That bites!: the transport of Bothrops asper venom in leg.

Computer Aided Engineering: Applications to Biomedical Processes
BEE 4530

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EXECUTIVE SUMMARY

Snake venom has been known to be a deadly toxin for centuries. Although many studies about the dangerous effects of snake venom have been conducted, the spread of venom throughout human tissue has not yet been modeled. The goal of this study is to examine the contributions of relevant modes of mass transport on the spread of venom in human skeletal muscle tissue.

In this study, computational models that mimic the localized propagation of venom were developed using parameter values for diffusivity, injection pressure, and injection volume determined from available research papers, empirical formulas, and clinical case studies. COMSOL Multiphysics 4.3b software was used to simulate the dissemination of venom from a fang into human flesh.

A simplified 2D axisymmetric geometry was used initially to model the human tissue punctured by a single snake fang. This model allows us to examine the diffusion of BAP1 metalloprotease in human tissue. The results were compared to data from in vivo snake bite case studies and a study of the diffusion of similarly sized non-toxic protein. It was determined that diffusion alone could not be responsible for the expected extent of venom spread.

Therefore, additional modes of mass transport, such as convection and Darcy's flow, were evaluated and integrated in a 3D model of a human leg. Darcy flow at the point of injection simulated the effect of the injection pressure pushing venom out of the fang into the tissue. The model results were compared to findings in a series of studies on venom mechanics and metering in rattlesnakes and were found to be consistent. The convection of venom away from the injection site due to circulation of blood and interstitial fluid in the leg was modeled as a source term. Sensitivity analyses were performed in order to study how sensitive the solutions are to these input parameters.

These models enable researchers to gain insight into how the different modes of mass transport influence the progression of edema. Knowing this can allow researchers to develop more effective treatment methods and antidotes for snakebites. Researchers can also modify these input parameters in order to model venom transport for different species of snakes, allowing them to tailor treatment methods for a variety of snakes.

INTRODUCTION

In many parts of the world, various species of venomous snakes are a threat to human safety. Their venoms are thought to be highly evolved, 'weaponized' saliva that are used to incapacitate prey, in addition to serving as a potent defense mechanism against predators. These venoms are complex mixtures of chemicals, amino acids, and enzymes that react with blood, body tissues, and nervous system signaling in order to produce detrimental local and systemic effects [1]. Though humans are not snakes' prey, deliberate attempts to handle them and accidental wild encounters with these exotic creatures can result debilitating injuries and even death from cardiac and/or respiratory failure.

Venomous snakes can be broadly divided by their venom apparatus which primarily consists of a pair of venom glands connected to hollowed fangs. It is the location, shape, and size of theses fangs that determines the delivery of venom. Elapids, such as cobras, mambas and kraits, have small, fixed fangs at

the front of their mouths. Colubrids, such as the African boomslang and vine snakes, have their venom delivering fangs at the rear of the mouth. Viperids, rattlesnakes, adders and vipers have hinged fangs at the front of their jaws that flare out when they strike [2]. Some of the most complex concoctions of toxins can be found among the venoms of viperids. These complex mixtures present physicians with a special challenge when it comes to devising effective treatment strategies [3]. Of special interest are pit vipers, which earn their name from the pits beneath their eyes that contain heat-sensing receptors. The pit vipers of particular interest are the members of the South American lancehead pit vipers, genera Bothrops, Bothropoides, and Rhinocerophis, which are responsible for more deaths than any other kinds of snakes in the Western hemisphere. For this model, *Bothrops asper* was chosen.

Bothrops asper, commonly referred to as the terciopelo, can be found throughout the lowlands of much of Central America, Ecuador, Colombia, and Venezuela. Its excellent camouflage ability, combined with its nasty temper, long fangs, farther than average strike range, as well as highly potent venom delivered in large doses, give this snake a well-deserved reputation as one of the world's most dangerous snakes [2]. As the venom of the *Bothrops asper* travels through the body, enzymes rupture cells and destroy the tissue fibers, commonly resulting in pain, hemorrhage, edema, loss of feeling or function of limbs, blistering, tissue necrosis, nausea, vomiting, and, without adequate treatment, loss of limbs. [4].

Due to the tenacious disposition of terciopelo, much research has been conducted regarding the make-up of its venom, and how these individual components affect the body. While the crude, or whole, venom, of terciopelo is composed of multiple components, the chief enzymes responsible for hemorrhage, and thus swelling, are zinc-containing metalloproteases, in particular an enzyme called BAP1. Because BAP1 is the most highly abundant metalloprotease in *Bothrops asper* venom, it is treated as the only active toxin in this study. BAP1 induces endothelial-cell lysis and attacks the fibrin of the extra-cellular matrix, resulting in hemorrhage, inflammation, blistering, and edema [5]. Combined with the deleterious effects of the other components of Bothrops asper venom, victims commonly experience very painful swelling of the limb, ischemia, and eventually tissue necrosis [4].

The proliferation of venom and the resultant local effects in vivo are not well understood, consequently it is the process of venom transport through the muscle tissues that we wish to model. For this project we are interested in modeling the development of edema, or swelling, as a representation of the extent of venom spread with the body. We hope to understand how this transport varies based on the pressure of venom injection, and what effect the concentration of venom has on swelling around the injection site.

PROBLEM STATEMENT

Upon examination of current research, no computational models of snakebite tissue damage are available. Developing this model will help give some insight into the rate of symptom development as an indication of envenomation severity and toxin spread. This knowledge can be used to develop an understanding of snake envenomation as a complex poisoning that should be treated as an acute medical emergency. Further, this model could be expanded as a method to test the efficacy of treatment protocols without the added uncertainty of individual sensitivity to the venom or treatment.

DESIGN OBJECTIVE

COMSOL software was used to model a fang pierced into human tissue. The model simulated the spread of venom and the development of cell death around the injection site.

1. Determine the parameter values (diffusivity constants, blood flow rates, pressure of injection, volume of injection) by using medical journals, research papers, and clinical studies on venom injection.
2. Design a procedure for representing convection due to blood flow that the computer model, COMSOL, will be able to use (reaction term).

3. Examine how sensitive the solutions are to these parameters.
4. Analyze the effect that each mode of species transport has on the transport of venom following snakebite.
5. Validate models of venom transport by comparing solutions to data from medical journals and clinical case studies.

ASSUMPTIONS

- The effects of the snake bite can be simulated using only one fang, as opposed to the two fangs that are involved in actual venom injection.
- BAP1 is the most abundant SVMP in *Bothrops asper* venom. It contributes to the development of edema. Therefore, data from BAP1 was used in order to analyze the spread of venom. In the time period of one hour after a snakebite, the effects of venom are localized and restricted to edema.
- BAP1 is the primary toxin in the *Bothrops asper* venom responsible for the development of edema.
- The effects of other components in *Bothrops asper* venom are negligible, unrelated, or secondary to the activity of BAP1.
- The diffusivity of BAP1 in venom is the same as in water; $D_{BAP1-Venom} = D_{BAP1,aq}$
- The tissue is homogenous. Diffusivity of venom and convective velocity are uniform and constant throughout the domain.
- Velocity of blood flow is constant throughout the leg.
- In this time frame, we assume insignificant cell death.
- Trace amounts of venom are metabolized during this time frame, resulting in negligible effects of venom degradation in the body.

2D AXISYMMETRIC PROBLEM SCHEMATIC

Figure 1 shows the 2D axisymmetric geometry that was used to model human tissue punctured by a single snake fang, with the axis of symmetry stemming through the center of the fang. A pool of venom beneath the fang served as the venom reservoir and simulated the injected volume of venom. The venom travels from the reservoir into the tissue through passive diffusion. The volume of the pool of venom represents the injection volume from a snakebite. The basis for using this volume is discussed in the Results and Discussion of the 2D Simplified Model. This 2D axisymmetric model was used to assess the extent of local venom transport due to only the effect of species diffusion.

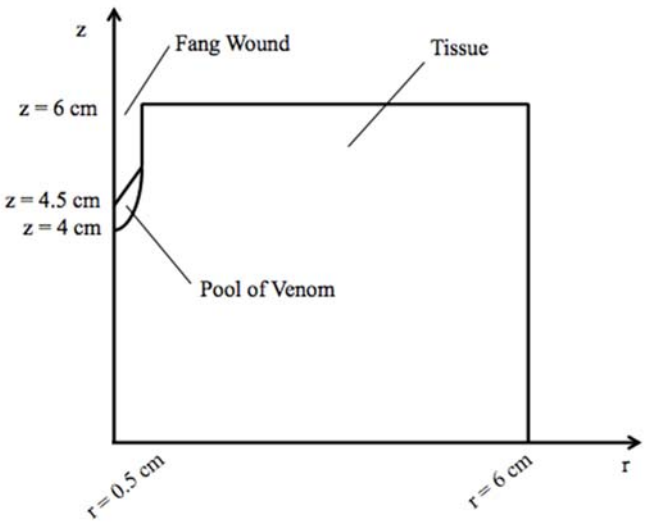

Figure 1: Pooling of venom in human tissue after initial injection from one snake fang. There are two domains in our 2D axisymmetric model: the venom reservoir, and human tissue. The void in the schematic represents the snakebite fang wound. The axis of symmetry goes through the center of the fang wound. The venom reservoir has a volume of 384 μL. Diffusion was considered the only mode of transport in this schematic. In the reservoir, $D_{BAP1-Venom}$ = 7.89646E-11 m^2/s. In the tissue, $D_{BAP1-Tissue}$ = 7.10681E-11 m^2/s.

2D AXISYMMETRIC GOVERNING EQUATIONS

In this simulation, diffusion is the only term in the mass transport equation. This model provides insight to the role of passive diffusion on the effect of venom dispersion.

$$\frac{\partial c}{\partial t} = D\left(\frac{1}{r^2}\frac{\partial}{\partial r}\left(r^2\frac{\partial c}{\partial r}\right) + \frac{\partial^2 c}{\partial z^2}\right)$$

Where c is the concentration of the venom in the tissue, r is the radius of the section of tissue, z is the depth of penetration into the tissue and D is the diffusivity of the venom through the tissue. The 2D axisymmetric model uses cylindrical coordinates, with no change in the θ direction.

A Time-Dependent study was implemented using the "Transport of Diluted Species" physics. In the reservoir, $D_{BAP1-Venom}$ = 7.89646E-11 m^2/s. In the tissue, $D_{BAP1-Tissue}$ = 7.10681E-11 m^2/s.

2D AXISYMMETRIC BOUNDARY CONDITIONS, INITIAL CONDITIONS

All exterior boundaries have zero flux. Domain is large enough that the venom would not travel far in the time frame that was used (see Appendix B for time stepping). Initially, there is no venom in the tissue, and a concentration of 500 g/m^3 of venom in the pool of venom.

$$c|_{Tissue\ Domain}(t = 0) = 0$$

$$c|_{Venom\ Reservoir\ Domain}(t = 0) = 500 g/m^3$$

The venom diffuses from the reservoir into the tissue, which is initially venom-free. The basis for determining the initial concentration of venom in the reservoir and the volume of injection are outlined in the Results and Discussion of the 2D Simplified Solution.

The process of determining the value of the diffusivity coefficient of BAP1 and the examination of its validity is outlined in Appendix A.

3D PROBLEM SCHEMATIC

The transport of venom was next modeled in a 3D model of a human leg, shown in Figure 2. A sphere indentation in the lower leg represents the puncture wound from the snake fang. This model considers the effects of diffusion, Darcy flow, and convection on the spread of venom.

The injection of venom is represented by a mass flux of venom at the bite boundary. This mass flux exponentially decays and reaches zero at 0.254 seconds, the injection time of a snakebite. The procedure for developing this mass flux term is outlined and justified in Appendix A. The effect of convective blood flow on the circulation of venom is included as a source term.

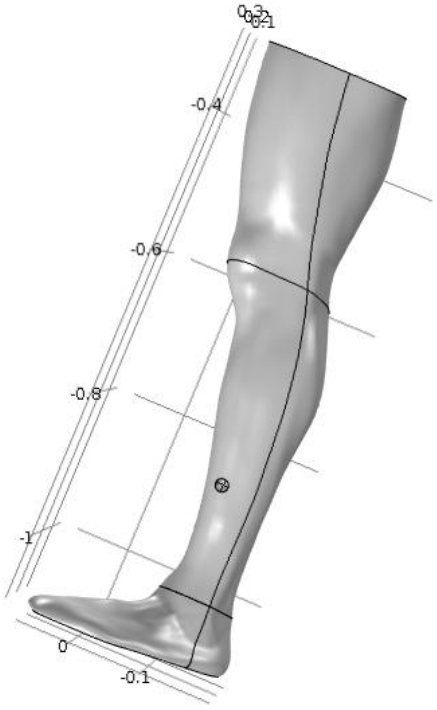

Figure 2: GrabCad model of human leg. The sphere indentation in the leg represents the puncture of the snake fang. This fang wound has a surface area of 6.2026E-4 m^2. There is an inward mass flux of BAP1 at the fang wound boundary, simulating the influx of BAP1. In the leg, $D_{BAP1-Tissue}$ = 7.10681E-11 m^2/s.

3D GOVERNING EQUATIONS

The second Time-Dependent study was implemented using "Darcy Flow" physics, which describe the flow of liquid through a porous media, coupled with "Transport of Diluted Species" from the 2D model,. Darcy flow is used to simulate the effects of pressure of the injection of venom and provide the initial driving force to move venom into the tissue. An inward mass flux in the Darcy Flow, simulated to the injection of venom, and generated a pressure gradient. A second mass flux in Transport of Diluted Species accounted for the concentration of BAP1 in the venom flow. Below are the governing equations for the gradients of concentration and pressure respectively, where c is concentration of venom, D is the diffusivity of BAP1 in the tissue, ∇P is the pressure gradient, μ is initial concentration of venom, ν is the entrance velocity, and K is permeability of the tissue.

$$\frac{\partial c}{\partial t} = D\nabla^2 c$$

$$\nabla P = -\frac{\mu}{K} v$$

This pressure gradient is coupled to the mass transfer equation and it further transports the venom.

Next, convective transport due to the circulation of bodily fluid was added to the governing equation. Due to limitations within the COMSOL software, convective transport could not be included as a convection term in the governing equation. Instead, convective transport is included in the governing equation as a reaction term.

$$\frac{\partial c}{\partial t} = D\nabla^2 c + R$$

This reaction term is designed to carry the BAP1 to areas where there is no BAP1 with a velocity.

3D BOUNDARY CONDITIONS, INITIAL CONDITIONS

There is initially no venom in the leg. The venom is injected through the boundary of the bite simulated by a BAP1 mass flux and carried by the pressure gradient simulated by Darcy flow.

$$c|_{Tissue\ Domain}(t = 0) = 0$$

The external boundary of the leg has an impermeable boundary condition, and there is an inward mass flux of venom at the bite boundary, simulating the entrance of liquid venom. This injection exponentially decays to zero, representing an injection time of 0.254 seconds. The flux of venom N_0 through the bite boundary is implemented as an inward mass flux in Darcy's law physics to represent the injection of venom that produces a pressure gradient.

$$N_0 = 9.52 \times e^{-15t}\ kg/m^2 s$$

Another mass flux $N_{0,c}$ below is implemented at the boundary condition in Transport of Diluted Species to account for the concentration of BAP1in the venom solution.

$$N_{0,c} = 0.0319 \times e^{-15t}\ mol/m^2 s$$

It is important to note that N_0 accounts for the total mass of crude venom, where $N_{0,c}$ only accounts for BAP1. This is necessary to create the proper pressure gradient that will move the BAP1 further into the tissue.

The of process of developing and adjusting the expression for the inward mass fluxes of venom, and determining the aforementioned reaction term is outlined in Appendix A.

2D SIMPLIFIED SOLUTION

Considering only diffusion as the primary mode of species transportation, the plot of venom concentration at 30 minutes can be found in Figure 3.

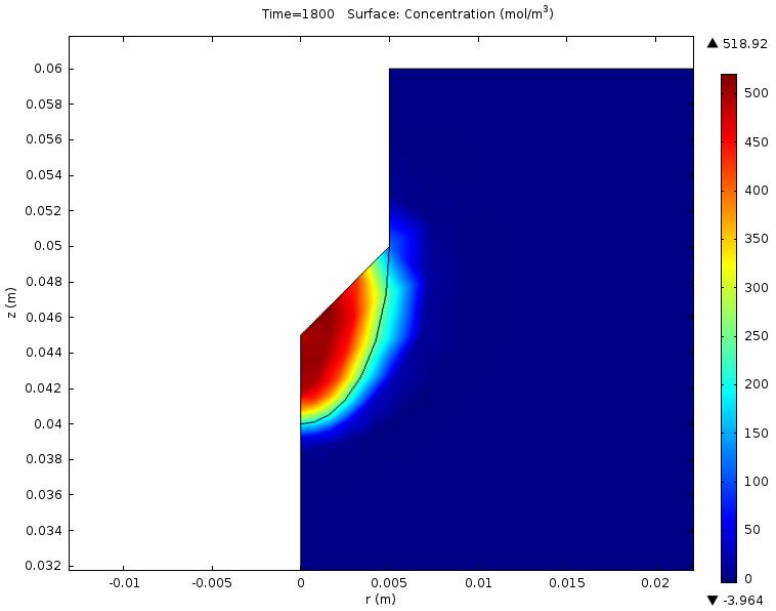

Figure 3: Concentration profile of venom in human tissue at 30 minutes. Only a portion of the tissue domain is shown. Species is transported by only diffusion. The void in the geometry represents the snake fang puncture. Venom diffuses from the reservoir domain into the tissue domain.

This simulation was developed to model a study conducted with pigs. In this study, pigs were injected with venom of concentration 0.5 μg/μL and injection volume of 200 μL. After 30 minutes, an area of induration of 3.8cm^2 is observed [6]. Assuming that the injury is circular, the radius of the induration would be about 1.1cm.

An injection volume of 384 μL and initial venom reservoir concentration of 500 grams/m^3 (=0.5 μg/μL) were used. The diffusion mass transfer term does not carry the venom as far as the experimental values have predicted. Therefore, other terms of the mass transport equation must be considered to explain the dispersion of venom. Next, a 3D leg model was created to include convection and Darcy flow.

Considering diffusion and Darcy flow as modes of species transportation, the plot of venom concentration at one hour can be found in Figure 4.

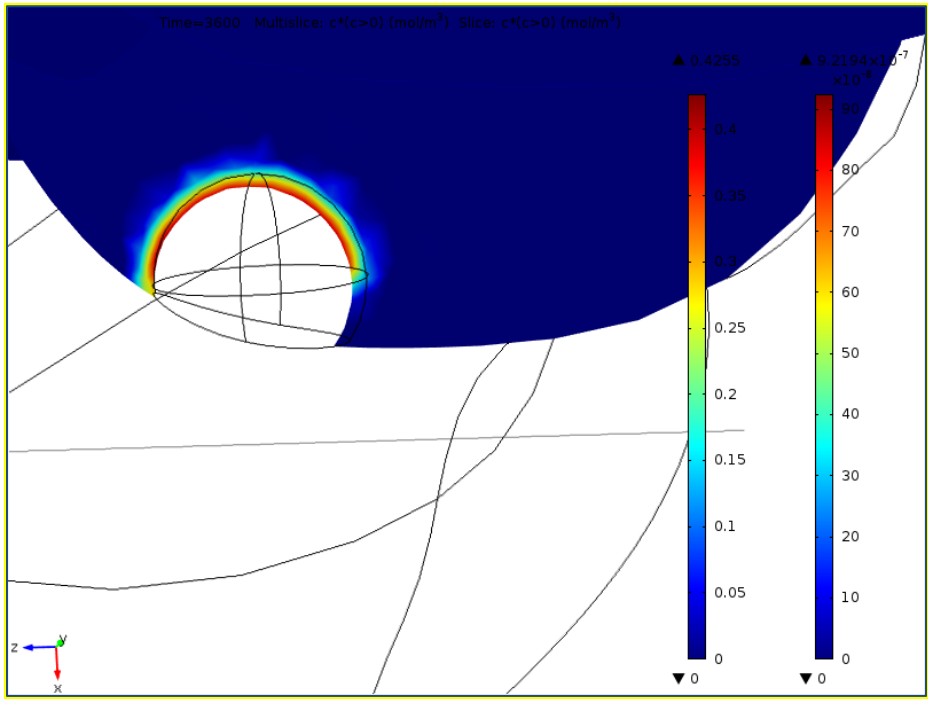

Figure 4: Concentration profile near bite wound at one hour after injection. Indicated here is a short distance of venom spread in this time period. The void represents the venom bite as a sphere. Only the tissue surrounding the bite is included. This model includes the effects of Darcy flow and diffusion.

The venom concentration near the bite is expected to increase drastically at early times, and slowly decay to steady state as the venom travels throughout the leg [7]. Although the pressure gradient did make the venom travel further, the distribution of venom did not spread farther than fractions of a centimeter. This is because the time of injection is only 0.254 seconds, so the pressure gradient does not make a big difference in the transport of venom.

Next, the effects of convective transfer in muscle were analyzed. Considering diffusion, convection, and Darcy flow as modes of species transportation, the plot of venom concentration v. time at a cut point that is about one centimeter away from the boundary can be found in Figure 5.

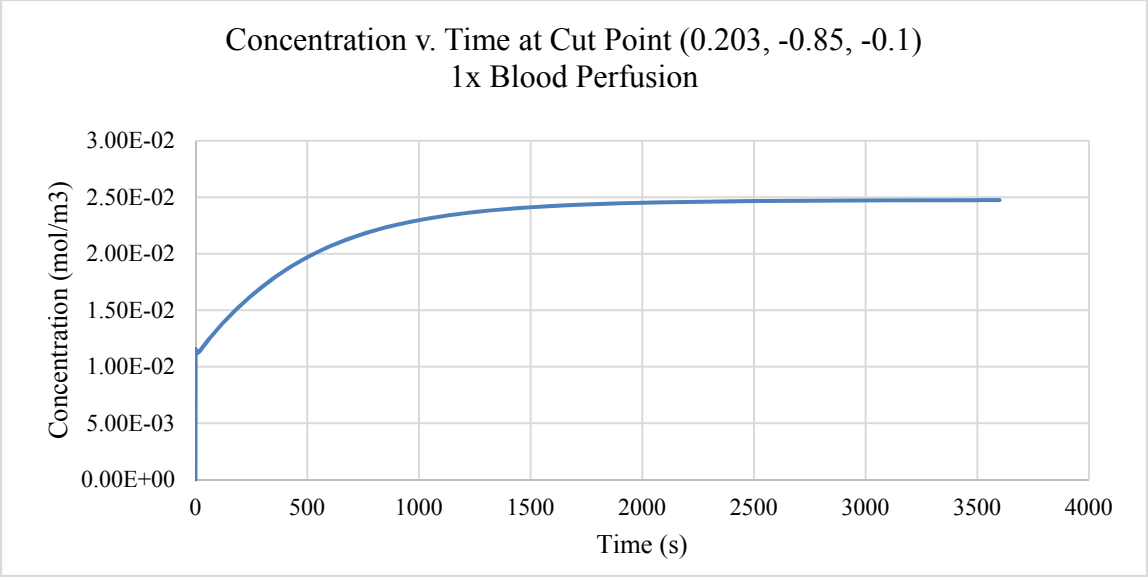

Figure 5: Concentration v. time at cut point (0.203, -0.85, -0.1), a point that is approximately one centimeter away from the bite. This model was implemented with the calculated reaction term. Initially, concentration at this cut point spikes and then equilibrates after approximately 30 minutes. This model considers the effects of diffusion, Darcy flow, and convection.

At this location near the snake bite, concentration equilibrated after approximately 30 minutes of transport. The pattern of venom spread did not behave in a manner that was expected, the venom was not transported away from the bite boundary with this blood perfusion value. Convection is ultimately the main bodily process that is responsible for the distribution of venom throughout the leg. This meant that the convective transfer reaction term was too high. A sensitivity analysis of the convection term was performed and after reducing the convection term by a factor of 100, the spread of venom was slowed and the behavior of venom travel was consistent with the expected pattern. The concentration v. time plot at the same cut point used previously, implementing this adjusted reaction term, is shown in Figure 6.

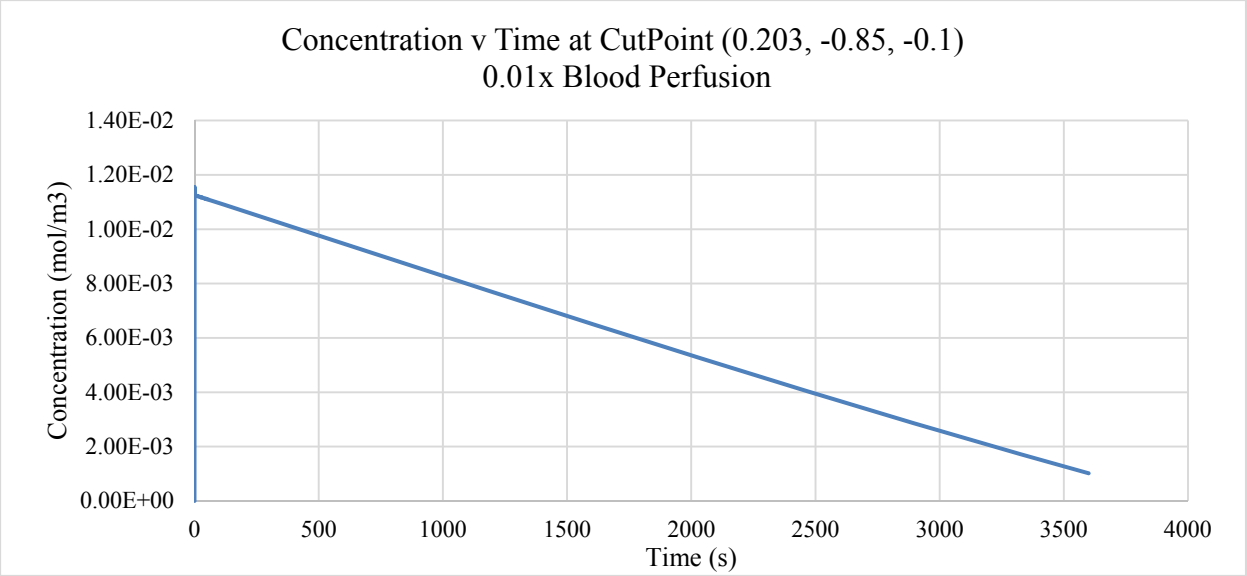

Figure 6: Concentration v. time at cut point (0.203, -0.85, -0.1), about one centimeter away from the bite. Here, the reaction term is lowered by a factor of 1000. Initially, concentration spikes and then slowly decreases with a steady slope over the period of implementation. This model considers the effects of diffusion, Darcy flow, and convection.

The addition of a convection term increased the accuracy of the model, proving to be the driving force behind the spread of venom in human tissue. The inaccuracy of the reaction term shows that there are other factors that must be considered while calculating the effective convection of venom due to bodily fluids. This includes a more accurate blood mass transfer factor, the time constant represented by ω in Appendix A, which is currently over-simplifying the convective process. In the current model, this constant is designed with the assumption that blood flow is the primary contributor of mass transfer. However, in reality, uptake or sequestration by other systems, such as the lymphatic system, may need to be considered as well. Another issue is that it is constant throughout the leg, with extent of convection dependent only on the venom concentration at that point. Pooling of the venom in less vascularized regions may also occur, so for better accuracy, this rate may need to vary depending on the location in the 3D model.

SENSITIVITY ANALYSIS

Sensitivity of input values was analyzed for mass flux, diffusivity, and blood perfusion. Analysis of diffusivity was performed using the 3D model prior to the addition of the source term. Mass flux and blood perfusion were analyzed using the 3D model that implemented coupled diffusion, Darcy flow, and a convective reaction term.

MASS FLUX

The inward BAP1 mass flux of $N_{0,c} = 0.0319 \times e^{-15t}\ mol/m^2 s$ was varied by ±20%, ±40%, and ±60%, while the source term and the diffusivity were held constant. The Darcy flow mass flux was also adjusted accordingly. This value range was used because our mass flux parameter was calculated using the average venom yield. Therefore, mass injected would lie in this range. Figure 7 shows the concentration profile at a point close to the injection site.

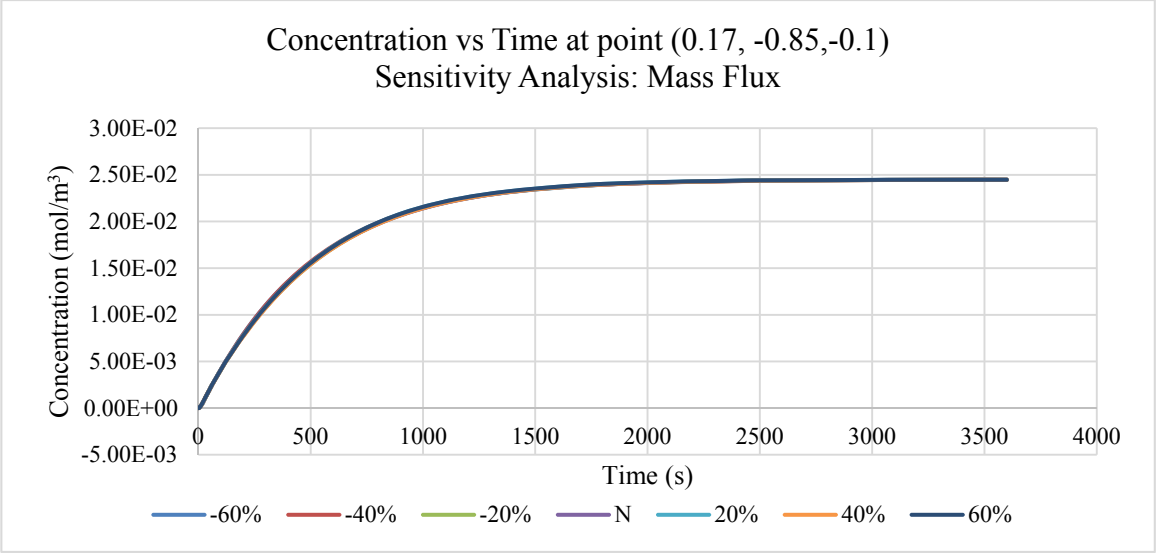

Figure 7: Concentration v. Time at point, (0.17, -0.85, -0.1) with varying Inward mass flux values. The source term and diffusivity are held constant. Mass flux of BAP1 was increased and decreased by ±20%, ±40%, and ±60%. The Darcy flow mass flux was also adjusted accordingly.

Overlapping data in Figure 7 indicates that our solution is not sensitive to mass flux. Therefore, the solution is not dependent on the accuracy of the mass flux input parameter.

DIFFUSIVITY

The diffusivity constant of $D_{BAP1-Tissue}$ = 7.10681E-11 (m^2/s) was varied by ±20% and ±40%. This range of values falls within known diffusivity values of proteins through porous media. To analyze the effect diffusivity changes had on our venom spread, we looked at concentration values at a single point for five different diffusivity values in this range over one hour, as seen Figure 8.

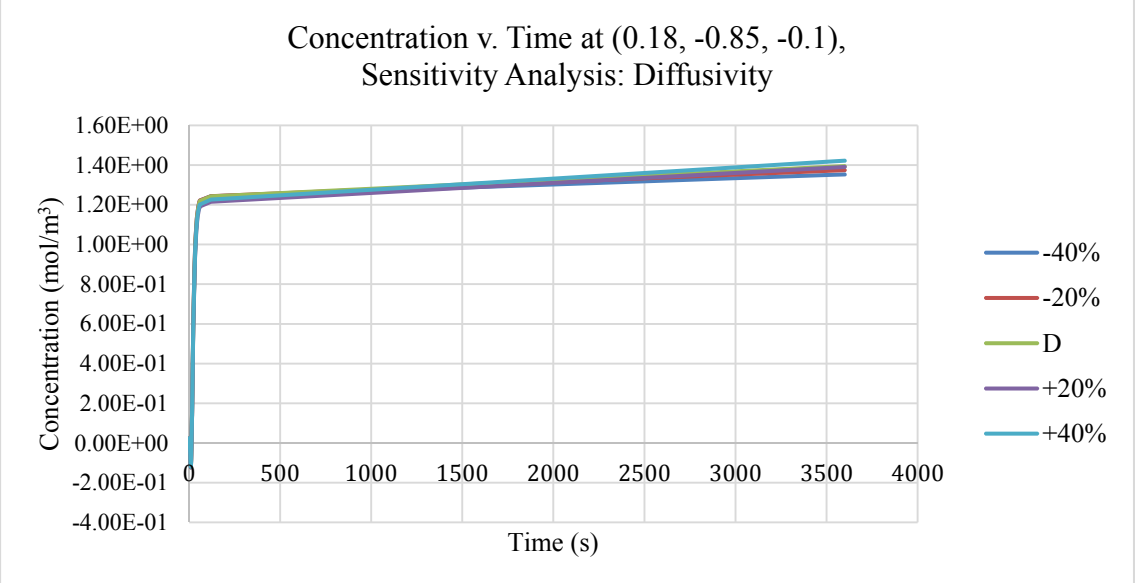

Figure 8: Concentration v. Time at cut point, (0.18, -0.85, -0.1). Diffusivity was varied by ±20% and ±40%, while other input parameters were held constant.

Data from Figure 8 indicates that the solutions are not particularly sensitive to diffusivity values at later times. Results indicate that the solution is not very sensitive to diffusivity.

BLOOD PERFUSION

The blood perfusion constant, determined as a value of $\omega = .00178s^{-1}$ was varied by factors of ten, with the extent being two factors of ten in either direction. This range of values is consistent with data obtained from medical journals.

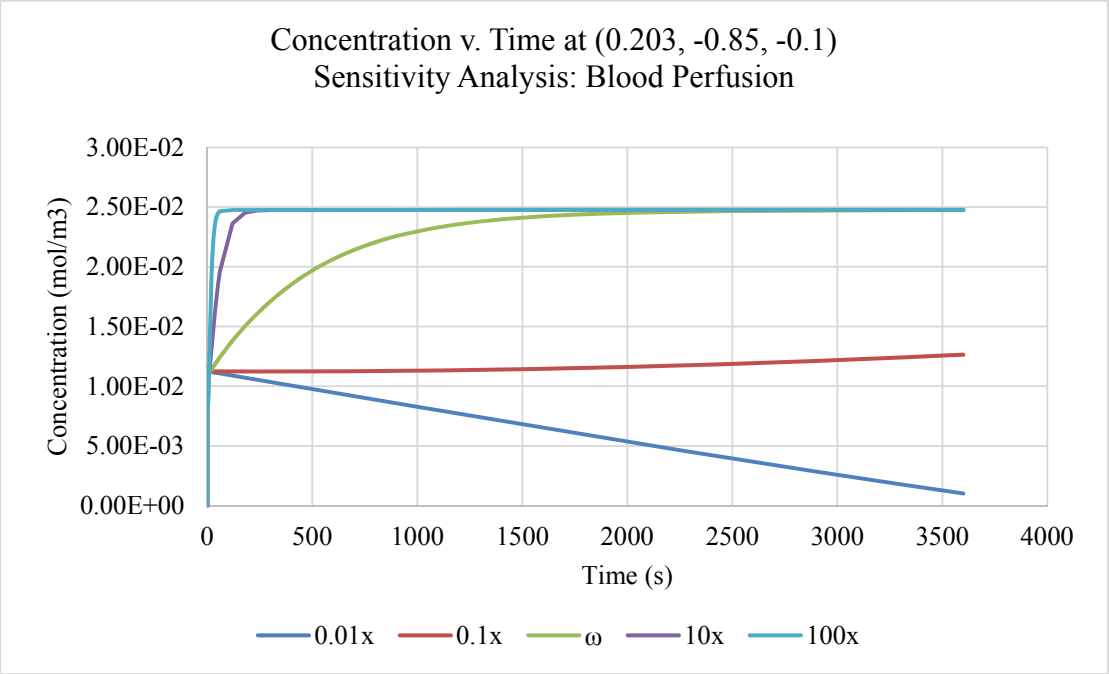

Figure 9: Concentration v. Time at point, (0.2038, -0.85, -0.1). Blood perfusion was varied by ±10x and ±100x while other input parameters were held constant.

Data from Figure 9 indicates that our model is the most sensitive to the blood perfusion value, with the most realistic model being obtained from the smallest values available in the range. This further supports the conclusion that the convective fluid transfer is the most influential mode of transportation for the transport of venom.

CONCLUSIONS

In generating a model that implemented a snake bite, we hoped to simulate the transport of venom through a human body during the initial hour after the bite. We wanted to consider the different modes of transport that contribute to the spread of venom throughout the body, leading to the formulation of models that simulate venom spread via diffusion alone, diffusion coupled to Darcy flow, and diffusion coupled to Darcy flow and a convective reaction term.

Our initial implementation, which relied solely on the physics of diffusion, suggested that after an hour the venom remained concentrated at the boundary of injection and did not travel far throughout the body. These results were not aligned with our expectations derived from clinical trials in literature, indicating that diffusion alone was not responsible for the expected spread of venom throughout the body.

In order to simulate the effects of the pressure of injection, we then coupled diffusion to Darcy flow as the movement of a fluid through a porous medium. We implemented Darcy flow as a boundary

condition through the boundary of the spherical injection site, using values that were adjusted for the surface area of the sphere that clearly differed from the surface area of a snake fang injection site. The pressure of injection was set to last for the length of a snake bite, 0.254 seconds, rendering the implementation of Darcy flow as short on the scale of our model. Because of the short injection time, the effects of Darcy flow were not as extreme or apparent as we had expected. The resulting concentration plots were not significantly different from those observed with diffusion alone, so we knew that the spread of venom must be attributed to a different physics.

Because of limitations of domain in COMSOL, we could not simply implement convection as its own mode of transport. Instead, we had to consider the transport of venom by fluid flow with a velocity as a reaction term. This reaction term was formulated to carry, with a velocity, venom from locations with a high concentration to locations with a low concentration. The initial results of this implementation appeared inaccurate, with concentration at a point near the injection site increasing and equilibrating over time, rather than increasing rapidly and decreasing slowly over time. Upon performing sensitivity analysis, we determined that the value for blood perfusion we calculated based on literature values was too high, suggesting that there is some resistance to fluid flow or additional physics that was unaccounted for. A blood perfusion value that was reduced by two factors of ten, or by 100, yielded results that were far more suitable to the expectations that we had for the process.

Assuming that with experimental values this blood perfusion term could be corrected to a more accurate value, convection was determined to account for the majority of the transport of venom from a snake bite in the leg throughout the remainder of the body.

DESIGN RECOMMENDATIONS

To improve the current model, more research must be done into the factors affecting the bulk flow of venom tissues, in order to improve the reliability of the final step of our model. Once the model accurately simulates the dispersion of venom, a deformed geometry physics should be implemented to simulate the resulting swelling that we originally wished to incorporate in our model. This deformed geometry physics must take into account the concentration of venom present at a given location in the domain of the leg, as well as the amount of time that concentration level is present at said location, as swelling in real life is affected by both the amount of venom present as well as the length of time it has had to act on the bodily tissues. At this point the model would be considered complete in the original intent of the researchers.

Further improvements could take into account different types of tissue and other regions of the body, such as the peritoneal tissue of the abdomen, as well as other metalloproteases and enzymes present within the crude Bothrops asper venom. If it is possible to produce a more generalized model that could simulate the dispersal of crude venom in the body, future researchers can endeavor to catalog the properties of viperid snakes and potentially create a module that could allow for the comparison of different venom compositions, thus supporting ecological efforts to understand the evolutionary development of venom, and medical efforts to understand complex envenomation and develop better guidelines for managing and treating snakebite.

APPENDIX A: MATHEMATICAL STATEMENT

EMPIRICALLY DETERMINED VALUES

DIFFUSIVITY

Diffusivity coefficients for both Bothrops asper venom and BAP1 are unavailable. We assumed that BAP1 is the only active toxin in the Bothrops asper venom and the results of edema and caused by the diffusion of BAP1 only. Therefore, empirical formulas were used to estimate the values of $D_{BAP1-Venom}$ and $D_{BAP1-Tissue}$. Snake venom has the similar consistency and viscosity to water. Therefore $D_{BAP1-Venom}$ was assumed to be equal to $D_{BAP1,aq}$. Two methods were used to find D_{aq} empirically.

Stokes-Einstein Correlation:

$$D_{AB} = \frac{k_B T}{6\pi\mu R}$$

Polson Correlation:

$$D_{AB} = \frac{9.40 \times 10^{-15} T}{\mu M_W^{1/3}}$$

Effective hindered diffusivity of a solute in a pore:

$$D_{eff} = \phi D_{aq}$$

Table A1: Parameter values used to calculate D_{aq} and D_{eff} of BAP1 using the Stokes-Einstein and Polson methods.

Parameter	**Variable**	**Value**
Boltzmann's Constant	k_B (J/K)	1.38054E-23
Molecular weight of solute	M_W (g/mole)	24071 [6]
Viscosity of water	$\mu_{water,\ 37°C}$ (kg/m•s)	6.92E-4
Temperature	T (K)	310
Diameter of solute	d_{BAP1} (m)	8.31E-9 [7]
Porosity of Tissue	Φ (%)	90 [8]

Table A2: Empirically determined diffusivity coefficients determined by the Stokes-Einstein and Polson methods. Effective diffusivity values were calculated considering the porosity of human tissue.

Method	**D_{aq}=** $D_{BAP1-Venom}$ (m^2/s)	**D_{eff}=** $D_{BAP1-Tissue}$ (m^2/s)
Stokes-Einstein	7.89646E-11	7.10681E-11
Polson	1.45843E-10	1.31259E-10

These two methods yielded two similar diffusion coefficient values, leading us to have confidence in the predicted values.

The molecular weight of BAP1 is 24071 g/mole. Green Florescent Protein is about the same size, with a molecular weight of 26900 g/mole. The diffusivity constant of GFP is 8.7E-11 m^2/s [9]. Since the calculated diffusivity values for BAP1 are similar to that of GFP, we have confidence that the predicted diffusivity values of BAP1 are in the correct range. The diffusivity coefficients found using the Stokes – Einstein method were used because it is the most general empirical formula for diffusivity coefficients.

INPUT PARAMETERS

Table A3: Parameter values used to model the spread of venom in human tissue in the simplified 2D model. The volume of the reservoir was determined by performing a Surface Integration of Domain 2 and having COMSOL compute the volume integral.

Parameter	**Value**
$c_{initial,\ reservoir}$ (g/m^3)	500
$c_{initial,\ tissue}$ (g/m^3)	0
$V_{reservoir}$ (μL)	384
$D_{BAP1\ -\ Venom}$ (m^2/s)	7.89646E-11
$D_{BAP1\ -\ Tissue}$ (m^2/s)	7.10681E-11

Table A4: Parameter values used to model the spread of venom in human tissue in the 3D leg model. The volume (V) and length (l) of the leg were determined by performing a volume and line integral, respectively. Treating the leg as a cylinder and utilizing its volume and length, the average diameter of the leg was found.

Parameter	**Value**
$c_{initial,\ tissue}$ (g/m^3)	0
$D_{BAP1\ -\ Tissue}$ (m^2/s)	7.10681E-11
Φ (%)	90
V_{leg} (m^3)	0.01126
l_{leg} (m)	0.82181
κ (m^2)	1E-17
ρ_{venom} (kg/m^3)	1084 [10]
μ_{venom} $(Pa \bullet s)$	0.044 [10]

INWARD MASS FLUX

Inward mass flux was derived using mean venom yield (dry mass) of Bothrops asper, and venom flow data from a similarly sized relative pit-viper, Crotalus atrox. As the amount of venom a snake ejects is dependent on the context of the bite, we have assumed the bite would be defensive in nature. The inward flux was calculated scaled to the size of our inject hemisphere, as opposed to the orifice of a snake's fang, to keep the total amount of venom entering the leg domain consistent with expected values seen in nature.

$$Mass\ Flux\ (N_0) = \frac{Mass\ of\ Venom\ (kg)}{Flow\ Time(s)\ \times\ Surface\ Area\ of\ Injection\ (m^2)}$$

$$N_{0,} = 9.52 \times e^{-15t}\ kg/m^2 s$$

The exponential term in this mass flux is designed to let the flux decay to zero after 0.254 seconds, the duration of venom injection in a snakebite [14]. This mass flux is implemented in the Darcy's law physics and represents the injection of venom that causes a pressure gradient. Another mass flux must be implemented into the Transport of Diluted Species physics. This mass flux would represent the inward flux of BAP1. The inward flux of BAP1 was calculated by dividing the mass flux of crude venom by the density of the crude venom and multiplying by the concentration of BAP1 in the injected volume of venom.

$$Drug\ Velocity = \dot{v} = \frac{\frac{9.52kg}{m^2-s}}{\frac{1084kg}{m^3}} = 0.0088\frac{m}{s}$$

$$Drug\ Flux = C_0\dot{v}\left[\frac{mol}{m^3}\right]\left[\frac{m}{s}\right] = [\frac{mol}{m^2-s}]$$

$$N_{0,c} = 0.0319 \times e^{-15t}\ mol/m^2s$$

Table A5: Parameter values used to calculate the mass flux of venom through muscle tissue. Surface area of injection was found by performing a surface integration in COMSOL.

Parameter	**Value**
ρ_{venom} (kg/m^3)	1084 [12]
$t_{ejection}$ (s)	0.254 [14]
m_{venom} (kg)	1500mg [4]
$C_{BAP1\text{-}venom}$ (mol/m^3)	12.1 [15]
$A_{injection}$ (m^2)	6.2026E-4

CONVECTION

Another input parameter that was estimated was the velocity of blood circulation in the muscle tissue of the leg that contributes to the convective mass transfer of the venom. Because the velocity would drive the venom to the skin boundary, and the skin has a no flux boundary condition, COMSOL solver would crash if we used a convective term to implement circulation. Thus, the parameter is integrated into the 3D model by adding a reaction term. This reaction term is composed of three parameters: a blood flow velocity, assumed to be a constant, the concentration of the blood and the concentration at a given point in the leg, values inputted and updated by COMSOL. The difference between these concentrations multiplied by the constant blood flow velocity makeup the reaction term, R. The time constant blood flow rate was represented as ω and was determined by relating blood flow in the leg to the legs entire volume. The blood concentration was computed by subtracting the current venom mass in the leg from the total venom mass flux into the system to account for venom leaving the leg and being transported to other areas in the body. To adjust for the different volume of blood in the leg compared to the body, this value was then multiplied by a leg/body mass ratio (m). This reaction term, while relatively simplistic, was designed to model the composite convective transport within the entire volume of the leg. These are the equations used in COMSOL for the reaction term.

$$R = \omega(c - c_{blood})$$

ω = blood perfusion time constant:

$$\omega = \frac{total\ blood\ flow\ in\ leg}{total\ leg\ volume}$$

c_{blood} = concentration in blood:

$$c_{blood} = m\frac{\left(\int_0^t mass\ flux\ in\right) - total\ mass\ in\ the\ leg}{total\ leg\ volume}$$

m = leg/body mass ratio

Table A6: Parameter values used to calculate the reaction term that represents the convective fluid transfer of venom through muscle tissue. Volume of the leg was found by performing a volume integration in COMSOL.

Parameter	**Value**
$\dot{V}_{blood,in\ leg}$ (m^3/s)	1084 [11]
V_{leg} (m^3)	.01126 (Calculated in COMSOL)
Blood perfusion time constant, ω (s^{-1})	.00178
m	.1755 [17]

APPENDIX B: SOLUTION STRATEGY

TIME STEP AND TOLERANCE

2D AXISYMETTRIC SOLUTION

Time step range: (0,5,3600)

Direct solver: PARDISO

Time – Dependent Solver (BDF): Absolute tolerance = 0.001

3D LEG MODEL

Time step range: (0, 0.25, 1), (2, 1, 59), (60, 60, 3600)

Direct solver: MUMPS

Time – Dependent Solver (BDF): Absolute tolerance = 0.001

2D AXISYMMETRIC MESH STRUCTURE

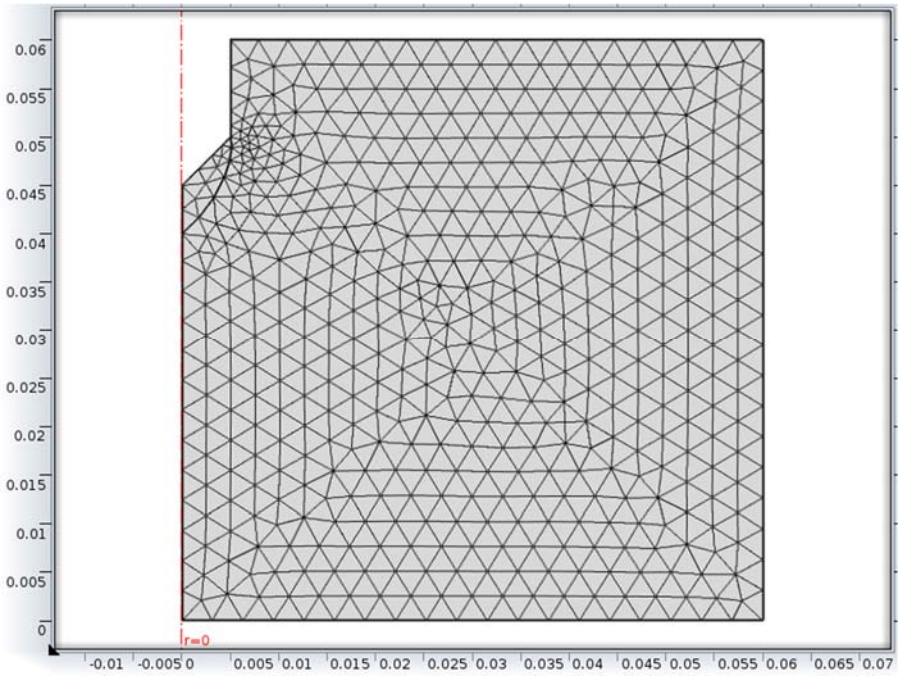

Figure B1: 2D axisymmetric geometry mesh. Free-triangular meshing utilizing the predefined size, Fine. Smaller elements are located near the venom reservoir. Mesh Convergence for this 2D simplified model is located below.

2D MESH CONVERGENCE

A mesh for the 2D simplified solution was created using Free Triangular elements. Normal sized elements produce a mesh with 696 elements. Fine produces 1013, Finer produces 2007, and Extra Fine produces 6470. Concentration v. Time at a point on the tissue domain close to the reservoir (r = 0.051m, z = 0.04m) was analyzed.

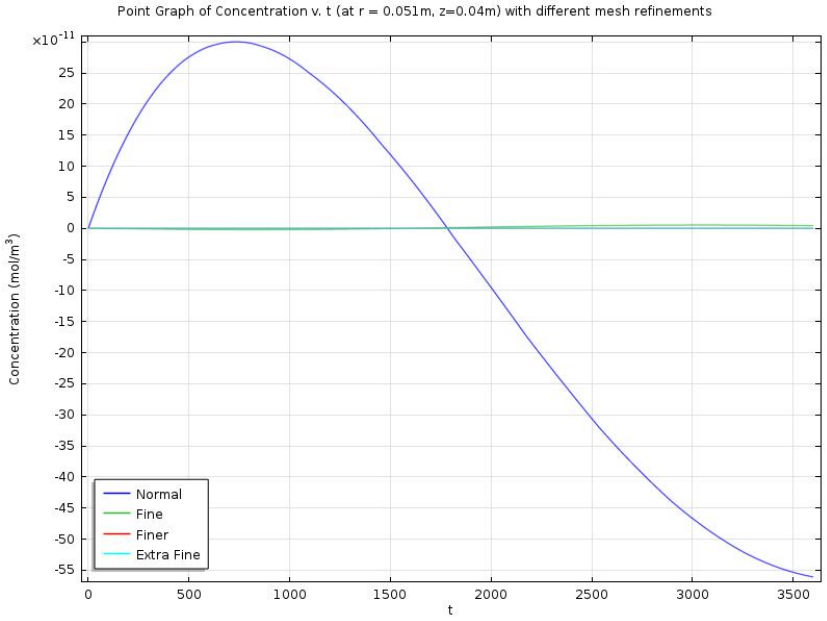

Figure B2: Mesh convergence done with a parametric sweep of mesh values corresponding to Normal, Fine, Finer, and Extra Fine settings built into COMSOL. This mesh convergence was done based on a CutPoint 2D location of r = 0.051m, z = 0.04m. Concentration v. Time at this point converges on Fine, Finer, and Extra Fine.

Figure B2 indicates that using the mesh converges between the Fine and Extra Fine setting. Thus, the Fine setting was used for the mesh sizing (Figure B1). This setting uses 1013 elements, with more elements concentrated near the pool of venom.

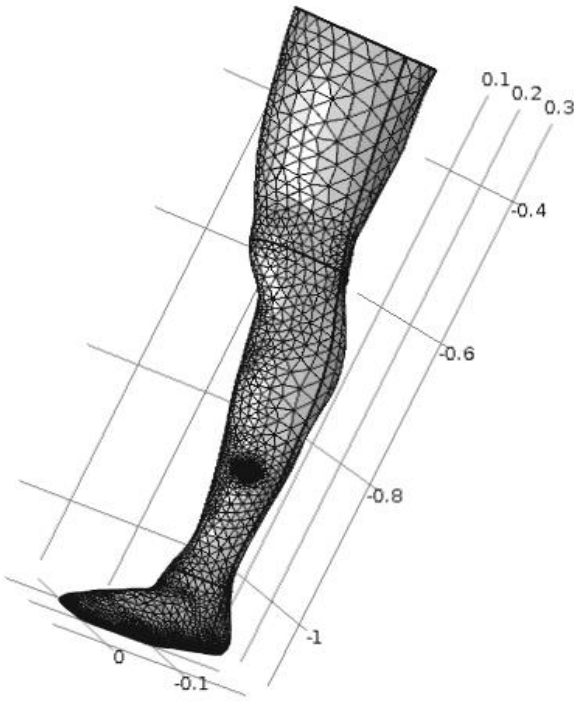

Figure B3: 3D model mesh structure of CAD leg used in the 3D implementation. Free Tetrahedral mesh utilizing the predefined size, Coarse (50427 elements), with more elements located near the injection site.

3D MESH CONVERGENCE

A mesh for the 3D leg was created using Free Tetrahedral elements. Mesh convergence was analyzed using the predefined sizes, Extremely Coarse, Coarser, Coarse, Fine, and Extremely Fine.

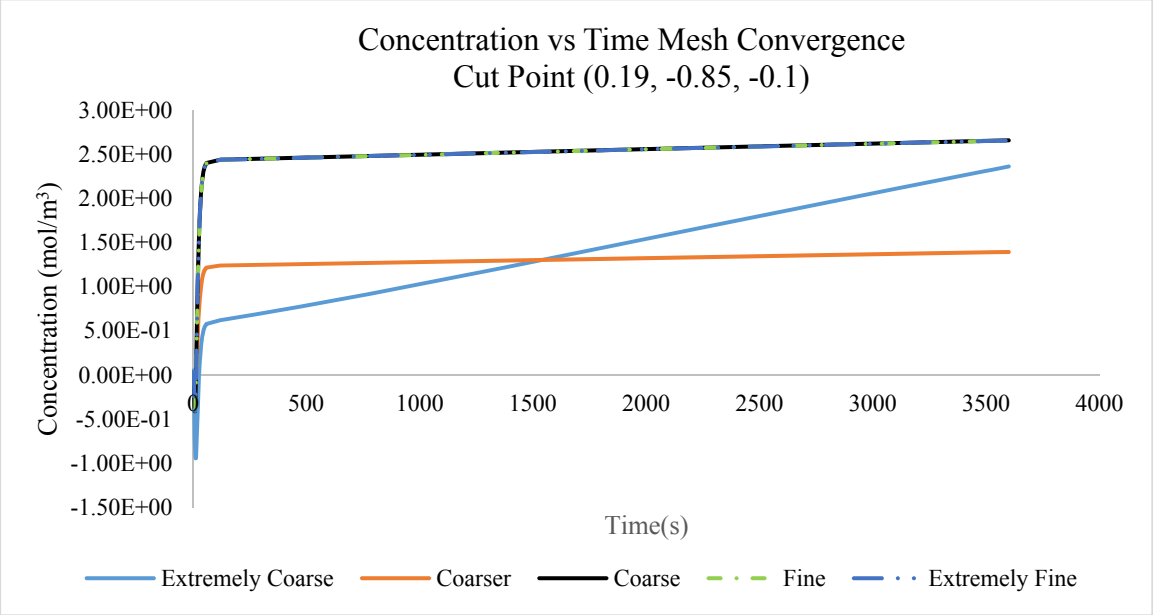

Figure B4: Concentration v. Time at point (0.19, -0.85, -0.1) using different element sizing. Solutions indicate mesh convergence at Normal sized meshing (50427 tetrahedral elements). Normal mesh results overlap with Extra Fine results.

Figure B4 indicates that the mesh converges at the Coarse setting. Thus, the Coarse setting was used for the mesh sizing for the 3D model. This setting uses 50427 elements, with elements concentrated near the pool of venom.

APPENDIX C: REFERENCES

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APPENDIX D: TEAM RESPONSIBILITIES

Team member name	Nick	Victoria	Marek	Melanie	NOT DONE
Wrote abstract		X	X		
Edited abstract			X	X	
Wrote introduction			X	X	
Edited introduction		X	X	X	
Wrote method section	X	X			
Edited method section			X	X	
Wrote results section	X	X		X	
Edited results section			X	X	
Wrote discussion section	X	X	X	X	
Edited discussion section			X	X	
Wrote summary and conclusion section			X	X	
Edited summary and conclusion section			X	X	
Wrote bibliography section		X	X	X	
Edited bibliography section		X	X	X	
Prepared processed data table for appendix		X		X	
Checked data in processed data table in appendix		X		X	
Prepared figures or tables for main text				X	
Checked figures or tables in main text		X	X	X	
Assigned tasks to group members					X
Put the report together from the parts provided by others		X	X	X	
Read and edited entire document to check for consistency		X	X	X	