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Enabling safer, more potent oligonucleotide therapeutics with bottlebrush polymer conjugates

Peiru Chen [a,1], Yun Wei [a,1], Tingyu Sun [a], Jiachen Lin [a], Ke Zhang [a,b,*]

[a] Department of Chemistry and Chemical Biology, Northeastern University, Boston, MA 02115, USA
[b] Department of Chemical Engineering and Bioengineering, Northeastern University, Boston, MA 02115, USA

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ABSTRACT

Oligonucleotide therapeutics have the unique ability to address traditionally undruggable targets through various target engagement pathways. However, despite advances in chemically modified oligonucleotides and carrier-assisted delivery systems such as lipid nanoparticles and protein/peptide conjugates, the development of oligonucleotide drugs is still plagued with lackluster potency, narrow therapeutic window, poor delivery to non-liver target sites, and/or high potential for toxicity and unwanted immune system activation. In this perspective, we discuss an unconventional delivery solution based upon bottlebrush polymers, which overcomes many key challenges in oligonucleotide drug development. We address the molecular basis of the polymer's ability to enhance tissue bioavailability and drug potency, reduce side effects, and suppress anti-carrier immunity. Furthermore, we discuss the potential of the technology in advancing oligonucleotide-based therapies for non-liver targets.

1. Challenges facing the oligonucleotide drug modality

Oligonucleotides hold significant promise as genetic therapeutics across various disease domains, including oncology [1–3], hereditary and genetic diseases [4–6], as well as infectious diseases [7,8]. In principle, they can be tailored to selectively target specific genes, with minimal or, at the very least, predictable off-target effects. Furthermore, they can be personalized to target patient-specific alterations, specific alleles [9], distinct transcript isoforms [10], traditionally "undruggable" targets [11–13].

As of October 2023, eighteen oligonucleotide drugs have been approved by the United States Food and Drug Administration (FDA) (Table 1). Despite the progress, major drawbacks in the development of oligonucleotide therapeutics, such as poor cytosolic delivery, stability of the oligonucleotides in vivo, and distribution into non-liver tissues, still remain [14,15]. On the cellular level, oligonucleotide faces entry barriers due to their large size and hydrophilicity, rendering cellular uptake and endosomal escape difficult. Unmodified oligonucleotides are also prone to enzymatic degradation [16,17]. On the physiological level, oligonucleotide formulations tend to accumulate in the kidney and the liver, leading to adverse events such as kidney inflammation and liver toxicity [18]. The distribution to non-liver/kidney organs is generally much less prevalent, differing by 2–3 orders of magnitude [19]. Therefore, to achieve a therapeutic concentration in a target organ with systemic administration often means unacceptable toxicity to the liver/kidney. Compounds that managed to accomplish a reasonable therapeutic window and regulatory approval often require frequent dosing at high quantities, dampening patient satisfaction and compliance. Therefore, a more efficient, side effect-free delivery strategy for non-liver targets is still very much a bottleneck.

Presently, the core of oligonucleotide drug development hinges on chemical modifications. As of now, oligonucleotide therapeutics introduced to the market contain a few types of first- and second-generation modifications, which include 2′-fluoro-RNA, 2'-*O*-methyl RNA, and phosphorothioate, a modification with a history spanning more than half a century [20]. Additionally, two other specific chemical entities, 2'-O-(2-methoxyethyl)-RNA (MOE) and phosphoramidomorpholine (PMO), play a pivotal role in conferring high target affinity, metabolic stability, and favorable pharmacokinetic properties to oligonucleotides [21,22].

In parallel, a range of carriers are being developed to facilitate delivery, among which lipid nanoparticles (LNPs) are the first to gain

* Corresponding author at: Department of Chemistry and Chemical Biology, Northeastern University, Boston, MA 02115, USA.
E-mail address: k.zhang@northeastern.edu (K. Zhang).
[1] The authors shared equal contribution.

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Table 1
FDA approved oligonucleotide drugs.

Name	Active Ingredient	Category	Approval Date	Indications
Vitravene	Fomivirsen	ASO	1998.08	Cytomegalovirus Retinitis
Macugen	Pegaptanib	Aptamer	2004.12	Age-Related Macular Degeneration
Kynamro	Mipomersen	ASO	2013.01	Homozygous Familial Hypercholesterolemia
Defitelio	Defibrotide	Mixture of ss-DNA and ds-DNA	2016.03	Hepatic Veno-Occlusive Disease
Exondlys 51	Eleplinsen	ASO	2016.09	Duchenne Muscular Dystrophy
Spinraza	Musinersen	ASO	2016.12	Spinal Muscular Atrophy
Onpattro	Patisiran	siRNA	2018.08	Heterotrophic Transthyretin Amyloidosis
Tegsedi	Nolersen	ASO	2018.01	Hereditary Transthyretin Amyloidosis, Polyneuropathy
Givlaari	Givosiran	siRNA	2019.11	Acute Hepatic Porphyrias
Vyondlys 53	Golodirsen	ASO	2019.12	Duchenne Muscular Dystrophy
Viltepso	Viltolarsen	ASO	2020.08	Duchenne Muscular Dystrophy
Oxluno	Umasiran	siRNA	2020.11	Primary Hyperoxaluria Type 1
LeqvioTM	Inelisiran	siRNA	2021.12	Hypercholesterolemia
Amondys 45	Casimersen	ASO	2021.02	Duchenne Muscular Dystrophy
Amvuttra	Vutrisiran	siRNA	2022. 06	Hereditary Transthyretin Amyloidosis, Polyneuropathy
Qalsody	Tofersen	ASO	2023.04	Amyotrophic Lateral Sclerosis
Izervay	Avacincaptad Pegol	Aptamer	2023.08	Geographic Atrophy
Rivfloza	Nedosiran	siRNA	2023.09	Primary Hyperoxaluria Type 1

clinical relevance [23,24]. A notable LNP example is Lipofectamine, often recognized as the gold standard for in vitro gene transfection agents. In 2018, LNP-encapsulated siRNA gained FDA approval, marking the first siRNA product, patisiran [25]. Moreover, LNPs have been utilized to produce COVID-19 mRNA vaccines [26]. Still, LNPs exhibit constraints in delivering therapeutics to extrahepatic sites [27].

Cationic polymeric materials, such as polyethylenimine (PEI) [28] and poly(β-amino-esters) (PBAE) [29], represent another important class of carriers for nucleic acids. Polymeric nanoparticles exhibit a relatively substantial nucleic acid loading capacity, primarily owing to their high charge density and molecular weight [30]. Their advantages include high tunable release kinetics and remarkable transfection efficiencies. However, unlike LNPs, which can disassemble once their purpose of delivery is served, thereby reducing toxicity, the polycationic nature of polymeric materials is persistent, which substantially increases their toxicity. Also, being exogenous materials, they are subject to adaptive immunity, which potentially makes redosing difficult. To date, these challenges have impeded the progress of electrostatic polyplexes as viable delivery systems for human therapeutic applications.

A third strategy to impart oligonucleotides with improved properties is through bioconjugation. Small molecule ligands such as GalNAc, aptamers, cholesterol, squalene, fatty acids, and nucleolipids, as well as macromolecule including antibodies, peptides, and polymers are promising examples [31,32]. These ligands can alter the cell-materials interactions and thereby improve uptake by certain cell populations, promote endosomal escape, alter subcellular localization, or change biodistribution profile. For example, the GalNAc conjugate exhibits a strong hepatic affinity and extended activity, presenting the potential to address a broad spectrum of conditions linked to genes expressed in the liver [33]. Mannose 6-phosphate, another carbohydrate, has been linked to siRNA for precise delivery to hepatic stellate cells, which play a pivotal role in liver fibrosis [34]. In addition, various cell-penetrating peptides (CPPs), which are the short basic amino acid-rich peptides, have been used to enhance the cellular uptake, endosomal escape, cell membrane receptor binding, and nuclear localization of drugs, with some reaching the clinical stage [35,36].

In this prospective, we detail an alternative delivery technology using bottlebrush polymers, one that is based on the idea of making oligonucleotide binding selective: the oligonucleotide-polymer conjugate resists protein binding but can still hybridize to complementary nucleic acid targets. We will discuss the molecular mechanism on how such selectivity is achieved, and how this approach delivers a compelling package of features that makes it a strong platform to enable a new generation of safer, more potent oligonucleotide drugs.

2. pacDNA: a bottlebrush polymer-based oligonucleotide vector for in vivo delivery

In 2015, Lu et, al. reported a bottlebrush polymer-DNA conjugate known as pacDNA (polymer-assisted compaction of DNA) for oligonucleotide delivery [37]. The conjugate consists of a bottlebrush-structured polymer with ~30 polyethylene glycol (PEG) side chains and 1–5 strands of oligonucleotide covalently tethered to a central polymer backbone. Upon inspecting the chemical structure of the conjugate (Fig. 1. A), it is not immediately apparent why efficient delivery can be accomplished, as the structure lacks all key components critical to prototypical non-viral vectors, such hydrophobic tails, cationic (ionizable) moieties, and surfactant-like characteristics (micellar, liposomal, etc.). In contrast to cationic polymer carrier systems or lipid particle-based approaches that rely on electrostatic bindings or oligonucleotide encapsulation, pacDNA stands out as a charge-neutral and fully covalent entity. In fact, >90% of the molecular weight of the conjugate is PEG, which by itself is not known to be transfection-active. Yet, the pacDNA is surprisingly effective at transfection at the cellular level and often more so in vivo, without the toxicity and immunological side effects associated with many cationic or protein/peptide-based transfection systems in development. What are the key factors driving its functionality? The following three main features, which gradually came to light during the ensuing years, may be important to the observed phenomena:

2.1. Binding selectivity

Unlike typical PEGylated macromolecular therapeutics where a single, linear/slightly branched strand of PEG is used, pacDNA utilizes multiple shorter PEG chains and creates a more significant entropic barrier that reduces specific/non-specific oligonucleotide-protein interactions [38]. For example, when using DNase I to digest double stranded (ds) DNA, the pacDNA exhibits 10–20 times longer half-life compared with free dsDNA, while a linear, high molecular weight PEG (40 kDa) does not noticeably prolong enzymatic half-life (Fig. 2.B). The entropic shielding effect is sensitive to the size of the PEG side chains and the oligonucleotide. The further away a nucleotide is from the brush backbone, the more accessible it is, leading to faster degradation. Therefore, longer PEG chains and shorter oligonucleotides are conducive to enhanced shielding. Using a series of "length probes", we determined that for every nucleotide's length toward the brush backbone, roughly one half-life of free dsDNA is gained (with 10 kDa side chain) [39]. It is possible to increase steric protection by adjusting the conjugation site, which modifies the relative distance between the distal terminus of the oligonucleotide and brush polymer backbone without altering the pacDNA sequence [40]. An optimally designed pacDNA can

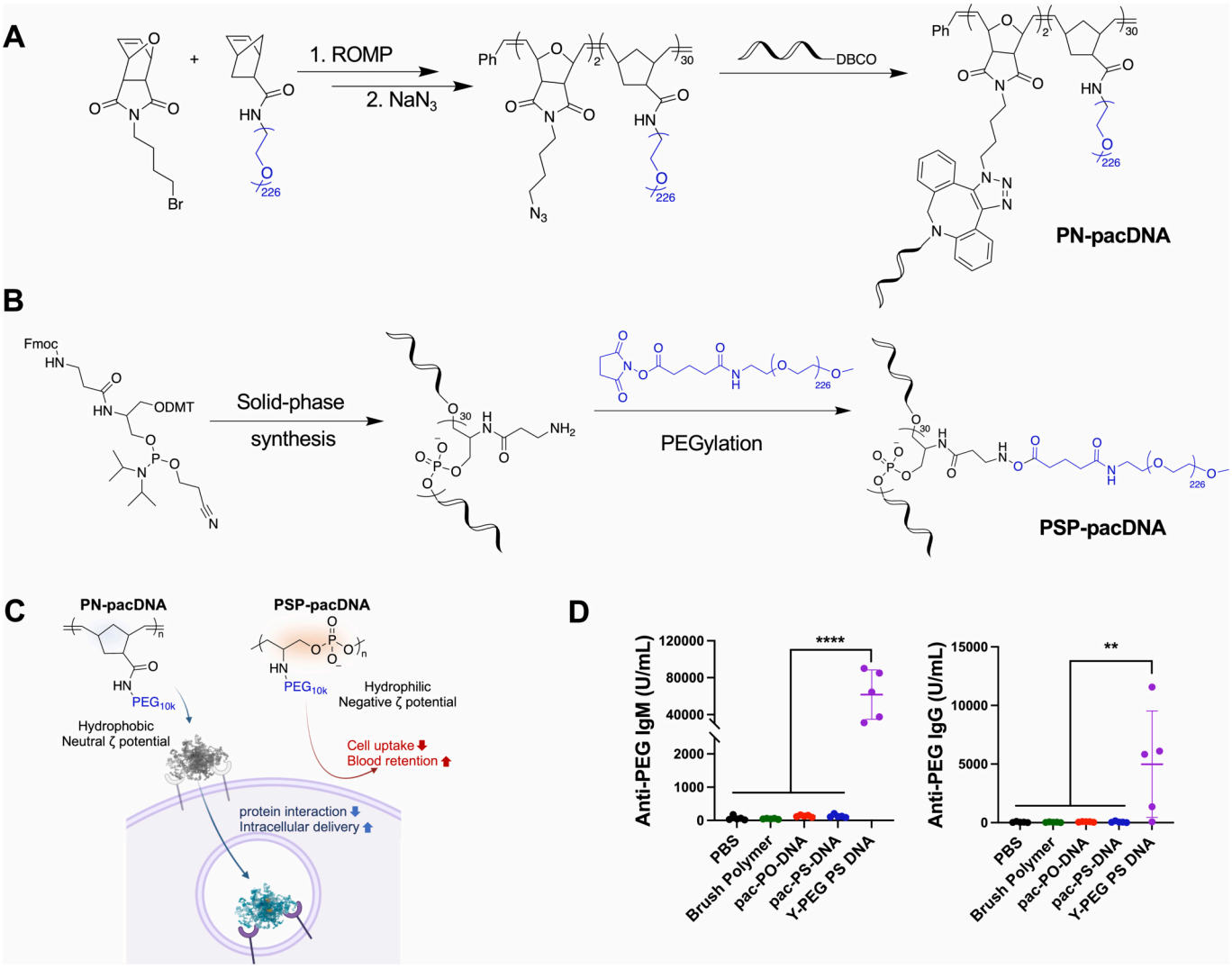

Fig. 1. (A) Synthetic scheme of PN-based pacDNA; (B) Synthetic scheme of PSP-based pacDNA; (C) Opposing cellular uptake propensities for PN and PSP-based pacDNA; created with BioRender.com; (D) Anti-PEG immunoglobin production in mice following repeated (12) i.v. dosing of pacDNA or Y-shaped PEG_{40k}-ASO conjugate.

provide steric protection to a 40-mer oligonucleotide, which is a sufficient length for most oligonucleotide modalities such as antisense oligonucleotides (ASOs) and small interfering RNAs (siRNAs).

Interestingly, for the oligonucleotide within the pacDNA to hybridize to a complementary nucleic acid strand, volume exclusion appears more dominant. This effect makes DNA hybridization more favorable in the presence of macromolecules than in the absence of them. However, the effect is quite small, and in practicality, the hybridization of pacDNA with its target is nearly indistinguishable from free DNA kinetically and thermodynamically (Fig. 2.C). [39]. Taken together, the bottlebrush polymer gives the pacDNA a preferred selectivity toward binding with a complementary sequence without the oligonucleotide being released from the polymer, while suppressing specific and non-specific oligonucleotide-protein interactions.

2.2. Biodistribution

Whereas intravenously (i.v.) dosed free oligonucleotides are often rapidly cleared by the kidney via glomerular filtration, the pacDNA conjugates are sufficiently large (~300 kDa) to evade renal clearance, which is commonly observed for naked oligonucleotides and some co-carrier systems. Phagocytic clearance is also retarded due to the dense PEG coverage, which creates a hydration layer that reduces protein corona formation, which is common for conventional polyplex systems [41]. These properties render the pacDNA highly persistent in plasma, with bioavailability that is 1–2 orders of magnitude greater than unconjugated oligonucleotides. The prolonged circulation times and reduced phagocytic clearance in turn affords a broader biodistribution profile, showing accumulation in hard-to-deliver sites such as the lung, muscle, and the skin.

The more even distribution across various organs has important implications in therapeutic window. For example, the ratio for the uptake of pacDNA in the kidney (often the dose-limiting organ) and the muscle is within one order of magnitude, which is significantly lower than the 2–3 orders of magnitude difference for free oligonucleotides or oligonucleotide-peptide conjugates [19]. Therefore, with pacDNA, it is much easier to raise the oligonucleotide concentration in the muscle to a therapeutic level without causing excessive toxicity or inflammation to the kidney. When it comes to liver distribution, conventional polyplex systems and lipid nanoparticle-based oligonucleotide delivery systems tend to result in the formation of a surface protein corona, leading to a significant portion of the dose accumulating in the liver. However, pacDNA circumvents protein corona formation, thereby limiting its distribution within liver tissue resulting in more evenly distribution

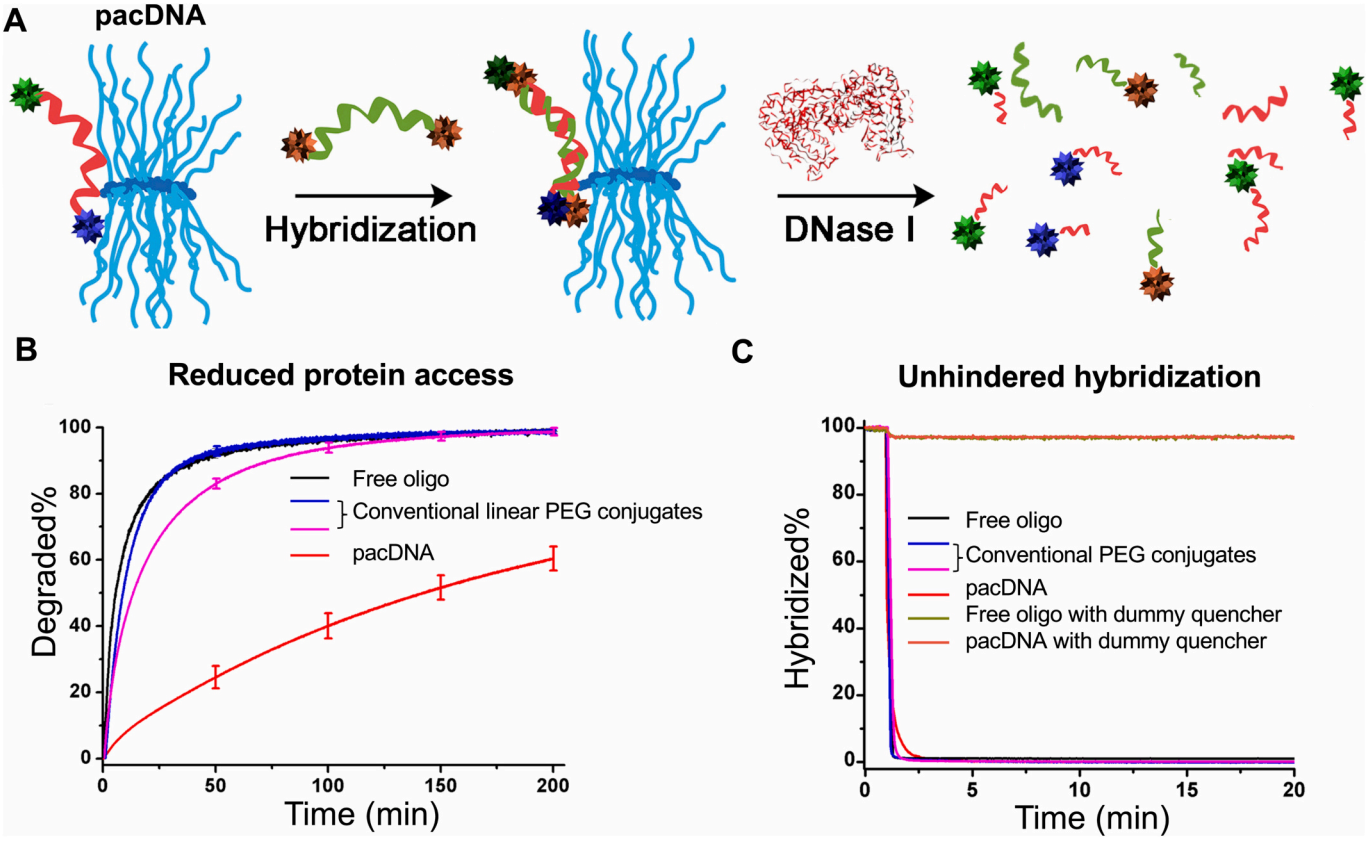

Fig. 2. (A) Schematics of assays for determining DNA hybridization and nuclease degradation kinetics. (B) Nuclease degradation kinetics for pacDNA vs free DNA. (C) Hybridization kinetics for pacDNA vs free DNA.

among tissues. Even if the pacDNA is less potent than an alternative delivery approach such as protein or peptide conjugates on a per-molecule basis, the elevated concentration in the target organ can very plausibly make up for the difference in activity. This philosophy in vector design allows one to bypass the use of the traditional polycationic materials that drive toxicity, and instead adopt safer materials that primarily improve distribution. In addition, the broad distribution opens new disease areas previously inaccessible to the oligonucleotide modality.

2.3. Backbone effect

While it was quickly clear that the bottlebrush polymer side chains were important for the binding selectivity of the conjugated oligonucleotide and the improved pharmacological properties, the function of the bottlebrush polymer backbone was thought to be merely structural until recently. The pacDNA backbone discussed so far is synthesized by ring-opening metathesis polymerization (ROMP), a polymerization process that generates a poly(norborneyl) (PN) backbone, which is slightly polydisperse (Fig. 1. A). In an effort to prepare molecularly pure bottlebrush backbones, we designed serinol-based phosphoramidite building blocks, which can be used to synthesize poly(serinol phosphodiester) (PSP) bottlebrush backbones in a step-wise fashion using automated solid-phase synthesis (Fig. 1. B) [42]. This method creates well-defined bottlebrush polymers with precise size, position/number of the oligonucleotide, and polymer architecture. However, we discovered that the seemingly small change in the structure of the polymer backbone (only ~4% in overall MW of the pacDNA) can greatly alter the biological characteristics despite that in most conformations (as revealed by molecular dynamics simulation), the backbone is not directly exposed to the cell due to dense PEG coverage (Fig. 1. C). Cellular uptake and antisense gene regulation of the PSP pacDNA is all but eliminated while the plasma PK is enhanced relative to PN bottlebrushes.

Examining the chemical structure of the two types of pacDNAs, two main differences can be noted: the backbone ionization (anionic vs. neutral) and hydrophobicity. This observation suggests to us a possible mechanism for cell uptake: parts of the PEG transiently adopt a crown ether-like conformation and bind with passing cations such as K^+ and Mg^{2+}. This temporary interaction then mediates the adsorption of the polymer onto the plasma membrane, which has a negative potential (−40 to −70 mV). The contact is short-lived and unstable, but increases the chance for the polymer backbone to be exposed to the membrane. With PN-based pacDNA, the near-neutral ζ potential promotes membrane adsorption, and the hydrophobic polymer backbone can further increase adhesion strength, and thus polymer residence time on the plasma membrane, allowing for increased uptake via macropinocytosis and endocytosis (~half of uptake was found to be via macropinocytosis in NCI-H358 cells; the rest by endocytosis) [43]. In contrast, the more negative ζ potential and the completely hydrophilic backbone of the PSP pacDNA should reduce the transient polymer-membrane interactions, and therefore cell uptake would not be enhanced compared to free DNA.

This observation suggests that some of the key properties of the pacDNA, i.e. cellular uptake, antisense gene regulation, biodistribution, and tissue retention, may indeed rest upon the backbone structure. The subtle distinction in backbone chemistry has a profound impact on cellular behavior, offering the possibility for fine-tuning the properties of pacDNA to fulfill disease-specific delivery requirements.

3. pacDNA in action

How do the characteristics of pacDNA make it an effective non-viral transfection vector in vivo? Here, using an example where a pacDNA containing a clinical ASO targeting the Kirsten Rat Sarcoma Virus

(KRAS) mRNA is tested in non-small cell lung carcinoma mouse models, we provide an account of the path that pacDNA takes in vivo, and demonstrate that pacDNA can be superior in many important ways to more established approaches in oligonucleotide delivery [44].

Upon entering the blood stream following i.v. dosing, the pacDNA resists protein corona formation and enzymatic digestion (in the case of phosphodiester [PO] oligonucleotides), allowing the pacDNA to bypass hepatic clearance. The large size of the pacDNA also reduces renal clearance. With the two major clearance pathways for oligonucleotide retarded, the pacDNA can remain in blood circulation for several days and be more slowly absorbed by other organs owing to the weak polymer backbone-cell membrane interactions. Indeed, examining the concentration of the pacDNA in tumor xenograft tissues (established with NCI-H358 cells in immunocompromised mice), peak levels were reached one week after the i.v. injection, which persisted for approximately three weeks [44]. Levels in the blood pool organs (peripheral blood, lung, heart, and kidney) saw notable decreases over a 2-week period, while levels in other organs (liver, spleen, GI tract, muscle, and skin) remained relatively constant over the same period.

Do the improved tissue uptake and retention lead to better target engagement? Using western blot and immunohistostaining, we have confirmed target downregulation (KRAS) in vivo in a dose-dependent manner. Outside the tumor tissue, we have also demonstrated target engagement in the skin, lung, spleen, muscle and liver using additional animal models (data to be published separately). How does the pacDNA overcome key barriers represented by the cell plasma membrane and endosome? We studied the mechanism of cellular uptake with NCI-H358 cells using different endocytosis inhibitors were used to block key pathways. Flow cytometry results confirmed that intracellular delivery of pacDNA was inhibited by low temperature (4 °C), dynasore (an inhibitor of dynamin), amiloride (an inhibitor of the epithelial sodium channel, ENaC), and fucoidan (a competitive ligand for scavenger receptor Class A, SR-A). These findings suggest that SR-A-mediated endocytosis (including both clathrin- and caveolae-dependent pathways) and macropinocytosis were the primary mechanisms of pacDNA uptake [43]. The extent of endosomal escape is unknown and likely at a low level, as the pacDNA does not contain moieties that promote endosomal disruption and escape.

We also investigated the mechanism for the inhibition of target proteins by pacDNA. Interestingly, although the downregulation of the target protein is apparent through western blot analysis, oftentimes there is no corresponding change at the transcript level, which suggests that pacDNA generally inhibits protein expression through the steric block mechanism. This observation may be attributed to the bottlebrush structure blocking RNase H from accessing the mRNA/oligonucleotide duplex.

With moderate cell uptake and endosomal escape but strong tissue uptake and retention, the potency of the pacDNA can still be massively increased compared with chemically modified oligonucleotides. Upon systemic administration to mice with human non-small-cell lung carcinoma xenografts ($KRAS^{G12C}$), the pacDNA showed remarkable potency, resulting in a significant reduction in both KRAS protein expression level and tumor growth rate at a very low dosage level [44]. When compared with a clinical ASO (AZD4785), which targeted the same transcript region, the pacDNA achieved higher levels of tumor suppression even with non-modified (PO) oligonucleotides [45]. Notably, this was accomplished using only a fraction of the dose (2.5%) and with reduced dosing frequency compared to AZD4785. Furthermore, the pacDNA also demonstrated antitumor activity against various KRAS-mutated isoforms. In a $KRAS^{G13D}$ xenograft model, even with only 10% the dosage of AZD4785, pacDNA was able to achieve a comparable level of antitumor response and increase overall animal survival rates.

While conjugation of PEG to biopharmaceuticals has been a widely used strategy to improve the pharmacology of conjugated therapeutic agents, anti-PEG immunoglobins can develop following repeated dosages particularly when the therapeutic agent itself is immunogenic. Indeed, when we administered i.v. a linear PEG-ASO (PS) to mice repeatedly (12 doses over 36 days), high levels anti-PEG IgM and moderate amounts of IgG were developed. On the other hand, the pacDNA generated no measurable immunoglobins when given with same dosage and dosing schedule (Fig. 1.D). Why is the pacDNA uniquely non-immunogenic? The cross-linking of multiple anti-PEG B cell receptors on a PEG-specific B cell can induce a T cell independent response, which is weak and does not usually generate anti-PEG immunoglobins. However, the response can be amplified by co-stimulatory signals via interactions with toll-like receptors (TLRs). Our hypothesis is that the PS oligonucleotide component can provide the co-stimulatory signal. Because the pacDNA's unique binding selectivity greatly reduces TLR activation in B cells, the co-stimulatory signals provided by the PS oligonucleotide is suppressed only for the pacDNA but not for the linear PEG-oligonucleotide conjugate [46]. Indeed, the binding selectivity has proven to be important for reducing most non-hybridization side effects such as coagulopathy and unwanted activation of the immune system.

4. Outlook

In this Perspective, the structures, features, and applications of pacDNA have been discussed. Many of these aspects and concepts are still in their in their early stages of development, presenting both obstacles and novel prospects. Here, we delve into potential future directions of pacDNA, including the possibility of expanding routes of administration, targeting a broader therapeutic area and delivering non-oligonucleotide payloads.

First, in our pursuit to unlock the full potential of pacDNA, it is important to explore alternative routes of administration that extend beyond the predominant i.v. injection. For instance, to tackle the formidable challenges presented by the blood-brain barrier (BBB), which impedes pacDNA's distribution within the central nervous system (CNS) following systemic administration, it is possible to use direct intrathecal (i.t.) administration into the cerebrospinal fluid (CSF) via lumbar puncture. pacDNA is anticipated to offer faster cellular uptake and an extended half-life in comparison to free oligonucleotides, potentially permitting reduced dosages and less frequent administration. This approach will provide an intriguing avenue for utilizing pacDNA's efficacy in treating a wide range of CNS diseases with a genetic basis.

Second, with the ability to target a diverse array of non-liver tissues, pacDNA holds promise for a broad spectrum of therapeutic areas. Currently, pacDNA has predominantly been studied in oncology settings. However, pacDNA's adaptability paves the way for new horizons in a wide range of medical domains to include metabolic diseases, neurology, ophthalmology, muscular disorders, and virology, where oligonucleotide therapeutics exhibit a promising future. It is worth noting that most of FDA-approved oligonucleotide drugs are designed to address rare genetic diseases (Table 1), indicating a compelling research direction for pacDNA.

Third, the pacDNAs discussed so far utilize ASOs as the functional payload. Can it work with other similarly sized payloads, and is there a good reason to do so?

4.1. siRNA

siRNA is a potential therapeutic payload for regulating gene expression with often better specificity, potency, and durability of action than ASOs. However, the mode of action of siRNA appears to prevent it from working with bottlebrush polymers, as the guide strand must engage with the RNA-induced silencing complex (RISC), which can be blocked by the bottlebrush polymer. However, this challenge can be designed around by having the guide strand hybridized to pacDNA that is covalently tethered to the passenger strand. Alternatively, a cleavable linker can be used to release the siRNA from the polymer under a

triggering condition. Using an siRNA that targets *BCL2* mRNA, we designed a bioreductively cleavable pacDNA, which exhibited improved nuclease stability, plasma pharmacokinetics, and tumor uptake/retention, similar to ASO-based pacDNA [47]. The conjugate effectively reduced the expression of BCL2 in vivo, induced apoptosis, and suppressed tumor growth in an SKOV3 xenograft mouse model.

4.2. Aptamers

Aptamers are oligonucleotides that fold into well-defined secondary structures having high binding affinities for their molecular targets. Aptamers are a powerful alternative to antibodies, especially regarding scalability, development cost, and susceptibility to biological contamination. However, aptamers face significant challenges for use outside the ideal conditions in which they are developed. As a result, aptamers have seen limited success in the therapeutics space with pegaptanib as the only approved drug in the U.S. Aptamers are an ideal class of oligonucleotides to combine with pacDNA, as poor blood retention times and unwanted interactions with non-targeted proteins are two of the key difficulties for the in vivo use of aptamers [48]. For aptamers, ideally the pacDNA should minimize cellular uptake and maximize blood retention. On the other hand, one may use aptamers or other targeting ligands to generate tissue tropism for the pacDNA, which may be feasible as the pacDNA can be considered as a stealth nanoparticle.

The optimization of pacDNA structure has led to the discovery that slight modifications to the backbone chemistry can have profound impact on the biological characteristics of pacDNA. This finding has led to the development of a PSP bottlebrush polymer-aptamer conjugate, which maximizes extracellular target binding in vivo. The hydrophilic phosphodiester backbone of the PSP pacDNA resists cellular uptake, and the high-density PEG environment reduces non-specific binding, leading to increased blood retention times and productive binding. Consequently, using a thrombin-binding aptamer (HD1), the PSP pacDNA exhibits superior performance in two anticoagulation mouse models compared to the free aptamer and the PN-based pacDNA [42,49]. Additionally, the anti-coagulation properties of pacDNA are fully reversible with an antidote (complementary sequence to the aptamer), which cannot be easily accomplished with antibodies. These results open up new possibilities for engaging with extracellular targets by combining pacDNA and aptamers.

4.3. Therapeutic peptides

The versatility of pacDNA extends beyond oligonucleotides, allowing for the enhancement of therapeutic potency in peptide drugs. This is particularly beneficial as the application of peptides oftentimes has been limited by dose-dependent toxicity and potential liver/kidney-related damage.

As proof of concept, we conducted a study with melittin (Mel) peptides, which are naturally occurring cytolytic peptides derived from bee venom. Mel has garnered attention as an antitumor agent due to its ability to bind to phospholipids and create pores in plasma and organelle membranes, regardless of cell types [50,51]. Despite the potent antitumor effects of Mel, its clinical application is hindered by significant difficulties, such as inadequate tissue distribution, hemolysis, rapid metabolism, and acute toxicity. For these reasons, Mel is an excellent drug candidate to benefit from the pacDNA delivery technology.

Similar to the bottlebrush structure of prototypical pacDNA, pacMel greatly inhibits the tetramer formation of Mel and its interaction with blood components, resulting in sharply lower hemolysis compared to free Mel at identical concentrations. When dosed systemically, pacMel exhibits prolonged plasma PK and uptake by tumor tissues, and effectively reduces renal and hepatic toxicity of Mel. Remarkably, at doses where free Mel has negligible effects on tumor growth, pacMel demonstrates significant tumor suppressive activity. Furthermore, pacMel shows a more benign toxicity profile, free from typical side effects such as immune system activation and liver damage. Taken together, these results demonstrate that the general pacDNA approach can be extended to non-nucleic acid payloads and provide them a more desirable pharmacological and safety profile while enhancing target-specific activity.

5. Conclusion

In summary, we have shared insights of a novel oligonucleotide delivery platform, pacDNA, which holds promise for developing non-toxic, non-immunogenic, and highly potent oligonucleotide drug candidates. The bottlebrush structure of pacDNA provides it with binding selectivity which reduces the propensity of non-specific and specific protein-oligonucleotide interactions. This feature enables pacDNA to evade recognition by the immune system, thereby reducing the possibility of acute immune system activation and PEG-specific immunity. Additionally, the reduced binding with plasma proteins results in reduced coagulopathy as well as decreased clearance and enzymatic degradation. These features improve the plasma PK of pacDNA, allowing significantly prolonged in-blood circulation. Notably, pacDNA has been observed to effectively reach organs that were previously difficult to deliver oligonucleotides to and engage with intracellular targets. In addition to its enhanced tissue distribution and retention, the increased potency of pacDNA is also attributed to its unique backbone, which enhances cellular uptake by improving materials-membrane interactions. These properties make pacDNA an attractive candidate for achieving efficient gene regulation for a wide range of biomedical use cases.

Initial optimization of the pacDNA structure has revealed a remarkable and unexpected discovery, highlighting the profound impact that minor modifications to the backbone chemistry can have on the biological properties of pacDNA. The tunable nature of pacDNA's structure has opened up avenues for exploring different designs and optimizations to cater to target-specific applications. Structural optimization is an actively ongoing research area and holds promise for advancing the field of pacDNA and its potential as therapeutic development platform. We have strong optimism that the field will experience accelerated growth in the near future.

CRediT authorship contribution statement

Peiru Chen: Writing – review & editing, Writing – original draft. **Yun Wei:** Writing – review & editing, Writing – original draft. **Tingyu Sun:** Writing – original draft. **Jiachen Lin:** Writing – original draft. **Ke Zhang:** Writing – review & editing, Writing – original draft.

Declaration of Competing Interest

KZ has financial interest in pacDNA LLC, a company developing the technology described in the article.

Data availability

Data will be made available on request.

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