Effects of E-liquids on M1 Macrophage Phagocytosis of *Aggregatibacter Actinomycetemcomitans*

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Abstract

Background: A 2020 CDC study revealed that 19.6% of US high school students are self-reported vapers, or users of aerosol releasing electronic-cigarette devices (ECIGs). ECIGs vaporize e-liquids containing various flavors into the oral cavity -- the entry point of the vapor. The oral cavity houses a microbiome of bacterial species that may be implicated in oral disease. *Aggregatibacter actinomycetemcomitans,* a gram-negative pathogenic species of bacteria, occurs in 90% of juvenile periodontitis, a chronic inflammatory disease characterized by the destruction of tooth-supporting tissues. M1 Macrophages are host white blood cells responsible for phagocytosis -- a cell ingesting process -- of pathogenic microbes. The purpose of this study is to determine the immunological effects of e-liquids -/+ flavors on M1 Macrophage phagocytosis of *A. actinomycetemcomitans.*

Methods: THP-1 human monocyte cell lines were first grown in RPMI, stimulated with 200nM phorbol 12-myristate 13-acetate at 37℃, 5% CO_2 (standard conditions) and later differentiated to M1 macrophages with 100ng/mL lipopolysaccharide and 20ng/mL interferon γ. Then, M1 macrophages were exposed to both flavorless and flavored (cinnamon, menthol, strawberry, and tobacco) e-liquids at a final concentration of 1% (v/v) in RPMI (standard conditions) as well as untreated control cells. Treated and control M1 macrophages were then exposed to *A. actinomycetemcomitans* at an MOI of 100, allowing macrophage phagocytosis to take place. Excess bacteria were removed by washing and M1 cells were incubated with RPMI antibiotic media to kill extracellular bacteria. M1 cells were then washed and lysed with sterile water. Lysates from each well were serially diluted and plated on agar for CFU counts.

Results: We expect that -/+ flavored e-liquids will decrease M1 Macrophage phagocytosis of *A. actinomycetemcomitans* compared to control. This study may indicate that e-liquids and vaping increase the risk of developing periodontal disease via modulation of host M1 macrophage phagocytosis efficacy, particularly among children and adolescents.

Introduction

- The oral cavity is home to a microbiome of hundreds of bacterial species, including pathogenic species like *Aggregatibacter actinomycetemcomitans* -- a gram-negative facultative anaerobe frequently associated with localized aggressive periodontitis, especially among young children and adolescents (1).
- M1 Macrophages are known to play a critical role in the host's immunological response in performing phagocytosis of oral pathogens (2).
- Cigarette smoke has been shown to impair alveolar macrophage phagocytosis (3), but few studies have been conducted evaluating the effects of e-liquids upon macrophage phagocytic ability.
- The aim of this study is to test the effects of E-liquids upon macrophage differentiation, survival, and the ability of M1 Macrophages to phagocytose oral pathogen *A. actinomycetemcomitans*.

Materials & Methods

Cytotoxicity Assay

- THP-1 human monocyte cells were grown in Roswell Memorial Park Institute (RPMI) media containing 10% Fetal Bovine Serum (FBS) + penicillin and streptomycin (P/S) at 37° C, 5% CO_2. The cells were cultured to about 90% confluency (4 days) and then passaged to achieve a cell concentration of about 200,000 cells/mL (determined via hemocytometer counts).
- Phorbol 12-myristate 13-acetate (PMA) was introduced to THP-1 cells to M0 Macrophage differentiation at a final [PMA] = 200 nM. One milliliter (200,000 cells) mixtures were pipetted into a 24-well plate and incubated for 24 hours.
- M0 morphologies were captured via light microscopy (100X)
- Ten microliters of M1 cocktail was added to each corresponding wells according to Figure 1, consisting of:
  - 2 µL of 100 ng/mL K12 *E. coli* LPS
  - 5 µL of 20 ng/mL human rIFN-γ
  - 3 µL of RPMI media
- E-liquid ingredients are described in Figure 3.
- E-liquid flavors: tobacco, cinnamon, menthol, strawberry, and flavorless E-liquids were added to their corresponding wells (Figure 1) at 1% and incubated for 24 hours.
- Well morphologies were subsequently taken via light microscopy (100X).
- Supernatants were collected to determine cell death, quantified via CyQUANT™ LDH Cytotoxicity Assay (Invitrogen) following manufacturer instructions.

M1	M1 + E-liq (Flavorless)	M1 + E-liq + Tobacco	M1 + E-liq + Cinnamon	M1 + E-liq + Menthol	M1 + E-liq + Strawberry
M1	M1 + E-liq (Flavorless)	M1 + E-liq + Tobacco	M1 + E-liq + Cinnamon	M1 + E-liq + Menthol	M1 + E-liq + Strawberry
M1	M1 + E-liq (Flavorless)	M1 + E-liq + Tobacco	M1 + E-liq + Cinnamon	M1 + E-liq + Menthol	M1 + E-liq + Strawberry
M1	M1 + E-liq (Flavorless)	M1 + E-liq + Tobacco	M1 + E-liq + Cinnamon	M1 + E-liq + Menthol	M1 + E-liq + Strawberry

Figure 1: 24-well plate map for Cytotoxicity Assay

Results

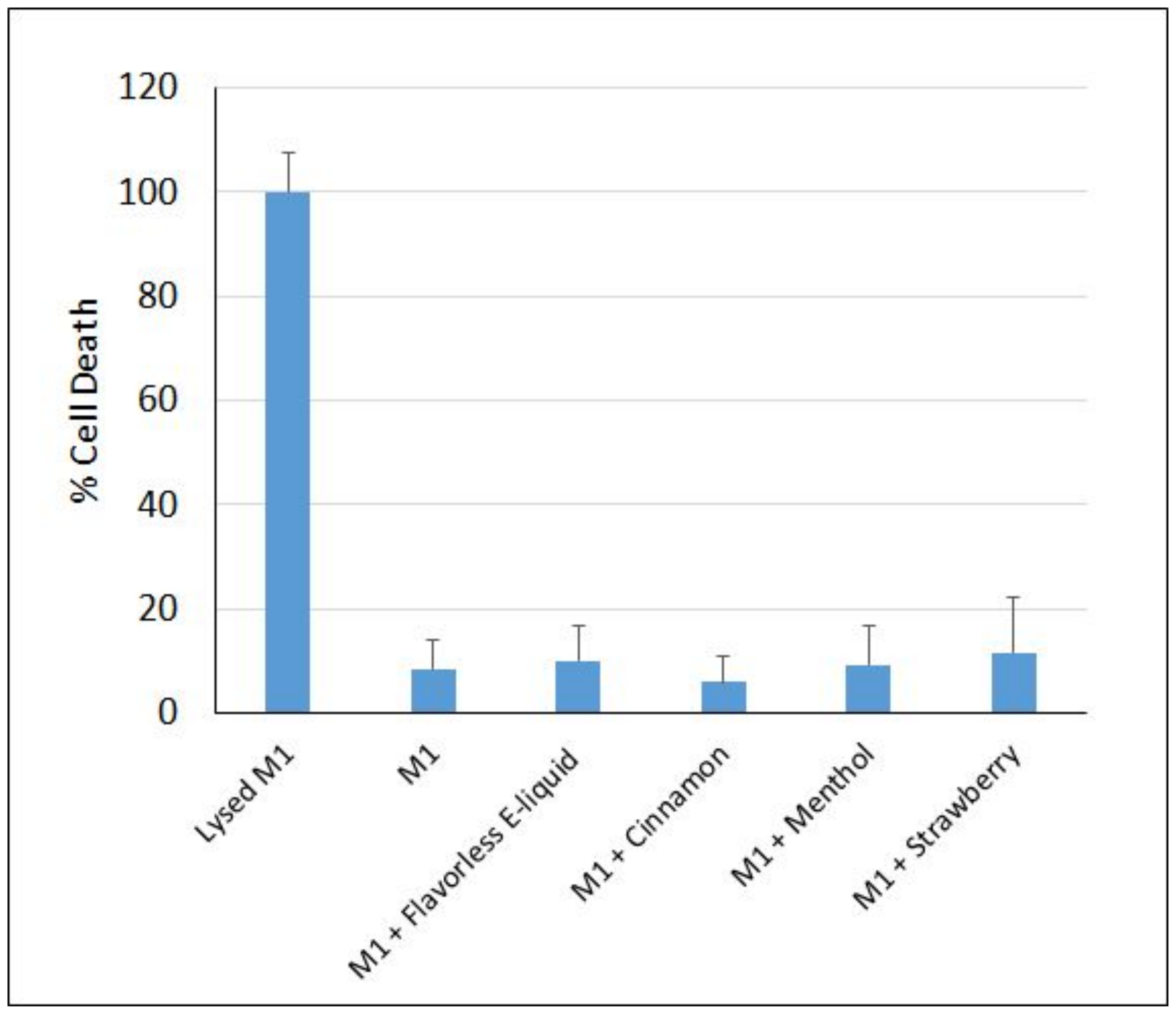

Figure 2: Cytotoxicity Assay. M1 Macrophage cells + 1% E-liquids +/- flavoring cell death quantification. Lysed and unlysed M1 Macrophages serve as controls.

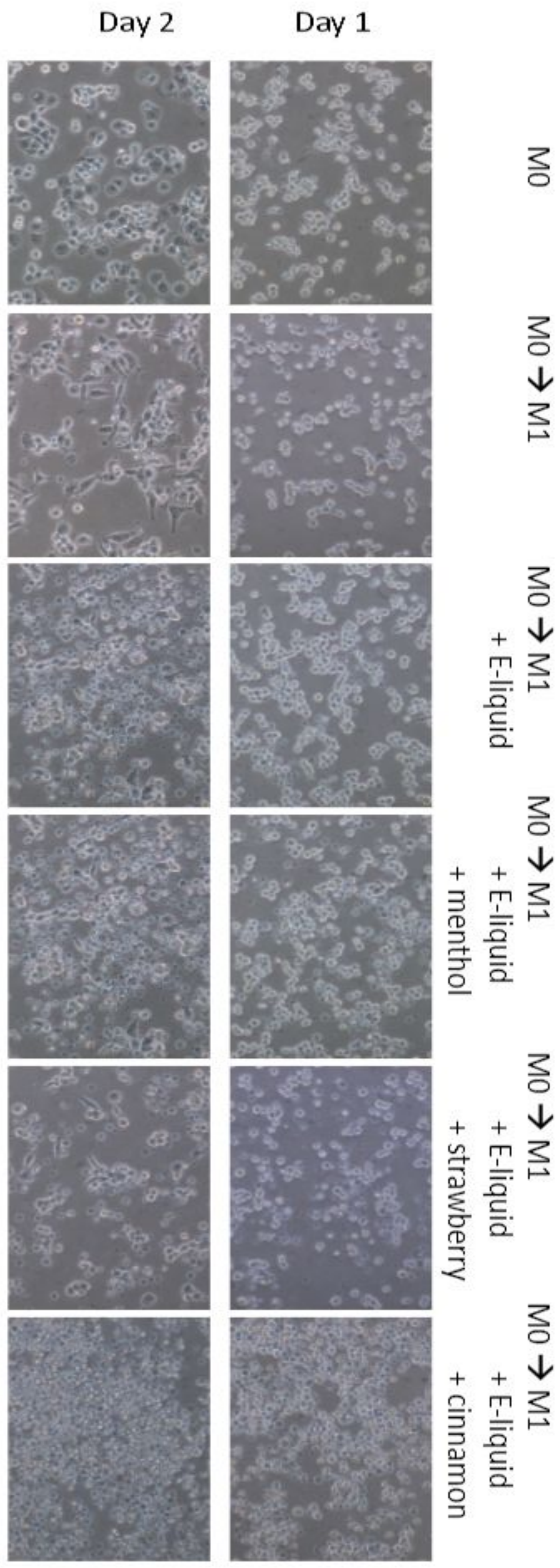

Figure 5: Light Microscopy Captured Cell Morphologies

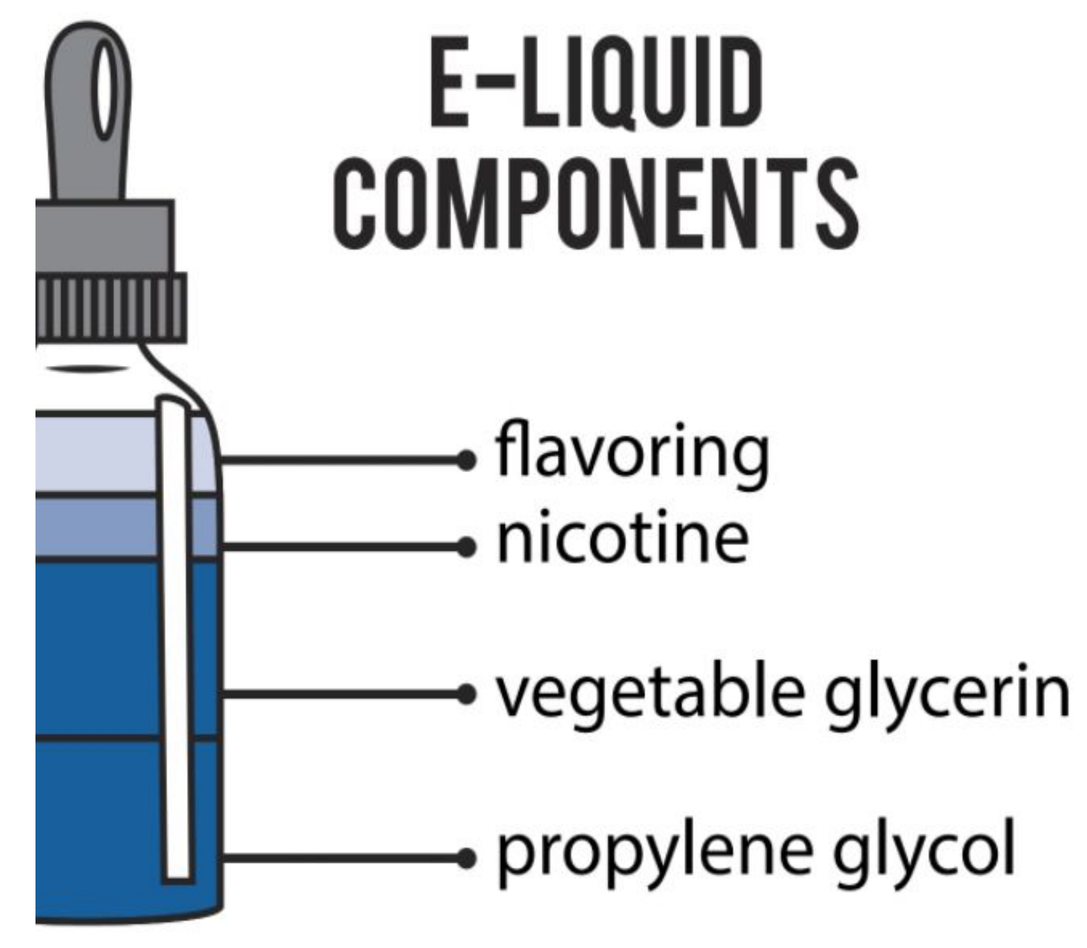

Figure 3: E-liquid Mixture Components

Conclusion

- Cytotoxicity Assay data successfully indicated that flavorless and flavored 1% E-liquids do not have a statistically significant impact on M1 cell death.
- Thus, 1% E-liquids are insufficient in compromising M1 cell membranes, suggesting that 1% E-liquids used for the Phagocytosis Assay may stress, but do not kill, M1 Macrophages.

Future Directions

- Determine the effects of 1% E-liquids on M1 Macrophages over the course of 3 days.
- Conduct Phagocytosis Assays with *A. actinomycetemcomitans* and M1 Macrophages to evaluate the effects of E-liquids on Macrophage phagocytic function (quantified via CFU counts).
- In addition to *A. actinomycetemcomitans,* oral pathogen *Porphyromonas gingivalis* can be similarly studied to determine their ability to modulate M1 Macrophage phagocytic function.

References

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Acknowledgements

- Muhlenberg College Biology Department and members of The Cuadra Lab
- THP-1 cells provided by Dr. Angela Brown from Lehigh University