Undergraduate Thesis Prospectus

A Novel Production Method for Industrial Manufacturing of an Inactivated COVID-19 Vaccine Using Vero Cells

(Technical Research Project in Chemical Engineering)

Opposition to Vaccination in The United States

(Sociotechnical Research Project)

by

Mucui Lin

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Technical Project Collaborators:

Aba Acquaah
Kevin Macera
Paul Imbrogulio
Sara Richardson

On my honor as a University student, I have neither given nor received unauthorized aid on this assignment as defined by the Honor Guidelines for Thesis-Related Assignments.
Mucui Lin

Technical advisor: Eric Anderson, Department of Chemical Engineering

STS advisor: Peter Norton, Department of Engineering and Society

General Research Project

How can the spread of infectious diseases in the United States be prevented?

The rise of urbanization has been tied to the increased infectious disease incidences (Mayer, 2007). Substandard living conditions and travel networks allow exotic pathogens to flourish and to be transported to new geographic locations. With the increased chance of an epidemic, the United States must possess ample medical supplies to respond. The feasibility of scaling up a vaccine production process depends on the virus characteristics and the existing production process. Yet, vaccination resistance has hampered the response.

A Novel Production Method for Industrial Manufacturing of An Inactivated COVID-19 Vaccine Using Vero Cells

How can the COViD-19 vaccine be produced more effectively?

The Coronavirus Disease-19 (COVID-19) was declared a pandemic by the World Health Organization on March 11, 2020 (Cucinotta & Vanelli, 2020). This disease is caused by Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2), a novel human coronavirus, that has spread to almost every country in the world. To date, there have been 46.3 million cases of COVID-19 across the globe, 1.2 million of which have resulted in death (Dong, Du, & Gardner, 2020). In addition to the severe death toll, the coronavirus has posed enormous economic, environmental, and social challenges to the entire human population. Business shutdowns due to the pandemic have led to the highest unemployment rate in the US since the Great Depression (Dadyan, L. et al., 2020); many have struggled to afford housing and food, and have lost employer-sponsored health insurance. The negative effects of the pandemic have disproportionately impacted minority populations concerning race, ethnicity, age, socioeconomic status, geographic location, chronic illness, presence of a disability, and employment status (George, 2020). Therefore, it is paramount that an equitable solution is made widely accessible as soon as possible.

Vaccinations offer a safer method of achieving herd immunity in a population than direct exposure to the virus itself. The design team will focus on an inactivated viral vaccine as a solution to the COVID-19 epidemic. Inactivated viral vaccines gained popularity once they could be synthesized outside of a host organism, referred to as in vitro, which increased the scale of manufacturing. All of these vaccines follow a similar method of production that includes pathogen cultivation, inactivation, and purification.

The development of a safe and effective vaccine has become a global priority as nations struggle to slow disease transmission. Currently, 47 vaccine candidates are in the human trial phase of development, however, meeting the global need for billions of doses of COVID19 vaccines is an additional challenge (WHO, 2020). This technical project aims to scale-up the production process of a promising vaccine candidate to accommodate global demand to eventually end this pandemic.

The design goal of the project is to create a novel production process for the inactivated virus bulk of a whole virion inactivated SARS-CoV-2 vaccine being developed by Bharat Biotech in India (Ganneru, 2020). The process will encompass upstream and downstream processing. Upstream processing will include a stepwise cell growth process from a cell seed train and bioreactor to produce SARS-CoV-2 virus particles in Vero cells CCL-81 (Ganneru, 2020). Vero cells CCL-81 are derived from

African Green Monkey kidney cells (Kiesslich, 2020). Vero cells for a cell seed train will come from frozen stock and will be scaled using multiple scale-up apparatus such as NUNC cell factories. NUNC cell factories provide a platform for cell growth due to the necessity for Vero cells to adhere to a surface during growth (Kiesslich, 2020). Media for cells will be based on low serum and low protein solution to simplify downstream processing. Once the cell density is sufficient, the inoculation of a microcarrier based bioreactor for monolayer growth of mammalian cells will occur (GE, 2016). Microcarriers are small bead-like particles allowing for anchorage-based growth for suspended cell cultures (GE, 2016). The bioreactor will be optimized by adjusting parameters such as agitation rate, oxygenation rate, and temperature for scale-up purposes. Post cell growth that meets the criteria for inoculation of the virus, the virus stock seed will be added to infect the Vero cells in solution (Kiesslich, 2020). Virus particles will be extracellularly produced due to Vero cells bursting making an extra cell lysing unnecessary. The solution containing virus particles will be passed to downstream processing for purification and inactivation of the virus.

The lysed cell solution will first undergo centrifugation to remove cell debris. The virus-containing supernatant is retained. This solution is passed through a depth filtration system for further clarification from cell debris and residual particles. Depth filtration is utilized as opposed to traditional microfiltration due to the absence of cake accumulation in the membrane, thus allowing higher throughput while maintaining a requisite purity in the effluent. Simulation analysis for membrane-based alternatives will be investigated. In either case, the principle of separation is size exclusion; the particle size of SARS-CoV-2 is approximately 50-200 nm (Chen, Zhou, et. al, 2020), which will be used to guide filter/membrane selection. The filtration effluent is concentrated and further purified using an anion exchange chromatography column, and subsequently precipitated with polyethylene glycol (Hagan, 2000). This pellet is then repeatedly resuspended with inactivation solvent, rendering a concentrated virus stock. Specifically, the stock is coprecipitated with aluminum hydroxide adjuvant, allowed to settle, decanted, then resuspended again. The inactivation component utilized in the Bharat Biotech formulation is β-propiolactone (Ganneru, 2020). Further, Bharat reported the generation of increased levels of neutralizing antibodies in their aluminum adjuvanted vaccines, improving antigen immunogenicity. Thus, the inactivated virus stock is treated with aluminum hydroxide diluent to single-dose volumes. Dosages currently being studied are 3 and 6 μg.

Monitoring of virus inactivation is essential at each step in the purification process for product safety, quality, and FDA compliance (U.S. Food and Drug Administration, 2019) for US market distribution. Inactivated virus samples will be assessed for cytopathic effects. Transmission electron microscopy (TEM) is used to validate the intact structure and presence of inactivated virus particles. Western blot analyses will be used to characterize the identity of specific antibodies from the inactivated virus. Samples drawn from various processing stages will be probed for anti-Spike (S1 and S2), anti-RBD, and anti-N proteins, with their corresponding bands used to validate high product purity.

The design team will investigate the COVID-19 vaccine production process during the fall and spring semesters. A Design Basis Memorandum (DBM) will be produced by the end of the fall semester, which contains a summary of the design work and the problem statement for the spring semester. The data necessary for the design will be based on prior research, several provided by Professor Michael King, and the purification process will be designed with recommendations from Professor Giorgio Carta. The work will be divided into different sections and they are assigned according to each person's strengths. There will be weekly meetings within the group to update the team on the progress and to ask for help on any problems that may arise.

Opposition to Vaccination in The United States

How do social groups use social media to influence public opinion on vaccines in the United States?

Infectious diseases have plagued mankind since the beginning of time, but the invention of vaccines has reduced the incidence of morbidity and mortality considerably (Poland et al., 2001). Despite its advantages, vaccination is still a controversial topic. Anti-vaccination movements have existed almost since the origin of vaccination in the 1800s, when the first smallpox vaccine was invented (Stern et al., 2005). After significant smallpox case reduction in the 1830s, a vociferous anti-vaccination movement emerged in the United States and Europe (Kaufman, 1976). Consequently, preventable diseases remerged, resulting in the loss of herd immunity. Herd immunity is the resistance to the spread of an infectious disease within a community that is based on vaccination and/or prior illness. To protect those that are medically exempt from vaccination, 85-95% of the population needs to be vaccinated for herd immunity to be effective (Stern et al., 2005).

Before the internet, information was spread through newspapers, telegraphs, or word of mouth. Since the emergence of the internet, news spreads almost instantaneously, regardless of its validity. Social media platforms, such as Facebook, allow people to spread their views on a global scale with a single post, and to create virtual communities of like-minded individuals who seek out information sources with which they feel comfortable. This causes confirmation bias, polarizing the view even further (Larson et al., 2014; Joubert et al., 2019). To complicate matters, most social media do not enforce quality control due to a lack of rewards (Joubert et al., 2019). Consequently, contents are produced by experts and quacks alike, and opinions and facts become blurred, impairing judgment. To improve user experiences, major social media platforms like Facebook and Twitter have set community standards and updated policies. Despite policy reforms, social media platforms are still filled with misinformation, rising skepticism, and contributing to vaccine misinformation (Doustmohammadi et al., 2020).

Relevant social groups include anti-vaccination groups, pro-vaccination groups, pharmaceutical companies that produce vaccines, and government agencies. Many of the anti-vaccination arguments are based on incorrect explanations of immunity, parental responsibilities, or a general distrust of expertise (Kata, 2009). Anti-vaxxers often fall into confirmation bias, selecting and sharing scientific information from open-access journal articles on social media to escalate uncertainty in the broader population. Parental responsibilities frequently led to a rise of safety concerns and a desire for additional vaccine information: “what the FDA fails to include is whether it’s okay to inject multiple vaccines simultaneously” (Morcan et al., 2019). Occasionally even doctors themselves question the validity of vaccines: Dr. Leonard G. Horowitz stated that “The greatest lie ever told is that vaccines are safe and effective” (Connecticut General Assembly, 2019).

To many critics, vaccines have many side effects that outweigh the benefits, but to others, vaccines are safe and necessary. To prevent the reemergence of infectious diseases and to maintain herd immunity, pro-vaccination groups, such as the Immunization Alliance, have committed to raising immunization levels by increasing vaccine awareness and optimizing communication between the public and the health officials (AAP, n.d.). Regulatory organizations and companies have provided additional information to the public to guarantee vaccine safety. WHO has stated that one of the common misconceptions about immunization is that "vaccines cause many harmful side effects, illness, and even death – not to mention possible long-term effects we don't even know about" (WHO, 2013). Pharmaceutical companies disclose vaccine information for transparency demands (Mellow et al., 2012). Both regulatory organizations and pharmaceutical companies will continue to share information to dispute misconceptions.

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