INVESTIGATION OF INFLAMMATION BEHAVIOR OF AMINOGLYCOSIDE-BASED POLYMER LIBRARY

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Introduction

Transfection as we know it can be broken up into two distinct methods. The first is physical. This transfection method typically takes advantage of physical techniques such as cell injection and electroporation. The drawbacks to physical methods of transfection are that it can lead to disruption of cellular membrane, and even cellular death. A second type of transfection includes chemical transfection. This method is widely used, as it is inexpensive and user-friendly. However, the drawbacks include low transfection efficiency, which can negatively impact achieving the target. Typically, transfection is either physical, or chemical. However, in the more recent years, a hybrid method of *in vitro* transfection has been developed. As time progresses, and more research is conducted, polymer-based transfection has the possibility to become the gold standard. However, in order to achieve that goal, significant research needs to be conducted on various synthetically derived polymers. This is the gap of information that the Rege Bioengineering lab intends to fill through investigations into various polymers for a variety of applications.

Objective

The main objective of this study is to synthesize Aminoglycoside-derived parental polymers and lipopolymers to study the interaction these polymers have in vivo. A mouse derived macrophage-like cell line (J774-Dual Cells) was used to investigate the following polymers: Neomycin-GDE Polymer (NG), Paromomycin-GDE Polymers (PG), Apramycin-GDE Polymers (AG), Neomycin-RDE Polymer (NR), Paromomycin-RDE Polymers (PR), Apramycin-RDE Polymers (AR). Interactions will be quantified using both a Quant-blue and Quant-luc assay, along with a LAL endotoxin assay.

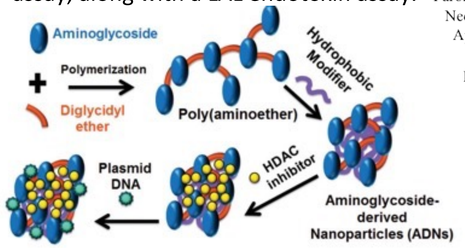


Figure 1: *Illustration depicting lipopolymer formation through utilization of aminoglycoside-derived polymers [1].*

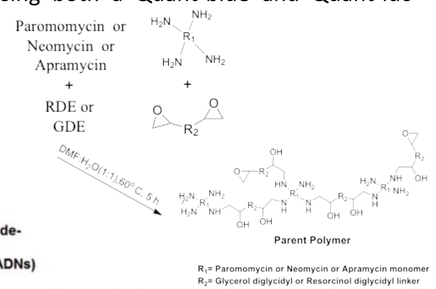
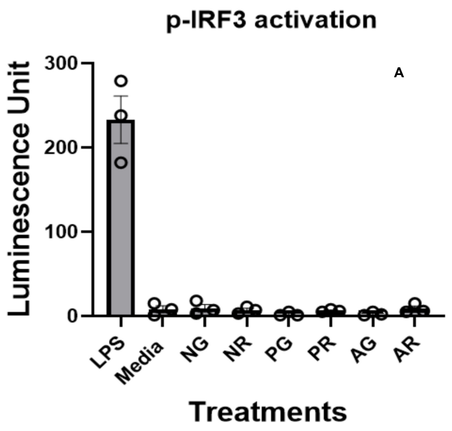
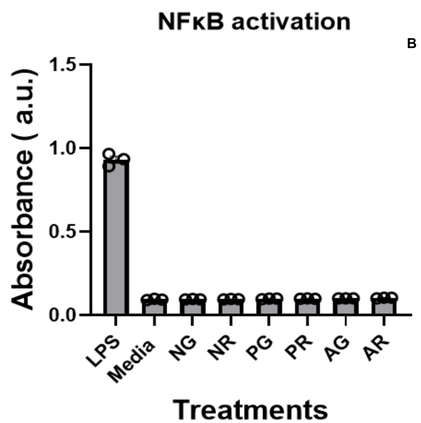


Figure 2: *Illustration depicting a schematic of lipopolymer formations through lipid conjugation on aminoglycoside-derived parental polymers (sch as NG, NR, PG, PR, AR and AG) with different alkanoyls chlorides [2].*

Preliminary Results





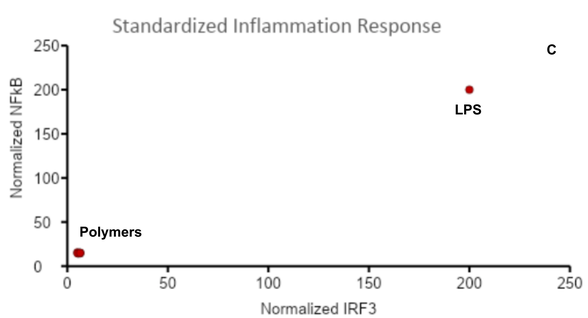


Figure 5: (a) *p-IRF3 activation (b), NF-kB activation, (c) Normalized Inflammation response at 5 ug/uL of polymer.*

Research Methods

J774-Dual Cells were cultured according to the manufacturer's specifications. When cells reached 90% confluency, the cells were harvested and utilized for the Quant-blue and Quant-luc assays to respectively quantify the NF-kb and IRF-luc activation response. After 3 polymer batches are collected, statistical analysis will be conducted to determine the optimal concentration of polymers to use that evoke the lowest inflammation response. During these assays, media was set aside for endotoxin analysis using an LAL assay (figure 3). J774-Dual cells are also NF-kb and IRF-Luc reporter cell lines. LPS and cGAMP serve as positive controls, with free media as a negative control.

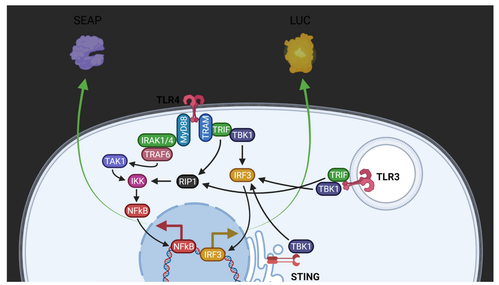


Figure 3: *Illustration depicting immune activation mechanisms (left) [3].*

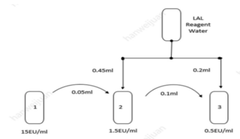


Figure 4: *Illustration of endotoxin assay (LAL assay) protocol (above) [3].*

Further Work

Our goal is to push the understanding of polymer-based transfection with the goal to use this technology for delivery of nucleic acid and small molecule drugs simultaneously *in vitro.* These investigations will lay the foundation for future investigations into other applications, such as use for endoscopic imaging, but will also bring us one step closer to understanding how to treat debilitating diseases, such as Huntington's disease.

Acknowledgements

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References

[1] Godeshala, Sudhakar, et al. "A Library of Aminoglycoside-Derived Lipopolymer Nanoparticles for Delivery of Small Molecules and Nucleic Acids." *Journal of Materials Chemistry B*, The Royal Society of Chemistry, 13 Aug. 2020, https://pubs.rsc.org/en/content/articlelanding/2020/tb/d0tb00924e.
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[3] Turley, Joanna L., et al. "Chitin-Derived Polymer Deacetylation Regulates Mitochondrial Reactive Oxygen Species Dependent Cgas-Sting and NLRP3 Inflammasome Activation." *Biomaterials*, Elsevier, 16 June 2021, https://www.sciencedirect.com/science/article/pii/S0142961221003173.

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