Development and Optimization of a High-Throughput Assay for Quantifying Phagocytosis in a Human Neutrophil-Like Cell Line

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Poster: #P184

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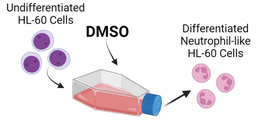
Background

- Phagocytosis, a central effector function of neutrophils, is essential for proper innate immune defense.
- Due to a lack of high-throughput methods for quantifying phagocytosis, it is often not assessed when determining the safety of a chemical.
- To test environmental chemicals for potential immunotoxicity, we developed a high-content imaging assay that quantifies the phagocytic ability of neutrophil-like HL-60 cells.

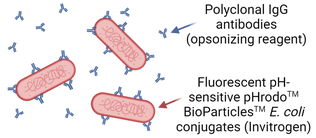
Methods

1. Differentiate HL-60s with DMSO

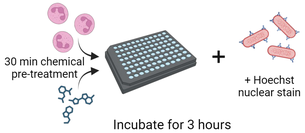


Allow cells to differentiate for 5 days

2. Prepare pHrodo BioParticles



3. Add pHrodo BioParticles to plated cells that have been untreated or pre-treated with chemicals



Incubate for 3 hours

4. Image on Opera Phenix + and quantify fluorescence

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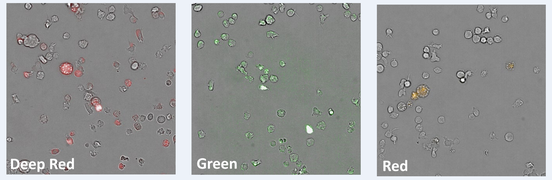
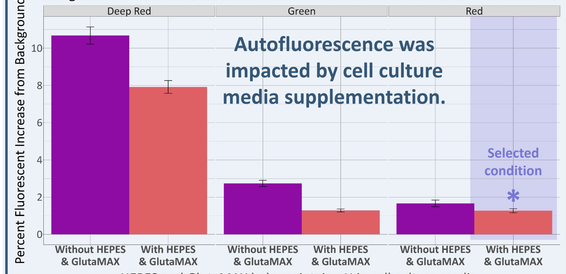
Assay Optimization & Parameter Selection

Autofluorescence was strongest in the deep red channel.

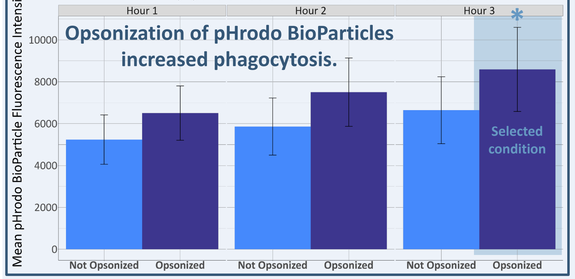


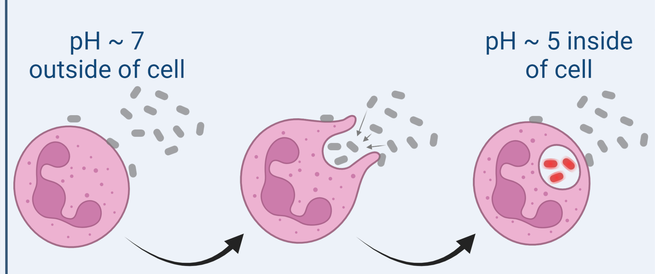
Quantification of HL-60 Autofluoresence in Deep Red, Green, and Red Channels Using Iscove's Modified Dulbecco's Medium With and Without HEPES and GlutaMAX



HEPES and GlutaMAX help maintain pH in cell culture media.

Phagocytosis of Opsonized and Non-opsonized pHrodo BioParticles 1, 2, and 3 Hours Post Addition to HL-60 Cells





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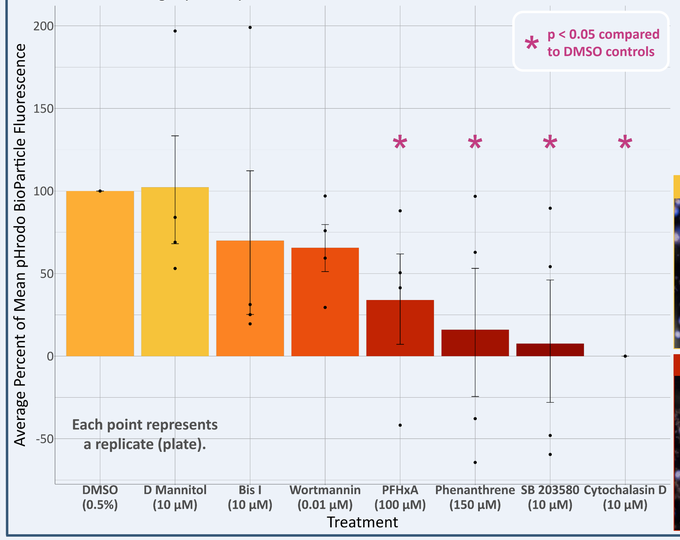
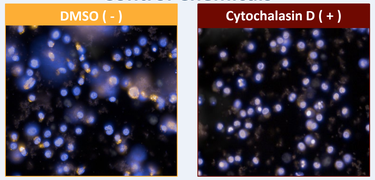
Percent Phagocytosis Quanitified by Normalized Red Fluorescence of Phagocytosed pHrodo BioParticles in Differentiated HL-60 Cells

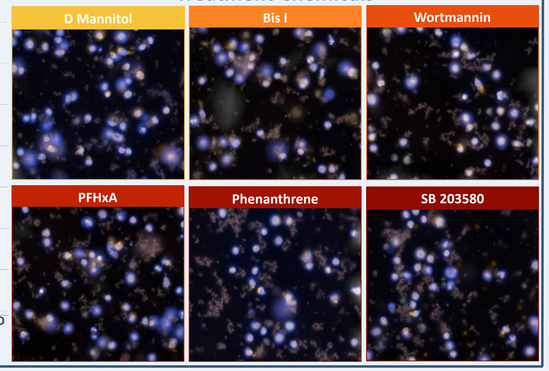


Chemical	Rationale
Cytochalasin D (positive control)	Actin polymerization inhibition and disruption of actin microfilaments
D Mannitol	Expected negative control (no inhibition)
Bisindolylmaleimide I (Bis I)	Highly selective protein kinase C (PKC) inhibitor
Wortmannin	Irreversible phosphatidylinositol 3-kinase (PI3K) inhibitor
SB 203580	Irreversible p38 mitogen-activated protein (MAP) kinase inhibitor
Phenanthrene	Polycyclic aromatic hydrocarbon shown to inhibit neutrophil intracellular redox balance
Perfluorohexanoic Acid (PFHxA)	Unknown, selected for future project considerations

Control Chemicals



Treatment Chemicals



Conclusions

- Differentiated HL-60 cells autofluoresced most strongly in the deep red channel, and this fluorescence was affected by cell culture media supplementation.
- Opsonization of pHrodo BioParticles increased phagocytosis.
- High-content imaging is a successful method for analysing HL-60 cell phagocytosis of red pHrodo BioParticles.
- This *in vitro* high-throughput assay will be used to screen chemicals for their immunotoxic potential via phagocytosis inhibition (i.e., immunosuppression) or activation (i.e., hypersensitization).

Next Steps

- Continue testing reference chemicals, including more PFAS compounds
- Characterize differentiated neutrophil-like HL-60 cells via immunophenotyping
- Compare Opera Phenix + results to phagocytosis results from flow cytometry

Imaging-based analysis provides a high-throughput approach for quantifying chemically-induced changes to phagocytosis in neutrophil-like HL-60 cells.

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