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RESEARCH ARTICLE

Development of a Novel Polymer-based Drug Delivery System

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ABSTRACT: Oligonucleotide drugs hold transformative potential in therapeutics by addressing a range of diseases with unmet medical needs. However, clinically, they are hindered by limitations in membrane permeability and biodistribution, underscoring the need for optimized delivery platforms. In this study, we developed a novel bottlebrush polymer-based delivery system featuring cholesterol molecules on its side chains to facilitate cellular uptake and broaden biodistribution. The design involved the conjugation of a single cholesterol molecule per bottlebrush polymer, followed by PEGylation to increase solubility and biocompatibility. Gel permeation chromatography (GPC) confirmed a consistent molecular weight of approximately 300 kDa of the sample. At the same time, Transmission Electron Microscopy (TEM) revealed a uniform spherical nanostructure with a core diameter of around 20 nm. Cellular uptake assays demonstrated significantly improved uptake of Cy3-labeled bottlebrush polymers compared to unmodified controls, validating the efficacy of cholesterol incorporation. These findings suggest that the cholesterol-modified bottlebrush polymer platform represents a promising and adaptable delivery system for oligonucleotide-based therapies.

KEYWORDS: Biomedical Engineering, Biomaterials and Regenerative Medicine, Bottlebrush Polymer, Oligonucleotide, Cellular Uptake.

Introduction

Oligonucleotide drugs hold significant promise for revolutionizing disease therapeutics due to their ability to target a broad spectrum of diseases with high molecular specificity.[1] These therapies have enabled breakthroughs in previously intractable conditions, exemplified by approved drugs such as Spinraza for spinal muscular atrophy and investigational treatments for myotonic dystrophy type 1 (DM1). Such advancements highlight the potential of oligonucleotide-based therapies for precise modulation of genes and molecular pathways.[2] However, the clinical translation of oligonucleotide drugs remains limited by inherent structural and physicochemical properties, such as a polyanionic backbone that confers a strong negative charge.[3] This feature severely restricts membrane permeability and cellular uptake, posing substantial challenges to effective systemic delivery. As a result, the development of optimized and versatile delivery platforms remains a critical focus in advancing oligonucleotide-based therapeutics.

To address these challenges, recent studies have explored various nanoparticle-based delivery systems. Lipid nanoparticles (LNPs), for instance, have shown promise by encapsulating oligonucleotides within a lipid shell, enhancing their stability and facilitating uptake into target cells.[4] However, LNPs tend to accumulate in the liver and spleen due to their size and composition, which can restrict therapeutic reach to non-hepatic tissues and limit their utility in targeting diverse organ systems.[5] To address this problem, oligonucleotides can be conjugated to targeting moieties such as small molecules, peptides, and antibodies, which can greatly enhance target specificity and mitigate off-target effects.[6] Antibody-conjugated delivery systems represent further refinement, leveraging the specificity of antibodies to target oligonucleotides to particular cell types or tissues. While this approach improves targeted delivery, it is in some cases hampered by limited blood circulation times—often due to linker instability and Fc-mediated clearance—as well as challenges in maintaining oligonucleotide stability. This results in decreased efficacy in delivering therapeutics to certain tissues or in conditions that require prolonged systemic exposure.[7]

To address these limitations, a bottlebrush polymer delivery system was developed to improve cellular uptake of oligonucleotide drugs and prolong blood circulation time.[8] Unlike traditional oligonucleotide delivery systems that predominantly target the liver, this platform demonstrates potential for broader organ targeting, expanding its therapeutic applications.[9,10] To further enhance cellular uptake and biodistribution, a new generation of bottlebrush polymer was designed and synthesized. This updated platform was thoroughly characterized to confirm its structural properties and optimize its suitability for targeted delivery.

Methods

Oligonucleotides and poly(serinol phosphodiester) monomer synthesis:

The synthesis (Figure 1) of serinol phosphoramidite started from β-alanine-serinol (compound 1), where 1 mmol of compound 1 was dissolved in 30 mL of pyridine. 4,4 -Dimethoxytrityl chloride was added dropwise under an ice bath. The reaction was carried out overnight and was quenched with methanol. The resulting mixture was purified by column chromatography using an ethyl acetate: hexane solvent system (2:1

DOI: 10.36838/IJHSR816.18

volume ratio) to give rise to serinol-DMT (compound 2) as a white crystal with a 63% yield.

The synthesized compound 2 (1 mmol) was subsequently dissolved in dichloromethane at room temperature, followed by the addition of *N*, *N*-diisopropylethylamine (5 mmol). 2-cyanoethyl-*N*, *N*-diisopropylchlorophosphoramidite (2 mmol) was added dropwise under an ice bath. The reaction was stirred for 1 hour and quenched with 1 mL of ethyl acetate. The resulting mixture was purified by column chromatography (gradient elution using ethyl acetate: hexane from 1:2 to 2:1 v/v) to afford serinol phosphoramidite (compound 3) as a white crystal with a 75% yield.

Using a similar procedure, Compound 5 was synthesized from Compound 4 with a yield of 65%.

Oligonucleotides and poly(serinol phosphodiester) backbone synthesis:

All oligonucleotides, with or without poly(serinol phosphodiester) backbones, were synthesized using solid-phase synthesis on a Model 391 DNA synthesizer (Applied Biosystems, Inc., Foster City, CA). Both natural and modified DNA phosphoramidites were sourced from Glen Research Co. (Sterling, VA, USA). Following synthesis, these oligonucleotides underwent on-column deprotection with 20% piperidine in DMF three times to remove the phosphate protection groups, followed by two washes with DMF.[11] Each oligonucleotide strand was cleaved from the CPG column and deprotected in an aqueous ammonium hydroxide solution (28-30% NH_3) at room temperature for 24 hours. The sequence of the DNA oligonucleotide is *CAG AGG AGT ATT AGG ACT*.

Purification in the Glen-Pak™ DNA purification cartridge:

All oligonucleotide strands were purified using Glen-Pak™ DNA purification cartridges. Following purification, the purified oligonucleotide solution was lyophilized in preparation for the subsequent PEGylation reaction.

PEGylation of pacDNA:[11]

To conjugate the PEG side chains, 100 nmol of the oligonucleotides with poly(serinol phosphodiester) modified backbones and 30 mg of N-hydroxysuccinimide (NHS)-terminated 10 kDa mPEG were dissolved together in 1 mL of phosphate-buffered saline (PBS, pH 7.4). The solution was gently shaken at 4˚C overnight and then lyophilized into a white powder, which was dissolved in 1 mL of anhydrous DMF as well as 42 µL triethylamine. Subsequently, 30 mg of NHS-PEG in 1 mL DMF was added in 5 aliquots at 12-hour intervals. After the last addition, the reaction mixture was shaken at 25 ˚C for an additional 12 h, followed by lyophilization.

Purification in GPC:[12]

After complete PEGylation, the molecular weight of pacDNA can reach approximately 250 kDa, allowing it to be separated from free 10 kDa PEG and unreacted free oligonucleotides via fractionation during aqueous gel permeation chromatography (GPC).

Transmission electron microscope:[13]

Following purification by GPC, transmission electron microscopy (TEM) was performed, which provided visual evidence of uniform, spherical nanostructures with an average core diameter of 20 nm, confirming the controlled assembly achieved through the synthetic process.

Cellular uptake assay:[14]

Cellular uptake of the samples and controls was evaluated via flow cytometry. Cells were plated in 24-well plates at a density of 2 × 10^5 cells per well in 1 mL of complete growth medium and incubated for 24 hours at 37 ˚C with 5% CO_2. After washing with 1×PBS, conjugate samples and controls (concentrations ranging from 250 nM to 5 µM DNA equivalents) were prepared in 400 µL serum-free culture medium and added to each well. Cells were then incubated at 37˚C for an additional 4 to 6 hours. Following incubation, cells were washed twice with PBS and treated with 60 µL of trypsin per well. Afterward, 1 mL of PBS was added to each well to resuspend the cells. Finally, cells were analyzed using an Attune™ NxT flow cytometer (Invitrogen, MA), with data collected from 10,000 gated events per sample.

Results and Discussion

Results:

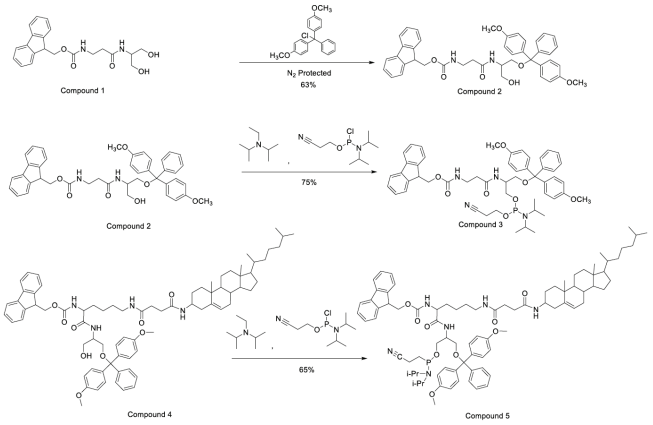

Figure 1: Synthesis of serinol and cholesterol phosphoramidite monomers enabling bottlebrush polymer functionalization. These monomers provide the chemical basis for controlled incorporation of cholesterol into the polymer backbone.

In collaboration with lab colleagues, serinol phosphoramidite (compound 3) was synthesized in a two-step organic synthesis from a protected diol (compound 1), while cholesterol phosphoramidite (compound 5) was prepared via a one-step phosphitylation reaction. The synthetic routes are shown in Figure 1. These monomers were subsequently used in solid-phase synthesis to generate bottlebrush polymer–oligonucleotide conjugates with defined cholesterol content. Successful synthesis enables precise structural modification of the polymer platform for downstream characterization and biological evaluation.

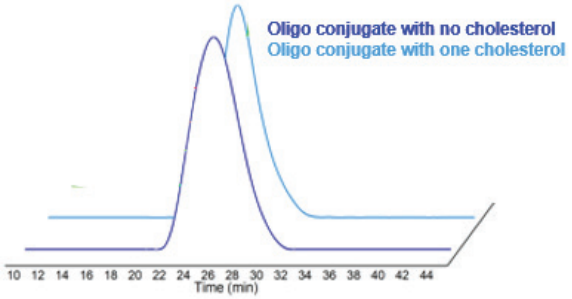

Figure 2: GPC analysis confirming successful cholesterol conjugation and purification of bottlebrush oligonucleotide conjugates. The chromatographic profiles indicate formation of uniform polymer populations following conjugation.

Following conjugation, the cholesterol-modified conjugate was purified by GPC. Two oligo conjugates were generated: one without cholesterol and one containing a single cholesterol unit, as shown in Figure 2. Elution profiles are plotted as detector response versus retention time. Both conjugates exhibit narrow, single peaks, indicating uniform molecular weight distributions. The retention shift observed for the cholesterol-modified construct confirms successful incorporation of the hydrophobic moiety without evidence of aggregation or degradation.

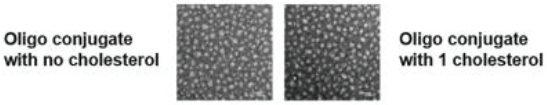

Figure 3: TEM of selected oligo cholesterol conjugates demonstrating uniform nanoparticle morphology of cholesterol-modified bottlebrush conjugates. The observed structures confirm consistent nanoscale assembly after cholesterol incorporation.

Oligonucleotide conjugates with and without cholesterol modification were imaged by TEM for particle size and morphology analysis, as shown in Figure 3. Images represent purified samples following GPC. Both constructs form uniform spherical nanostructures with an average core diameter of approximately 20 nm. Particle size and morphology were consistent across samples. These results demonstrate that cholesterol incorporation preserves nanoparticle structure and does not disrupt controlled assembly of the bottlebrush platform.

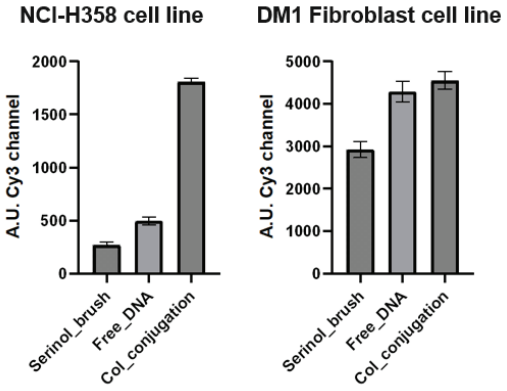

Figure 4: Enhanced cellular uptake of cholesterol-conjugated bottlebrush polymers in NCI-H358 and DM1 fibroblast cells. Cholesterol modification improves intracellular delivery compared to unmodified controls.

The cholesterol-conjugated oligonucleotide was evaluated in NCI-H358 and DM1 fibroblast cells using an unloaded serinol brush and free DNA as controls, as shown in Figure 4. Cellular uptake was quantified by flow cytometry as Cy3 fluorescence intensity following 4–6 hours of incubation. Flow cytometry analysis revealed significantly increased fluorescence intensity for cholesterol-modified constructs compared to controls in both cell lines. These results confirm that cholesterol conjugation substantially enhances cellular uptake and improves the delivery efficiency of the bottlebrush polymer system.

Discussion:

In this study, we successfully synthesized a bottlebrush polymer monomer featuring cholesterol modification on its side chain, followed by the conjugation of one cholesterol unit per polymer. Subsequent PEGylation was completed, and gel permeation chromatography (GPC) analysis confirmed that all resulting polymers exhibited a molecular weight of approximately 300 kDa, indicating successful conjugation and size consistency across samples.

Transmission Electron Microscopy (TEM) provided visual confirmation of the bottlebrush polymers' uniform, spherical nanostructures with an average core diameter of 20nm, reflecting the controlled assembly achieved through our synthetic approach. This spherical morphology, combined with cholesterol conjugation, likely plays a key role in enhancing cellular uptake, as cholesterol's affinity for cellular membranes facilitates closer interaction and internalization.[15]

Flow cytometry-based cellular uptake assays further supported the efficacy of this design. Cy3-labeled bottlebrush polymers demonstrated notably higher cellular uptake compared to controls, demonstrating that the synergistic effect of PEGylation and cholesterol modification improves delivery efficiency. This study focused on constructs containing a single cholesterol unit per polymer; future work could explore multiple cholesterol incorporations to evaluate potential effects on uptake and biodistribution. Overall, this study underscores the potential of cholesterol-modified bottlebrush polymers as effective carriers for oligonucleotide therapeutics, offering improved membrane permeability, favorable biodistribution, and opportunities for targeted, non-hepatic delivery.

■ Conclusion

A cholesterol-modified bottlebrush polymer platform was developed and characterized for the enhanced delivery of oligonucleotide drugs. The synthesis process involved conjugating one cholesterol unit per bottlebrush polymer monomer, followed by PEGylation, yielding polymers with consistent molecular weights of approximately 300 kDa, as confirmed by GPC. TEM analysis revealed uniform, spherical nanostructures, indicating stable and controlled assembly. Cellular uptake assays further demonstrated significantly enhanced uptake, likely due to the membrane affinity imparted by cholesterol conjugation.[15] This innovative bottlebrush polymer delivery system addresses the challenges related to membrane permeability and has the potential to improve biodistribution,

DOI: 10.36838/IJHSR816.18

offering a promising approach for targeted oligonucleotide delivery beyond hepatic tissues and expanding the potential for therapeutic applications.

Acknowledgments

I would like to thank my mentors, Professor Ke Zhang, Tingyu Sun, and Jiachen Lin, for their guidance, advice, and patience. I would also like to thank my lab mates and collaborators for sharing their experiences and collaborations.

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Alex Wang is currently a junior in high school at The Governor's Academy. Alex has developed a great interest and passion in biology, chemistry, and engineering.