Mathematical Modeling and Analysis of Inflammation and Tissue Repair: Lung Inflammation and Wound Healing in Corals Under Stress

Quintessa Hay

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MATHEMATICAL MODELING AND ANALYSIS OF INFLAMMATION AND TISSUE REPAIR: LUNG INFLAMMATION AND WOUND HEALING IN CORALS UNDER STRESS

A dissertation submitted in partial fulfillment of the requirements for the degree of Doctor of Philosophy at Virginia Commonwealth University.

by

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May, 2024
Acknowledgements

Thank you to my advisor Dr. Angela Reynolds who always provided me with the best opportunities and advice. Thank you for always supporting me and knowing that I could do this every time that I questioned myself along the way.

Thank you to Dr. Rebecca Segal, committee member and collaborator, who provided instrumental advice and guidance. Thank you for always being a kind face to see around campus.

Thank you to Dr. Nastassja Lewinski, committee member and collaborator, who introduced me to the fascinating world of coral biology. It was a joy caring for the corals and watching snails roam about the tank. Thank you to Dr. Lewinski’s lab, particularly Dr. Liza Roger, for providing guidance on experimental methods and coral biology. Thank you to all the undergraduate students in the lab who helped with the extensive data collection involved in tracking coral wound healing.

Thank you to Dr. Harold Ogrosky, committee member, for your support and expertise. I appreciate all your effort in reviewing my work and offering kind support.

Thank you to collaborator Dr. Rebecca Heise and lab for your guidance and effort in models of lung inflammation. Your biological insight greatly improved my models and I am so grateful for all your students who helped in collecting data.

Thank you to my undergraduate advisors and mentors Dr. Colton Sawyer, Dr. Adam Gilbert, and Dr. William Jamieson. Your support gave me the confidence to pursue graduate school. To Dr. Sawyer, thank for introducing me to mathematical biology and for all your support as an undergraduate advisor. To Dr. Gilbert, thank you for teaching yourself R so that you could teach it to us at SNHU. This was my first introduction into any mathematics-based software and has helped me greatly in my graduate studies. Thank you to both Dr. Gilbert and Dr. Jamieson for easing my
worries about succeeding in graduate school. I frequently thought about your kind words and advice when I was doubting my place here.

Thank you to my partner Eduardo Moreno, who has been my biggest cheerleader throughout this whole process. Your love and support has given me strength and healed parts of me I didn’t know were broken. You’ve picked me up off the ground and dried my tears more times than I can count. I look forward to figuring out real adult life with you. Thank you to your family as well for cheerleading for me along with you and offering a home away from home.

Finally, thank you to my parents, Jocelyn and Bill, for your continued support throughout my long academic career. Thank you to my mother who believed math would be important in my life even when I said “I don’t want to study math.” You always know me better than I know myself. Both of you have given me the tools to be successful and have always encouraged me to pursue my goals. Thank you for your love and support. Thank you as well to my brothers Brenden and Caleb for being built in friends (and sometimes rivals) who know what it was like to grow up in our house. It was a pleasure growing up by your side.
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Abstract

MATHEMATICAL MODELING AND ANALYSIS OF INFLAMMATION AND TISSUE REPAIR: LUNG INFLAMMATION AND WOUND HEALING IN CORALS UNDER STRESS

By Quintessa Hay

A Dissertation submitted in partial fulfillment of the requirements for the degree of Doctor of Philosophy at Virginia Commonwealth University.

Virginia Commonwealth University, 2024.

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A variety of insults, including tissue injury and/or exposure to pathogen, elicit an immune response in many organisms. An improperly regulated immune response can result in deleterious effects to the organism. Here we present models for lung injury in young and old mice and models for wound healing in coral reefs. It is well known that the immune response becomes less effective in older individuals. This is of particular interest in pulmonary insults such as ventilator induced lung injury (VILI) or lung infection. We extended a mathematical model for the inflammatory response to VILI and used experimental data to select parameters and perform model analysis. We then modified this model to include a bacterial insult and specific cytokine populations, performing a similar process for parameter selection and model analysis. In both cases, parameters involved in macrophage activity primarily drove observed differences associated with the young or old data. Coral reefs regularly
experience wounding events some corals have exhibited an immune response similar to that of humans. The effects of climate change stresses the reef which may affect wound healing processes. To address this, we formulated a mathematical model for wound healing in corals in normal conditions and heat stressed conditions. We further paired this model with a dynamic energy budget model, to show how corals must balance energy reserves between growth and tissue repair. The models presented here serve as a first step to modeling the immune response to tissue damage in corals in various environments.
CHAPTER 1

INTRODUCTION

Cell damage and/or exposure to foreign matter elicits an immune response in many organisms. The immune response is an essential component of wound healing and defense against foreign infiltrates. In a healthy body, wound healing consists of four overlapping, yet distinct phases including hemostasis, inflammation, proliferation, and remodeling. The immune cells are mainly responsible for the processes involved in inflammation making them key components in wound healing as well as defensive mechanisms. Severe insults or a compromised immune response may result in disproportionate levels of inflammation, inability to clear infections or resolve tissue damage, or even uncontrolled inflammation resulting in organ damage. Biological systems, including those involving the immune response, have historically been modeled using mathematics to supplement experimental data or provide insight when experiments are difficult or costly. Here we present methods for mathematically modeling the immune response to a variety of insults in healthy and compromised individuals, focusing primarily on the inflammatory phase, which include the primary cellular actors and mediators involved in the innate inflammatory response.

Inflammation in the lungs can occur from stress or tissue strain resulting in cell damage, exposure to foreign bacteria or viruses, chronic diseases, or systemic inflammation. Chronic inflammation of the lungs can be particularly detrimental, so a rapid and well-regulated response is necessary. Inability to resolve inflammation leads to acute respiratory distress syndrome, acute lung injury, multi-system organ failure, or even death. Particularly pervasive injuries or infections may necessitate mechanical
ventilation potentially resulting in further lung injury referred to as ventilator-induced lung injury (VILI). Clinical research has observed marked differences in the immune response associated with age, evidenced by higher rates of severe disease and increased inflammation in older individuals. Due to the difficulties associated with experimental trials, mathematical models can be used to better understand these disparities. Specifically, we model the inflammatory phase and cellular repair following lung injury originating from various insults with a focus on age related differences.

Models of inflammation and tissue repair can also be applied to other species. Coral reefs currently face major threats to their survival, mainly from the effects of climate change, which can affect the organism’s ability to recover from damage. Recent reef restoration practices have also involved intentional wounding by systematically breaking coral fragments and relocating them to revitalize damaged reefs, a practice known as microfragmentation. Despite its importance, very little research has explored the immunological response to wounding in coral species, mainly due to difficulties associated with in situ, in vivo, and in vitro studies in corals. To our knowledge, no known mathematical models exist for the immune response to wounding in reef-building corals, thus we developed a baseline model for this response in normal environmental conditions. Perturbations in the environment have been observed to affect wound healing in corals, so we explored extensions of this baseline model to include wound healing in adverse conditions.

In Chapter 2 we present a model for VILI adapted from Minucci et al. to include terms for excess influx of inflammatory cells and mediators from the blood vessels into the lung as a result of damage to the alveolar epithelium. We also perform parameter sampling by fitting model outputs to experimental data for young and old mice and perform statistical analysis on the resulting parameter sets. Using these results, we perform pseudo-interventions in silico to estimate the potential for
improvement of alveolar health in specific groups. This work has been published in PLOS Computational Biology [2].

In Chapter 3, we present a model for lung injury induced by bacterial infection (exposure to lipopolysaccharides). The mathematical model is an extension of the VILI model presented in Chapter 2 to replace the ventilator damage with a bacterial insult and include specific cytokine populations to replace the general pro- and anti-inflammatory mediators in the previous model. Similar to the methods presented in Chapter 2, we perform parameter sampling paired with experimental data from young and old mice and analyze the resulting sets using a variety of statistical methods.

In Chapter 4, we present a novel model for the inflammatory response and tissue repair to injury in reef-building corals. The model includes debris and foreign infiltrates, amoebocytes (phagocyte cells), fibroblasts, and epithelial tissue. We perform parameter sampling and estimation methods using experimental data in the reef-building coral Pocillopora damicornis. We also perform sensitivity analysis and use the results to find an identifiable set of parameters. This work is currently in revisions for publication.

In Chapter 5, we extend the model formulated in Chapter 4 to model wound healing of corals in adverse environments. In Section 5.2, we sample parameter values of the mathematical model presented in Chapter 4 using new experimental data for normal and stressed environments. Parameter values were initially fit to the wound healing data under optimal environmental conditions, and then modified to fit those experiencing heat stress (+3°C). In Section 5.3, we pair the wound healing model with a dynamic energy budget model adapted from Cunning et al. [3]. This iteration of the model accounts for reef growth dynamics with and without a wound in optimal conditions and stressed conditions (increased light).

Finally in Chapter 6, we summarize our findings and present future directions
for the various models. Code for simulating each model is provided in the appendix.
CHAPTER 2

AGE-DEPENDENT VENTILATOR INDUCED LUNG INJURY

The work presented in this chapter has been published in PLOS Computational Biology [2]. All code used in this model is available on Zenodo (https://doi.org/10.5281/zenodo.8253722).

2.1 Introduction

A variety of inhaled pathogens and other pulmonary insults illicit an immune response that causes inflammation in the lung tissues. Intense or persistent inflammation can damage the delicate alveolar tissue and result in acute respiratory distress syndrome (ARDS). This can progress to complete respiratory failure in some cases. To increase the probability of survival, the clinical intervention for ARDS is the use of mechanical ventilation (MV) [4]. While MV is often a necessary procedure, prolonged use or misuse of the ventilator may result in ventilator-induced lung injury (VILI). The damage caused to alveolar sacs (clusters of alveolar cells) during MV can lead to volutrauma (extreme stress/strain), barotrauma (air leaks), atelectrauma (repeated opening and closing of alveoli), and biotrauma (general severe inflammatory response). The culmination of these injuries can result in ventilator dependence, multi-system organ failure, or even death [5, 6].

Although VILI can occur in patients regardless of prior lung health [5], there is a higher incidence of critical disease as well as observable differences in the inflammatory response of older individual [7, 8, 9]. Past research has shown increased risk of lung injury following ventilation for older mice [10, 11]. Most recently, infections
associated with the novel coronavirus have also exhibited an increased risk of mortality and severe disease in older patients [12]. One particular study found that 6.6% of participants aged 60 years of age and older developed critical disease following a SARS-CoV-2 infection; this is approximately twelve times higher than in younger participants (0.54%) [9]. Studies have also reported increased levels of circulating inflammatory cytokines and altered macrophage function in older mice [13]. These observed discrepancies in the inflammatory response and increased rate of mortality and severe disease in elderly patients stress the need for further studies of VILI in regard to aging.

Ninet al. [14] found increased susceptibility and severity for pulmonary and vascular dysfunction associated with age during high tidal volume ventilation in mice. Older mice also exhibited increased levels of inflammation marked by a higher concentration of interleukin-6, a pro-inflammatory cytokine, and aspartate aminotransferase, a non-specific marker of cell injury. This intrinsic decline in the effectiveness of the innate immune response has been studied extensively [15, 16, 17, 18]. Most notably, Dace and Apte [18] found that aging affected the polarization of macrophages, an immune cell that can exhibit a range of pro- and anti-inflammatory properties. An effective immune response relies on both a pro-inflammatory response to rid the insult of foreign cells and other debris and an anti-inflammatory response to regulate the pro-inflammatory response, promote repair, and remove debris incurred by early-response phagocytes. With aging, polarization of macrophages was observed to be skewed toward the alternatively-activated, M2, phenotype. Decreased activation of the classically-activated, M1, phenotype, generally associated with pro-inflammatory activities, could result in a decreased ability to clear infections and debris, thus prolonging the inflammatory response and inhibiting later stages of healing.

An imbalance in the pro- and anti-inflammatory responses can cause additional
complications for the individual during various injuries and insults. Macrophages in particular play a significant role in the impact of aging on the immune response \[13, 19, 20\]. Therefore, to develop interventions to mitigate the effects of VILI, it is important to study the immune response to lung injury and the interplay between various types of cells. We are focused on the innate immune cells, neutrophils and macrophages, their associated cytokines, and the alveolar epithelium, which consists of alveolar type I and type II cells. Alveolar type I cells make up about 95% of the alveolar surface and are primarily responsible for facilitating gas exchange. Type II cells cover the other 5% of the surface and are important in the innate immune response. In the presence of damage, these cells proliferate to repair the epithelium and can also differentiate to type I cells \[21, 22\]. In the present study, we examine these cellular populations in 2-3 month old mice (young) and 20-25 month old mice (old) exposed to high-pressure MV for up to 2 hours. We present broad macrophage sub-phenotypes, M1 and M2, obtained from flow cytometry and quantitative measures of lung damage at the alveolar epithelial-endothelial barrier.

We use mathematical modeling and statistical methods to investigate the differences in the pulmonary innate immune response and predicted outcomes for the model simulations associated with either young or old experimental data. At this stage of exploration of VILI, we focus on epithelial damage and immune system interactions in young and old mice. It is difficult to clinically isolate the local epithelial and inflammatory response in the lung during VILI and often expensive to collect quality data. For this reason, we rely on \textit{in silico} modeling of experimental data to supplement the available \textit{in vivo} data. These \textit{in silico} approaches provide insight into the immune response and the nonlinear dynamics of the system. The resulting analysis is used to identify important factors and generate hypotheses \[23\].

Minucci \textit{et al.} \[1\] developed a model for VILI including major immune cell
interactions involved in the inflammatory response to tissue damage and epithelial variables encompassing healthy, damaged, and dead epithelial cellular states. The current model is an expansion of the model built in Minucci et al. by including terms modeling epithelial barrier breakdown leading to increased cytokines and immune cells in the alveolar compartment. The resulting model has 19 variables and 64 parameters. In this study we use the young and old experimental data to select plausible parameter sets and explore age-dependent outcomes and dynamics.

These mechanistic, equation-based models are often used in conjunction with statistically-based methods and models to understand the possible dynamics associated with varying parameter sets. Parameter sampling and post-analysis of the mechanistic data obtained from the model are just two examples of processes that benefit from a statistical approach. To sample large parameter spaces, numerous aptly named ‘space-filling designs’ have been developed since the advent of computer experiments in the 1970s. Perhaps the most commonly used design is Latin hypercube sampling, but many others are used, including uniform sampling and maximin designs. Many others have built off these general designs that work only for continuous data, and created variants and alternatives more specifically geared towards individual use-cases, such as the sliced Latin hypercube design for categorical inputs and the fast flexible filling algorithm, designed for non-rectangular spaces. Machine learning algorithms have also aided in the analysis of mechanistic models. Methods such as random forest, neural networks, and principal components analysis continue to be used in congruence with mathematical models and biological systems. These methods work well to process the large amounts of data obtained from a mechanistic model and identify abstract features of the system. These algorithms can also identify nonlinear interactions between factors within the model, adding crucial insight into parameters affecting the response.
Sensitivity analysis is a useful tool for models with a large number of parameters where baseline values are unknown or difficult to measure. This approach measures changes to the model outputs from perturbations in the model inputs and includes both local and global methods. In local sensitivity analysis, the change in the model output is observed when only one model parameter is varied around a selected nominal value and all other parameters are held constant. Global methods examine the sensitivity of parameters within the entire parameter space. Global techniques are usually implemented using Monte Carlo simulations, giving them the description of sampling-based methods. This includes methods like Pearson correlation coefficient and partial correlation coefficients for linear trends and Spearman rank correlation coefficient and partial rank correlation coefficient for nonlinear trends with a monotonic relationship between inputs and outputs. Nonlinear non-monotonic trends require slightly different methods based on decomposition of model output variance. These methods include the Sobol method and its extended version based on (quasi) random numbers and an ad hoc design, and the Fourier amplitude sensitivity test and its extended version. The methods have been implemented in various models involving wound healing and the inflammatory response.

To determine plausible parameter sets for our model using both the young and old experimental data, we initially sampled using a beta distribution to favor lower parameter values, and then performed an iterative stochastic local search around likely candidates. The model variables were then simulated from the resulting parameter sets and model variables were compared with in vivo data to determine young or old presenting behavior in the resulting transients before and after ventilation. Various transient features were also calculated and analyzed including epithelial qualities and inflammatory cell quantities. Analysis of the resulting parameter sets included identifying parameters associated with young or old data, analyzing differences in lung
health states between the young or old sets, and determining parameters associated with poorer lung health both including and excluding age classification. Further investigation of the identified parameter sets included local sensitivity analysis to assess model output sensitivity to variations in the parameters. The results from classification and sensitivity analysis were used to simulate pseudo-interventions to determine parameters that may be modulated to improve epithelial health during MV. This can help inform potential therapeutic targets for patients that are considered high risk before ventilation or even for patients that present signs of distress during ventilation.

2.2 Materials and methods

2.2.1 Experimental data

Experimental samples were collected from male C57BL/6J wild-type mice at 2-3 months and 20-25 months of age to represent young and old groups, respectively. Mice were mechanically ventilated for 2 hours using a high-pressure controlled mechanical ventilation protocol. Pulmonary function and tissue mechanics were measured and collected at baseline and every hour during the 2-hour ventilation. A separate group of mice was maintained on spontaneous ventilation for 2 hours as a control group. Following ventilation, lung tissue was used to provide cell counts for macrophage cells exhibiting varying levels of activation and phenotype expression. Lung tissue was also used for histology analysis measuring tissue damage.

2.2.2 Mathematical model and analysis methods

2.2.2.1 Model equations

The model uses differential equations to track the transition from a healthy lung state to a state with damage to the epithelial cells in response to ventilation. This
models a direct transition from a healthy state to a damaged state at the cellular level. That is, we do not explicitly model the stress and strains at the tissue level that give rise to epithelial tissue damage. In our model damaged cells produce mediators that activate innate immune cells, neutrophils and macrophages. Immune cell influx causes additional damage to the lung epithelial cells. The epithelial cells can 1) return to a healthy state via repair, which is regulated by repair mediators, or 2) transition to the death/empty state. The portion of the population that is in the death/empty state represents the portion of the lung that needs to be replaced via proliferation of healthy epithelial cells. A full model schematic including the dynamics is given in Figure 1, model variables are in Table 1, and the parameters with brief descriptions are in Table 2.
Fig. 1. **Model schematic for ventilator induced lung injury.** The model has two compartments: lung tissue and blood. The various circles and boxes represent the different inflammatory cells, mediators, and epithelial cell states. Black arrows represent upregulation or transition and black lines with bars represent inhibition or down-regulation. The blue arrows represent movement between the two compartments, either diffusion based or at a constant rate. The red arrows represent movement from the blood into the lung compartment as a result of epithelial barrier degradation.
Table 1. **Ventilator-induced lung injury model variables with descriptions.** The epithelial variables $E_h$, $E_d$ and $E_e$ are proportions, such that $E_h + E_d + E_e = 1$. The remaining variables have arbitrary units for simulation. The variables $M_0$, $M_1$, and $M_2$ are used to calculate percentages for each phenotype, in order to compare to experimental flow cytometry data.

<table>
<thead>
<tr>
<th>Bloodstream</th>
<th>Lung</th>
<th>Description</th>
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<tbody>
<tr>
<td>$E_h$</td>
<td></td>
<td>Healthy epithelial cells</td>
</tr>
<tr>
<td>$E_d$</td>
<td></td>
<td>Damaged epithelial cells</td>
</tr>
<tr>
<td>$E_e$</td>
<td></td>
<td>Dead epithelial cells/empty space</td>
</tr>
<tr>
<td>$p_b$</td>
<td>$p$</td>
<td>Pro-inflammatory mediators</td>
</tr>
<tr>
<td>$a_b$</td>
<td>$a$</td>
<td>Anti-inflammatory mediators</td>
</tr>
<tr>
<td>$M_{0b}$</td>
<td>$M_0$</td>
<td>Naive macrophages</td>
</tr>
<tr>
<td>$M_{1b}$</td>
<td>$M_1$</td>
<td>M1 classically-activated macrophages</td>
</tr>
<tr>
<td>$M_{2b}$</td>
<td>$M_2$</td>
<td>M2 alternatively-activated macrophages</td>
</tr>
<tr>
<td>$N_{0b}$</td>
<td>$N_0$</td>
<td>Unactivated neutrophils</td>
</tr>
<tr>
<td>$N_b$</td>
<td>$N$</td>
<td>Activated neutrophils</td>
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<tr>
<td>$AN$</td>
<td></td>
<td>Apoptotic neutrophils</td>
</tr>
<tr>
<td>$R$</td>
<td></td>
<td>Repair mediators</td>
</tr>
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</table>
Table 2: Ventilator induced lung injury model parameters with descriptions.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Description</th>
<th>Units</th>
<th>Young Min.</th>
<th>Young Max.</th>
<th>Old Min.</th>
<th>Old Max.</th>
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<tbody>
<tr>
<td>$a b_\infty$</td>
<td>Relative effectiveness of $a_b$ at inhibiting $M_{0b}$ differentiation to $M_{1b}$</td>
<td>$a$-units</td>
<td>$1.33 \times 10^{-4}$</td>
<td>84.5325</td>
<td>3.2248</td>
<td>39.0498</td>
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<tr>
<td>$a_\infty$</td>
<td>Relative effectiveness of $a$ at inhibiting $M_0$ differentiation to $M_1$</td>
<td>$a$-units</td>
<td>0.0108</td>
<td>72.5097</td>
<td>0.2902</td>
<td>57.2817</td>
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<tr>
<td>$b_d$</td>
<td>Baseline decay of damaged cells</td>
<td>$h^{-1}$</td>
<td>$8.25 \times 10^{-5}$</td>
<td>76.1677</td>
<td>3.0326 $\times 10^{-3}$</td>
<td>65.1373</td>
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<tr>
<td>$b_p$</td>
<td>Baseline self-resolving repair of epithelial cells</td>
<td>$h^{-1}$</td>
<td>0.8312</td>
<td>69.7958</td>
<td>0.0311</td>
<td>44.2681</td>
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<tr>
<td>$b_r$</td>
<td>Baseline repair of damaged cells</td>
<td>$h^{-1}$</td>
<td>$6.96 \times 10^{-4}$</td>
<td>72.1149</td>
<td>0.2169</td>
<td>47.1704</td>
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<tr>
<td>$d_a$</td>
<td>Rate of diffusion for $a$</td>
<td>$h^{-1}$</td>
<td>0.0125</td>
<td>82.1587</td>
<td>0.1035</td>
<td>67.5635</td>
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Table 2 – continued from previous page

<table>
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<th>Old Min.</th>
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<td>$d_{m0}$</td>
<td>Rate of diffusion for $M_0$</td>
<td>$h^{-1}$</td>
<td>0.1717</td>
<td>84.7813</td>
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<td>$d_p$</td>
<td>Rate of diffusion for $p$</td>
<td>$h^{-1}$</td>
<td>$1.264 \times 10^{-3}$</td>
<td>81.5292</td>
<td>6.8524 $\times 10^{-3}$</td>
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<td>$k_{am1}$</td>
<td>Production rate of $a$ by $M_{1b}$ &amp; $M_1$</td>
<td>$a$-units $\cdot$ $M$-units$^{-1}$ $\cdot$ $h^{-1}$</td>
<td>0.0144</td>
<td>69.6048</td>
<td>10.9822</td>
<td>72.1224</td>
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<tr>
<td>$k_{am2}$</td>
<td>Production rate of $a$ by $M_{2b}$ &amp; $M_2$</td>
<td>$a$-units $\cdot$ $M$-units$^{-1}$ $\cdot$ $h^{-1}$</td>
<td>$6.4797 \times 10^{-3}$</td>
<td>71.1447</td>
<td>4.6606 $\times 10^{-3}$</td>
<td>63.1206</td>
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<td>$k_{an}$</td>
<td>Rate at which neutrophils become apoptotic</td>
<td>$h^{-1}$</td>
<td>0.0516</td>
<td>62.276</td>
<td>0.0403</td>
<td>37.3451</td>
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<td>$k_{amm1}$</td>
<td>Rate of $M_1$ phagocytosis of $AN$</td>
<td>$M$-units$^{-1}$ $\cdot$ $h^{-1}$</td>
<td>$1.73 \times 10^{-5}$</td>
<td>68.8604</td>
<td>0.2398</td>
<td>54.0499</td>
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<td>$k_{amm2}$</td>
<td>Rate of $M_2$ phagocytosis of $AN$</td>
<td>$M$-units$^{-1}$ $\cdot$ $h^{-1}$</td>
<td>$1.4828 \times 10^{-3}$</td>
<td>92.023</td>
<td>7.8663</td>
<td>56.181</td>
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<th>Old Min.</th>
<th>Old Max.</th>
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<tr>
<td>$k_{em1}$</td>
<td>Rate of phagocytosis of damaged cells by $M_1$</td>
<td>M-units$^{-1} \cdot h^{-1}$</td>
<td>1.2397 × 10$^{-3}$</td>
<td>85.7842</td>
<td>16.4332</td>
<td>98.4928</td>
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<td>$k_{en}$</td>
<td>Rate of phagocytosis of damaged cells by $N$</td>
<td>N-units$^{-1} \cdot h^{-1}$</td>
<td>2.2547 × 10$^{-3}$</td>
<td>82.9453</td>
<td>4.1329 × 10$^{-3}$</td>
<td>66.7115</td>
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<td>$k_{ep}$</td>
<td>Rate of self-resolving repair mediated by $p$</td>
<td>p-units$^{-1} \cdot h^{-1}$</td>
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<td>76.1888</td>
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<td>$k_{er}$</td>
<td>Rate of repair of damaged cells by $R$</td>
<td>h$^{-1}$</td>
<td>0.0176</td>
<td>61.7146</td>
<td>5.5664 × 10$^{-3}$</td>
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<td>$x_{er}$</td>
<td>Regulates effectiveness of repair of damaged cells by $R$ (Hill-type constant)</td>
<td>R-units</td>
<td>0.0217</td>
<td>73.0257</td>
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<td>$k_{m0a}$</td>
<td>Rate of differentiation of $M_0$ induced by $a$</td>
<td>h$^{-1}$</td>
<td>9.2296 × 10$^{-3}$</td>
<td>75.3057</td>
<td>3.8383 × 10$^{-3}$</td>
<td>42.3501</td>
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<td>Regulates effectiveness of $a$-units to induce differentiation of $M_0$ by $a$ (Hill-type constant)</td>
<td>1.75 × 10^{-4}</td>
<td>83.8636</td>
<td>0.4768</td>
<td>54.2981</td>
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<td>$k_{m0ab}$</td>
<td>Rate of differentiation of $M_{0b}$ $h^{-1}$ induced by $a_b$</td>
<td>1.757 × 10^{-3}</td>
<td>76.8485</td>
<td>0.0343</td>
<td>68.9449</td>
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<tr>
<td>$x_{m0ab}$</td>
<td>Regulates effectiveness of $a_b$ $a$-units to induce differentiation of $M_{0b}$ (Hill-type constant)</td>
<td>5.28 × 10^{-4}</td>
<td>73.6692</td>
<td>0.0149</td>
<td>64.5509</td>
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<td>$k_{m0p}$</td>
<td>Rate of differentiation of $M_0$ $h^{-1}$ induced by $p$</td>
<td>2.4 × 10^{-4}</td>
<td>80.7515</td>
<td>0.6304</td>
<td>46.5157</td>
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<td>$x_{m0p}$</td>
<td>Regulates effectiveness of $p$ $p$-units to induce differentiation of $M_0$ (Hill-type constant)</td>
<td>0.0996</td>
<td>54.3827</td>
<td>0.5422</td>
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<tr>
<td>$k_{m0pb}$</td>
<td>Rate of differentiation of $M_{0b}$ $h^{-1}$ induced by $p_b$</td>
<td>2.3817 × 10^{-3}</td>
<td>91.7174</td>
<td>3.3674</td>
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<td>Regulates effectiveness of $p_b$ to induce differentiation of $M_{0b}$ (Hill-type constant)</td>
<td>$p$-units</td>
<td>0.2125</td>
<td>70.6259</td>
<td>$4.19 \times 10^{-4}$</td>
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<td>$k_{man}$</td>
<td>Rate of $M_1$ switch to $M_2$ by $AN$</td>
<td>$M$-units · $N$-units$^{-1}$</td>
<td>$1.0217 \times 10^{-3}$</td>
<td>85.4308</td>
<td>0.9625</td>
<td>58.4498</td>
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<td>$k_{mne}$</td>
<td>Rate of collateral damage to epithelial cells by macrophages and neutrophils</td>
<td>$h^{-1}$</td>
<td>0.0144</td>
<td>87.410</td>
<td>4.3902</td>
<td>55.8505</td>
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<tr>
<td>$x_{mne}$</td>
<td>Regulates effectiveness of macrophages and neutrophils to damage epithelial cells (Hill-type constant)</td>
<td>$(M + N)$-units</td>
<td>0.0895</td>
<td>68.5317</td>
<td>$3.05 \times 10^{-3}$</td>
<td>10.2302</td>
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<tr>
<td>$k_n$</td>
<td>Rate of infiltration of $N_b$ to lung</td>
<td>$h^{-1}$</td>
<td>0.0342</td>
<td>92.8295</td>
<td>0.8702</td>
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<td>$k_{n0pb}$</td>
<td>Rate of activation of $N_b$ induced by $p_b$</td>
<td>$h^{-1}$</td>
<td>$5.4571 \times 10^{-3}$</td>
<td>79.0613</td>
<td>0.0686</td>
<td>71.3452</td>
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<tr>
<td>$x_{n0pb}$</td>
<td>Regulates effectiveness of $p_b$ to induce activation of $N_b$</td>
<td>$p$-units</td>
<td>$4.83 \times 10^{-3}$</td>
<td>80.1988</td>
<td>0.0708</td>
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<td>$k_{pe}$</td>
<td>Production rate of $p$ by $E_d$</td>
<td>$p$-units · $h^{-1}$</td>
<td>5.0833</td>
<td>78.5846</td>
<td>0.0576</td>
<td>45.4633</td>
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<td>$k_{pm1}$</td>
<td>Production rate of $p$ by $M_1$ &amp; $M_{1b}$</td>
<td>$p$-units · $M$-units$^{-1}$ · $h^{-1}$</td>
<td>$1.6489 \times 10^{-3}$</td>
<td>75.4175</td>
<td>0.0208</td>
<td>68.4131</td>
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<td>$k_{pn}$</td>
<td>Production rate of $p$ and $p_b$ by neutrophils</td>
<td>$N$-units$^{-1}$ · $h^{-1}$</td>
<td>$3.62 \times 10^{-5}$</td>
<td>75.2844</td>
<td>0.0167</td>
<td>37.4531</td>
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<td>$k_{rm2}$</td>
<td>Production rate of $R$ by $M_2$</td>
<td>$R$-units · $M$-units$^{-1}$ · $h^{-1}$</td>
<td>$2.0303 \times 10^{-3}$</td>
<td>76.6348</td>
<td>7.235 $\times 10^{-3}$</td>
<td>42.9838</td>
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<td>$\mu_a$</td>
<td>Decay rate of $a$</td>
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<td>0.0550</td>
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<td>Decay rate of $M_1$</td>
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<td>83.7439</td>
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<td>$\mu_{m2b}$</td>
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<td>$4.9563 \times 10^{-3}$</td>
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<th>Young Max.</th>
<th>Old Min.</th>
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<td>$s_a$</td>
<td>Source rate of background $a_b$</td>
<td>$a$-units · h$^{-1}$</td>
<td>0.0315</td>
<td>90.5490</td>
<td>8.8694</td>
<td>81.6087</td>
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<tr>
<td>$s_d$</td>
<td>Rate of damage from ventilator</td>
<td>h$^{-1}$</td>
<td>1.6823</td>
<td>82.2663</td>
<td>0.9770</td>
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<td>$s_m$</td>
<td>Source rate of $M_{0b}$</td>
<td>$M$-units · h$^{-1}$</td>
<td>0.0649</td>
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<td>Source rate of $N_{0b}$</td>
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<td>$s_p$</td>
<td>Source rate of background $p_b$</td>
<td>$p$-units · h$^{-1}$</td>
<td>0.1903</td>
<td>60.7942</td>
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<td>$k_{ee*}$</td>
<td>Rate of inflammatory cell and mediator leakage into alveolar compartment</td>
<td>h$^{-1}$</td>
<td>$2.0545 \times 10^{-3}$</td>
<td>79.59</td>
<td>9.0485</td>
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<tr>
<td>$x_{ex^*}$</td>
<td>Regulates effectiveness of inflammatory cell leakage into alveolar compartment (Hill-type constant)</td>
<td>Unitless</td>
<td>0.75</td>
<td>0.75</td>
<td>0.75</td>
<td>0.75</td>
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<tr>
<td>$x_{emm^*}$</td>
<td>Regulates effectiveness of inflammatory mediator leakage into alveolar compartment (Hill-type constant)</td>
<td>Unitless</td>
<td>0.5</td>
<td>0.5</td>
<td>0.5</td>
<td>0.5</td>
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<tr>
<td>$k_{m1^*}$</td>
<td>Rate of $M_1$ infiltration into alveolar compartment</td>
<td>h$^{-1}$</td>
<td>1.8228</td>
<td>74.9223</td>
<td>25.1536</td>
<td>60.8061</td>
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<tr>
<td>$k_{m2^*}$</td>
<td>Rate of $M_2$ infiltration into alveolar compartment</td>
<td>h$^{-1}$</td>
<td>$4.6 \times 10^{-4}$</td>
<td>91.5074</td>
<td>7.1409</td>
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Table 2 – continued from previous page

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<th>Young Max.</th>
<th>Old Min.</th>
<th>Old Max.</th>
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<tr>
<td>$k_{np}$</td>
<td>Rate of activation of $N$ induced by $p$ in the alveolar compartment</td>
<td>h$^{-1}$</td>
<td>0.1285</td>
<td>85.1315</td>
<td>0.0144</td>
<td>46.1535</td>
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<td>$x_{np}$</td>
<td>Regulates effectiveness of $p$ to induce $N$ activation in the alveolar compartment</td>
<td>$p$-units</td>
<td>0.0355</td>
<td>65.0746</td>
<td>2.0399</td>
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<td>$\mu_{an}$</td>
<td>Decay rate of $AN$</td>
<td>h$^{-1}$</td>
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<td>81.2026</td>
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<td>$\mu_{n0}$</td>
<td>Decay rate of $N_0$</td>
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<td>0.0393</td>
<td>85.9090</td>
<td>0.0119</td>
<td>48.7474</td>
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Parameters indicated with an asterisk (*) are novel to this iteration of the model and were not utilized in the Minucci et al. model [1]. Model variable units are arbitrary and indicated as related to the general cell type. Therefore, $N$-units represent neutrophils, $M$-units represent all phenotypes of macrophages, $p$-units represent pro-inflammatory mediators, $a$-units represent anti-inflammatory mediators, and $R$-units represent repair mediators. The time unit is hours, $h$. Parameter ranges are determined by the maximum and minimum value achieved by each parameter over the plausible parameter sets associated with the young or old experimental data. The process for obtaining these ranges is explained in the following sections.
We account for macrophage phenotype on a population level. Therefore, our variables track the overall level of M1 type activity (classically activated) versus M2 type activity (alternatively activated). Activation of naive macrophages (M0) by pro-inflammatory mediators (PIMs) give rise to the M1 phenotype, phagocyte cells producing PIMs and anti-inflammatory mediators (AIMs). M1 cells phagocytize both damaged epithelial cells and apoptotic neutrophils. Neutrophils also phagocytize damaged epithelial cells and produce PIMs.

Conversely, activation of naive cells by AIMs gives rise to the M2 phenotype, producing AIMs and repair mediators. M1 cells can transition to M2 cells in response to phagocytising apoptotic neutrophils. The full equations for our model are in Appendix B. The main reference for the equation derivations are given in Minucci et al., since our current model is an adaptation of that model.

The main change from Minucci et al. in this model was the introduction of a breakdown in the epithelial barrier integrity that leads to an increase in inflammatory cell and mediator movement between the systemic blood compartment into the lung that is not diffusion driven. This mechanism is illustrated in Figure 2. We show this in the model equations with an immune cell equation, the lung $M_0$ equation (Equation 2.1), and a mediator equation, the lung PIMs ($p$, Equation 2.2), but this type of term occurs for all the immune cells and mediators (see Appendix B for the full model equations). The second to last term in the $M_0$ equation (Equation 2.1) is a nonlinear Hill-type term that allows naive cells to move from the blood into the lung when the epithelial lung barrier is degraded significantly. Therefore, the term is dependent on $E_e$ with the parameter $x_{ee}$ controlling the level of $E_e$ at which this term achieves its half maximum. $x_{ee}$ is fixed to 0.75 for all immune cell equations. The second to last term of the $p$ equation (Equation 2.2) has the same form but with the parameter defined as $x_{eem}$ which is fixed to 0.5 for all mediator equations. These parameters are
fixed at these levels to ensure that the smaller mediators pass through the degraded alveolar-capillary barrier at lower value of $E_e$ than the immune cells. The values of $x_{ee}$ and $x_{eem}$ at this point create a partitioning of the range of values for $E_e$, $[0, 1]$ which is a first step to mapping the epithelial variables to clinically relevant lung injuries, such as pulmonary edema. These values were chosen so that $x_{eem}$ is smaller than $x_{ee}$ and that substantial damage (half of the range) is needed before reaching the half maximum for the mediator influx term. Mediator flux is associated with severe lung damage and immune cell leakage into the lung due to barrier disruption which would lead to ventilation failure as the alveoli fill with fluid instead of air. The third from last term in both of these equations model the diffusion driven infiltration between compartments.

The first term in the $M_0$ equation (Equation 2.1) models the activation of the naive macrophages into the M1 and M2 phenotypes with down-regulation of the M1 phenotype via inhibition by AIMs represented by the variable $a$. The first three terms of the $p$ equation (Equation 2.2) models production of PIMs by damaged epithelial cells, M1 cells (inhibited by $a$) and neutrophils, respectively. The last term in both equations model intrinsic decay.
Fig. 2. Diagram of inflammatory cell and mediator influx from the bloodstream into the alveolar compartment resulting from damage to the alveolar epithelium.
\[
\frac{dM_0}{dt} = -M_0 \left[ \frac{k_{m0b}p_b^2}{x_{m0b}^2 + p_b^2} \right] + \left[ \frac{1}{1 + \left( \frac{a}{a_\infty} \right)^2} \right]
\]

\[
= \frac{k_{m0a}a^2}{x_{m0a}^2 + a^2} + \frac{k_{m1}E_e^4}{x_{e1}^4 + E_e^4} - \frac{k_{m2}E_e^4}{x_{e2}^4 + E_e^4} - \frac{k_{m2}E_e^4}{x_{e3}^4 + E_e^4} - \frac{k_{m2}E_e^4}{x_{e4}^4 + E_e^4}
\]

Inhibition by AIMs

\[
\frac{dp}{dt} = k_{pe}E_d + \frac{k_{pm1}M_1}{1 + \left( \frac{a}{a_\infty} \right)^2}
\]

\[
= \frac{k_{pm}N}{1 + \left( \frac{a}{a_\infty} \right)^2} - \frac{k_{pm}N}{1 + \left( \frac{a}{a_\infty} \right)^2}
\]

Diffusion

\[
\frac{dp}{dt} = \frac{k_{pe}E_d}{x_{e1}^4 + E_e^4} - \frac{k_{pe}E_d}{x_{e2}^4 + E_e^4} - \frac{k_{pe}E_d}{x_{e3}^4 + E_e^4} - \frac{k_{pe}E_d}{x_{e4}^4 + E_e^4}
\]

Decay

\[
= \frac{\mu_{M_0}M_0}{x_{m0a}^2 + a^2} - \frac{\mu_{M_0}M_0}{x_{m0a}^2 + a^2}
\]

2.2.2.2 Sampling and parameter selection

The model variables were simulated using MATLAB (R2021a) to determine plausible in silico parameter sets associated with each experimental group. Plausible parameter sets were those where, 1) non-ventilated simulated variables reached a numerical steady state, 2) the associated steady state value fell within the range of in vivo data for either the young or old data at \( t = 0 \) hours, and 3) ventilated simulations starting from steady state fell within the range of the associated young or old data at \( t = 2 \) hours. During this selection process \( M_0, M_1, M_2, \) and \( E_e \) were compared to the experimental data for unactivated macrophages, classically-activated macrophages, alternatively-activated macrophages, and airspace enlargement, respectively.

The data ranges used for each experimental group and cell are plotted in Figure 3. Note from Figure 3 that some data ranges overlap significantly, but given that there is separation in the M0 and M2 data for time zero, there are no parameter sets that satisfy both young and old conditions.
Fig. 3. **Summary of data ranges used to select plausible parameter sets for the ventilator induced lung injury model.** The plot gives the range of experimental values for M0, M1, and M2 macrophages and airspace enlargement used to assess the numerical simulations for biological plausibility. Each range consists of 3-6 experimental observations since some data points were excluded as outliers. Outliers were defined as those being more than two standard deviations outside the mean.

Model variables were simulated for each parameter set to reach numerical steady state to ensure that the dynamics observed during ventilation were only a product of the ventilation and not a result of the variables attempting to return to steady state. For these simulations, the parameter $s_d$, a parameter used to describe the damage caused by ventilation, was set to zero. Each parameter set was simulated starting at an initial condition where all model variables were set to zero excluding M1 macrophages in the lung and healthy epithelial cells which were set to 50 and 1, respectively. This initiates a return to steady state from an inflammatory insult. Simulations were run for 800 hours and classified as achieving numerical steady state if the Euclidean norm of the difference between the end values of the variables and the value at each numerical time step within the last 20 hours of simulation was less than 0.001. If the model variables did not reach steady state, the model was simulated
starting at an additional initial condition where all model variables were set to zero excluding healthy epithelial cells and damaged epithelial cells which were set to 0.75 and 0.25, respectively. This initiates a return to steady state from an epithelial damage-induced insult. Parameter sets that did not produce model transients that reach steady state for either initial condition were excluded from further sampling and analysis. Variable transients that achieved steady state and fell within the range of either the young or old initial data values were then simulated starting at their numerical steady state for a total of 200 hours with ventilation occurring during the first two hours ($s_d > 0$) to observe transient behavior during and after ventilation. The resulting model variables were then compared to the data at 2 hours and the respective parameter sets were classified as young or old depending on their ability to satisfy the respective data ranges. Those that did not fall within the range of either the young or old data at both time points were excluded from the final accepted parameter sets.

Parameter sets for this process were found using a three-step parameter sampling process in R [43]. In the initial step, a large number of samples were generated using a scaled $Beta(1, 3)$ distribution with a large scale parameter to sample between 0 and 120 (to include multiple orders of magnitude). Parameter sets with associated transients that fell within the range of the data for any of the macrophage variables or $E_e$ at either time point for either age group were used to define the ranges of each parameter during the next stage of sampling. Uniform sampling was used over this refined space for the second stage. The final step was iterative stochastic local search; using four iterations, successively restricting the space by adding more components of the criteria until in the end all remaining sets matched every criterion for one of the age groups.
2.2.2.3 Classification, regression and sensitivity

Several methods were fitted on various subsets of the parameter sets which fell in the range for either the young or old data. A suite of model fitting algorithms were applied using the R package H2O [44], including Generalized Linear Models, Distributed Random Forests, Gradient Boosting Machines, and Neural Networks. The best model in terms of 5-fold cross-validated F1 score (for classification) or root mean squared error (for regression) was chosen and the variable importance values were calculated for each. In classification methods, the importance values rank the relative importance of each data feature in the defined statistical model. Thus, an importance value is calculated for each parameter based on their relative influence in separating the parameter sets into the young or old associated groups. In order to justify the importance metric for classification models where the data was not balanced (e.g., predicting young versus old), the same models were fitted on down-sampled data with balanced labels.

Local sensitivity analysis was also used to measure the relative sensitivity of the model parameters for each experimental group. The methods were implemented using the SimBiology package available in MATLAB [42] which calculates the time-dependent derivatives of the model sensitivity to each parameter evaluated at specified time points. Details about the calculations performed can be found in Martins et al. [45]. Default settings were used for the sensitivity matrix. The overall relative sensitivities for each parameter were calculated by taking the root mean square of the sensitivity values at the chosen time points for the chosen variables. The resulting values for each parameter were then normalized by scaling each to the maximum overall sensitivity value in each group.
2.3 Results

2.3.1 Plausible parameter sets and transients

Iterative sampling used for the large sampling space produced 19,202 plausible parameter sets associated with either the young or old experimental data. Of these sets, 17,477 were associated with the young data and 1,725 were associated with the old data. Average model behavior for the variables $E_h$, $E_e$, $M_0$, $M_1$, $M_2$, $N$, and $AN$ is shown in Figure 4. Additionally, the percentage of the macrophage activity that was $M_0$, $M_1$, or $M_2$ were plotted.
Fig. 4. **Average young and old transients.** The solid blue and red lines plotted within the corresponding shaded bands represent the mean response for the transients associated with each experimental group at each time point. The borders of the surrounding bands encompass the 10th and 90th percentile at each time point. $M_0\%, M_1\%, \text{ and } M_2\%$ represent the percentage of the macrophage activity that is M0, M1, and M2, respectively. These percentages along with the $E_e$ variable were validated by the experimental data at 0 hours and 2 hours. Due to differences in scale, the variables $N$ and $AN$ also include overlays with just the young mean and percentiles plotted.
Parameter sets were separated using the proportion of their associated $E_h$ variable value before ventilation ($t = 0$) and directly after the 2 hour ventilation ($t = 2$). Parameter sets were defined at each selected time point as healthy when $E_h > 0.9$, moderate when $0.5 < E_h < 0.9$, and severe when $E_h < 0.5$. The resulting groups are shown in Figure 5.

![Parameter sets separated by age group and epithelial health](image)

Fig. 5. **Plausible parameter sets separated by age group and epithelial health.** Separation of plausible sets was made using the $E_h$ variable proportion before and after 2 hours of ventilation. Transients were classified as healthy when $E_h > 0.9$, moderate when $0.5 < E_h < 0.9$, or severe when $E_h < 0.5$. Corresponding percentages were calculated at each level of separation in the flowchart as a percentage of the previous bin.

This separation scheme created a natural division in the plausible parameter sets into the following groups by age and ventilation response: young sets that started healthy and became moderate after ventilation (Young H2M), young sets that started healthy and became severe after ventilation (Young H2S), old sets that started healthy and became moderate after ventilation (Old H2M), old sets that started healthy and became severe after ventilation (Old H2S), old sets that started moderate and stayed moderate after ventilation (Old M2M), and old sets that started moderate and became severe after ventilation (Old M2S). We will exclude the one young set that remained
healthy after ventilation due to the sample size.

A representative set was determined for each age and ventilation response group such that it was one of the parameter sets in the group and its behavior was most inline with the mean of the key variable transients for all the parameter sets in its group. The mean of the transients was calculated for each group using the average values at each time point for the model variables $E_h$, $E_d$, $E_e$, $M_0$, $M_1$, $M_2$, $N$, and $AN$. A representative set from each group was then chosen by minimizing the residual sum of squares for all six mean transients. The representative sets are plotted for each variable in Figure 6.
Fig. 6. **Plots of selected model variables for each representative set.** Transients are plotted for $E_h$ (plot A), $E_e$ (plot B), $N$ (plot C), $M_0$ (plot D), $M_1$ (plot E), and $M_2$ (plot F). Due to the differences in scale, plot C also includes a zoomed in plot of just the young transients for the variable $N$. 
2.3.2 Importance factors for age classification

Importance values for the various classification methods are shown in Figure 7. To account for the difference in sample size between the young and old associated parameter sets, classification methods were performed on random subsets of the parameter sets associated with the young data. For each classification method, down-sampled data yielded similar results to those calculated using the full parameter sets; the ordering of parameters often varied, but the parameters most important for each classification remained unchanged. Plot A exhibits ranked parameters in the classification of young and old parameter sets. In terms of scaled importance, the first four parameters are of interest since the numerical value decays significantly after the fourth parameter. Thus, the following parameters had high importance in classifying between the young and old parameter sets: $k_{em1}$, the rate of phagocytosis of damaged cells by $M_1$, $x_{er}$, a Hill-type constant regulating the effectiveness of repair of damaged cells by repair mediators, $k_{m1}$, the rate of $M_1$ infiltration into the alveolar compartment, which is novel to this iteration of the model, and $x_{mne}$, a Hill-type constant regulating the effectiveness of macrophages and neutrophils to damage epithelial cells.
Fig. 7. Scaled variable importance for top 10 predictors. Plots A-D exhibit scaled importance values for all observations (predicting young or old), the old class (predicting healthy or moderate at time 0), the healthy, young class (predicting moderate or severe after 2 hours), and the healthy, old class (predicting moderate or severe after 2 hours), respectively.

The parameters $k_{em1}$, $x_{er}$, and $x_{mne}$ specifically involve repair and damage of the epithelial cells. The experimental data (Figure 3, ‘Airspace Enlargement’) and in silico simulations (Figure 4, ‘$E_e$’) both exhibited a discrepancy in the epithelial variables. In terms of the actual data, the experimental range for airspace enlargement (Figure 3, ‘Airspace Enlargement’, correlated with the model variable $E_e$), does overlap between young and old mice, but a general increase in this value is observed in the old mice. However, simulations show a clear distinction in the variable $E_e$ (Figure 4, ‘$E_e$’). Therefore, the classification methods found parameters associated with epithelial dynamics important in separating parameter sets between the two age groups. Why other parameters affecting this variable were not found to be as important is unclear.

The parameters $k_{m1}$, $k_{em1}$, and $x_{mne}$ involve macrophages, specifically the M1
phenotype. This was not the expected phenotype to be associated with age classification given that the experimental groups have non-overlapping ranges for M0% and M2% (Figure 3) and for the in silico simulations (Figure 4). However, the M1 phenotype changes are driven by the ventilation and their percentages are directly linked to the M0 and M2, thus activation of the M1 phenotype directly affects population numbers of the M0 and M2 phenotypes.

An additional classification suite was performed for the old parameter sets prior to ventilation, shown in Figure 7B, since there was variety in the starting states of these simulations. The parameter $s_d$ holds the largest importance value as it contributes directly to damage of the epithelial cells by the ventilator. The parameters $k_{er}$, the rate of repair to damaged cells by repair mediators, and $x_{mne}$, a Hill-type constant regulating the effectiveness of macrophages and neutrophils to damage epithelial cells, are both parameters in the epithelial equations contributing to damage and repair processes. The parameters $k_{m0ab}$, the rate of differentiation of $M_0b$ by $a_b$, and $s_a$, the source rate of background $a_b$, also had high importance factors. Both parameters are involved in the function of AIMs that have not yet been discussed in the topic of lung health classification. AIMs as well as their cellular counterparts, namely M2 macrophages, help regulate the pro-inflammatory response and are crucial to preventing a feedback loop of chronic inflammation. As discussed earlier, both the pro- and anti-inflammatory responses are needed to ensure effective healing. Thus, despite their absence in the epithelial equations, AIMs are crucial to controlling cellular damage and promoting repair. The classification results reflect this relationship.

2.3.3 Importance factors for response

Figure 7C exhibits classification for a moderate or severe state after two hours of ventilation for young parameter sets. The most important parameters in classifying
moderate or severe lung health were \( s_d \), the rate of epithelial damage from the ventilator, \( k_{n0p} \), the rate of activation of \( N \) in the alveolar compartment, novel to this iteration of the model, \( k_{m0p} \), the rate of differentiation of \( M_0 \) by \( p, b_r \), baseline repair of damaged cells, and \( b_d \), baseline death rate for damaged cells.

For old sets that started healthy, classification methods were used to determine importance factors for a moderate versus severe response to ventilation, Figure 7D. The most important parameters for classification were \( s_d, k_{ep} \), the rate of self-resolving repair mediated by \( p, b_p \), baseline self-resolving repair of epithelial cells, \( b_r \), and \( x_{m0pb} \), a Hill-type constant regulating the effectiveness of differentiation of \( M_{0b} \) by \( p_b \).

The actual parameters with high importance factors differ between young and old parameter sets but generally involve rates of damage and repair. The parameter \( s_d \) was the top parameter for each group. This parameter contributes directly to decreasing \( E_h \) and thus influences the resulting classification of lung health. The parameters \( b_r, b_d, k_{ep}, \) and \( b_p \) all contribute directly to the epithelial variables and thus influence the level of \( E_h \) as well. The remaining parameters \( k_{n0p}, k_{m0p}, \) and \( x_{m0pb} \) are not directly involved in the epithelial variables but do contribute indirectly to increased damage. All are involved in activation of inflammatory cells by PIMs. The pro-inflammatory phagocytic cells contribute to additional cellular damage. This is a well-established phenomenon of the inflammatory stage \[46, 47\].

### 2.3.4 Local sensitivity analysis for representative sets

Local sensitivity analysis was performed to measure the sensitivity of the variables \( E_h \) and \( E_e \) to the model parameters. For each of the representative sets a local sensitivity analysis was performed and the top ten sensitive parameters for each representative set are plotted with their normalized value in Figure 8.
Fig. 8. Normalized local sensitivity values for the top ten parameters in each representative set. Plots A-F are the sensitivities for the parameter sets grouped in Young H2M, Young H2S, Old H2M, Old H2S, Old M2M, and Old M2S, respectively. Parameters indicated with an asterisk had sensitivities greater than 10% of the maximum sensitivity value for all representative sets.

The model variables were considered to be sensitive to a parameter if the sensi-
tivity value was larger than 10% of the maximum value for each group. Multiple parameters were identified above this threshold for all of the representative sets, namely; $b_p$, baseline self-resolving repair of epithelial cells, $s_d$, the rate of damage from the ventilator, $b_r$, baseline repair of damaged cells, $k_{ep}$, the rate of self-resolving repair mediated by $p$, and $\mu_p$, decay rate of $p$. These parameters mainly affect accumulation of damage, ability to repair, and dynamics involving PIMs. Since $E_h$ and $E_e$ were the variables used in the sensitivity calculations, it is unsurprising that the parameters in those equations had high sensitivity values. We also again see parameters involved in the pro-inflammatory response to be important.

Additional parameters were found to be sensitive in the majority of the groups. Parameters $s_p$, source of PIMs, $s_n$, source of neutrophils, and $k_{en}$, rate of phagocytosis of damaged cells by neutrophils, had sensitivity values larger than 10% of the maximum in all groups except Young H2M. The parameter $x_{n0p6}$, a Hill-type parameter involved in the activation of neutrophils by PIM, had a sensitivity value larger than 10% of the maximum in all the old groups. Again, we see parameters involved in PIMs and inflammatory cell activation as well as their processes. Interestingly, the parameter $k_{em1}$, phagocytosis of damaged cells by M1 macrophages, had small sensitivity values for the majority of the groups. This is interesting since it was the top parameter identified in separating parameter sets associated with the young or old data. However, while the parameter values clearly differ between the parameter sets associated with either the young or old data, the parameter itself does not appear to have a major affect on the proportion of healthy or dead epithelial cells in the lung tissue. This is consistent with previous research which has hypothesized that a sustained inflammatory insult is primarily mediated by neutrophils rather than macrophages, contributing to the development of acute lung injury and ARDS.\[48.\]
2.3.5 Modulating response to ventilation

The results of the local sensitivity analysis were used to simulate a pseudo-intervention for the parameter sets associated with a severe state after 2 hours of ventilation; Young H2S, Old H2S, and Old M2S.

The parameters chosen as influential across all groups from the sensitivity analysis were \( b_p, s_d, b_r, k_{ep}, \) and \( \mu_p \). These parameters were increased or decreased by 10\% one at a time and the percent increase or decrease in the variables \( E_h \) and \( E_c \) at 2 hours was calculated for each parameter set within the Young H2S, Old H2S, and Old M2S groups. Table 3 shows the minimum, mean, and maximum percent change for each variable for each of these three groups. The supplementary materials include this same procedure with the additional parameters identified as influential in the majority of the groups as well as an intervention starting after one hour of ventilation rather than prior to ventilation as shown here.
Table 3. Variable effects of modulating parameters in the ventilator-induced lung injury model.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Young H2S</th>
<th></th>
<th></th>
<th>Old H2S</th>
<th></th>
<th></th>
<th>Old M2S</th>
<th></th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Min.</td>
<td>Mean</td>
<td>Max.</td>
<td>Min.</td>
<td>Mean</td>
<td>Max.</td>
<td>Min.</td>
<td>Mean</td>
<td>Max.</td>
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<td>-2.46%</td>
<td>-0.48%</td>
<td>-0.82%</td>
<td>0.51%</td>
<td>0.00%</td>
<td>-1.50%</td>
<td>-5.73%</td>
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<tr>
<td></td>
<td>+0.52%</td>
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<td>0.44%</td>
<td>0.81%</td>
<td>2.00%</td>
<td>2.53%</td>
<td>-2.93%</td>
<td>-0.60%</td>
<td>-0.36%</td>
</tr>
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<td>0.17%</td>
<td>-0.67%</td>
<td>0.22%</td>
<td>-0.01%</td>
<td>10.93%</td>
<td>-11.65%</td>
<td>0.03%</td>
<td>-3.25%</td>
</tr>
<tr>
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<td>+0.01%</td>
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<td>0.59%</td>
<td>-4.60%</td>
<td>2.72%</td>
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<td>0.02%</td>
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<td>3.08%</td>
<td>0.21%</td>
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<td>9.11%</td>
<td>-7.64%</td>
<td>-2.29%</td>
<td>-2.14%</td>
</tr>
</tbody>
</table>

Minimum, mean, and maximum change in the variables $E_h$ and $E_e$ from a 10% decrease (indicated by “-”) or a 10% increase (indicated by “+”) in the listed parameters. Values are shaded on a sliding scale where darker colors represent numbers with a larger magnitude and lighter colors represent numbers with a smaller magnitude. Maximum and minimum values for color gradient were defined separately for the minimum, mean, and maximum columns. For the minimum in each group, induced decreases in the value of $E_h$ and $E_e$ are shades of orange and induced increases in the value of $E_h$ and $E_e$ are shades of blue.
Parameters had varying effects on the variables, with some increasing $E_h$ while simultaneously increasing $E_e$ as well; thus not necessarily improving health outcome. However, an increase in the parameters $b_p$, $b_r$, and $k_{ep}$ consistently increased $E_h$ and decreased $E_e$ with a decrease in the parameter producing the opposite effect. The parameter $s_d$ exhibited the inverse relationship where an increase generally produced a decrease in $E_h$ and an increase in $E_e$ with a decrease in this parameter producing the opposite effect. It is also clear by the color intensities that variation in the parameters generally had the most impact on the parameter sets in the Old M2S group. The largest impact was specifically observed in the variable $E_e$. Since only $E_h$ was used to define the possible health states, a large change in $E_e$ may not result in a change in the health state as we have defined it.

Figure 9 shows some example transients for the variations of the parameters $b_r$ and $s_d$. The variations use the same magnitude shown in Table 3 of a 10% increase or 10% decrease in the selected parameter. The parameter changes appear to have a greater effect on the parameter set associated with the Old M2S representative set (Figures 9C & 9F) which was previously observed in Table 3. Model transients of the variable $E_e$ also show larger differences compared to the transients of $E_h$ as inferred from Table 3. Also note that the general shape of the transients did not change significantly when parameters were varied. Instead the value at which the model variable plateaus changed.
Fig. 9. $E_h$ and $E_e$ model transients with varying parameters for example parameter sets from selected groups. Each model variable was simulated for the baseline value, a 10% increase, and a 10% decrease for the selected parameter. Plots A-C exhibit transients for the varied parameter $b_r$ using randomly selected parameter sets from the representative groups Young H2S, Old H2S, and Old M2S, respectively. Plots D-F exhibit transients for the varied parameter $s_d$ using a randomly selected parameter set from the representative groups Young H2S, Old H2S, and Old M2S, respectively.
The intent of modulating parameters was to explore potential targets for therapeutic interventions prior to or during MV. The results in Table 3 demonstrate the various outcomes as well as differences among groups. A range of responses is expected in practice since patients have unique health profiles, however, the mean provides a reasonable expectation for the average response. The results shown in Table 3 exhibit the wide range of behavior possible with the model and parameter sets provided and specific groups and parameter combinations produced larger ranges of possible values. Specifically, the Old M2S parameter sets had a high level of variability in the observed maximums, especially for the variable $E_e$. The parameters $b_p$, $k_{ep}$, $s_d$, and $\mu_p$ produced a possible difference of around 40-50% in the variable $E_e$. Potential large decreases were also observed for the same parameters producing a decrease in $E_e$ at similar magnitudes. Generally, overall averages were not as considerable and were all less than 10% in absolute value, with some producing average differences close to zero. This suggests that on average, we would not expect a significant change in the defined health state, unless the simulation was already relatively close to the set threshold. Despite this, even a 2-3% increase or decrease in the amount of healthy epithelial cells available for gas exchange may affect clinical presentation. More research would need to be done to assess this claim.

2.4 Discussion

Age-dependent responses to ventilation are of medical interest given the increased need for ventilation and increased mortality rates of ventilated patients associated with age. Further, despite the clinical need for better understanding of age-related VILI, there is no consensus on VILI models that are used experimentally, and few existing experimental models have tested aged subjects. Using mathematical modeling and statistical methods, we analyzed plausible ventilator responses associ-
ated with experimental groups for young and old mice with 2 hours of ventilation using macrophage phenotype and lung integrity data. Our mathematical model calibrated with data from one commonly used mouse VILI model, high-pressure mechanical ventilation for 2 hours, may be used to test future VILI models and plan experiments with clinically meaningful results.

The experimental data was used as acceptance criteria to identify parameter sets associated with the young or old data. Thus, differences observed in the *in silico* model transients of $M_0$, $M_1$, $M_2$, and $E_e$ match those observed in the experimental data. Neutrophil counts, however, were not included in data collection, but do exhibit an observable difference in the model simulations as seen in Figure 4 and Figure 6. The old transients exhibited much higher levels of neutrophils compared with the young sets. This is consistent with *in vivo* results where increased neutrophil infiltration and alveolar damage were correlated \[50\]. Higher neutrophil infiltration has also been observed in older individuals following lung injury \[51\].

Classification results revealed that parameters involved in repair and damage of epithelial cells were important in separating parameter sets into the young or old experimental groups. The parameters involved in repair and damage of epithelial cells were expected results given the discrepancies observed in the airspace enlargement variable of the experimental data as well as past research that has shown poorer lung health in aged subjects. The potential for repair and damage in the epithelial tissue offers some insight into what may be driving major differences in the response to ventilation for young and old patients. Parameters involved in macrophage function were also ranked highly in separating parameter sets between the two age groups. These were expected to be associated with differences in parameter sets associated with the young and old data based on past research as well as our experimental findings. Classification results highlighted macrophage parameters specifically relating to the M1
phenotype which was not observed to have discernible differences in the experimental
data. However, changes in activation of the M1 phenotype directly affect the popu-
lations of the M0 and M2 phenotypes which did have significantly different ranges in
the experimental data at baseline. Based on the data, differences are observed in the
M0 and M2 phenotype populations at baseline but the classification results indicate
that this difference may be related to underlying M1 dynamics.

Classification results based on lung health state identified parameters involved
in activation of inflammatory cells and mediators, and parameters involved in dam-
age and repair to be important. These results are expected from the model since
repair and damage directly contribute to the variables used to classify the health
state. Additionally, the processes involved in the pro-inflammatory response are
known to affect local tissue health. Local sensitivity results identified similar pa-
rameters involved in damage and repair as well as parameters directly related to the
pro-inflammatory response, namely PIMs and neutrophils. Past research has identi-

fied a pro-inflammatory response, mainly mediated by neutrophils, to be significant
in the development of acute lung injury and ARDS. Our model suggests that neu-

trophils likely play a role in age-related differences as well since increased neutrophil
activation was observed in the parameter sets associated with the old data as well as
generally correlating with poorer epithelial health.

To explore how targeted interventions could change the poor responses to venti-
lation we modulated parameters that model outputs were sensitive to and evaluated
changes in the model-predicted epithelial cell health. The local sensitivity results
were used to select parameters to modulate and simulations were performed for all
parameter sets in each of the representative groups with a severe health state after
ventilation. In some cases, a wide range of responses were observed. The greatest
effects were observed in the old representative sets, specifically for the variable $E_e$. 48
For the old representative set classified as moderate before ventilation and severe after ventilation, some parameter modulations produced a potential increase or decrease in $E_e$ of about 40-50%. Overall, the mean response to modulation of each parameter had a magnitude less than 10%, with some near 0. Despite this, the targeted parameters offer potentially large improvements, particularly in the old parameter sets with poorer lung health. Additionally, an intervention performed mid-way through ventilation also produced potential improvements in the transients but to a lesser extent.

The exploration of age differences in VILI expresses early attempts to create more personalized medical interventions. Despite the observed differences in MV response with age, clinical use and interventions for MV remain a one-size-fits-all approach, ignoring underlying immunological variance in patients. Our model suggests that parameters controlling M1 macrophage dynamics and pro-inflammatory mediators show distinct separation between young and old associated parameter sets. These could be potential targets for further research to help identify the cause of the differing responses to ventilation in young and old subjects. Simulated interventions indicated damage and repair parameters showed the potential to improve tissue health during ventilation, with these being most influential for old associated parameter sets with poorer lung health. However, the wide range of potential responses indicates there are more components involved in alveolar tissue health not accounted for in this analysis.

This model can be adapted to account for non-VILI associated lung injury, such as an infection or inhaled toxins. The statistical and mathematical method used with this model can then determine key components of the immune and repair responses for those insults. Insults could be combined with ventilation to better explore the age-dependent response to VILI while accounting for co-factors that lead to the necessity for ventilation.
3.1 Introduction

Lipopolysaccharides (LPS) are outer membrane components of gram-negative bacteria and are biomarkers in the host-pathogen interaction, mainly facilitating the infection process \[52\]. LPS induces an innate immune response and its recognition is essential for clearing infections. Lysis of the bacterial cells initiated by the immune response clears the bacterial cells but leaves behind fragments of the membrane that trigger inflammatory cells inducing fever, diarrhea, and in more extreme cases, septic shock. LPS is a common culprit of this process in acute lung injury (ALI) and has prompted many experimental studies \[53\-58\,59\,60\]. Due to its role in activating the innate immune response, LPS-induced ALI is dependent on the neutrophil and macrophage response \[53\-58\,59\,60\]. To model ALI induced by the introduction of LPS to a murine system, we adapted the model described in Chapter 2 to an LPS induced injury and its interaction with the innate immune response and epithelial cells in the lungs.

Specific cytokines play a large role in ALI and are up-regulated by LPS exposure. Cytokines are extracellular signaling proteins secreted by cells that can modify the behavior of surrounding cells \[61\]. Cytokines are important actors in the inflammatory response and help to up-regulate and subsequently down-regulate the expression of inflammatory cells, guiding the progression to subsequent phases of tissue repair. In the previous model described in Chapter 2 cytokines were included as general cell
mediators described as either pro-inflammatory, anti-inflammatory, or repair mediators. Research into LPS-induced ALI, has identified specific cytokines as well as chemokines (cell proteins that signal chemotaxis) that play important roles in lung injury. Modeling specific cytokines and other mediators in the inflammatory response may aid in a better understanding of the progression of LPS-induced ALI. The collection of cytokines and other mediators involved in the immune response to tissue damage and infection is extensive; however, specific cytokines are often identified in clinical trials. Common pro-inflammatory cytokines associated with LPS-induced ALI include tumor necrosis factor alpha (TNF-\(\alpha\)) and interleukin 6 (IL-6) \[53, 60, 58, 62, 57, 59, 63, 64\].

TNF-\(\alpha\) is mainly produced \textit{in vivo} by monocytes, fibroblasts, and endothelial cells \[62\]. Once activated, TNF-\(\alpha\) is also produced by macrophages, T-cells, B-lymphocytes, granulocytes, eosinophils, mast cells, and others. Administration of LPS has been shown to induce high levels of TNF-\(\alpha\) production in animal models, correlating with features of septic shock and severe pro-inflammatory reactions \[65\]. The high levels of TNF-\(\alpha\) have also been reproduced in human subjects as a response to bacterial endotoxin \[66\] and complicates many diseases \[67, 68, 69, 70, 71\]. TNF-\(\alpha\) has also been shown to stimulate production of reactive oxygen species (ROS), a toxic byproduct of phagocytic cells \[72\]. ROS production has been observed in pulmonary and non-pulmonary tissues from human endothelial cells \[73\] and neutrophils \[74\]. TNF-\(\alpha\) has been suggested as an important early mediator of ALI \[75\]; although, the exact influence remains unclear \[62\]. Some studies suggest the effects of TNF-\(\alpha\) may be related to its ability to down-regulate surfactant proteins \[76, 77\], increasing surface tension along the alveolar epithelium and increasing the risk of alveolar damage associated with volutrauma and atelectrauma \[78\].

IL-6 has been shown to exhibit both pro- and anti-inflammatory properties \[63\].
and is produced by certain types of lymphocytes, monocytes, fibroblasts, and endothelial cells, among others. IL-6 is described as the chief activator for the production of other cytokines and mediators involved in inflammation and has been observed to play an important role in instances of chronic inflammation. Despite the observed pro-inflammatory effects, it is also involved in regulatory processes of other pro-inflammatory mediators, representing its anti-inflammatory role. IL-6 has been evidenced to help the transition from neutrophil-driven to monocyte-driven inflammation. Both primary roles of IL-6 make it an important mediator of inflammation in the lungs and has been evidenced to both ameliorate and exacerbate lung inflammation in certain settings.

Another primary cytokine involved in lung inflammation is the anti-inflammatory cytokine interleukin 10 (IL-10). Anti-inflammatory cytokines play an important role in controlling the inflammatory response, particularly to bacterial stimulants, which work to prevent excess tissue damage from pro-inflammatory processes. IL-10 is produced by a variety of immune cells but the primary sources in vivo are T helper cells, monocytes, macrophages, and dendritic cells. IL-10, as well as other anti-inflammatory mediators, are essential for resolving inflammation and suppression or dysregulation of these signals results in chronic inflammation and other detrimental effects.

Since we are able to identify specific cytokines involved in inflammation in the lung tissue involved in LPS-induced ALI, we include these specific populations in our model. Each protein is represented by an ODE so that we can track the resulting levels over time following LPS-induced lung injury. A well-defined model will offer insight into the dependencies of lung injury with the associated cytokines. Additionally, as we have demonstrated with the model in Chapter 2, we incorporate age differences utilizing experimental data from different age groups of laboratory mice to identify...
and assess parameter sets associated with the different age groups. Similar to Chapter 2, we also use classification methods to identify important parameters in classifying between parameter sets associated with the young or old data.

3.2 Methods

The model developed in this chapter expands the model introduced in Chapter 2 to include specific cytokine populations in place of the general mediator variables $p$ and $a$, representing pro-inflammatory and anti-inflammatory mediators, respectively. In this model, injury is induced by LPS rather than mechanical ventilation. We pair this model with experimental data for LPS-induced lung injury in young and old mice to identify parameter sets associated with each age group.

3.2.1 Experimental data

Experimental data was obtained from collaborators in the Biomedical Engineering department at Virginia Commonwealth University (VCU). LPS was administered intratrachially through inhalation to mice in both young (2-3 months) and old (18-24 months) age groups. Data was collected for various cytokine levels (TNF-$\alpha$ and IL-10) and inflammatory cell counts (neutrophils and macrophages) from the lavage fluid collected from the lungs. Lung resistance measures were also collected. The data was used to identify biologically plausible parameter sets for the mathematical model. The relevant data ranges for the mathematical model are plotted in Figure 10. Due to the separation in the macrophage counts at time 0 between the young and the old data sets, we can identify parameter sets that are exclusively associated with either the young or old data.
Fig. 10. Plot of experimental data ranges for TNF-α (pg/mL), IL-10 (pg/mL), neutrophils (cells/mL), macrophages (cells/mL), and resistance in the young (blue) and old (red) groups. The measure for resistance plotted here is normalized based on the raw resistance values reported in the data. All resistance values were initially scaled to the average of the 0 hours data for each group (young or old). Measures were then normalized to the minimum and maximum of these scaled values where the minimum is mapped to 0 and the maximum is mapped to 0.6. We assume that LPS exposure of this level can result in at most a damage state of 0.6 (60% of lung epithelium compromised).
3.2.2 Mathematical model and analysis methods

3.2.2.1 Model equations

The LPS-induced injury model is an expansion of the VILI model formulated in Chapter 2. The LPS model modifies the VILI model by including specific populations of the inflammatory mediators TNF-α, IL-6, and IL-10 rather than generalized variables for pro- and anti-inflammatory mediators. We also include a variable representing damage signals from damaged epithelial cells (p) and a variable for LPS introduced into the lung compartment. Interactions between the innate immune cells, inflammatory mediators, and LPS are included in Figure 11. The full model variables are described in Table 4 and model parameters are described in Table 5.
Fig. 11. **Model schematic of inflammatory cells and inflammatory mediators.**
The solid black arrows represent activation or up-regulation and the dotted
lines with bars represent inhibition or down-regulation. This schematic specifi-
cally represents interactions between the inflammatory cells and inflammatory
mediators within the alveolar compartment but these same interactions are
observable in the blood vessels without the presence of LPS.
Table 4. **LPS-induced lung injury model variables with descriptions.**

The epithelial variables $E_h$ and $E_d$ and $E_e$ are proportions, such that $E_h + E_d + E_e = 1$. The remaining variables have arbitrary units for simulation. The variables $N_0$, $N$, $M_0$, $M_1$, and $M_2$ are verified against experimental data for neutrophil and macrophage counts. The variables $T_\alpha$ and $IL_{10}$ are also verified by experimental data for TNF-α and IL-10, respectively.

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<th>Description</th>
</tr>
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</tr>
<tr>
<td>$E_d$</td>
<td>Damaged epithelial cells</td>
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</tr>
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<td>$E_e$</td>
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<td>$N_b$</td>
<td>$N$</td>
<td>Activated neutrophils</td>
</tr>
<tr>
<td>$AN$</td>
<td>Apoptotic neutrophils</td>
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<tr>
<td>$R$</td>
<td>Repair mediators</td>
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Table 5.: LPS-induced lung injury model parameters with descriptions.

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<tr>
<th>Parameter</th>
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<tbody>
<tr>
<td>$b_d$</td>
<td>Baseline decay of damaged cells</td>
<td>h⁻¹</td>
<td>0.0051</td>
<td>119.1629</td>
<td>0.0080</td>
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<tr>
<td>$b_p$</td>
<td>Baseline self-resolving repair of epithelial cells</td>
<td>h⁻¹</td>
<td>0.0047</td>
<td>118.4912</td>
<td>0.0006</td>
<td>119.8872</td>
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<tr>
<td>$b_r$</td>
<td>Baseline repair of damaged cells</td>
<td>h⁻¹</td>
<td>0.0002</td>
<td>117.9420</td>
<td>0.0034</td>
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<tr>
<td>$d_{m0}$</td>
<td>Rate of diffusion for $M_0$</td>
<td>h⁻¹</td>
<td>0.0020</td>
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<td>$k_{an}$</td>
<td>Rate at which neutrophils become apoptotic</td>
<td>h⁻¹</td>
<td>0.0026</td>
<td>119.0606</td>
<td>0.0041</td>
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<tr>
<td>$k_{anm1}$</td>
<td>Rate of $M_1$ phagocytosis of $AN$</td>
<td>(cells/nL)⁻¹· h⁻¹</td>
<td>0.0020</td>
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<td>0.0575</td>
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<tr>
<td>$k_{anm2}$</td>
<td>Rate of $M_2$ phagocytosis of $AN$</td>
<td>(cells/nL)⁻¹· h⁻¹</td>
<td>0.0001</td>
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<td>$k_{ee}$</td>
<td>Rate of inflammatory cell and mediator leakage into alveolar compartment</td>
<td>h$^{-1}$</td>
<td>0.0010</td>
<td>119.5388</td>
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<td>$x_{ee}$</td>
<td>Regulates effectiveness of inflammatory cell leakage into alveolar compartment (Hill-type constant)</td>
<td>Unitless</td>
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<td>$x_{eem}$</td>
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<td>$k_{em1}$</td>
<td>Rate of phagocytosis of damaged cells by $M_1$</td>
<td>(cells/nL)$^{-1}$ · h$^{-1}$</td>
<td>0.0002</td>
<td>119.0105</td>
<td>0.0021</td>
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<td>$k_{en}$</td>
<td>Rate of phagocytosis of damaged cells by $N$</td>
<td>(cells/nL)$^{-1}$ · h$^{-1}$</td>
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<td>$k_{ep}$</td>
<td>Rate of self-resolving repair mediated by $p$</td>
<td>p-units$^{-1}$ · h$^{-1}$</td>
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<td>0.0038</td>
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<td>$k_{man}$</td>
<td>Switch of $M_1$ to $M_2$ induced by $AN$</td>
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<td>$k_{m1}$</td>
<td>Rate of $M_1$ infiltration into alveolar compartment</td>
<td>h$^{-1}$</td>
<td>0.0101</td>
<td>117.0232</td>
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<tr>
<td>$k_{mne}$</td>
<td>Rate of collateral damage to epithelial cells by macrophages and neutrophils</td>
<td>h(^{-1})</td>
<td>0.2887</td>
<td>119.9389</td>
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<td>$x_{mne}$</td>
<td>Regulates effectiveness of macrophages and neutrophils to damage epithelial cells (Hill-type constant)</td>
<td>(cells/nL)</td>
<td>0.0003</td>
<td>16.3808</td>
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<td>$k_{n}$</td>
<td>Rate of infiltration of $N_{b}$ to lung</td>
<td>h(^{-1})</td>
<td>0.0837</td>
<td>117.1025</td>
<td>0.6200</td>
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<td>$k_{pc}$</td>
<td>Production rate of $p$ by $E_{d}$</td>
<td>p-units $\cdot$ h(^{-1})</td>
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<td>$k_{rm2}$</td>
<td>Production rate of $R$ by $M_{2}$</td>
<td>R-units $\cdot$ (cells/nL)$^{-1}$ $\cdot$ h(^{-1})</td>
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<td>Decay rate of $AN$</td>
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<td>$\mu_{m2b}$</td>
<td>Decay rate of $M_{2b}$</td>
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<td>$\mu_{nb}$</td>
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<td>$\mu_R$</td>
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<td>$s_m$</td>
<td>Source rate of $M_{0b}$</td>
<td>(cells/nL) · $h^{-1}$</td>
<td>0.0497</td>
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<tr>
<td>$s_n$</td>
<td>Source rate of $N_{0b}$</td>
<td>(cells/nL) · $h^{-1}$</td>
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<td>118.9370</td>
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Table 5 – continued from previous page

<table>
<thead>
<tr>
<th>Parameter</th>
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<th>Description of IL-6 effect on TNF-α production by M₁ and M₁₀ in the blood</th>
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<tbody>
<tr>
<td>$d_{IL6}^*$</td>
<td>Diffusion rate of IL-6</td>
<td>$h^{-1}$</td>
</tr>
<tr>
<td>$d_{IL10}^*$</td>
<td>Diffusion rate of IL-10</td>
<td>$h^{-1}$</td>
</tr>
<tr>
<td>$d_p^*$</td>
<td>Diffusion rate of damage mediators</td>
<td>$h^{-1}$</td>
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<tr>
<td>$d_t^*$</td>
<td>Diffusion rate of TNF-alpha</td>
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<tr>
<td>$IL_{6,∞}^*$</td>
<td>Parameter controlling the effectiveness of IL-6 at inhibiting the production of TNF-α by $M_1$</td>
<td>$(pg/\mu L)$</td>
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<td>$IL_{6,∞}^*$</td>
<td>Parameter controlling the effectiveness of IL-6 at inhibiting the production of TNF-α by $M_{16}$ in the blood</td>
<td>$(pg/\mu L)$</td>
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<td>$IL_{10\infty}^*$</td>
<td>Parameter controlling the effectiveness of IL-10 at inhibiting the production of TNF-α by $M_1$</td>
<td>(pg/µL)</td>
<td>0.0038</td>
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<td>0.0217</td>
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<td>$IL_{10\infty}^{*b}$</td>
<td>Parameter controlling the effectiveness of IL-10 at inhibiting the production of TNF-α by $M_{1b}$ in the blood</td>
<td>(pg/µL)</td>
<td>0.0017</td>
<td>119.7475</td>
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<td>$k_{IL6m1}^*$</td>
<td>Production rate of IL-6 by $M_1$ (pg/µL) · (cells/nL)$^{-1}$ · h$^{-1}$</td>
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<td>0.0119</td>
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<td>$k_{IL6n}$</td>
<td>Production rate of IL-6 by $N$</td>
<td>(pg/$\mu$L) · (cells/nL)$^{-1}$ · h$^{-1}$</td>
<td>0.0020</td>
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<td>$k_{IL6mb}$</td>
<td>Production rate of IL-6 by $N_b$ in the blood</td>
<td>(pg/$\mu$L) · (cells/nL)$^{-1}$ · h$^{-1}$</td>
<td>0.0036</td>
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<td>$k_{IL10m1}$</td>
<td>Production rate of IL-10 by $M_1$</td>
<td>(pg/$\mu$L) · (cells/nL)$^{-1}$ · h$^{-1}$</td>
<td>0.0034</td>
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<td>$k_{IL10m1b}$</td>
<td>Production rate of IL-10 by $M_{1b}$ in the blood</td>
<td>(pg/$\mu$L) · (cells/nL)$^{-1}$ · h$^{-1}$</td>
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<td>(pg/$\mu$L) · (cells/nL)$^{-1}$ · h$^{-1}$</td>
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<td>$k^{*}_{IL10m2b}$</td>
<td>Production rate of IL-10 by $M^*_2$ in the blood</td>
<td>(pg/µL) · (cells/nL)$^{-1}$ · h$^{-1}$</td>
<td>0.0003</td>
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<td>$k^{*}_{m0a}$</td>
<td>Differentiation rate of $M_0$ to $M_2$ induced by IL-10</td>
<td>h$^{-1}$</td>
<td>0.0020</td>
<td>118.4945</td>
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<td>$x^{*}_{m0a}$</td>
<td>Controls the effectiveness of IL-10 at inducing differentiation of $M_0$ to $M_2$ (Hill-type function)</td>
<td>(pg/µL)</td>
<td>0.0034</td>
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<tr>
<td>$k^{*}_{m0ab}$</td>
<td>Differentiation rate of $M_{0b}$ to $M^*_2b$ induced by IL-10 in the blood</td>
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<td>Controls the effectiveness of IL-10 at inducing differentiation of $M_{0b}$ to $M_{2b}$ in the blood (Hill-type function)</td>
<td>(pg/µL)</td>
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<td>$k_{m0i}^*$</td>
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<td>$k_{m0p}^*$</td>
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<td>Differentiation of $M_0$ to $M_1$ induced by damage mediators in the blood</td>
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<td>$k^{*}_{m0\text{t}}$</td>
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<td>$x^{*}_{m0\text{p}}$</td>
<td>Controls the effectiveness of pro-inflammatory mediators at inducing differentiation of $M_0$ to $M_1$ (Hill-type function)</td>
<td>$p$-units</td>
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<td>$k^{*}_{m0\text{bt}}$</td>
<td>Differentiation of $M_0b$ to $M_1b$ induced by TNF-α in the blood</td>
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<th>Parameter</th>
<th>Description</th>
<th>Units</th>
<th>Young Min.</th>
<th>Young Max.</th>
<th>Old Min.</th>
<th>Old Max.</th>
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<tr>
<td>$x_{m_{0p}}^*$</td>
<td>Controls the effectiveness of pro-inflammatory cytokines at inducing differentiation of $M_{0b}$ to $M_{1b}$ in the blood (Hill-type function)</td>
<td>$p$-units</td>
<td>0.0091</td>
<td>119.5618</td>
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<td>$k_{n_{0bi}}^*$</td>
<td>Activation rate of $N_{bi}$ induced by IL-6 in the blood</td>
<td>Unitless</td>
<td>0.0019</td>
<td>119.4686</td>
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<td>$k_{n_{0i}}^*$</td>
<td>Activation rate of $N$ induced by IL-6</td>
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<td>119.3924</td>
<td>0.0038</td>
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<td>$k_{n_{0p}}^*$</td>
<td>Activation rate of $N$ induced by damage mediators</td>
<td>Unitless</td>
<td>0.0035</td>
<td>116.9338</td>
<td>0.0026</td>
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<td>$k_{n_{0bp}}^*$</td>
<td>Activation rate of $N$ induced by damage mediators in the blood</td>
<td>Unitless</td>
<td>0.0006</td>
<td>119.3920</td>
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<th>Old Min.</th>
<th>Old Max.</th>
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<tr>
<td>$k_{n0t}^*$</td>
<td>Activation rate of $N$ induced by LPS</td>
<td>Unitless</td>
<td>0.0029</td>
<td>119.6248</td>
<td>0.0017</td>
<td>117.3235</td>
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<td>$k_{n0t}^*$</td>
<td>Activation rate of $N$ induced by TNF-α</td>
<td>$h^{-1}$</td>
<td>0.0002</td>
<td>119.9273</td>
<td>0.0063</td>
<td>118.0517</td>
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<tr>
<td>$x_{n0p}^*$</td>
<td>Controls the effectiveness of pro-inflammatory mediators at inducing activation of $N$</td>
<td>$p$-units</td>
<td>0.0008</td>
<td>118.8525</td>
<td>0.0297</td>
<td>118.4887</td>
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<tr>
<td>$k_{n0bt}^*$</td>
<td>Activation rate of $N$ induced by TNF-α</td>
<td>$h^{-1}$</td>
<td>0.0322</td>
<td>119.7579</td>
<td>0.0560</td>
<td>119.3138</td>
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<td>$x_{n0bp}^*$</td>
<td>Controls the effectiveness of pro-inflammatory mediators at inducing activation of $N_b$ in the blood</td>
<td>$p$-units</td>
<td>0.0006</td>
<td>115.6708</td>
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<th>Young Max.</th>
<th>Old Min.</th>
<th>Old Max.</th>
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<tr>
<td>$k_{tn1}$</td>
<td>Production of TNF-α by $M_1$</td>
<td>(pg/µL) · (cells/nL)$^{-1}$ · h$^{-1}$</td>
<td>0.0136</td>
<td>119.9697</td>
<td>0.0064</td>
<td>119.2525</td>
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<tr>
<td>$k_{tn1b}$</td>
<td>Production of TNF-α by $M_{1b}$ in the blood</td>
<td>(pg/µL) · (cells/nL)$^{-1}$ · h$^{-1}$</td>
<td>0.0031</td>
<td>119.6095</td>
<td>0.0012</td>
<td>119.2994</td>
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<td>$k_{tn}$</td>
<td>Production of TNF-α by $N$</td>
<td>(pg/µL) · (cells/nL)$^{-1}$ · h$^{-1}$</td>
<td>0.0010</td>
<td>119.8310</td>
<td>0.0145</td>
<td>114.2052</td>
</tr>
<tr>
<td>$k_{tnb}$</td>
<td>Production of TNF-α by $N_b$ in the blood</td>
<td>(pg/µL) · (cells/nL)$^{-1}$ · h$^{-1}$</td>
<td>0.0123</td>
<td>119.4667</td>
<td>0.0291</td>
<td>115.2628</td>
</tr>
<tr>
<td>$\mu_{IL6}$</td>
<td>Decay rate of IL-6</td>
<td>h$^{-1}$</td>
<td>0.0063</td>
<td>119.2901</td>
<td>0.0198</td>
<td>118.7615</td>
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<tr>
<td>$\mu_{IL6b}$</td>
<td>Decay rate of IL-6 in the blood</td>
<td>h$^{-1}$</td>
<td>0.0027</td>
<td>119.5945</td>
<td>0.0106</td>
<td>117.1510</td>
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<table>
<thead>
<tr>
<th>Parameter</th>
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<th>Old Max.</th>
</tr>
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<tbody>
<tr>
<td>$\mu_{IL10}^s$</td>
<td>Decay rate of IL-10</td>
<td>h⁻¹</td>
<td>0.0355</td>
<td>118.6184</td>
<td>0.0216</td>
<td>119.5572</td>
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<tr>
<td>$\mu_{IL10b}^s$</td>
<td>Decay rate of IL-10 in the blood</td>
<td>h⁻¹</td>
<td>0.0060</td>
<td>119.5421</td>
<td>0.0047</td>
<td>119.8978</td>
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<tr>
<td>$\mu_{LPS}^s$</td>
<td>Decay rate of LPS</td>
<td>h⁻¹</td>
<td>1.7035</td>
<td>1.7035</td>
<td>0.4545</td>
<td>0.4545</td>
</tr>
<tr>
<td>$\mu_p^s$</td>
<td>Decay rate of damage mediators</td>
<td>h⁻¹</td>
<td>0.0030</td>
<td>118.2140</td>
<td>0.0083</td>
<td>118.7814</td>
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<tr>
<td>$\mu_{pb}^s$</td>
<td>Decay rate of damage mediators in the blood</td>
<td>h⁻¹</td>
<td>0.0028</td>
<td>115.1025</td>
<td>0.0076</td>
<td>114.7611</td>
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<tr>
<td>$\mu_{t}^s$</td>
<td>Decay rate of TNF-α</td>
<td>h⁻¹</td>
<td>0.0050</td>
<td>119.9337</td>
<td>0.0030</td>
<td>109.4998</td>
</tr>
<tr>
<td>$\mu_{tb}^s$</td>
<td>Decay rate of TNF-α in the blood</td>
<td>h⁻¹</td>
<td>0.0057</td>
<td>119.6182</td>
<td>0.0202</td>
<td>119.4405</td>
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<tr>
<td>$s_{IL10}^s$</td>
<td>Background production of IL-10</td>
<td>(pg/µL) · h⁻¹</td>
<td>0.0001</td>
<td>119.0005</td>
<td>0.0032</td>
<td>108.3125</td>
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Continued on next page...
Parameters indicated with an asterisk (*) are novel to this iteration of the model and were not utilized in the VILI model presented in Chapter 2. Inflammatory cells units (neutrophils and macrophages) and inflammatory mediator units (TNF-α, IL-6, and IL-10) were scaled from those in the experimental data to ensure that the model outputs could reflect these values using the sampled parameter range. Raw cells values were scaled by $10^{-6}$ to the units cells/nL and mediators were scaled by $10^{-3}$ to the units pg/µL. Damage mediators ($p$) and repair mediators ($R$) are not fit to experimental data, so they are represented by the general units $p$-units and $R$-units, respectively. All epithelial variables are scaled to one, so their variables are unitless. The time unit is hours, $h$. Parameter ranges are determined by the maximum and minimum value achieved by each parameter over the plausible parameter sets associated with young and old experimental data. The process for obtaining these ranges is explained in Section 3.2.2.2.

Table 5 – continued from previous page

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Description</th>
<th>Units</th>
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<th>Young Max.</th>
<th>Old Min.</th>
<th>Old Max.</th>
</tr>
</thead>
<tbody>
<tr>
<td>$s_p^*$</td>
<td>Background production of damage mediators</td>
<td>$p$-units $\cdot$ h$^{-1}$</td>
<td>0.0046</td>
<td>119.8860</td>
<td>0.0439</td>
<td>118.7975</td>
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</tbody>
</table>

Parameters indicated with an asterisk (*) are novel to this iteration of the model and were not utilized in the VILI model presented in Chapter 2. Inflammatory cells units (neutrophils and macrophages) and inflammatory mediator units (TNF-α, IL-6, and IL-10) were scaled from those in the experimental data to ensure that the model outputs could reflect these values using the sampled parameter range. Raw cells values were scaled by $10^{-6}$ to the units cells/nL and mediators were scaled by $10^{-3}$ to the units pg/µL. Damage mediators ($p$) and repair mediators ($R$) are not fit to experimental data, so they are represented by the general units $p$-units and $R$-units, respectively. All epithelial variables are scaled to one, so their variables are unitless. The time unit is hours, $h$. Parameter ranges are determined by the maximum and minimum value achieved by each parameter over the plausible parameter sets associated with young and old experimental data. The process for obtaining these ranges is explained in Section 3.2.2.2.
Model equations for the inflammatory cytokines mirror those for the pro- and anti-inflammatory mediators in the VILI model described in Chapter 2. Examples of the inflammatory mediator equations in the lung compartment are shown in Equations 3.1-3.4 and the equation for LPS is shown in Equation 3.5. For the inflammatory mediator equations, the first term represents diffusion across the epithelial barrier from the blood vessels. The next term, or terms, describe production of the given mediator. For TNF-α (Equation 3.1), the second term describes production of TNF-α by M1 macrophages and the third term describes production by neutrophils. Both production rates are inhibited by IL-6 and IL-10. For IL-6 (Equation 3.2), the second term describes production of IL-6 by M1 macrophages and the third term describes production by neutrophils. Both production rates are inhibited by IL-10. For epithelial damage mediators (Equation 3.3), damage mediators are produced by damaged epithelial cells and thus are directly proportional to the amount of damaged cells. For IL-10 (Equation 3.4), the second term describes production of IL-10 by M1 macrophages and the third term describes production by M2 macrophages. These rates are assumed to be uninhibited. The second to last terms describe the leak into lung that occurs when the epithelial barrier is significantly degraded. This term was introduced in Chapter 2. A saturating hill function with a sufficiently large exponent is used to act as a switch once the barrier has surpassed a critical damage value. The last term in each equation describes intrinsic decay. The corresponding equations in the blood compartment have similar structures (Equations F.1-F.4 in Appendix F). The final equation represented here is for LPS which just decays over time.
\[
\frac{dT_\alpha}{dt} = -d_e(T_\alpha - T_{ab}) + k_{lam} M_1 \left( \frac{1}{1 + \left( \frac{IL_6}{IL_6T_{10}} \right)^2} \right) + \frac{1}{1 + \left( \frac{IL_10}{IL_10T_{10}} \right)^2}
\]
\[
+ k_{tan} N \left( \frac{1}{1 + \left( \frac{IL_6}{IL_6T_{10}} \right)^2} \right) \left( \frac{1}{1 + \left( \frac{IL_10}{IL_10T_{10}} \right)^2} \right)
\]
\[
+ T_{ab} \frac{k_{ee} E_e^4}{x_{eem} E_e^4} + \frac{1}{1 + \left( \frac{IL_6}{IL_6T_{10}} \right)^2} - \mu_{tab} T_\alpha
\] (3.1)

\[
\frac{dIL_6}{dt} = -d_{IL6}(IL_6 - IL_{6b}) + k_{IL6m1} M_1 \left( \frac{1}{1 + \left( \frac{IL_10}{IL_10T_{10}} \right)^2} \right) + \frac{1}{1 + \left( \frac{IL_10}{IL_10T_{10}} \right)^2}
\]
\[
+ \frac{k_{ee} E_e^4}{x_{eem} E_e^4} \left( \frac{1}{1 + \left( \frac{IL_6}{IL_6T_{10}} \right)^2} \right) + IL_{6b} \frac{k_{ee} E_e^4}{x_{eem} E_e^4} - \mu_{IL6} IL_6
\] (3.2)

\[
\frac{dp}{dt} = -d_p(p - p_b) + k_{pe} E_d + p_b \frac{k_{ee} E_e^4}{x_{eem} E_e^4} - \mu_{p} p
\] (3.3)

\[
\frac{dIL_{10}}{dt} = -d_{IL10}(IL_{10} - IL_{10b}) + k_{IL10m1} M_1 + k_{IL10m2} M_2
\]
\[
+ \frac{k_{ee} E_e^4}{x_{eem} E_e^4} \left( \frac{1}{1 + \left( \frac{IL_6}{IL_6T_{10}} \right)^2} \right) - \mu_{IL10} IL_{10}
\] (3.4)

\[
\frac{dLPS}{dt} = -\mu_{LPS} LPS
\] (3.5)

In addition to the equations for inflammatory mediators, the mediator variables also appear in activation terms for the various inflammatory cells. The individual cytokines and mediators essentially replace the pro- and anti-inflammatory mediators from the previous model. LPS, TNF-\(\alpha\), IL-6, and \(p\) are included in the activation
terms for neutrophils and M1 macrophages. IL-10 is included in the activation term of M2 macrophages.

3.2.2.2 Sampling and parameter selection

The parameter sampling methods are similar to those described in Chapter 2. Candidate parameter sets were initially drawn from a scaled Beta(1, 3) distribution with range 0 to 120 to include multiple orders of magnitude. Parameter sets were then simulated in MATLAB to determine if the parameter sets fit any of the data. This process is the same as those described in Chapter 2 where parameter sets were simulated from 1 or 2 initial conditions to determine if they reached a numerical steady state. Simulated transients were then compared to experimental data. Parameter sets were selected for further investigation if they fit all the variables included in the experimental data ($T_a$, IL-10, $N$, $M$, and $E_d + E_e$) for at least one time point in either age group (0, 4, or 24 hours for the young mice and 0 or 24 hours for the old mice). Data used for parameter sampling is plotted in Figure 10. These parameter sets were then sampled around their nominal value to find parameter sets that satisfied all of the time points for either the young or the old data. Each parameter set chosen from the first simulation was sampled using Latin hypercube sampling (LHS). LHS is a Monte-Carlo sampling method that can accurately capture the full parameter space with fewer samples than simple random sampling. LHS uses stratified sampling without replacement where the random parameter distributions are divided into $N$ equal probability intervals. It is recommended that $N$ be at least the number of parameters plus one, but a much larger number is often chosen. A parameter range and probability distribution is defined for each parameter, but, since little is known of the distribution and range for each parameter, a uniform distribution was used for each. Parameters were sampled within 50% of their initial value for 500 sets.
each. This resulted in a total of 371,000 sets generated using LHS in this second sampling. The resulting sets were then simulated again as described before where parameter sets were simulated to steady state and then model outputs were compared to experimental data. Final sets chosen from this step were those that satisfied the maximal number of time points for one of the age groups.

Parameter sampling was performed on all parameters except \( x_{ee}, x_{eem}, \) and \( \mu_{LPS} \). The parameters \( x_{ee} \) and \( x_{eem} \) control the half maximum of the function that models influx of immune cells and mediators due to damage to the epithelium. Like the model in Chapter 2, these are fixed at \( x_{ee} = 0.75 \) and \( x_{eem} = 0.5 \). These values are set such that \( x_{ee} > x_{eem} \) which ensures that mediator influx occurs for lower levels of damage than cell influx since the mediators are smaller in size than the cells. The parameter \( \mu_{LPS} \) is the rate at which LPS decays in the lung space. This value differs between young and old mice, so this value was set separately for the two groups based on experimental data. For these simulations, we use rates for mucociliary clearance in young and old mice [92]. Age ranges for young and old mice specimens are the same as those selected for the experimental data discussed in Section 3.2.1, so this is applicable to our model. The clearance rates presented in Grubb et al. [92] are given as percent clearance per 15 minutes. These are converted to parameter estimates for the ODE model by integrating the differential equation for \( LPS \) and using the percent clearance to solve for the parameter \( \mu_{LPS} \). Details of these calculations are given in Appendix G. The values for \( \mu_{LPS} \) were set to 1.7035 h\(^{-1}\) for the young simulations and 0.4545 h\(^{-1}\) for the old simulations.

### 3.2.2.3 Classification methods

Similar to the methods explained in Chapter 2, several methods were fitted on various subsets of the parameter sets which fell in the range for either the young or
old data using the R package H2O [44]. Parameters were assigned relative importance values based on these results.

3.3 Results

3.3.1 Plausible parameter sets and transients

After sampling, we selected 1,900 parameter sets associated with the young data and 919 parameter sets associated with the old data. The data points used for parameter selection were TNF-α which was compared with the model variable $T_\alpha$, IL-10 which was compared to the model variable $IL_{10}$, neutrophils which were compared to $N_0 + N$, macrophages which was compared to $M_0 + M_1 + M_2$, and resistance which was compared to $E_d + E_e$. Some of this data was difficult to satisfy, so we accepted young sets that satisfied at least 9 of the 15 data ranges and old sets that satisfied at least 5 of the 10 data ranges (satisfying at least 50% of the total data points in each group). The total parameter sets that fit each of the data ranges are shown in Figures 12,13.
Fig. 12. Number of parameter sets that satisfied each data range for LP-S-induced lung injury in the young parameter sets.
Fig. 13. Number of parameter sets that satisfied each data range for LP-S-induced lung injury in the old parameter sets.

As is shown in the plots, there was particular difficulty satisfying variables at baseline (T=0 h) in both groups. TNF-α also had very few parameter sets that satisfied this flag for T=4 h in the young group. None of the parameter sets satisfied all the flags for either age group. Because we were not able to satisfy macrophages at T=0 h for either group, which served as the point of separation between the two groups, there were some parameter sets that fit both the young and old data for our selection criteria. Fortunately, only 10 of the sets overlapped, so these were removed. The total number of plausible sets for each group was 1,890 parameter sets associated with the young experimental data and 909 parameter sets associated with the old experimental data.

There was generally a significant amount of overlap in the parameter ranges
selected for the accepted sets in each age group. The majority of parameters had selected values ranging across the entire sampled range $[0,120]$, which is likely due to the nonlinearity of the equations resulting in significant parameter interactions. Thus, a diverse set of parameter combinations is able to produce the desired model outputs. Additionally, the majority of parameter values show little distinction in overall distributions. Histograms of the parameter values were plotted. Those with significant visual separation are shown in Figure 14. The selected parameters were $s_n$, the source rate of neutrophils, $s_m$, the source rate of macrophages, and $x_{mne}$, a parameter controlling the effectiveness of $M1$ and $N$ at causing damage to the epithelium.
Fig. 14. Histograms of selected parameters from the accepted parameter sets. The three parameters with significant separation were $s_n$ (plot A), the source rate of neutrophils, $s_m$ (plot B), the source rate of macrophages, and $x_{mne}$ (plot C), a parameter controlling the effectiveness of M1 and N at causing damage to the epithelium.

3.3.2 Importance factors for age classification

H2O \cite{44} was run in R \cite{43} to fit a suite of machine learning algorithms and select the best performing model separating young and old parameter sets. The best model selected by H2O was a Gradient Boosting Model. The resulting variable importance values are plotted in Figure \ref{fig15}. As expected from the distributions shown in Figure \ref{fig14}, the parameters $x_{mne}$ and $s_m$ were assigned the top two largest importance factors. Since there was a difference in the number of accepted sets between the two
groups, we randomly down-sampled the old parameter sets to match the number of young parameter sets and reran H2O for 5 random samples. The best chosen model stayed consistent for these resampling. The exact ranking did differ for some runs ($s_m$ first and $x_{mne}$ second) but overall, these two parameters were selected as the top two and parameters selected in the top ten remained static. Other top ranked parameters included $s_{IL10}$, the source rate of IL-10, $k_{mne}$ which controls the maximum rate that M1 macrophages and neutrophils cause damage to the epithelium, $s_n$, the source rate of neutrophils, $d_{IL10}$, the diffusion rate of IL-10 from the blood compartment into the lung compartment, $\mu_{IL10}$, decay rate of IL-10, $k_{an}$, the rate at which neutrophils become apoptotic, $\mu_n$, the decay rate of neutrophils, and $b_r$, baseline repair of damaged cells, $\mu_{IL10_b}$, decay rate of IL-10 in the blood, and $\mu_{m0b}$, decay rate of naive macrophages in the blood. After this parameter, the remaining importance values are less than 5% of the maximum. In general, important parameters tended to involve macrophages, neutrophils, and IL-10.
Fig. 15. **Ranked variable importance factors determining age classification.**

The variable importance factors were calculated from a Gradient Boosting Model, chosen by H2O as the best model, for classifying between the young and old parameter sets. Importance values were normalized to the maximum.

It is clear that overwhelmingly parameters involved in neutrophil and macrophage dynamics were significant in classifying between the two age groups. Since these variables were fit to data, these are expected to differ between the two age groups. This also explains why parameters involved in IL-10 and activation of inflammatory cells by TNF-α show up to be important as well.
3.4 Discussion

We modified the model for lung inflammation developed in Chapter 2 to be initiated by a bacterial insult, namely LPS, and include specific cytokines involved in inflammatory signalling. Experimental data for TNF-α, IL-10, neutrophils, macrophages, and lung resistance were all used to select plausible parameter estimates for the model. The experimental data revealed subtle differences in each of these variables between the young and old experimental groups. Exact separation of experimental ranges was observed only in the T=0 h data point for macrophages. The experimental data was used to select plausible parameter sets associated with either the young or old data. Some of the data were difficult to fit, resulting in some parameter sets that satisfied both the young and the old data using our selection criteria. These were excluded from classification methods.

Using the selected plausible parameter sets, we performed a suite of machine learning algorithms to select the best classification model separating the young and old parameter sets which assigns importance values to each parameter. Since the importance values decayed relatively quickly, we showed only the top fifteen parameter values used in classification. The majority of the parameters identified in the top fifteen are related to terms controlling neutrophil and macrophage dynamics. Given the experimental results, this is expected since we fit the model specifically to those cell populations. Although we show results for the top fifteen ranked parameters in the classification model, it is clear from the importance values that the classification overwhelmingly relies on the top two ranked parameters $x_{mne}$ and $s_m$. The parameter $x_{mne}$ was also ranked relatively high in the classification of parameter sets in the VILI model. The parameter $\mu_n$ also showed up in the top importance values for the VILI model as well as in the present model. This model, however, did not rank
parameters involved in the epithelial variables as of high importance in classifying parameter sets between young and old associated sets. This reflects how VILI is generally characterized by mechanical damage of the epithelium while LPS-induced ALI is primarily characterized by an intense inflammatory response.

Overall, the model developed here appears to support experimental results which suggest that the innate inflammatory response, specifically macrophage and neutrophil dynamics drive the progression of LPS-induced lung injury and exhibit significant differences between the young and old specimens. The results presented in Chapter 2 reflect the importance of macrophages as well in age differences regarding VILI.
CHAPTER 4

A MATHEMATICAL MODEL FOR WOUND HEALING IN A REEF-BUILDING CORAL

The work presented in this Chapter is in revisions for publication. We thank the VCU Accelerate Fund which supported the development and analysis of the mathematical model and the VCU Undergraduate Research Opportunities Program summer fellowship which supported the collection of the wound healing data. All code used in this model is available on Github (https://github.com/quintessahay/coralwoundmodel).

4.1 Introduction

Coral reefs are biodiversity hot spots and essential components of tropical and subtropical marine ecosystems. They provide food and shelter to approximately 30% of all marine fish, despite only covering 0.5% of the seabed worldwide [93]. Coral reefs also provide coastal protection to 450 million people (from storms and erosion) and a number of goods and services (food, income, medicines, tradition and culture) valued at 375 billion U.S. dollars a year [94, 95, 96, 97]. Warming of sea-surface temperatures are precipitating the loss of coral reefs worldwide with serial bleaching events of growing severity [98]. Pollution, unsustainable fishing practices, and other human activities exacerbate the stress brought on by climate change [96]. These changes in the environment result in physiological stress to the organism [96].

Coral reefs frequently experience wounding events resulting in significant allotment of bioenergy to restore and repair lost biomass [99]. Wounds are defined as
epithelial breaks and can include extensive tissue damage and/or skeletal damage and are commonly caused by wave action and storm damage, predatory fish species, and boring organisms (e.g. worms, sponges, mollusks), as well as anthropogenic activities (e.g. large vessels, debris, tourism). Recent practices in reef restoration have also involved systematically breaking coral fragments and relocating them to revitalize damaged reefs, a practice known as microfragmentation. In addition to increased energy expenditure to repair the wound, a wounding event also exposes the reef to potential foreign infiltrates such as bacterial or fungal infections and/or colonization by predatory algae species. Despite its importance, the majority of available research on wounding events in marine invertebrates, such as stony corals, has focused on surface level mechanics (e.g. wound closure times and tissue dynamics at the macro level) without examining the underlying cellular process.

Wound healing in humans and other vertebrates has been studied extensively at the cellular level and some invertebrates have also been observed to have similar processes. The wound healing process consists of four distinct yet overlapping phases: (1) hemostasis, (2) inflammation, (3) proliferation, and (4) remodeling. Hemostasis occurs immediately after wounding to stop any bleeding and form a clot, which serves as a scaffold for inflammatory cell infiltration. The inflammatory phase is the body’s defense mechanism where inflammatory cells (mainly neutrophils and macrophages in humans) remove debris and foreign bacteria from the wound. This typically occurs immediately following a wounding event and resolves within a few days. The proliferation phase consists of epithelialization and wound contraction mediated by fibroblasts. This stage typically occurs over the course of a few weeks. Remodeling is the final phase and can last anywhere from a few months to years, depending on a number of factors. This phase...
is mainly marked by collagen deposition in an organized way to achieve acceptable integrity and flexibility in the new tissue.

Although a generalized theoretical model of wound healing has yet to be categorized for many marine invertebrates, including reef-building corals, Palmer et al. [111] found that wound healing in the scleractinian species *Porites cylindrica* was comparable to the process observed in humans and other vertebrates. Palmer observed wound healing in *P. cylindrica* at the cellular level. Both tissue and skeletal composition during the healing process were observed throughout the wound healing process by decalcifying fragments and preserving the tissue for thin section histology. Wound healing in *P. cylindrica* showed an overlapping four-phase process similar to that observed in humans and other vertebrates, even involving similar cell types and mediators [111]. Researchers categorized an initial plug formation similar to the clot formation that occurs during hemostasis, an inflammatory phase mediated by phagocytic cells called amoebocytes, a proliferation phase mediated by fibroblasts, and remodeling marked by collagen deposition [111]. The density of Symbiodiniaceae within the coral tissue was also reported throughout the healing process since reef-building corals maintain a complex endosymbiosis with these dinoflagellate algae which provide the majority of the coral’s energy for bioprocesses [116].

Experimental studies on wound healing in corals have predominantly focused on characterizing dynamics at the polyp or colony scale, leaving the cellular-level aspects under-explored [117]. *In vitro* studies in mammalian systems have greatly advanced our understanding of the complex cellular interactions involved in wound healing [118]. Wound healing has also been observed at the cellular level in some invertebrates (e.g. drosophilia) [112], but coral *in vitro* research has been more challenging [119]. Common issues in coral cell culture include cell viability and culture media composition [120], cell identification (type and function) and separation [121].
Recent research has made large strides in coral cell culture practices and cell sorting and identification, such as fluorescence based labeling, but until more progress is made in the field of coral in vitro research, alternative methods to investigate the underlying mechanisms, such as mathematical modeling, can be beneficial.

Mathematical modeling has been a useful tool for biological systems that are difficult or expensive to test experimentally. Mathematics has been used to model coral reef dynamics, including coral-algal competition, factors related to bleaching, and other processes. The immune response to injury and other insults in mammalian systems has historically been modeled using various mathematical approaches including ordinary differential equations (ODEs), partial differential equations, and other models. Primarily, mathematical models of wound healing have focused on the inflammatory phase and early stages of proliferation, as these tend to harbor the most potential for issues to arise regarding infection and non-healing wounds, but mathematical models of remodeling have also been explored.

In this Chapter, we propose a mathematical model using a system of ODEs to describe the cellular mechanisms of wound healing in a scleractinian coral, *Pocillopora damicornis*. We develop the model using the mathematical terms described in previous mathematical models of wound healing in mammals for cellular activation as well as the identified cellular actors and mechanisms of wound healing in a scleractinian coral presented in Palmer *et al.*. The model is coupled with experimental data for wounds with two geometric shapes and different positions on a coral fragment (base, middle, and branch) to obtain plausible param-
eter estimates. The experimental data acts as acceptance criteria imposed on the model variables to ensure the outputs of the mathematical model represent observed biological trends. We also perform a local sensitivity analysis to identify influential parameters and obtain an identifiable set used to further optimize the parameter estimates. The parameters selected for the identifiable set include the source rate of precursor cells for amoebocytes and fibroblasts, the growth or accumulation rate of debris and foreign matter in the wound, the rate of removal of debris and foreign matter by amoebocytes, and the rate at which fibroblasts can facilitate new tissue formation in the wounded space. We explore additional model behavior by simulating variations in wound size and geometry, dynamics involved in debris and foreign organisms, and access to immune cell precursors. These simulations demonstrate the model’s ability to fit experimental data and produce transients associated with various environmental conditions and colony health.

4.2 Methods & model development

The mathematical model was built using common methods for modeling the inflammatory response to injury and foreign infiltrates \cite{136,137,138,139,140} as well as observed wound healing dynamics in \textit{P. cylindrica} \cite{111}. The mathematical model includes equations modeling debris and foreign infiltrates, the phagocytic cell identified in scleractinians (amoebocytes), fibroblasts, and new epithelial cells in the wounded space. Parameters were chosen for the mathematical model using Latin-hypercube sampling and optimization methods. Parameter selection relied on experimentally obtained data from wound closure times observed in \textit{P. damicornis} fragments. Sensitivity analysis was also performed to select an identifiable set, aiding in parameter selection. Model behavior was further explored for varying environmental and coral health conditions.
4.2.1 Experimental data

Six wounds of circular or linear geometric shape were applied to a *P. damicornis* fragment at various locations (approximate locations shown in Figure 16). Wound size was measured over time providing data for the mathematical model. Data was used to fit plausible parameter sets by fitting the variable $C$, a variable describing the formation of new tissue.

![Image](image_url)

Fig. 16. *P. damicornis* fragment with scale and approximate wound locations. Circular wounds are marked with a dashed circle and linear wounds are marked with a dashed line. These represent approximate locations for the wounds at the base (blue), middle (red), and top (yellow) of the fragment.

4.2.2 Model equations

The mathematical model is a system of four nonlinear differential equations modeling the populations of debris and/or foreign organisms (bacteria and invasive algae) ($M$), amoebocytes ($A$), fibroblasts ($F$), and new tissue ($C$). Basic interactions of the four variables can be seen in Figure 17. There are a total of seventeen unknown pa-
Parameters that were selected over the parameter space using Latin Hypercube sampling. Brief explanations of the model parameters are included in Table 6.

Fig. 17. Model schematic for the system of differential equations. The four variables are debris and foreign matter (M), amoebocytes (A), fibroblasts (F), and newly formed tissue (C). $s_c$ represents the source rate of multipotent cells ($S$) that can differentiate into amoebocytes or fibroblasts. More details about this rate and the population of multipotent cells are included in Section 4.2.2. Arrows indicate upregulation or transition and dashed lines with a bar indicate downregulation or inhibition. The arrows and bars are labeled with the main parameter driving the interaction.
Table 6: **List of parameters and wound attributes for the coral wound healing model.**

<table>
<thead>
<tr>
<th>Parameter $k_m$</th>
<th>Description</th>
<th>Estimated Value</th>
<th>Units</th>
</tr>
</thead>
<tbody>
<tr>
<td>Accumulation of debris and foreign matter (bacteria and algae)</td>
<td>6.2121</td>
<td>$M$-units · h$^{-1}$</td>
<td></td>
</tr>
<tr>
<td>$k_{ma}$</td>
<td>Rate of removal of debris and foreign matter by amoebocytes</td>
<td>25.7689</td>
<td>$A$-units · h$^{-1}$</td>
</tr>
<tr>
<td>$\mu_m$</td>
<td>Natural decay rate of debris and foreign matter</td>
<td>0.3294</td>
<td>h$^{-1}$</td>
</tr>
<tr>
<td>$s_c$</td>
<td>Source of mutlipotent cells that can differentiate into amoebocytes or fibroblasts</td>
<td>2.3617</td>
<td>S-units · h$^{-1}$</td>
</tr>
<tr>
<td>$k_{am}$</td>
<td>Differentiation of resting cells to amoebocytes initiated by debris and foreign matter</td>
<td>19.0942</td>
<td>$M$-units$^{-1}$·h$^{-1}$</td>
</tr>
<tr>
<td>$k_{aa}$</td>
<td>Differentiation of multipotent resting cells to amoebocytes initiated by amoebocytes</td>
<td>12.4464</td>
<td>$A$-units$^{-1}$ · h$^{-1}$</td>
</tr>
<tr>
<td>$\mu_{sc}$</td>
<td>Decay rate of multipotent resting cells</td>
<td>0.9582</td>
<td>h$^{-1}$</td>
</tr>
<tr>
<td>$k_{af}$</td>
<td>Transition of amoebocytes to fibroblasts</td>
<td>0.0643</td>
<td>h$^{-1}$</td>
</tr>
</tbody>
</table>

Continued on next page...
<table>
<thead>
<tr>
<th>Parameter</th>
<th>Description</th>
<th>Estimated Value</th>
<th>Units</th>
</tr>
</thead>
<tbody>
<tr>
<td>$M_\infty$</td>
<td>Inhibition constant of transition of amoebocytes to fibroblasts by debris and foreign matter</td>
<td>35.4939</td>
<td>$M$-units</td>
</tr>
<tr>
<td>$k_{fa}$</td>
<td>Transition of fibroblasts to amoebocytes</td>
<td>1.5449</td>
<td>h$^{-1}$</td>
</tr>
<tr>
<td>$\mu_a$</td>
<td>Natural decay rate of amoebocytes</td>
<td>11.8697</td>
<td>h$^{-1}$</td>
</tr>
<tr>
<td>$k_f$</td>
<td>Differentiation of multipotent resting cells to fibroblasts</td>
<td>0.6858</td>
<td>h$^{-1}$</td>
</tr>
<tr>
<td>$\mu_f$</td>
<td>Natural decay rate of fibroblasts</td>
<td>1.4694</td>
<td>h$^{-1}$</td>
</tr>
<tr>
<td>$k_c$</td>
<td>Rate that new tissue forms or migrates into the wound space</td>
<td>20.8144</td>
<td>$F$-units · h$^{-1}$</td>
</tr>
<tr>
<td>$x_c$</td>
<td>Hill constant for new tissue formation</td>
<td>19.6923</td>
<td>$F$-units</td>
</tr>
<tr>
<td>$p_f$</td>
<td>Proliferation rate of fibroblasts induced by the missing tissue</td>
<td>90.5915</td>
<td>$F$-units$^{-1}$ · h$^{-1}$</td>
</tr>
<tr>
<td>$k_a$</td>
<td>Baseline rate of differentiation of multipotent cells to amoebocytes</td>
<td>0.7318</td>
<td>h$^{-1}$</td>
</tr>
<tr>
<td>$C_\infty$</td>
<td>Initial wound size</td>
<td>-</td>
<td>mm$^2$</td>
</tr>
</tbody>
</table>

Continued on next page...
Table 6 – continued from previous page

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Description</th>
<th>Estimated Value</th>
<th>Units</th>
</tr>
</thead>
<tbody>
<tr>
<td>$max_{pd}$</td>
<td>Maximum distance between polyps along the shortest dimension in the initial wound</td>
<td>-</td>
<td>mm</td>
</tr>
</tbody>
</table>

Estimated parameter values were selected through Latin-hypercube sampling and an optimization routine using experimental data. Details on how parameter sampling and optimization were performed are given in Sections 4.2.4 and 4.2.5. Units for the parameter values are given as general units pertaining to each model variable where $M$-units, $A$-units, and $F$-units represents the units for debris and foreign matter ($M$), amoebocytes ($A$), and fibroblasts ($F$), respectively. The variable $C$ is fit to the experimental wound data in mm$^2$ and the time variable is in hours, $h$. 
The equation for the rate of change of the variable $M$ represents the population of foreign organisms (bacteria and algae), damaged cells, and other debris that can enter the wounded area (Equation 4.1).

\[
\frac{dM}{dt} = k_m \left(1 - \frac{C}{C_\infty}\right) - k_{ma} MA - \mu_m M
\]  
(4.1)

Debris is assumed to accumulate in the wound at a rate $k_m$. This rate is multiplied by the term \((1 - C/C_\infty)\), where $C_\infty$ is the initial size of the wound in mm$^2$. This term ensures that the accumulation rate achieves its maximum value when $C = 0$ and decreases to zero as $C$ approaches $C_\infty$. The debris, $M$, is removed by activated amoebocytes at a rate of $k_{ma}$. Following mass action kinetics, this rate is multiplied by the populations of $M$ and $A$. Since the bacteria and algae in the wound may be removed from the system in other ways (i.e. removal by other organisms or dislodging into the surrounding seawater), the foreign matter $M$ also has an intrinsic decay rate of $\mu_m$, proportional to the population of $M$.

The equation for the rate of change for the variable $A$ (Equation 4.2), representing the population of amoebocytes (immune cells observed in corals) and the equation for the rate of change for the variable $F$ (Equation 4.3), representing the population of fibroblasts are derived together due to the similarity in activation pathways.

\[
\frac{dA}{dt} = \left(\frac{s_c P_d(C) R_A(M, A)}{R_A(M, A) + R_F(F, C) + \mu_{sc}}\right) - \frac{1}{1 + \frac{M}{M_\infty}} + \frac{k_{fa} F}{k_{fa} F - \mu_a A}
\]  
(4.2)
Due to morphological consistencies, it is believed that both granular amoebocytes and fibroblasts originate from a common multipotent cell [111]. Therefore, they have similar first terms in their equations and changes in the different rates of activation in one equation affects the other. The activation term for each is derived by tracking the activation of resting multipotent stem cells ($S$) and then using a quasi-steady state assumption on this variable. We first derived the differential equation of the resting population, Equation 4.4. This equation describes the activation of multipotent cells from a resting state to the activated forms of immunological cells with activation to amoebocytes and fibroblasts, $R_A(M, A)$ and $R_F(F, C)$, respectively.

\[
dSdt = \frac{dc}{dt}P_d(C) + \frac{R_A(M, A) + R_F(F, C) + \mu_s}{1 + M/M_\infty} - \frac{k_{af}A}{1 + M/M_\infty} - \frac{k_{fa}F}{1 + M/M_\infty} - \mu_sS \]

(4.3)

where

\[
R_A(M, A) = k_a + k_{am}M + k_{aa}A \]

(4.5)

\[
R_F(F, C) = k_f + \frac{p_f F(1 - C/C_\infty)}{1 + max_{pd}(1 - C/C_\infty)} \]

(4.6)

\[
P_d(C) = \frac{1}{1 + max_{pd}(1 - C/C_\infty)} \]

(4.7)
The rate of differentiation to amoebocytes ($R_A(M,A)$, Equation 4.5) is assumed to have a small background rate, $k_a$. When an injury occurs, additional activation and recruitment occurs through interaction with damage mediators, $k_{am}$, and from mediators released by other amoebocytes, $k_{aa}$. For this model, we assume that the cellular variables encompass the cell populations as well as their respective mediators which represent chemical signals used for communication between cells. The rate of differentiation to fibroblasts ($R_F(F,C)$, Equation 4.6) is assumed to have a background constant rate of $k_f$. When a wound occurs, the rate of differentiation is increased by a rate of $p_f$. Similar to Equation 4.1, the increased differentiation to fibroblasts represented by $p_f$, decays as $C$ approaches $C_\infty$, so that $R_F(F,C)$ approaches its background rate, $k_f$, as the wound heals.

The parameter $s_c$ (Equation 4.4) is the source rate of multipotent stem cells ($S$) from the surrounding tissue; the function $P_d(C)$ (Equation 4.7) modulates the available source of cells based on the initial wound geometry. The form is an inhibitory function where the wound size acts as the direct inhibitor of access to multipotent cells. The parameter $max_{pd}$ controls the effectiveness of the remaining wound size at inhibiting cell availability. The value of $max_{pd}$ is calculated by measuring the maximum distance between viable wound perimeters along the shortest dimension. It is assumed that the wound heals from the healthy tissue inward, maintaining the geometric orientation. As $C$ approaches $C_\infty$, this function will approach a value of 1, resulting in no inhibition. The final parameter, $\mu_{sc}$ represents the intrinsic decay rate.

Initial formulations of the rate of change of the variables $A$ and $F$ would include activation of the form $R_A(M,A)S$ and $R_F(F,C)S$, respectively, requiring us to include the equation for $S$ (Equation 4.4) in the system. However, it is assumed that the rate of activation occurs very quickly, thus we apply the quasi-steady state assumption.
and assume that $\frac{dS}{dt} \approx 0$. Using this assumption, the variable $S$ can be solved for (shown in Equation 4.8) and substituted into the activation terms for the variable $S$. This substitution yields the final form given in the first terms of Equation 4.2 and Equation 4.3.

$$S = \frac{s_c P_d(C)}{R_A(M, A) + R_F(F, C) + \mu_s} \quad (4.8)$$

The remaining terms in Equation 4.2 and Equation 4.3 model the transition between the two cell types and intrinsic decay. The inclusion of cellular transitions between phenotypes was included from the inflammation model in Torres et al. [140] for classically and alternatively activated macrophages. Similar to the multipotent cell population found in corals, the differentially activated macrophage cells originate from multipotent monocytes and are assumed to be able to switch between phenotypes based on the potency of cellular mediators at the wound site. Therefore, we used the same modeling approach for the amoebocytes and fibroblasts in the second and third terms in each equation, Equation 4.2 and Equation 4.3, respectively. The presence of debris and foreign matter signal a need for amoebocytes and inhibit the transition to fibroblasts. This is modeled by the function $1/(1 + M/M_\infty)$ which reduces the rate of transition from amoebocytes to fibroblasts, $k_{af}$, nonlinearly such that $M_\infty$ controls the effectiveness of the $M$ variable at inhibiting this process. $k_{fa}$ is the transition rate for fibroblasts becoming amoebocytes and currently little is known about this process. The intrinsic decay of amoebocytes and fibroblasts have rates $\mu_a$ and $\mu_f$, respectively.

The final equation, (Equation 4.9), represents the rate of change in new tissue, mainly epidermal and gastrodermal cells, through proliferation and migration into the wound space. This process is assumed to be mediated by fibroblasts. For wound heal-
ing in humans and other vertebrates, fibroblasts are involved in laying down the extracellular matrix as well as collagen production that is needed for re-epithelialization \cite{150, 151, 152, 153}. Palmer et al. \cite{111} found fibroblasts were similarly involved in re-epithelialization as well as collagen-like deposition in the imperforate coral species \textit{P. cylindrica}, and thus this process has been applied to the present model.

\[
\frac{dC}{dt} = \frac{k_c F}{x_c + F} \left( 1 - \frac{C}{C_\infty} \right) \tag{4.9}
\]

The first multiplier in the term is a Michaelis-Menten type term with a maximum rate of \(k_c\) and a half-max of \(x_c\). The second multiplier causes this rate to decay as \(C\) approaches \(C_\infty\) and models the contact inhibition signal that the fibroblasts would receive as the wound is closed.

4.2.3 Steady state

The system has only one equilibrium point, which corresponds to the healed state when \(C = C_\infty\). The full equilibrium point is \([0, A^*, F^*, C_\infty]\), where \(A^*\) and \(F^*\) are positive values given by Eqs. 4.10 - 4.12. Calculation of the equilibrium point is given in \cite{111}

\[
A^* = \frac{-b - \sqrt{b^2 - 4ac}}{2a} \tag{4.10}
\]

where

\[
a = -k_{aa}((k_{fa} + \mu_f)(k_{af} + \mu_a) - k_{fa}k_{af})
\]

\[
b = (-k_{fa} + \mu_f)((\mu_{sc} + k_a + k_f)(k_{af} + \mu_a) - s_c k_{aa}) + k_{fa}k_{af}(\mu_{sc} + k_a + k_f)) \tag{4.11}
\]

\[
c = s_c(k_a(k_{fa} + \mu_f) + k_{fa}k_f)
\]
\[ F^* = \frac{s_c k_f}{(\mu_s + k_a + k_{aa} A + k_f)(\mu_f + k_{fa})} + \frac{k_{af} A^*}{\mu_f + k_{fa}} \] (4.12)

Steady state for the variables \(A\) and \(F\) were used to select plausible parameter sets since we assume that the amount of amoebocytes in healthy tissue is less than or equal to the amount of fibroblasts. For the baseline parameter values given in Table 6, the steady state values for \(A\) and \(F\) were \(A = F = 0.1353\).

### 4.2.4 Parameter sampling to determine initial parameter set for optimization

To sample the large parameter space, Latin hypercube sampling (LHS) was used. LHS is a Monte-Carlo sampling method that can accurately capture the full parameter space with fewer samples than simple random sampling [91]. LHS uses stratified sampling without replacement where the random parameter distributions are divided into \(N\) equal probability intervals. It is recommended that \(N\) be at least the number of parameters plus one, but a much larger number is often chosen. For this model 100,000 samples were used. A parameter range and probability distribution is also defined for each parameter. Since little is known of the distribution and range for each parameter, a uniform distribution on the interval \([0,100]\) was used for each. The resulting parameter sets were then simulated to analyze the resulting model outputs. Parameter sets were simulated from the initial condition \([M, A, F, C] = [0,0,0,0]\) since no tissue remains in the wounded area.

The experimental data from healing wounds were used to assess resulting model outputs and select plausible parameter sets. The ranges used for model assessment were defined from the experimental wound area data within each experimental group (linear and circular wounds) over the experimental time window in order to determine...
acceptable dynamics for the $C$ variable. Parameter sets were initially considered plausible if the associated model output for variable $C$ fell within the range of the experimental data for either group at any collected time point. The parameter set that fell within these ranges most often in both experimental groups was used as the initial parameter set for the optimization method.

Fig. 18. **Raw wound healing data for the linear (A) and circular (B) wounds.** Wounds were created in a circular or linear shape at either the base (blue), middle (red) or top (yellow) of the fragment. Wound closure was measured by overall wound area using image analysis at various time points. On average, wounds healed within 800-900 hours. One wound (plot B, red) did not heal in the recorded time. This wound appeared to be deeper than the other wounds and was excluded from parameter estimation.

### 4.2.5 Parameter estimation with sensitivity analysis

A parameter set was chosen to fit both the linear and circular wound simultaneously with only the parameters related to the initial wound size varying ($C_\infty$ and $max_{pd}$). Parameter estimation was performed using `fmincon` in MATLAB (R2021a)\[42\] to optimize the parameter set. Again, the linear and circular data were both fit simultaneously using a scaled average of the data (shown in Figure\[18\]) for each (linear
or circular) and optimized using the residual sum of squares (RSS) as the objective function. The default tolerances for the objective function and the parameter vector were used to determine solution convergence.

The variables \(A\) and \(F\) were also assessed to ensure that \(A(0), F(0) < 10\) and \(A(0) < F(0)\). The model is constructed so that \(A\) and \(F\) are non-zero at steady state, but it is assumed that these numbers are small at baseline. However, units for these equations are relative levels of cellular units, so the choice of small numbers is quantitatively arbitrary. It is also assumed that the number of amoebocytes is smaller than the number of fibroblasts at time zero. Amoebocyte and fibroblast ratios at steady state reflect known dynamics between fibroblasts and inflammatory cells in humans and other vertebrates as well as unpublished observations in the coral tissue.

Local sensitivity analysis was applied using the optimized parameter set. The partial derivatives with respect to each parameter were calculated, utilizing the one-at-a-time sensitivity method. This was performed for both the linear and circular initial conditions. The partial derivatives used a full scale normalization method. The sensitivity values were then aggregated for each parameter using the root mean square (RMS). This calculation is described in Equation \(4.13\) where \(\delta_j\) is the sensitivity value for each parameter \(j\), \(\frac{\partial y_i}{\partial p_j}\) is the time dependent partial derivative, \(y_i\) is the value of the variable \(C\) at each time point \(i\), \(p_j\) is the value of each parameter \(j\), and \(n\) is the total number of time points (as described in [154]). These values were then scaled by the maximum sensitivity index such that \(0 \leq \delta_j \leq 1\).

\[
\delta_j = \sqrt{\frac{1}{n} \sum^n_i \left(\frac{p_j \partial y_i}{y_i \partial p_j}\right)^2}
\]  

(4.13)

Sensitivity values less than 0.05 were classified as insensitive and fixed at their
nominal values. A reduced sensitivity matrix was formed from the remaining parameters. The reduced sensitivity matrix was used to find an identifiable set of parameters. For this method, pairwise correlations and the collinearity index of the various sets is used to identify the optimal set. The set with the lowest collinearity index which included relevant parameters was chosen as the identifiable set. This set was then used to further optimize the parameter estimates and explore other possible behavior of the model.

4.3 Results

4.3.1 Sensitivity analysis & identifiable set

Sensitivity values are plotted in Figure 19. Any parameter with $\delta_j < 0.05$ was considered insensitive, resulting in four parameters considered to be insensitive in the model. These parameters were $mu_{sc}$, the decay rate of multipotent cells, $k_{aa}$, the activation rate of amoebocytes by other amoebocytes, $k_u$, the background activation rate of amoebocytes, and $M_\infty$, a parameter controlling the effectiveness of debris and foreign matter at inhibiting the switch of amoebocytes to fibroblasts. These parameters were excluded from further calculations. Removing these parameters yielded a reduced sensitivity matrix consisting of the remaining thirteen parameters.
Fig. 19. **Normalized sensitivity values from local sensitivity analysis on the wound healing model.** Sensitivity values were calculated for the variable $C$ at each time point for the linear and circular wounds. The plotted value is the root mean square (RMS) of all the time points for each wound (described in Equation 4.13). The value was then normalized to the maximum observed sensitivity value.

The reduced sensitivity matrix ($S_{n \times p}$), where $p$ is the total number of parameters and $n$ is the total number of time points, was used to find the identifiable set. Pairwise correlations were calculated iteratively where highly correlated parameters (correlation coefficient greater than 0.95) were removed at each step. The resulting set had four parameters remaining, suggesting an identifiable set of size four.

Another measure of correlation between parameters is the collinearity index described in Brun et al. [154]. The value is given by $CI = 1/\sqrt{\lambda_k}$, where $\lambda_k$ is the largest eigenvalue of the matrix $\tilde{S}_{n \times p}^T \tilde{S}_{n \times p}^*$ for $p^* \leq p$ and $\tilde{S}_{n \times p}^*$ is a subset of the sensitivity matrix $S_{n \times p}$. The collinearity index is an additional measure of how changes in a parameter propagate to changes in the model output. In general, the change in model output caused by a change in a parameter $p_j$ can be compensated for
by other parameters up to $\frac{1}{e}$. Following past researchers, a cutoff of $\text{CI} = 20$ was used to select subsets of parameters with low collinearity. Since the pairwise correlation algorithm selected a set of size four, we calculated the CI values for all combinations of parameters of size four. The rank of the Fisher Information Matrix (FIM), calculated from the reduced sensitivity matrix where $\text{FIM} = \left(\bar{S}^*_{nxp}\right)^T\left(\bar{S}^*_{nxp}\right)$, can also help guide the selection of the size of the identifiable set. The FIM was full rank (all non-zero eigenvalues), but the condition number was large ($>10^{11}$), suggesting that some of the eigenvalues may not actually be non-zero and can be attributed to accumulation of numerical error. The value of the eigenvalues decays significantly after the first four. The remaining eigenvalues do not change significantly in value and are near-zero. A plot of the eigenvalues on a log scale is shown in Figure 4.3.1. This further supports an identifiable set of size four, since the rank for the FIM can be assumed to be four.
Fig. 20. **Eigenvalues for the Fisher Information Matrix (Log scale).** The rank of the Fisher Information Matrix can help determine the size of the identifiable set. The exact matrix was full rank (all non-zero eigenvalues), but the condition number was 1.7069\(^{11}\) indicating that some of these eigenvalues may not truly be non-zero. The value of each eigenvalue decays significantly after the fourth eigenvalue, suggesting the true rank may be close to 4.

All possible sets of size four from the remaining thirteen parameters were calculated and ranked from smallest to largest by the value of CI. Potential sets were further reduced by excluding sets where any pairwise parameter set had a correlation coefficient larger than 0.95. The RMS of the sensitivity values for each parameter set of size four was also calculated since past works suggest minimizing CI while simultaneously maximizing the RMS \(^{155}\). Table 7 contains parameter combinations of the 20 smallest CI values which have pairwise correlation coefficients less than 0.95. The maximum RMS value across all sets was 9.8512, so we can select a set closer to this RMS value while only slightly compromising on the CI value. (shown in Table 7).

The set selected as the identifiable set (indicated in bold in Table 7) contained \(s_c\), source rate of multipotent cells that can differentiate to amoebocytes and fibroblasts, \(k_m\), the accumulation rate of debris and foreign matter in the open wounded space,
$k_{ma}$, the rate of removal of debris and foreign matter by amoebocytes, and $k_c$, the rate that new tissue forms in the wound space. This set had a CI of 0.5726 and RMS of 8.0347. This set also had no decay rates, which are unlikely to vary significantly across individual organisms.
Table 7. **Table of selected parameter sets of size four where the collinearity index is less than 20 and pairwise correlation coefficients are less than 0.95.** This table shows 20 parameter combinations with the largest RMS value. The identifiable set and corresponding CI value and RMS value are indicated in bold text.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>$CI = 1/\sqrt{\lambda_k}$</th>
<th>RMS</th>
</tr>
</thead>
<tbody>
<tr>
<td>$k_{am}$, $k_{ma}$, $p_f$, $\mu_f$</td>
<td>0.5352</td>
<td>3.8768</td>
</tr>
<tr>
<td>$k_m$, $k_{ma}$, $p_f$, $\mu_f$</td>
<td>0.5353</td>
<td>6.3830</td>
</tr>
<tr>
<td>$k_{am}$, $k_{ma}$, $\mu_f$, $k_f$</td>
<td>0.5439</td>
<td>4.0790</td>
</tr>
<tr>
<td>$k_m$, $k_{ma}$, $\mu_f$, $k_f$</td>
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<td>6.5852</td>
</tr>
<tr>
<td>$k_m$, $\mu_a$, $\mu_f$, $k_f$</td>
<td>0.5483</td>
<td>5.3289</td>
</tr>
<tr>
<td>$k_{am}$, $\mu_a$, $\mu_f$, $k_f$</td>
<td>0.5498</td>
<td>2.8226</td>
</tr>
<tr>
<td>$k_m$, $\mu_a$, $\mu_f$, $x_c$</td>
<td>0.5575</td>
<td>5.1514</td>
</tr>
<tr>
<td>$k_m$, $\mu_a$, $\mu_f$, $k_c$</td>
<td>0.5577</td>
<td>5.1858</td>
</tr>
<tr>
<td>$k_{am}$, $k_{ma}$, $\mu_f$, $k_{af}$</td>
<td>0.5585</td>
<td>5.1098</td>
</tr>
<tr>
<td>$k_{am}$, $\mu_a$, $\mu_f$, $x_c$</td>
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<td>2.6451</td>
</tr>
<tr>
<td>$k_{am}$, $\mu_a$, $\mu_f$, $k_c$</td>
<td>0.5643</td>
<td>2.6796</td>
</tr>
<tr>
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<td>0.5702</td>
<td>7.6161</td>
</tr>
<tr>
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<td>8.0003</td>
</tr>
<tr>
<td>$s_c$, $k_m$, $k_{ma}$, $k_c$</td>
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<td><strong>8.0347</strong></td>
</tr>
<tr>
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<td>5.1267</td>
</tr>
<tr>
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<td>3.9014</td>
</tr>
<tr>
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<td>3.9359</td>
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<tr>
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<td>6.4422</td>
</tr>
<tr>
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<td>0.5830</td>
<td>2.6205</td>
</tr>
</tbody>
</table>
4.3.2 Final parameter set

The final fit obtained from estimating only the parameters in identifiable set and fixing other parameters at their baseline value identified in the initial optimization routine is plotted in Figure 21 with residuals plotted in Figure 22. Numerical values for the final parameter set are in Table 6. Recall that the two simulations only differed in the initial wound size \( (C_\infty) \) and maximum distance between polyps \( (max_{pd}) \). The circular wound was over double the total size of the linear wound, but had a smaller value for \( max_{pd} \) so the wounds healed at about the same time and the variable \( C \) exhibits a similar shape for both.

![Figure 21](image)

**Fig. 21.** Parameter estimation for linear and circular wounds. Both plots use the same parameter values (listed in Table 6) and only differ in the value of \( C_\infty \), the initial wound size (mm\(^2\)) and \( max_{pd} \), the maximum distance between polyps along the shortest dimension (mm). The data plotted here is the inverse of the mean data plotted in Figure 18. The linear wound (A) has a mean square error of 0.1570 and the circular wound (B) has a mean square error of 1.3686.
Fig. 22. **Residuals for the circular and linear wound data.** The optimization routine was run using the summed residual squared error for both data sets as the objective function.

Figure 23 plots the different variables overlayed with each wound type. The variable representing new tissue in the wound space (C, Figure 23A) is normalized to show the progression of the variable over time on a comparable scale for each wound type. As shown in the plot, the transients look very similar with some difference in the rate of healing visible around 400-700 hours where the circular wound exhibits accelerated healing compared to the linear wound. The debris variable (M, Figure 23C) has similar trends for both wounds, with a slightly prolonged presence and larger magnitude in the linear wound between 400-700 hours. This correlates with the accelerated rate of epithelial growth for the circular wound, shown in plot A. The variables A and F, Figure 23C and 23D, respectively, show increased values for the circular wound and delayed peaks for the linear wound. An increased inflammatory response in the circular wound is expected due to the larger size of the wound. The slightly delayed inflammatory response in the linear wound correlates with the
slightly prolonged presence of debris observed in Figure 23B.

Fig. 23. **Plot of model transients for both the linear (solid line) and circular (dashed line) wounds.** Plot A shows the variable $C$ which has been normalized to show comparisons between the differently sized wounds. Plot B shows the variable $M$. Plot C shows the variable $A$. Plot D shows the variable $F$. The variables $A$ and $F$ are plotted in the same column to show the timing in the onset of the inflammatory phase (plot C) and the proliferation phase (plot D).

### 4.3.3 Varying model parameters

Other model simulations were performed by varying different parameters to demonstrate the model’s capacity for diverse behavior. Initial wound conditions were explored first by varying the values for $C_{\infty}$, the initial wound size (mm$^2$) and $max_{pd}$, the maximum distance between polyps in the initial wound, calculated on the shortest dimension (mm). As noted in the model development and in the experimental
results, both values influence healing times.

Various values for $C_{\infty}$ and $max_{pd}$ are plotted in Figure 24 and points are colored by the simulated healing time in hours. As expected, longer healing times were observed for larger wound sizes. At the smallest polyp distance (0.05 mm), healing times ranged from 21 hours for the smallest wound (1 mm$^2$) to 582 hours for the largest wound (30 mm$^2$). Similar trends were observed throughout the range for polyp distance. Differing polyp distance at constant wound size also drastically changed the healing time. At the largest wound size (30 mm$^2$), the healing time ranged from 582 hours for the smallest polyp distance (0.1 mm) to over 2,000 hours for the largest polyp distance (3.05 mm).

**Fig. 24.** Healing times (in hours) for varying initial wound size ($C_{\infty}$, in mm$^2$) and maximum distance between polyps in the initial wound, calculated on the shortest dimension ($max_{pd}$, in mm). Model estimates for heal time are colored on a gradient indicated by the scale to the right of the plot (in hours). The two large markers represent the initial wound size and maximum distance between polyps, measured along the shortest dimension, for the linear wound (♦) and circular wound (×).
Another mechanism of interest is debris accumulation and removal. The main parameters involved are $k_m$ (rate of debris accumulation) and $k_{ma}$ (rate of debris removal by amoebocytes). Transients for each variable with varying values for these parameters are presented in Figure 25. The values for the initial wound size ($C_\infty$) and maximum distance between polyps on the shortest dimension ($max_{pd}$) are set to the values associated with the linear wound.

![Fig. 25](image)

**Fig. 25. Modulating parameters involved in debris and foreign matter.** The plots show the model results of varying the parameters $k_m$, accumulation/growth rate of debris and foreign matter, and $k_{am}$ removal of debris and foreign matter by amoebocytes. A 50% decrease or 50% increase was used for each parameter. Plot A shows transients for the new tissue formation ($C$), plot B shows transients for debris and foreign matter ($M$), plot C shows transients for amoebocytes ($A$), and plot D shows transients for fibroblasts ($F$).

The accumulation term involves a simple multiplication by the parameter ($k_m$) and the percent of open wound space ($1 - C/C_\infty$) in the equation for the rate of
change of debris and foreign matter ($M$). Thus, the observed effects on the variables are generally very straightforward. As observed by the variable for new tissue (Figure 25A), a decrease in $k_m$ produces a faster healing wound and an increase produces a slower healing wound. For the debris variable (Figure 25B), a decrease in $k_m$ results in a smaller amount of debris over a shorter period of time and an increase in $k_m$ results in a larger amount of debris over a longer period of time compared to the baseline. For amoebocytes (Figure 25C) a decrease in $k_m$ produces an earlier peak and an increase produces a later peak. For fibroblasts (Figure 25D), transients produced varying results. A decrease in $k_m$ produced a fast healing wound, thus there was a large, quick peak in $F$. The total number of $F$-units over the course of healing was slightly larger than baseline. An increase in $k_m$ produces an extremely delayed peak in the variable $F$, that does not rise above the background value in the tissue. Despite this, the number of $F$-units during healing is similar to those required for the baseline case, just recruited over a longer period of time.

The removal parameter ($k_{ma}$), also produced similarly expected results. A decrease in this parameter produced a slightly faster healing wound and an increase produced a slower healing wound (Figure 25A). A decrease in $k_{ma}$ produced a larger value and prolonged resolution of the variable $M$ while an increase produced a smaller, shorter resolution (Figure 25B). These correlate with the slower healing wound and the faster healing wound, respectively. For the variable $A$ (Figure 25C), a decrease in $k_{ma}$ produced delayed peak, while an increase produced an earlier peak than the baseline value. For the variable $F$ (Figure 25D), a decrease in $k_{ma}$ produced a delayed, but smaller peak and an increase produced an earlier but larger peak than the baseline value. Again, both of these results correlate with the transients shown in Figure 25A and 25B.

Finally, the immune cell response was explored by varying the source rate of stem
cells available to differentiate into amoebocytes or fibroblasts, $s_c$. This parameter had the highest sensitivity index, meaning it has the potential for inducing significant variations in model output. This also drives availability of immune cell precursors, driving subsequent levels of immune cells in the healed tissue. Transients for the four variables are plotted in Figure 26. Again, the results are straightforward where a decrease in $s_c$ produces a slower healing wound (Figure 26A), a larger peak and delayed resolution of $M$ (Figure 26B), a delayed peak in $A$ (Figure 26C), and a smaller, delayed response in $F$ that does not exceed the background value during the course of healing (Figure 26D). Conversely, an increase in this parameter produces a slightly faster healing wound (Figure 26A), a slightly quicker resolution of $M$ (Figure 26B), an earlier peak in $A$ (Figure 26C), and a larger, earlier peak in $F$ (Figure 26D). Even a 10% decrease in this parameter produces the same behavior observed in fibroblasts ($F$) shown in Figure 25 with a 50% increase in $k_m$, the accumulation rate of debris. The value of $F$ remains below the background value during the course of healing. This is likely due to the parameter $k_{fa}$, which allows available fibroblasts to transition to amoebocytes if needed to remove debris and foreign organisms. For both of these transients, a prolonged presence of debris is observed, prompting the need for an extended amoebocyte response.
Fig. 26. Modulating the source of multipotent cells that can differentiate into amoebocytes or fibroblasts. The parameter $s_c$ was varied using either a 10% decrease or 10% increase. Plot A shows transients for new tissue formation ($C$), plot B shows transients for debris and foreign matter ($M$), plot C shows transients for amoebocytes ($A$), and plot D shows transients for fibroblasts ($F$).

4.4 Discussion

The exact mechanisms that reef-building corals utilize in response to a wounding event are relatively understudied. Using the process observed experimentally in some Cnidarians and known immune interactions in humans and other vertebrates, we formulated a mechanistic mathematical model for the immune response to a wounding event in reef-building corals. Immunological responses have been observed in Cnidarian species such as *P. cylindrica* [111] that parallel those observed in humans and other vertebrates including phagocytic cells involved in cellular lysis of bacterial organisms and other wound debris as well as fibroblasts involved in re-epithelialization.
and collagen deposition at the wound site. Many mathematical models have been created for the inflammatory response and early fibroblast activity in humans and other vertebrates \[137, 136, 138, 140, 139\], lending a framework for a model in corals. These past models combined with the immunological observations in \textit{P. cylindrica} led to the formation of the present model.

Experimental data were collected for wound healing in a \textit{P. damicornis} fragment by observing the wound area over time (Figure 18) at various locations. This data was used to fit model transients for wound closure (Figure 21) and obtain plausible parameter estimates. The present data offers only polyp-level observations of the healing tissue and does not include cellular population counts for amoebocytes, fibroblasts, and levels of debris and foreign organisms. Thus the remaining model variables relied on known dynamics categorized in humans and other vertebrates and qualitative observations in \textit{P. cylindrica} \[111\]. There are some notable differences between \textit{P. cylindrica} and \textit{P. damicornis} that will influence tissue regeneration overall and the reinitialization of biomineralization (growth), however very little data is available on coral tissue regeneration and the model for \textit{P. cylindrica} developed by Palmer \textit{et al.} \[111\] is the only study detailed enough to be used. Additionally, \textit{P. cylindrica} appears to produce more mucus than \textit{P. damicornis} in a laboratory setting (based on our observations) so we did not visually observe a mucus plug as was seen in \textit{P. cylindrica}; although, \textit{P. damicornis} has been observed to form an algal/sand plug in field experiments \[106\]. Sand was not present in our laboratory tanks, and algal levels were likely not large enough to colonize the wounded area, as has been observed in field experiments. To build the model, some initial assumptions had to be made, such as comparisons to \textit{P. cylindrica} and vertebrates, and we expect to keep refining these as our work progresses.

To parameterize the model, we used experimental data, local sensitivity analysis,
and collinearity indices. The sensitivity analysis was performed for the fitted variable, $C$, and indicated that the model variable was sensitive to thirteen of the seventeen total parameters (Figure 19). Sensitivity results indicated that the model is highly sensitive to parameters involved in inflammation and proliferation as well as removal of debris and foreign organisms. The matrix rank of the sensitivity matrix as well as other measures suggested an identifiable set of size four. The identifiable set of influential parameters was ultimately chosen by minimizing the CI and maximizing the RMS. This ensures that the parameters were both minimally correlated and maximally influential in determining model output. The final identifiable set was chosen containing the parameters $s_c$, the source rate of multipotent cells that can differentiate into amoebocytes or fibroblasts, $k_m$, the rate of accumulation of debris and foreign matter, $k_{ma}$, the rate of removal of debris and foreign matter by amoebocytes, and $k_c$, the rate at which new tissue forms in the wound space. These parameters are all related to amoebocytes and fibroblasts, supporting the importance of these cellular populations in the healing process.

The identifiable set was used to run a reduced estimation of the model parameters and define the final parameter set. Simulations produced transients that fit the experimental data with low residual values, demonstrating the model’s ability to capture the behavior of the healing tissue. Transients of the variables for amoebocytes and fibroblasts mirrored temporal trends in *P. cylindrica* (Figure 23). Additional numerical simulations demonstrated diverse model behavior for relevant parameter variations (Figs. 25 & 26). The model was simulated for various initial wound sizes and maximum distance between polyps in the initial wound, providing a measure for differences in wound geometry (Figure 24). Results demonstrated the interaction between these values in affecting wound healing times.

Parameters involved in debris dynamics and immune cell precursors were also
explored through numerical simulation. Increased debris accumulation can indicate competitive algal overgrowth (from seawater or the underlying skeleton for endolithic algae) in the wounded area or an aggressive bacterial or fungal infection. Variation in the debris removal parameter demonstrates variation in amoebocyte effectiveness at removing certain infiltrates. Variation in the availability of multipotent cells that can differentiate to amoebocytes or fibroblasts may also occur in the natural environment since immune cell processes require cellular energy\cite{156}. Various environmental stressors may affect energy availability in corals, resulting in varying availability and effectiveness of immunological cells.

The experimental results presented here indicate healing trends for *P. damicornis* fragments in a laboratory setting. However, the process of photographing the wounds each day involved removing the coral fragment from the tank and photographing the wounds in open air. This significantly disturbs the polyps and stresses the healing tissue, akin to scraping off a scab. Thus, it is assumed that the present data represents a delayed timeline of healing. Alternative methods for monitoring the wound size during the experiment are currently being tested to reduce disruption and provide more reasonable estimates for healing trends in corals. Despite this, the data does provide an expected timeline for a disturbed fragment that the mathematical model was able to reproduce. Healing times are likely also extended due to the fact that all six wounds were placed on a single fragment.

Additional experiments will need to be performed for other model variables including phagocytic amoebocytes and fibroblasts, which have not yet been observed empirically during healing in scleractinians. Small tissue samples were collected and viewed under the microscope, but reliable counts or proportions in healthy and wounded tissue have yet to be gathered. In the present model, findings published for *P. cylindrica* \cite{111} were used; however, morphological differences may contribute to
model imprecision. One distinction between coral species involves the organization of the tissue relative to the skeleton: perforate versus imperforate. Perforate corals (e.g. massive *Porites* spp.) have an extensive gastrovascular canal system which allows them to have a large fluid volume for buffering and colony-wide exchanges for chemical signaling. The gastrovascular canal system extends into the skeleton. Imperforate corals on the other hand, do not. The tissue simply covers the surface of the skeleton and polyps are only connected to their nearest neighbor through a single gastrovascular canal. Our observations of the two species type in a laboratory setting indicated that healing was much more efficient in *P. damicornis*, an imperforate coral, compared to *P. cylindrica*, a perforate coral (visual observations only, data was not presented here) for similar wound types (severed branch tip). Thus, deep injuries seem to affect perforate corals more than imperforate corals. Additionally, polyps farther from the injury in imperforate corals may be unaffected by the event compared to perforate corals due to the aforementioned anatomy differences. Further research would need to be performed to understand the differences in healing between the two types of corals. The fact that *P. damicornis* is imperforate may also have been beneficial for the present experimental methods since, hypothetically, wounds placed on the same fragment should not interact significantly. However, this does not account for byproducts of phagocytosis that may leech into the local environment or the physiological stress resultant from loss of healthy tissue.

In addition to skeletal structure, wound geometry also affects wound healing behavior. Despite attempting to capture variations in wound shape with a simple parameter (*max*$_{pd}$) this only accounts for two-dimensional differences in wound types. The extent and severity of injury can also vary depending on the wound depth. In the natural environment, corals frequently sustain wounds of varying shapes and depths. Some predatory fish may only scrape the surface, creating a shallow wound, while
others may break off pieces, creating a deeper wound. The present model does not account for depth of the wound which may be a crucial component, especially in perforate corals due to the underlying polyp structure. One of the circular wounds applied during our experiment did not heal in the observed time frame, and it is postulated that this is due to the larger wound depth. Future models will need to account for wound attributes in a three-dimensional space.

The present model also does not account for environmental factors such as other symbiotic partners of the coral microbiome. Tropical-subtropical reef-building corals are holobionts, consisting of the coral host, the endosymbiotic dinoflagellates (Family Symbiodiniaceae) and associated microorganisms including bacteria, archaea, fungi, viruses, and protists [157, 158]. Scientists are just beginning to understand how these microorganisms contribute to overall reef health. Competitive microorganisms also exist in the natural environment mainly in the form of endolithic algae. Endolithic algae competes with the coral soft tissue for space on the skeleton and can even grow over a wound or cause tissue recession in some cases [159]. Any tissue break can result in algal infiltration in the skeleton that will spread to the surface as it competes for sunlight. Although it is impossible to model all of the complexities involved in the reef ecosystem, the inclusion of other micro-organisms and endolithic algae are important for gauging how wound healing would occur in the natural habitat.

Environmental perturbations, such as increased water temperatures, should also be considered in future models as many stressors can disrupt the relationship with the symbiotic dinoflagellate algae. Disruption of this relationship (breakdown of symbiosis, also known as coral bleaching), results in decreased access to cellular energy and increased risk of mortality. Reduced bioavailability of energy can also affect other homeostatic processes such as wound healing if these events occur concomitantly. Bonesso et al. [160] demonstrated that increased temperatures, below bleaching lev-
els, decreased the corals’ (*Acropora aspera*, perforate) ability to recover from damage. Heat stress alone has also been observed to induce tissue loss in *Acropora hyacinthus* [161].

The present model will help integrate wound healing into ongoing coral research and act as a foundational model for wound healing in reef-building corals. Future iterations of the model will focus on incorporating environmental factors that may affect wound healing rate and efficacy. Despite the frequency of wounding effects sustained on reefs, wound healing has frequently been omitted from coral restoration and management efforts. Future models will be able to predict expected recovery times after major wounding events such as tropical storms and damage from large vessels. Better understanding of the intricacies of the immune response can even help optimize purposeful wounding involved in microfragmentation. Math models of this kind will be able to inform the frequency of wounding events, the amount of wounded tissue, and potentially other environmental factors that will result in the greatest success of fragment survival. The model formulated here will support the development of these future models, resulting in a better understanding of coral healing dynamics and advancements in restoration practices.
CHAPTER 5

CORAL WOUND HEALING UNDER STRESS OR LINKED WITH ENERGY MODEL

5.1 Introduction

Climate change is negatively affecting coral reefs resulting in long periods of stress and even death of the world’s networks of coral reefs \[162\]. The most evident effect of climate change and thermal stress is the occurrence of bleaching events. Warm-water tropical corals are mixotrophic organisms receiving as much as 95% of their energy from a photosynthetic endosymbiotic algae of the genus Symbiodiniceae \[163\]. The environmental stress from climate change can sever this relationship resulting in a bleaching event where the symbiotic algae are removed from the coral tissue and the organism is left to reveal its stark white skeleton through translucent tissue \[164, 165\]. These bleaching events result in stressed corals with reduced energy reserves.

Environmental stress has been shown to influence the rate of healing and regeneration \[166\]. Bonesso et. al \[160\] demonstrated that increased temperatures decreased the ability to recover from damage in \textit{A. aspera}. Heat stress alone has also been observed to induce tissue loss in \textit{A. hyacinthus} \[161\]. Burmester et. al investigated the effects of symbiont density along with heat stress to attempt to sparse the exact cause of heat related healing disruption \[167\]. Researchers concluded that the positive effects associated with symbiont density could be attributed to the higher energy reserves and tissue content at the time of wounding, added energy availability during healing due to the active photosynthesis, or potentially their direct contribution to the healing pathway, noted in Fine et. al \[168\]. The effects of major bleaching events
may even extend after recovery due to the reduced energy reserves \[169\]. Thus the
effects of bleaching events and diminished energy on healing are extensive.

Given that access to and allocation of energy is of great concern during bleaching
events, Cunning \textit{et. al} developed a dynamic energy budget (DEB) model for coral
host growth and symbiont growth based on relevant external inputs \[3\]. The DEB
model focuses on shared elements between the coral host and the symbiont, namely,
carbon and nitrogen. The symbiont fixes carbon through photosynthesis and receives
nitrogen from the host. The host acquires nitrogen through the environment from
prey and dissolved inorganic nitrogen (DIN) in the environment and receives carbon
from the symbiont. Both organisms use the carbon and nitrogen building blocks to
synthesize biomass described in the model as synthesizing units (SUs). The model
contains only two state variables, the symbiont biomass and the coral biomass, and
a large amount of flux rates and associated parameters describing the exchange and
synthesis of biomaterials. The model uses the light source and prey availability as
inputs to model environmental stressors on the process of biomass synthesis. The pop-
ulation of symbiont also allows for the simulation of a bleaching event by decreasing
the population within the model.

To explore the effects of environmental stress and bleaching events on wound
healing, we first explore fitting our initial wound healing model to experimental data of
tissue repair under stressed conditions. This will allow us to identify parameters in the
wound healing model that produce results associated with healing under stress. We
then combine the wound healing model with the DEB model formulated in Cunning
\textit{et. al} \[3\] to model how energy is allocated during a wounding event. Past studies
have noted that growth and regeneration related to wound healing may compete,
particularly during times of stress \[170\]. Thus, the coupling of the energy model
with the wound healing model will allow us to explore growth and healing dynamics
associated with normal and stressed environmental conditions.

5.2 Coral wound healing under stress

5.2.1 Methods

We pair the wound healing model formulated in Chapter 4 with experimental data for wound healing in *P. damicornis* fragments under various environmental conditions. We refit the wound healing model for the control data and then fit modified parameter values to the data for wound healing during heat stress.

5.2.1.1 Experimental data

*P. damicornis* fragments were used to procure experimental wound healing data under various conditions. Wounds were inflicted by clipping off small branches, revealing a bare skeleton wound. Fragments were kept in tanks filled with artificial seawater made using FritzPRO Reef Pro Mix Salt and reverse osmosis deionized water. Artificial light was provided by Aqua Illumination Prime™ 16HD LED light (light-dark cycle: 10h/14h). To measure wound area, fragments were photographed with a scale each day using a macro-lens attachment on an iPhone 8 cellular device. ImageJ software was used to estimate the wound size at each time point from the resulting images.

Experiments were performed for two water temperatures of 25°C, average temperature, and 28°C, moderate heat stress. Plots of the data for each experimental setting are shown in Figure 27.
Fig. 27. **Wound healing data from selected experiments.** Plot A shows wound healing data for two of the control experiments (25° C). Plot B shows wound healing data for a heat stress experiment (28° C).

### 5.2.1.2 Parameter sampling & estimation

Parameter sampling was performed using LHS. Methods for LHS are explained in Sections 3.2.2.2 and 4.2.4. Parameter sets for LHS were sampled using a range of [0,100] and a uniform sampling distribution for each parameter. A sample size of 100,000 parameter sets was used. Parameter sets were then simulated using the analytical steady state calculated for the wound healing model in Section 4.2.3. Model outputs were compared to experimental data from the control wound healing experiments described in Section 5.2.1.1 (25°C, average temperature). Parameter sets were selected if they fit the majority of the data points across all experimental fragments; thus parameter sets were selected if they fit at least 16 of the 31 total data points (across 6 fragments in total).

Sensitivity analysis was then performed on the accepted sets from the initial simulation to identify the most influential parameters driving model behavior. The specific sensitivity analysis methods used are described in Section 5.2.1.3. The results of the sensitivity analysis were used to select parameters to modulate to fit the heat
stress experimental data. Heat stress is generally assumed to increase reaction rates and healing rates, so we fit the heat stress experiment using a positive addition to each parameter identified as highly sensitive from the sensitivity analysis. Thus, parameter values for the heat stressed experiments were modified to \( p_i + c_i \) where \( p_i \) is the parameter value fit to the control data and \( c_i > 0 \). Both the initial parameter value and the positive constant were estimated using the methods explained in Section 4.2.5 for \texttt{fmincon} in MATLAB to minimize the SSE between the model and the data. A total of six fragments from the control experiments and three fragments from a moderate heat stress experiment were fit simultaneously.

5.2.1.3 Sensitivity analysis

Sensitivity analysis was performed on the accepted parameter sets from the initial LHS simulations to identify influential parameter driving model output. Specifically, extended Fourier Amplitude Sensitivity Test (eFAST), a global sensitivity analysis method, was used. eFAST was developed by Saltelli et al. \cite{Saltelli1999, Saltelli2002, Saltelli2008, Saltelli2010}, which is an expansion of the original FAST method developed by Cukier et al. \cite{Cukier1973, Cukier1977, Cukier1984}. eFAST is a variance-based sensitivity method where variance in model output is quantified using the statistical definition of variance and then partitioned to each parameter, generating a quantitative measure of model output variability attributed to each parameter. The output of interest from eFAST for a non-linear model is the total sensitivity index \( (S_{Ti}) \) for each \( i \)th parameter which includes both the parameter’s individual influence on model output variability and its interactions with other parameters in the model.

Implementation of eFAST requires the number of samples per search curves \( N_S \) and the resampling number \( N_R \). eFAST uses sinusoidal functions of a specific frequency in its sampling procedures which is related to the value chosen for \( N_S \). It
is recommended that this value be at least 65 \[^{34}\]. Since sinusoidal functions are symmetric, the samples obtained from the search curves will eventually repeat. To combat this, a resampling scheme is performed \[^{178}\], specified by the value \(N_R\), which repeats the eFAST algorithm \(N_R\) number of times.

Marino \textit{et al.} \[^{149}\] presented a methodology for performing eFAST in MATLAB and further improved the conclusions by included statistical significance tests on the resulting \(S_{Ti}\) values. In this implementation, a dummy parameter (a parameter not included in the model) is included in the sampling and eFAST algorithm which will be assigned a non-zero \(S_{Ti}\) based on the algorithm. Model parameter \(S_{Ti}\) values are then compared with the \(S_{Ti}\) assigned to the dummy parameter using a T-test to determine whether the model parameters \(S_{Ti}\) values are statistically significant compared to random variation. This method makes it favorable to include larger values of \(N_R\) to increase the number of samples assessed in the T-test. It is also often recommended that eFAST be performed for multiple values of \(N_S\) and \(N_R\) to ensure that the parameters remain influential irrespective of \(N_S\) and \(N_R\) values.

To assess parameter sensitivity indices across multiple values for \(N_S\) and \(N_R\), eFAST was performed using three separate combinations of \(N_S\) and \(N_R\): (1) \(N_S = 65, N_R = 5\), (2) \(N_S = 129, N_R = 10\), and (3) \(N_S = 193, N_R = 15\).

5.2.2 Results

5.2.2.1 Sensitivity analysis for control data

The total sensitivity index for each parameter at the selected time points were calculated from the eFAST results for each run \((N_S = 65, N_R = 5; N_S = 129, N_R = 10; N_S = 193, N_R = 15)\). The results for each parameter are plotted over time in Figure \[^{28}\] for eFAST run with \(N_S = 193\) and \(N_R = 15\). Details on these values can
Fig. 28. **Total sensitivity indices for each parameter over time.** Total sensitivity indices were calculated from eFAST for $N_S = 193$ and $N_R = 15$. The parameters $s_c$, $\mu_f$, $k_c$, and $x_c$ consistently had larger values over time, indicating that the variable $C$ in the model is sensitive to these parameters.

The parameters that consistently had larger total sensitivity indices were $s_c$, the source rate of multipotent cells that can differentiate to amoebocytes or fibroblasts, $\mu_f$, the decay rate of fibroblasts, $k_c$, the rate at which new tissue forms in the wounded space, and $x_c$, a parameter controlling the effectiveness of fibroblasts at supporting new tissue formation.

To quantify the selection of sensitive parameters, we also used the T-test suggested by Marino *et al.* [35]. Total sensitivity indices for each parameter were compared to the total sensitivity index of the dummy parameter. A p-value less than 0.05 indicates that the given parameter was statistically different than random variation.
in the model. Table 8 show the number of time points for which each parameter was statistically different than the dummy parameter for each eFAST run. Sensitive parameters were parameters that had a value larger than 5 for at least two of the eFAST runs. This includes the parameters \( s_c \), the source rate of multipotent cells that can differentiate into amoebocytes or fibroblasts, \( k_{af} \), transition rate of amoebocytes to fibroblasts, \( k_{fa} \), transition rate of fibroblasts to amoebocytes, \( \mu_f \), decay rate of fibroblasts, \( k_c \), the rate at which new tissue forms in the wounded space, and \( x_c \), a parameter controlling the effectiveness of fibroblasts to supporting new tissue formation. These are written in bold in Table 8.
Table 8. **Table of the number of significant total sensitivity indices over time for each eFAST run for each parameter.** Sensitive parameter were those that fit more than half of the time points ($n_{sens} \geq 5$) in at least two of the eFAST runs. Highly sensitive parameters are written in bold.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>$N_S = 65$</th>
<th>$N_S = 129$</th>
<th>$N_S = 257$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$N_R = 5$</td>
<td>$N_R = 10$</td>
<td>$N_R = 20$</td>
</tr>
<tr>
<td>$k_m$</td>
<td>5</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>$k_{ma}$</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>$\mu_m$</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>$s_c$</td>
<td>8</td>
<td>9</td>
<td>9</td>
</tr>
<tr>
<td>$k_{am}$</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>$k_{aa}$</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>$\mu_{sc}$</td>
<td>0</td>
<td>0</td>
<td>9</td>
</tr>
<tr>
<td>$k_{af}$</td>
<td>0</td>
<td>6</td>
<td>9</td>
</tr>
<tr>
<td>$M_\infty$</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>$k_{fa}$</td>
<td>2</td>
<td>6</td>
<td>9</td>
</tr>
<tr>
<td>$\mu_a$</td>
<td>0</td>
<td>3</td>
<td>9</td>
</tr>
<tr>
<td>$k_f$</td>
<td>0</td>
<td>0</td>
<td>8</td>
</tr>
<tr>
<td>$\mu_f$</td>
<td>9</td>
<td>9</td>
<td>9</td>
</tr>
<tr>
<td>$k_c$</td>
<td>8</td>
<td>9</td>
<td>9</td>
</tr>
<tr>
<td>$x_c$</td>
<td>9</td>
<td>9</td>
<td>9</td>
</tr>
<tr>
<td>$p_f$</td>
<td>0</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>$k_a$</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>
5.2.2.2 Parameter estimation for control and moderate heat stress data

Parameter values for the control data and heat stress data are shown in Table 9. Parameter estimates for the control data are contained in the column $p_i$ and estimates for the heat stress data are contained in the column $c_i$ such that the corresponding parameter in the heat stress equation equals $p_i + c_i$. Only parameters selected from the sensitivity results in Section 5.2.2.1 were estimated for the heat stress data. All values $p_i$ and $c_i$ were restricting to the range [0,100].
Table 9. Table of estimated parameters for the control and heat stress data.
Parameters for the control data are contained only in the column $p_i$. Parameters for the heat stress data are those in the $p_i$ column plus the corresponding value in the $c_i$ column. $c_i$ was only estimated for the parameters selected in Section 5.2.2.1.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>$p_i$</th>
<th>$c_i$</th>
</tr>
</thead>
<tbody>
<tr>
<td>$k_m$</td>
<td>2.8264</td>
<td></td>
</tr>
<tr>
<td>$k_{ma}$</td>
<td>6.8066</td>
<td></td>
</tr>
<tr>
<td>$\mu_m$</td>
<td>0.1739</td>
<td></td>
</tr>
<tr>
<td>$s_c$</td>
<td>69.5864</td>
<td>99.8332</td>
</tr>
<tr>
<td>$k_{am}$</td>
<td>35.1501</td>
<td></td>
</tr>
<tr>
<td>$k_{aa}$</td>
<td>65.1986</td>
<td></td>
</tr>
<tr>
<td>$\mu_{sc}$</td>
<td>87.2964</td>
<td></td>
</tr>
<tr>
<td>$k_{af}$</td>
<td>55.9633</td>
<td>97.8956</td>
</tr>
<tr>
<td>$M_\infty$</td>
<td>35.4117</td>
<td></td>
</tr>
<tr>
<td>$k_{fa}$</td>
<td>1.0156</td>
<td>1.3597</td>
</tr>
<tr>
<td>$\mu_a$</td>
<td>2.7214</td>
<td></td>
</tr>
<tr>
<td>$k_f$</td>
<td>19.9022</td>
<td></td>
</tr>
<tr>
<td>$\mu_f$</td>
<td>14.7272</td>
<td>0.0761</td>
</tr>
<tr>
<td>$k_c$</td>
<td>0.3463</td>
<td>1.4498</td>
</tr>
<tr>
<td>$x_c$</td>
<td>0.5496</td>
<td>4.8014\times10^{-4}</td>
</tr>
<tr>
<td>$p_f$</td>
<td>2.8323</td>
<td></td>
</tr>
<tr>
<td>$k_a$</td>
<td>47.9670</td>
<td></td>
</tr>
</tbody>
</table>

The resulting simulations for the chosen parameter sets are plotted in Figure 29. The simulations produced good fits to the data. The MSE for the control data was
0.1393 and 1.7800 for the heat stress data.

Fig. 29. **Parameter fits for control (A) and moderate heat stress data (B).**
Average experimental data was fit for the control environment (25° C) and moderate heat stress (28° C) using the wound healing model. Parameter values were first estimated using an optimization routine for the control data. Parameter values were then estimated for the moderate heat stress by adding positive values to sensitive parameters selected in Section 5.2.2.1. The MSE for the control data was 0.1393 and the MSE for the heat stress data was 1.7800.

5.2.3 Discussion

Using experimental data for wound healing in *P. damicornis* fragments, we refit the wound healing model formulated in Chapter 4. We started by building a cohort of parameter sets that satisfied the majority of data points for the control experiments (ideal light and heat conditions). This cohort was used to calculate the total sensitivity index for each parameter using the global sensitivity method eFAST. This allowed us to identify parameters most likely to influence model outcome. The identified parameters were then used for a parameter estimation algorithm estimating parameters associated with the control data and then additional parameters associated with the moderate heat stress data. Moderate heat stress has generally been
observed to increase healing rates and reactions rates, so parameters estimates for the moderate heat stress data used the estimates found for the control data plus a positive constant.

Sensitive parameters included $s_c$, the source rate of multipotent cells that can differentiate into amoebocytes or fibroblasts, $k_{af}$, transition rate of amoebocytes to fibroblasts, $k_{fa}$, transition rate of fibroblasts to amoebocytes, $\mu_f$, decay rate of fibroblasts, $k_c$, the rate at which new tissue forms in the wounded space, and $x_c$, a parameter controlling the effectiveness of fibroblasts at supporting new tissue formation. By only modulating these selected parameters, we were able to fit the data associated with wound healing in a heat stress environment. The current method for modulating parameters is adding a constant to the selected parameter estimates. In future iterations we may consider different modulations such as multiplying by a constant. This method could also be applied to different environmental conditions such as photo-oxidative stress (excess light), water pollution, or feeding.

5.3 Coral wound healing linked with energy model

5.3.1 Methods

The model for coral wound healing linked with an energy model consists of the coral wound healing model first introduced in Chapter 4 combined with a DEB model developed in Cunning et al. [3].

A model schematic for the paired DEB and wound healing model can be viewed in Figure 30. Combining the two models required the addition of one variable describing the symbiont population in the newly formed tissue at the wound site as well as a few novel parameters and fluxes describing the interactions between the wounded tissue compartment and the host with symbiont. Model variables are described in Table 10.
For model fluxes and parameters from the DEB model, the reader is referred to the original model formulation in Cunning et al. For additional details on parameters involved in wound healing, the reader is referred to Chapter. Parameters and fluxes related to new model terms are discussed in the sections below.

**Fig. 30.** Model schematic of dynamic energy budget model paired with wound healing model. The solid black arrows represent biomass construction, the red arrows represent carbon exchange, the blue arrows represent nitrogen exchange, and the yellow arrows represent light energy. Dashed arrows represent excess energy exchanged between the host and symbiont. Black boxes on the outer host and symbiont perimeter indicate uptake of matter from the external environment. Dashed lines with bars indicate inhibition and dashed lines with circles indication acceleration.
Table 10. **Merged energy wound healing model variables with descriptions.**

<table>
<thead>
<tr>
<th>Symbol</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>S</td>
<td>Symbiont biomass</td>
</tr>
<tr>
<td>H</td>
<td>Host biomass</td>
</tr>
<tr>
<td>M</td>
<td>Debris and foreign matter</td>
</tr>
<tr>
<td>Am</td>
<td>Amoebocytes</td>
</tr>
<tr>
<td>F</td>
<td>Fibroblasts</td>
</tr>
<tr>
<td>C</td>
<td>New tissue</td>
</tr>
<tr>
<td>CA</td>
<td>New tissue with symbiont</td>
</tr>
</tbody>
</table>

5.3.1.1 Model formulation

The merged DEB and wound healing model builds off of the original formulation for these two models. Merging the two models required the introduction of some new variables, model terms, and parameters which are discussed below.

**New tissue formation.** The wound healing model accounts for new tissue formation using the variable $C$. This is regulated in the model only by the fibroblast population in the tissue since fibroblasts are responsible for forming the extracellular matrix that acts as a scaffold for new tissue formation to occupy the wound space. When pairing this model with the DEB model, we assume that some energy originally allocated for tissue growth will need to be allocated to regeneration at the wound space. This is consistent with current theories of coral growth where growth tends to downregulate during periods of healing or stress [182]. Thus the differential equation describing host growth is modified to include a term describing the allocation of host growth SUs to regeneration of new tissue at the wound site. Additionally, we assume that regenerated coral tissue containing symbiont becomes host biomass. We account
for this by changing host mass to be $H + k_{hca}CA$. Note that since we are using previously estimated parameters for the DEB and wound healing models, $H$ and $CA$ have different parameters. The parameter $k_{hca}$ is necessary for this reason and has units C mol/mm$^2$. The updated equation for coral host biomass is given in Equation 5.1 where new terms are written in red.

$$\frac{dH}{dt} = (H + k_{hca}CA)(\hat{HG} - \hat{HT} - \hat{JT})$$  \hspace{1cm} (5.1)

where

$$j_{NT} = k_{NT}(1 - (C + CA)/C_\infty)$$  \hspace{1cm} (5.2)

**Symbiont acquisition in newly formed tissue and host.** The DEB model reaches a strict steady state for the host to symbiont ratio and small perturbations in this value has the potential to lead the system to host death. When wounding occurs, the ratio would remain static in a perfect system since both the symbiont and tissue are removed in our wounding procedure. However, as the wound begins to heal, new tissue is formed without the symbiont present, resulting in a decrease in the host to symbiont ratio. This occurs only briefly as the wound heals and new tissue is later colonized by the symbiont. To include the effects of wound healing in the symbiont equation we developed a new term to be added to the equation originally defined in Cunning et. al [3]. We include a positive flux that accounts for additional symbiont proliferation caused by the presence of newly formed tissue without symbiont ($C$). We also include an additional positive term in the symbiont equation which allows for symbiont uptake from the environment [183]. This would help model a coral reef that has suffered from a massive bleaching event. The uptake term is only triggered
if the ratio of symbiont to host \((S/H)\) is below the steady state value \((SH_\infty)\) in Equation \(5.3\). This term is also inhibited by the presence of \(c_{ROS}\), which will be explained further in the paragraphs below. Both terms are shown in Equation \(5.3\), which includes original model fluxes from the Cunning \textit{et al.} model \(3\). The new terms are written in red.

\[
\frac{dS}{dt} = S(\underbrace{jSG - jST}_\text{Symbiont growth rate} + \underbrace{jSP}_\text{Symbiont turnover rate} + \underbrace{jUP}_\text{Symbiont proliferation initiated by new tissue}) + \underbrace{jUP}_\text{Symbiont uptake from the environment} \tag{5.3}
\]

where

\[
j_{SP} = k_{SP}C, \tag{5.4}\]

\[
j_{UP} = k_{UP}(1 - (S/H)/SH_\infty) + Hf_i(c_{ROS}; k_{si}), \tag{5.5}\]

and

\[
f_i(x; k) = \frac{1}{1 + kx} \tag{5.6}\]

In addition to changes in the DEB model, to account for symbiont colonization in the newly formed tissue, a new variable was added to the wound healing model (introduced in Table \(10\)). This equation contains a single term for colonisation at the wound site resulting from the interaction of new tissue without symbiont \((C)\) with symbiont in the host \((S)\). This is illustrated in Equation \(5.7\).

\[
\frac{dCA}{dt} = k_{ca}CS \tag{5.7}
\]

Additional production of reactive oxygen species (ROS). ROS is included
in the DEB model as a result of photo-oxidative stress, but immune cells also produce ROS during phagocytosis \[184\]. ROS has been evidenced in phagocytic immune cells of other invertebrates \[185, 186\] and can have negative effects on healthy tissue \[187, 188\]. Past researchers have correlated oxidative stress with coral bleaching events \[188\], however other studies have failed to identify a causal relationship \[189\]. In the DEB model, ROS does not directly induce bleaching but excess ROS production reduces the production rate of SUs produced from photosynthesis, inhibiting symbiont growth and reducing excess SUs shared to the host. We include additional production of ROS from amoebocyte phagocytosis of foreign matter with an additional term in the flux equation for \(c_{ROS}\). The updated equation for \(c_{ROS}\) is given in Equation \[5.8\] where new terms are written in red. Notice that the additional ROS production is a function of both \(M\) and \(A\) representing ROS production as a result of phagocytosis of debris and foreign matter.

\[
c_{ROS} = \frac{\text{Baseline production}}{1} + \frac{(j_{eL} + j_{NPQ})_+}{k_{ROS}} + \frac{\text{Excess ROS production from phagocytosis}}{k_{AROS}MA} \tag{5.8}
\]

**Effects of reactive oxygen species (ROS).** ROS, at low concentrations, is beneficial to inflammation \[190\]. It acts as a regulator of inflammation and other cell processes associated with optimal resolution of the inflammatory phase. Higher concentrations, however can be deleterious, even resulting in direct damage to surrounding cells. Overproduction can also result in chronic inflammation, preventing progression to subsequent phases of wound healing. ROS overproduction is implemented into the model by both preventing progression towards proliferation and contributing to damage and tissue loss at the wound site. Specifically, high levels of ROS act as inhibiting terms of fibroblast activation and transition of amoebocyte-like cells.
to fibroblast-like cells. To account for potential tissue damage, ROS interacts with new tissue cells and contributes to damage and debris and inhibits the regrowth of new tissue. The equations effected by ROS are given in Equation 5.9-5.12. New terms are written in red. Recall from Equation 5.5, symbiont uptake from the environment is also inhibited by the presence of excess ROS.

\[
\frac{dM}{dt} = k_m(1 - (C + CA)/C_\infty) - k_{ma}MA + g(c_{ROS}, C) \tag{5.9}
\]

\[
\frac{dA}{dt} = \frac{scP_d(C, CA)R_A(M, A)}{R_A(M, A) + f_i(c_{ROS}; k_{RFi})R_F(F, C) + \mu_{sc}} - \frac{scP_d(C, CA)R_A(M, A)}{R_A(M, A) + f_i(c_{ROS}; k_{RFi})R_F(F, C) + \mu_{sc}} \tag{5.10}
\]

\[
\frac{dF}{dt} = \frac{scP_d(C, CA)\tilde{f}_i(c_{ROS}; k_{RFi})R_F(F, C)}{R_A(M, A) + f_i(c_{ROS}; k_{RFi})R_F(F, C) + \mu_{sc}} + \frac{scP_d(C, CA)\tilde{f}_i(c_{ROS}; k_{RFi})R_F(F, C)}{R_A(M, A) + f_i(c_{ROS}; k_{RFi})R_F(F, C) + \mu_{sc}} \tag{5.11}
\]

\[
\frac{dC}{dt} = \frac{k_cF}{x_c + \tilde{F}} \left(1 - \frac{C + CA}{C_\infty}\right) f_i(c_{ROS}; k_{RFi}) - g(c_{ROS}, C) \tag{5.12}
\]
5.3.2 Simulations

The merged energy model was initially simulated with no wound to ensure that the DEB dynamics did not significantly differ from the original model formulated in Cunning et al. [3]. We confirmed that outputs for the variables $H$ and $S$ matched those from the Cunning et al. [3] model and variables from the wound healing model reached a steady state consistent with unwounded tissue.

5.3.2.1 Healing and growth under normal conditions

To demonstrate model behavior we simulated the merged model at baseline (no wound) and for two different wound sizes (12 mm$^2$ and 24 mm$^2$). The wound variables are shown in Figure 31.

The DEB variables ($S$ and $H$) and the new flux rates $j_{NT}$ and $j_{SP}$ are plotted in Figure 31. As observed previously [191], we see slower growth rates in both the symbiont (Figure 31A) and host (Figure 31B). The flux term $j_{SP}$, plotted in Figure 31C, represents induced proliferation of the symbiont in response to the wounding event and new tissue without symbiont. The observed inflection point in both wounds corresponds with the point at which the wound heals, thus symbiont growth switch back to normal levels sustaining the baseline level in the tissue. The flux term $j_{NT}$, plotted in Figure 31D, shows the prolonged demand for energy necessary to heal the wound. Recall that this rate is negative in the derivative for $H$, so this term is the main driver of the decreased growth in $H$.

As expected from dynamics observed in the original formulation of the wound healing model in Chapter 4, the larger wound produced a longer healing time (Figure 31E) and an increased magnitude and presence of debris and foreign matter (Figure 31F). Differences were also observed in the immune response variables (Figure 31G...
& H).
Fig. 31. Simulations of the merged energy model variables and fluxes with and without a wound. The model was simulated for a smaller wound ($C_\infty = 12$, $max_{pd} = 1.5$) (red) and a larger wound ($C_\infty = 24$, $max_{pd} = 3$) (yellow). Outputs in the variable $H$ indicate how wound healing may affect the overall growth of the reef. The plotted variables and fluxes are symbiont biomass (A), host biomass (B), $j_{SP}$ (C), $j_{NT}$ (D), new coral tissue (E), amoeboocytes (F), debris and foreign matter (G), and fibroblasts (H).
5.3.2.2 Healing and growth under stress

The merged energy wound healing model was also simulated for varying environmental conditions, namely, normal light conditions and increased light conditions.

The DEB model variables and associated novel fluxes are plotted in Figure 32. From these variables we can observe a large discrepancy in the symbiont and host biomass in the stressed environment (Figure 32 A & B). With the introduction of excess photo-oxidative stress alone (No Wound + Stress), we observe a significantly attenuated growth rate. When photo-oxidative stress is combined with a wound (Wound + Stress), we actually observe a decay in both the symbiont and host biomass. Depending on the degree to which growth is affected, the decay in biomass may continue until the host eventually dies, or it may recover when excess ROS induced by phagocytosis at the wound site and increased metabolic load for tissue regeneration returns to baseline.

For the wound healing variables (Figure 32E-H), the wound healing process is only marginally affected by the photo-oxidative stress induced by the increased light. This was mainly incorporated into the model using inhibition terms associated with increased production of ROS. The wound healing process can also be affected if the photo-oxidative stress results in significant tissue loss in the host. On this time scale, we do see some decrease in host biomass (Figure 32B) for the wound with stress scenario, but it does not appear to be significant enough to affect the wound healing process.
Fig. 32. Simulations of the merged energy model variables and fluxes in normal and light stressed environments. The model was simulated with a wound for two environmental conditions corresponding to normal conditions and adverse conditions due to photo-oxidative stress (increased light). The plotted variables and fluxes are symbiont biomass (A), host biomass (B), $j_{SP}$ (C), $j_{NT}$ (D), new coral tissue (E), amoebocytes (F), debris and foreign matter (G), and fibroblasts (H).
5.3.3 Discussion

Allocation of energy in the coral organism during wound healing is known to affect growth rates \[191, 182\]. To address this, we paired a DEB model for coral growth with our wound healing model developed in Chapter 4. To pair the two models, we introduced a new term in the wound healing model \(CA\) which represents the newly formed tissue colonized by endosymbiotic microalgae. We also introduced a collection of new parameters and model fluxes representing interplay between the two models. Specifically, we incorporate reallocation of energy typically used for growth of the host to new tissue formation at the wound site. We also introduced novel dynamics to the symbiont population including induced proliferation caused by the formation of new tissue without symbiont as well as uptake of symbiont from the environment after times of stress.

The generation of ROS also plays an important role in both the DEB model as well as wound healing dynamics. Cellular phagocytosis produces ROS as a byproduct, potentially restricting wound healing if produced in excess \[190\]. In the DEB model, excess ROS inhibits symbiont growth, subsequently decreasing coral host growth. In the merged model, we account for additional production of ROS from phagocytosis of debris and foreign matter as well as the effects of ROS on wound healing. Primarily, we inhibit fibroblasts processes with excess ROS production and induce damage to new tissue from the toxic environment.

To demonstrate model behavior, we simulated this merged model with and without a wound. Model outputs showed attenuated growth in both the symbiont and coral host during a wound event. This results in less biomass generated in the host over time. We also simulated the model with and without a wound and with and without photo-oxidative stress induced by an increase in light in the environment.
Photo-oxidative stress resulted in a significant decrease in growth dynamics for both the symbiont and host. This effect was further increased when a wound was present. For the simulations shown, the presence of the wound in addition to photo-oxidative stress results in decay of the host while photo-oxidative stress alone induces a significantly attenuated growth rate.

Current simulations of this merged energy wound healing model are only demonstrative of potential variable dynamics since parameter values have not been optimized to data and model outputs are not validated by known values. Future work will include identifying known parameter values and estimating those that cannot be mapped to experimental data. Once biologically relevant parameters have been identified, predictions obtained from the model could aid in predicting growth dynamics and reef survival during major wounding events.
CHAPTER 6

CONCLUSION

6.1 Summary

In this work we developed models for inflammation in the lungs from various insults and models for wound healing in reef-building corals. The models for inflammation in the lungs were built off of a framework previously developed for VILI [1], by first extending the VILI model to include new terms and parameter sets informed by data and second modifying the model to a bacterial insult, namely LPS-induced lung injury, and selected parameter sets based on data. The initial model for wound healing in reef-building corals is, to our knowledge, a novel model for this process in corals. The initial model formulation includes both phagocytic cells (amoebocytes) and fibroblasts observed in wound healing in some scleractinian corals [111]. Extensions of this included refitting the model to new control data and experimental data with moderate heat stress (+3° C) and combining the model with a previously formulated DEB model for growth of a coral host [3].

The model developed for VILI was built to further explore age-related differences in patient response to MV and assess potential interventions to improve lung health during MV. There are few existing models assessing the effects of MV on aged subjects [49], so there is still more to be explored. Our model identified parameters involved in repair and damage to the epithelium and parameters involved in macrophage function to be important in both age classification as well as classification for lung health. The importance of macrophage dynamics was mirrored by the differences observed in the experimental data and parameters involved in repair
and damage to the epithelium likely reflect age-related differences in the airspace enlargement, a lung health related metric. We also performed pseudo-interventions by simulating the model with modified parameter values and observed large potential improvements in overall lung health. Further development of this model could inform clinically relevant interventions for older patients undergoing MV.

The model for LPS-induced lung injury was formulated to address age-related differences in another lung injury model. Like the model developed for VILI, we selected parameter sets for this model by comparing simulations to experimental data and applied machine learning methods to identify important parameters driving age-related differences. Macrophage-related parameters were identified as important predictors separating the young and old parameter sets. This is similar to results found for the VILI model, but since LPS-induced lung injury mainly induces an intense inflammatory response, in contrast to mechanical injury of the epithelium, parameters involved in repair and damage to the epithelium did not show up in the classification results. The classification results did however identify a parameter involved in damage to the epithelium specifically from macrophages and neutrophils. This reflects the inflammatory nature of LPS-induced lung injury.

The model developed for wound healing in reef-building corals is, to our knowledge, a novel area of development in regard to mathematical modeling of this process. There is still much debate about the wound healing process in corals, as they tend to differ greatly across different taxa, but here we specifically model the wound healing process observed in some scleractinian corals [111]. The model was paired with wound healing samples collected from fragments of the coral species *P. damicornis*. The experimental data was utilized to estimate parameter values and ensure biological applicability of the model output. This model also included wound initial conditions regarding initial wound size and wound geometry. We performed simulations by
varying these wound initial conditions and observing heal times. We also performed
additional simulations by varying parameters associated with the accumulation of
debris and foreign matter and the immune response. These serve as preliminary ex-
ploration into variations of these parameters that may occur due to variations in the
organisms themselves or by changes in the environment. Environmental conditions
such as heat stress could result in changes to both the debris and foreign matter and
the overall immune response. This model serves as an initial formulation, for which
future iterations will search to improve the model and inform overall understanding
of the wound healing process in corals.

As was explored initially in the wound healing model, variations in regard to en-
vironmental conditions are particularly important for understanding wound healing
in corals in the natural environment. Climate change in particular has resulted in in-
creased instances of periods of heat stress which affect the wound healing process and
the overall coral health. We performed experiments on coral fragments by wounding
fragments and observing healing dynamics for various environmental conditions. Us-
ing this data, we refit the wound healing model to these new experiments for normal
conditions and moderate heat stress conditions. The resulting parameter estimates
demonstrate the wound healing models ability to fit a wide range of data, including
those with perturbations in the environment.

Finally, to further address the effects of adverse environmental conditions on
wound healing, we paired the wound healing model with a previously developed DEB
model [3] which models organism growth as a function of environmental inputs. It
has been observed that during wound healing, coral reefs need to divert energy away
from growth to tissue regeneration [170]. This makes a DEB model a great option to
assess the impact of wound healing on coral growth dynamics. To pair the models
we included a variety of new terms mainly regarding diversion of growth resources
to tissue regeneration in the wound, symbiont colonization of the newly formed tissue, and the effects of increased ROS production as a result of inflammatory cell phagocytosis of debris and foreign matter. We showed simulations of the model for reef and symbiont growth while healing different sized wounds and under different environmental conditions. One interesting result from this is the possibility that heat stress combined with a wound could induce host death where the heat stress alone does not induce host death and instead only inhibits growth.

6.2 Future Directions

The lung inflammation model developed for both VILI and LPS-induced lung injury could be further expanded to include a variety of other insults. The current framework could be adjusted to include different types of exposure to a variety of bacterial or viral infections, allowing for exploration of specific insults. The selected cytokine populations included in the LPS-induced model could also be adjusted to reflect the most relevant cytokines for specific insults, as these may differ. The current models have also only been fit to the local inflammatory response (within the lung tissue), and thus could benefit from the inclusion of data from the systemic inflammatory response (in the blood). This would allow us to better assess inflammation throughout the body which can affect other organs.

The coral wound healing model presented in this work serves as a first step to understanding the wound healing process in corals and successfully modeling this mathematically. It could be discussed ad infinitum the different variations that could be added to increase model complexity with one example being wound depth. Wound depth appears to be the most relevant factor to add first especially since the experimental data used here included a deep wound that was not included in model parameter selection and estimation as the wound did not heal during the observed time
frame. Perhaps the most prudent addition without changing the model structure is the addition of time series data for the amoebocyte and fibroblast populations. Current dynamics for these variables are based on those observed in humans and other vertebrates. Data specific to the species of coral explored would help to better restrict the parameter space and improve model outputs.

The merged energy wound healing model also serves as an initial attempt at modeling the interplay observed between reef growth and healing. In its current form, this model has not been compared to any experimental data or optimized to any known values of reef growth. This model would thus benefit from parameter estimation using experimental data or validation of model outputs from known data or trends. Additionally, the merged wound healing model has currently only been explored for adverse environmental conditions regarding light, but has the potential to incorporate heat through the wound healing model or prey and nitrogen levels directly in the DEB structure. This model should serve as a stepping stone for future models to further utilize the underlying structures for modeling healing and growth in a variety of environments.

Overall, our models serve as initial scaffolding for future variations in model utility and applicability. We hope that these models can offer both insight into the underlying processes themselves, in regard to both inflammation involved in lung injury and wound healing corals, as well as possible avenues for improvement of organism health. Ultimately, these models seek to define how inflammation and wound healing progress in different hosts and environments.
Appendix A

ABBREVIATIONS

AIM Anti-inflammatory mediator
ALI Acute lung injury
ARDS Acute respiratory distress syndrome
DEB Dynamic energy budget
DIN Dissolved inorganic nitrogen
IL-6 Interleukin 6
IL-10 Interleukin 10
LHS Latin-hypercube sampling
LPS Lipopolysaccharides
MV Mechanical ventilation
ODE Ordinary differential equation
PIM Pro-inflammatory mediator
ROS Reactive oxygen species
RSS Residual sum of squares
SU Synthesizing unit
TNF-α Tumor necrosis factor alpha
VILI Ventilator-induced lung injury
Appendix B

VENTILATOR-INDUCED LUNG INJURY MODEL EQUATIONS

M0 macrophage equations

\[
\frac{dM_{0b}}{dt} = d_{m0}(M_0 - M_{0b}) + \frac{\text{Source}}{s_m} - M_{0b} \frac{k_e E^4}{x_e + E^4} - \mu M_{0b} M_{0b} - \frac{\text{Decay}}{\mu_{M0b} M_{0b}}
\]

\[
- M_{0b} \left[ \frac{k_{m0b} E^2}{x_{m0b} + p_b^2} \left( \frac{1}{1 + \left( \frac{a}{a_{b\infty}} \right)^2} \right) + \frac{k_{m0a} a^2}{x_{m0a} + a^2} \right]
\]  

\[
\frac{dM_0}{dt} = - d_{m0}(M_0 - M_{0b}) + M_{0b} \frac{k_e E^4}{x_e + E^4} - \frac{\text{Decay}}{\mu M_{0} M_{0}}
\]

\[
- M_0 \left[ \frac{k_{m0b} E^2}{x_{m0b} + p_b^2} \left( \frac{1}{1 + \left( \frac{a}{a_{b\infty}} \right)^2} \right) + \frac{k_{m0a} a^2}{x_{m0a} + a^2} \right]
\]  

M1 macrophage equations

\[
\frac{dM_{1b}}{dt} = M_{0b} \left[ \frac{k_{m0b} E^2}{x_{m0b} + p_b^2} \left( \frac{1}{1 + \left( \frac{a}{a_{b\infty}} \right)^2} \right) - M_{1b} \frac{k_e E^4}{x_e + E^4} - \frac{\text{Migration}}{k_{m1} M_{1b}} - \frac{\text{Decay}}{\mu M_{1b} M_{1b}} \right]
\]

\[
\frac{dM_1}{dt} = M_0 \left[ \frac{k_{m0p} E^2}{x_{m0p} + p_b^2} \left( \frac{1}{1 + \left( \frac{a}{a_{b\infty}} \right)^2} \right) - k_{man} (k_{anhM1} AN M_1) \left( \frac{1}{1 + \left( \frac{a}{a_{b\infty}} \right)^2} \right) \right]
\]
M2 macrophage equations

\[
\frac{dM_{2b}}{dt} = M_{0b} \left( \frac{k_{m0a}a^2}{x_{m0a}^2 + a^2} \right) - M_{2b} \frac{k_{ee}E_e^4}{x_{ee}^4 + E_e^4} - k_{m2b}M_{2b} - \mu_{M2b}M_{2b} \tag{B.5}
\]

\[
\frac{dM_2}{dt} = M_0 \left( \frac{k_{m0a}a^2}{x_{m0a}^2 + a^2} \right) + M_{2b} \frac{k_{ee}E_e^4}{x_{ee}^4 + E_e^4} + k_{m2b}M_{2b} - \mu_{M2}M_2 \tag{B.6}
\]

Neutrophil equations

\[
\frac{dN_{0b}}{dt} = -N_{0b} \left( \frac{k_{n0b}p^2}{x_{n0b}^2 + p^2} \right) + \frac{1}{1 + \left( \frac{a}{a_{\infty}} \right)^2} - N_{0b} \frac{k_{ee}E_e^4}{x_{ee}^4 + E_e^4} - \mu_{N_{0b}}N_{0b} \tag{B.7}
\]

\[
\frac{dN_b}{dt} = N_{0b} \frac{k_{n0b}p^2}{x_{n0b}^2 + p^2} - N_b \frac{k_{ee}E_e^4}{x_{ee}^4 + E_e^4} + N_b \frac{k_{n0b}p^2}{x_{n0b}^2 + p^2} - \mu_{N_b}N_b \tag{B.8}
\]

\[
\frac{dN}{dt} = \frac{k_{nN_b}}{1 + \left( \frac{a}{a_{\infty}} \right)^2} + N_b \frac{k_{ee}E_e^4}{x_{ee}^4 + E_e^4} - \mu_{N}N \tag{B.9}
\]

\[
\frac{dAN}{dt} = k_{an}N - k_{an1AN}M_1 \frac{1}{1 + \left( \frac{a}{a_{\infty}} \right)^2} - k_{anm2AN}M_2 - \mu_{AN}AN \tag{B.10}
\]
Pro- and anti-inflammatory mediators equations

\[
\frac{dp_b}{dt} = \frac{\text{Diffusion}}{dp} \left( p - p_b \right) + \frac{\text{Production via M1}}{k_{pm1} M_b} \left( \frac{1}{1 + \left( \frac{a_b}{a_{\text{bc}}^\infty} \right)^2} \right) + \frac{\text{Production via neutrophils}}{k_{pa} N_b}
\]

\[
\frac{dp}{dt} = -\frac{\text{Diffusion}}{dp} \left( p - p_b \right) + \frac{\text{Production via M1}}{k_{pm1} M_1} \left( \frac{1}{1 + \left( \frac{a}{a_{\text{bc}}^\infty} \right)^2} \right) + \frac{\text{Production via neutrophils}}{k_{pa} N}
\]

\[
\frac{da_b}{dt} = \frac{\text{Diffusion}}{da} \left( a - a_b \right) + \frac{\text{Production via M1}}{k_{am1} M_1} + \frac{\text{Production via M2}}{k_{am2} M_2} + \frac{\text{Background production}}{s_a} - \frac{\text{Leak into lung}}{-a_b \frac{k_{ee} E_e^4}{x_{eem}^4 + E_e^4}} - \frac{\text{Decay}}{\mu a_b}
\]

\[
\frac{da}{dt} = -\frac{\text{Diffusion}}{da} \left( a - a_b \right) + \frac{\text{Production via M1}}{k_{am1} M_1} + \frac{\text{Production via M2}}{k_{am2} M_2} + \frac{\text{Leak into lung}}{a_b \frac{k_{ee} E_e^4}{x_{eem}^4 + E_e^4}} - \frac{\text{Decay}}{\mu a a}
\]
Repair and epithelial equations

\[ \frac{dR}{dt} = \frac{\text{Upregulation by M2}}{k_{rm2}M_2} - \frac{\text{Decay}}{\mu_R R} \]  \hspace{1cm} (B.16)

\[ \frac{dE_h}{dt} = \left( b_p + k_{ep}P \right) \left( E_h + E_d \right) E_e + E_d \left( b_r + \frac{k_{er}R}{x_{er} + R} \right) \]  

\begin{align*}
&\text{Baseline repair} & \text{Upregulation via repair mediators} \\
&\text{Damage via M1 & neutrophils} \quad \text{Damage from ventilator} \\
&\text{Proliferation of healthy cells, upregulated by PIMs} \quad s_d E_h \\
&\text{Baseline repair} & \text{Upregulation via repair mediators} \\
&\text{Damage via M1 & neutrophils} \quad s_d E_h \\
&\text{Proliferation of healthy cells, upregulated by PIMs} \quad s_d E_h - b_d E_d \]  \hspace{1cm} (B.17)

\[ \frac{dE_d}{dt} = - E_d \left( b_r + \frac{k_{er}R}{x_{er} + R} \right) + E_h \left( \frac{k_{mne}(M_1 + N)^2}{x_{mne}^2 + (M_1 + N)^2} \right) \]  

\begin{align*}
&\text{Phagocytosis of damaged cells by M1} & \text{Death} \\
&\text{Inhibition by AIFs} & \text{Damage from ventilator} \\
&\text{Phagocytosis of damaged cells by N} & \text{Death} \\
&\text{Proliferation of healthy cells, upregulated by PIMs} \quad s_d E_h - b_d E_d \]  \hspace{1cm} (B.18)

\[ \frac{dE_e}{dt} = - (b_p + k_{ep}P) \left( E_h + E_d \right) E_e \]  

\begin{align*}
&\text{Phagocytosis of damaged cells by M1} & \text{Death} \\
&\text{Inhibition by AIFs} & \text{Damage from ventilator} \\
&\text{Phagocytosis of damaged cells by N} & \text{Death} \\
&\text{Proliferation of healthy cells, upregulated by PIMs} \quad s_d E_h - b_d E_d \]  \hspace{1cm} (B.19)
Appendix C

VENTILATOR-INDUCED LUNG INJURY MODEL MATLAB SCRIPTS

C.1 Run File

```matlab
function x = solve_cci_odes(tspan,ics,params)

% Solve system of ODEs representing ventilator-induced lung injury
% Input a single set of corresponding parameters and initial conditions.

vent_time=2; % hours of simulation, starting at 0

 [~,x]=ode15s(@(t,y) ccimodel_rhs(t,y,params,vent_time),tspan,ics,[])
end
```

C.2 ODE File

```matlab
function [dxdt] = ccimodel_rhs(t,y,param,vent_time)

% Equations for compartmental model of macrophages
% November 2023
```
% multiply this by vent terms in Eh and Ed when vent is turned on

    if vent_time==0
        vent_factor=0;
    elseif t<=vent_time
        vent_factor=1;
    else
        vent_factor=0;
    end

% parameters

dp = param(1); % PIM diffusion

da = param(2); % AIM diffusion

dm0 = param(3); % M0 diffusion

xm0pb = param(4); % differentiation of M0b by pb (MM)

xm0ab = param(5); % differentiation of M0b by ab (MM)

kpm1 = param(6); % production of p by M1

kpe = param(7); % production of p by damaged cells

kam1 = param(8); % production of a by M1

kam2 = param(9); % production of a by M2

xm0p = param(10); % differentiation of M0 by p (MM)

xm0a = param(11); % differentiation of M0 by a (MM)

xer = param(12); % repair of damaged cells by R (MM)
kep = param(13); % self-resolving repair mediated by p

xn0pb = param(14); % activation of neutrophils by PIM (MM)

kpn = param(15); % production of PIM by neutrophils

kn = param(16); % migration of activated neutrophils to lung

kman = param(17); % upregulation of M1 switch by AN

kan = param(18); % neutrophils become apoptotic

knn = param(19); % neutrophils decay

kanm1 = param(20); % phagocytosis by M1

kanm2 = param(21); % phagocytosis by M2

km0pb = param(22); % differentiation of M0b by pb (MM)

km0ab = param(23); % differentiation of M0b by ab (MM)

km0p = param(24); % differentiation of M0 by p (MM)

km0a = param(25); % differentiation of M0 by a (MM)

krm2 = param(26); % production of R by M2 (MM)

ker = param(27); % repair of damaged cells by R (MM)

kem1 = param(28); % further damage by M1 (MM)

kn0pb = param(29); % activation of neutrophils by PIM (MM)

abinf = param(30); % maximum amount of ab for inhibition
ainf = param(31); % maximum amount of a for inhibition
mupb = param(32); % decay rate of pb
muab = param(33); % decay rate of ab
mum0b = param(34); % decay rate of M0b
mum1b = param(35); % decay rate of M1b
mum2b = param(36); % decay rate of M2b
mup = param(37); % decay rate of p
mua = param(38); % decay rate of a
mum0 = param(39); % decay rate of M0
mum1 = param(40); % decay rate of M1
mum2 = param(41); % decay rate of M2
muR = param(42); % decay rate of R
mun0b = param(43); % decay rate of Nu
munb = param(44); % decay rate of Na
sm = param(45); % source of M0b
sn = param(46); % source of Nu
bd = param(47); % baseline decay of damaged cells
br = param(48); % baseline repair of damaged cells
bp = param(49); % baseline self-resolving repair of epithelial cells
sd= param(50); % possible function for constant damage
kmne = param(51);
sp = param(52); % source of PIM
sa = param(53); % source of AIM
xmne = param(54); % damage to healthy epithelial cells
due to M1 & N
ken = param(55); % rate of phagocytosis of damaged
cells by neutrophils

% LPS parameters
mulps = 0;
km0l = 0;
kn1 = 0;

% new diffusion parameters
kee = param(59); % rate at which cells leak into lung
xee = param(60); % hill-type parameter that controls
cell leak based on $E_e$ level
mun0 = param(61); % decay rate of N0
kn0p = param(62); % activation rate of neutrophils in
alveolar space
xn0p = param(63); % hill-type parameter that regulates
activation of neutrophils in alveolar space
km1 = param(64); % rate at which differentiated M1
macrophages migrate to alveolar space
km2 = param(65); % rate at which differentiated M2 macrophages migrate to alveolar space

exem = param(66); % hill-type parameter that controls PIM/AIM leak based on $E_e$ level

muan = param(67); % rate at which apoptotic neutrophils decay

% rename variables

pb = y(1);
ab = y(2);
m0b = y(3);
m1b = y(4);
m2b = y(5);
n0b = y(6);
nb = y(7);
p = y(8);
a = y(9);
m0 = y(10);
m1 = y(11);
m2 = y(12);
n = y(13);
an = y(14);
R = y(15);
eh = y(16);
ed = y(17);
ee = y(18);

% new variables
lps = y(19);
n0 = y(20);

% equations
dxdt = zeros(length(y), 1);

%%% new variable - stays 0 for VILI model
% 19. LPS (blood compartment)
dxdt(19) = - mulps * lps;

% 1. pb / PIM
dxdt(1) = sp + dp *(p - pb) + kpm1 * m1b * (1/(1 + (ab/abinf)^2)) + kpn * nb - pb *(kee * ee^4)/(xeem^4 + ee^4) - mupb * pb;

% 2. ab / AIM
dxdt(2) = sa + da *(a - ab) + kam1 * m1b + kam2 * m2b - ab *
*(kee * ee^4)/(xeem^4 + ee^4) - muab * ab;

% 3. M0b
ndxdt(3) = sm + dm0 *(m0 - m0b) - m0b * ((km0pb * pb^2)/(xm0pb^2 + pb^2) * (1/(1 + (ab/abinf)^2)) + ((km0ab * ab^2)/(km0ab * ab^2)) + ((km0ab * ab^2) /
xm0ab^2+ab^2) - m0b*(kee*ee^4)/(xee^4+ee^4) - mum0b*m0b;

\% 4. M1b
\[
\frac{dx}{dt}(4) = m0b*((km0pb*pb^2)/(xm0pb^2+pb^2))*(1/(1+(ab/abinf)^2)) - m1b*(kee*ee^4)/(xee^4+ee^4) - km1*m1b - mum1b*m1b;
\]

\% 5. M2b
\[
\frac{dx}{dt}(5) = m0b*(km0ab*ab^2/(xm0ab^2+ab^2)) - m2b*(kee*ee^4)/(xee^4+ee^4) - km2*m2b - mum2b*m2b;
\]

\% 6. N0b
\[
\frac{dx}{dt}(6) = sn - n0b*((kn0pb*pb^2)/(xn0pb^2+pb^2))*(1/(1+(ab/abinf)^2)) - n0b*(kee*ee^4)/(xee^4+ee^4) - mun0b*n0b;
\]

\% 7. Nb
\[
\frac{dx}{dt}(7) = n0b*((kn0pb*pb^2)/(xn0pb^2+pb^2))*(1/(1+(ab/abinf)^2)) - nb*(kee*ee^4)/(xee^4+ee^4) - kn*nb - munb*nb;
\]

\% LUNG COMPARTMENT

\% 8. p / PIM
dxdt(8) = - dp*(p-pb) + kpm1*m1*(1/(1+(a/ainf)^2)) + kpe*ed + kpn*n + pb*(kee*ee^4)/(xem^4+ee^4) - mup*p;

% 9. a / AIM

dxdt(9) = - da*(a-ab) + kam1*m1 + kam2*m2 + ab*(kee*ee^4)/(xem^4+ee^4) - mua*a;

% 10. M0

dxdt(10) = - dm0*(m0-m0b) - m0*(((km0p*p^2/(xom0p^2+p^2))+(km0l*lps))*(1/(1+(a/ainf)^2)) + (km0a*a^2/(xmoa^2+a^2))) + m0b*(kee*ee^4)/(xem^4+ee^4) - mum0*m0;

% 11. M1

dxdt(11) = m0*(((km0p*p^2/(xom0p^2+p^2))+(km0l*lps))*(1/(1+(a/ainf)^2)) - (kman*(kanm1*an*m1))*(1/(1+(a/ainf)^2)) + m1b*(kee*ee^4)/(xem^4+ee^4) + km1*m1b - mum1*m1;

% 12. M2

dxdt(12) = m0*(((km0a*a^2)/(xmoa^2+a^2)) + (kman*(kanm1*an*m1))*(1/(1+(a/ainf)^2)) + m2b*(kee*ee^4)/(xem^4+ee^4) + km2*m2b - mum2*m2;
%% new variable

% 20. N0
dxdt(20) = n0b*(kee*ee^4)/(xee^4+ee^4) - n0*(((kn0p*p^2)/(xn0p^2+p^2))+(kn1*lps))*(1/(1+(a/ainf)^2)) - mun0*n0;

% 13. N
dxdt(13) = kn*nb - kant*n + nb*(kee*ee^4)/(xee^4+ee^4) + n0*(((kn0p*p^2)/(xn0p^2+p^2))+(kn1*lps))*(1/(1+(a/ainf)^2)) - knn*n;

% 14. AN

dxdt(14) = kant*n - kanm1*an*m1*(1/(1+(a/ainf)^2)) - kanm2*an*m2 - muan*an;

% 15. R
dxdt(15) = krm2*m2 - muR*R;

% 16. Eh

dxdt(16) = (bp+kep*p)*(eh+ed)*ee + ed*(br+(ker*R/(xer+R)) - eh*(kmne*(m1+n)^2/(xmne^2+(m1+n)^2)) - sd*eh
*vent_factor;

% 17. Ed
\[ \text{dxdt}(17) = - ed \cdot (br + \left( \frac{\text{ke} \cdot R}{\text{xer} + R} \right)) + eh \cdot \left( \frac{\text{kmne} \cdot (m1 + n)}{\text{xmne}^2 + (m1 + n)^2} \right) - ed \cdot \text{ken} \cdot n - kem1 \cdot m1 \cdot ed \cdot \frac{1}{1 + \left( \frac{a}{a\inf} \right)^2} + sd \cdot eh \cdot \text{vent\_factor} - bd \cdot ed; \]

% 18. Ee

\[ \text{dxdt}(18) = - (bp + kep \cdot p) \cdot (eh + ed) \cdot ee + kem1 \cdot m1 \cdot ed \cdot \frac{1}{1 + \left( \frac{a}{a\inf} \right)^2} + ed \cdot \text{ken} \cdot n + bd \cdot ed; \]
### Full Table Modulating Parameters Prior to Ventilation

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<th>Parameter</th>
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<th></th>
<th></th>
<th></th>
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<th></th>
<th></th>
<th>Old MDS</th>
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<td>Max.</td>
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</tbody>
</table>

Minimum, mean, and maximum change in the variables $E_h$ and $E_e$ from a 10% decrease (indicated by “-“) or a 10% increase (indicated by “+“) in the listed parameters. Values are shaded on a sliding scale where darker colors represent numbers with a larger magnitude and lighter colors represent numbers with a smaller magnitude. For the minimum in each group, induced decreases in the value of $E_h$ and $E_e$ are orange and induced increases in the value of $E_h$ and $E_e$ are blue.
## Appendix E

### FULL TABLE MODULATING PARAMETERS AFTER 1 HOUR OF VENTILATION

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Young HfS</th>
<th>Mean</th>
<th>Max</th>
<th>Old HfS</th>
<th>Mean</th>
<th>Max</th>
<th>Old MfS</th>
<th>Mean</th>
<th>Max</th>
</tr>
</thead>
<tbody>
<tr>
<td>$E_h$</td>
<td>$E_h$</td>
<td>$E_h$</td>
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<td>$E_h$</td>
<td>$E_e$</td>
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<td>$E_e$</td>
<td>$E_h$</td>
<td>$E_e$</td>
</tr>
<tr>
<td>$E_b$</td>
<td>-1.10%</td>
<td>-0.83%</td>
<td>-0.51%</td>
<td>-1.37%</td>
<td>-0.94%</td>
<td>-0.63%</td>
<td>-0.26%</td>
<td>-1.82%</td>
<td>-1.42%</td>
</tr>
<tr>
<td>$E_a$</td>
<td>-0.78%</td>
<td>-0.60%</td>
<td>0.00%</td>
<td>-0.68%</td>
<td>-0.40%</td>
<td>0.00%</td>
<td>-0.68%</td>
<td>-0.40%</td>
<td>-0.68%</td>
</tr>
<tr>
<td>$E_r$</td>
<td>-1.10%</td>
<td>-0.78%</td>
<td>-0.51%</td>
<td>-1.82%</td>
<td>-1.42%</td>
<td>-1.82%</td>
<td>-1.42%</td>
<td>-1.82%</td>
<td>-1.42%</td>
</tr>
</tbody>
</table>

Minimum, mean, and maximum change in the variables $E_h$ and $E_e$ from a 10% decrease (indicated by “−”) or a 10% increase (indicated by “+”) in the listed parameters after 1 hour of ventilation. Values are shaded on a sliding scale where darker colors represent numbers with a larger magnitude and lighter colors represent numbers with a smaller magnitude. For the minimum in each group, induced decreases in the value of $E_h$ and $E_e$ are orange and induced increases in the value of $E_h$ and $E_e$ are blue.
Appendix F

LPS-INDUCED LUNG INJURY MODEL EQUATIONS

F.1 Blood Compartment

Pro- and Anti-Inflammatory Mediators

\[
\begin{align*}
\frac{dT_{ab}}{dt} &= \text{Diffusion} + \frac{dT}{dt}(T_\alpha - T_{ab}) + \kappa_{M1} M_{1b} \left( \frac{1}{1 + \left( \frac{IL_{6b}}{IL_{6\infty}} \right)^2} \right) + \kappa_{M1} M_{1b} \left( \frac{1}{1 + \left( \frac{IL_{10b}}{IL_{10\infty}} \right)^2} \right) \\
&\quad + \kappa_{M1} M_{1b} N_b \left( \frac{1}{1 + \left( \frac{IL_{6b}}{IL_{6\infty}} \right)^2} \right) + \kappa_{M1} M_{1b} N_b \left( \frac{1}{1 + \left( \frac{IL_{10b}}{IL_{10\infty}} \right)^2} \right) \\
&\quad - T_{ab} \frac{k_{ee} E^4}{x_{eem} + E^4} - \mu_{T}\alpha (F.1) \\
\frac{dIL_{6b}}{dt} &= \text{Diffusion} + \frac{dIL_{6}}{dt}(IL_{6} - IL_{6b}) + \kappa_{M1} M_{1b} \left( \frac{1}{1 + \left( \frac{IL_{6b}}{IL_{6\infty}} \right)^2} \right) + \kappa_{M1} M_{1b} \left( \frac{1}{1 + \left( \frac{IL_{10b}}{IL_{10\infty}} \right)^2} \right) \\
&\quad - IL_{6b} \frac{k_{ee} E^4}{x_{eem} + E^4} - \mu_{IL_{6b}} IL_{6b} (F.2) \\
\frac{dp_b}{dt} &= \text{Diffusion} + \frac{dp}{dt}(p - p_b) + s_p \frac{p_b}{x_{eem} + E^4} - p_b \frac{k_{ee} E^4}{x_{eem} + E^4} - \mu_{p_b} p_b (F.3) \\
\frac{dIL_{10b}}{dt} &= \text{Diffusion} + \frac{dIL_{10}}{dt}(IL_{10} - IL_{10b}) + \kappa_{M1} M_{1b} + \kappa_{M2} M_{2b} + \kappa_{M2} M_{1b} + \kappa_{M2} M_{2b} + s_{IL_{10}} \frac{k_{ee} E^4}{x_{eem} + E^4} - \mu_{IL_{10b}} IL_{10b} (F.4)
\end{align*}
\]
Inflammatory Cells

\[
\frac{dM_{0b}}{dt} = d_{M0}(M_0 - M_{0b}) - M_{0b} \left[ \left( \frac{k_{m0b}Pb_{m0}^2}{x_{m0b}^2 + Pb_{m0}^2} \right) + \left( \frac{1}{1 + \left( \frac{IL_{10b}}{IL_{10b_{\infty}}} \right)^2} \right) + \left( \frac{k_{m0a}IL_{10b}^2}{x_{m0ab}^2 + IL_{10b}^2} \right) \right]
\]

\[
dM_{1b} = M_{0b} \left[ \left( \frac{k_{m0b}Pb_{m0}^2}{x_{m0b}^2 + Pb_{m0}^2} \right) + \left( \frac{1}{1 + \left( \frac{IL_{10b}}{IL_{10b_{\infty}}} \right)^2} \right) \right] - M_{1b} - k_{m1}M_{1b} - M_{0b} \left( \frac{k_{ee}E_4}{x_{ee}^2 + E_4^2} \right) - \mu M_{1b}M_{1b}
\]

\[
dM_{2b} = M_{0b} \left( \frac{k_{m0b}IL_{10b}^2}{x_{m0ab}^2 + IL_{10b}^2} \right) - k_{m2}M_{2b} - M_{2b} \left( \frac{k_{ee}E_4}{x_{ee}^2 + E_4^2} \right) - \mu M_{2b}M_{2b}
\]

\[
dN_{0b} = -N_{0b} \left( \frac{k_{n0b}Pb_{n0}^2}{x_{n0b}^2 + Pb_{n0}^2} \right) + \frac{1}{1 + \left( \frac{IL_{10b}}{IL_{10b_{\infty}}} \right)^2} + sN
\]

\[
dN_{b} = N_{0b} \left( \frac{k_{n0b}Pb_{n0}^2}{x_{n0b}^2 + Pb_{n0}^2} \right) + \frac{1}{1 + \left( \frac{IL_{10b}}{IL_{10b_{\infty}}} \right)^2} - k_{n}N_{b}
\]

where

\[
pb_{m0} = T_{ab} + k_{n0b}IL_{10b} + k_{m0b}Pb
\]

\[
pb_{n0} = T_{ab} + k_{n0b}IL_{10b} + k_{m0b}Pb
\]
F.2 Lung Compartment

Pro- and Anti-Inflammatory Mediators

\[
\begin{align*}
\frac{dT_\alpha}{dt} &= -\left(\text{Diffusion} + \text{Production via } M_1 \right) + \left(\text{Inhibition by IL-10} \right) + \left(\text{Inhibition by IL-6} \right) \\
&= d_t(T_\alpha - T_{ab}) + k_{tn1}M_1 \left(\frac{1}{1 + \left(\frac{IL_{10}}{IL_{10}\infty}\right)^2}\right) \left(\frac{1}{1 + \left(\frac{IL_6}{IL_6\infty}\right)^2}\right) \\
&+ \left(\text{Leak into lung} \right) + \left(\text{Decay} \right) \\
&= T_{ab}x_4^4E_e^4 + \mu T_\alpha \quad (F.10)
\end{align*}
\]

\[
\begin{align*}
\frac{dIL_6}{dt} &= -\left(\text{Diffusion} + \text{Production via } M_1 \right) + \left(\text{Inhibition by IL-10} \right) + \left(\text{Inhibition by IL-6} \right) \\
&= -d_{IL6}(IL_6 - IL_{6b}) + k_{IL6m1}M_1 \left(\frac{1}{1 + \left(\frac{IL_{10}}{IL_{10}\infty}\right)^2}\right) + \left(\text{Leak into lung} \right) + \left(\text{Decay} \right) \\
&+ IL_{6b}x_4^4E_e^4 + \mu IL_6 \quad (F.11)
\end{align*}
\]

\[
\begin{align*}
\frac{dp}{dt} &= -\left(\text{Diffusion} + \text{Production via } \text{ep. damage} \right) + \left(\text{Leak into lung} \right) + \left(\text{Decay} \right) \\
&= -d_p(p - p_b) + k_{pe}E_d + p_bx_4^4E_e^4 + \mu p \quad (F.12)
\end{align*}
\]

\[
\begin{align*}
\frac{dLPS}{dt} &= -\mu_{LPS}LPS \\
&= \quad (F.13)
\end{align*}
\]

\[
\begin{align*}
\frac{dIL_{10}}{dt} &= -\left(\text{Diffusion} + \text{Production via } M_1 \right) + \left(\text{Production via } M_2 \right) \\
&= -d_{IL10}(IL_{10} - IL_{10b}) + k_{IL10m1}M_1 + k_{IL10m2}M_2 + \left(\text{Leak into lung} \right) + \left(\text{Decay} \right) \\
&+ IL_{10b}x_4^4E_e^4 + \mu IL_{10} \quad (F.14)
\end{align*}
\]
Inflammatory Cells

\[
\begin{align*}
\frac{dM_0}{dt} &= - \frac{dM_0}{dt} (M_0 - M_{0b}) \\
&- M_0 \left[ \left( \frac{k_{m0}p_0}{x_{m0p}^2 + p_{m0}^2} \right) + \frac{1}{1 + (\frac{IL_{10}}{IL_{10\infty}})^2} + \frac{k_{m0a}IL_{10}^2}{x_{m0a}^2 + IL_{10}^2} \right] \\
&\quad + \frac{M_0}{E} \left( \frac{k_{ee}E^4}{x_{ee} + E^4} \right) - \mu_{M0}M_0 \\
\frac{dM_1}{dt} &= M_0 \left( \frac{k_{m0}p_0}{x_{m0p}^2 + p_{m0}^2} \right) + \frac{1}{1 + (\frac{IL_{10}}{IL_{10\infty}})^2} + \frac{k_{m1}IL_{10}^2}{x_{m1}^2 + IL_{10}^2} \\
&\quad - k_{man}(k_{anm1}ANM_1) \left( \frac{1}{1 + (\frac{IL_{10}}{IL_{10\infty}})^2} \right) + k_{man}(k_{anm1}ANM_1) \left( \frac{1}{1 + (\frac{IL_{10}}{IL_{10\infty}})^2} \right) + \frac{k_{m1}M_1}{E} - \mu_{M1}M_1 \\
\frac{dM_2}{dt} &= M_0 \left( \frac{k_{m0a}IL_{10}^2}{x_{m0a}^2 + IL_{10}^2} \right) + k_{man}(k_{anm1}ANM_1) \left( \frac{1}{1 + (\frac{IL_{10}}{IL_{10\infty}})^2} \right) \\
&\quad + \frac{M_2}{E} \left( \frac{k_{ee}E^4}{x_{ee} + E^4} \right) + k_{m2}M_2 - \mu_{M2}M_2 \\
\frac{dN_0}{dt} &= N_0 \left( \frac{k_{ee}E^4}{x_{ee} + E^4} \right) - N_0 \left( \frac{k_{n0}p_0}{x_{n0p}^2 + p_{n0}^2} \right) \frac{1}{1 + (\frac{IL_{10}}{IL_{10\infty}})^2} - \mu_{N0}N_0 \\
\frac{dN}{dt} &= N_0 \left( \frac{k_{ee}E^4}{x_{ee} + E^4} \right) + N_0 \left( \frac{k_{ee}E^4}{x_{ee} + E^4} \right) - \mu_{n}N \\
\end{align*}
\]
\[
\frac{dAN}{dt} = \text{Transition to apoptotic} - k_{an} N - k_{anm_1}ANM_1 \left( \frac{1}{1 + \left( \frac{IL_{10}}{IL_{10\infty}} \right)^2} \right) - k_{anm_2}ANM_2 - \mu_{an}AN \quad (F.20)
\]

where

\[
p_{m0} = \alpha + k_{m0i}IL_6 + k_{m0p}p + k_{m0l}LPS
\]

\[
p_{n0} = \alpha + k_{n0i}IL_6 + k_{n0p}p + k_{n0l}LPS
\]
Repair and Epithelial Cells

\[
\frac{dR}{dt} = \text{Upregulation by M2} \quad \frac{1}{k_{rm2}M_2} \quad \text{Decay} \quad \mu_R R
\]

\[
\frac{dE_h}{dt} = \left( b_p + k_{ep}(T_\alpha + I L_6 + p) \right) (E_h + E_d) E_e + E_d \left( b_r + \frac{k_{er}R}{x_{er} + R} \right)
\]

\[
\frac{dE_d}{dt} = -E_h \left( \frac{k_{mne}(M_1 + N)^2}{x_{mne}^2 + (M_1 + N)^2} \right) - \frac{k_{em1}M_1 E_d}{1 + \left( \frac{IL_{10}}{IL_{10\infty}} \right)^2} - \frac{k_{en}N E_d}{1 + \left( \frac{IL_{10}}{IL_{10\infty}} \right)^2}
\]

\[
\frac{dE_e}{dt} = -\left( b_p + k_{ep}(T_\alpha + I L_6 + p) \right) (E_h + E_d) E_e + \left( k_{em1}M_1 E_d \right) \left( 1 + \left( \frac{IL_{10}}{IL_{10\infty}} \right)^2 \right) - \frac{k_{en}N E_d}{1 + \left( \frac{IL_{10}}{IL_{10\infty}} \right)^2} + \frac{k_{en}N E_d}{1 + \left( \frac{IL_{10}}{IL_{10\infty}} \right)^2}
\]

\[F.21\]

\[F.22\]

\[F.23\]

\[F.24\]
Grubb et al. reported mucociliary clearance rates in the lung from young and old mice. The clearance rate for the young group was reported as 34.68% per 15 minutes and 10.47% per 15 minutes for the old group. These can be converted to estimates for the parameter $\mu_{LPS}$ in our model by integrating the differential equation for LPS and using this data to solve for $\mu_{LPS}$.

\[
\frac{dLPS}{dt} = -\mu_{LPS}LPS \quad \text{(G.1)}
\]

\[
\frac{dLPS}{LPS} = -\mu_{LPS}dt \quad \text{(G.2)}
\]

\[
\int \frac{dLPS}{LPS} = \int -\mu_{LPS}dt \quad \text{(G.3)}
\]

\[
\ln(LPS) = -\mu_{LPS}t + c \quad \text{(G.4)}
\]

\[
LPS(t) = Ce^{-\mu_{LPS}t} \quad \text{(G.5)}
\]

$C$ in this equation represents the initial dose, thus we can solve for $\mu_{LPS}$ using the Equation [G.6] where $CR$ is the clearance rate given for the young or old group and $t = 0.25$.

\[
\mu_{LPS} = \ln(1 - CR)/t \quad \text{(G.6)}
\]

Using Equation [G.6] the values for $\mu_{LPS}$ are 1.7035 h$^{-1}$ and for the young group and 0.4545 h$^{-1}$ for old group.
Appendix H

LPS-INDUCED LUNG INJURY MODEL MATLAB SCRIPTS

H.1 Run File

```matlab
function x = solve_lps_odes(tspan,ics,params)
% Solve system of ODEs representing LPS-induced lung injury.
% Input a single set of corresponding parameters and initial conditions.

y_lps_dose = 49.39130435;
% o_lps_dose = 60;

lps_dose = y_lps_dose;

[~,x]=ode23s(@(t,y) lpsmodel_rhs(t,y,params,lps_dose),
     tspan,ics,[]);
end
```

H.2 ODE File

```matlab
function [dxdt] = lpsmodel_rhs(t,y,param,lps_dose)
```

181
% Equations for compartmental model of LPS induced lung injury

% parameters

dt = param(1); % diffusion rate of TNF-alpha
ktm1b = param(2); % production of TNF-alpha by M1
il10binf = param(3); % parameter controlling the effectiveness of IL-10 at inhibiting production of TNF-alpha by M1 in the blood
il6binf = param(4); % parameter controlling the effectiveness of IL-6 at inhibiting the production of TNF-alpha by M1 in the blood
ktnb = param(5); % production of TNF-alpha by N
kee = param(6); % rate of leak into lung (Hill type)
xeem = param(7); % parameter controlling the half-max of leak into lung of mediators (Hill type)
mutb = param(8); % decay rate of TNF-alpha in the blood
dil6 = param(9); % diffusion rate of IL-6
kil6m1b = param(10); % production rate of IL-6 by M1
kil6nb = param(11); % production rate of IL-6 by N
muil6b = param(12); % decay rate of IL-6 in the blood
dp = param(13); % diffusion rate of damage mediators
sp = param(14); % background production rate of damage mediators
mupb = param(15); % decay rate of p in the blood
dil10 = param(16); % diffusion rate of IL-10
kil10m1b = param(17); % production rate of IL-10 by M1
kil10m2b = param(18); % production rate of IL-10 by M2
sil10 = param(19); % background production of IL-10
muil10b = param(20); % decay rate of IL-10 in the blood
dm0 = param(21); % diffusion rate of M0
km0bt = param(22); % differentiation of M0b by TNF-a (MM)
km0bi = param(23); % differentiation of M0b by IL-6 (MM)
km0bp = param(24); % differentiation of M0b by p (MM)
xm0pb = param(25); % controls differentiation of M0b by pro-inflammatories (MM)
km0ab = param(26); % differentiation of M0b by IL-10 (MM)
xm0ab = param(27); % controls differentiation of M0b by anti-inflammatories (MM)
sm = param(28); % source rate of M0b
xee = param(29); % controls leak into lung (MM)
mum0b = param(30); % decay rate of M0b
km1 = param(31); % migration rate of M1b into lung
mum1b = param(32); % decay rate of M1b
km2 = param(33); % migration rate of M2 into lung
mum2b = param(34); % decay rate of M2b
kn0bt = param(35); % differentiation of N0b by TNF-a
kn0bi = param(36); % differentiation of N0b by IL-6
kn0bp = param(37); % differentiation of N0b by pro-inflammatories
xn0bp = param(38); % controls differentiation of N0b
sn = param(39); % source of N0b
mun0b = param(40); % decay rate of N0b

kn = param(41); % migration rate of Nb into the lung
munb = param(42); % decay rate of Nb
ktm1 = param(43); % production of TNF-a by M1
il6inf = param(44); % inhibition by IL-6
il10inf = param(45); % inhibition by IL-10
ktn = param(46); % production of TNF-a by N
mut = param(47); % decay rate of TNF-a
kil6m1 = param(48); % production of IL-6 by M1
kil6n = param(49); % production of IL-6 by N
muit6 = param(50); % decay rate of IL-6

kpe = param(51); % production by epithelial damage
mup = param(52); % decay rate of pro-inflammatories
mulps = param(53); % decay rate of LPS
kil10m1 = param(54); % production of IL-10 by M1
kil10m2 = param(55); % production of IL-10 by M2
muil10 = param(56); % decay rate of IL-10
km0t = param(57); % differentiation of M0 by TNF-a
km0i = param(58); % differentiation of M0 by IL-6
km0p = param(59); % differentiation of M0 by pro-
    inflammatories
km0l = param(60); % differentiation of M0 by LPS
xm0p = param(61); % controls differentiation of M0b (MM)
kmoa = param(62); % differentiation to M2 by IL-10
xm0a = param(63); % controls the differentiation of M0
    by IL-10 (MM)
mum0 = param(64); % decay rate of M0
kman = param(65); % switch M1 to M2
kanm1 = param(66); % phagocytosis rate of AN by M1
mum1 = param(67); % decay rate of M1
mum2 = param(68); % decay rate of M2
kn0t = param(69); % rate of differentiation of NO by
    TNF-a
kn0i = param(70); % rate of differentiation of NO by
    IL-6
kn0p = param(71); % rate of differentiation of N0 by pro-inflammatory

kn0l = param(72); % rate of differentiation of N0 by LPS

xn0p = param(73); % controls differentiation of N by pro-inflammatory (MM)

mun0 = param(74); % decay rate of N0

kan = param(75); % transition of N to AN

mun = param(76); % decay rate of N

kanm2 = param(77); % phagocytosis of AN by M2

muan = param(78); % decay rate of AN

krm2 = param(79); % upregulation of R by M2

muR = param(80); % decay rate of R

bp = param(81); % proliferation of healthy cells

kep = param(82); % upregulation of proliferation by PIM

br = param(83); % baseline repair

ker = param(84); % upregulation of repair by R

xer = param(85); % controls repair by R (MM)

kmne = param(86); % epithelial damage via M1 and N

xmne = param(87); % controls epithelial damage via M1 and N

kem1 = param(88); % phagocytosis of damaged cells by m1
ken = param(89); % phagocytosis of damaged cells by N
bd = param(90); % death of damaged cells

% LPS parameters
if lps_dose==0
    mulps = 0; % decay rate of LPS
    km0l = 0; % rate at which LPS differentiates M0 macrophages to M1 phenotype
    kn0l = 0; % rate at which LPS activates neutrophils
end

% rename variables

% blood compartment
tab = y(1);
il6b = y(2);
pb = y(3);
il10b = y(4);
m0b = y(5);
m1b = y(6);
m2b = y(7);
n0b = y(8);
b = y(9);

% lung compartment
\begin{verbatim}
125 ta = y(10);
126 il6 = y(11);
127 p = y(12);
128 lps = y(13);
129 il10 = y(14);
130 m0 = y(15);
131 m1 = y(16);
132 m2 = y(17);
133 n0 = y(18);
134 n = y(19);
135 an = y(20);
136 R = y(21);
137 eh = y(22);
138 ed = y(23);
139 ee = y(24);

140 \% equations
141 dxdt = zeros(length(y),1);
142
143 \% blood compartment
144
145 \% 1. tab / TNF-alpha
146 dxdt(1) = dt*(ta-tab)\% diffusion
147 + ktm1b*m1b * (1/(1+(il6b/il6binf)^2)) * 
148 (1/(1+(il10b/il10binf)^2)) \% production
\end{verbatim}
via M1 * inhibition by IL-6 * inhibition by IL-10
+ ktnb*nb * (1/(1+(il6b/il6binf)^2))... %
production via N * inhibition by IL-6
- tab*(kee*ee^4)/(xeem^4+ee^4)... % leak into lung
- mutb*tab; % decay

% 2. il6b / IL-6
\[ dxdt(2) = dil6*(il6-il6b)\ldots \% \text{diffusion} \]
+ kil6m1b*m1b * (1/(1+(il10b/il10binf)^2))... %
production via M1 * inhibition by IL-10
+ kil6nb*nb... % production via N
- il6b*(kee*ee^4)/(xeem^4+ee^4)... % leak into lung
- muil6b*il6b; % decay

% 3. pb / damage mediators
\[ dxdt(3) = dp*(p-pb)\ldots \% \text{diffusion} \]
+ sp... % background production
- pb*(kee*ee^4)/(xeem^4+ee^4)... % leak into lung
- mupb*pb; % decay

% 4. il10b / IL-10
\[
\frac{dx}{dt} (4) = d_{l10} \cdot (i_{l10} - i_{l10b}) \quad \% \text{diffusion}
\]
\[
+ \; k_{l10m1b} \cdot m_{1b} \quad \% \text{production via M1}
\]
\[
+ \; k_{l10m2b} \cdot m_{2b} \quad \% \text{production via M2}
\]
\[
+ \; s_{l10} \quad \% \text{background production}
\]
\[
- \; i_{l10b} \cdot \left( \frac{\text{kee} \cdot e^4}{x_{eem}^4 + e^4} \right) \quad \% \text{leak into lung}
\]
\[
- \; m_{u110b} \cdot i_{l10b} \quad \% \text{decay}
\]
\[
\% 5. M_{0b}
\]
\[
\frac{dx}{dt} (5) = d_{m0} \cdot (m_{0} - m_{0b}) \quad \% \text{diffusion}
\]
\[
- \; m_{0} \cdot \left( \frac{(k_{m0bt} \cdot (t_{ab} + k_{m0bi} \cdot i_{l6b} + k_{m0bp} \cdot p_{b})^2)}{(x_{m0pb}^2 + (t_{ab} + k_{m0bi} \cdot i_{l6b} + k_{m0bp} \cdot p_{b})^2)} \cdot \frac{1}{1 + \left( \frac{i_{l10b}}{i_{l10binf}} \right)^2} \right) + \left( \frac{(k_{m0ab} \cdot i_{l10b}^2)}{(x_{m0ab}^2 + i_{l10b}^2)} \right) \quad \% \text{differentiation to M1 via PIM} \quad \% \text{inhibition by IL-10} \quad \% \text{differentiation to M2 by IL-10}
\]
\[
+ \; s_{m} \quad \% \text{source}
\]
\[
- \; m_{u0b} \cdot m_{0b} \quad \% \text{decay}
\]
\[
\% 6. M_{1b}
\]
\[
\frac{dx}{dt} (6) = m_{0} \cdot \left( \frac{(k_{m0bt} \cdot (t_{ab} + k_{m0bi} \cdot i_{l6b} + k_{m0bp} \cdot p_{b})^2)}{(x_{m0pb}^2 + (t_{ab} + k_{m0bi} \cdot i_{l6b} + k_{m0bp} \cdot p_{b})^2)} \cdot \frac{1}{1 + \left( \frac{i_{l10b}}{i_{l10binf}} \right)^2} \right) \quad \% \text{differentiation to M1 via PIM} \quad \% \text{inhibition by IL-10} \quad \% \text{differentiation to M2 by IL-10}
\]
IL-10

- \( m_{1b} \)\((\text{ke} \times \text{ee}^{-4})/\text{xee}^{-4}+\text{ee}^{-4}) \) \( \% \) leak into lung
- \( \text{km}_{1b} \) \( m_{1b} \) \( \% \) migration into lung
- \( \text{mum}_{1b} \) \( m_{1b} \) \( \% \) decay

% 7. M2b

dxdt(7) = \( m_{0b} \)\(((\text{km}_{0ab} \times \text{il10b}^{-2})/\text{xm}_{0ab}^{-2}+\text{il10b}^{-2})\) \( \% \) differentiation to M2

- \( \text{km}_2 \)\( m_{2b} \) \( \% \) migration into lung
- \( m_{2b} \)\((\text{ke} \times \text{ee}^{-4})/\text{xee}^{-4}+\text{ee}^{-4}) \) \( \% \) leak into lung
- \( \text{mum}_{2b} \) \( m_{2b} \) \( \% \) decay

% 8. N0b

dxdt(8) = \(- \text{n0b} \)\(((\text{kn}_{0bt} \times (\text{tab}+\text{kn0bi} \times \text{il6b}+\text{kn0bp} \times \text{pb})^{-2})/\text{xn}_{0bp}^{-2}+(\text{tab}+\text{kn0bi} \times \text{il6b}+\text{kn0bp} \times \text{pb})^{-2})\)*(\(1/(1+(\text{il10b}/\text{il10binf})^{-2})\)) \( \% \) activation by PIM * inhibition by IL\(-10\)

+ \( \text{sn} \) \% source
- \( \text{n0b} \)\((\text{ke} \times \text{ee}^{-4})/\text{xee}^{-4}+\text{ee}^{-4}) \) \( \% \) leak into lung
- \( \text{mum}_{0b} \)\( n_{0b} \) \( \% \) decay

% 9. Nb
\[ \begin{align*}
\text{dxdt}(9) &= n_0 b \left( \frac{\left( k_{0b} t (a + l_{10} b + k_{0b} p b)^2 \right)}{\left( n_{0b} p + (a + l_{10} b + k_{0b} p b)^2 \right)} \right) \frac{1}{1 + \left( \frac{l_{10}}{l_{10,\text{inf}}} \right)^2} \tag{9} \quad \% \text{activation by PIM * inhibition by IL-10} \\
- k_n n_b &\quad \% \text{migration into lung} \\
- n_b (k_{e0} e_0^4)/(x_{ee}^4 + e_0^4) &\quad \% \text{leak into lung} \\
- m_{0b} n_b; &\quad \% \text{decay} \\
\end{align*} \]

\% LUNG COMPARTMENT

\% 10. ta / TNF-alpha

\[ \begin{align*}
\text{dxdt}(10) &= -d t (t_a - t_{ab}) \quad \% \text{diffusion} \\
+ k_{tm1} m_1 \left( \frac{1}{1 + \left( \frac{l_6}{l_6,\text{inf}} \right)^2} \right) &\quad \% \text{production via M1 * inhibition by IL-6 * inhibition by IL-10} \\
+ k_{ln} n_b \left( \frac{1}{1 + \left( \frac{l_6}{l_6,\text{inf}} \right)^2} \right) &\quad \% \text{production via N * inhibition by IL-6} \\
+ t_{ab} (k_{ee} e_0^4)/(x_{ee}^4 + e_0^4) &\quad \% \text{leak into lung} \\
- m_{ut} t_a; &\quad \% \text{decay} \\
\end{align*} \]

\% 11. il6 / IL-6

\[ \begin{align*}
\text{dxdt}(11) &= -d_{il6} (i_{il6} - i_{il6,b}) \quad \% \text{diffusion} \\
+ k_{il6,m1} m_1 \left( \frac{1}{1 + \left( \frac{l_{10}}{l_{10,\text{inf}}} \right)^2} \right) &\quad \% \text{production via M1 * inhibition by IL-10} \\
\end{align*} \]
+ kil6n*nb... % production via N
+ il6b*(kee*ee^-4)/(xeem^-4+ee^-4)... % leak into lung
- mul6*il6; % decay

% 12. p / damage mediators
dxdt(12) = -dp*(p-pb)... % diffusion
    + kpe*ed... % production via epi. damage
    + pb*(kee*ee^-4)/(xeem^-4+ee^-4)... % leak into lung
    - mup*p; % decay

% 13. lps
dxdt(13) = -mulps*lps; % decay

% 14. il10 / IL-10
dxdt(14) = -dil10*(il10-il10b)... % diffusion
    + kil10m1*m1... % production via M1
    + kil10m2*m2... % production via M2
    + il10b*(kee*ee^-4)/(xeem^-4+ee^-4)... % leak into lung
    - muil10*il10; % decay

% 15. M0
dxdt(15) = -dm0*(m0-m0b)... % diffusion
- \( m_0 \times \frac{((km_0t \times (ta+km_0i \times il6+km_0p \times p+km_0l \times lps)^2)/(xm_0p^2+(ta+km_0i \times il6+km_0p \times p+km_0l \times lps)^2))+(km_0l \times lps) \times (1/(1+(il10/\text{il10inf})^2))}{1/(1+(il10/\text{il10inf})^2))} \) ... % differentiation to M1 by PIM and LPS * inhibition by IL-10

+ \( (km_0a \times \text{il10}^2/(xm_0a^2+\text{il10}^2)) \) ... % differentiation to M2

+ \( m_0b \times (kee \times ee^4)/(xee^4+ee^4) \) ... % leak into lung

- \( mum_0 \times m_0; \) % decay

% 16. M1
dxdt(16) = \( m_0 \times ((km_0t \times (ta+km_0i \times il6+km_0p \times p+km_0l \times lps)^2)/(xm_0p^2+(ta+km_0i \times il6+km_0p \times p+km_0l \times lps)^2)) \times (1/(1+(il10/\text{il10inf})^2)) \) ... % differentiation to M1 via PIM and LPS * inhibition by IL-10

- \( (kman \times (kanm_1 \times an \times m_1)) \times (1/(1+(il10/\text{il10inf})^2)) \) ... % M1 switch to M2 by phagocytosis

+ \( m_1b \times (kee \times ee^4)/(xee^4+ee^4) \) ... % leak into lung

+ \( km_1 \times m_1b \) ... % migration from blood

- \( mum_1 \times m_1; \) % decay

% 17. M2
\[ \text{dxdt(17)} = m0* \left( \frac{(km0a*il10^2)/(xm0a^2+il10^2)}{1} \right) \ldots \% \text{differentiation to M2} \\
+ (kman*(kanm1*an*m1))*(1/(1+(il10/il10inf)^2)) \ldots \% \text{M1 switch to M2 by phagocytosis...} \\
+ m2b*(kee*ee^4)/(xee^4+ee^4) \ldots \% \text{leak into lung} \\
+ km2*m2b \ldots \% \text{migration from blood} \\
- mum2*m2; \% \text{decay} \\
\]

\[ \% 18. \, N0 \]
\[ \text{dxdt(18)} = - n0*e \left( \frac{(kn0t*(ta+kn0i*il6+kn0p*p+kn0l*lps)^2)/(xn0p^2+(ta+kn0i*il6+kn0p*p+kn0l*lps)^2)+(kn0l*lps)}{1+(il10/il10inf)^2} \right) \ldots \% \text{activation by PIM * inhibition by IL-10} \\
+ n0b*(kee*ee^4)/(xee^4+ee^4) \ldots \% \text{leak into lung} \\
- mun0*n0; \% \text{decay} \\
\]

\[ \% 19. \, N \]
\[ \text{dxdt(19)} = kn*nb \ldots \% \text{migration into lung} \\
- kan*n \ldots \% \text{transition to AN} \\
+ nb*(kee*ee^4)/(xee^4+ee^4) \ldots \% \text{leak into lung} \\
+ n0*e \left( \frac{(kn0t*(ta+kn0i*il6+kn0p*p+kn0l*lps)^2)/(xn0p^2+(ta+kn0i*il6+kn0p*p+kn0l*lps)^2)}{1+(il10/il10inf)^2} \right) \]
\*(1/(1+((il10/il10inf)^2))) \ldots \% activation by PIM \* inhibition by IL-10

\[dx_{t=20} = kan*n \ldots \% transition to AN\]

\[dx_{t=21} = krm2*m2 \ldots \% upregulation by M2\]

\[dx_{t=22} = (bp+kep*(ta+il6+p))*(eh+ed)*ee \ldots \%
proliferation of healthy cells, upregulated by PIM
+ ed*(br+(ker*R/(xer+R))) \ldots \% baseline repair, upregulation via repair mediators
- eh*(kmne*(m1+n)^2/(xmne^2+(m1+n)^2)); \%
damage via M1 and N\]

\[dx_{t=23} = \ldots \% 23. Ed\]
dxdt(23) = - ed*(br+(ker*R/(xer+R)))... % baseline repair
  with upregulation via repair mediators
    + eh*(kmne*(m1+n)^2/(xmne^2+(m1+n)^2))... %
      damage via M1 and N
    - ed*ken*n... % phagocytosis of damaged cells
      by N
    - kem1*m1*ed*(1/(1+(il10/il10inf)^2))... %
      phagocytosis of damaged cells by M1 *
      inhibition by IL-10
    - bd*ed; % death

% 24. Ee

dxdt(24) = - (bp+kep*(ta+il6+p))*(eh+ed)*ee... %
  proliferation of healthy cells upregulated by PIM
    + kem1*m1*ed*(1/(1+(il10/il10inf)^2))... %
      phagocytosis of damaged cells by M1 *
      inhibition by IL-10
    + ed*ken*n... % phagocytosis of damaged cells
      by N
    + bd*ed; % death

end
Appendix I

CALCULATION OF EQUILIBRIUM POINT FOR WOUND HEALING MODEL IN CORALS

We will start by setting \( \frac{dC}{dt} = 0 \).

\[
\frac{dC}{dt} = \frac{k_c F}{x_c + F} (1 - C/C_\infty) = 0 \quad \rightarrow \quad \frac{k_c F}{x_c + F} = 0 \quad \text{or} \quad (1 - C/C_\infty) = 0 \quad (I.1)
\]

\[
(1 - C/C_\infty) = 0 \quad \rightarrow \quad C = C_\infty \quad (I.2)
\]

\[
\frac{k_c F}{x_c + F} = 0 \quad \rightarrow \quad F = 0 \quad (I.3)
\]

Fibroblasts are known to have resting populations in uninjured tissue, so we assume \( F \neq 0 \), thus the only solution is \( C = C_\infty \). Next we will work with \( \frac{dM}{dt} \).

\[
\frac{dM}{dt} = k_m (1 - C/C_\infty) - k_{ma} A - \mu_m M = 0 \quad (I.4)
\]

We substitute \( C = C_\infty \) and get

\[
-k_{ma} A - \mu_m M = 0 \quad \rightarrow \quad M = 0 \quad \text{or} \quad -k_{ma} A - \mu_m = 0 \quad (I.5)
\]

\[
-k_{ma} A - \mu_m = 0 \quad \rightarrow \quad A = -\frac{\mu_m}{k_{ma}} \quad (I.6)
\]

\( A(t) \geq 0 \) for all \( t \) and \( \mu_m, k_{ma} > 0 \), so the only solution is \( M = 0 \). Now we will work with \( \frac{dF}{dt} \).

\[
\frac{dF}{dt} = \left( \frac{s_c \left( \frac{1}{1 + \max_{p=1}(1 - C/C_\infty)} \right) \left( k_f + p_f F(1 - C/C_\infty) \right)}{\mu_s + k_a + k_{am} M + k_{aa} A + k_f + p_f F(1 - C/C_\infty)} \right) + k_{af} A \left( \frac{1}{1 + M/M_\infty} \right) - k_{fa} F - \mu_f F = 0 \quad (I.7)
\]
We can substitute in our known variables $C = C_{\infty}$ and $M = 0$. After making our substitutions, the equation reduces to Equation I.8 and we solve for $F$ in Equation I.9.

\[
\frac{dF}{dt} = \left( \frac{s_c k_f}{\mu_{sc} + k_a + k_{aa} A + k_f} \right) + k_{af} A - k_{fa} F - \mu_f F = 0 \tag{I.8}
\]

\[
F = \frac{s_c k_f}{(\mu_{sc} + k_a + k_{aa} A + k_f)(\mu_f + k_{fa})} + \frac{k_{af} A}{\mu_f + k_{fa}} \tag{I.9}
\]

Finally we can substitute all known solutions into $\frac{dA}{dt}$ and solve for $A$.

\[
\frac{dA}{dt} = \left( \frac{s_c \left( \frac{1}{1 + \max_p(1-C/C_{\infty})} \right) (k_a + k_{am} M + k_{aa} A)}{\mu_{sc} + k_a + k_{am} M + k_{aa} A + k_f + p_f F(1-C/C_{\infty})} \right) - k_{af} A \left( \frac{1}{1 + M/M_{\infty}} \right) + k_{fa} F - \mu_a A = 0 \tag{I.10}
\]

\[
\frac{dA}{dt} = \left( \frac{s_c (k_a + k_{aa} A)}{\mu_{sc} + k_a + k_{aa} A + k_f} \right) - k_{af} A - \mu_a A \tag{I.11}
\]

\[
+ k_{fa} \left( \frac{s_c k_f}{(\mu_{sc} + k_a + k_{aa} A + k_f)(\mu_f + k_{fa})} + \frac{k_{af} A}{\mu_f + k_{fa}} \right) = 0
\]

When we rearrange Equation I.11, we get a function with a quadratic form (Equation I.12). Thus, we solve for $A$ using the quadratic equation and get two possible roots (Equation I.13).

\[-k_{aa}((k_{fa} + \mu_f)(k_{af} + \mu_a) - k_{fa}k_{af})A^2 \tag{I.12}
\]

\[+ (-k_{fa} + \mu_f)((\mu_{sc} + k_a + k_f)(k_{af} + \mu_a) - s_c k_{aa}) + k_{fa}k_{af}(\mu_{sc} + k_a + k_f))A \]

\[+ s_c(k_a(k_{fa} + \mu_f) + k_{fa}k_f) = 0\]
\[ A_1 = \frac{-b - \sqrt{b^2 - 4ac}}{2a} \quad A_2 = \frac{-b + \sqrt{b^2 - 4ac}}{2a} \] (I.13)

where

\[ a = -k_{aa}((k_{fa} + \mu_f)(k_{af} + \mu_a) - k_{fa}k_{af}) \]

\[ b = (-k_{fa} + \mu_f)((\mu_{sc} + k_a + k_f)(k_{af} + \mu_a) - s_c k_{aa}) + k_{fa}k_{af}(\mu_{sc} + k_a + k_f)) \] (I.14)

\[ c = s_c(k_a(k_{fa} + \mu_f) + k_{fa}k_f) \]

Out of 100,000 samples, the steady calculation using \( A_1 \) was within \( 10^{-4} \) of those calculated numerically.
Appendix J

CORAL WOUND HEALING MODEL MATLAB SCRIPTS

J.1 Run File

```matlab
function x = solve_coral_odes(tspan,ics,params)
    % Solve system of ODEs representing coral wound healing
    % Input a single set of corresponding parameters and
    % initial conditions.

    [~,x]=ode23s(@(t,y) coral_ODEs_rhs(t,y,params),tspan,ics);
end
```

J.2 ODE File

```matlab
function dy = coral_ODEs_rhs(t,y,params)

    % Rename the parameters to their actual names
    km=params(1); % rate that foreign bacteria and debris/damaged cells accumulates in the wound
    kma=params(2); % rate that amoebocytes remove foreign bacteria and debris/damaged cells
```
\texttt{mum=params(3);} \% decay rate of foreign bacteria/ other debris

\texttt{sc=params(4);} \% source rate of stem cells that can become amoebocytes or fibroblasts

\texttt{kam=params(5);} \% rate that stem cells differentiate to amoebocytes (activated by M)

\texttt{kfa=params(10);} \% rate that fibroblasts transition to amoebocytes

\texttt{mfa=params(12);} \% rate that stem cells differentiate to fibroblasts

\texttt{muc=params(7);} \% decay rate of stem cells

\texttt{kfa=params(10);} \% rate that fibroblasts transition to amoebocytes

\texttt{mua=params(11);} \% decay rate of amoebocytes

\texttt{muf=params(13);} \% decay rate of fibroblasts

\texttt{kc=params(14);} \% rate of epidermal coral cells maturation (activated by F)

\texttt{xc=params(15);} \% value that controls the rate that epidermal coral cells mature (MM)

\texttt{pf=params(16);} \% fibroblast proliferation from other fibroblasts and lack on contact inhibition
ka=params(17); % baseline rate at which cells differentiate to amoebocytes

cinf=params(18); % initial wound size (mm^2)

maxpd=params(19); % maximum distance between polyps in the wound (mm)

% Establish our model variables
M=y(1); A=y(2); F=y(3); C=y(4);

% Initialize the dy vector for the model variables
dy=zeros(4,1);

% Define the odes

dy(1)=km*(1-C/cinf)-kma*M-A-mum*M; % debris

dy(2)=(sc*(1/(1+(maxpd*(1-C/cinf)))))*ka/km+M+kaa*A)/(musc+ka+kam*M+kaa*A+pf*F*(1-C/cinf))-kaf*A*(1+(M/minf))'+kfa*F-mua*A; % amoebocytes

dy(3)=(sc*(1/(1+(maxpd*(1-C/cinf))))*(kf+pf*F*(1-C/cinf)))/(musc+(kf+pf*F*(1-C/cinf))+ka+kam*M+kaa*A)+kfa*F-muf*F; % fibroblasts

dy(4)=(kc*F)/(xc+F)*(1-C/cinf); % coral epithelial cells
end
Appendix K

ADDITIONAL PLOTS OF TOTAL SENSITIVITY INDICES FOR THE CORAL WOUND HEALING MODEL

Total sensitivity indices were calculated from eFAST for $N_s = 65$ and $N_R = 5$. The parameters $s_c$, $\mu_f$, $k_c$, and $x_c$ consistently had larger values over time, indicating that the variable $C$ in the model is sensitive to these parameters.
Total sensitivity indices were calculated from eFAST for $N_S = 129$ and $N_R = 10$. The parameters $s_c, \mu_f, k_c,$ and $x_c$ consistently had larger values over time, indicating that the variable $C$ in the model is sensitive to these parameters.
Appendix L

MERGED ENERGY WOUND HEALING MODEL MATLAB SCRIPTS

```matlab
function [out]=merge_DEB_WH(h,yrs,initS,cinf,maxpd,
     wound_switch)

% function for simulating merged energy wound healing model

%%%%% INPUTS %%%%%
% h = step size
% yrs = number of years for simulation
% initS = initial value for S - H is hard coded with an initial value of 50
% for these values, S > 3 results in survival and S < 3 results in death
% cinf = initial wound size - if you are running a wound, you may set to any positive value
% maxpd = initial wound measurement finding the maximum distance on the shortest dimension (used to account for wound geometry)
% wound_switch = 0 for no wound or 1 for wound
```
% % % % OUTPUT % % % % %

% out = matrix of variable outputs
% S(:,i) = out(:,1); % symbiont biomass
% H(:,i) = out(:,2); % host biomass
% ratio(:,i) = out(:,3); % S/H
% dH_Hdt(:,i) = out(:,4); % host biomass derivative
% dS_Sdt(:,i) = out(:,5); % symbiont biomass derivative
% jsg(:,i) = out(:,6); % symbiont growth flux
% jst(:,i) = out(:,7); % symbiont turnover flux
% jcp(:,i) = out(:,8); % photosynthesis rate
% rhon(:,i) = out(:,9); % Nitrogen shared with the symbiont
% jhg(:,i) = out(:,10); % host growth flux
% jht(:,i) = out(:,11); % host turnover flux
% rhoc(:,i) = out(:,12); % fixed carbon shared with host
% L(:,i) = out(:,13); % light input
% N(:,i) = out(:,14); % nitrogen input
% X(:,i) = out(:,15); % prey input
% jx(:,i) = out(:,16); % prey assimilation (feeding) rate
% jn(:,i) = out(:,17); % nitrogen uptake rate
% jl(:,i) = out(:,18); % light absorption rate
% jco2(:,i) = out(:,19); % CO2 input to photosynthesis
% jel(:,i) = out(:,20); % light energy in excess of
  photochemistry
% jnpq(:,i) = out(:,21); % total capacity of NPQ
% cros(:,i) = out(:,22); % ROS production proportional
  to baseline
% jec(:,i) = out(:,23); % excess carbon used to
  activate host CCMs
% M(:,i) = out(:,24); % debris and foreign matter in
  the wound
% Am(:,i) = out(:,25); % amoebocytes in the wound
% F(:,i) = out(:,26); % fibroblasts in the wound
% C(:,i) = out(:,27); % new tissue formed at the wound
  site
% CA(:,i) = out(:,28); % new tissue with symbiont
% dM_dt(:,i) = out(:,29); % debris and foreign matter
  derivative
% dAm_dt(:,i) = out(:,30); % amoebocyte derivative
% dF_dt(:,i) = out(:,31); % fibroblast derivative
% dC_dt(:,i) = out(:,32); % new tissue derivative
% dCA_dt(:,i) = out(:,33); % new tissue with symbiont
  derivative
% jnt(:,i) = out(:,34); % energy diverted to wound
  healing
% jsp(:,i) = out(:,35); % proliferation of symbiont
  induced by wound
```plaintext
52  \% jup(:,i) = out(:,36); \% uptake of symbiont from the environment
53
54 \n55 tspan=(0:h:yrs*365);
56
57 \% Allocate space - DEB vars and time-dep fluxes
58 S=zeros(length(tspan),1); H=zeros(length(tspan),1);
59 dS_Sdt=zeros(length(tspan),1); dH_Hdt=zeros(length(tspan),1);
60 A=zeros(length(tspan),1); jl=zeros(length(tspan),1); jec=
61 \% Allocate space - DEB vars and time-dep fluxes
62 rhoc=zeros(length(tspan),1); jhg=zeros(length(tspan),1);
63 rhon=zeros(length(tspan),1);
64 rch=zeros(length(tspan),1); rcs=zeros(length(tspan),1);
65 jel=zeros(length(tspan),1);
66 jnpq=zeros(length(tspan),1); cros=zeros(length(tspan),1);
67 \% Allocate space - WH variables
68 M=zeros(length(tspan),1); Am=zeros(length(tspan),1);
69 F=zeros(length(tspan),1); C=zeros(length(tspan),1);
70```

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\[ dM_{dt} = \text{zeros}(\text{length}(t\text{span}),1); \quad dAm_{dt} = \text{zeros}(\text{length}(t\text{span}),1); \]
\[ dF_{dt} = \text{zeros}(\text{length}(t\text{span}),1); \quad dC_{dt} = \text{zeros}(\text{length}(t\text{span}),1); \]

% Allocate space - new variables and fluxes
CA=\text{zeros}(\text{length}(t\text{span}),1); \quad \text{dCA}_{dt}=\text{zeros}(\text{length}(t\text{span}),1);
\]
\text{jnt}=\text{zeros}(\text{length}(t\text{span}),1);
\text{jsp}=\text{zeros}(\text{length}(t\text{span}),1); \quad \text{jup}=\text{zeros}(\text{length}(t\text{span}),1);

% environment vals
\text{X=prey}(0,0,0,t\text{span})'; \quad \text{L}=\text{light}(25,25,1,t\text{span})';
\text{N}=\text{DIN}(1e^{-7},1e^{-7},0,t\text{span})';

% parameters - DEB
jht0 = 0.03; % Host specific biomass turnover rate (d^{-1})
nnh = 0.18; % N:C ratio in host biomass (-)
nnx = 0.2; % N:C ratio in prey biomass (-)
signh = 0.9; % Proportion of host nitrogen turnover recycled (-)
sigch = 0.1; % Proportion of host carbon turnover recycled (-)
jxm = 0.13; % Maximum specific host feeding rate (molX/CmolH/d)
jnm = 0.035; % Maximum specific host DIN uptake rate (molN/CmolH/d)
jhgm = 1; % Maximum specific host growth rate (CmolH/CmolH/d)
kco2 = 10; % Rate of host CCM's (molCO2/molC/d)
kn = 1.5e-6; % Half-saturation constant for host DIN uptake (molN/L)
kk = 1e-6; % Half-saturation constant for host feeding (CmolX/L)
initH = 50; % Initial host biomass (CmolH)
yc = 0.8;
jst0 = 0.03; % Symbiont specific biomass turnover rate (d^-1)
nns = 0.13; % N:C ratio in symbiont biomass (-)
ycl = 0.1; % L:C ratio in fixed carbon (=quantum yield) (molC/mol ph)
knpq = 112; % capacity of non-photochemical quenching (mol ph/CmolS/d)
% calculated as 4x max. photochemical quenching (Gorbunov et al. 2001)
kros = 80; % amount of excess light beyond NPQ capacity (e.g., jeL-jNPQ) that doubles ROS production relative to baseline (mol ph/CmolS/d)
k = 1; % exponent on ROS production (-)
astar = 1.34; % Symbiont specific cross-sectional area (m
^2/C-molS)
signs = 0.9; % Proportion of symbiont nitrogen turnover
recycled (-)
sigcs = 0.9; % Proportion of symbiont carbon turnover
recycled (-)
jcpm = 2.8; % Maximum specific photosynthate production
rate (Cmol/CmolS/d)
jsgm = 0.25; % Maximum specific symbiont growth rate (CmolS/CmolS/d)
%initS = 0.5; % Initial symbiont biomass (CmolS)
b = 5; % Scaling parameter for bleaching response

% parameters - WH
km=6.2121*24; % rate that foreign bacteria and debris/
damaged cells accumulates in the wound
kma=25.7689*24; % rate that amoebocytes remove foreign
bacteria and debris/damaged cells
mum=0.3294*24; % decay rate of foreign bacteria/ other
debri
sc=2.3617*24; % source rate of stem cells that can become
amoebocytes or fibroblasts
kam=19.0942*24; % rate that stem cells differentiate to
amoebocytes (activated by M)
$kaa = 12.4464 \times 24$; % rate that stem cells differentiate to amoebocytes (activated by A)

$musc = 0.9582 \times 24$; % decay rate of stem cells

$kaf = 0.0643 \times 24$; % rate that amoebocytes transition to fibroblasts

$minf = 35.4939$; % value that controls inhibition from M

$kfa = 1.5449 \times 24$; % rate that fibroblasts transition to amoebocytes

$mua = 11.8697 \times 24$; % decay rate of amoebocytes

$kf = 0.6858 \times 24$; % rate that stem cells differentiate to fibroblasts

$muf = 1.4694 \times 24$; % decay rate of fibroblasts

$kc = 20.8144 \times 24$; % rate of epidermal coral cells maturation (activated by F)

$xc = 19.6923$; % value that controls the rate that epidermal coral cells mature (MM)

$pf = 90.5915 \times 24$; % fibroblast proliferation from other fibroblasts and lack on contact inhibition

$ka = 0.7318 \times 24$; % baseline rate at which cells differentiate to amoebocytes

% new parameters

$knt = 0.005$; % rate of H SU diverged to new tissue formation

$ksp = 1$; % rate of S proliferation induced by wound
\begin{verbatim}
% rate of uptake of S from environment
kup=1;

% controls uptake of symbiont from the environment (calculated by SS of DEB)
SHinf=0.1;

% controls effectiveness of ROS at inhibiting uptake of S from environment
ksi=1;

% ROS production from phagocytosis of debris and foreign matter
karos=0.1;

% rate at which ROS damages new tissue
keros=0.1;

% controls effectiveness of ROS at inhibiting fibroblast activation
krosi=0.05;

% controls effectiveness of ROS at inhibiting amoebocyte switch to fibroblasts
krisi=0.05;

% controls effectiveness of ROS at inhibiting new tissue formation
krfi=0.05;

% rate at which new tissue is colonized by S
kca=3;

% proportion of new tissue that contributes to host (C mol/mm^2)
khca=0.5;

% Host fluxes
jx  = jxm.*X./(X+kx);

jn  = jnm.*N./(N+kn);

rnh = (jht0*nnh*signh)*ones(length(tspan),1);

rhon(1) = jn(1);

jec(1) = 10;
\end{verbatim}
jco2(1) = kco2*jec(1);
jhg(1) = 0.25;
jht = jht0;
rch(1) = jht0*sigch;

dH_Hdt(1) = jhgm;
H(1) = initH;

% Symbiont fluxes
rns = (jst0*nns*signs)*ones(length(tspan),1);
jl(1) = L(1)*astar;
jcp(1) = max(0,synth(jl(1)*ycl, jco2(1)*H(1)/initS, 
             jcpm));
jel(1) = max(0,jl(1)-jcp(1)*(1/ycl));
jnpq(1) = knpq;
jsg(1) = jsgm/10;
rhoc(1) = jcp(1);
jst(1) = jst0;
rcs(1) = jst0*sigcs;
cros(1) = 1;

dS_Sdt(1) = jsgm;
S(1) = initS;
% Wound healing initialization
M(1)=0;
if wound_switch==1
    Am(1)=0;
    F(1)=0;
else
    [Am(1),F(1)]=steadystate_analytical(params);
end
C(1)=0;
if wound_switch==1
    CA(1)=0;
else
    CA(1)=cinf;
end
dM_dt(1)=km*(1-(C(1)+CA(1))/cinf)-kma*M(1)*Am(1)-mum*M(1)
    + kcros*C(1)*cros(1);
dAm_dt(1)=((sc*(1/(1+(maxpd*(1-(C(1)+CA(1))/cinf)))))*(ka+ 
    kam*M(1)+kaa*Am(1)))/(musc+ka+kam*M(1)+kaa*Am(1)+(1/(1+
    krfi*cros(1)))*(kf+pf*F(1)*((1-(C(1)+CA(1))/cinf)) 
    -(1/(1+kafi*cros(1)))*kaf*Am(1)*(1/(1+(M(1)/minf))))+kfa 
    *F(1)-mua*Am(1);
dF_dt(1)=((sc*(1/(1+(maxpd*(1-(C(1)+CA(1))/cinf)))))*(1/(1+
    krfi*cros(1)))*(kf+pf*F(1)*((1-(C(1)+CA(1))/cinf))) /
    (musc+(1/(1+krfi*cros(1)))*(kf+pf*F(1)*((1-(C(1)+CA(1))/cinf)) 
    +ka+kam*M(1)+kaa*Am(1))+(1/(1+kafi*cros(1)))*kaf*
\[
\begin{align*}
\text{Am}(1) & \cdot \frac{1}{1 + (M(1)/\text{minf})} - kfa \cdot F(1) - muf \cdot F(1); \\
\text{dC}_d(1) & = \frac{k_c \cdot F(1)}{x_c + F(1)} \cdot (1 - (C(1) + CA(1))/\text{cinf}) - k_{cros} \cdot cros(1) \cdot C(1) - k_{ca} \cdot C(1) \cdot S(1); \\
\text{dCA}_d(1) & = k_{ca} \cdot C(1) \cdot S(1); \\
\end{align*}
\]

% New variables and fluxes
\[
\begin{align*}
\text{jnt}(1) & = 0; \\
\text{jsp}(1) & = 0; \\
\text{jup}(1) & = 0; \\
\end{align*}
\]

% Update functions and solve ODEs
\[
\begin{align*}
\text{for } t & = 2: \text{length}(\text{tspan}) \\
\end{align*}
\]

% Symbiont fluxes
\[
\begin{align*}
\text{S}_\text{tot} & = S(t-1); \ % \text{Get total symbiont abundance from prev time step} \\
\% \text{Photosynthesis} \\
\% \text{-------------} \\
\% \text{Light input flux} \\
\text{jl}(t,:) & = (1.256307 + 1.385969 \cdot \exp(-6.479055 \cdot (S_\text{tot}/(H(t-1)+CA(t-1)))))*L(t)*\text{astar}; \\
\% \text{CO2 input flux} \\
\text{rcs}(t,:) & = \text{sigcs} \cdot (jst0+(1-yc) \cdot jsg(t-1,:)/yc); \ % \text{metabolic CO2 recycled from symbiont biomass}
\end{align*}
\]
% Production flux (photosynthetic carbon fixation)
jcp(t,:) = synth(jl(t)*ycl, (jco2(t-1) + rch(t-1))*(H(t-1)+CA(t-1))/S_tot + rcs(t,:), jcpm)./cros(t-1,:);

% Rejection flux: CO2 (wasted to the environment)
% jCO2w[t] <- max((H$jCO2[t-1] + H$&CH[t-1])*H$H[t-1]/
% S.t + rCS[t] - jCP[t], 0)

% Rejection flux: excess light energy not quenched by carbon fixation
jel(t,:) = max(jl(t,:) - jcp(t,:)/ycl, 0);

% Amount of excess light energy quenched by NPQ
jnpq(t,:) = (knpq^(-1) + jel(t,:)^(-1))^(1); % single substrate SU

% Scaled ROS production due to excess excitation energy (=not quenched by carbon fixation AND NPQ)
cros(t,:) = 1 + (max(jel(t,:) - jnpq(t,:), 0)/kros)^k
  + karos*M(t-1)*Am(t-1);

% Symbiont biomass

% Production flux (symbiont biomass formation)
jsg(t,:) = synth(yc*jcp(t,:), (rhom(t-1)*(H(t-1)+CA(t-1))/S_tot + rns(t,:))/nns, jsgm);

% Rejection flux: carbon (surplus carbon shared with the host)
rhoc(t,:) = max(jcp(t,:) - jsg(t,:)/yc, 0);
% Symbiont biomass loss (turnover)
\[ j_{st}(t,:) = j_{st0} \times (1 + b \times (c_{ros}(t,:) - 1)) \];

% Total amount of carbon shared by all symbionts
\[ r_{hoc\_tot} = r_{hoc}(t,:)\times S(t-1) \];

% Host fluxes
% ============
% Production flux (host biomass formation)
\[ j_{hg}(t) = \text{synth}(y_{c}\times r_{hoc\_tot}/(H(t-1)+CA(t-1)) + j_{x}(t)), \]
\[ \left( j_{n}(t) + n_{nx}\times j_{x}(t) + r_{nh}(t) \right) / n_{nh}, j_{hgm} \];

% Rejection flux: nitrogen (surplus nitrogen shared with the symbiont)
\[ r_{hon}(t) = \max(j_{n}(t) + n_{nx}\times j_{x}(t) + r_{nh}(t) - n_{nh}\times j_{hg}(t), 0) \];

% Rejection flux: carbon -- given back to symbiont as CO2 input to photosynthesis
\[ j_{ec}(t) = \max(j_{x}(t) + r_{hoc\_tot}/(H(t-1)+CA(t-1)) - j_{hg}(t)/y_{c}, 0) \];

% Host biomass loss
\[ r_{ch}(t) = \text{sigch} \times (j_{ht0} + (1-y_{c})\times j_{hg}(t)/y_{c}); \]
% metabolic CO2 recycled from host biomass turnover
\[ j_{co2}(t) = k_{co2} \times j_{ec}(t); \]
% carbon not used in host biomass is used to activate CCM's that deliver CO2 to photosynthesis
% New fluxes

\[ jnt(t) = knt \cdot \left(1 - \frac{C(t-1) + CA(t-1)}{cinf}\right) \]
\[ jsp(t) = ksp \cdot C(t-1) \]
\[ jup(t) = kup \cdot \max\left(\frac{1 - \left(S(t-1)/H(t-1)\right)}{SHinf}, 0\right) \cdot H(t-1) \cdot \frac{1}{1 + ksi \cdot \text{cros}(t)} \]

% DEB State equations

\[ dS_Sdt(t,:) = S(t-1,:)*\left(jsg(t,:) - jst(t,:) + jsp(t)\right) + jup(t); \] % Specific growth rates (Cmol/Cmol/d)
\[ S(t,:) = S(t-1,:) + dS_Sdt(t,:)*h; \] % Biomass (Cmol)

\[ dH_Hdt(t) = \left(H(t-1) + khca \cdot CA(t-1)\right) * \left(jhg(t) - jht - jnt(t)\right); \] % Specific growth rates (Cmol/Cmol/d)
\[ H(t) = H(t-1) + dH_Hdt(t) * h; \] % Biomass (Cmol)

%WH state equations

\[ dM_dt(t) = km \cdot \left(1 - \frac{C(t-1) + CA(t-1)}{cinf}\right) - kma \cdot M(t-1) \cdot \text{Am}(t-1) - mum \cdot M(t-1) + kcros \cdot C(t-1) \cdot \text{cros}(t); \]
\[ M(t) = M(t-1) + dM_dt(t)*h; \]

\[ dAm_dt(t) = \left(sc \cdot \left(\frac{1}{1+\left(maxpd\cdot(1-(C(t-1)+CA(t-1))/cinf)\right)}\right) \cdot \left(kaa + \text{Am}(t-1)\right) + kcros \cdot C(t-1) \cdot \text{cros}(t)\right) \]
\[ \times \left(\frac{(1-\left(1+(1+krfi*\text{cros}(t))\right) \cdot \text{F}(t-1) \cdot \left(1-(C(t-1)+CA(t-1))/cinf\right)}{(1+kafi*\text{cros}(t))} \right) \]
kaf*Am(t-1)*(1/(1+(M(t-1)/minf))) + kfa*F(t-1) - mua*Am(t-1);

Am(t) = Am(t-1) + dAm_dt(t)*h;

dF_dt(t) = (sc*(1/(1+(maxp*(1-(C(t-1)+CA(t-1))/cinf)))) *(1/(1+krfi*cros(t)))*(kf+pf*F(t-1)*((1-(C(t-1)+CA(t-1))/cinf))*(1/(1+krfi*cros(t))))*(1/(1+kafi*cros(t)))*kaf*Am(t-1)*(1/(1+(M(t-1)/minf)))) - kfa*F(t-1) - muf*F(t-1);

F(t) = F(t-1) + dF_dt(t)*h;

dC_dt(t) = (kc*F(t-1))/(#/F(t-1))*(1-(C(t-1)+CA(t-1))/cinf) *(1/(1+kcfi*cros(t)))*kca*C(t-1)*S(t-1);

C(t) = C(t-1) + dC_dt(t)*h;

dCA_dt(t) = kca*C(t-1)*S(t-1);

CA(t) = CA(t-1) + dCA_dt(t)*h;

end

ratio = S./H;

jht = ones(length(tspan),1)*jht0;

out = [S H ratio dH_Hdt dS_Sdt jsg jst jcp rhon...
function L = light(min, max, func, tspan)
    if func == 0
        L = linspace(min, max, length(tspan));
    elseif func == 1
        L = linspace(max, min, length(tspan));
    elseif func == 2
        L = 0.5 * (max - min) * sin(0.0172 * tspan) + min + 0.5 * (max - min);
    elseif func == 3
        L = 0.5 * (max - min) * sin(0.0172 * (tspan - 182)) + min + 0.5 * (max - min);
    elseif func == 4
        f = 0.5 * (max - min) * sin(0.0172 * tspan);
        f = rescale(f, 50, max);
        ff = rescale(1 + (0.01 / (1 + exp(0.05 * (tspan - 200)))) , 0.6, 1);
        L = f * ff;
function N = DIN(min,max,func,tspan)
    if func==0
        N=linspace(min,max,length(tspan));
    elseif func==1
        N=linspace(max,min,length(tspan));
    else
        N=0.5*(max-min)*sin(0.0172*tspan)+min+0.5*(max-min);
    end
end

function X = prey(min,max,func,tspan)
    if func==0
        X=linspace(min,max,length(tspan));
    elseif func==1
        X=linspace(max,min,length(tspan));
    else
        X=0.5*(max-min)*sin(0.0172*tspan)+min+0.5*(max-min);
    end
end
function out = synth(x, y, m)
    out = 1 / ((1 / m) + (1 / x) + (1 / y) - (1 / (x + y)));
end
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Quintessa Hay, originally from Bennington, Vermont, spent most of her schooling excelling at math but never thinking that she would ever want to do it as a career. After graduating high school she began attending Southern New Hampshire University as a small business major with big dreams to open up a bakery some day. After realizing a business degree was not for her, she chose to pursue her other interests in college including psychology and mathematics. Halfway through her undergraduate career, she became interested in biology and briefly considered pursuing a degree in biology. It was at this same time that she discovered from an academic advisor that biology can be studied through the lens of mathematics. She was interested in attending graduate school and was excited by this new area of academia to explore called mathematical biology. In attempts to flee the cold long winters of Vermont, she applied to schools further south and got accepted to Virginia Commonwealth University to pursue a masters in Statistics and a Ph. D. in Systems Modeling & Analysis. This allowed her to explore more areas of mathematical biology which included learning some pretty cool things about coral reefs and their wound healing mechanisms. She hopes to continue her post-graduate career modeling biological systems and continuing to bake for friends and family in her free time 😊.