Flavor Classification and Differential Toxicity of Oral Nicotine Pouches(ONPs) in Lung Epithelial Cells

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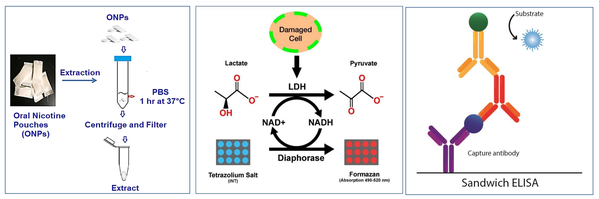
INTRODUCTION

- Nicotine-containing products (NCPs) such oral nicotine pouches (ONPs) are substitutes for traditional tobacco products, and are becoming increasingly popular due to their perceived safety and extensive use of various flavors (menthol, fruit, drink, and tobacco etc.)
- Lack of a standardized classification of ONPs and increased use of synthetic nicotine compounds has complicated regulatory oversight, leading to the unregulated manufacture of ONPs.
- The potential systemic toxicity and negative effect from consuming ONPs is poorly understood.

HYPOTHESIS

Flavored ONPs are unsafe and likely to cause increased reactive oxygen species production and inflammation

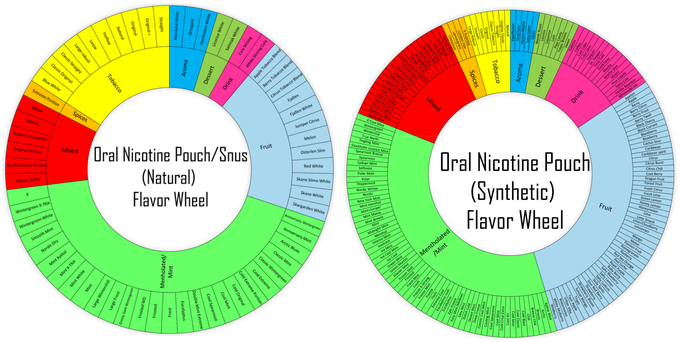
METHODS



- ONP pouches were classified by flavor and type of nicotine (tobacco derived vs synthetic) in a wheel diagram
- ONPs with similar nicotine concentrations (menthol, tobacco and fruit flavors) were agitated in PBS (1:10 w/v) for 1h at 37°C. Extract was filtered (100%)and used to treat lung epithelial cells (Beas-2b and 16HBE) at 0.25% and 1% concentrations.
- 4 h following treatment, reactive oxygen species production (ROS) was measured by CellROX Green. Mean image intensity was calculated in Image J.
- 24 h following treatment, cytotoxicity (LDH release) by Roche kit and inflammation (IL-8 and IL-6) were measured by ELISA

RESULTS

Flavor Classification Wheel



ONP Extraction

Table 1. Selected ONPs

Product-type	Brand	Flavor	Nicotine-type	Nicotine Concentration (mg)
ONP	ON	Original	TFN	8
ONP	Rogue	Mango	TFN	6
ONP	Velo	Black Cherry	TFN	7
ONP	Zyn	Cool Spearmint	TFN	6
Snus	SKOAL	Spearmint	TDN	5

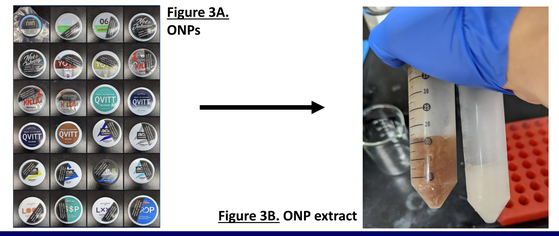
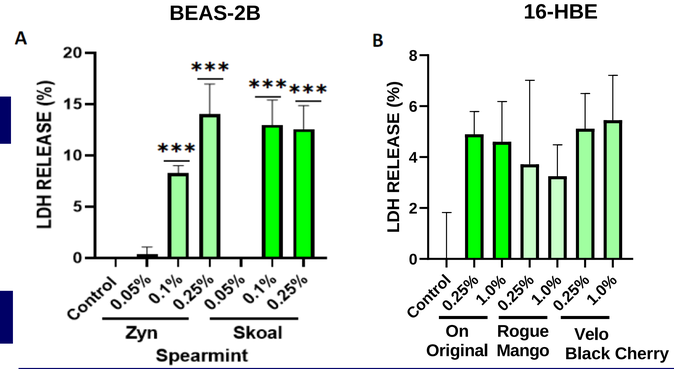


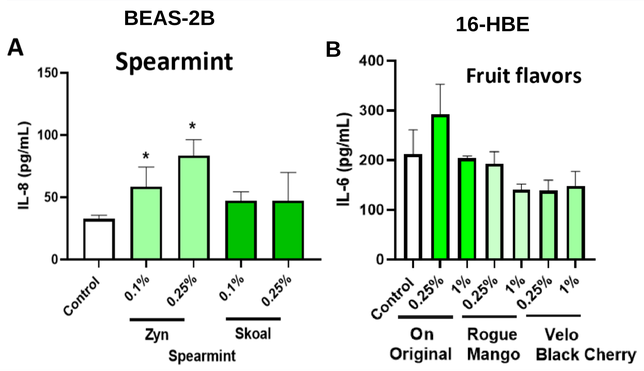
Figure 3A. ONPs

Figure 3B. ONP extract

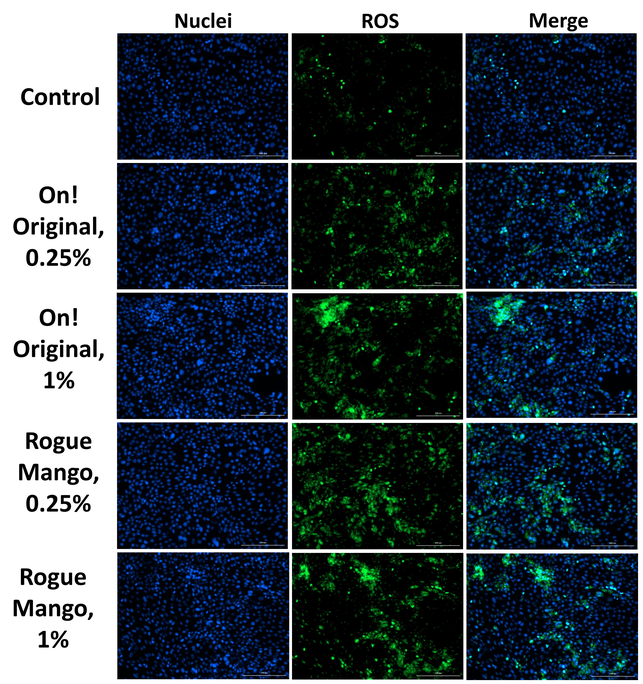
Differential Cytotoxicity of ONPs

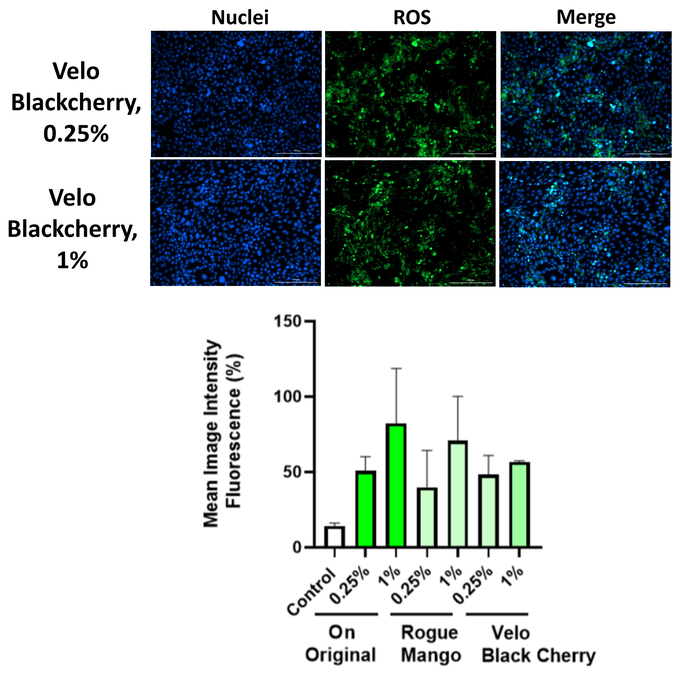


Differential Inflammatory Responses



Reactive Oxygen Species production in ONP treated 16HBE cells





SUMMARY

- 2 major categories of ONPs are identified
- 8 main categories of flavors
  - Tobacco, menthol, fruit, aroma, spices, drink, dessert and mixed.
- Cytotoxicity
  - BEAS-2B: 0.1% and 0.25% menthol flavor treatment show significant increase.
  - 16HBE: 0.25% and 1% tobacco, mango and black cherry flavor treatment show differential LDH release.
- Inflammatory Response
  - BEAS-2B: 0.1% and 0.25% spearmint flavor treatment show significant increase in IL-8 level for ZYN but not for Skoal
  - 16HBE: IL-6 level does not show significant changes
- Oxidative Stress
  - Treatment groups show higher ROS level, albeit these difference is not significant.

CONCLUSION

- Biomarkers including inflammatory cytokines, oxidative stress and cytotoxicity show different levels of toxicity induced by ONP to bronchial epithelial cells.
- Further studies would be done on oral epithelial cells
  - TFN product vs TDN product
  - Flavor comparisons
  - Different concentrations

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