

# THE DEVELOPMENT OF A NEUTROPHIL REPROGRAMMING DEVICE FOR THE TREATMENT OF SEPSIS

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## INTRODUCTION

Sepsis is a system wide inflammatory condition caused by severe infection in the blood. The condition is widespread and potentially fatal, with millions of individuals being affected around the world and killing one in four persons. [1] Moreover, the rate of incidence is steadily on the rise, with hospitalizations with sepsis and hospitalizations for sepsis both increasing in recent years [2].

Sepsis is generally initiated by a bacterial infection and complications begin to arise when the body's immune system attempts to compensate and defend against the infection. However, this innate immunological response is often too drastic and can lead to damage of healthy tissue and eventually organ failure. The main mediator for this response are leukocytes called neutrophils, which have increased chemotaxis to sites of infection in times sepsis [3].

The development of a device to treat sepsis begins with a method to reduce the effective neutrophil chemotactic response. The main objective is to regulate the response in an extracorporeal device and return the neutrophil to the body in a state at which the neutrophil no longer responds to chemotactic signals in the body. To achieve this, an ideal device would consist of hollow fibers immobilized with the naturally occurring chemokine, Interleukin-8 (IL8), on the inner surface. Neutrophil chemotactic response has been shown to decrease with exposure to this chemokine. As blood flows through the hollow fibers, receptors on neutrophil bind to these chemokines, internalize, and are returned to the body in a primed state. Several factors of this device need to be considered, including the characterization of the neutrophil response as it passes through the fibers and the amount of chemokine immobilized on the surface of the fibers to produce that response.

## OBJECTIVE

The objective of the project that was assigned was to quantify the amount of chemokine immobilized on the inner surface of the hollow fibers by using various protein quantification assays, such as the  $\mu$ BCA assay or the Bradford assay. Once quantified, the amount of protein immobilized may be optimized to deliver the optimal chemotactic response for neutrophils.

## SUCCESS CRITERIA

The success criteria for this study is to successfully obtain a quantitative measurement of how much protein is deposited onto the inner fiber surface during the immobilization process and to find consistency among the results.

## METHODS

Aminated polysulfone fibers were set inside of a thin, cylindrical plastic module with diameter 5mm and length 15cm. Several washes with detergents such as SDS and PBS took place in order to ensure the fibers were cleansed of foreign protein prior to the immobilization. Immobilization of the IL-8 took place by circulating glutaraldehyde and subsequently IL-8 to covalently attach the protein to the surface of the fiber.

In order to quantify the amount of protein deposited on the fibers, a mass balance approach was used. That is,

$$[\text{Protein Pre}] - [\text{Protein Post}] = [\text{Protein Deposited}]$$

The Protein Pre quantity was the concentration of the IL-8 solution prior to recirculation and the Protein Post quantity was the concentration of the IL-8 solution after recirculation. The difference of these two, adjusted for the volumes, would provide the amount of protein deposited on the fiber surface.

The actual quantification of the protein involved using a spectrophotometric protein quantification assay called the  $\mu$ BCA assay. Several samples of the solutions were taken from the two effluents and measured using the assay. Finally, the concentrations were averaged, adjusted for the total volume of the solution, and summed together to provide the total amount of protein in the effluents.

## RESULTS

The results of the experiment demonstrated that the final sample of protein solution contained 200 $\mu$ g of protein and the initial sample contained 150 $\mu$ g of protein. This result was opposite of the expectation that the final sample would have less protein than the initial sample of protein. One such result is shown in the graph on the next page, which demonstrates the total amount of protein that was in the Final Protein effluent and the Initial Protein effluent [Figure 1].

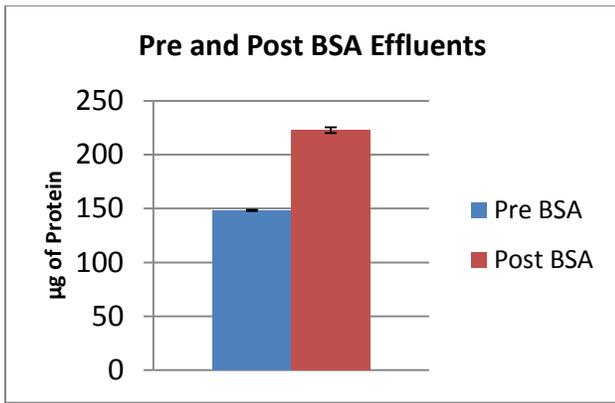


Figure 1 Total Amount of Protein in Pre and Post Effluents

The amount of protein deposited on the fibers was not reported due to the inconsistency in the results and the intention of the experimental design.

### DISCUSSION

According to the results, the Post Effluent showed more protein than the Pre Effluent. The error bars are very small in this case due to the fact that the  $\mu$ BCA assay is extremely precise and the consistency of the low error means that these values should have a high degree of accuracy. The result shown is in contrast to the expectation of the Pre Effluent containing more protein than the Post Effluent as some of the protein in the solution was to be deposited onto the surface of the fiber. There are several interpretations of this result. One possibility is that the modules contained some substance that was deposited into the final solution that triggered the  $\mu$ BCA assay and caused it to show a higher signal. Another possibility is that the fibers were initially contaminated with foreign particles that ended up in the final solution as solutions were recirculating. A third possibility is that the assay that was used may be sensitive to steps that were taken in the immobilization process.

In order to solve some of these issues, there are a few directions that can be taken in terms of the experimentation. One option is to ensure that all detergents, adhesives, plastics, and manufacturing components are all compatible with the assay; that is, these items should not cause a signal or inhibit a signal in the assay. Another option is to optimize the cleansing of the fibers prior to immobilization to ensure that there are not foreign contaminants as the immobilization is taking place. A third solution would be to make use of a different assay such as a Bradford Assay or an ELISA that would be more compatible due to the mechanism of protein detection or the entire process.

Due to the difficulty in assessing the amount of protein deposited on the fibers, another option has been considered to use a different type of fiber that would have the immobilized protein on the surface of the fiber. This would change the design of the device, but it is an option that has been considered due to the fact that other devices have used such a fiber and have had success in determining amounts of protein deposited

on the surface. These options are not of priority, however, due to the other implications that such a change may elicit.

### CONCLUSION

The intent of the experiment was not achieved and thus the success criterion was not met. There are several other directions that will be taken to solve some of the potential problems that may have arisen in the experimentation. A drastic approach would be to change an aspect of the device, but there are design implications that must be considered before taking such a route. Further analysis on how the device performs as an effective treatment for sepsis will be assessed via different experiments in the future, but for the present, the quantification remains an important goal to meet.

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### REFERENCES

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