Proposal for Wound Healing Assay (Scratch Test) on Endothelial EaHy.926 Cells Using Caffeine

Hypothesis

We hypothesize that caffeine will influence endothelial cell migration and wound healing rates. If low to moderate doses of caffeine is used to assist in cell cellular proliferation and migration, then the endothelial cells should accelerate in closing the wound. However, we would expect that high doses of caffeine will slow down the rate of cell migration.

Objective

We aim to determine whether caffeine enhances or impairs endothelial wound healing. We want to determine the most efficient amount of caffeine required for the most effective wound healing rate for endothelial cells. To test our hypothesis, we will perform a wound healing assay (scratch test) on endothelial cells with and without caffeine treatment. The experimental procedure will include the following steps:

1) Endothelial cells will be cultured under standard conditions in complete growth medium in wells
2) Once the cells have covered the bottom on the entire well, a razor will be used to make an 'X' on each of the bottom of the wells.
3) A sterile pipette tip will be used to create a uniform scratch in the confluent cell monolayer.
4) Cells will be treated with different concentrations of caffeine ranging from 0 to 0.5mM to study dose-dependent effects. The negative control will not have any caffeine.
5) Wound closure will be monitored throughout 48 hours. We chose to use 48 hours due to our previous laboratory session on February 3, 2025, showing that without caffeine, the cells completely heal from the wound by the 48-hour mark. Pictures will be taken throughout the process at hours 0, 6, 12, 18, 24, 30, 36, 42, and 48 using a phase contrast inverted microscope with 4x objective (camera for image acquisition).
6) The images captured will allow for the analysis of the cells to quantify the migration and closure rate of the wound area during the different time intervals.
7) We will then compare the wound healing rates to the different treatment groups.

Literature Resources

Ojeh et al 2014 studied the effects of caffeine on wound healing. They used human epidermal keratinocytes and HaCaT cells that were cultured in complete medium and placed in an incubator to control temperature levels. The MTT assay was used to measure proliferation. The cells were treated with varying caffeine doses ranging from 0.1 to 5 mM. The controls they used included untreated cells, positive with EGF (epidermal growth factor) and KGF (keratinocyte growth factor), and negative controls used to detect contaminations and unexpected results. The cells were grown to confluency and scratched with a pipette tip. Caffeine was applied and the wound closure was monitored over 27 hours. Jonkman et al 2014 wrote an article on wound healing assay and describe the different ways to statistically graph and analyze the wound healing rate. We will use this article as a guide when determining how to resent our data. Another article written by Wang et al studied how Caffeine (50 μM) promotes endothelial cell motility through the signaling pathways of cAMP/PKA/AMPK. This article mainly focused on the physiological aspects of cells undergoing the scratch wound healing assay with caffeine.

References:

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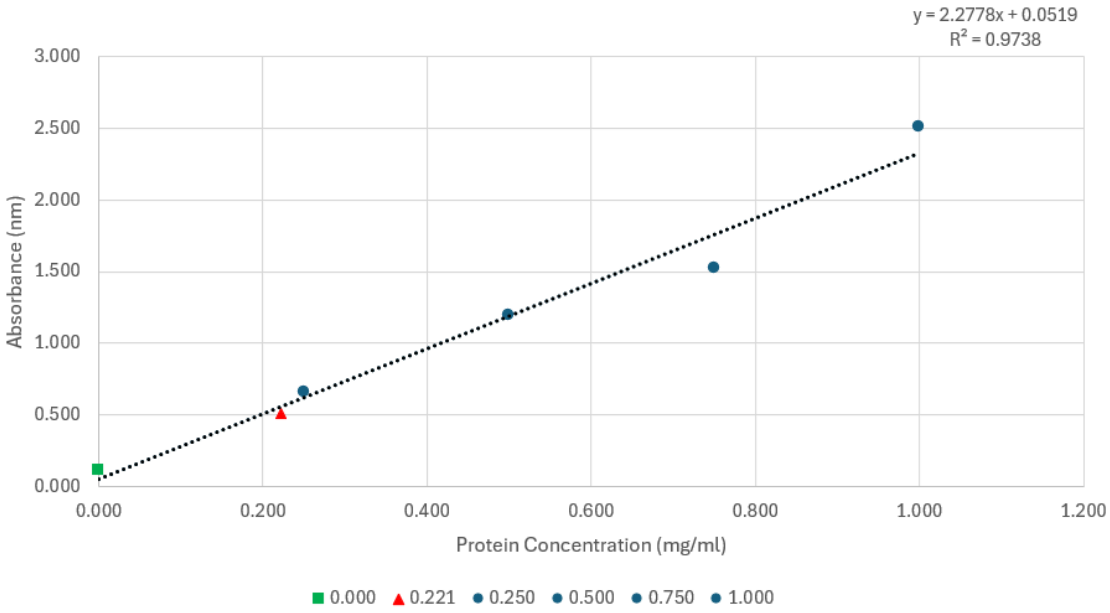

Figure 1. The Average Protein Concentration (mg/ml) vs. Absorbance (nm) of three trials for each Load. The Loads include the Reference (green square), Sample (red triangle) and Standards (blue circle).