the poly(*N*-methacryloxysuccinimide) and PPFMA systems.[29, 33] In comparison, polymers with relatively high reactivity, such as PVDMA and poly(acryloyl hydrazide), that are functionalized without added catalysts or co-reagents to yield pure, functional polymers without the formation of byproducts, hold great promise to be used for delivery efficiency evaluation if the functional small molecules used for the post-modification can be exhausted.

Overall, the highly reactive azlactone- and hydrazide-functionalized polymers that can react with functional small molecules without added catalysts or co-reagents in aqueous, DMSO, ethanol, methanol or THF solutions to yield a combinatorial polymer library with a consistent degree of polymerization and significant chemical diversity are the most promising templates for *in situ* and high-throughput polymer synthesis and screening.

4.2. Computational approach in polymer design for therapeutic delivery

The rational selection of functional small molecules used for post-modification of reactive polymers is essential for combinatorial polymer synthesis and delivery vehicle screening.[17, 31] Although thousands of polymers with structural diversity can be synthesized in parallel via the high-throughput polymer synthesis method in a short time,[20] the cost to purchase so many functional small molecules cannot be neglected. Pre-screening can guide functional small molecule selection and facilitate the efficient fabrication of polymeric delivery vehicles for stable therapeutic loading. Computational chemistries, such as theoretical methods and molecular simulations, have been applied in nanoparticle systems to understand therapeutic-loading properties.[148] The computation of the interactions between therapeutics and large polymers is usually time-consuming. Instead, the binding energy between therapeutics and small molecular building blocks can be rapidly computed by molecular docking, which allows for high-throughput virtual screening of a library of chemically diverse small molecules.[17, 31] In libraries synthesized by post-modification of reactive polymers, the structural diversity of different polymers originates solely from the structural small molecules used for the post-modification. The interactions between therapeutics and structural small molecular building blocks will contribute to the interactions between therapeutics and the resulting polymers, which rationalize the parallel comparison of the polymers conjugated with different structural small molecules. Therefore, the approach of virtual screening of small molecules is particularly suitable for the optimization of polymers synthesized by the post-modification approach. This virtual screening approach could help us to pre-select top-ranking functional small molecules from a large library of thousands of samples for combinatorial polymer synthesis followed by further experimental screening. Such an approach can save time and effort by helping narrow the range of small molecules to be tested at the bench.

The approach of virtual screening of functional small molecules followed by reactive polymer-based combinatorial polymer synthesis and systematic evaluation/optimization was suggested by us in 2015.[17] The initial success of this approach was obtained in a linear-dendritic telodendrimer system for the delivery of an anticancer drug, doxorubicin (Fig. 11a).[17] In a subsequent study, this approach was demonstrated to be effective in structure-based telodendrimer design for the delivery of a therapeutic protein, insulin (Fig. 11b).[31] Reasonable agreement was acquired when employing different computational methods (i.e., molecular docking and molecular dynamic simulation) to evaluate the binding affinity between therapeutics and small-molecule binders (Fig. 11c and d).[17] Moreover, significant correlations between the computational predictions and experimental results were observed (Fig. 11e and f).[17, 31] It should be mentioned that the post-modification-based combinatorial polymer synthesis and characterization are essential for systematic validation of the computational prediction in guiding the design of therapeutic delivery polymers. The

optimized formulations identified by this computer-aided approach in these two studies showed affinity-controlled therapeutic release behaviors, leading to superior anticancer effects and improved blood glucose control, respectively.[17, 31] This novel bottom-up approach for the rational design and synthesis of therapeutic delivery polymers may dramatically accelerate the development of polymer-based therapeutics.

5. Conclusions and future perspectives

Herein, we have summarized the methods and recent advances in combinatorial polymer synthesis by post-modification of reactive polymers for the optimization of therapeutic delivery polymers. By using this post-modification approach, a library of polymers with distinct structural diversity and a consistent degree of polymerization can be synthesized in a combinatorial manner, which is critical for parallel comparison of different polymers for therapeutic delivery. The reactive polymer platforms allow for the integration of multiple kinds of functionalities in a controlled manner for efficient therapeutic encapsulation and delivery. The telodendrimer systems have provided a versatile blueprint for both structure-based polymer design and combinatorial polymer synthesis to synergistically accelerate the development and optimization of therapeutic delivery systems. In comparison to telodendrimers, the polysaccharides isolated from natural sources and conventional reactive polymers synthesized by single-step polymerization are generally more economic in developing vehicles for therapeutic delivery. The use of catalysts or co-reagents, and the formation of reaction byproducts during the post-modification processes in the above mentioned systems make them challenging to be adapted in a high-throughput manner when compared to the development of cationic polymers/lipidoids such as poly(β-amino ester)s for polynucleotide delivery. The *in situ* formation of functional polymers via traceless post-modification of highly reactive polymers (e.g., the azlactone- and hydrazide-functionalized polymers) provides an opportunity to introduce different building blocks sequentially or simultaneously in a combinatorial manner to generate a library of polymers for the screening and optimization of therapeutic delivery systems. The precise decorations, rational combinations, and tailored chemical components of the polar/nonpolar structural building blocks on these reactive polymers will be the key to the development of optimal delivery vehicles for a therapeutic. More studies on their biocompatibility, chemical and colloidal stability, and *in vivo* performance are necessary to further evaluate the potential for *in vivo* therapeutic delivery. Further, an approach of virtual screening of structural molecules followed by combinatorial polymer synthesis and systematic evaluation/optimization of therapeutic delivery polymers shows great promise in accelerating the development of therapeutic delivery systems via the combinatorial post-modification of reactive polymers and high-throughput screening processes.

The development of advanced reactive polymers is promising to increase the throughput of polymer synthesis. The establishment of fast and efficient screening methods for quantitative evaluations of the polymers used for the delivery of small-molecule drugs and protein therapeutics is also essential for high-throughput optimization of drug and protein delivery vehicles. The immobilization of reactive polymers on surfaces by bioprinting to form polymer arrays[149, 150], stepwise reactions to form brushes,[59, 151, 152] or layer-by-layer assembly to form multilayer films[53, 153–155] coupled with fast fluorescence or

other optical detection techniques[156] may be another interesting approach toward high-throughput polymer screening of therapeutic loading properties. Although the drug-binding properties on the immobilized surface may be different from the physiochemical properties of drug-loaded nanoparticles in solution, it provides the first criteria to narrow down the drug-binding polymer candidates for focused evaluation. In short, reactive polymers provide an efficient platform greatly applicable for combinatorial polymer synthesis and optimization for therapeutic delivery. It is expected that more useful polymers will be developed and identified for the efficient delivery of nucleic acids, small-molecule drugs, and protein therapeutics in the future by fully exploring the reactive polymer-based combinatorial polymer synthesis methods.

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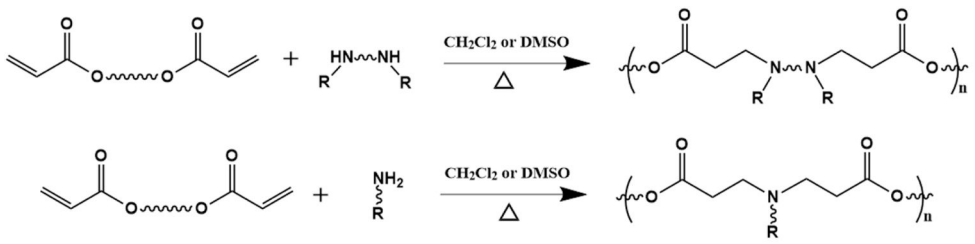

Fig. 1.

Synthesis of poly(β-amino ester)s by polymerization of functionalized monomers. The reactions can be done in a combinatorial manner by varying “R” functional groups.

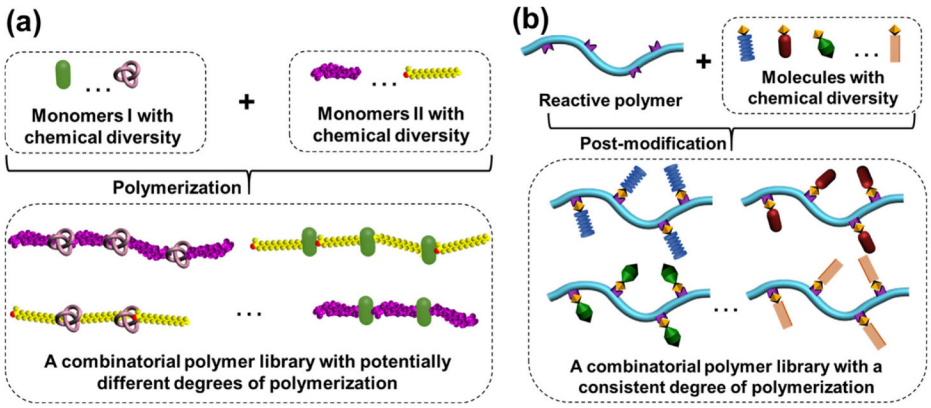

Fig. 2.

Schematic illustration of combinatorial polymer libraries synthesized by two approaches of (a) polymerization of functionalized monomers, and (b) post-modification of reactive polymers for the optimization of therapeutic delivery polymers.

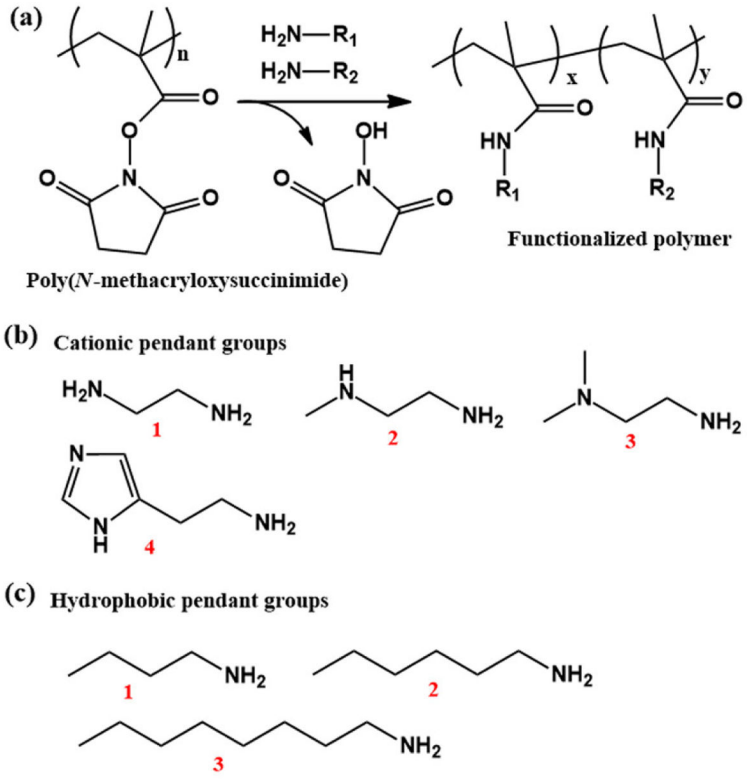

Fig. 3.

(a) Synthesis of mono- and bi-functionalized polymers by the post-modification of poly(*N*-methacryloxysuccimide). (b) Cationic pendant groups. (c) Hydrophobic pendant groups.

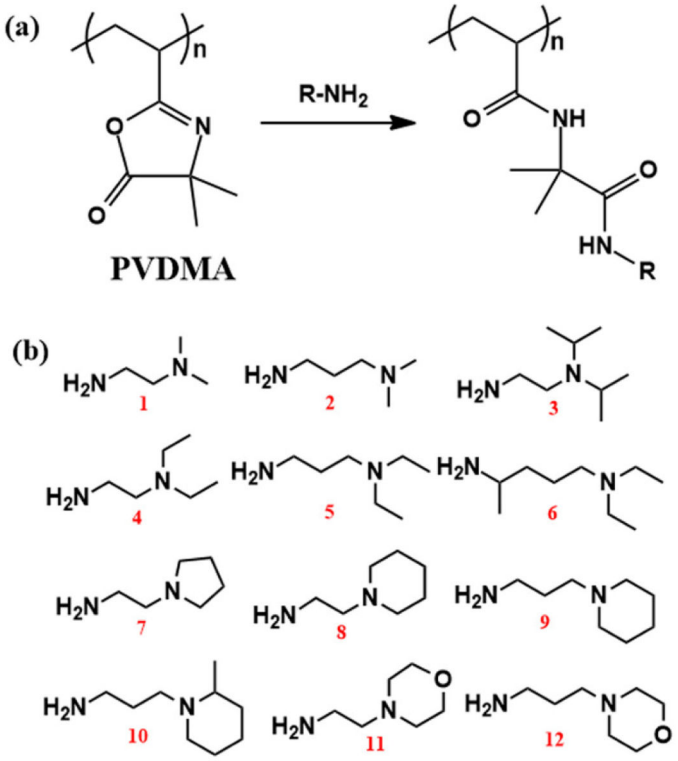

Fig. 4.

(a) Reaction of amines and poly(2-vinyl-4,4-dimethyl azlactone) (PVDMA). (b) Chemical structures of compounds 1–12 possessing both primary and tertiary amine functionalities.

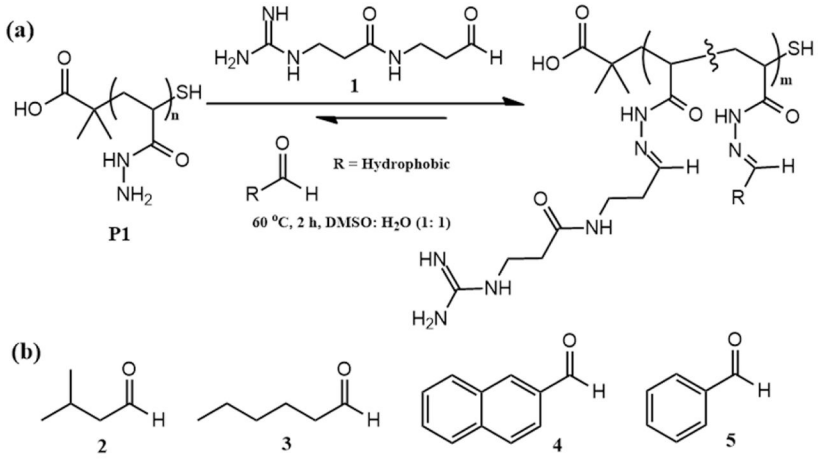

Fig. 5.

(a) Post-modification of poly(acryloyl hydrazide) with a cationic aldehyde and hydrophobic aldehydes. (b) Representative hydrophobic aldehydes for post-modification of reactive poly(acryloyl hydrazide).

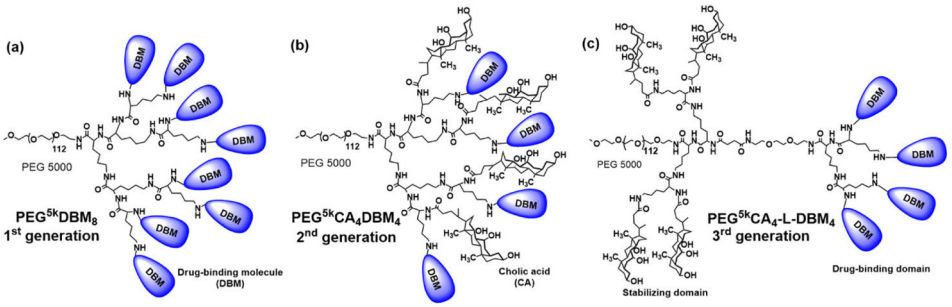

Fig. 6.

Chemical structures of telodendrimers of (a) first generation PEG^{5k} DBM_8 (G1), (b) second generation PEG^{5k} CA_4 DBM_4 (G2), and (c) third generation PEG^{5k} CA_4-L-DBM_4(G3).

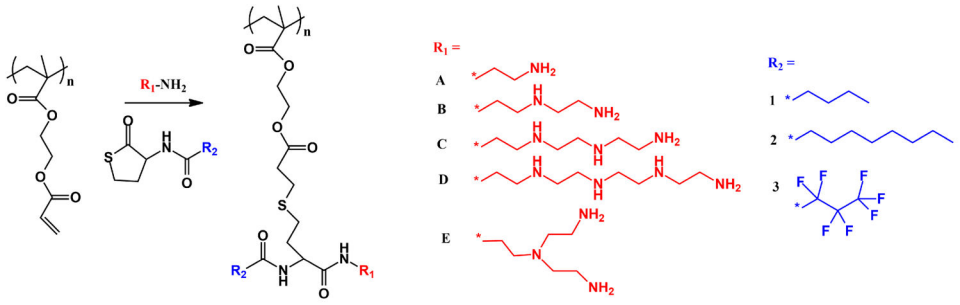

Fig. 7.

One-pot route for thiolactone chemistry-based combinatorial synthesis of multifunctional polymers for efficient gene delivery.

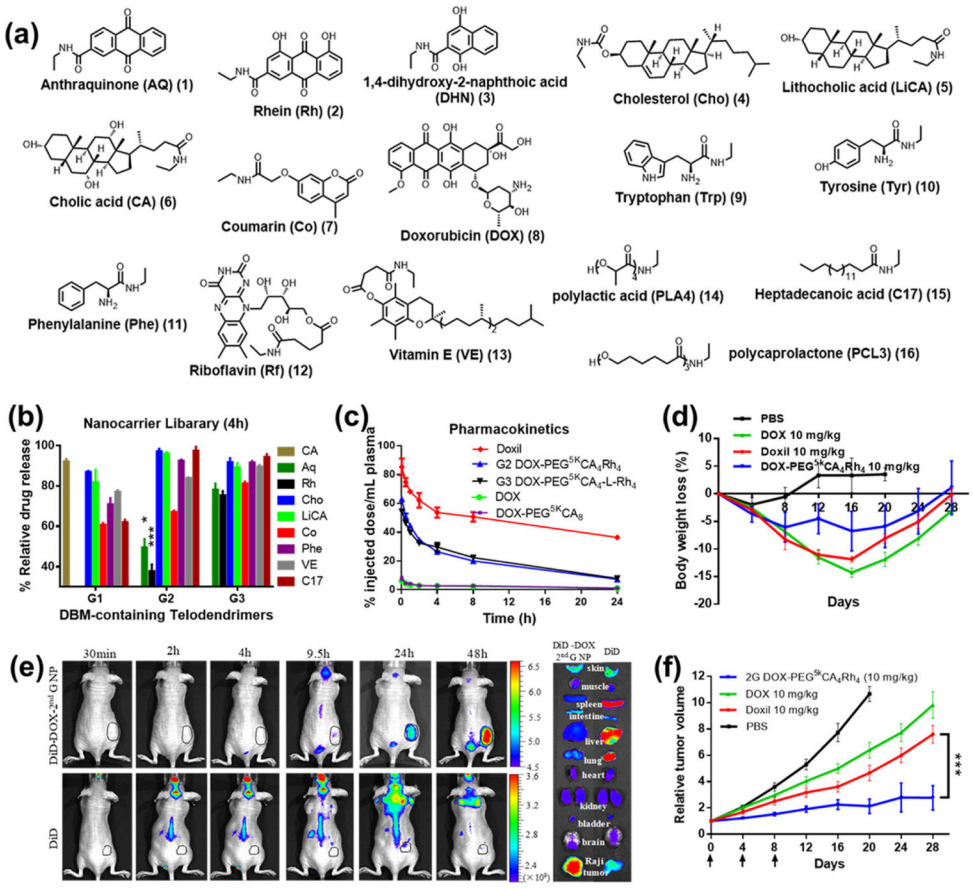

Fig. 8.

(a) Representative drug-binding molecules (DBMs) for doxorubicin (DOX). (b) Percentage of doxorubicin release from telodendrimer micelle library relative to the release rate of free doxorubicin and PEG^{5k} CA_8 at 4 h. (c) Pharmacokinetics profiles of doxorubicin concentration in plasma. (d) Body weight change of Raji lymphoma-bearing nude mice treated with different doxorubicin formulations. (e) *In vivo* and *ex vivo* near-infrared fluorescence optical images of Raji lymphoma-bearing nude mice injected intravenously with free DiD dye and DiD-doxorubicin-coloaded telodendrimer micelles. (f) *In vivo* tumor growth inhibition of Raji lymphoma-bearing nude mice treated with different doxorubicin formulations. Data were presented as mean±s.e.m. Student's t-test: $*P<0.05$; $**P<0.01$; $***P<0.001$. Reproduced with permission from ref. [17]. Copyright 2015 Nature Publishing Group.

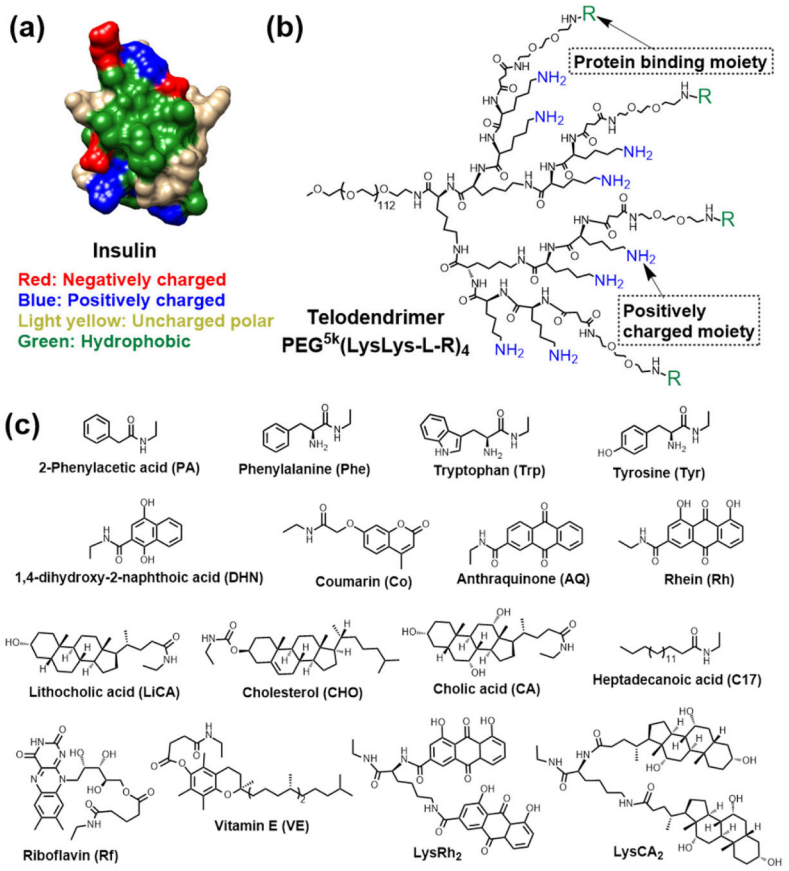

Fig. 9.

(a) Surface structure of human insulin. (b) Chemical structure of the telodendrimer with both charged and hydrophobic groups. (c) Representative protein binding molecules. Reproduced with permission from ref. [31]. Copyright 2017 American Chemical Society.

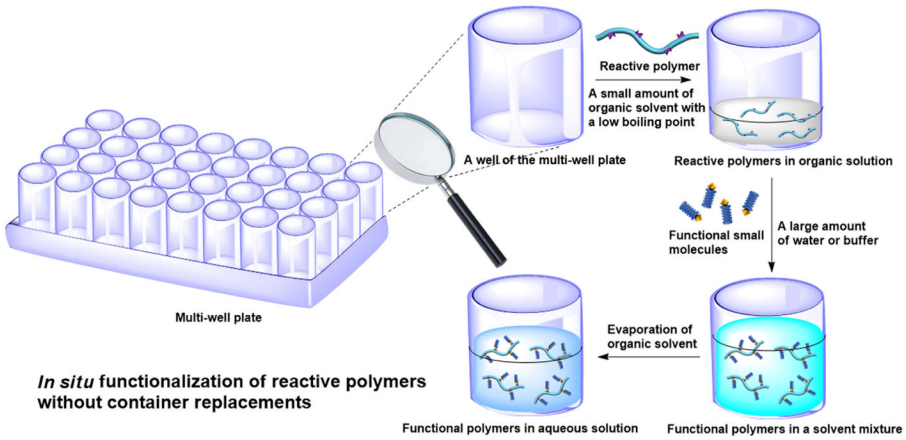

Fig. 10.

Schematic illustration of *in situ* functionalization of reactive polymers towards high-throughput polymer synthesis.

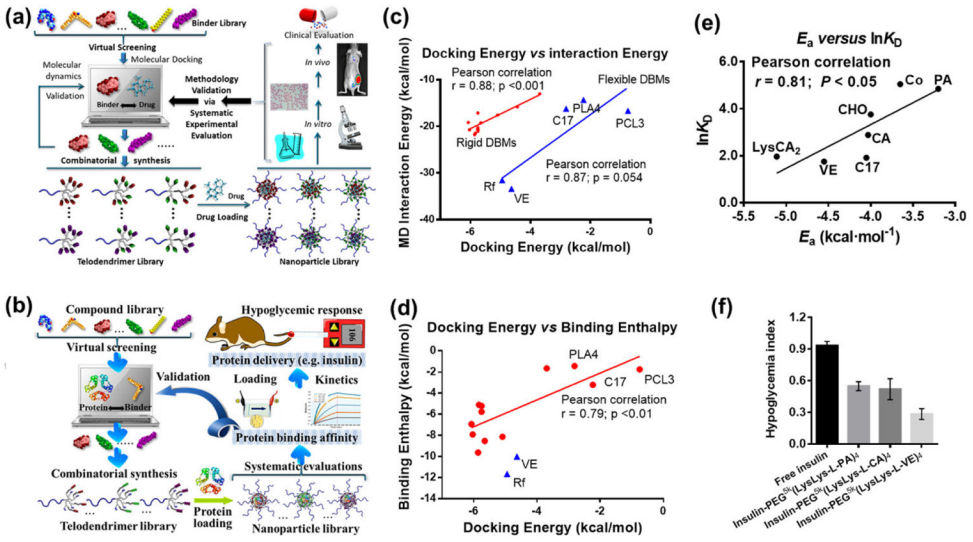

Fig. 11.

(a, b) Schematic illustrations of the rational design and combinatorial synthesis of telodendrimers for systematic evaluation and optimization for (a) drug delivery and (b) protein delivery. (c) Unbound interaction energy computed from molecular dynamics simulations plotted against the minimum docking energies. (d) The binding enthalpies analyzed by a solvent-balance method were plotted against the lowest docking energies. (e) Natural logarithm of equilibrium dissociation constant ($\ln K_D$) plotted against average docking energy (E_a). Linear regression was fit via Ordinary Least Square to calculate the Pearson correlation coefficient r and the associated P values for c, d and e. (f) Quantification of hypoglycemia index. Reproduced with permissions from ref. [17] and [31]. Copyrights to Nature Publishing Group and the American Chemical Society.

Table 1

Overview of the reactive polymers that have been used as templates for the post-modification-based optimization of therapeutic delivery polymers.

Category of reactive polymers	Representative reactive polymers	Compounds for post-modification	Conditions and results of post-modification			Polymer library size	Properties of functional polymers synthesized by post-modification			Therapeutic payloads	Ref.
			Need of catalyst or co-reagent	Reaction byproduct yielded	Need of additional purification steps		Well-defined chemical structure	Cost of polymer and synthesis	Biocompatibility/ biodegradability		
N-Hydroxysuccinimide-functionalized polyacrylates	Poly(*N*-methacryloxysuccinimide)	Primary or secondary amines	No	Yes	Yes	168	Fair	Low	Fair	DNA	[29]
Azlactone-functionalized polymers	Poly(2-vinyl-4,4-dimethylazlactone)	Primary amines	No	No	Yes[†]	12	Fair	Low	Unknow	DNA	[27]
Hydrazide-functionalized macromolecules	Poly(acryloyl hydrazide)	Aldehydes	No	No	No	10	Fair	Low	Unknow	siRNA	[28]
	Hydrazone-modulated peptide	Aldehydes	No	No	No	28	Yes	High	Good	DNA	[43]
Natural polysaccharides and derivatives	Hyaluronic acid	Amines	Yes	No	Yes	14	No	Low	Good	siRNA	[35]
	O-pentynyl dextran	Azides	Yes	No	Yes	8	No	Low	Good	Anticancer drugs	[36]
Oligo(amino acid)s-based platforms	Hydrazone-modulated peptide	Aldehydes	No	No	No	28	Yes	High	Good	DNA	[43]
	Telodendrimer	Carboxylic acids	Yes	No	Yes	24, 7	Yes	High	Good	Doxorubicin, insulin	[17], [31]

[†]In ref. [27], the additional purification step was performed to remove the excess, unreacted small-molecule reactants. However, purification steps will become unnecessary if equivalent units of small-molecule reactants are used for post-modification, which will enable high-throughput polymer synthesis for therapeutic delivery.

Table 2

Overview of the model therapeutic payloads used for the optimization of therapeutic delivery polymers synthesized by the post-modification approach.

Category of therapeutic payloads	Representative therapeutic payloads	Reactive polymers for post-modification	Compounds for post-modification	Delivery efficiency evaluation method	Ref.
Gene	DNA	Poly(2-vinyl-4,4-dimethylazlactone), poly(*N*-methacryloxysuccinimide), poly(2-(acryloyloxy)ethyl methacrylate)	Cationic and/or hydrophobic compounds	Cell-based high-throughput screening	[27], [29], [37]
Anticancer drug	Doxorubicin	Telodendrimer, *O*-pentynyl dextran	Hydrophobic compounds	Drug release profile, treatment efficacy	[17], [36]
Protein	Insulin	Telodendrimer	Charged and hydrophobic compounds	Binding affinity, treatment efficacy	[31]