**Fighting the Flu: Pain-free administration of a cross-protective subunit Influenza vaccine**

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**Statement of Purpose**:

The influenza virus is a respiratory pathogen that infects between 5-20% of Americans every year. For the 2018-2019 influenza season, the flu vaccine was reported to be 45% protective (Centers for Disease Control). Additionally, yearly flu vaccination coverage can be low due to the flu shot being given via a potentially painful intramuscular injection. Therefore, based on the performance of past years' marketed flu vaccines being administered via injection, the proposed project utilizes a universal influenza vaccine that was administered via laser ablation. The aim of the research was to investigate the efficacy and protectivity of a cross-protective (universal) matrix-2 protein (M2e) antigen, which is conserved in all Influenza A strains. To improve the immunogenicity of the protein, M2e was formulated into tandem repeats to produce M2e virus-like particles (VLPs). To improve uptake of the antigen by immune cells, the M2e VLPs were entrapped into a biodegradable microparticles, which are better engulfed by antigen-presenting cells (APCs). Additionally, the use of a sustained release polymer provides a long-term release of the antigen from the particulate matrix, which prolongs antigen-presentation in the body. The particles were assessed for their *in vitro* immunogenicity and cytotoxicity prior to being administered transdermally to assess the *in vivo* efficacy of the vaccine in a preclinical murine model.

**Methods**:

The antigens and adjuvants (MPL-A® and Alhydrogel®) were combined with pre-crosslinked bovine serum albumin (BSA) and encapsulated into polymeric microparticles using a Buchi spray dryer. The formulated particles were characterized for their physical characteristics and assessed *in vitro* for their nitric oxide production, cytotoxicity, and the ability to stimulate the expression of antigen-presenting molecules, major histocompatibility complexes (MHC) I and II and their respective co-stimulatory molecules CD 80 and CD 40. *In vivo*, mice were vaccinated transdermally using laser ablation technique. Serum samples were collected throughout the animal studies in order to assess immunoglobulin G (IgG) levels using enzyme-linked immunosorbent assay (ELISA). At Week 12, the mice were challenged with a live A strain of influenza (A/Philippines/H3N2). Post-challenge, the mice were sacrificed, and their lymph nodes and spleens were harvested to assess expression of immune markers such as CD4 an CD8. Lung homogenates will be assessed for the expression of CD4 and CD8 (cell-mediated immunity) using flow cytometry and IgA (humoral immunity) using ELISA. Influenza viral titers in lung homogenates will be evaluated in vaccinated and unvaccinated mice using a viral plaque assay.

**Results**:

The spray-dried M2e-loaded vaccine microparticles were approximately 3 microns in size. *In vitro* results confirmed that dendritic cells exposed to M2e vaccine microparticles led to the production of significant levels of nitric oxide. For assessment of vaccine safety, APCs were pulsed with varying concentration of vaccine microparticles to assess cytotoxicity. Cells treated with vaccine microparticles showed at least 80% viability across all concentrations. Cells that were exposed to M2e + adjuvant microparticles expressed significantly higher levels of MHC I/CD80 and MHC II/CD40 compared to the control and M2e particles without adjuvants. Vaccinated mice also demonstrated elevated levels of IgG at weeks 4, 7, and 10 that were statistically higher compared to unvaccinated mice.

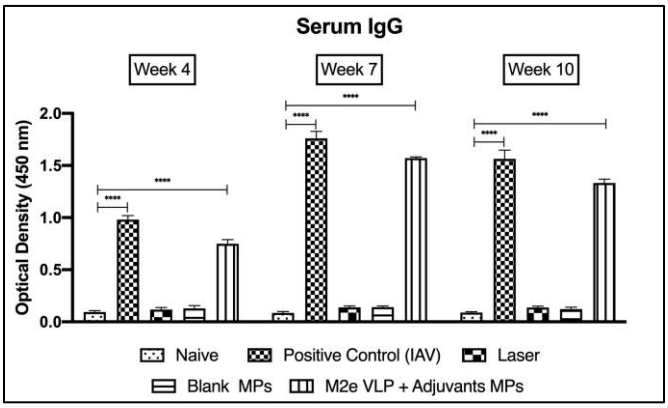


*Figure 1: Serum IgG levels in mice sera of various groups after immunization.*

**Conclusion**:

Currently, marketed influenza vaccines can face many hindrances pertaining to cross-protectivity, vaccination coverage, and cost. In contrast, the proposed influenza vaccine containing the M2e VLPs and adjuvants in biodegradable microparticles were easily administered transdermally in a preclinical mouse model. Additionally, the vaccine was shown to be stable and capable of producing M2e-specific antibodies. Furthermore, in a microparticulate form, the vaccine can be stable at room temperature eliminating the need for cold-chain storage. Lastly, the use of laser ablation was shown to be a non-invasive route for delivering the vaccine microparticles, which can eliminate the costs for cold-chain storage, vials and syringes.

**References:**

(Deng L. *Vaccines (Basel)*. 2015;3(1):105-136.) (Chen X. *Expert Rev Vaccines*. 2013;12(11):1313-1323.)