

## Introduction

Chronic wounds are wounds characterized by disordered wound healing processes and fail to heal after one month<sup>[1]</sup>. Though chronic wounds affect about 2% of people in the United States per year, they are difficult to track because they are often underlined by other health conditions, such as diabetes<sup>[1]</sup>. Because of the disordered wound healing process, chronic wounds are susceptible to bacterial infection. In particular, *Staphylococcus aureus* is a bacterium that commonly causes infections, and is one of the most prevalent bacteria in chronic wounds<sup>[2]</sup>. Typically, macrophages kill bacteria in wounds, however studies show that *S. aureus* can evade macrophage killing and can alter macrophage activity<sup>[5][6]</sup>. We hypothesize that *S. aureus* infection changes the progression of chronic wounds, and our study explores this progression using adapted simulations from a previously constructed agent-based model (ABM) of a healing wound<sup>[3]</sup>. Our study will increase understanding of the role of bacterial infection in chronic wounds, contributing to the search for mitigation of these wounds.

In order to address the above-stated hypothesis, modifications of the previously constructed ABM were made, with particular emphasis on the relationship between macrophage activity and the progression of wounds into chronic wounds, as well as the relationship between the administration of antibiotics and the same progression of wounds to chronic wounds. From these 2 areas of exploration, the 2 primary questions that were used to investigate the above-stated hypothesis are as follows:

1. Can altered macrophage activity (specifically, dysregulated functionality) lead to the formation of a chronic wound from an initial, non-chronic wound with bacteria present?
2. Which role do antibiotics play in treating wounds with dysregulated macrophage activity?

## Methods

### Agent-based model

The Netlogo<sup>TM</sup> agent-based model simulates a wound's healing process. To represent the bacteria in a wound, the model accounts for the initial amount of bacteria in the wound and how quickly it divides. Similarly, the model has an initial amount of *S. aureus* and includes the proliferation rate of 2 hours. The model represents neutrophils, which target and kill the bacteria, and macrophages, which also kill bacteria. Additionally, these leukocytes endocytose debris from dead bacteria in the wound. To simulate dysregulated macrophage activity, we included the ability to change the probability of macrophages killing bacteria when the two come into contact. The model also accounts for antibiotic treatment, which is used to treat *S. aureus* infection. Antibiotic treatment was simulated as a single dose administered at the onset of infection.

### Assumptions Made

Several assumptions were made in the development of this model. These include assuming that the bacteria had a proliferation rate of 2 hours, assuming that antibiotic-resistant strains of the bacteria would *not* develop, assuming that one "molecule/patch" of antibody was enough to result in the death of a bacterium, assuming that the ability of a macrophage to engulf a bacterium is represented by a probabilistic outcome, assuming that bacterial persistence is a measurement for wound prognosis, and the assumption that this simulation is a semi-closed-system (bacterium cannot invade other tissues or enter the bloodstream - this results in an ceiling limit to the bacteria population).

### Wound Simulations

We simulated 10 wounds for 100 days each. The first set of five simulations were with no antibiotic treatment. The neutrophil and macrophage responses were low, the initial number of bacteria was 25, and the doubling time for the bacteria was 2 hours. The probability that macrophages kill bacteria was set to 0.2, 0.4, 0.6, 0.8, and 1 for the 5 simulations, respectively. The second set of five simulations were the same as the first five, but with antibiotic treatment. For each simulated wound, we average data across 10 trials.

### Analyses/Statistics

We create a measure of how chronic the wound is called bacterial persistence. Bacterial persistence is defined in Figure 1.

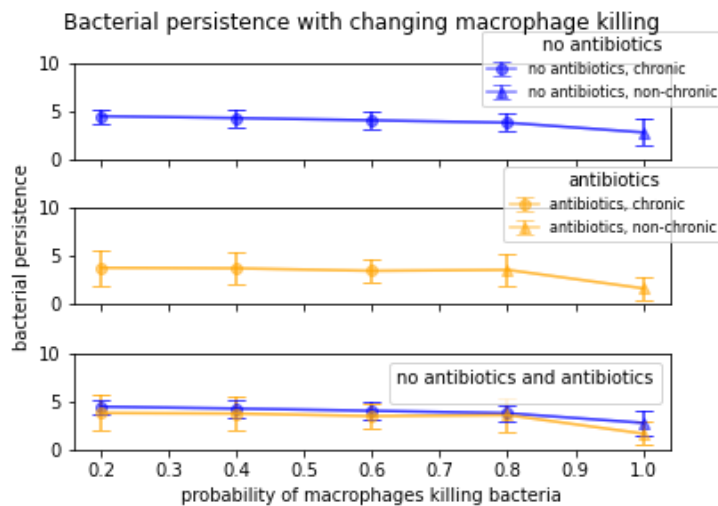
$$bacterial\ persistence = \frac{\sum_{time} \# of\ bacteria}{\sum_{time} \# of\ neutrophils + \sum_{time} \# of\ macrophages}$$

**Figure 1.** The above figure is an equation for bacterial persistence. Each wound simulation, averaged over 10 trials, has a bacterial persistence. The amount of bacteria, summed over time points, is normalized to the amount of neutrophils and macrophages, also summed over time points.

We plotted bacterial persistence with their corresponding probabilities of macrophage killing and calculated a Spearman correlation coefficient and p-value for this coefficient. For this analysis, we set  $\alpha = 0.05$ . We performed a 2-tailed t-test to compare the wounds with no antibiotic treatment to the wounds with antibiotic treatment. For this analysis, we set  $\alpha = 0.05$ . We determine that a wound is non-chronic if more than half of the trials have 0 bacteria by the last time point.

### Results

A.



B.

	Without antibiotics	With antibiotics
Spearman's correlation coefficient	-0.999	-0.899
p-value	$1.40 \times 10^{-24}$	$3.74 \times 10^{-2}$

**Figure 2.** Panel A of the figure shows the probability of macrophages killing bacteria on the x-axis against bacterial persistence, defined in the Methods section. Panel A distinguishes between chronic and non-chronic wounds by shape, as written in the legend. Panel B shows Spearman's correlation coefficient and the corresponding p-value for both of the curves in Panel A. Performing a 2-tailed t-test to compare wounds with no antibiotic treatment to wounds with treatment, we fail to reject the null hypothesis with p-value=0.256.

Figure 2 shows that with both no antibiotics and with antibiotics, bacterial persistence decreases as the probability of macrophages killing bacteria increases. There is a strong negative correlation between the probability of macrophage killing bacteria and bacterial persistence, as shown by the correlation coefficients. And at the  $\alpha$  we set, both of these correlations are statistically significant. But according to the t-test, the difference between the two groups of wounds is not statistically significant. Using probability of macrophage killing as a proxy for macrophage function, our analyses support the hypothesis that dysregulated macrophage activity affects the prognosis of wounds, but that there is not a significant difference between the sets of wounds.

### Conclusions/Future directions

According to our model and in line with our assumptions, we conclude that there is evidence to support that a chronic wound can arise by dysfunctional macrophage activity, and there is a positive correlation between decreasing macrophage functionality and poor wound progression. Additionally, we conclude that the difference in wound progression between wounds with and without antibiotic

treatment is not significant. While antibiotics may aid in the treatment of chronic wounds, our analyses suggest that antibiotics are not sufficient.

In regard to future directions for this model, it would be interesting to implement different dosing antibody trials, such as simulating how the wound would progress (either healing or transitioning to a chronic-wound state) if treated at different time points along the progression timeline at varying doses when compared to a control.

## **References**

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