Adjuvant Conjugated Microparticles for Vaccine Delivery: A Robust Method to Promote Immune Response

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Statement of Purpose: The underlying scientific question we are asking is "how can we make subunit vaccines and make them more effective?". Subunit vaccines have been accepted more readily for their solid level of safety. The tradeoff for using a subunit vaccine has been that the level of immune response is dramatically lessened. It is for this purpose that we look to biomimicry. We have created a particle that from the human body's perspective, it looks like an offensive microbe. After our particle creates an immune response, it will then release the subunit vaccine and create immunity.

Methods: Using the Layer by Layer technique, various polymers and ceramics were tested for their suitability. The microparticle is created using a sacrificial Calcium Carbonate core and layered with alternating polyallylamine hydrochloride (PAH) and polyacrylic acid (PAA). The adjuvant Lipopolysaccharide was isolated and then, using a carbodiimide, chemically conjugated to the microoparticle. Once constructed, the vaccine was characterized and tested.

Results: The existing construction technique has been characterized using multiple methods. Quantitatively, the capsule has been tested using Bradford Assays, Limulus Amebocyte Lysate (LAL) Assays, XTT Cell Proliferation Assays, Zeta Potential and Dynamic Light Scattering (DLS). Furthermore, imaging techniques such as Scanning Electron Microscopy (SEM), Transmission Electron Microscopy (TEM) and Fluorescent Microscopy have been used.

The Bradford Assay has been used to quantify protein diffusivity and protein form. The rate in relation to the amount of ionic polymeric bilayers was determined. LAL Assays were used to measure toxicity levels and the concentration of lipopolysaccharide. In addition, these tests helped to determine the concentration levels of the vaccine. XTT Cell Proliferation Assays to measure the cell viability when the vaccine was exposed to a model cell line. Various concentrations of the vaccine were exposed to the cells for varying lengths of time. The Zeta Potential was used to quantify the surface charge of the particle and to confirm successful application of the Layer by Layer technique.

Microscopy techniques were used quantitatively to measure the size of the vaccine particles, the size of the polymer bilayers, as well as the batch variability of size. Qualitatively, SEM, TEM and Fluorescent Microscopes were used to evaluate the overall shape of the particle. By conjugating the subunit protein to a fluorescent marker, fluorescent microscope techniques were used in order to confirm that the protein was encapsulated successfully. Results show optimal characteristics for cellular uptake. *In vitro* cell studies using HeLa cells showed that the cells remained viable at several concentration levels. These results support our hypothesis: conjugating an adjuvant to a polymeric capsule assembled by the Layer by Layer technique allows for the use of a subunit vaccine to be successfully delivered without the use of a viral delivery system. Furthermore, we can support that using these techniques shows promise in promoting adaptive immunity.

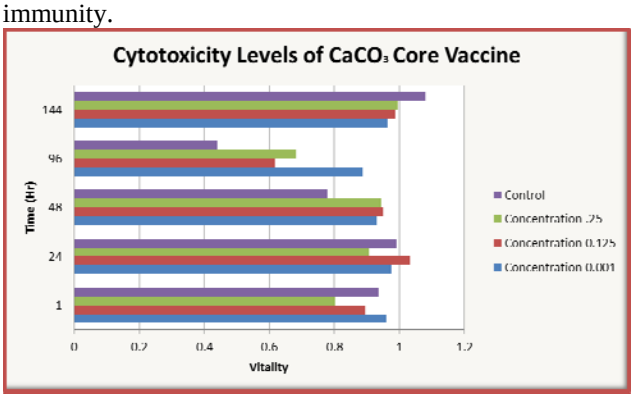

Figure 1. XTT Cell Proliferation Assay on a vaccine particle containing a lipopolysaccharide termination. These results show positive results of the cell's exposure to the vaccine over the course of several days.

Conclusions: Construction of a unique microparticle for vaccine delivery has been developed in this study. The use of this microparticle system for vaccine administration offers a more effective vaccine system. This study can conclude that further testing of this vaccine vehicle is appropriate. Further testing will include looking at cellular cytokine production, and antibody production.

The relevance of this study is that a safer and more effective vaccine delivery system is possible. Throughout its history, vaccines had first been constructed using live attenuated viruses. These treatments often caused patients side effects that today would be considered severe and unacceptable, but it saved lives from even more deadly consequences. As time progressed, vaccines had been created from viruses and bacterium that were dead. This allowed the adaptive immune system to be able to create an adaptive immunity as well as to drastically reduce side effects. Public outcry however calls for a safer method. Subunit vaccines have since been created where no part of the pathologic had been in contact with the vaccine. The problem with subunit vaccine is that often the immune system does not recognize them as dangerous and therefore no effective long term immunity is retained. Our research aims to combine the safety of the subunit vaccine while creating effective, long-term immunity. We do this by the use of adjuvant. In this study, isolated lipopolysaccharide is chemically conjugated to the surface of our vaccine particle in order to mimic an offensive pathogen. Once the immune system is alerted to the presence of the vaccine system, the subunit protein is then released, and the adaptive immunity is then recorded. Furthermore, the subunit vaccine can be used for both

cancers and pathogenic agents that we wish to promote an immune response and disease prevention.