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Application of Controlled Radical Polymerization for Nucleic Acid Delivery

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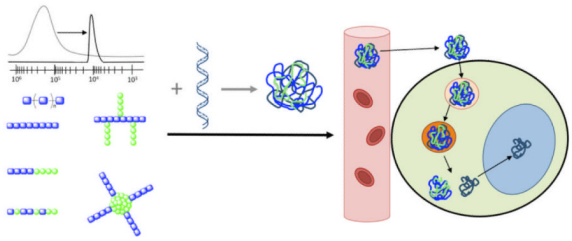
CONSPECTUS



Nucleic acid-based therapeutics can potentially address otherwise untreatable genetic disorders and have significant potential for a wide range of diseases. Therapeutic gene delivery can restore protein function by replacing defunct genes to restore cellular health while RNA interference (RNAi) can mask mutated and harmful genes.

Cationic polymers have been extensively studied for nucleic acid delivery applications due to their self-assembly with nucleic acids into virus-sized nanoparticles and high transfection efficiency *in vitro*, but toxicity and particle stability have limited their clinical applications. The advent of controlled radical polymerization has improved the quality, control and reproducibility of synthesized materials. Controlled radical polymerization yields well-defined, narrowly disperse materials of designable architectures and molecular weight, allowing study of the effects of polymer architecture and molecular weight on transfection efficiency and cytotoxicity for improved design of next-generation vectors. Robust methods such as atom transfer radical polymerization (ATRP), reverse addition-fragmentation chain transfer polymerization (RAFT), and ring-opening metastasis polymerization (ROMP) have been used to engineer materials that specifically enhance extracellular stability, cellular specificity, and decrease toxicity. This Account reviews findings from structure-function studies that have elucidated key design motifs necessary for the development of effective nucleic acid vectors. In addition, polymers that are biodegradable, form supramolecular structures, target specific cells, or facilitate endosomal release are also discussed. Finally, promising materials with *in vivo* applications ranging from pulmonary gene delivery to DNA vaccines are described.

I. Introduction

Nucleic acid-based therapeutics have been investigated as treatments for hereditary, acquired, and infectious disease. Nucleic acid-based drug candidates include plasmids that induce gene expression and small, interfering RNA (siRNA) which silences target genes.

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Viral-based delivery methods utilize engineered viruses that provide high efficiency nucleic acid delivery. However, concerns regarding vector immunogenicity, oncogenicity, and nucleic acid loading capacity limit clinical potential. Synthetic materials offer advantages such as high loading capacity, ease of synthesis and formulation, and low immunogenicity but suffer from poor delivery efficiency and significant toxicity; as such, there are no clinically-approved materials to date. Ideal synthetic vectors emulate viruses, packaging nucleic acids for cellular uptake and protection from nucleases, targeting cells of interest, escaping the endo-/lysosomal degradative pathway, and releasing the payload in the cytosol or nucleus.

Living radical polymerization techniques provide flexible methods for synthesizing well-defined polymers with narrow polydispersity, controllable molecular weight (MW), complex architecture, and multifunctional properties. Tight control over polymer structure allows study of structure-function relationships and identification of key variables.

II. Controlled Radical Polymerization

Recent advances in state-of-the-art controlled radical polymerization methodology have the potential to revolutionize the development of “smart” multifunctional drug delivery systems. The most widely investigated CRP techniques include stable free radical polymerization (SFRP), atom transfer radical polymerization (ATRP), and reversible addition-fragmentation chain transfer (RAFT) (Figure 1). SFRP, best exemplified by nitroxide-mediated systems (NMP), is based on the persistent radical effect which employs a stable free radical to reversibly terminate the majority of active propagating chains in a dormant non-propagating species.[1,2] While SFRP has been shown to provide good control for a range of styrenic monomers, poor control is often observed for other monomer classes. In addition, long polymerization times and high temperatures are often required in order to reach high monomer conversions.

ATRP is generally considered to be more widely applicable than SFRP in that most monomers containing an active double bond are amenable to controlled polymerization. Similar to SFRP, this technique employs a reversible deactivation step in order to reduce the instantaneous concentration of active propagating radicals.[3] In the case of ATRP, this equilibrium is established through the reversible homolytic cleavage of a terminal alkyl halide initiator catalyzed by a transition metal catalyst. ATRP provides a powerful method for preparing complex polymer architectures (e.g. blocks, brushes, stars) from commercially available starting materials. Additionally, polymerizations can be conducted over a wide range of temperatures and conditions to yield telechelic materials suitable for secondary conjugation steps. Perhaps the most significant limitation of ATRP at present is the need for a metal catalyst, which for many biotechnology applications must be reduced to very low levels.

In contrast to SFRP and ATRP that impart living character to radical polymerizations by establishing a low equilibrium concentration of active propagating radicals, RAFT is based on a rapid degenerate chain transfer process.[4-7] The versatility of RAFT lies in the elegant simplicity of the technique, broad chemical compatibility, and ease of use. RAFT employs a thiocarbonyl thio compound as a degenerate chain transfer agent (CTA). Through a series of chain transfer steps, polymerizations proceed in a controlled process with most polymer chains containing well-defined end groups at their alpha and omega chain termini. By simple manipulation of the initial monomer, CTA, and initiator stoichiometry, it is possible to prepare near-monodisperse materials over a range of predefined MWs. Following polymerization of a given monomer(s), the resultant macroCTA can be isolated for use in subsequent block (co)polymerization steps. Because this methodology does not require the

use of any toxic metal catalysts, it is particularly well-suited for use in biotechnology applications. The RAFT process also provides a means by which polymers with discrete orthogonal chemical functionalities can be easily prepared by employing functionalized RAFT chain transfer agents.

III. Polymer structure and delivery

In this section, we summarize work investigating the relationship between polymer structure and the effectiveness of materials as nucleic acid carriers. While many of these topics have been addressed using materials synthesized by conventional polymerization (e.g. Wolfert et al),[8] this review focuses on conclusions drawn from materials prepared by living polymerization.

a. Polymer composition

The chemical composition of polycations has been reported extensively to be a major factor in gene delivery efficiency and cytotoxicity.[9,10] Some key factors include the type of cationic charge center, charge density, presence of hydrogen bonding partners for nucleic acids, and balance between hydrophobicity/hydrophilicity.

Some commonly used monomers in living polymerization of polycations include APMA (primary amine) and DMAEMA (tertiary amine). Table 1 lists full names and structures of monomers discussed in this review. Monomers containing primary amines can be easily guanidinylated to introduce an alternative charge center.[11] DMAEMA is one of the most frequently used monomers; polymers of DMAEMA (pDMAEMA) have average pKa ~ 7.5 and are partially protonated at physiologic pH, allowing for self-assembly with nucleic acids through electrostatic complexation and buffering of acidifying endosomes, thereby delaying or even preventing lysosome degradation of vehicles. One study compared polycations with varying ratios of primary (AEMA) and tertiary (DMAEMA) amine-containing monomers that were synthesized by RAFT and showed similar transfection efficiencies for all polycations.[12] Another study compared the gene delivery efficiencies of polycations composed of tertiary and quaternary amines by using DMAEMA monomers followed by alkylation to quaternary amines.[13,14] Conversion of tertiary amines to quaternary amines resulted in stronger nucleic acid binding, higher zeta potential of polyplexes, higher toxicity, and lower transfection efficiency. The decreased transfection efficiency was attributed to reduced buffering capacity of the resulting polymers.

The effect of spacing between charge group and polymer backbone has been investigated by comparing polymers synthesized using AEMA, APMA and AHMA monomers that contain 2-, 3-, and 6-carbon spacers, respectively.[12,15] As spacer group lengths increase, polymers become both more effective at gene delivery and also more cytotoxic, likely due to increased accessibility of the charge group. Zhuo and colleagues synthesized pAEMA and pAEAEMA polymer analogues that introduced hydroxyl groups and showed that polymers containing hydroxyl groups exhibited stronger nucleic acid binding and reduced cytotoxicity.[16] The observed effects were attributed to hydrogen bonding to nucleic acids and decreased surface charge, respectively. Hydrogen bonding between hydroxyl groups and nucleotide base amines has been shown to be a dominant thermodynamic contributor to DNA binding.[17] Binding free energy contributions from hydrogen bonding are unaffected by salt concentration while electrostatic contributions significantly decrease with increasing salt.

Finally, incorporation of hydrophilic, charge-neutral monomers such as carbohydrates, HEMA or OEGMA in polycations has been shown to reduce polymer cytotoxicity.[15,18] In some cases, increases in transfection efficiency can also be achieved because higher charge ratios can be used without compromising cell viability.[18]

b. Polymer molecular weight

The effect of MW on gene delivery has been tested using several DMAEMA-based polycations synthesized by ATRP, including linear block copolymers,[18] grafted DMAEMA block copolymers,[14,19,20] and star-shaped copolymers.[21] The effective polycation MW can be varied by changing the DMAEMA block length[14,18,19,21] or by increasing the number of grafted DMAEMA blocks of a specific size.[20] In all studies, DNA condensation and optimized gene transfection efficiency increased with the DMAEMA block length until toxicity became a limiting factor. Cytotoxicity of polymers also generally increased with polymer MW.[14,18,19] These trends were observed with DMAEMA blocks copolymerized with poly(caprolactone) (PCL),[19] pHEMA,[20] or hydroxypropyl cellulose (HPC),[14] indicating that the cationic DMAEMA segment drives both the delivery efficiency and cytotoxicity of the materials.

The trends of higher transfection efficiency and cytotoxicity with increasing MW have been reported for other polycations synthesized by living polymerization, including pAEMA and pAPMA polymers.[15,22] The Pun and Emrick groups have also shown similar trends in polymers synthesized from oligolysine macromonomers.[23,24] Poly(cyclooctene-*g*-pentalysine) synthesized by ring-opening metathesis polymerization (ROMP) are more effective at gene delivery with increasing MW. The highest MW copolymer tested (202 kDa) gave the best overall gene expression with efficiencies comparable to commercial reagents such as jetPEI, SuperFect and Lipofectamine 2000 while exhibiting low cytotoxicity.[23] A panel of HPMA-oligolysine copolymers with varying MW synthesized by RAFT polymerization demonstrated transfection efficiency comparable to PEI at high MW (78kDa) but also trends of increasing cytotoxicity with higher MW.[24]

In summary, transfection efficiency and cytotoxicity tends to increase with polymer MW; polymer length therefore generally needs to be optimized for these two factors. Synthesis of materials by living polymerization can reproducibly produce well-defined materials with desired MW. Shi et al compared HPMA-oligolysine polymers synthesized by free radical and RAFT polymerization and found similar transfection efficiency at the target mass to charge ratios; however, the IC_{50} values of the free radical polymers was nearly 10-fold lower than that of the RAFT polymers.[25] Jonsson and Linse showed by Monte Carlo simulations that high MW polyelectrolytes can form increased interaction with macroions; thus, polyplexes from higher MW polycations may prevent premature release of DNA by providing additional stabilization.[26] However, another observed trend is the associated increase in cytotoxicity as polymer MW is increased. Polyplexes formed with lower MW polymers are more easily reversible, while complexes from high MW polymers are less dynamic, which can then lead to crosslinking of DNA and exposure of excess positive charge.[8] Microinjected polyplexes formed with high MW polymers showed poor transfection efficiency attributed to highly stable polyplexes that may inhibit transcription. Scales et al showed that as the length of DMAPMA in DMAPMA-HPMA block copolymers increase, there is a decreased amount of soluble complex due to cross-bridging of complexes, leading to aggregation.[27] Interestingly, as the HPMA block increased in length, shorter DMAPMA blocks were required for efficient complexation into soluble particles. Furthermore, Kissel and co-workers showed that high MW polymers adsorbed highly to the cell surface due to high binding affinity towards the plasma membrane, leading to aggregate formation, membrane disruption, and eventually cell death by necrosis.[28]

c. Polymer architecture and transfection efficiency

Polymers with controlled architectures including homopolymers, block copolymers, random or statistical copolymers, graft copolymers and star-shaped polymers can also be synthesized by living polymerization (Figure 2). The reader is referred to a recently published and

thorough review that focuses on the preparation of various polymeric architectures using living radical polymerization.[29] This review will emphasize effects of polymer architecture on nucleic acid delivery.

The effect of block versus random copolymer architectures has been recently investigated by the Narain group (Figure 3).[15,30] Primary amine-containing monomers were used to copolymerize with either carbohydrate monomer GAPMA or zwitterionic phospholipid MPC. With both systems, random copolymers were less cytotoxic than their block copolymer analogues, likely due to reduced charge density. Random copolymers containing GAPMA were more effective for gene delivery compared to corresponding block copolymers but the opposite was observed with MPC monomers. The block glycopolymers were hypothesized to form core-shell structures with buried charge, as opposed to polyplexes formed from random copolymers with more accessible surface charge. The accessible charge facilitates cellular uptake of polyplexes.[15] Certain zwitterionic polymers have been previously reported to exhibit high resistance to protein adsorption due to formation of hydration shells.[31] Polyplexes formed from statistical MPC polymers may similarly resist protein interactions necessary for cellular uptake and transfection. Therefore, while random copolymers of hydrophilic monomers and cationic monomers may offer reduced toxicity compared to block copolymers, the chemical structure of the hydrophilic monomer also significantly affects *in vitro* nucleic acid delivery efficiency.

Graft or branched polymer structures generally have shown higher transfection efficiencies and lower toxicities than their linear counterparts. Reports of grafting pDMAEMA onto backbones of pHEMA,[20] HPC,[14] dextran,[13] and chitosan[32] via ATRP show these grafted structures transfected better and were less cytotoxic than high MW pDMAEMA. In addition, Xu et al showed that HPC-*g*-pDMAEMA copolymers were more effective even with lower cation incorporation compared to linear pDMAEMA.[14] One potential reason for this phenomenon could be that these grafted structures can enhance the electrostatic interaction of the cationic pDMAEMA side chains with nucleic acids or the cell membrane. Hyperbranched DMAEMA also showed much higher transfection than linear DMAEMA of a similar MW without increased cytotoxicity.[33] Polymers with graft oligolysines also exhibit higher transfection efficiency compared to linear poly-L-lysine (PLL).[23,34]

IV. Supramolecular Structures

Most polycations have been shown to condense nucleic acids into compact spherical or toroidal nanoparticles. Block copolymers that form micellar structures may provide advantages such as increased particle stability, decreased surface charge resulting in reduced cytotoxicity, and the ability to co-deliver additional therapeutics encapsulated in the micellar core.

Several groups investigated the synthesis of micellar systems for nucleic acid delivery including mPEG-*b*-(PCL-*g*-DMAEMA),[19] DMAEMA-*b*-(DMAEMA-*co*-PAA-*co*-BMA),[35] and DMAEMA-*b*-PCL-*b*-DMAEMA.[36] mPEG-*b*-(PCL-*g*-DMAEMA) micelles showed efficiency comparable to polyethylenimine (PEI) in HeLa cells and exceeded PEI and Lipofectamine 2000 in HepG2 cells. However, these materials demonstrated substantial DMAEMA-dependent toxicity.

DMAEMA-*b*-(DMAEMA-*co*-PAA-*co*-BMA) micelles efficiently delivered siRNA to cells with over 90% knockdown of target genes at optimized conditions.[32] High siRNA delivery efficacy is attributed to the pH-responsive, membrane-lytic second block as a means of efficient endosomal escape. The stability of the micelle is also pH dependent, with destabilization occurring with decreasing pH. It was shown that the micellar structure encourages siRNA binding, and that loss of micelle structure leads to weak siRNA binding.

Thus, the high efficiency can be attributed to simultaneous release of siRNA during micelle destabilization and activation of membrane-lytic activity in the endosomes.

Zhu et al (2010) demonstrated dual drug delivery by encapsulating a small molecule drug (paclitaxel) with anti-neoplastic activity in the core of micellar nucleic acid delivery vehicles composed of DMAEMA-*b*-PCL-*b*-DMAEMA triblocks.[36] When paclitaxel was co-delivered with VEGF siRNA, VEGF knockdown was close to 85% and was higher than siRNA or paclitaxel alone.

V. Polymer Degradability

Polymer degradability is an important feature for *in vivo* applications. Three main methods of introducing degradability into polymers synthesized via controlled radical polymerization are: (1) disulfide linkages, which can be degraded by glutathione in the reducing environment of the cytosol; (2) acid-labile linkages, such as esters, which can be hydrolyzed in the endo-/lysosomal compartments; and (3) enzymatically-degradable elements, which can be degraded by specific proteolytic enzymes.

Disulfide bonds are the most commonly employed degradable linkage in controlled polymerization reactions due to the ease of introducing thiols or disulfide bonds into the initiator or chain transfer agent. For example, reducible pDMAEMA polymers were synthesized by oxidation of thiol-terminated DMAEMA oligomers prepared by RAFT polymerization using a difunctional CTA. In another approach, reducible linkages were introduced in HPMA-oligolysine copolymers via an amino acid analogue containing an internal disulfide.[25] Polyplexes of the reducible copolymers were much less toxic than their non-reducible analogue, but suffered decreased transfection efficiency.

Other strategies for introducing degradability into the polymer structure include the use of acid-labile linkages and enzymatically-degradable segments. Lin et al. synthesized via ATRP block copolymers of poly(ethylene glycol) (PEG) and pDMAEMA connected through a cyclic orthoester linkage.[37] Transfection with acid-labile PEG-*a*-pDMAEMA polyplexes was better than with its acid-stable counterpart, PEG-*b*-pDMAEMA, at high amine to phosphate (N/P) ratios. Enzymatically-degradable polymers, such as chitosan, can also be attractive alternative for more specific and controlled degradation. Chitosan, which can be degraded by lysozyme, was used as a backbone and copolymerized with side chains of pDMAEMA via ATRP for endosomal buffering.[32] Additionally, the use of enzymatically-cleavable linker sequences has shown the possibility of specific enzymatic degradation within polyplex formulations. Recently, copolymers of HPMA and oligolysine monomers linked by an amino acid sequence that is recognized by the endo-/lysosomal protease cathepsin B were reported.[38] Transfection of these copolymers in HeLa and NIH/3T3 cells were comparable to non-degradable copolymers synthesized with (D)-amino acids but with reduced cytotoxicity.

In general, polymers synthesized with these degradable features show decreased cytotoxicity *in vitro*, but transfection efficiency is only on par or even reduced compared to non-degradable analogues. Therefore, optimization of these degradable segments requires further study.

VI. Polymer Functionalities

There are several barriers to the successful delivery of nucleic acids (Figure 4). Non-viral vectors must overcome the following obstacles: (1) stability in serum for intravenous injection, (2) specificity for the cell- or tissue-of-interest, (3) escape from endo-/lysosomal degradation, and (4) successful release of active payload. Thus, multiple strategies have

been explored for building in polymer functionalities to improve transfection efficiency, specifically addressing several of these barriers.

a. Serum stability

Colloids instability and resulting aggregation should be avoided in applications requiring systemic delivery of polyplexes. Salt and serum-induced polyplex aggregation can lead to opsonization of particles or pulmonary embolism.

The major strategy to improve polyplex stability is the incorporation of hydrophilic polymers (such as HPMA, PEG or zwitterionic polymers) that serve as steric stabilizers,[39] minimizing protein adsorption to particles and particle aggregation.[23,30,40-42] In general, these copolymers, regardless of specific architecture and form of hydrophilic modification, show significantly decreased cytotoxicity and enhanced colloidal stability relative to their respective homopolymers. For example, MPC-AEMA and MPC-APMA copolymers showed low protein adsorption likely due to the zwitterionic MPC component.[30] However, *in vitro* transfection efficiency relative to their respective homopolymers is also typically reduced.[40,41,43]

While the hydrophilic shielding limits *in vitro* efficacy, *in vivo* it has proven valuable. One aerosolized formulation of OEGMA-*co*-DMAEMA demonstrated 7-fold greater bulk pulmonary transfection compared to PEI.[41] pDMAEMA-*b*-PEG used for intranasal delivery of DNA vaccines showed immune response comparable to and higher than PEI and DMAEMA homopolymer, respectively.[40]

b. Targeting

Effective cell targeting of polyplexes increases specific delivery to target tissues thereby reducing off-target effects. Polyplexes, typically with a positive surface charge, electrostatically associate with electronegative cellular membranes, leading to non-specific uptake.[44] Modifying polymers with ligands while including domains for charge shielding targets polyplexes to receptors expressed on the cell or tissue of interest.

Folate is a small molecule ligand of the folate receptor that is commonly overexpressed in cancer and has been shown to be an effective targeting ligand for drug carriers.[45] Several groups have incorporated folate into polymers synthesized by living polymerization.[46-49] Folate-targeted polyplexes showed enhanced siRNA delivery in cells with upregulated folate receptor expression compared to control cells, with specific targeting demonstrated by decreased silencing when treated competitively with free folate.

Biotin-DMAEMA-*b*-(DMAEMA-*co*-PAA-*co*-BMA) micelles, functionalized with streptavidin-Anti-CD22 monoclonal antibody conjugates, demonstrated highly specific targeting against CD22+ lymphoma.[50] These micelles were used to deliver siRNA and specifically reduced target gene expression by 70% (Figure 5).

c. Endosomal release

Another major barrier to efficient transfection is entrapment of polyplexes in endo-/lysosomal organelles. Some approaches to increasing endosomal release are the use of protonatable amines to buffer the acidifying endosome, often referred to as the “proton sponge effect,”[14,32,51] and the incorporation of hydrophobic groups to destabilize endosomal membranes.[52,53]

Endosomal buffering is the result of the accumulation of protons brought into the endosome via an ATPase.[54] Tertiary amine-containing polymers, such as PEI, are increasingly

protonated in acidifying endosomes, causing osmotic swelling of the endosome and ultimate release of endosomal contents. For example, Ma et al synthesized PEG-*b*-PGMA diblock copolymers via ATRP and grafted small oligoamines, such as low MW PEI, to encourage endosomal buffering of these vectors.[51] Although the transfection of these polymers was less efficient than PEI alone, high buffering capacity and low binding ability was correlated to high transfection ability. As discussed in section IIIa, DMAEMA monomers contain a tertiary amine that also buffers in endosomal pH. This review has highlighted select examples of DMAEMA-containing polymers synthesized for nucleic acid delivery.[12-14,18,32,55] Because polymerized DMAEMA also induces cytotoxicity, DMAEMA content in polycations needs to be optimized to balance between transfection efficiency and cytotoxicity.

Another promising method for endosomal release is the use of hydrophobic groups that become membrane-lytic via a pH-sensitive trigger. Convertine et al synthesized copolymers of DMAEMA, PAA, and hydrophobic BMA via RAFT that undergo a pH-induced conformational change.[52] At pH 7, positively-charged DMAEMA and negatively-charged PAA mask the hydrophobicity of BMA; at lower pH, the polymer undergoes a conformational change, exposing the BMA hydrophobic residues and thus rendering the polymer hydrophobic and hemolytic. These polymers showed significant activity of delivered siRNA.

VII. In vivo results

Despite extensive development of polymeric nucleic acid delivery systems over the past two decades, clinical translation of these materials is in its nascency. Noted drawbacks of tested polymers include poor *in vivo* stability, high cytotoxicity, and low delivery efficiency. The development of living radical polymerization techniques for gene delivery allows for controlled architectures and rationally-designable materials. This section highlights some examples of promising and recent *in vivo* results using such materials.

Ternary complexes of PCL-*g*-pDMAEMA polyplexes coated with folate-targeted poly(glutamic acid)-*g*-mPEG yielded enhanced transgene delivery to tumor xenograft models compared to untargeted ternary complexes.[49] Administration of binary complexes of PCL-*g*-pDMAEMA polyplexes without the mPEG coating resulted in animal death within several hours of administration likely due to erythrocyte toxicity, demonstrating the importance of hydrophilic shielding with targeting. In another report, OEGMA-*co*-DMAEMA polyplexes were aerosolized and administered directly to the pulmonary tract. The optimal copolymer formulation showed a 7-fold bulk increase in luciferase transgene expression compared to PEI polyplexes (Figure 6).[41] A similar material, DMAEMA-*b*-PEG, was used for DNA delivery for vaccination against HIV-1.[42] When administered intranasally to mice, DMAEMA and DMAEMA-*b*-PEG copolymers showed stimulation of TNF-α and IL-10 production, enhancing the immunogenicity of the DNA vaccine, while PEI did not. Since DMAEMA-*b*-PEG *in vitro* was shown to be significantly less toxic than homoDMAEMA, this makes for an attractive DNA vaccine vector.

VIII. Conclusions/Future Perspectives

Application of controlled radical polymerization techniques has allowed for detailed structure-function analysis and optimization of existing vectors. More importantly, these approaches allow for reproducible and controlled synthesis of desired materials. With increasing understanding of effective motifs, controlled radical polymerization provides a robust means to design and implement future materials meeting the properties of low

cytotoxicity, high stability, and high transfection efficiency required for nucleic acid delivery applications.

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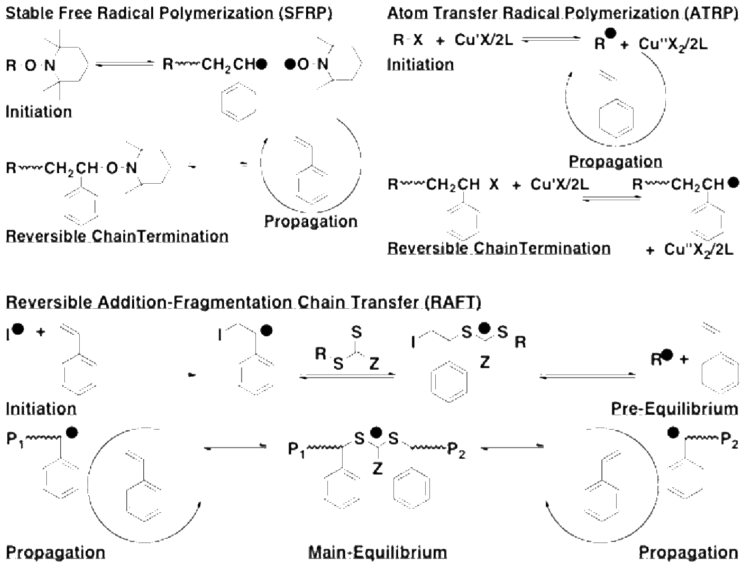


Figure 1.

Controlled radical polymerization of styrene by (a) stable free radical polymerization (SFRP) in the presence of the nitroxide mediator TEMPO (b) copper-catalyzed atom transfer radical polymerization (ATRP) and (c) reversible addition-fragmentation chain transfer (RAFT) in the presence of a dithiobenzoate-based chain transfer agent (CTA).

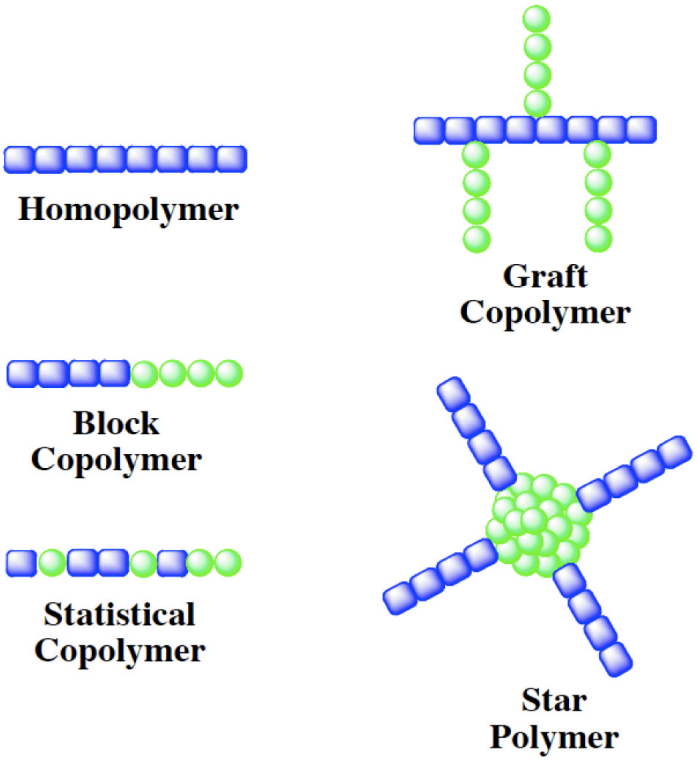


Figure 2.
Various polymer architectures.

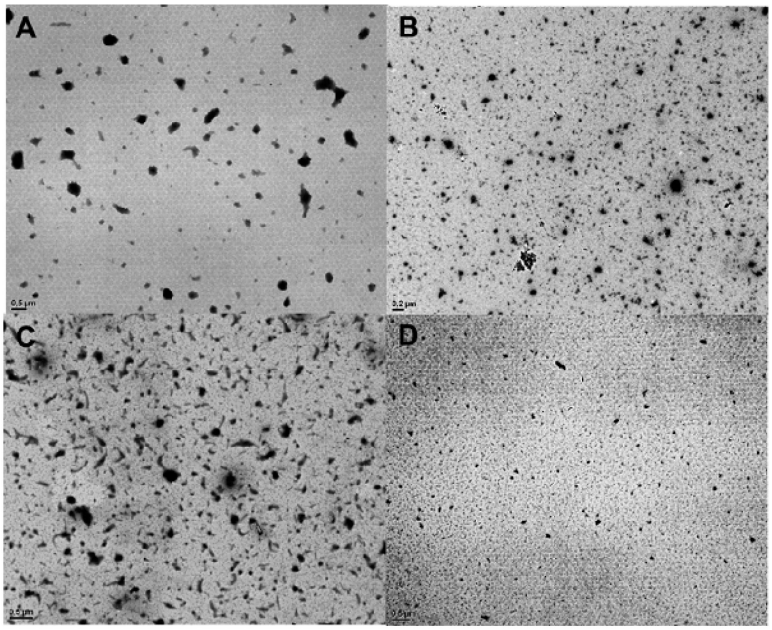


Figure 3.
TEM images of polyplexes comparing polyplexes formulated with statistical (A, C) and block (B, D) phospholipid-containing polycations.[30]

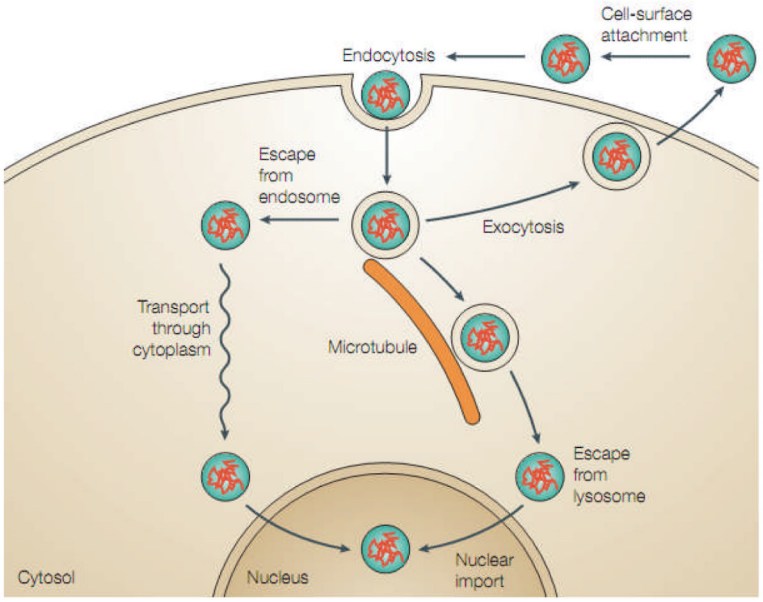


Figure 4.
Barriers to gene delivery.[56]

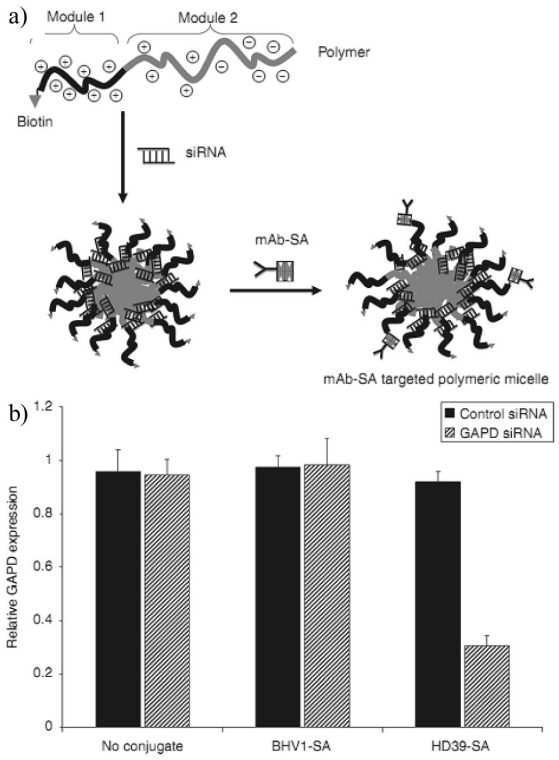


Figure 5.
(a) Structure and (b) GAPD knockdown efficacy of antibody-conjugated polymeric micelles in CD22+ lymphoma cells measured using real time RT-PCR.[50]

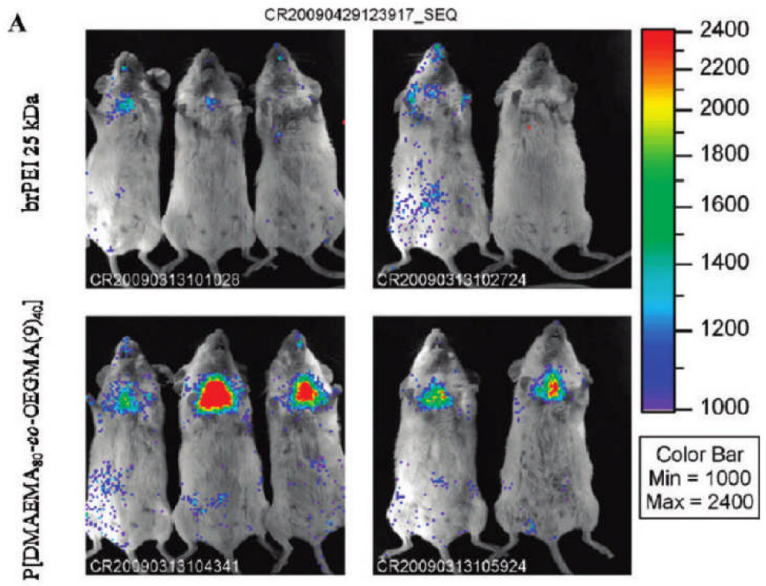
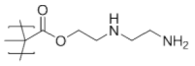
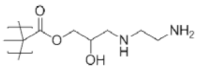
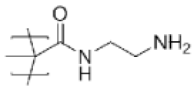
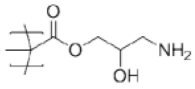
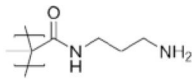
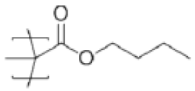
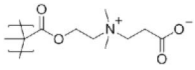
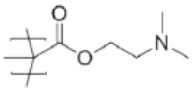
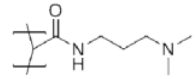
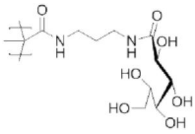
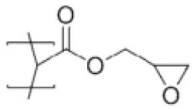
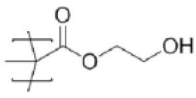


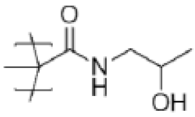
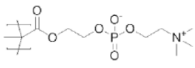
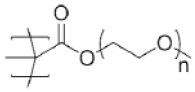
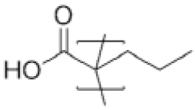
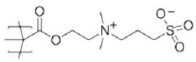
Figure 6.

In vivo bioluminescence images of Balb/c mice after intratracheal application of OEGMA-*co*-DMAEMA block copolymers delivering luciferase transgene.[41]

Table 1

Some common monomers used in living polymerizations

Abbreviation	Structure	Full Name	Poly. Type	References
AEAEMA		2-(2-aminoethylamino) ethyl methacrylate	ATRP	16
AEAHPMA		3-(2-aminoethylamino) 2-hydroxylpropyl methacrylate	ATRP	16
AEMA		2-aminoethyl methacrylamide	ATRP RAFT	12,15,16,30
AHPMA		3-amino-2-hydroxypropyl methacrylate	ATRP	16
APMA		3-aminopropyl methacrylamide	RAFT	15,30,46,47
BMA		butyl methacrylate	RAFT	35,48,50,52
CBMA		carboxybetaine methacrylate	ATRP	31
DMAEMA		2-(dimethylamino) ethyl methacrylate	ATRP RAFT	12-14,18-21,32,33,35,37,40-43,48-50,52,53,55
DMAPMA		*N*-[3-(dimethylamino)propyl] methacrylamide	RAFT	27,47
GAPMA		3-gluconamidopropyl methacrylamide	RAFT	15
GMA		glycidyl methacrylate	ATRP	51
HEMA		2-hydroxyethyl methacrylate	ATRP	18,20,22

Abbreviation	Structure	Full Name	Poly. Type	References
HPMA		*N*-(2-hydroxypropyl) methacrylamide	RAFT	24,25,27,34,38,46,47
MPC		2- methacryloyloxyethyl phosphorylcholine	ATRP RAFT	30,43
OEGMA		oligo(ethylene glycol) methacrylate	ATRP	31,41
PAA		propylacrylic acid	RAFT	35,48,50,52
SBMA		sulfobetaine methacrylate	ATRP	31