Development of needle free transdermal microparticulate vaccine for Coronavirus Disease

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Statement of Purpose:

Coronaviruses are zoonotic viruses known to cause mild to serious upper respiratory tract illnesses in both animals as well as humans. The causal agent of COVID-19, the SARS CoV-2 is a novel pandemic strain, which has caused global chaos. Consequently, there exists an immediate need for an effective vaccine that is reproducible, safe, and scalable. This study aims to test the immunogenicity of a novel heat-inactivated coronavirus microparticulate (MP) vaccine administered via dissolving microneedles (MN). The vaccine formulation utilizes a microparticulate matrix that provides protection of the antigen and improved antigen uptake in antigen-presenting cells. Furthermore, the addition of adjuvants – alum and MF59, can enhance the overall immune response to the antigen. To enhance patient compliance and to eliminate painful needle administration, a novel transdermal route of administration via microneedles was chosen for delivering coronavirus antigen in a microparticulate matrix.

Methods:

The MPs were formulated as a $W_1/O_1/W_2$ emulsion using probe homogenization. In brief, the W_1 phase consisted of the antigen in an aqueous phase, which was added to an oil phase consisting of PLGA (poly (lactic-co glycolic acid)) to produce the primary emulsion. The primary emulsion was then added to the W_2 phase, which contained PVA (polyvinyl alcohol). Next, solvent evaporation was carried out to remove the excess organic solvent thereby hardening the MPs which was then concentrated by centrifugation. Finally, the antigen-loaded PLGA MPs were lyophilized to obtain a free-flowing dry powder. The formulated MP were characterized for their physical properties including size, charge, and morphology. In vitro studies were performed to assess the innate immunity using a nitrite assay to determine the production of nitric oxide. The ability of the antigen to elicit an adaptive immune response was assessed in vitro using flow cytometry. Here, the expression of antigen presenting molecules MHC I and MHC II and their corresponding co-stimulatory molecules CD80 and CD40, on the surface of dendritic cells were evaluated upon exposure to the MP vaccine. For in vivo assessment of the vaccine formulation, the mice were immunized with the MPs via the skin (transdermal) using dissolving MN patches, formulated using Hyaluronic acid (HA). The efficacy of MP vaccine is being tested in 6-8 weeks old Swiss Webster mice. A prime, followed by two booster doses of vaccine were administered to the mice at weeks 0, 2, and 4 respectively. Sera of mice were collected at various points to determine the presence of total IgG antibodies using ELISA. At the end of week 12, the animals will be sacrificed and the immune organs including spleen and lymph nodes will be isolated to analyze the expression of CD4+ and CD8+ T-cells to evaluate the induction of humoral and cellular adaptive immune response.

Results:

The MP were less than 5 microns in size. The Griess's nitrite assay showed a significantly higher ($p<0.05$) release of nitric oxide (NO) by antigen presenting cells (APCs), in response to being stimulated by the vaccine, with and without adjuvants, as compared to untreated cells. Flow cytometry analysis confirms, significantly high ($p<0.05$) expression of antigen presenting molecules MHC I and MHC II and their co-stimulatory molecules CD80 and CD40 respectively on the surface of APCs, in response to being pulsed with the MP with and without the adjuvant, as compared to untreated cells. ELISA was done to test the IgG levels in sera of immunized mice. The total IgG levels of the vaccine + adjuvant groups were significantly higher ($p<0.05$) than the group that received no treatment.

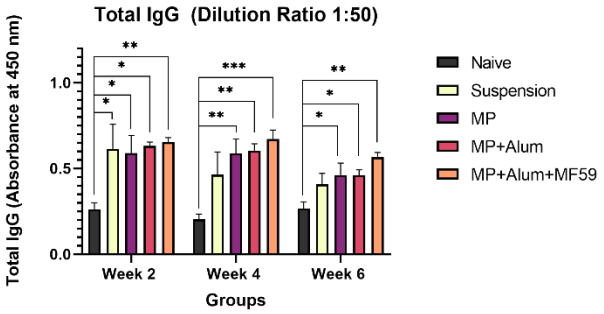

Figure 1: Total IgG levels in immunized mice sera.

Conclusion:

The microparticulate vaccine produces an effective innate and adaptive immune response as indicated by the significant release of nitric oxide and expression of antigen-presenting molecules by the APCs. Additionally, the microparticulate vaccine enhances the immunogenicity of the heat-inactivated coronavirus, by conferring cross-presentation of the antigen on the surface of the APCs. Moreover, the vaccine is also capable of producing neutralizing antibodies which is crucial for vaccine development. Further analysis of serum and T-cell phenotypes from immune organs in mice will confirm if the vaccine can produce a robust immune response.

References:

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