Acta Biomaterialia 50 (2017) 407–416

Contents lists available at ScienceDirect

Acta Biomaterialia

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

Full length article

Efficient tuning of siRNA dose response by combining mixed polymer nanocarriers with simple kinetic modeling

Chad T. Greco [a,1], Victoria G. Muir [a,1], Thomas H. Epps III [a,b,*], Millicent O. Sullivan [a,*]

[a] Department of Chemical and Biomolecular Engineering, University of Delaware, Newark, DE 19716, USA
[b] Department of Materials Science and Engineering, University of Delaware, Newark, DE 19716, USA

ARTICLE INFO

Article history:
Received 9 September 2016
Received in revised form 1 December 2016
Accepted 3 January 2017
Available online 4 January 2017

Keywords:
Polyplexes
Photo-responsive
siRNA dosing
Block copolymers
Gene silencing

ABSTRACT

Two of the most prominent challenges that limit the clinical success of siRNA therapies are a lack of control over cargo release from the delivery vehicle and an incomplete understanding of the link between gene silencing dynamics and siRNA dosing. Herein, we address these challenges through the formulation of siRNA polyplexes containing light-responsive polymer mixtures, whose varied compositions and triggered release behavior provide enhanced gene silencing and controlled dose responses that can be predicted by simple kinetic models. Through the straightforward mixing of two block copolymers, the level of gene knockdown was easily optimized to achieve the maximum level of GAPDH protein silencing in NIH/3T3 cells (~70%) using a single siRNA dose. The kinetic model was used to describe the dynamic changes in mRNA and protein concentrations in response to siRNA treatment. These predictions enabled the application of a second dose of siRNA to maximally suppress gene expression over multiple days, leading to a further 50% reduction in protein levels relative to those measured following a single dose. Furthermore, polyplexes remained dormant in cells until exposed to the photo-stimulus, demonstrating the complete control over siRNA activity as well as the stability of the nanocarriers. Thus, this work demonstrates that pairing advances in biomaterials design with simple kinetic modeling provides new insight into gene silencing dynamics and presents a powerful strategy to control gene expression through siRNA delivery.

Statement of Significance

Our manuscript describes two noteworthy impacts: (1) we designed mixed polymer formulations to enhance gene silencing, and (2) we simultaneously developed a simple kinetic model for determining optimal siRNA dose responses to maintain silencing over several days. These advances address critical challenges in siRNA delivery and provide new opportunities in therapeutics development. The structure-function relationships prevalent in these formulations were established to enable tuning and forecasting of nanocarrier efficiency *a priori*, leading to siRNA dosing regimens able to maximally suppress gene expression. Our advances are significant because the mixed polymer formulations provide a straightforward and scalable approach to tailor siRNA delivery regimens. Moreover, the implementation of accurate dosing frameworks addresses a major knowledge gap that has hindered clinical implementation of siRNA.

1. Introduction

Therapeutic approaches exploiting RNA interference (RNAi) for post-transcriptional sequence-specific gene silencing offer unique opportunities in the treatment of a wide range of acquired and hereditary diseases [1]. Advances in the design of small interfering RNA (siRNA) have facilitated activation of this gene silencing pathway against nearly any target of interest. Additionally, the development of chemical modification strategies for the RNA backbone has conferred enhanced resistance to enzymatic hydrolysis and reduced immune responses through Toll-like receptor pathways [2]. siRNA delivery approaches already have enabled robust knockdown of aberrantly expressed genes in human clinical trials for the

* Corresponding authors.
E-mail addresses: thepps@udel.edu (T.H. Epps III), msullivan@udel.edu (M.O. Sullivan).
[1] These authors contributed equally to this work.

http://dx.doi.org/10.1016/j.actbio.2017.01.003
1742-7061/

treatment of metastatic melanoma [3]. Furthermore, other siRNA-based approaches are under clinical investigation for the treatment of conditions as varied as diabetic macular edema and Ebola [4], indicating the enormous promise of siRNA platforms for a range of applications in human health.

Although the use of siRNA to modulate gene expression holds tremendous promise, numerous delivery challenges have hindered clinical impact. Effective nanocarriers must satisfy seemingly contradictory demands to control binding *vs.* release of the siRNA at various points along the delivery pathway [5]. In particular, several reports have highlighted the importance of maintaining carrier stability in the presence of anionic proteoglycans and nucleases in the extracellular environment to maintain siRNA integrity [6,7]. At the same time, additional studies have reported inefficient intracellular siRNA release as a major bottleneck facing delivery to the cytoplasm to enable interaction with the RNA-induced silencing complex (RISC) [8–11].

To overcome these challenges, many approaches have employed cationic block copolymers (BCPs) that electrostatically bind siRNA and self-assemble into nanoscale complexes (polyplexes). BCPs offer numerous appealing properties including excellent control over chain length, synthetic versatility, low dispersities, and biocompatibility [12,13]. These unique characteristics have been harnessed to improve control over siRNA release using several different strategies. One of the most common strategies is to tune the nucleic acid binding capacity through systematically varying the molecular weight and/or charge density of the cationic block [14,15]. Generally, increasing the number of cationic groups increases the polymer binding efficiency and cellular uptake of the polyplexes; however, increased positive charge also hinders siRNA release in the cytoplasm and results in greater cytotoxicity of the nanocarriers [16,17]. Most studies must compromise and use polymers with intermediate binding forces that balance the above factors to improve nucleic acid delivery [16]. Furthermore, such systematic approaches often require the synthesis of small libraries of polymers, a process that can be both tedious and costly [18,19].

An alternative and more flexible strategy for improving the control of siRNA release is the use of mixed polyplexes assembled from polymers with different block compositions [20]. Modulations to the net cationic charge can be achieved simply by changing the molar ratios of as few as two polymers, allowing for rapid determination of structure-function relationships [21–23]. For example, Omedes Pujol et al. prepared polymeric nanoparticles with varying ratios of two amphiphilic diblock copolymers that differed in their hydrophilic blocks (cationic or neutral) [24]. Mixed micelles that contained greater amounts of the cationic polymer mediated higher levels of gene knockdown, but also were found to be cytotoxic, presumably due to insufficient shielding of the cationic charge in the corona.

Perhaps the most promising strategy to control siRNA release involves the use of responsive materials, whose binding affinity for siRNA can be altered by application of a stimulus [25–27]. Photo-sensitive nanocarriers offer unique advantages such as rapid response, exquisite spatial control with minimal diffusive effects, and tunability of light wavelength and intensity [28–32], ideal for topical wound repair and other regenerative medicine applications [33]. However, although photo-responsive biomaterials have enhanced spatiotemporal control over siRNA delivery, a limited understanding of the dynamic silencing response persists [34]. RNAi depends on numerous biological parameters, such as cell doubling time, as well as mRNA and protein half-lives [35,36]. Diseases characterized by rapid cell division, such as cancer, are difficult to treat with siRNAs because dilution effects exclude the possibility of sustained protein knockdown with a single dose [36]. Thus, multiple doses must be administered to maintain robust gene silencing over a prolonged period of time.

The majority of RNAi protocols and dosing schedules reported in the literature are chosen on the basis of precedence, or through trial and error. Multiple experiments must be conducted to screen for conditions that achieve the desired level and/or duration of knockdown, yet this tedious approach often fails to identify improved dosing regimens [37]. Kinetic modeling can provide critical insights into the underlying causes for these shortcomings. For example, Bartlett et al. used modeling approaches to identify a dosing frequency to induce tumor inhibition in a syngeneic mouse cancer model [37], and these approaches later guided the design of dosing regimens employed in clinical trials [38]. Several other such models also have been published [34–36,39–42]. However, modeling approaches typically require knowledge of numerous kinetic parameters, and modeling has most often been applied to commercial gene delivery systems (e.g. Lipofectamine, Oligofectamine, PEI) [36,39,42], which are incapable of precisely controlled and tunable nucleic acid activity. The development of delivery vehicles capable of externally-triggered siRNA release would provide greater versatility in the timing and magnitude of gene silencing, thereby facilitating the use of streamlined models to predict dosing schedules in regenerative medicine.

Herein, we report the use of mixtures of novel and tailorable mPEG-*b*-poly(5-(3-(amino)propoxy)-2-nitrobenzyl methacrylate) [mPEG-*b*-P(APNBMA)$_n$] BCPs combined with simple kinetic modeling for improved control over gene silencing. The polymers have tunable molecular weights, low dispersities, and photocleavable moieties that permit light-induced charge reversal to initiate nucleic acid release [43]. A nonfouling PEG block was incorporated to provide stability in physiological environments and resistance to opsonization. These BCPs have proven biocompatibility and protect siRNA in salt, serum, and nuclease solutions, while simultaneously stimulating siRNA release and gene-specific knockdown upon application of a cytocompatible photo-stimulus [44].

These properties were exploited to predict and regulate siRNA dosing effects through the formulation of polyplexes containing varying ratios of two different photo-responsive mPEG-*b*-P(APNBMA)$_n$ polymers with cationic block lengths of n = 7.9 and n = 23.6 average repeat units. By tuning polyplex composition and application of the photo-stimulus, the extent of gene silencing was easily controlled and maximized. Furthermore, temporal control over siRNA release, combined with the use of our kinetic model, facilitated the accurate prediction of dynamic changes in mRNA and protein concentrations in response to one dose or multiple doses of siRNA. Thus, we show accurate gene modulation through integration of model-based design and stimuli-responsive control over siRNA application regimens, highlighting a unique method for overcoming limitations prevalent in a variety of RNAi applications.

2. Materials and methods

2.1. Materials

The mPEG-*b*-P(APNBMA)$_n$ (M_n = 7900 g mol^{-1}, n = 7.9; M_n = 13,100 g mol^{-1}, n = 23.6) polymers were synthesized *via* atom-transfer radical polymerization as described elsewhere [43]. All siRNA molecules were purchased from GE Healthcare Dharmacon, Inc. (Chicago, IL). ON-TARGETplus non-targeting siRNAs and glyceraldehyde 3-phosphate dehydrogenase (GAPDH) siRNAs were used as received. Custom-made siRNA (both Dy547- and Dy647-labeled) targeting GAPDH were designed and terminally altered with 5′-P and a fluorophore (sense: 5′ Dy547/Dy647-GUGUGAAC CACGAGAAAUAUU 3′; antisense: 5′5′-P-UAUUUCUCGUGGUUCACA