



Characterization of the Effects of Aging on the THP-1 Human Monocyte ▶ Cell Line

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Outline

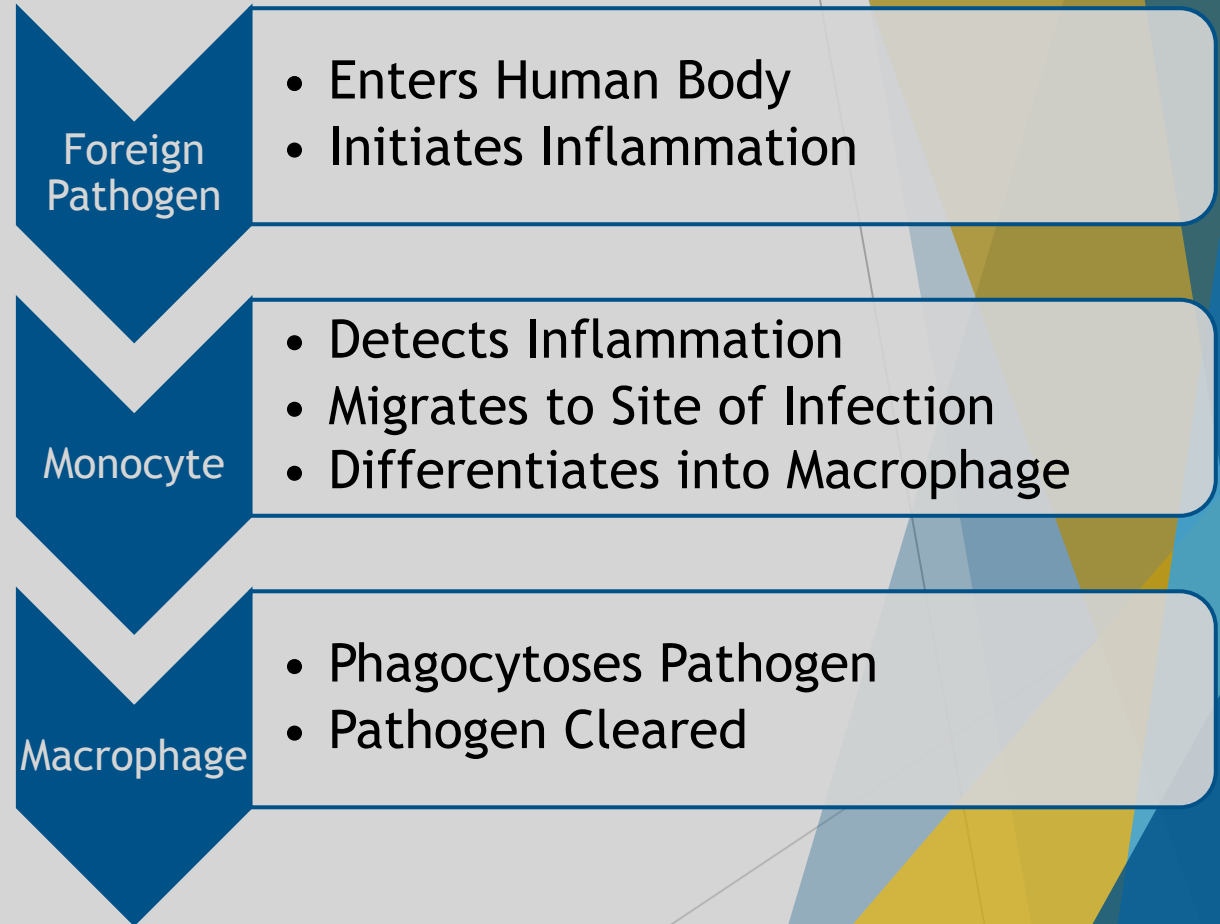
- ▶ Introduction
 - ▶ Human Monocyte
 - ▶ THP-1 Cell Line
 - ▶ Project Objective
 - ▶ Methodology
- ▶ Analysis of Endpoints
 - ▶ TNF- α
 - ▶ Migration
 - ▶ Phagocytosis
 - ▶ Viability
 - ▶ Growth Rate and Doubling Time
- ▶ Acknowledgments

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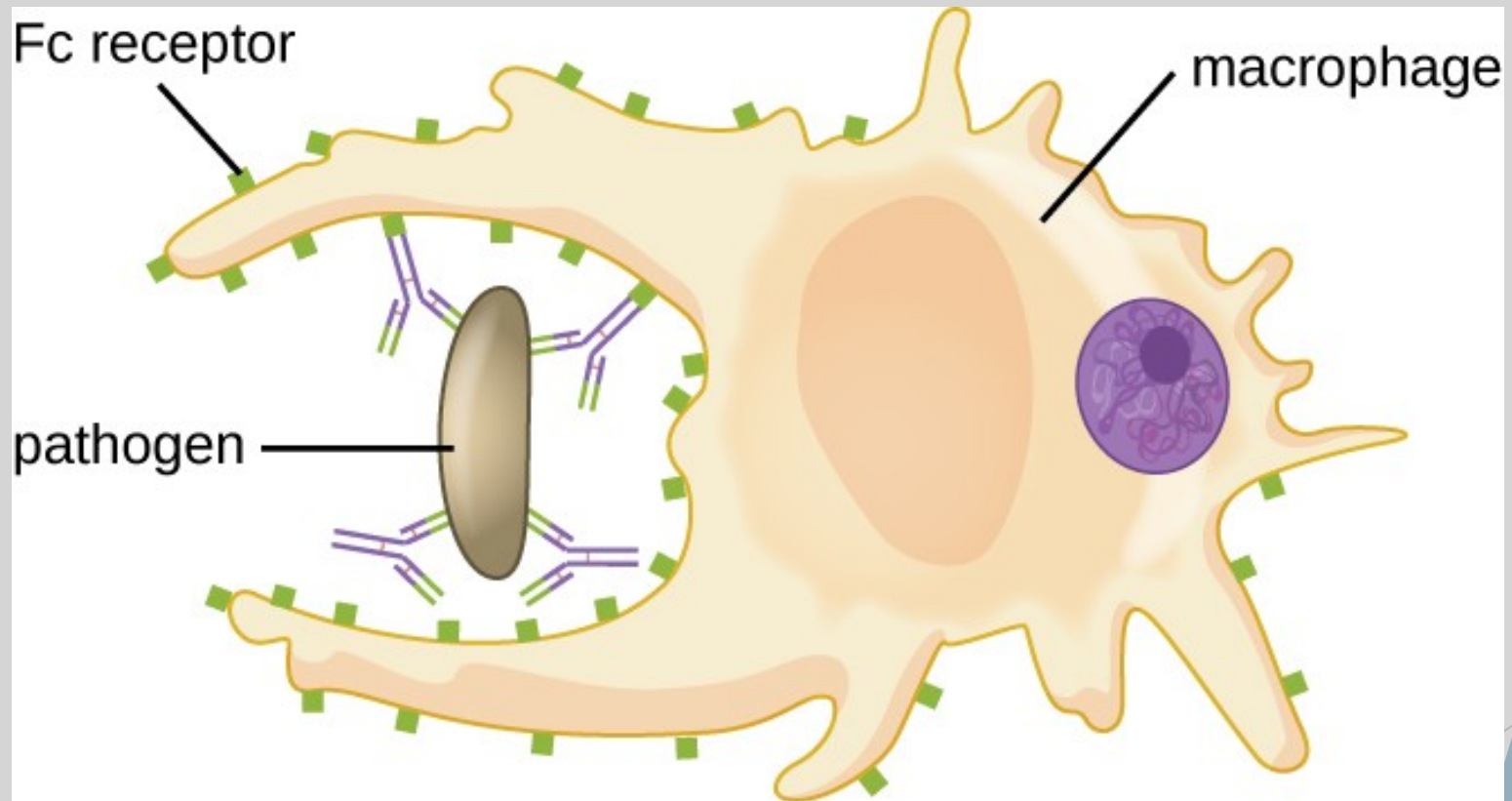
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Human Monocyte

- ▶ Leukocyte (White Blood Cell)
 - ▶ Functions in Innate Immunity
- ▶ Differentiate into Macrophage
 - ▶ Secretes TNF- α
 - ▶ Vascular Permeability
 - ▶ Engulfs Pathogen via Phagocytosis
 - ▶ Destroys Pathogen

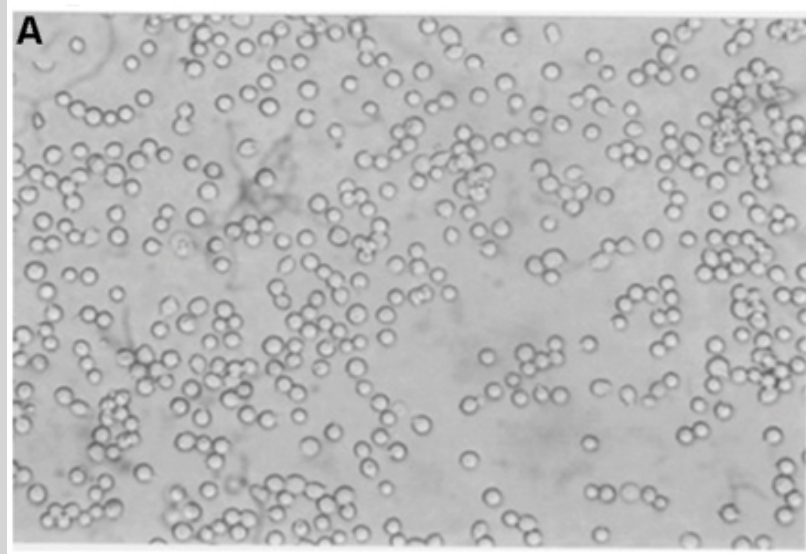


Human Monocyte



THP-1 Cell Line

- ▶ Monocytic Cell Line
- ▶ Derived from an Acute Monocytic Leukemia Patient in 1980
- ▶ Commonly Used for Immunological Translational Research



Project Objective

- ▶ To characterize the effects of continuous culture on the THP-1 cell line while also looking at the effects of added glucose at concentrations of 0 mM, 5 mM, and 25 mM as a model for uncontrolled diabetes.

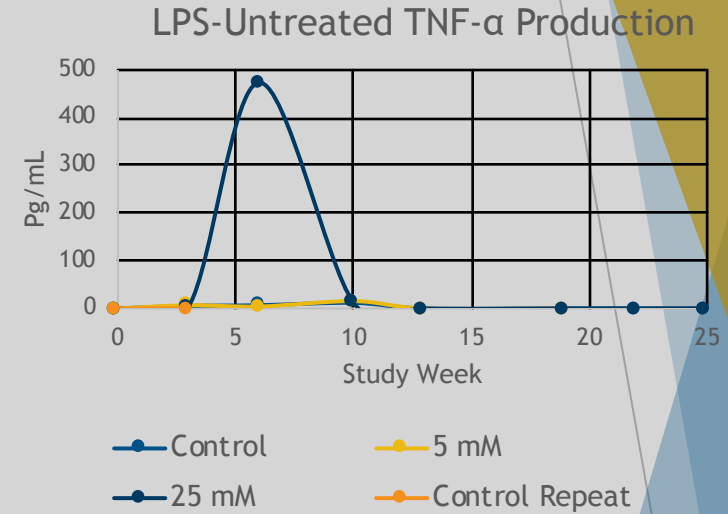
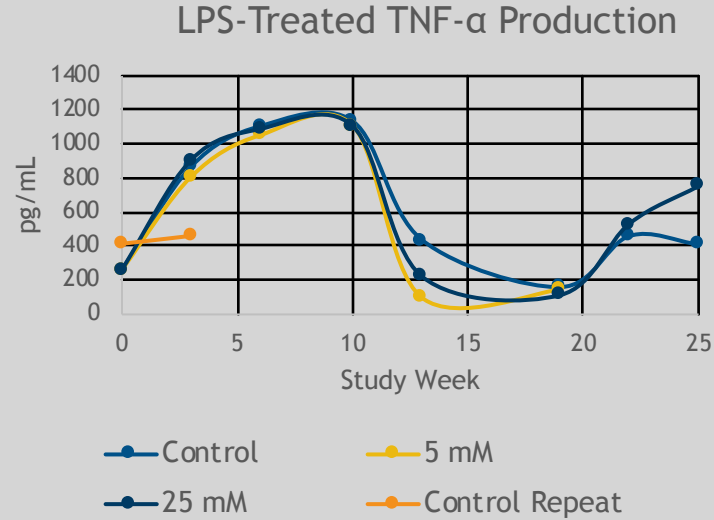
Methodology

- ▶ 3 Different Cultures
 - ▶ 0 mM of Additional Glucose (Control)
 - ▶ 5 mM of Additional Glucose
 - ▶ 25 mM of Additional Glucose (Hyperglycemia)
- ▶ Experiments Completed
 - ▶ Weekly
 - ▶ Cell Count/Replication Rate
 - ▶ Cell Viability
 - ▶ Tri-weekly
 - ▶ Phagocytosis
 - ▶ Migration
 - ▶ TNF- α Production
- ▶ Study Duration
 - ▶ 26 Weeks

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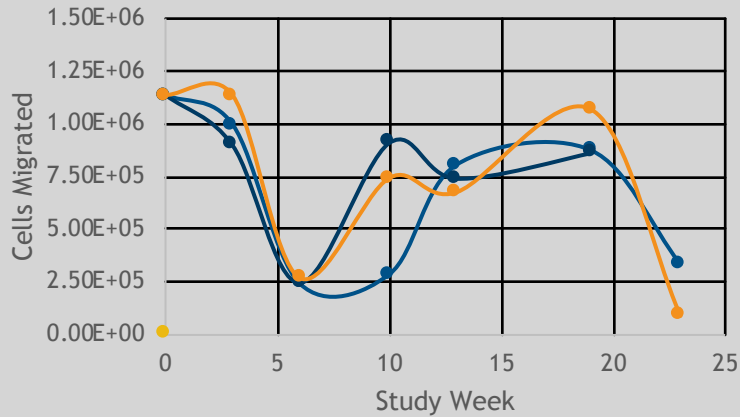
TNF- α Production



- ▶ LPS-Treated
 - ▶ No Consistent Trend
 - ▶ Beginning Study Week 10, Less TNF- α is Produced
- ▶ LPS-Untreated Control
 - ▶ Consistently Negligible
 - ▶ Exception of Study Week 6
- ▶ Conclusion
 - ▶ Media Glucose Concentration and Culture Age Do Not Appear to Impact TNF- α Production

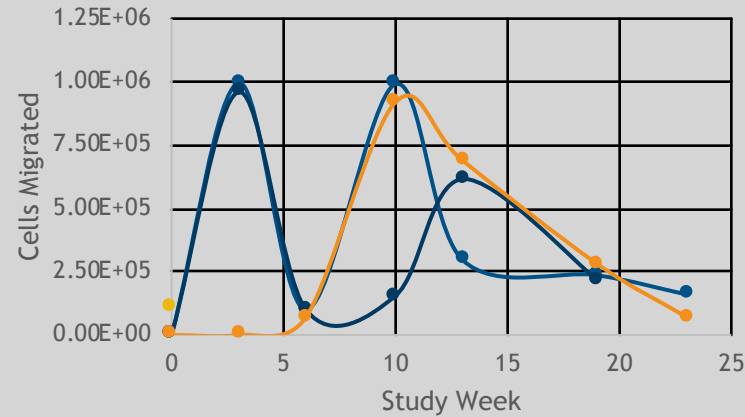
Migration

MCP1-Treated Migration



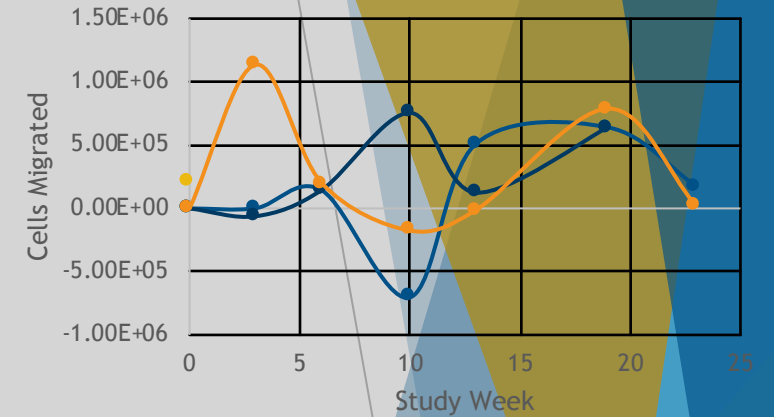
Control Control Repeat 5 mM 25 mM

MCP1-Untreated Control Migration



Control Control Repeat 5 mM 25 mM

Net Migration



Control Control Repeat 5 mM 25 mM

▶ MCP1-Treated

- ▶ Inverse of Results Seen for TNF- α
- ▶ Migration Decreases in Later Weeks

▶ MCP1-Untreated Control

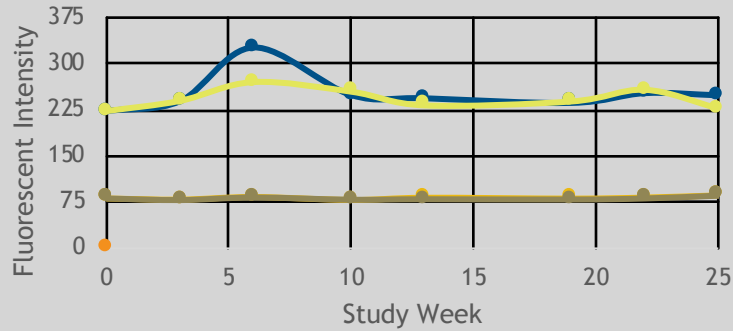
- ▶ No Consistent Trend Prior to Study Week 13
- ▶ Net Migration is Impacted

▶ Conclusion

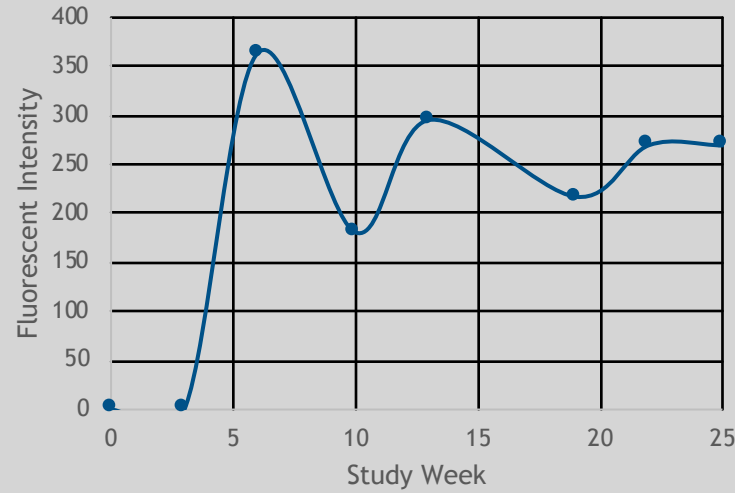
- ▶ Media Glucose Concentration and Culture Age Do Not Appear to Impact Migration Ability

Phagocytosis

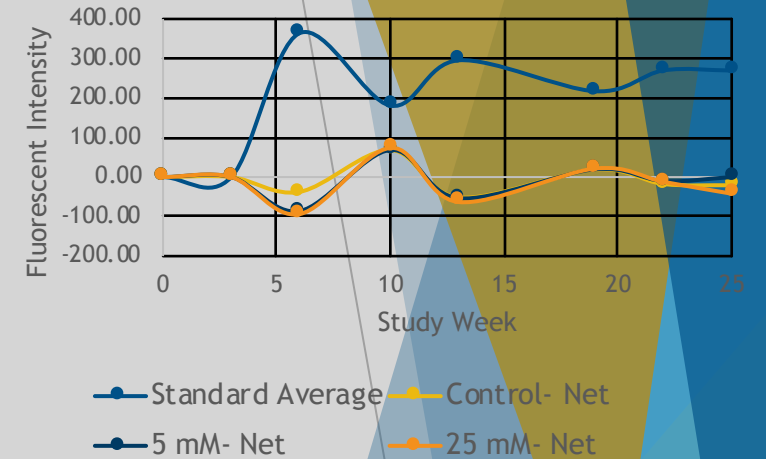
PMA-Treated vs PMA-Untreated



Fluorescent Standard

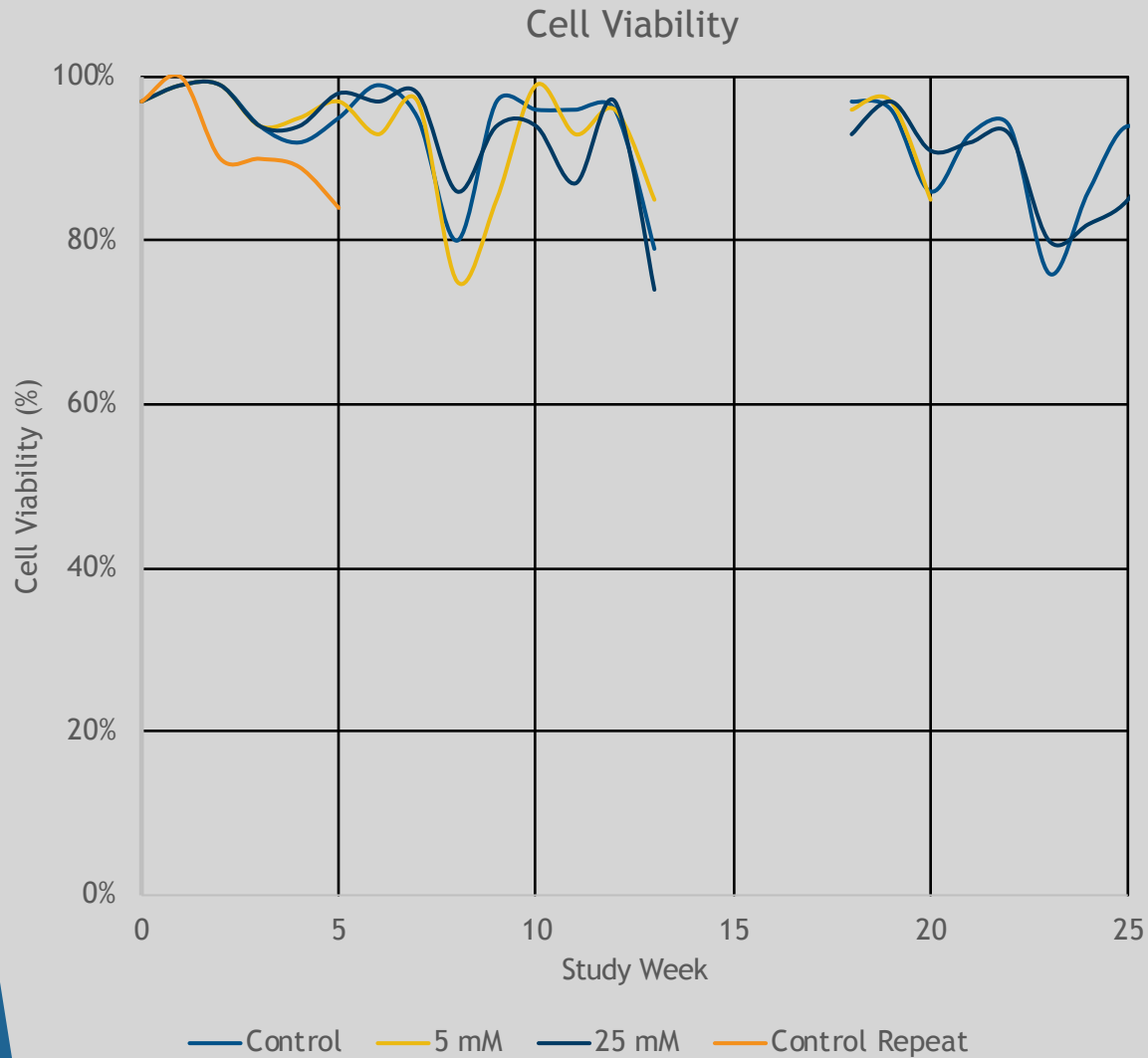


Net Fluorescence vs Fluorescent Standard



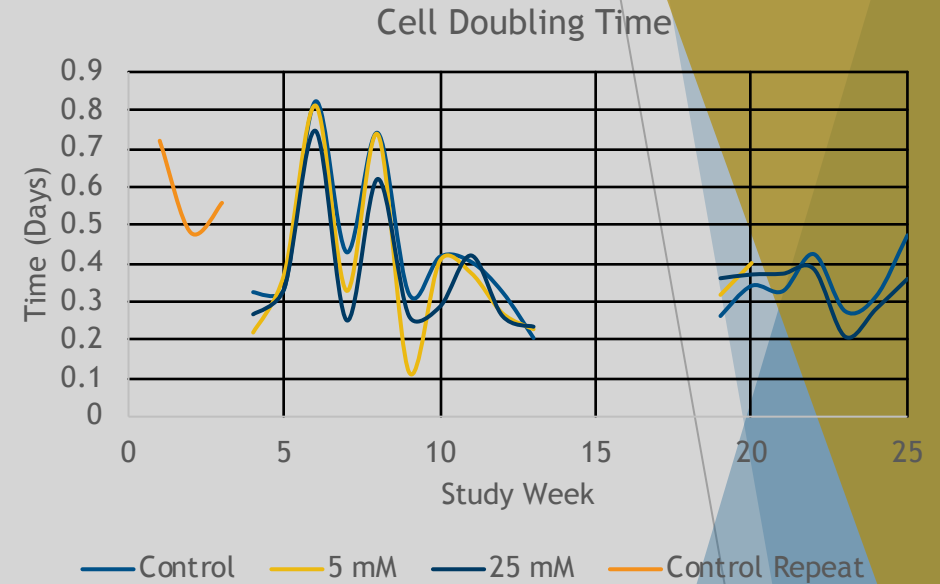
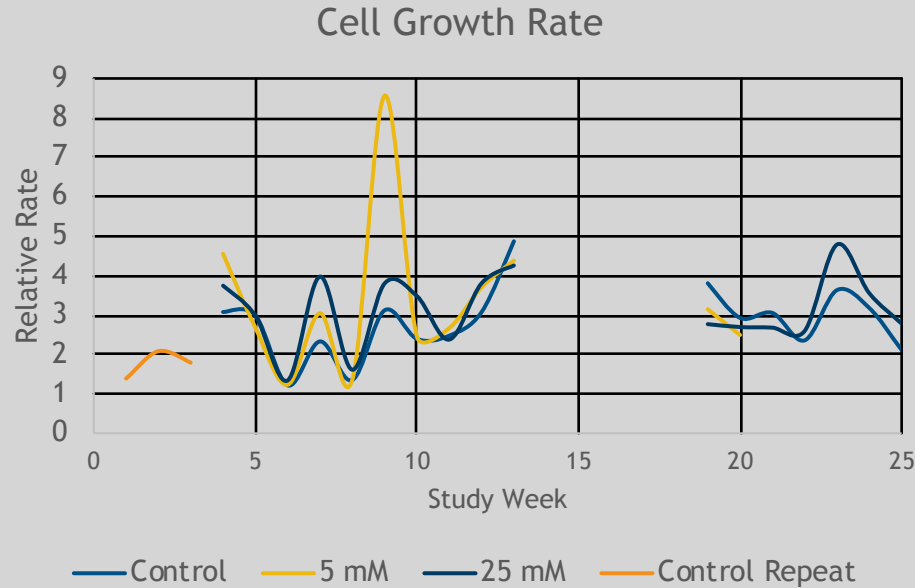
- ▶ PMA-Treated
 - ▶ Relatively Constant Throughout Study
- ▶ PMA-Untreated Control
 - ▶ Consistently Minimal Autofluorescence
- ▶ Conclusion
 - ▶ Media Glucose Concentration and Culture Age Do Not Appear to Impact Phagocytotic Ability
 - ▶ Standard Inconsistency Inversely Correlates to Net Fluorescence

Viability



- ▶ Data Not Reported For Study Weeks 14 Through 18
- ▶ Viability Consistently Within Acceptable Range
- ▶ Slower Rate of Recovery in Later Weeks
 - ▶ Not Statistically Significant

Growth Rate and Doubling Time



- ▶ Weekly Cell Counts Used
 - ▶ Data Not Reported For Study Weeks 14 Through 18
- ▶ Doubling Time is Inverse of the Growth Rate
- ▶ No Consistent Trends Prior to Study Week 10
 - ▶ Growth Rate Increases, Then Decreases Beginning Study Week 19
 - ▶ Not Statistically Significant

Conclusion

- ▶ The THP-1 cell line was found to be stable in continuous culture for up to 26 weeks regardless of media glucose concentration.
- ▶ Upon Review of Data, Many Results Changed Following Study Week 10
 - ▶ During Study Week 9, Incubator CO₂ Levels Adjusted by Another Researcher Due to Difficulty Growing Another Cell Line
 - ▶ CO₂ Concentration May Impact Monocyte Health and Immunological Abilities

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- ▶ **Acknowledgments**

Thank You

- ▶ Dr. Kaleb Pauley
- ▶ Katie Flint
- ▶ Claire Burton
- ▶ Marie Butts

References

- ▶ Devaraj, S., Venugopal, S. K., Singh, U., & Jialal, I. (2005). Hyperglycemia Induces Monocytic Release of Interleukin-6 via Induction of Protein Kinase C- α and $-\beta$. *Diabetes*, 85-91.
- ▶ Kuppan, G., Balasubramanyam, J., Monickaraj, F., Srinivasan, G., Moha , V., & Balasubramanyam, M. (2010). Transcriptional Regulation of Cytokines and Oxidative Stress by Gallic Acid in Human THP-1 Monocytes. *Cytokine*, 229-234.