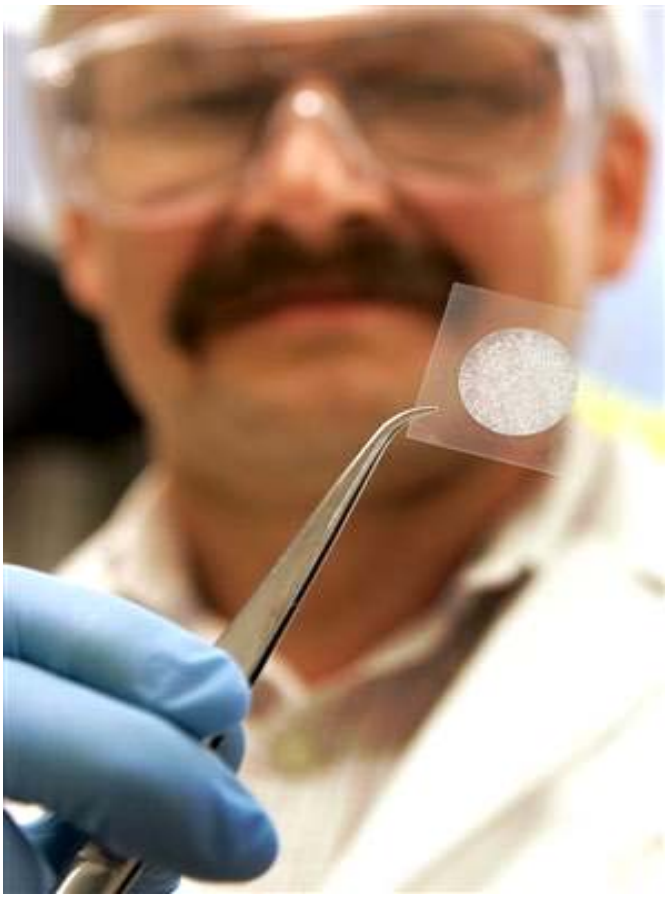
Patch Immunization:

Transcutaneous Vaccination for the Cholera Toxin and Optimization of Immunization Cycles



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BEE/MAE 453

Table of Contents

Executive Summary

The main point of this analysis was to investigate the diffusion of the cholera vaccination through specific layers of the skin. The antigen was initially modeled through the skin directly to the blood stream. The antigen was also modeled with the presence of a network of Langerhans cells. There was a smooth concentration profile in the skin after one week of patch exposure in the absence of the LC network. However, there was discontinuity in the concentration profile when the LC network was present. The LC network functioned as a large enough sink term that the flux into the bloodstream was virtually zero. Therefore, we concluded that the LC network alone can create a cutaneous immune response. The LC network was enhanced with the presence of *Imiquimod*, a typical immune response modifier. The modifier increased the activity of the LC network, thus increasing the reaction rate of the LC cells. With Imiquimod there was a sharper discontinuity in the concentration profile at the LC network and the antigen flux into the blood stream is zero.

The most effective enhancer tested was the MEMs microneedles, which increased the porosity of the skin and thus the diffusivity of the antigen through the skin. Contour plots of the skin showed absolute diffusion and consumption of the antigen into the LC network, while only partial consumption with the other enhancers tested. Concentration gradients were present in the ultrasonically and photo mechanically enhanced skin because they had weaker enhancing capabilities compared to the MEMs needles. The MEMs needles are the most effective in mass transfer, but are also the most evasive.

Vaccines are usually given in cycles to increase the concentration of the antigen in the skin and bloodstream. When the patch was applied to the skin with no enhancer, the maximum concentration was achieved after 2.3 days. However, the maximum concentration in the skin is achieved sooner with the various adjuvants. For example, when the patch is applied with MEMs needles, the maximal concentration is achieved in the skin only after 1.2 hours of exposure. Immunization cycles presented in Glenn et al were simulated to determine the approximate concentration of the antigen at the center of the skin needed for an immune response. This concentration is 0.0038 mol/m^3. Therefore, it was assumed that if the concentration in the skin is close to this value, then an immune response will be initiated. The immunization cycles for each adjuvant used were then optimized.

Introduction and Design Objective

This project is aimed towards analyzing and optimizing an effective method for human transcutaneous immunization against the cholera toxin. The causative agent of cholera, *Vibrio cholerae*, generates acute diarrhea in infected individuals in which, without proper treatment, death is inevitable. A relatively ancient disease, the first acute cases were documented in Calcutta, India in the early 1800's. Since then, over seven epidemics have occurred in densely populated areas of South America, Great Britain, China and sub-Saharan Africa. Over the last century, Cholera occurrences in the Western world are rare, but it continues to plague Third world countries, especially in South Asia. Today, over eight million patients suffer from Cholera in just South India alone. With high mortality rates, augmented by unsanitary water, it is essential to improve vaccines and delivery.

Vibrio cholerae infects humans by consumption of raw meats, drinking of infected water, or contamination from fecal matter. The curved-rod shaped bacteria, once inside the host, colonize the lumen of the small intestine. There, the secreted Cholera toxin initiates a cascade that produces a rapid flux of bodily fluids into the small intestine, resulting in massive diarrhea. Due to the loss of fluids, patients exhibit dehydration, vomiting and muscle cramps. Intravenous solutions are used to hydrate patients and intense antibiotic therapy is immediately commenced.

The Cholera toxin, shown in Figure 1, belongs to a family of structurally similar bacterial enterotoxins. These toxins are hexameric and consist of AB_5 subunit structures. The A subunit usually has enzymatic properties that play an essential role in the disease pathway. The pentameric B subunits usually play a targeting role. Toxins that are included in this family include: shiga toxin, pertusis toxin, and diphtheria toxin. Also, the heat-labile E. coli toxin has AB_5 morphology and has 80% sequence homology to the Cholera toxin. (Zhang et al., 1995)

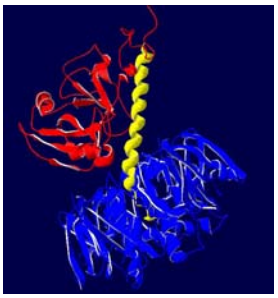


Figure 1: Side view of the cholera toxin. The red subunit, designated as the A1, has a catalytic domain, which is connected to the yellow A2 α-helix. A1 and A2 are produced from one polypeptide chain, but they are cleaved during toxin processing. The A2 polypeptide penetrates the core of the blue pentameric B subunit.

Transcutaneous immunization involves the application of a thin patch to skin to expose a patient to an antibody proliferating antigen. Studies conducted by Glenn et al reveal that a mixture of the dissociated subunits of the cholera toxin when administered as a vaccine produces a strong immune response in mice. In this study we will use the beta subunit as our antigen. The presence of the beta subunit in the skin will cause an immune response and the production of antibodies. For details on a transcutaneous immune response, refer to Figure 2 in the appendix.

***Situation I*-Antigen Modeled to the Blood Stream**

The patch will be placed on the epidermis of the skin and the antigen will diffuse through the dermis and into the bloodstream. As depicted in figure 3, we modeled the patch as a circle, so that we are able to simplify the model using axisymmetric properties. The circular path dimensions will have the same area as the square path used by Glenn et al. in their study. We will include an additional area of skin around the patch in our analysis to understand the effects of lateral diffusive mass transfer, as shown in Figure 4. We will assume that the skin acts as a porous material and we will use Fick's law of diffusion to model mass transfer.

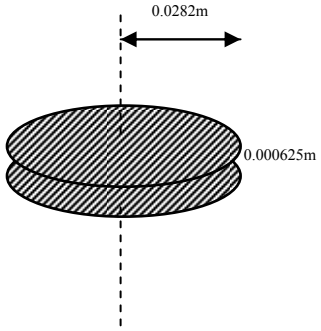


Figure 3: The patch size designated in Glenn et al. is a square patch with 0.05m side. The area of the square path was converted to a circular patch with radius of 0.0282m and thickness of 0.000625m.

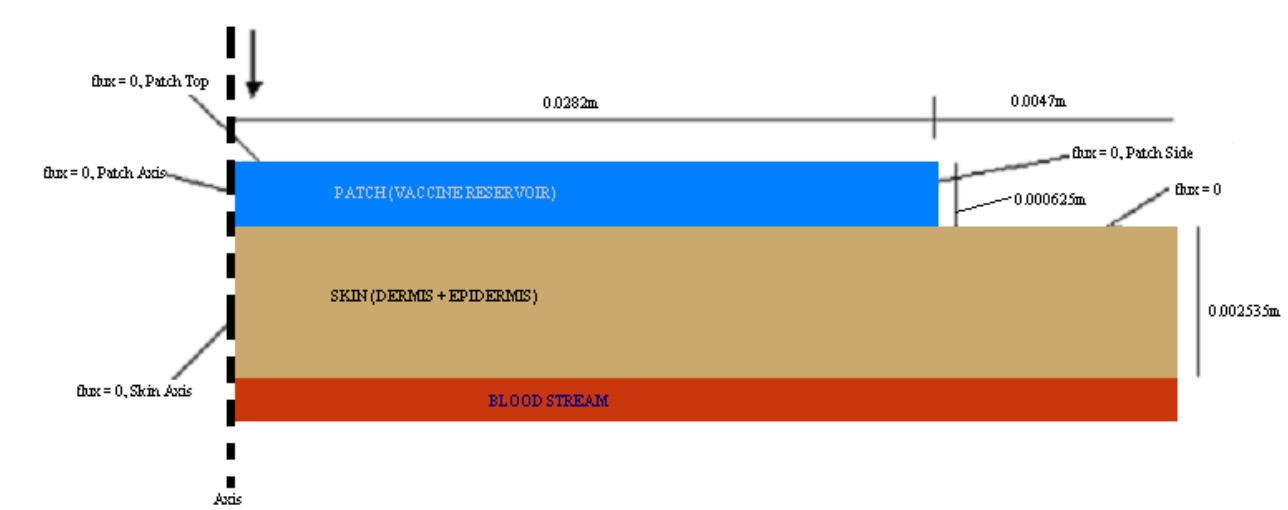


Figure 4: Schematic of simulation with a patch containing an initial concentration of the vaccination, the skin, and the blood stream. Boundary conditions and layer dimensions are specified.

We made numerous assumptions in modeling the diffusion of the antigen to the blood stream. We assumed that the diffusivity coefficients were constant within each layer. The epidermis is the rate-limiting layer in the skin. There is no metabolic/consumption reaction within the patch and skin. Skin dermis and epidermis dimensions are average values. The patch occupies very small area of overall skin surface. The system is isothermal. Blood flow at the skin-dermis interface immediately removes the antigen. The diffusion coefficient in the drug reservoir is equal to the diffusion coefficient in adhesive layer.

***Situation II*-Antigen Modeled to the Langerhans Cell Network**

Studies by Suzuki et al reveals that the antigen does not need to reach the blood stream for an immune response. Active immunity can occur at the cutaneous level. The dermis is replete with immune cells called Langerhans Cells (LCs) which are in close proximity to the most powerful superficial layer of the skin called the stratum corneum. These cells are derived from a macrophage-monocyte precursor in the bone marrow. This allows us to model the LCs with macrophage cellular kinetics (including the rate of antigen uptake and the reaction kinetics that follow). These Langerhans Cells form a monolayer network of immune cells that are evenly distributed throughout the suprabasal layer of the epidermis. The network underlies 25% of the skin's total surface area and has no direct physical contact between dendritic processes. When LCs carry antigen to the draining lymph nodes, a systemic immune response is induced. To achieve immunity, the cholera antigens must migrate and bind to regularly ordered networks of langerhans cells of density of 800 cells/mm^2. The Langerhans cells carry antigens to the draining lymph nodes where systemic immune responses are induced. Therefore, as long as the concentration of antigen is enough in the superficial layers of the skin, active immunization will occur. Figure 5 is a schematic of situation two, where the langerhans cell network is depicted by the yellow mesh layer.

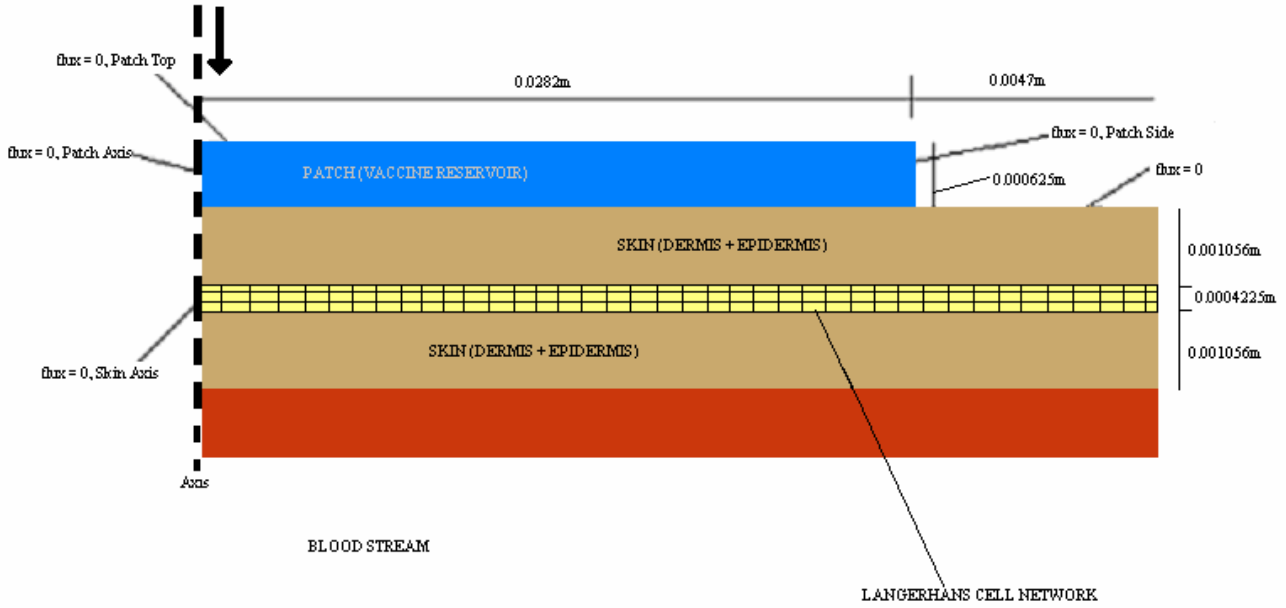


Figure 5: Schematic of situation 2 where the patch is placed on the skin and the antigen diffuses through the first layer of skin to the Langerhans Skin Network, then passes through the rest of the skin into the blood stream.

Situation III-Antigen Modeled to Langerhans Cells with *Imiquimod*

Immunological adjuvants were originally described as substances that will be used in combination with a specific antigen that can produce more immunity that the antigen alone. In medicine, adjuvants are special agents that can modify the effect of other agents while having no direct effects when given by themselves. An adjuvant can be roughly thought of as a chemical catalyst for a reaction. In immunology, they are often used to augment the efficacy of the vaccine by stimulating the immune system to respond to the vaccine with much more vitality that it would without the adjuvant. The presence of adjuvant followed by vaccination will greatly increase the innate immune responses to the antigen by augmenting the activities of such biological components as the dendritic cells, lymphoctyes, and macrophages. Imiquimod as a adjuvant has shown to enhance the cuteneous immune response while having anti-viral and anti-tumor effect. After treatment of imiquimod, the density of the Langerhans Cells decreased by 43%. This decreased number of langerhans cells in the skin is the direct result of enhanced migration of the cells from the skin to the lymph nodes. Even though the density of the LC network has been so drastically reduced, the immunization level will remain the same or will increase since the LC sensitivity is greater.

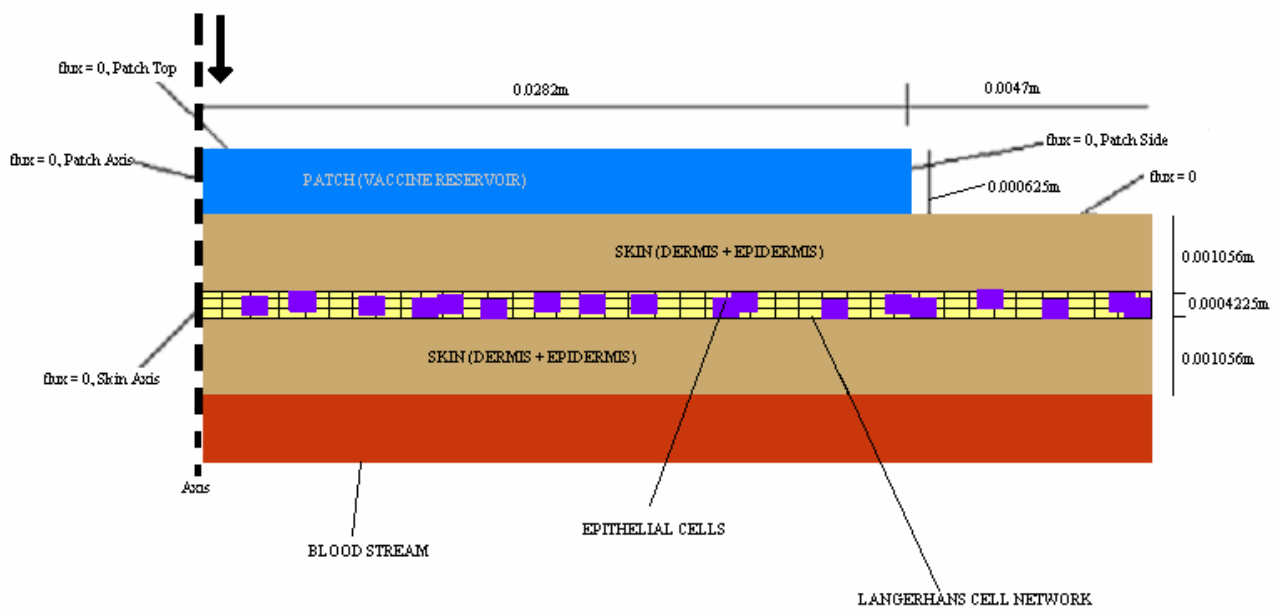


Figure 6: Schematic of situation 3 where a patch is placed on skin. The antigen diffuses through the first skin layer into the Langerhans Cell Network. Here, it is picked up by highly active cells while passing epithelial cells that have taken the place of the moving langerhans cells. The antigen then passes through the rest of the skin into the bloodstream.

Situation IV-Antigen Modeled with Physical Disruption of the Patch-Skin Barrier

MEMS-based fabrications allows for the development of micron-scale projections (approximately 150mm) which can penetrate the outermost layers of the skin. Devices containing microfabricated silicon projections called microenhancer array (MEA) have been used to deliver vaccines epidermally. The device mechanically breaches the skin barrier and enables the topical delivery of the vaccine without the need for complex or potentially unsafe formulations. In this simulation, the device is used to mechanically disrupt the skin, then putting our patch over the mechanically disrupted section of skin. The permeability of the skin is being effectively increased by increasing the poreosity of the skin.

By applying ultrasound at therapeutic frequencies (~1 MHz), growth and oscillation of air pockets present in the keratinocytes of the stratum corneum is induced. This proven phenomenon is known as cavitation. The oscillations produced will disorganize the stratum corneum lipid bilayers. This disorganization enhances transdermal transport. In order to apply the ultrasound, a transducer is applied to a patch of skin (about 1 cm^2 at a time) while oriented perpendicular to the skin. A safe distance of 1 cm from the skin was used to hold the transducer. Human skin permeability to substances such as insulin was found to be much higher after the ultrasound was applied.

When rapid transcutaneous delivery is desired, a mechanical stress pulse generated by a single laser pulse is shown to transiently increase the permeability of the stratum corneum in vivo. After the application, the stratum corneum is shown to have fully recovered within minutes. Within those few minutes, the increase in permeability of a section of skin allows macromolecules to diffuse through the epidermis and dermis of the skin. Whereas the action of the ultrasound (above) is primarily mediated by heat and cavitation, photomechanical wave effects are caused by mechanical forces.

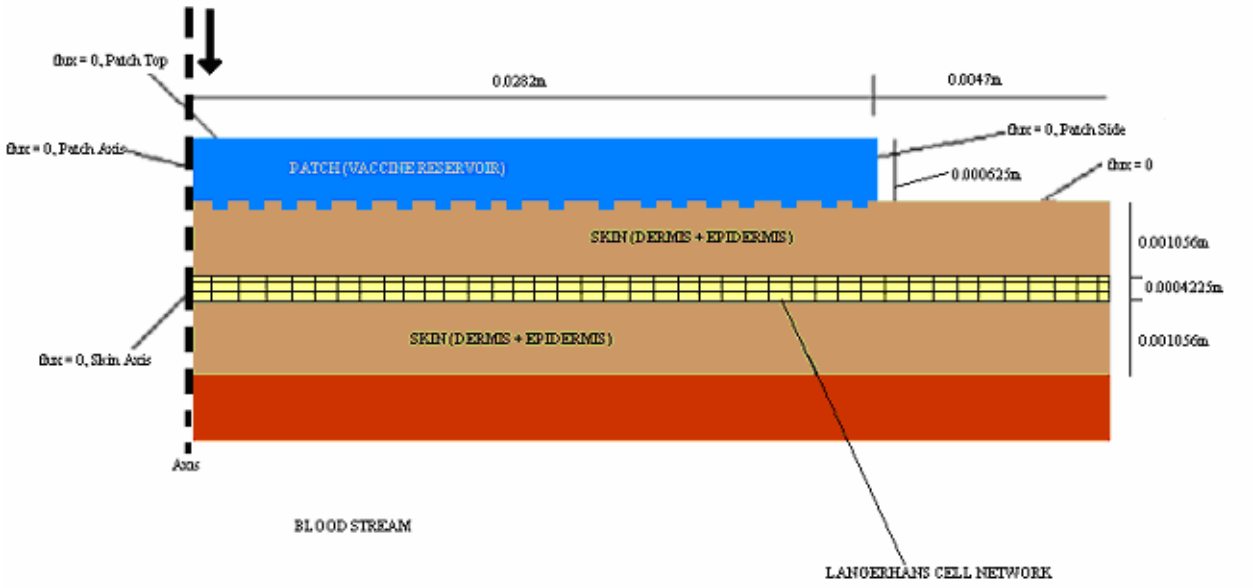


Figure 7: Schematic (in general) of the 3 different types of the situation in which the skin barrier is penetrated. The antigen has a higher diffusivity through the skin because of this decreased barrier.

Table 1 below summarizes the input parameters in modeling the four situations and their respective references.

Parameter	Value	Reference
Diffusivity antigen in patch	$D_P = 1.15 \times 10^{-10}$ m^2/s	Antigen modeled through hydro gel. Value taken from previous BEE 453 reports.
Diffusivity antigen in Skin	$D_D = 6 \times 10^{-12}$ m^2/s	Value was taken from BEE 453 reports from previous years for the diffusion of large proteins/drugs in skin.
Diffusivity antigen through LC network	$D_{LC} = 3 \times 10^{-12}$ m^2/s	Modeled as equivalent situation of drug diffusing through neuronal networks in brain. (Robinson et al, 1990)
Diffusivity of skin after MEMs needles	$D_{MEMs} = 6 \times 10^{-9}$ m^2/s	Mikszta et al., 2002
Diffusivity of skin after ultrasonic enhancement	$D_{ULTRA} = 3 \times 10^{-11}$ m^2/s	Mitragotri et al., 1995
Diffusivity of skin after photomechanical enhancement	$D_{PHO} = 1.68 \times 10^{-11}$ m^2/s	Lee et al., 1998
1st order rate constant, K for LC network	$K = 5.5 \times 10^{-6}$ m^2/s	Modeled from first order kinetics of phagocytes. (Leijh et al., 1979)
1st order rate constant, K for LC network with the presence of Imiquimod	$K = 1.1 \times 10^{-5}$ m^2/s	Modeled from data presented in publication. (Suzuki et al., 2000)
Initial concentration of antigen in skin	$C_{D, initial} = 0$ mol/m^3	There is no toxin in the skin before application of the patch.
Initial concentration of antigen in patch	$C_{D,patch} = 0{,}01739$ mol/m^3	Modeled as the patch used in Glenn et al.

Table 1 summarizes the input parameters.

Design Objectives

The purpose of this design project is to model the diffusion of the cholera antigen in the human skin to improve our understanding of the transcutaneous immunization. We will first characterize the diffusion of the antigen from the patch to the blood stream, where the concentration of the antigen is considered zero. We will then determine how this process is affected by the inclusion of a Langerhans network, which acts as a sink term for the antigen. To further characterize the Langerhans network's affects on mass transfer, we will incorporate the adjuvant, Imiquimod, into our model. Various other adjuvants or enhancers will be incorporated into our model to see how changing diffusivity of the skin affects mass transfer with the presence of a Langerhans network.

After characterizing the different situations presented above, we will shift our focus to analyzing immunization cycles for the cholera vaccine. We start by modeling the immunization cycles presented in Glenn et al. Patients received 500 μg of cholera antigens in 500μl of saline, adsorbed on a 0.05 x 0.05 m^2 single-ply polyester-rayon gauze pad with polyethylene backing covered by a 10 x 12 cm^2 Tegaderm dressing. The patch was placed on the upper arm for 24 hours, after which it was removed and the site was rinsed with 500mL of sterile saline. Individuals were re-immunized after 24 hours. From modeling this situation, it is possible to determine the maximum concentration in the skin necessary for an immune response, since Glenn et al observed

a four fold increase in antibody concentration in the blood. Then, we will model the same immunization cycles for the adjuvants and enhancers. We then optimize the immunization cycles for these various adjuvants.

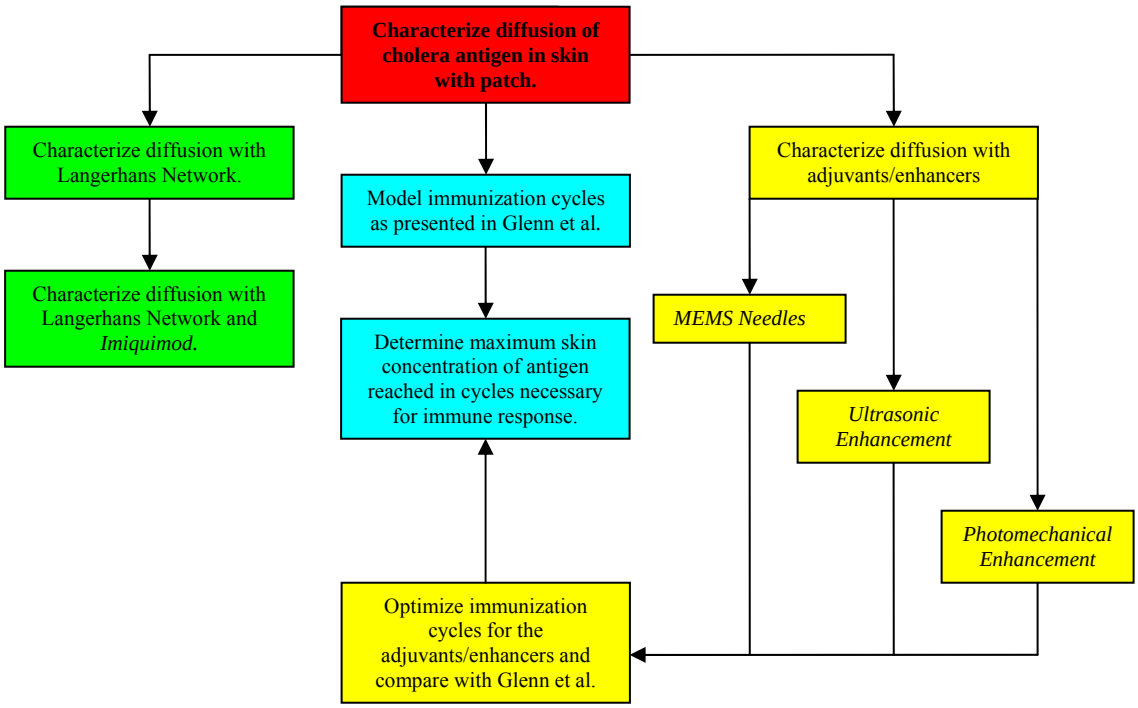


Figure 8: Overall scheme of project.

Results and Discussion

Figure 9 is the mesh we used to model the antigen diffusion through the skin. Higher densities of elements were used at the patch-skin interface to accurately model the transfer. We used a structured mesh to shorten computation times. Figure 10 is the mesh we used to model the antigen diffusion through the LC network and to the blood stream. We used an unstructured mesh to produce detailed contours in the LC network and patch-skin interface.

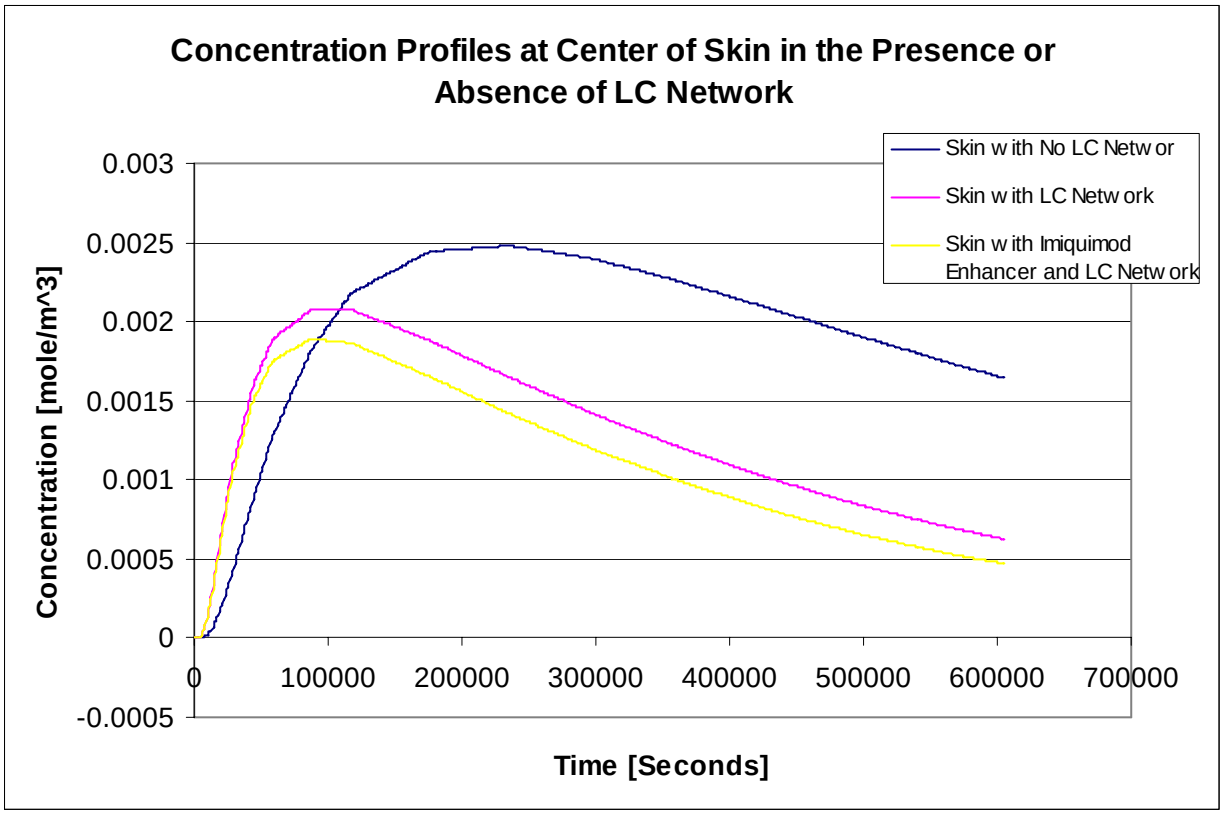


Figure 11: Cross sectional concentration profiles with the LC network included or excluded in the modeling analysis.

Figure 11 reveals the concentration profile at the center of the skin after the patch has been applied over a period of one week. When we do not include the LC network in our model, the concentration of the antigen peeks at about 2.3 days. When we include the LC network, the concentration profile is shifted to left, indicating that peak concentrations are achieved in the skin at short time periods. The decrease in peak antigen concentration is attributed to the sink nature of the LC network. When Imiquimod is added as an enhancer to the skin, the LC network is hyperactive and the sink nature of the network is increased. This is shown with a downward shift in the concentration profile shown in figure 11.

Figure 12 is a contour map of the diffusion of the antigen to the Langerhans Cells with the presence of *Imiquimod*, a typical immune response modifier. The modifier increases the activity of the LC network, thus increasing the reaction rate constant. According to figure 12, there is a sharper discontinuity in the concentration profile at the LC network and the antigen flux into the blood stream is virtually zero.

We now move to the second part of our results involving the maximization of immunization trials. Vaccines are usually given in cycles to increase the concentration of the antigen in the skin and bloodstream. It is important to note that by simply placing a patch on a patient for many weeks will only result in a certain maximum concentration. This concentration may not be sufficient for an immune response. Figure 13 exhibits this result. When the patch is applied to the skin with no enhancer, the maximum concentration is achieved after 2.3 days. However, the maximum concentrations in the skin are achieved sooner with the various adjuvants. For example, when the patch is applied with MEMs needles, the maximal concentration is achieved in the skin only after 1.2 hours of exposure.

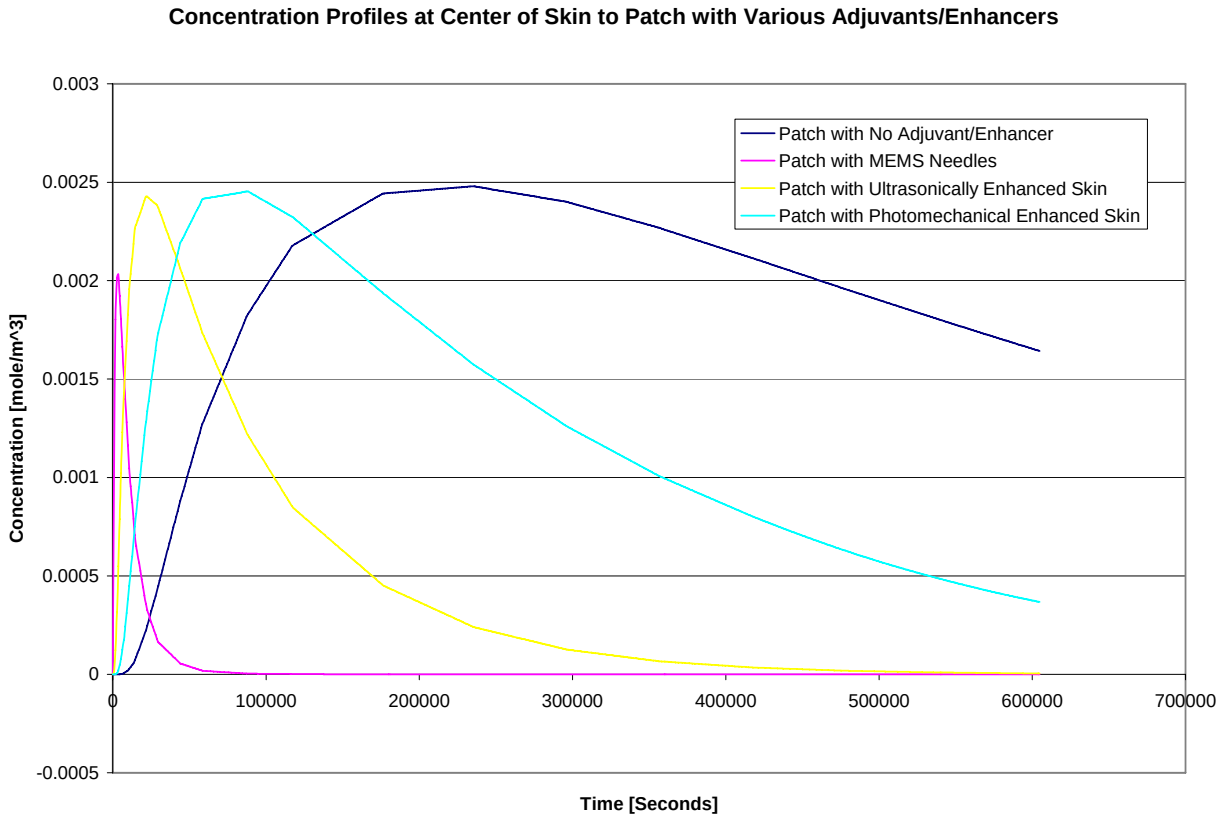


Figure 13: Concentration profile at center of skin region over 1 week of exposure the Cholera vaccine patch.

Figure 14 shows the concentration profile of the antigen at the center of the skin region during the immunization cycle outlined in Glenn et al. The researchers devised an immunization cycle to increase the concentration of the antigen in skin and bloodstream, enough so that a potent immune response was initiated. We simulated these cycles to determine the approximate concentration of the antigen at the center of the skin needed for an immune response. According to Figure 14, this concentration is 0.0038 mol/m^3. We therefore, assume that if the concentration in the skin is close to this value, then an immune response will be initiated. However, according to the Figure 14, if we use the same immunization cycles with the presence of the adjuvants, the maximum concentration reached is always below 0.0038 mol/m^3. We then optimized the immunization cycle for each adjuvant used to increase the maximal concentration.

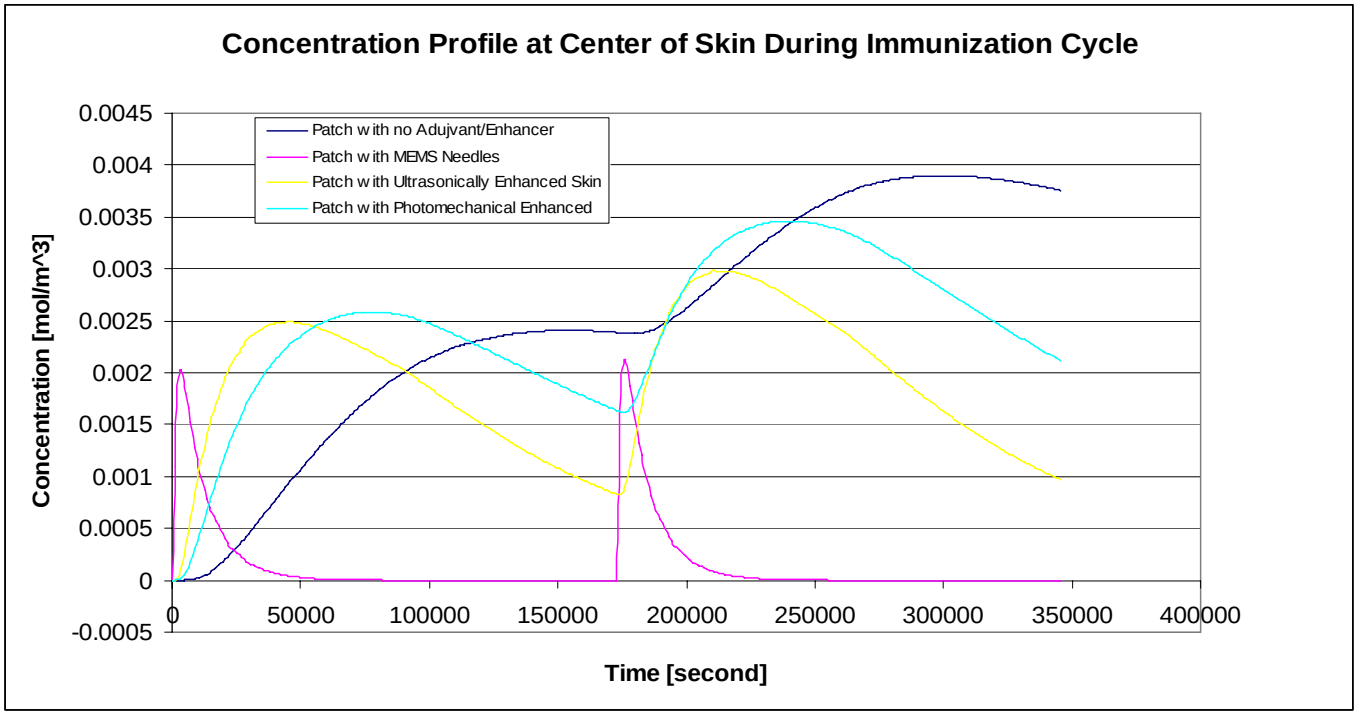


Figure 14: Concentration profile at the center of the skin region after exposure to immunization cycles outlined in Glenn et al. Patch was placed on patients arm for 24 hours, after which is was removed for 24 hours. A fresh patch was then applied again for 24 hours.

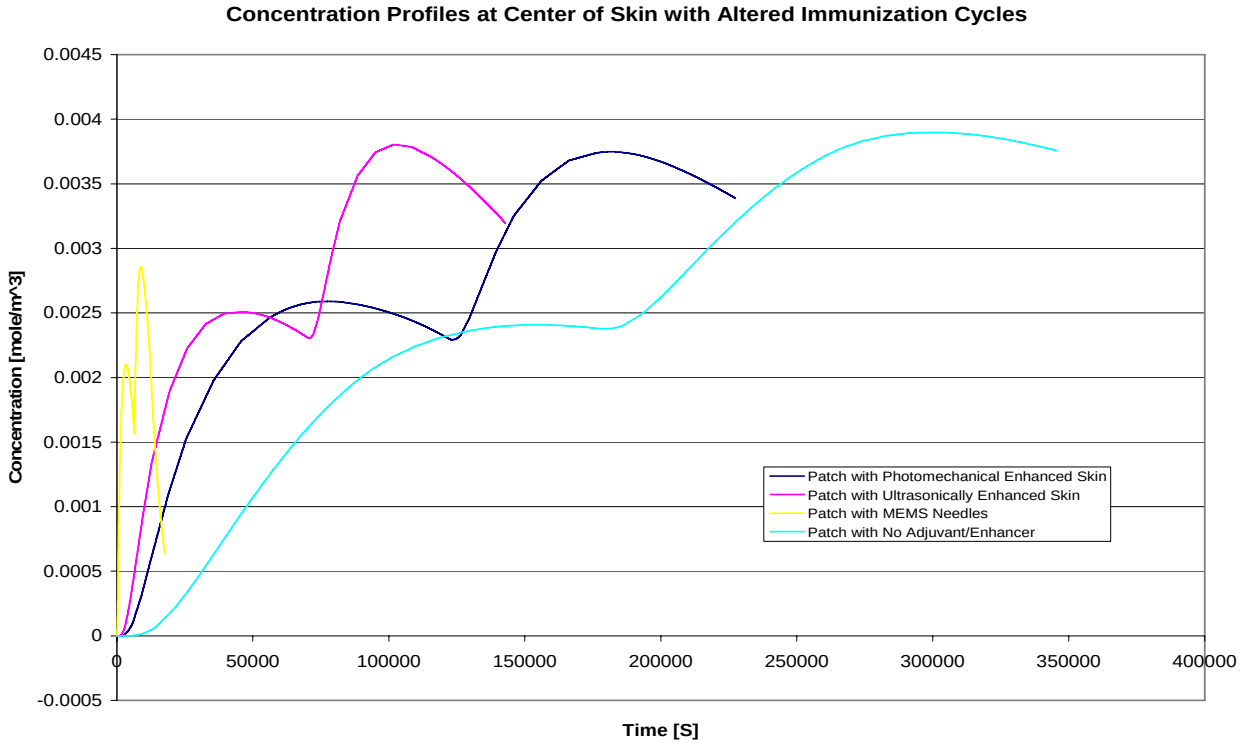


Figure 15: Concentration gradient of antigen over 1 week when the diffusivity is 1.68*10^(-11) after photomechanical forces have been applied to the skin.

We ran multiple trials with varying times for path exposure and patch removal times. We used the date presented in figure 15 to determine when the concentration peaks in each case. We determined that if we apply the patch to the skin just long enough until the concentration peaks and then remove the path and apply a fresh patch after a period of time, we can achieve a high concentration. Figure 15 is the result of several trials and represents the best immunization trials in the presence of the adjuvants.

Sensitivity Analysis

We conducted a sensitivity analysis to determine the affect on the rate constant, k, for the consumption of the antigen by the LC network (figure 16). This value was provided to use from publications based on first order enzymes kinetics. However, there was a range of values given, which indicates uncertainty with this value.

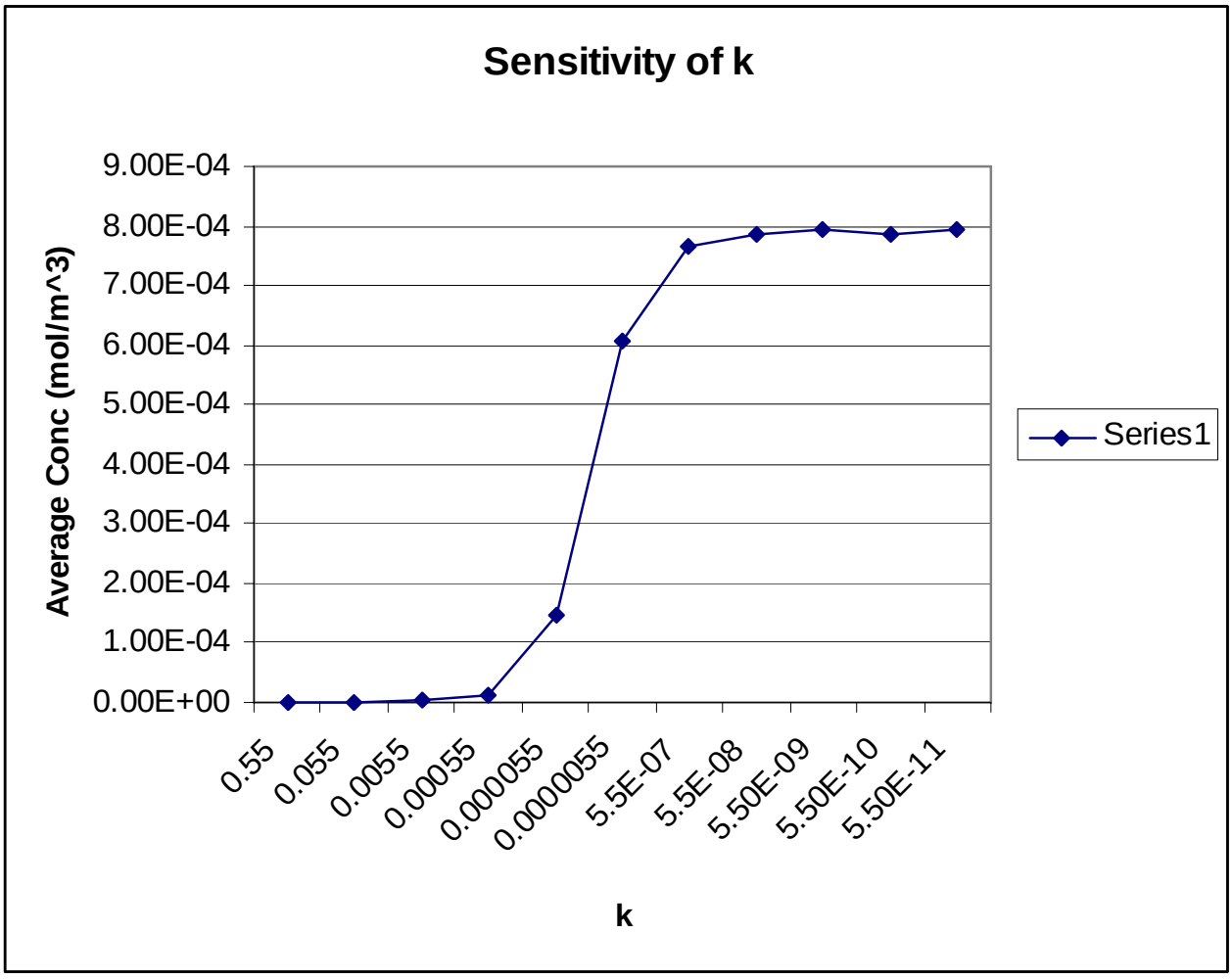


Figure 16: Sensitivity analysis results of k constant.

We also conducted a sensitivity analysis to determine the affect of the diffusivity of the antigen through the skin (figure 17). This value was based on previous case studies in BEE 453.

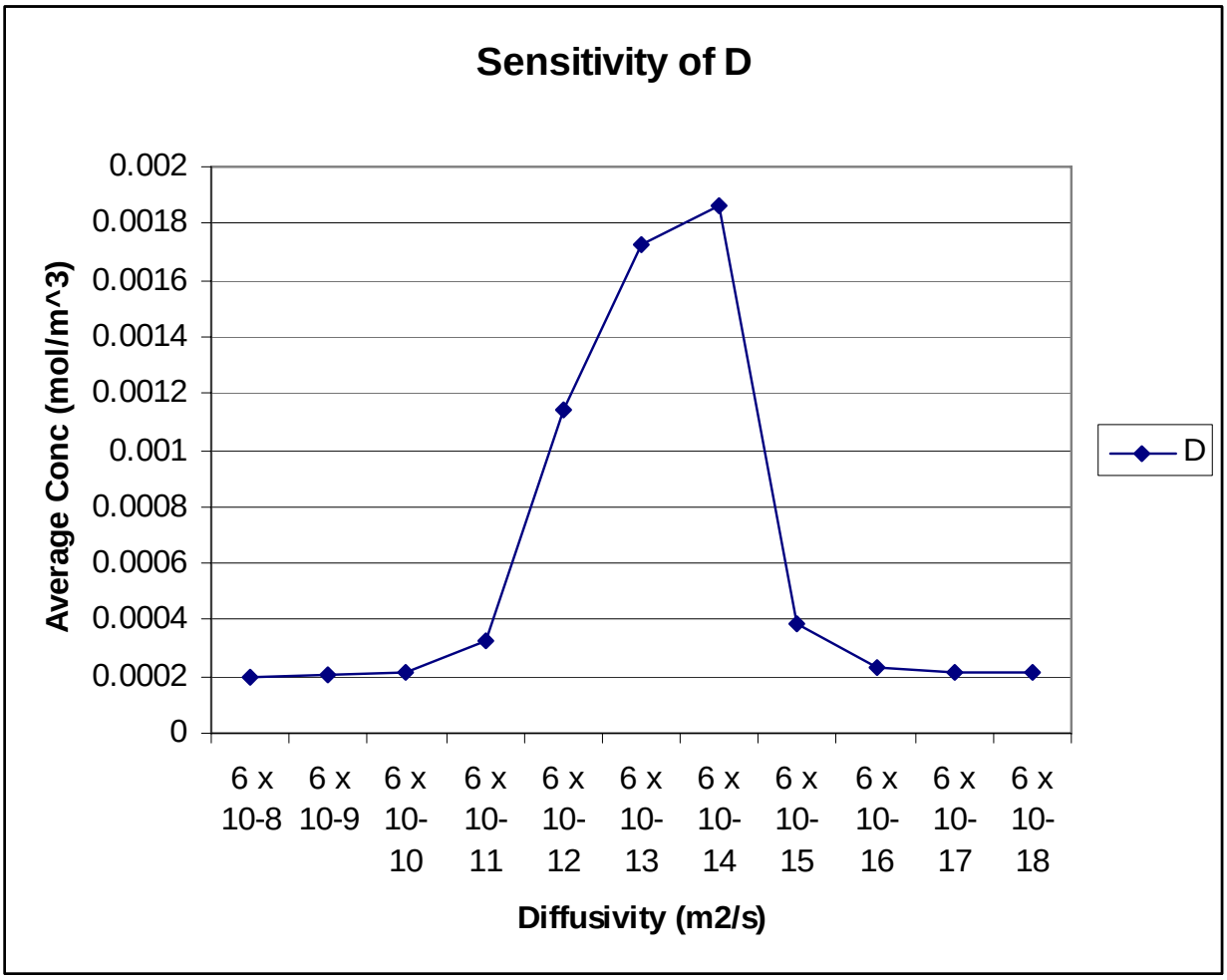


Figure 17: Sensitivity analysis results of diffusivity coefficient..

Conclusions and Design Recommendation

The main point of this analysis was to investigate the diffusion of the cholera vaccination through specific layers of the skin. This simulation consisted of a 2-D model of a piece of human skin and bloodstreams that imitates drug delivery via a patch that is placed onto the skin. It is assumed that all skin layers have the same diffusive properties. Since the patch will release the antigen in an intravenous fashion equivalent to doses injected manually, our method is advantageous over repeated doctor's visits to get the same treatment.

The antigen was initially modeled through the skin directly to the blood stream. The antigen was also modeled with the present of antigen presenting network of Langerhans cells. There was a smooth concentration profile in the skin after one week of patch exposure, but there was discontinuity when the LC network was present. The LC network functioned as a large enough sink term that the flux into the bloodstream was virtually zero. Therefore, we can say that the LC network can alone create a cutaneous immune response. The LC network was enhanced with the presence of *Imiquimod*, a typical immune response modifier. The modifier increased the activity of the LC network, thus increasing the reaction rate of the LC cells. With Imiquimod there was a sharper discontinuity in the concentration profile at the LC network and the antigen flux into the blood stream is zero.

The most effective enhancer tested was the MEMs microneedles, which increased the porosity of the skin and thus the diffusivity of the antigen through the skin. Contour plots of the skin shows absolute diffusion and consumption of the antigen into the LC network, while only partial consumption with the other enhancers. Concentration gradients were present in the ultrasonically and photo mechanically enhanced skin because they have weaker enhancer capabilities, compared to the MEMs needles. The MEMs needles are more effective, but more evasive.

Vaccines are usually given in cycles to increase the concentration of the antigen in the skin and bloodstream. When the patch was applied to the skin with no enhancer, the maximum concentration is achieved after 2.3 days. However, the maximum concentration in the skin is achieved sooner with the various adjuvants. For example, when the patch is applied with MEMs needles, the maximal concentration is achieved in the skin only after 1.2 hours of exposure. We simulated immunization cycles to determine the approximate concentration of the antigen at the center of the skin needed for an immune response. This concentration is 0.0038 mol/m^3. We therefore, assume that if the concentration in the skin is close to this value, then an immune response will be initiated. We then optimized the immunization cycle for each adjuvant used to increase the maximal concentration. Below are the recommended immunization cycles:

- **MEMS: Place patch on for 1.5 hours, then instantly place another patch for 1.5 hours.**
- **Ultrasonically enhanced skin: Place path on for 18.5 hours, then wait 30 minutes before applying another patch for 18.5 hours.**

- **Photo mechanically enhanced skin: Place patch on for 1.1 days, then wait 5.5 hours before applying another patch for 1.1 days.**

When designing a biomedical product, it is important to consider realistic constraints that may affect implementation of the product. One such concern in our design would be the health and safety of the patient using the patch. Typically with such patches, common side effects include things like nausea, blood clot, and fatigue. We would need to conduct further testing to ensure that the patch is safe for use and that these and other potential side effects are minimal and have little or no chance of escalating into a more serious problem. It is also crucial to test the effects of using the patch while being on other medication or while having other medical conditions. A related ethical concern would be in regards to experimentation on humans and animals. Furthermore, since the patch is designed for home use it should be made safe for disposal and manufactured from materials that meet environmental standards. In third world regions such as Africa and India, the patch would be safer than the traditional vaccination via needles. There is usually lack of hygiene in some of these underdeveloped areas, and improper use of needles can lead to transmission of other diseases like AIDS. Since the patch is meant for single use and does not come into contact with disease-transmitting fluids, it is a much safer way to become immunized.

Economically speaking, the cholera patch seems to be more cost effective than alternate methods of immunization. One such method is the oral vaccine called Dukoral. It is not available in the United States, but is available in other locations abroad. For the minimum two doses that travelers would be required to take, the cost would be about $110. Although we do not know what the exact cost of our patch would be, we estimate around $35 to $45, which is just slightly higher than a month's supply of the birth control patch. Another restriction with Dukoral is that food consumption cannot occur for one hour before and after consumption, whereas with the patch there are no such restrictions. The Dukoral doses also occur over a few months, so it is more likely that one would forget when they're supposed to take the medicine. The patch is worn short term, so it is easy to remember when to take it on and off. Dukoral also does not provide full immunity until one week after dosage begins, however the patch would provide immunity less than a week after it is put on. Once the patch becomes widespread, some cost could potentially be covered under health insurance plans. In addition, the patch is intended for home use which means the cost of medical personnel needed decreases since physicians do not need to administer doses. Socially speaking, people are more likely to be comfortable becoming immunized because there is a degree of fear associated with injection by needles, as opposed to the patch which is extremely user-friendly.

Appendix A

Governing Equations and Boundary Conditions

Situation 1

$$\frac{\partial C_D}{\partial t} + v\left(\frac{\partial C_D}{\partial x}\right) = D_D\left(\frac{\partial^2 C_D}{\partial x^2}\right) + C_D$$

(0 and 0)

$$\frac{\partial C_D}{\partial t} = D_D\left(\frac{\partial^2 C_D}{\partial x^2}\right)$$

C_D = concentration of antigen
t = time
x = distance ↓
D_D = Diffusivity = 6 x 10^{-12} m^2/s

Initial Conditions

C_D = (t = 0) = 0 in skin

Boundary Conditions

C_DAt, x = 0.002535m = 0
Assuming that the bloodstream carries away all of the antigen

Situation 2

$$\frac{\partial C_D}{\partial t} + v\left(\frac{\partial C_D}{\partial x}\right) = D_D\left(\frac{\partial^2 C_D}{\partial x^2}\right) + C_D$$

(0 and 0)

$$\frac{\partial C_D}{\partial t} = D_D\left(\frac{\partial^2 C_D}{\partial x^2}\right)$$

Initial Conditions

C_D = concentration of antigen
t = time
x = distance ↓
D_D = Diffusivity = 6 x 10^{-12} m^2/s
K = .55X10^-5

[8.3] – Situation 3

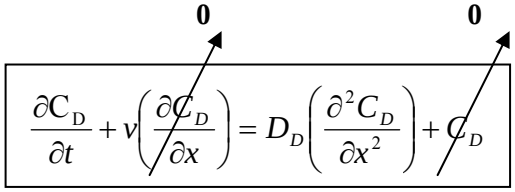
Governing Equation

$$\frac{\partial C_D}{\partial t} = D_D\left(\frac{\partial^2 C_D}{\partial x^2}\right)$$

C_D = concentration of antigen
t = time
x = distance ↓
D_D = Diffusivity = 6 x 10^{-12} m^2/s

Initial Conditions
C_D (t = 0) = 0 in skin
Boundary Conditions
1.047 x 10^{-11} mol/m^2s initial flux at patch-skin boundary

Situation 4



$$\frac{\partial C_D}{\partial t} = D_D\left(\frac{\partial^2 C_D}{\partial x^2}\right)$$

C_D = concentration of antigen
t = time
x = distance
D_D = Diffusivity = varied depending on enhancer used
Initial Conditions
C_D = (t = 0) = 0 in skin
Boundary Conditions
C_DAt, x = 0.002535m = 0
Assuming that the bloodstream carries away all of the antigen

Appendix B

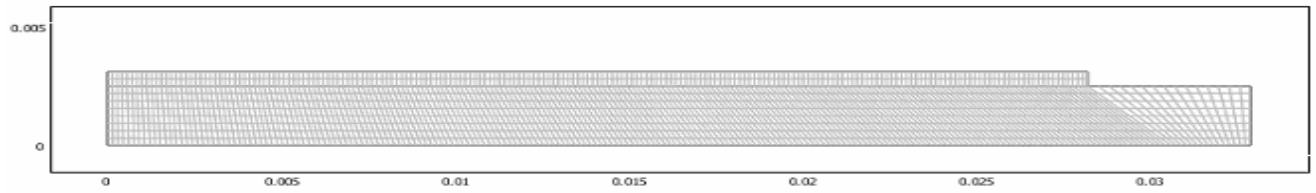


Figure 9: Mesh applied to Situation 1, a patch on top of the skin layer with antigen diffusing through the skin to the blood stream.

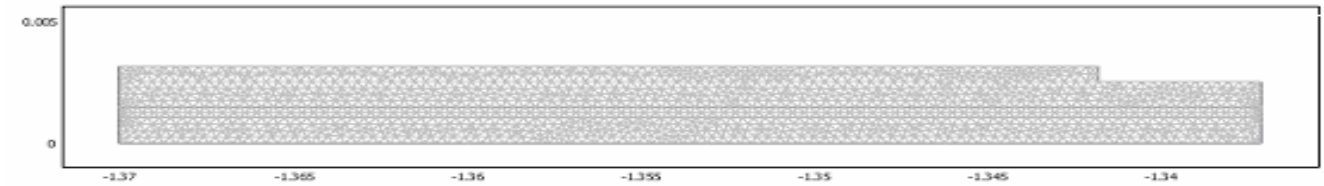


Figure 10: Mesh of situation 2, a patch on top of the skin layer with antigen diffusing through the LC network and to the blood stream.

and immunizes the human.

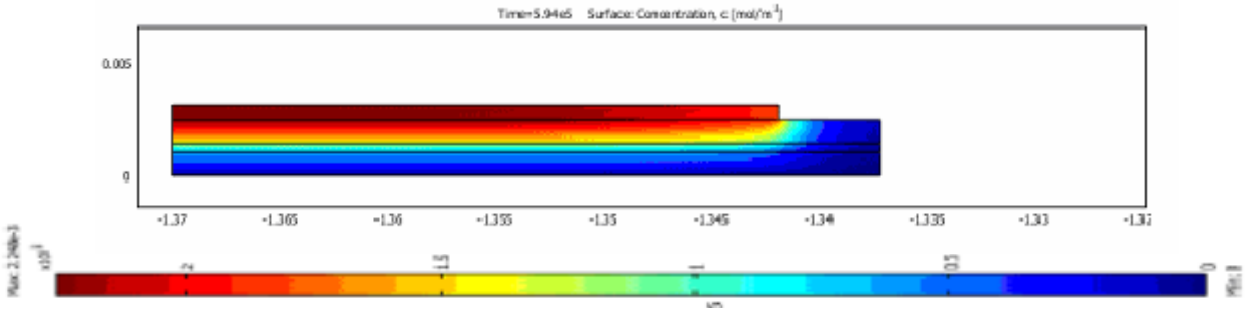


Figure 12: Concentration gradient of the antigen developed over one week during situation 3.

Below is a mesh convergence analysis (figure 18). We used convergence in relation to average concentration because this would give us a very accurate number of elements to use in our analysis. We used 77 or greater nodes in our analysis.

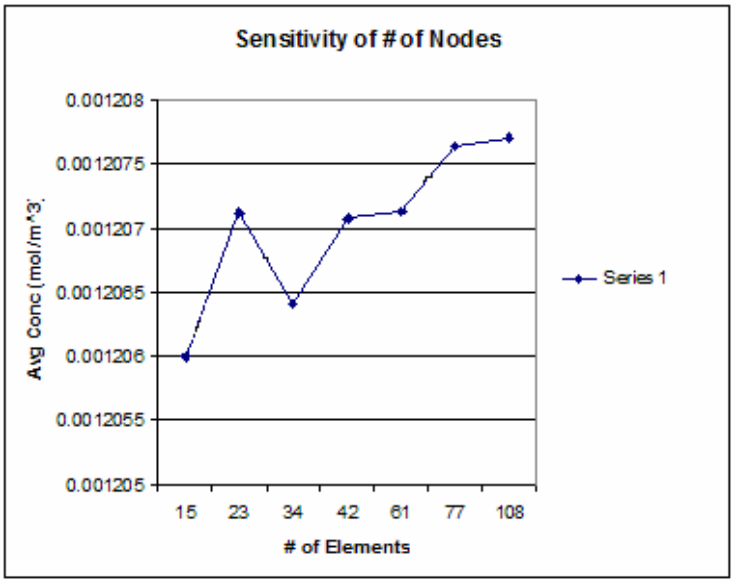


Figure 18: Sensitivity of number of nodes to average concentration in the skin.

Appendix C

Figure 2: Primary immune response cascade. http://www.transferfactorinstitute.com/ImmuneSystem/basics.cfm

The figure above depicts a primary immune response cascade that will occur after the patient has been exposed to cholera toxin antigen through the patch. (1) The antigen will diffuse through the skin layers, signaling an immune response from macrophages and other scavenger immune cells. (2) Mast cells release chemicals that trigger inflammation, allowing other immune cells to rush to the diffusing antigen. (3) Before reinforcements arrive, macrophages and other immune cells start engulfing the antigens. (4) The antigens are transported to the lymph nodes where these macrophages bind to B and T cells. B cells begin producing antibodies specifically for the particular cholera toxin antigen. (5) The antibodies tripper responses from certain immune cells like NK cells, macrophages and killer T cells to engulf and kill the antigen-infected cells. (6 & 7) Multiple other steps occur to ensure that there is an immune response to the antigen.

Appendix D

References:

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