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RAFT Synthesis and DNA Binding of Biodegradable, Hyperbranched Poly(2-(dimethylamino)ethyl Methacrylate

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Introduction. A number of human diseases have genetic origins, such as cancer and cystic fibrosis.[1−3] The insertion of a targeting gene into cells to replace or override defective genes, termed "gene therapy", is a promising and rapidly developing therapeutic approach. However, direct injection of naked DNA is unviable as enzymatic degradation and poor transfer efficiency negate effective therapeutic implementation. Viral and nonviral vectors have been developed to help the introduction of functional DNA.[4−8] Although viral vectors (viruses) are effective delivery agents, there are drawbacks such as cargo capacity, resistance to repeated infection, and safety concerns, all limiting application.[9−11] Synthetic materials such as cationic lipids, liposomes, chitosans, amino acid polymers, dendrimers, and inorganic nanoparticles have all been suggested as nonviral vectors,[12−15] to avoid viral vector limitations. Cationic polymers have attracted a lot of research interest as potential nonviral vectors as they have the potential to complex with negatively charged DNA or RNA generating neutral or positive polyplexes, with the ability to cross the negatively charged cell membrane.[16] As a result, a huge research area has emerged in an attempt to translate the potential of cationic polymers into dependable and safe vectors.

An important factor in nonviral gene delivery is the experimental design of polymer structures (MW, type and spacing of charged groups, and degree of polymer branching). The polymer design has a significant influence on the subsequent polyplex formation affecting gene transfer efficiency and polyplex toxicity; for example, when histidine/lysine copolymers (HK) were evaluated with a lipsome carrier, branched HK copolymers were much more effective than their linear counterparts in transformed cell lines, while the linear HK copolymer enhanced gene expression better in primary cell lines.[17] Thus, the synthesis of polymers with predesigned structures is critical to improve the application of synthetic polymers for gene delivery applications. Living radical polymerizations (LRPs) are versatile approaches to synthesize polymers with predetermined molecular weights and narrow dispersities. Reverse addition−fragmentation chain transfer (RAFT) polymerization can be applied to a wide range of functional monomers to generate well-defined polymeric structures.[18−21] Recently, RAFT has been utilized to prepared polymers for gene delivery.[22−26] Herein, we report the RAFT synthesis of a biodegradable hyperbranched cationic polymer, poly(2-(dimethylamino)ethyl methacrylate) (PDMAEMA). It is known that increasing the positive charge on a polymer improves cellular uptake and transfection efficiency, but with an accompanying detrimental toxicity effect originating from the destabilization and loss of integrity of cellular membranes. To overcome this drawback, it would seem appropriate to design biodegradable cationic polymers by cross-linking low molecular weight polymers (that have lower toxicity) generating high molecular weight polycationic carriers with biodegradable linkers. Thus, we hypothesize that biodegradable polymers might overcome the main two obstacles for using cationic polymers as nonviral vectors: polyplex unpacking and cytotoxicity.[27−29] PDMAEMA has been evaluated as a gene delivery agent and has shown promising transfection activity, but its high cytotoxicity and lack of biodegradability have hindered its potential as a DNA delivery agent.[30−32] Recently, biodegradable linear PMAEMA was tested as a gene delivery agent and exhibited significantly reduced cytotoxicity in a range of cell lines.[33] In this current paper, we report the RAFT synthesis of biodegradable hyperbranched PDMAEMA. Targeting high molecular weights (M_w∼290K) of the polymer should ensure effective DNA binding. The RAFT technique was chosen to control the polymerization process to obtain low molecular weight branch oligomers with similar chain lengths and low toxicity. A biodegradable disulfide-base dimethacrylate (DSDMA), 1,2-bis(2-(3-methylbuta-1,3-dien-2-yloxy)ethyl)disulfane, is employed as a cross-link agent to link branched oligomers into hyperbranched structures, with each branch point in the polymer linked by a disulfide bond—a well-known redox-sensitive linkage. Post-DNA delivery, the disulfide bond should be cleaved under cellular reducing conditions to yield small molecular weight oligomer chains, enhancing gene release and subsequent polymer excretion from the cells (Scheme 1). To our knowledge, this is the first description of a synthetic strategy for the creation of biodegradable hyperbranched PDMAEMA for potential use as a gene delivery agent.

Preparation of Biodegradable Hyperbranched PDMAEMA. Chain transfer agent (CTA), 2-((2-(methacryloyloxy)ethyl)disulfanyl)ethyl 4-cyano-4-(phenylcarbonothioylthio)pentanoate (**2**), was prepared by the reaction between 2-((2-hydroxyethyl) disulfanyl)ethyl 4-cyano-4-(phenylcarbonothioylthio)pentanoate (**1**) and methacryloyl chloride in the presence of triethylamine (TEA). When this CTA was directly employed to synthesize polymers from DMAEMA, hyperbranched polymers were formed, mixed with many linear polymer chains as shown in the GPC trace (Figure 1a). It is known that adding a cross-linking agent in a LRP reaction has the effect of increasing the degree of polymer branching while avoiding undissolvable gel formation.[34−38] Thus, DSDMA was added, and polymerizations with different ratios of the [CTA]/[DSDMA]/[monomer] were performed to optimize the reaction conditions. GPC chromatograms (Figure 1) confirmed that the ratio of branched polymer and molecular weight increased with DSDMA addition.

Subsequently, polymer was prepared with a ratio [CTA]/[DSDMA]/[monomer] = 1:2:40. The reactions were monitored over time as shown in Figure 2. As expected, both the MW and branch polymer ratio increased with increasing reaction times. As GPC was calibrated with polystyrene standards, it could not be used to quantitatively analyze the polymers. Consequently, static light scattering was

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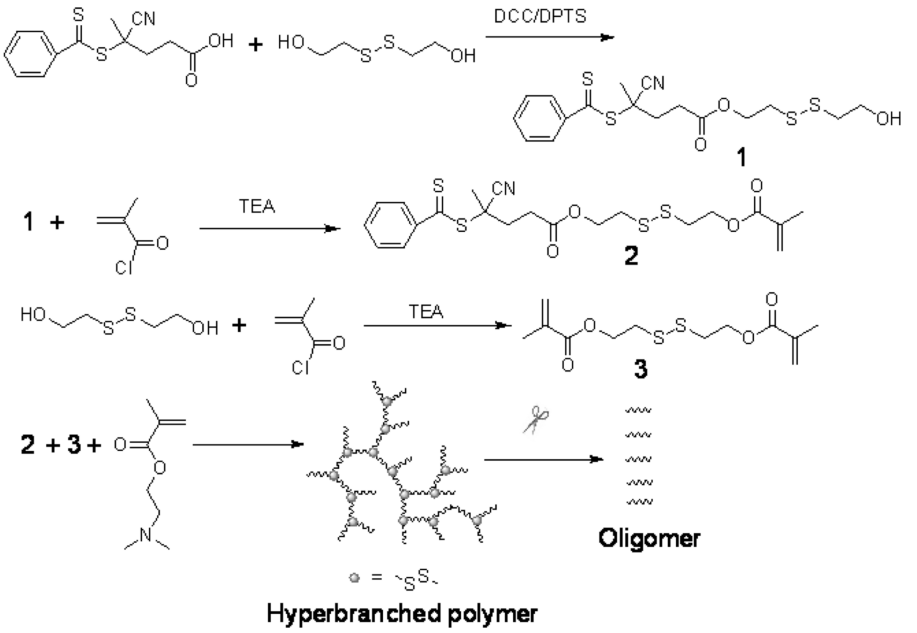
Scheme 1. Synthesis of Hyperbranched PDMAEMA and the Subsequent Cleavage Reaction



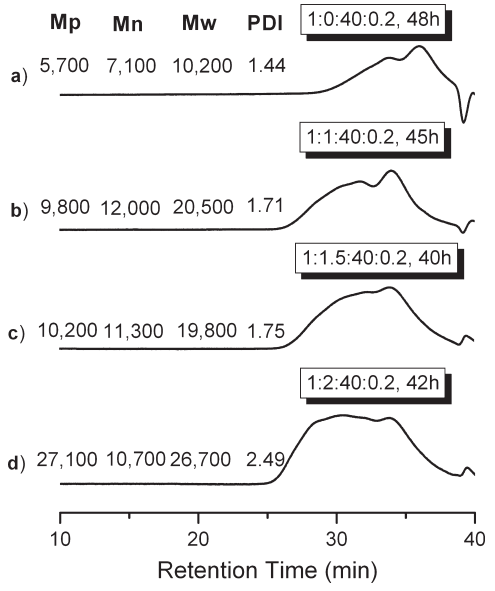


Figure 1. GPC traces of hyperbranched polymers generated from different [CTA]:[DSDMA]:[monomer]:[initiator] ratios at 70 °C.

employed to characterize the "true" molecular weight, yielding a weight-average molecular weight of $M_w \sim 290$K.

Cleavage of the Biodegradable Hyperbranched Polymer. The successful implementation of RAFT should ensure that all the chain branches should have a similar length. As every branch point is a disulfide linkage, the polymer is cleavable in the presence of reductants. Consequently, the polymer was treated with DTT (0.5 M) in DMAc solution for 6 h. The GPC chromatogram (Figure 3) indicated that the postreduction polymer possessed a much lower molecular weight (M_n(GPC) ~ 9900) than the original polymer. The narrow polydispersity of the cleaved polymer (PDI ~ 1.32) also confirmed reasonable uniformity, consistent with RAFT polymerization control, demonstrating a design control capacity over the final degraded polymer via the original synthesis protocol.

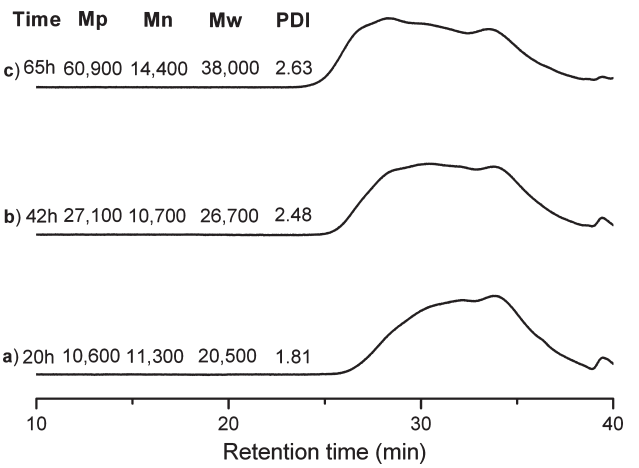


Figure 2. GPC traces of hyperbranched polymers generated from [CTA]:[DSDMA]:[monomer]:[initiator] ratio of 1:2:40:0.2 at 70 °C.

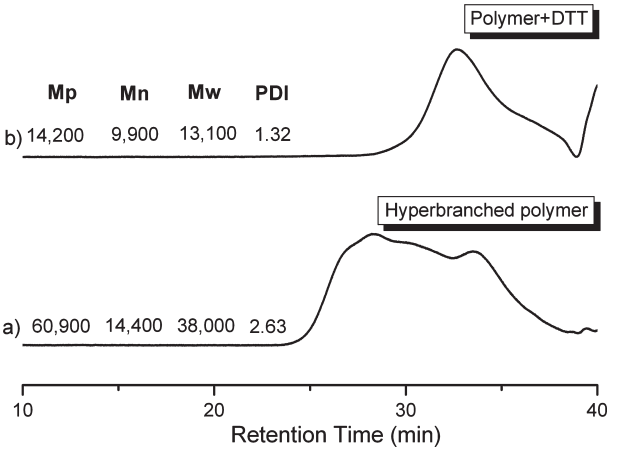


Figure 3. Polymers (a) before and (b) after adding DTT.

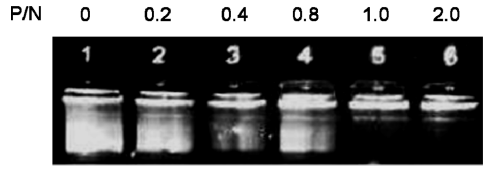


Figure 4. Agarose gel electrophoresis of the DNA bounded by hyperbranched polymer under different positive/negative (P/N) ratio.

DNA Binding to the Biodegradable Hyperbranched PDMAEMA. Deoxyribonucleic acid sodium salt from salmon testes was used as a model DNA. The hyperbranched degradable PDMAEMA was protonated prior to DNA binding. The protonated polymer was mixed with DNA having different positive/negative (P/N) ratio, and the binding was monitored by agarose gel electrophoresis. The negative signal from DNA decreased with an increasing P/N ratio, as shown in Figure 4. When the ratio reached 1:1, the DNA was neutralized perfectly and there was no DNA complex observed via the gel, consistent with an effective formation of a polymer/DNA complex.

Conclusions. We have described a straightforward methodology to synthesize new hyperbranched biodegradable PDMAEMA structures via RAFT polymerization. The designed polymer structure is hyperbranched and cleavable under reducing conditions. Cleavage tests confirmed that the branch chains were well-defined (consistent with RAFT control). This design strategy for cationic polymers could yield DNA polyplexes efficiently via multivalent electrostatic interactions, enabling the use of noncytotoxic gene delivery agents. The polymeric structures are inherently biodegradable producing oligomers with similar chain length on reduction. This makes it possible to optimize the synthetic protocol to minimize the toxicity of the oligomers during their excretion phase. This synthetic approach is quite general and represents a new, versatile method to prepare new cationic polymers for gene delivery.

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Supporting Information Available: Experimental details. This material is available free of charge via the Internet at http://pubs.acs.org.

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