Silicone Nanoparticles for DNA Drug Delivery

Kendall Pletcher

Bioengineering, Franklin W. Olin College of Engineering

NNIN iREU Site: National Institute for Materials Science (NIMS), Tsukuba, Ibaraki, Japan
NNIN iREU Principal Investigator: Dr. Nobutaka Hanagata, Biosystem Control Group, National Institute of Materials Science
NNIN iREU Mentor: Dr. Svetlana Chechetka, Biosystem Control Group, National Institute of Materials Science
Contact: kendall.pletcher@students.olin.edu, hanagata.nobutaka@nims.go.jp, chechetka.svetlana@nims.go.jp

Abstract:

Because of its role in immune-system activation, high-mobility group box protein (HMGB) has been targeted for treatment of auto-immune diseases. It has been found that single-stranded deoxyribonucleic acid (DNA) fragments, oligodeoxynucleotides (ODN), can competitively bind to HMGB, blocking apoptotic DNA responsible for prompting improper immune responses leading to autoimmune diseases. This study examined the immune response of cells that were stimulated with five ODN of different backbones, sequences, and lengths, either free or in a NP-ODN complex to examine the effect of nanoparticles (NP) on the efficacy of the DNA drug. The NP-ODN complex was found to enhance the suppressive effect of the ODN on interferon alpha (IFN-α) production, but had no effect on the production of chemokine ligand 5 (CCL5). These findings suggest that ODN-HMGB binding mode can effect downstream signal transduction and, additionally, that there is promise in NP design for the purpose of affecting the function of DNA drugs.

Introduction:

Pattern recognition receptors (PPRs) recognize conserved pathogen-associated molecular patterns and activate cells of the innate immune system, playing an integral role in protecting against microbial pathogens. However, the activation of PPRs can also result in harmful immune responses such as in the case of life-threatening inflammation and autoimmunity. One approach to curb such inappropriate immune responses has shown promise: HMGB targeted therapies may suppress innate immune responses due to the role of HMGB in triggering all nucleic acid receptor-mediated innate immune responses [1]. Consequently, it has been suggested that nucleic acids with high binding affinity for HMGB may function as suppressing agents for HMGB-mediated diseases. Thus, competitive binding of ODN to HMGB may have clinical applications for treatment of autoimmune diseases.

This study looks at the immune response-inhibiting effects of six different ODN, examining the effect of backbone, length, and sequence of the ODN on the inhibitory effect observed. Additionally, silicon nanoparticles (Si-NPs) are introduced as delivery agents for the ODN and examined to determine therapeutic effects. The inhibitory effects of each ODN in complexes with NPs and alone are shown and the significance of the findings are discussed.

Methods:

Six single-stranded ODN were used in this study:

1. CG PT (CG sequence, phosphorothioate backbone, 24 base length),
2. GC PT (GC sequence, phosphorothioate backbone, 24 base length),
3. x1GC PD (GC sequence, phosphodiester backbone, 24 base length),
4. x2GC PD (GC sequence, phosphodiester backbone, 48 base length),
5. x3GC PD (GC sequence, phosphodiester backbone, 72 base length), and
6. x3CG PD (CG sequence, phosphodiester backbone, 72 base length).

These ODNs were electrostatically bound onto allylamine-modified Si-NPs with average diameter of 3.4 nm.

Mouse fibroblast cells were treated with free ODNs or NP-ODN conjugates for 1 h, and then B-DNA was added into

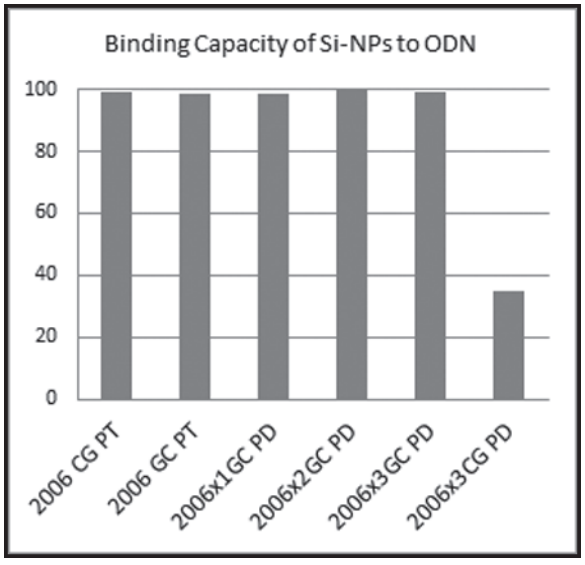

Figure 1: Binding Capacity. 2006x3 CG PD was found to have a much lower binding capacity (35%) than the other five ODNs, of which each sported nearly 100%.

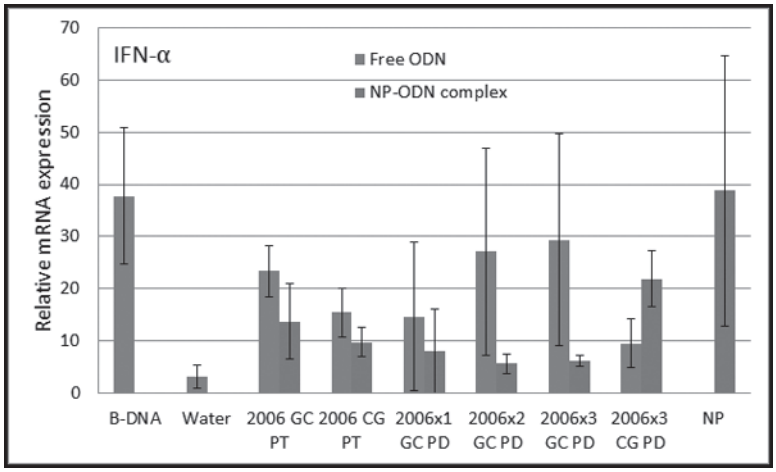

Figure 2: IFN-α Results. NP-ODN complexes were found to express lower levels of IFN-α with the exception of 2006x3 CG PD. No significant differences due to backbone, sequence, or length of ODN were found.

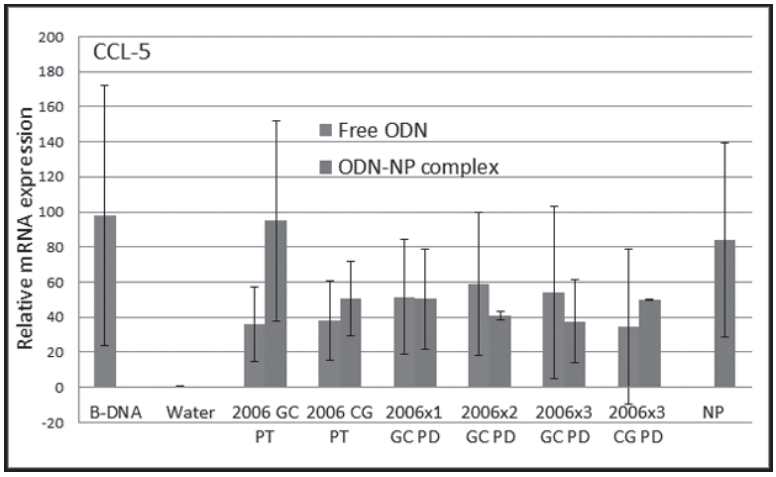

Figure 3: CCL5 Results. NP-ODN complexes were not found to significantly affect the results, as were differences due to backbone, sequence, and length of ODN.

culture medium to stimulate HMGB. After 24 h, total RNA was extracted to examine the expression level of IFN-α and CCL5 genes by using quantitative real time PCR (qRT-PCR).

Results and Discussion:

Binding capacity results showed that four of the ODN used featured nearly 100% binding capacity. However, 2006x3 CG PD that consisted of the 72 base phosphodiester backbone CG sequence featured a much lower binding capacity of 35% (Figure 1).

Results of IFN-α qRT-PCR showed that the cells stimulated with NP-ODN complexes produced less IFN-α relative mRNA expression than those stimulated with free ODN alone (Figure 2). The only exception to this trend was found to be 2006x3 CG PD. The solitary nature of this result implies that the significantly lower binding capacity of the 2006x3 CG PD ODN to Si-NPs affected the results.

Results of CCL5 qRT-PCR showed no significant differences between cells stimulated with NP-ODN complexes compared to those stimulated with free ODN (Figure 3). No significant differences in relative IFN-α or CCL5 mRNA expression were observed due to changes in backbone, sequence, or length of ODN in stimulation.

The effect of the NP-ODN complexes on IFN-α and CCL5 production is significant in its implication of the importance of interaction mode. Due to the difference in responses triggered by free ODN and NP-ODN complexes, there is evidence that the interaction mode between HMGB and ODN can affect the resulting downstream signal transduction. Thus, altering NP conformation and NP-ODN binding mode can be used to alter the production of a single cytokine. Additionally, because the complexes in this study affected production of only one of the cytokines of interest, there is evidence that a single stimulation method can be designed such that it affects the production of several different cytokines in predictable but dissimilar ways.

Conclusions:

Backbone, sequence, and length of the ODN used in stimulation were not found to affect the relative mRNA expression for either IFN-α or CCL5. However, the use of NP-ODN complexes in stimulation were found to increase the inhibitory effect of the ODN on IFN-α production while not clearly affecting the production of CCL5, signifying the importance on binding mode on downstream signal transduction in the HMBG-mediated mechanism of auto-immune disease.

Acknowledgements:

I would like to acknowledge the work of Chinnathambi Shanmugavel and Svetlana Chechetka, who aided the author throughout, and Dr. Nobutaka Hanagata, for his invaluable guidance. This work would not have been possible without funding and support from the National Science Foundation, the NNIN iREU Program, and the National Institute of Materials Science.

References:

[1] Yanai, Hideyuki et al. "Suppression of immune responses by nonimmunogenic oligodeoxynucleotides with high affinity for high-mobility group box proteins (HMGBs)"; Proc Natl Acad Sci USA. 2011 July 12; 108(28):11542-7.