CNTVac: HIV vaccine using Carbon Nanotubes
(Technical Project)

The difference in FDA regulation between CNT-based vaccines and conventional vaccine methods
(STS Project)

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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.

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Introduction

Human immunodeficiency virus 1 (HIV-1) is a virus that attacks the immune system by destroying CD4 T cells, a type of immune cell, rendering them ineffective in protecting the body (Chen, 2019). In 2022, there were 650,000 deaths from HIV-related causes, 39 million people living with the disease globally, and 1.3 million people who acquired it (World Health Organization, 2023). While the annual amount of people who acquire HIV has decreased by 38% since 2010, in which 2.1 million people acquired HIV, these statistics indicate the need for an effective vaccine for HIV. Currently, there is no such vaccine from conventional vaccine delivery methods. Therefore, it is important to explore new methods of vaccine delivery for HIV that work more effectively than current methods. The development of a new vaccine delivery method brings concerns about the policies implemented to regulate it.

Two main challenges arise when creating a vaccine for HIV-1. Firstly, the virus has a high rate of mutation during viral replication which means that antigen is highly variable (Ng'uni et al., 2020). The antigen is the part of the virus that the antibodies in the body target and therefore, this means that the virus is highly genetically diverse. This diversity makes it difficult to create a universal vaccine that can be used globally. Secondly, conventional vaccine types raise the risk of retaining the HIV virus upon administration (Ng'uni et al., 2020). While this risk is possible for other viruses such as influenza, the implications for contracting HIV from a vaccine are far more severe because there are cures for the flu but not HIV.

The technical aspect of this project researches one vaccine delivery candidate – carbon nanotubes – as a vehicle for a mRNA vaccine of HIV-1. Messenger ribonucleic acid (mRNA) is genetic material that can be coded into proteins of interest by the organism. Carbon nanotubes (CNTs) are nanoscale materials made up of carbon from graphite sheets and are rolled into

hexagonal mesh structures that can be layered into single-walled (SWCNTs) or multi-walled carbon nanotubes (MWCNTs) (Anzar et al., 2020). Additionally, since COVID-19, interest in mRNA vaccines has increased because these vaccine types take less time to design, test, and mass produce vaccines compared to deoxyribonucleic acid (DNA)- or viral-based vaccines (Xu et al., 2023). The technical project seeks to address the issue of no suitable vaccine for HIV.

The implementation of a new vaccine delivery method means that policymakers need to create new policies that assess the vaccine's safe use, manufacturing capabilities, and ethical impact. The Food and Drug Administration (FDA) is the US agency that assesses the safety of new medical drugs and devices such as vaccines. They create policies regarding use of the vaccines. The STS aspect of this project specifically explores how the FDA regulations of CNT-based mRNA vaccines differ from conventional vaccine delivery methods. For the vaccine developed in the technical project to be implemented, the FDA first needs to approve the vaccine and only then will the vaccine be able to address the larger issue of HIV prevention.

Technical Project

Currently, there are no viable HIV-1 vaccines on the market. Conventional vaccine types, such as live-attenuated vaccines and inactivated vaccines result in ineffective methods of preventing HIV-1 through narrow immune responses, retaining viral properties, and latent viral reservoirs. Live-attenuated vaccines are created by live viruses that are weakened which create a response by replicating in the body. These vaccines cause a milder infection and the immune response from these vaccines is identical to natural infections. Due to this property, live-attenuated viruses risk the ability to retain viral properties and cause severe infections in patients with a weakened immune system as in HIV-1 (Wodi & Morelli, 2021).

Additionally, due to the variation of the HIV-1 antigens, the human immune system cannot elicit a broad enough antibody response using live-attenuated vaccines (Hemelaar, 2013). Inactivated vaccines are not live and therefore cannot cause the disease. However, they elicit a weaker and shorter-lasting immune response than live-attenuated vaccines and mRNA vaccines and therefore require multiple doses (Wodi & Morelli, 2021). This is ineffective for HIV which requires a stronger response. Additionally, latent viral reservoirs allow inactive HIV-1 viruses to remain in the body undetected by the immune system until they are activated, upon which they can infect the person (Churchill et al., 2016).

In recent years, mRNA vaccines have become a viable alternative to these conventional methods for HIV-1. There are several advantages to using mRNA technology in this application. First, the body already has the machinery to translate mRNA into the glycoprotein antigens that are targeted by the immune system. The immune system is divided into two systems: innate and adaptive immunity. Innate immunity refers to the first line of defense and targets any foreign material in the body, making it nonspecific. Adaptive immunity takes over if the innate immune system cannot eradicate the substance and it takes on a more specific response through antibodies ("The Innate and Adaptive Immune Systems," 2020). Research shows that proper control over HIV in the body requires targeting not only the antibodies but also the innate immunity through a type of immune cells called CD8+ T-cells. mRNA can activate both immune systems in a balance to create a safe and effective response.

Short carbon nanotubes (CNTs) are a novel vaccine delivery approach being developed at Luna Labs for mRNA vaccines. They have demonstrated a non-toxic and effective delivery platform which is a potential alternative to other conventional vaccine delivery methods (Xu et al., 2022, 2023). The goal of the technical project is to develop an effective needle-free intranasal

HIV-1 vaccine candidate. My team and I will optimize the ratio of mRNA that encodes the VIV2 glycoprotein antigen on the delivery vehicle CNTs and will be tested for the effectiveness and cytotoxicity of the vaccine formulation. To accomplish this goal, we will size and functionalize the carbon nanotubes for optimal interaction with Calu-3 epithelial cells as the intranasal/mucosal delivery model. We will conjugate HIV-1 mRNA at varying ratios and test the level of cellular uptake and integration (transfection) of mRNA into the cell's genetic makeup with conventional methods, antigens alone, and CNTs alone. We will test the surface functionalization of the CNTs by analyzing various functional groups.

By experimenting with antigen ratios and surface functionalizations, we hope to develop both an injectable and intranasal vaccine that can be internalized into immune cells to provide a robust immune response against HIV-1, despite its biodiversity. By using CNTs, we hope to elicit a stronger immune response as compared to current vaccine methods as well as decrease the cytotoxicity of the vaccine. This vaccine will provide an important method of prevention for a disease that up until now could only be treated and not prevented.

STS Project:

CNTs are a new technology in the bioengineering field and their long-term effects are still unclear. This raises many concerns about what aspects the FDA focuses on to approve vaccines derived from these CNTs. FDA approval is needed for widespread use of a vaccine in the United States. Therefore, it is important to understand the implications of CNTs on the vaccine approval process. For the STS project, I ask the question: how do CNT-based vaccines compare to conventional vaccines in terms of FDA regulations? This question is important to ask because it addresses concerns of the relevant social groups involved in vaccine production, the implementation of the CNT-based vaccine, and the risk-benefit analysis of CNT-based vaccines.

The FDA is a political agency with multiple social groups that affect its vaccine policy. Technology is often constructed based on the interests of relevant social groups and this can be reflected in its approval processes as well (Pinch & Bijker, 1984). These social groups are the groups of people that use, make, and implement the technology. They give meaning to the technology. Relevant social groups that influence the FDA's vaccine approval process include high-risk individuals, biotechnology companies, and bioethics committees (Meissner et al., 2018). High-risk individuals have concerns about the stigma associated with HIV, vaccine hesitancy following COVID-19, and preferred method of delivery (Ramvikas et al., 2017). Addressing these concerns can affect how the vaccine is designed and therefore introduce new elements for the FDA to analyze. Biotechnology companies also influence the FDA because ultimately they are funding the vaccine production and manufacturing, both of which are typically assessed in vaccine approval (Center for Biologics Evaluation and Research, 2020). Bioethics committees are a relevant social group that influences the FDA's approval process. These committees have concerns over the ethics of the production procedures and animal testing (Rasmussen & Ebbesen, 2014). I will analyze the influence of each of these groups through a literature review and discourse analysis of each of these groups using the Social Construction of Technology (SCOT) framework. SCOT argues that people shape the technology that is made and implemented in society. Understanding how these groups affect the production of the vaccine leads to understanding areas of concern for the implementation of the vaccine.

Understanding how the FDA regulations of CNT-based vaccines compare to conventional vaccines is important because it governs how the vaccine is implemented for use. Implementation rests on understanding the safety of CNTs. The FDA expresses its interest in understanding CNT properties through studies to understand the effects, applications, and

efficacy of CNTs. The FDA has funded research concerning the toxicity of the CNTs in the body (Orecna et al., 2014). The FDA has also funded research looking into an application for CNTs in drug delivery for cancer (Mahmood et al., 2013). Research into the efficacy of CNTs in nanomedicine is also funded by the FDA (Zhang et al., 2014). I will analyze each of these studies through a literature review to generate a list of characteristics that the FDA specifically is concerned about in CNT technology and not in conventional methods. By comparing FDA regulations for CNT-based vaccines and conventional methods, I will understand the safety areas of concern for the FDA that determine how the vaccine is implemented.

It is important to understand in what ways FDA regulations of CNTs compare to conventional methods because it provides a risk-benefit analysis for this vaccine. Vaccine research turned to the use of CNTs because conventional methods failed. The FDA emphasizes that the introduction of an adjuvant such as CNTs should not be "introduced into a product unless there is satisfactory evidence that it does not affect adversely the safety or potency of the product" (*Guidance For Industry For the Evaluation of Combination Vaccines for Preventable Diseases; Production, Testing, and Clinical Studies*, 1997, p. 4). The FDA is not only looking for successful prevention of the virus of interest but also that it results in more benefits than risks. While this is also a concern for conventional methods, the addition of CNTs introduces more variables that can magnify the benefit or risk compared to conventional methods. Additionally, the risk-benefit analysis may differ when comparing different populations and different diseases (Da Silva et al., 2015). These differences can limit the use of CNT-based vaccines and affect the manufacturing and licensing of CNT-based vaccines. I will analyze the FDA regulations for conventional methods and adjuvant vaccines through policy analysis to

understand the main areas of difference. I will determine how different populations are affected by CNT technology and how this affects FDA approval through literature review.

Conclusion:

For the technical project, I will prepare CNTs with the proper size, antigen ratio, and surface functionalization molecules for best HIV-1 mimic and initiate cellular transfection studies of the optimized CNT vaccine. This is important to research to understand the potency of the HIV vaccine in comparison to other forms. The urgency for a vaccine is illustrated by the fact that there is no current vaccine for a high-prevalence disease. For the STS project, I will understand what considerations are taken into account when the FDA approves CNT-based vaccines in comparison to conventional vaccines. This is important because as more CNT-based vaccines are researched and produced, it is important to understand how policy will contribute to the distribution and availability of the vaccine. In the United States, vaccines need to be approved by the FDA. If I can understand what aspects of the vaccines will be analyzed with higher scrutiny, the production of vaccines can be tailored to target these goals. Ultimately, these two projects will increase the protection against the global disease of HIV.

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