Journal of Controlled Release 240 (2016) 302–311

Contents lists available at ScienceDirect

Journal of Controlled Release

journal homepage: www.elsevier.com/locate/jconrel

# Nanotechnology for delivery of peptide nucleic acids (PNAs)

Anisha Gupta [a,1], Raman Bahal [a,1], Meera Gupta [b,c], Peter M. Glazer [a,d,*], W. Mark Saltzman [b,**]

[a] Department of Therapeutic Radiology, Yale University, New Haven, CT, USA
[b] Department of Biomedical Engineering, Yale University, New Haven, CT, USA
[c] Department of Chemical Engineering, Indian Institute of Technology-Delhi, New Delhi, India
[d] Department of Genetics, Yale University, New Haven, CT, USA

ARTICLE INFO

*Article history:*
Received 25 August 2015
Received in revised form 10 December 2015
Accepted 4 January 2016
Available online 8 January 2016

*Keywords:*
Nanoparticles
Peptide nucleic acids
Gene editing
Antisense therapy

ABSTRACT

Over the past three decades, peptide nucleic acids have been employed in numerous chemical and biological applications. Peptide nucleic acids possess enormous potential because of their superior biophysical properties, compared to other oligonucleotide chemistries. However, for therapeutic applications, intracellular delivery of peptide nucleic acids remains a challenge. In this review, we summarize the progress that has been made in delivering peptide nucleic acids to intracellular targets. In addition, we emphasize recent nanoparticle-based strategies for efficient delivery of conventional and chemically-modified peptides nucleic acids.



## 1. Introduction

Nucleic acid structures play an important role in directing transcriptional as well as post-transcriptional events during protein synthesis and their subsequent effects on gene expression. Point mutations in DNA and RNA sequences lead to myriads of diseases, such as various forms of cancer and rare genetic disorders. Hence, small molecule based drugs targeting nucleic acids have attained significant attention as anticancer therapeutics [1–4]. Though promising results have been obtained, off target toxicities produced by small molecules often limit their clinical use. An alternate approach is to target diseases at the genetic level, using the specificity of complementary binding by nucleic acids to minimize off-target effects. Gene therapy approaches rely heavily on targeted reagents, which can recognize DNA and RNA sequences selectively.

Bio-organic reagents have been developed to mimic the chemical features of DNA and RNA but exhibit improved properties. Examples include phosphorothioates [5,6], morpholinos [7,8], and locked nucleic acids (LNA) [9,10]. Rigorous chemical structure optimization has been done on these nucleic acids analogs to provide improved features such as reduced off-target binding, resistance to enzymatic degradation, and improved aqueous solubility. One promising class of reagents is peptide nucleic acids (PNAs) [11,12]. PNAs, which were introduced by Nielsen and coworkers in 1991, are synthetic DNA analogs in which the phosphodiester backbone is replaced with unchanged 2-N-aminoethylglycine units (Fig. 1). Importantly, PNAs are resistant to enzymatic degradation by nucleases and proteases [13]. This feature makes PNAs attractive candidates for numerous therapeutic and biomedical based applications [14]. Additionally, unlike other reagents, the neutral PNA backbone confers strong hybridization properties with its complementary DNA/RNA targets in a sequence specific manner [15,16]. Over the last two decades, PNAs have been extensively studied as antisense (targeting mRNA or microRNAs) as well as antigene agents (targeting genomic DNA) to control the gene expression and regulation [17–19].

Intracellular delivery of PNAs is a challenge [20]. Therefore, a number of strategies have been investigated for enhancing PNA delivery into cells [21–23]. These strategies include electroporation, transfection-based methods, inclusion of cationic peptides, use of chemically modified PNAs, and entrapment of PNA in polymer nanoparticles [24,25]. Though all of these strategies have yielded some interesting results, issues such as selectivity, endosomal entrapment, and unwieldy synthetic procedures limit their broader applications. Here, we review current PNA delivery strategies and their limitations, focusing on the use of nanoparticle-based delivery strategies. Table 1 summarizes recent reports on nanoparticle-based approaches for PNA delivery.

## 2. Peptide nucleic acids and their delivery methods

PNA-based agents have shown promise *in vitro* due to their favorable biophysical properties, but broader applications of PNAs have

* Correspondence to: P.M. Glazer, Department of Therapeutic Radiology, Yale University, New Haven, CT, USA.
** Corresponding author.
*E-mail addresses:* peter.glazer@yale.edu (P.M. Glazer), mark.saltzman@yale.edu (W.M. Saltzman).
[1] The first two authors contributed equally to this work.

http://dx.doi.org/10.1016/j.jconrel.2016.01.005
0168-3659/

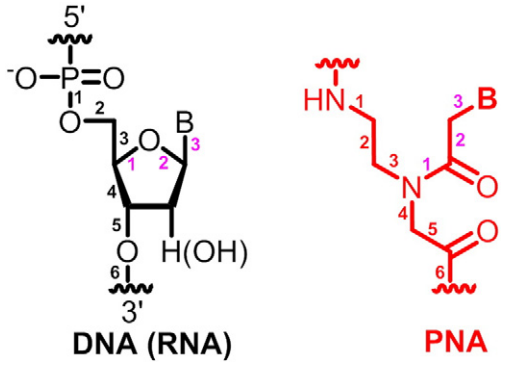


**Fig. 1.** Chemical structures of DNA (RNA) and PNA.

been more difficult to achieve due to their limited cellular uptake. To increase uptake, various methods have been tested for increasing PNA transport across the cell membrane (Fig. 2).

Hanvey et al. first demonstrated the antisense and antigene properties of PNAs by microinjecting the PNAs into fibroblast cells [19]. PNAs were delivered inside cells *via* electroporation for modulating alternative splicing [42] and for induction of human gamma globin gene [43]. Corey et al. found that co-transfection of PNA/DNA complexes can lead to significant uptake in cells [44–47]. Direct permeabilization of cells using streptolysin-O enhances permeability to PNAs, which leads to PNA based mutations in supFG1 reporter gene [48]. These direct delivery methods have been useful in showing the biological activity of PNAs when introduced into *via* mechanical or electrical transduction (microinjection, electroporation and transduction), but they appear to be restricted to small-scale experiments, and likely are not translatable into clinical applications.

Another promising strategy for PNA delivery involves conjugation to cell penetrating peptides (Table 2). However, PNAs delivered *via* conjugated peptides appear to be largely entrapped in endocytic vesicles, where they are degraded or recycled back to the cell surface: escape into the cytoplasm and transport to the nucleus appears limited [49]. On the other hand, one particular approach, using a peptide called pHLIP (pH low insertion peptide) [50,51], appears to be particularly promising for selective delivery to cancer cells. pHLIP undergoes uptake only under acidic conditions through a pH-dependent cellular translocation mechanism. This technology is promising, but is limited to delivery to cells in acidic microenvironments [18].

Further, PNAs have also been conjugated with lipophilic moieties such as adamantyl acetic acid to facilitate their cellular uptake [52]. In addition to adamantyl acetic acid, triphenyl phosphinum based cations have also been used for PNA delivery in the human fibroblast cells [53].

In a different strategy, chemical modifications are introduced in the PNA backbone to impart positive charge characteristics, which facilitates cellular uptake properties [59–61]. These positively-charged groups include guanidinium, which can be placed at the gamma or alpha position on the PNA backbone (Fig. 3).

Studies have shown that PNA-peptide conjugates are more toxic than GPNAs. This is likely due to the amphipathic nature of the PNA peptide conjugates, which result in disruption or permeabilization of cell membranes. Guanidinium containing PNAs are less toxic because they are less amphipathic than PNA peptide conjugates [60].

These strategies have shown promise in intracellular uptake of PNA and in targeting beta-catenin [62] as well as E-cadherin genes [60] which play an important role in WnT pathway. However, PNA backbone modification requires complex chemical synthetic procedures, and will require further optimization for more widespread use.

## 3. PLGA based nanoparticles for PNA delivery

Poly (lactic co-glycolic acids) (PLGA) is a commonly used biodegradable polymer for drug delivery systems and medical devices. It has been approved by both the US Food and Drug Administration (USFDA) and European Medical Agency [63] in a variety of clinical applications [64]. An appealing feature of PLGA is that it degrades by hydrolysis into endogenous non-toxic metabolites (lactic acid and glycolic acid), which enhances its biocompatibility for *in vivo* delivery [65]. Microparticles of PLGA have been used clinically for prostate cancer treatment in the form of Lupron ® and Trelstar ®. Another advantage of PLGA is that the properties of the material can be adjusted, to some extent, by variation of the copolymerization ratio. For example, PLGA 80:20 identifies a copolymer consisting of 80% lactic acid and 20% glycolic acid, which exhibits different physical properties and degradation rates, than homopolymers of poly(lactic acid) (PLA) or poly(glycolic acid) (PGA). Interestingly, PLGA nanoparticles have been shown to facilitate uptake of various macromolecules ranging from proteins, lipopeptides, peptides, viruses, cell lysates and the plasmid DNA into the cytoplasm [64]. PLGA nanoparticles can enhance the activity of vaccines, by providing controlled and targeted delivery of antigens [66]. In addition to delivering purified antigen, PLGA nanoparticles containing tumor lysates have been tested as anticancer vaccines based on tumor-associated antigens (TAAs) [67]. Numerous reports suggest that PLGA nanoparticles can be used to encapsulate antibiotics for more effective antibacterial applications [68].

PLGA-nanoparticles appear to permeate the cell membrane by a combination of fluid phase pinocytosis and clathrin-mediated endocytosis. Cells of the reticuloendothelial system (RES) can phagocytose PLGA nanoparticles and eliminate them from the blood stream, predominantly by uptake in the liver. To prevent this unwanted uptake, PLGA nanoparticles have been decorated with chemical functionalities

**Table 1**
Nanoparticles for delivery of PNA.

| Nanoparticles | Applications | Study design | Ref |
|---|---|---|---|
| PLGA | Gene editing | Cell culture (CD34+ and THP1)<br>Preclinical (in mice) | [26–28] |
| | Antisense | Cell culture (THP1 and HeLa) | [29] |
| Peptide coated PLGA | Antisense | Preclinical (in mice) | [30] |
| PBAE/PLGA | Gene editing | Preclinical (in mice) | [27] |
| MPG coated PBAE/PLGA | Gene editing | Preclinical (in mice) | [31] |
| Avidin-labeled nanoparticles | Delivery vehicle | Preliminary characterization | [32] |
| Zeolite-L-nanocrystals | Delivery vehicle | Cell culture (HeLa) | [33] |
| Mesoporous silica nanoparticles | Antisense | Cell culture (HeLa) | [34] |
| Cationic shell cross-linked knedel-like nanoparticles | Antisense | Preclinical (in mice) | [35,36] |
| | Splice correction | Cell culture (HeLa) | [37] |
| | Imaging mRNAs | Cell culture (RAW 264.7) | [38,39] |
| Gold nanoparticles | RNA detection | Cell culture (Vero cells) | [40] |
| Cobalt ferrite core/metallic shell | PNA/DNA based biosensors | Solution based *in vitro* assay | [41] |