Electronic Cigarette's Oxidizing Effects Between Stainless Steel and Nichrome Atomizers

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1. Abstract

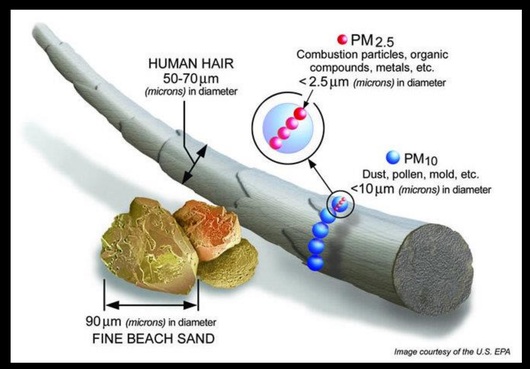
- The goal of this study is to identify the effects of e-cigarette particulate matter and demonstrate the oxidizing potential of its condensate. If e-cigarette particulate matter does result in high oxidizing effects, it can be recognized as a hazardous PM that can be a risk to health issues and lead to disease such as atherosclerosis.
- Macrophages mediate inflammatory responses by releasing super oxide in a process known as respiratory burst. Macrophages phagocytize the inhaled contaminants and generate reactive oxygen species (ROS), such as superoxide and peroxide in order to disinfect and destroy the contaminants. This can lead to localized oxidative stress when normal antioxidant defenses are overwhelmed.
- The ability of particles to elicit ROS production by macrophages may be a function of e-cigarette PM size or chemistry can be assessed in a cell-culture based model system. The model system will include e-cigarette PM with different atomizer components to directly analyze the difference between stainless steel and nichrome coils.
- A DTT oxidative potential assay is a cell free assay used to study the potential of each experimental sample to participate in oxidation chemistry and rapid reactions that lead to oxidative stress. A TOX-4 assay is a cell-based assay used to study the absorbance of natural red stain by living cells directly measuring cell viability. E-cig condensate promoted free radical production in the cell-free DTT assay and is toxic to cells. TOX-4 results determined decreased cell viability in RAW264.7 cells in e-cig condensates as concentration of the aerosol increased.

2. Introduction

- The use of e-cigarette (e-cig) has become popular with recent generations resulting in a two-fold increase in teenage use consisting about 1.78 million middle-school and high school users(2).
- Knowledge of the long-term toxicological and immunological effects of electronic cigarette vapor remains unclear due to its recent introduction into the market.
- E-cigarettes contain an atomizer component directly involved in producing vapor. The properties of the heating coils and atomizer can be customized by users and can be comprised of various metals.
- In this study, e-cigarette vapor generated by a stainless steel and nichrome coil was collected using a closed system allowing condensation to occur. The oxidative properties of the two different e-cig samples were assess in cell free and cell-based assays..
- A dithiothreitol (DTT) assay was performed to analyze the oxidative potential of each PM sample to participate in oxidation chemistry and rapid reactions that lead to oxidative stress (5).
- Cell viability after exposure was determined using the Neutral Red (Tox-4) uptake assay. It provides a quantitative estimation of the number of viable cells in a culture, based on the ability of viable cells to incorporate and bind the neutral red dye in the lysosomes(1).

Hypothesis:

- It's predicted that E-cig vapor condensate will have high levels of oxidative capacity. Additionally, chemical composition variations in e-cig vapor condensates from nichrome versus stainless-steel atomizers will be reflected in differences in cell viability after exposure.



Particulate matter drawn to scale to demonstrate PM 2.5 that results from combustion particles, organic compounds, metals, etc.

3.Methods

E-Cigarette Condensate Extraction:

- A box-mod e-cig device was used to generate vapor in a closed system.
- Nichrome and stainless-steel atomizer components were individually used per sample.
- Puff frequency was set to generate vapor every 30 seconds with 2 seconds of inhalation.
- The glycerol extract was doped with 0.9 g/mL of nicotine.

DTT Assay:

- The loss of DTT in phosphate buffer was measured over time at 37˚C.
- Aliquots of the reaction mixture were taken every ten minutes.
- Aliquots were modified to produce 2-nitro-5-thiobenzoic acid (TNB).
- TNB was quantified using a quartz cell in a spectrophometer.
- Absorption at 412 was measured.

E-Cig Exposure:

- RAW 264.7 cells were cultured in 5% CO2 in an incubator at 37˚C can kept alive with complete media.
- Pure condensate samples were standardized by diluting to 10%, 25%, and 50% in phosphate buffer saline (PBS).
- Cells were mounted on a 96 well plate and exposed to:
  - Stainless steel e-cig condensate
  - Nichrome e-cig condensate
  - DMEM (untreated)

Tox 4 Assay

- Cells are incubated for four hours with a medium containing 10% neutral red.
- The cells are subsequently washed, and the dye is extracted in each well.
- The absorbance is read at 550 nm using a spectrophotometer as indicated in the manufacturer's protocol.

4. Increased Oxidative Potential in E-cig Particulate Matter

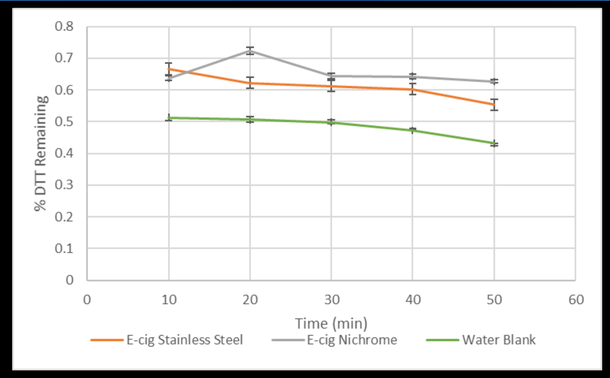


Figure 1. Oxidizing capacity of samples on DTT reagent. A.) Mean levels of % DTT remaining with absorption at 412 nm with +SEM of each particulate matter as a function of time .

- E-cigarette condensate from nichrome based resulted in higher % DTT remaining compared to stainless steel.
- Higher percentage of DTT indicates levels of oxidation occurred on DTT.

5. Decreased Cell Viability between E-cig Steel and Nichrome

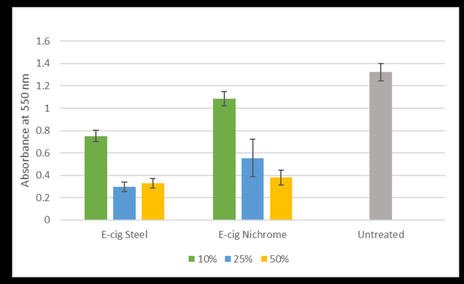


Figure 1. Cell viability trend during TOX-4 assay on RAW264.7. Mean measure of absorbances (+SEM) in varying concentrations (10%,25%,50%) of particulate matter from e-cig condensate and untreated control.

- Cell viability was lower in e-cigarette condensate from stainless steel atomizer compared to nichrome.
- Both e-cig condensates resulted in decreased cell viability as concentration increased.

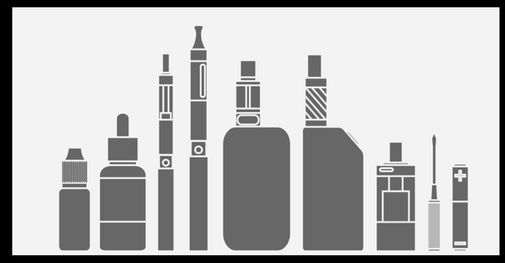


Illustration of various electronic cigarette devices. (3)

6. Conclusions

- E-cig condensate promotes free radical production in the cell-free DTT assay and is more toxic to cells.
- TOX-4 showed there were significant reductions in cell viability at all dose levels for the e-cigs with a trend showing that stainless steel condensate was more toxic than nichrome.
- Nickel is a group 1 carcinogen, is known to be a respiratory and skin irritant and has been associated in inducing lymph node damage and reduced acquired immunity (4).
- Potential source of nickel and chromium is found in the atomizer itself.
- Most nickel and chromium are used to make stainless steel and nichrome and may be important source of this metal being emitted in the aerosol (3).
- Future studies can include a third atomizer not composed of nickel and steel.
  - A Greiss reagent and LDH cytoxicity assay to detect nitrous dioxide and cytotoxicity in e-cig PM samples .

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8. References

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