Studying the Effects of Electronic Cigarette Exposure on DNA Mutation and Repair in *Tetrahymena thermophila*

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Introduction

- The unicellular, eukaryotic ciliate *Tetrahymena thermophila* (*T. thermophila)* is a popular model organism in molecular biology.
- *APN2* codes for an enzyme involved in single base excision DNA repair.
- Electronic cigarette exposure is suspected to cause point mutations.
- **Hypothesis:** If *T. thermophila* are exposed to electronic cigarette vapors, then *APN2* gene expression will increase and cell growth rates will decrease.

Methods

- **Primer synthesis:** Primers for *APN2* were designed using IDT Oligoanalyzer software.
- **Culturing:** *T. thermophila* cultures were maintained in NEFF media. Upon experimentation, all cultures were transferred into SPP media where experimental cultures were exposed to electronic cigarette aerosol puffs.
- **Electronic Vaping:** Starting at 0900 vapor was drawn from the electronic cigarette using a 12 mL syringe and exposed to the experimental groups every 3 minutes for 9 minutes. This process was repeated twice, once every 4 hours. The following day the entire process was repeated.
- **RNA extraction:** RNA was extracted using Qiagen's RNeasy Mini Kit.
- **Reverse transcription:** cDNA was synthesized using RevertAid RT Kit.
- **Quantitative PCR** was performed using PowerUp SYBR mix. *BTU1* gene expression was used as positive control.
- **Cell Counts** were performed with hemocytometers and light microscopes.

Results

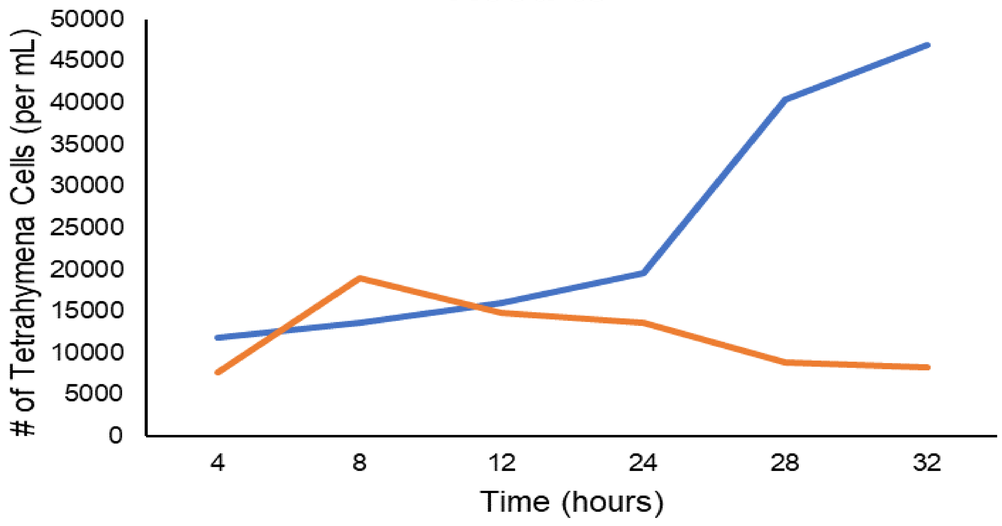

Figure 1: A growth assay was done comparing cell growth in the electronic cigarette vaped experimental group (orange) and the non-vaped control group (blue) over a timed period. P-values are in order as follows: 0. 74, 0.36, 0.61, 0.84, 0.15, 0.04. (n=4 for each group)

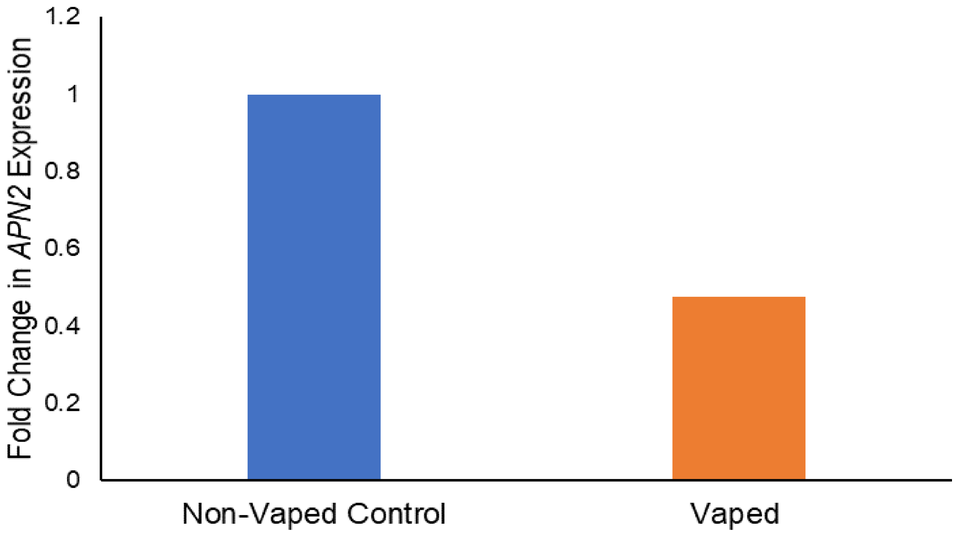

Figure 2: Graphical representation of the fold change in *APN2* expression in the control and experimental *Tetrahymena* cultures, respectively. A t-test was performed assuming unequal variances and yielded a p-value of 0.49. (n=4 for each group)

Conclusions

- The growth assay demonstrated a downward trend in cell longevity in the experimental electronic cigarette vaped cells.
- Both qPCR rounds demonstrated that aerosol exposure resulted in no major change in expression of *APN2*, as seen in Figure 2.
- A t-test performed comparing the gene expression of *APN2* showed there was not a significant statistical difference in expression. p-value: 0.49.
- The results disagreed with our hypothesis on gene expression, but agreed with our hypothesis for cell growth.
- The decline in cell growth does suggest that aerosol exposure is harmful to the cells.

References

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