10/31/24

My goal is to have this primer as a reference for our experimental design, terminology, and methods we’ve incorporated so far.

Biological terminology:

* Neutrophils – largest population of white blood cells in the body, “first responders” to fighting off an infection. Neutrophils are typically in an “unactivated” state as they flow through the circulatory system, but when they receive signals indicating a pathogen is present they will become activated. Activated neutrophils bind to the side wall of a blood vessel and “extravasate” between endothelial cells to leave the vessel, entering the surrounding tissue matrix. These neutrophils will then continue to migrate towards the source of infection, killing bacteria by eating them or releasing toxic chemicals.
* Endothelial cells – the primary cell that makes up the blood vessel wall. When these cells become activated by signals indicative of infection, they upregulate proteins on their surface that recruit neutrophils to bind and extravasate across the vessel wall. Endothelial cells are key activators in the neutrophil recruitment cascade, as well as serving as a barrier between the circulatory system and surrounding tissue.
* Extravasation – The process by which neutrophils squeeze between endothelial cells to exit a blood vessel and enter surrounding tissue.
* Extracellular matrix (ECM) – A collection of proteins and carbohydrates that if the foundation of non-cellular components of tissue. The extracellular matrix provides structural support for all tissues.
* Collagen – collagen is a protein that is found in extracellular matrix. In our model, we create collagen gels to represent the ECM environment.
* Lumen – This is the term we use for our model blood vessels. The lumen consists of a hollow cylinder in a matrix of collagen. This lumen wall is coated with endothelial cells to mimic a blood vessel, and the lumen is then loaded with neutrophils for our experiments.

Experimental setup:

Our experiment consists of adding a bacteria source to our devices, such that there is bacteria distal to the lumen. The bacteria give off activating signals that change the behavior of the endothelial cells and neutrophils. Our goal is to quantify how neutrophils become activated and extravasate from the lumen. In particular, the experiment we are analyzing data for consists of changing collagen concentrations to understand the impact on neutrophil extravasation.

Biological replicates: Each day of experimentation, endothelial cells and immune cells were all obtained from 1 source and used across all technical replicates performed on that day. Five separate biological replicates were performed, each with 2-4 technical replicates per experimental condition.

Technical replicates: Each experiment was run with 4 technical replicates, meaning 4 copies of the same condition were run to account for slight variations between lumens. Some of these technical replicates on a given day fail, so only successful replicates have been included in the datasets. 2-4 technical replicates per condition are included in the data.

Data quantification – To control for differences in neutrophil loading density, the extravasated neutrophil count is normalized against initial number of neutrophils present in the lumen at time t=0 hours. The normalized neutrophil count is not so much percentage of neutrophils extravasated as it is controlling for loading density, such that values above 1.0 are possible and not a concern for our analysis.

Data:

Chris\_Extrav.txt – Data for Chris’s original analysis. Split into 3 columns, one for experiment (biological replicate), one for combined collagen concentration + timepoint of data (e.g. 2mg/ml\_3 indicates 2 mg/mL collagen at hour 3), and one column with normalized neutrophil count. This data is called in the R code “Chris\_Extrav\_Analysis.R”

SplitData.xlsx – excel sheet containing the same data as Chris\_Extrav.txt but split out to include separate columns for collagen concentration and timepoint. Also includes some adjusted values (adjusted to remove zeros and allow logarithmic transform) and log values that I tested in some models. This is the code used in “ModelTrials.R”

Code:

Chris\_Extrav\_Analysis.R – Original analysis method, treats each variable as a factor and creates a simple linear regression model to perform emmeans statistical analysis and run pairwise comparisons across all conditions to check for statistical significance between results.

ModelTrials.R – My attempts at model creation, I played with several things such as splitting out variables, treating time as a continuous variable, using logarithmic transforms and fitting to a model, etc. None of the various models I tried improved the diagnostic plots meaningfully, and in most cases it seems like the fit got worse with my adjustments.