SUPPLEMENTAL MATERIALS

Chronic e-cigarette use impairs endothelial function on the physiological and cellular levels

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Supplemental methods:

Inclusion Criteria for all participants: 21-50 years old and healthy based on medical history (normal blood pressure, fasting lipid profile and glucose level)

Exclusion Criteria for all participants:

- Physician diagnosis of asthma, heart disease, hypertension, dyslipidemia, thyroid disease, diabetes, renal or liver impairment or glaucoma.
- Unstable psychiatric condition (such as current major depression, history of schizophrenia or bipolar disorder) or current use of more than two psychiatric medications
- Pregnancy or breastfeeding (by history)
- Systolic blood pressure >140 mm Hg
- Diastolic blood pressure >90 mm Hg
- Patient on Viagra, Levitra, or Cialis
- Abnormal lipid profile
- Alcohol, opiate, cocaine, amphetamine or methamphetamine dependence within the past 5 years
- BMI >35 or 18 kg/m^2
- Current opiate, cocaine, amphetamine or methamphetamine use (by history or urine test)
- On anti-coagulant therapy (warfarin, direct thrombin inhibitors, factor Xa inhibitors)
- Occupational exposure to smoke, dusts, or fumes
- Concurrent participation in another clinical trial

- Unable to communicate in English
- Any cardiac or serious conditions, laboratory abnormalities, or psychiatric illness, that is deemed ineligible at the discretion of the investigator
- Unable to withhold the following drugs or nutrients prior to each study visit:

Drug or Nutrient	Duration of hold
Steroid nasal spray	2 weeks
Anti-platelet drugs (aspirin)	7 days
Non-steroidal anti-inflammatory meds (e.g. ibuprofen and naproxen)	5 days
PDE-5 inhibitors (Viagra, Cialis, Levitra)	5 days
Oral antihistamines	5 days
Antioxidant vitamins or supplements	5 days
Sympathomimetic decongestants	3 days
Caffeinated beverages and foods	12 hours
Alcohol	12 hours

Additional Inclusion Criteria for Active Smokers:
Currently smoke >5 cigarettes per day
≥ 1 pack year

Additional Exclusion Criteria for Active Smokers:
Unable to hold marijuana for 1 week prior to each study visit.
Exhaled CO <10 ppm at each visit
Negative salivary cotinine test using a rapid-read, over the counter test with 30 ng/ml cutoff

Additional Exclusion Criteria for Non-smokers:
More than 1 pack year smoking history
Quit smoking < 5 years ago
Ever a daily marijuana smoker
Smoked anything within the last 3 months

Additional Inclusion Criteria for E-Cigarette Users:
Currently use of e-cigarettes > 5 times a week
Has used e-cigarettes for >3 months

Additional Exclusion Criteria for E-Cigarette Users:
Current use of other tobacco products
Unable to refrain from marijuana use for 1 week prior to each study visit

Useful Information

Amount of e-liquid in JUULpod: $700\ uL\ (equates\ to\ 200\ puffs)$

Number of puffs per session of JUUL use: $12 - 15\ puffs$

Amount of nicotine in JUULpod: $40\ mg\ of\ nicotine\ in\ JUULpod$

Amount of PG in JUULpod:

$$700 ul\ of\ e\text{-}liquid\ in\ JUULpod\ \times 0.3 = 210\ ul\ of\ PG \times \frac{1\ mL}{1000\ uL} \times \frac{1.04\ g}{1\ mL} \times \frac{1000\ mg}{1\ g} = 218.40\ mg$$

Amount of VG in JUULpod:

$$700\ ul\ of\ e\text{-}liquid\ in\ JUULpod\ \times 0.6 = 420\ ul\ of\ VG \times \frac{1\ mL}{1000\ uL} \times \frac{1.261\ g}{1\ mL} \times \frac{1000\ mg}{1\ g} = 529.62\ mg$$

Nicotine:

$$\frac{15\ puff\ of\ JUUL\ per\ session}{200\ puff\ in\ one\ JUULpod} \times 40\ mg\ of\ total\ nicotine = 3\ mg\ of\ nicotine\ per\ session$$

$$3\ mg\ of\ nicotine\ per\ session\ \times \frac{1\ g}{1000\ mg} \times \frac{1\ mol}{162.23\ g} \times \frac{1000\ mmol}{1\ mol}$$

$$= 0.018\ mmol \div 5\ L\ of\ blood\ in\ average\ human$$

$$= \frac{0.0037\ mmol}{L}\ of\ nicotine\ in\ circulation$$

Propylene Glycol (PG):

$$\frac{15\ puff\ of\ JUUL\ per\ session}{200\ puff\ in\ one\ JUULpod} \times 218.4\ mg\ of\ total\ PG = 16.38\ mg\ of\ PG\ per\ session$$

$$16.38\ mg\ of\ PG\ per\ session\ \times \frac{1\ g}{1000\ mg} \times \frac{1\ mol}{76.09\ g} \times \frac{1000\ mmol}{1\ mol}$$

$$= 0.215\ mmol \div 5\ L\ of\ blood\ in\ average\ human = \frac{0.043\ mmol}{L}\ of\ PG\ in\ circulation$$

Vegetable Glycerin (VG):

$$\frac{15\ puff\ of\ JUUL\ per\ session}{200\ puff\ in\ one\ JUULpod} \times 529.62\ mg\ of\ total\ VG = 39.72\ mg\ of\ VG\ per\ session$$

$$39.72\ mg\ of\ VG\ per\ session\ \times \frac{1\ g}{1000\ mg} \times \frac{1\ mol}{92.094\ g} \times \frac{1000\ mmol}{1\ mol}$$

$$= 0.431\ mmol \div 5\ L\ of\ blood\ in\ average\ human = \frac{0.086\ mmol}{L}\ of\ VG\ in\ circulation$$

Supplemental Figures:

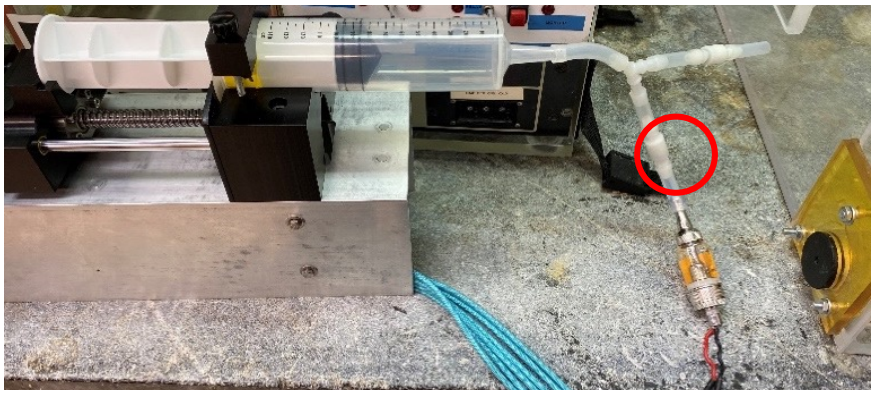

Figure S1. E-cigarette aerosol generation set-up for the collection of condensates. Photograph of the Gram system and the aerosol generation set-up. Red circle indicates the one-way valve where the e-cigarette aerosol condensates are collected.

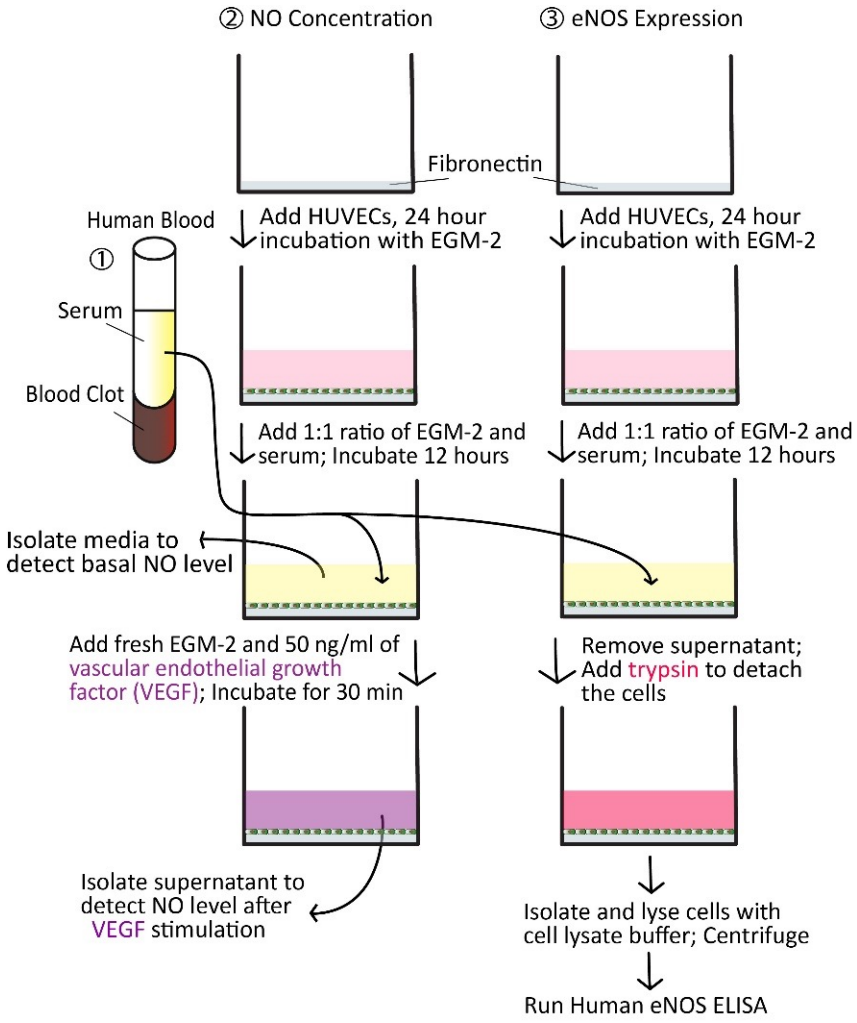

Figure S2. Sequence of events in the endothelial cell culture protocol for NO studies. Cells were plated in two parallel workflows as shown graphically in the two columns.

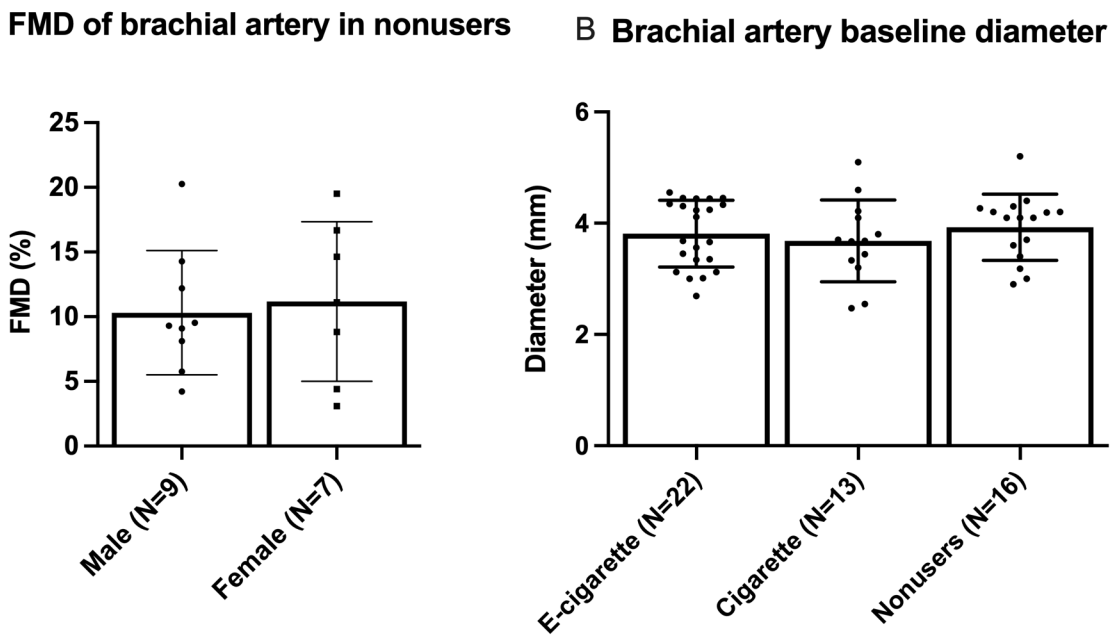

Figure S3. No significant change was observed in FMD between male and female nonusers. Also, there was no significant change in baseline diameter of brachial artery in all groups ($p<.05$ required for significance; all p values were >0.1). Group means were compared by Mann-Whitney test and Kruskall Wallis. Bars=SD.

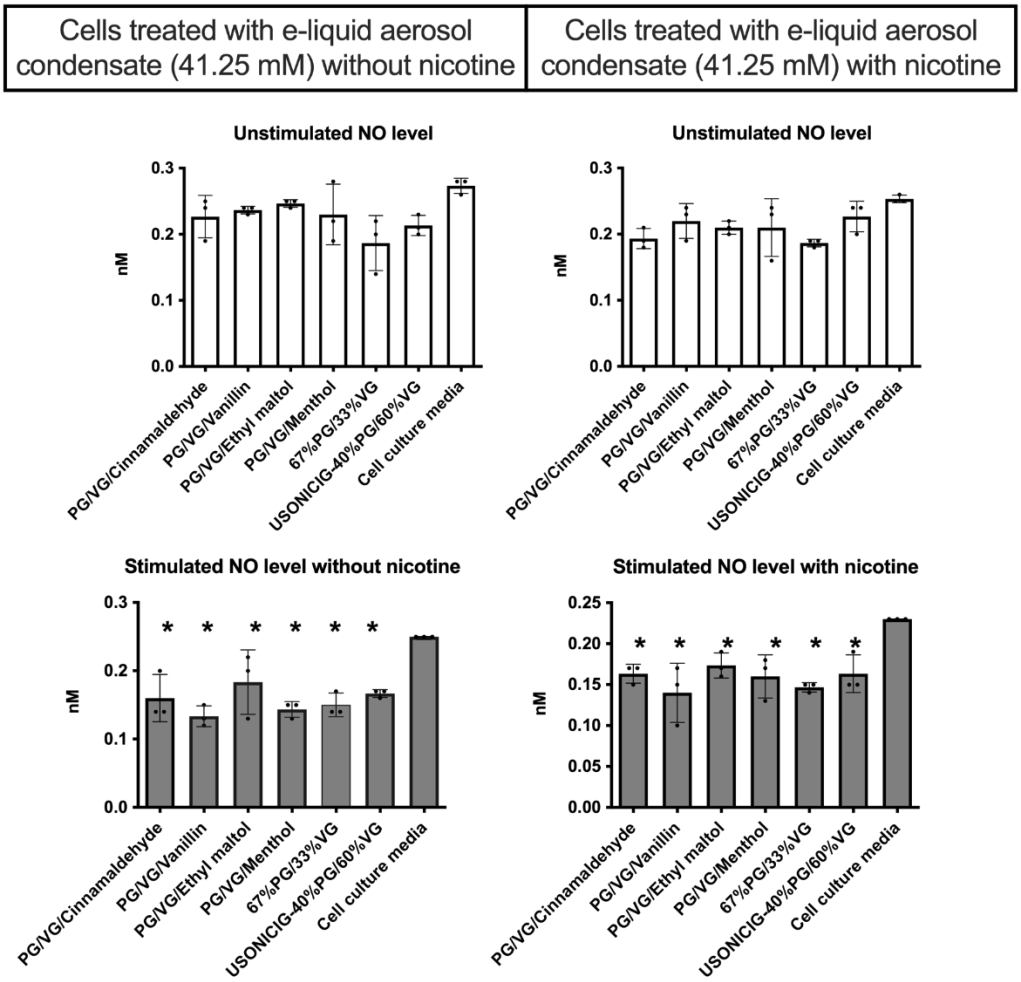

Figure S4. NO level released from HUVECs treated with 41.25 mM (0.3% v/v) aerosol condensate. E-liquid aerosol condensates at this concentration decreased VEGF-stimulated NO production compared to culture medium control (*P<0.05). Group means were compared by Kruskal-Wallis with Dunn's post-hoc adjustment. Bars=SD.

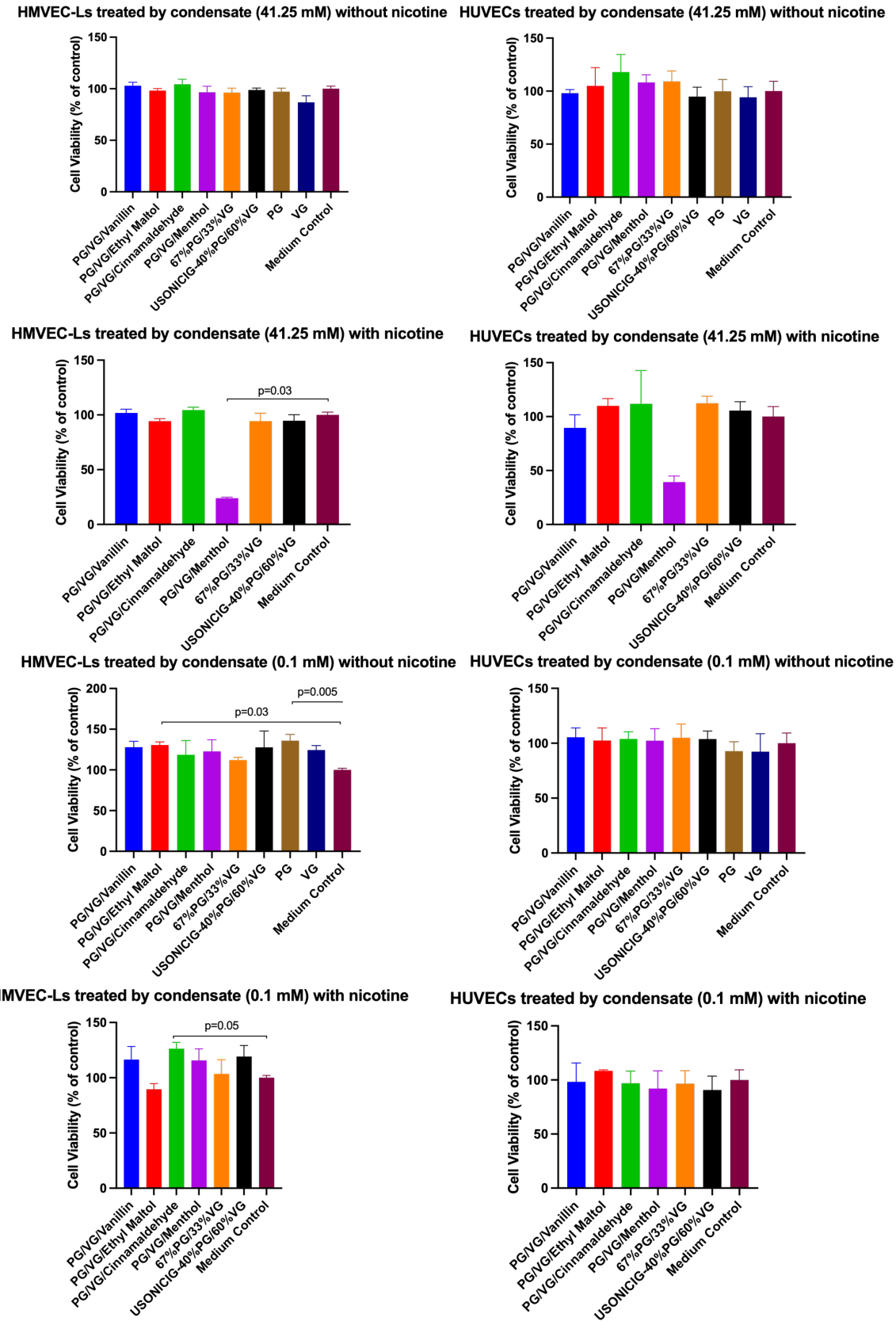

Figure S5. Viability assessment of endothelial cells for different aerosol condensate conditions using CCK8 kit. * is significance (p<.05) from Kruskal-Wallis test with Dunn's post-hoc adjustment. Bars=SD.

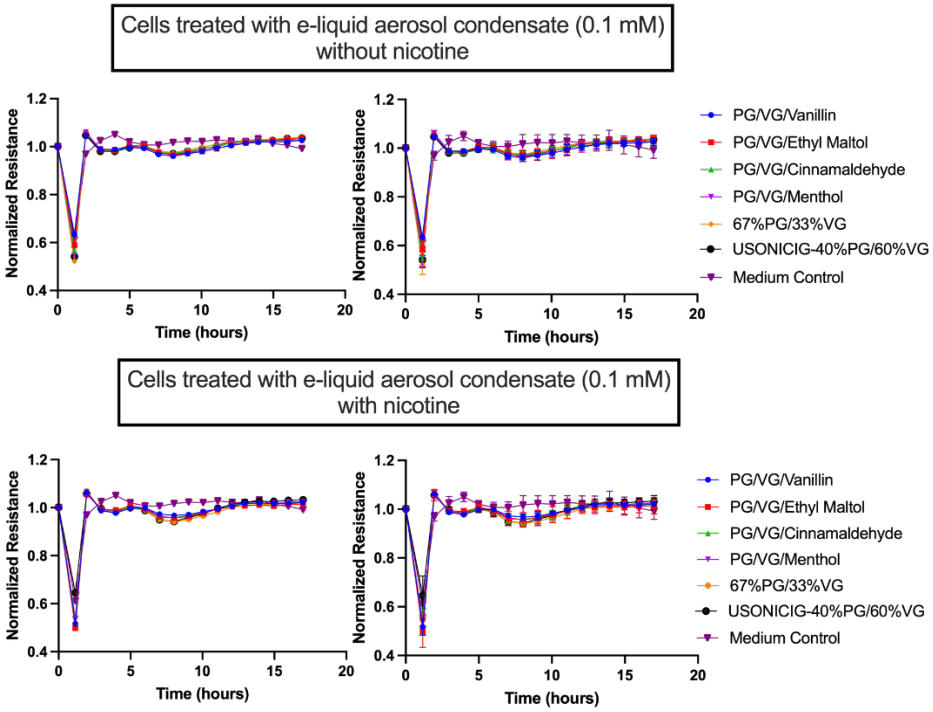

Figure S6. Endothelial cell permeability was not changed in cells treated with e-liquid aerosol condensate in the medium without human serum (0.1 mM). Graph shows resistance across a monolayer of HMVEC-Ls; lower resistance values correspond to greater permeability. $p<.05$ required for significance; all p values were >0.1. Group means at each timepoint were compared by repeated measures ANOVA with post-hoc adjustment. Bars=SD.

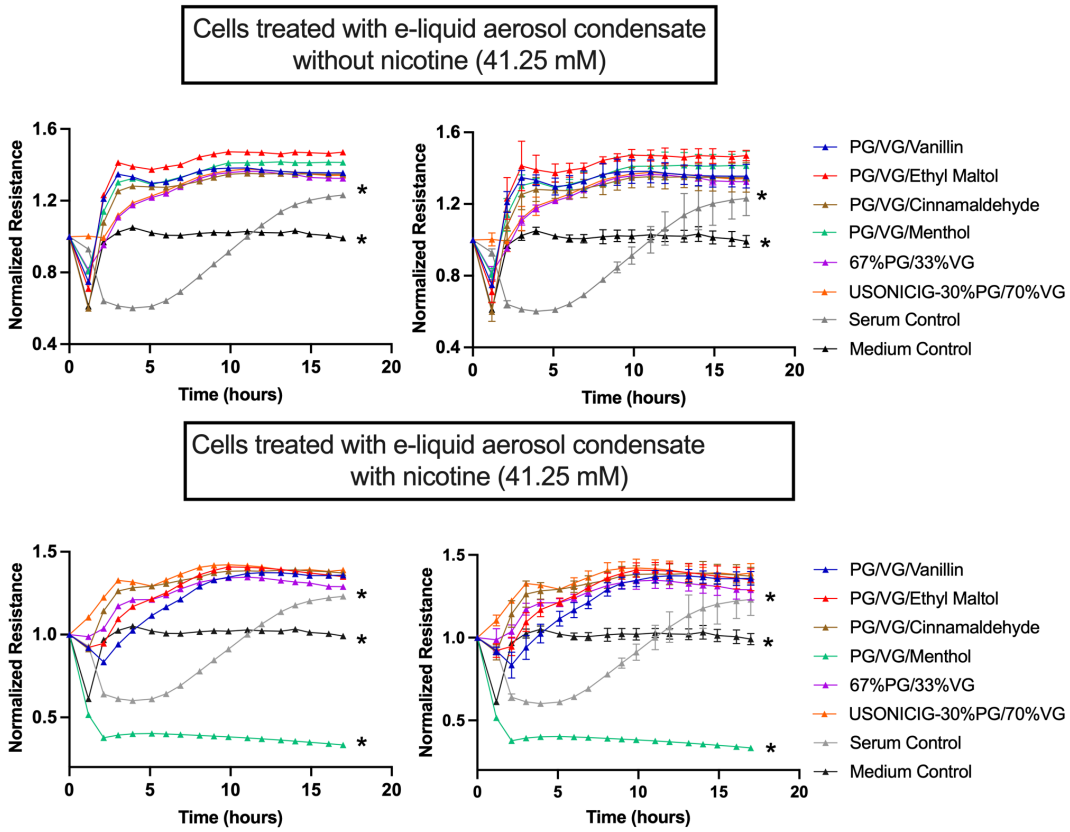

Figure S7. Endothelial cell permeability was decreased in cells treated with e-liquid aerosol condensate (41.25 mM). Graph shows resistance across a monolayer of HMVEC-Ls; lower resistance values correspond to greater permeability. (*p<0.05 for PG/VG/menthol starting at 3 hours, serum control starting at 6 hours and medium control starting at 2 hours compared to all other conditions.) Group means at each timepoint were compared by repeated measures ANOVA with Holm-Šidák post-hoc adjustment. Bars=SD.

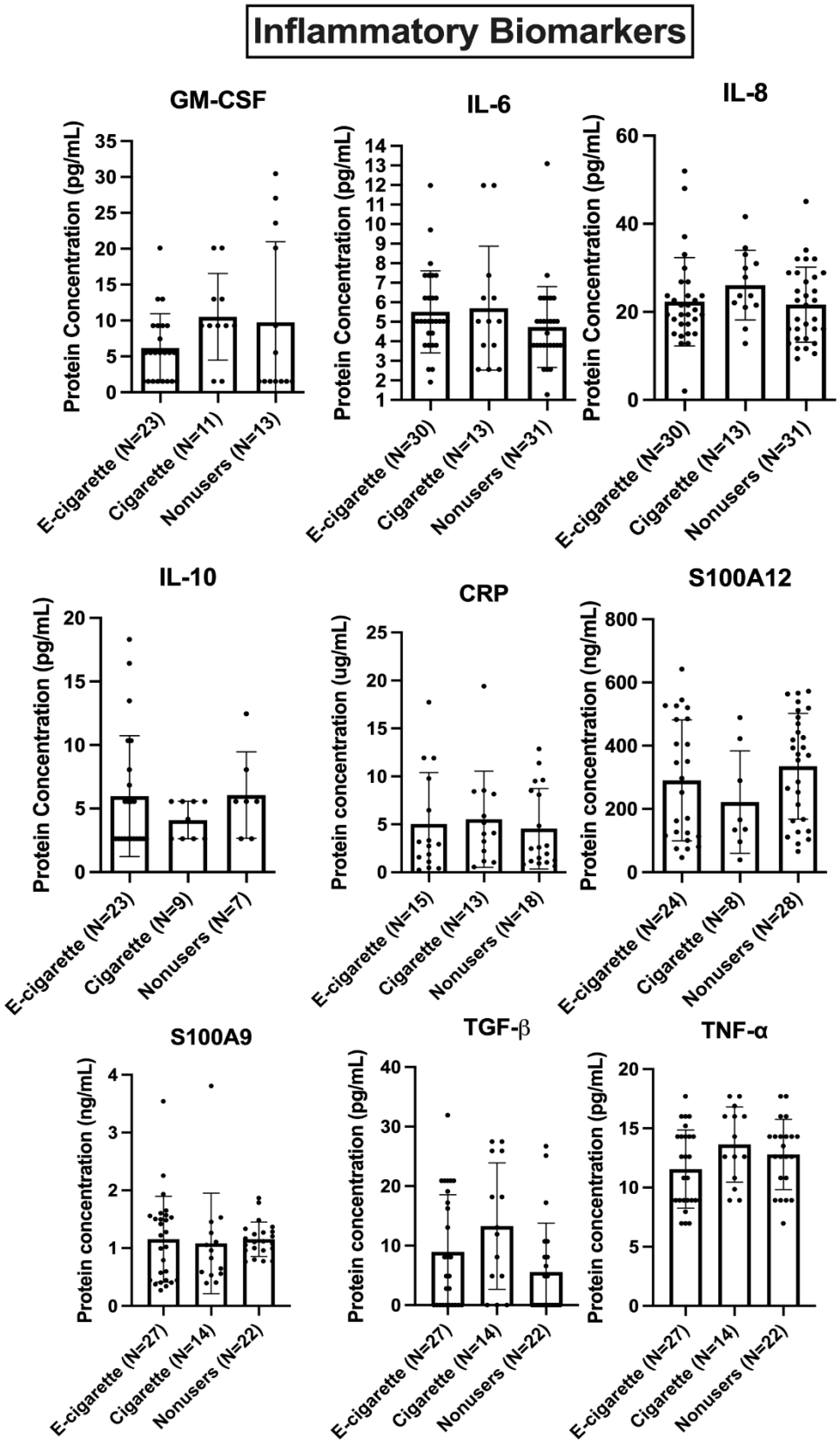

Figure S8. **No significant changes were observed in these inflammatory biomarkers between all groups.** p<.05 required for significance; all p values were >0.1. Group means were compared by Kruskal-Wallis test with Dunn's post-hoc adjustment. Bars=SD.

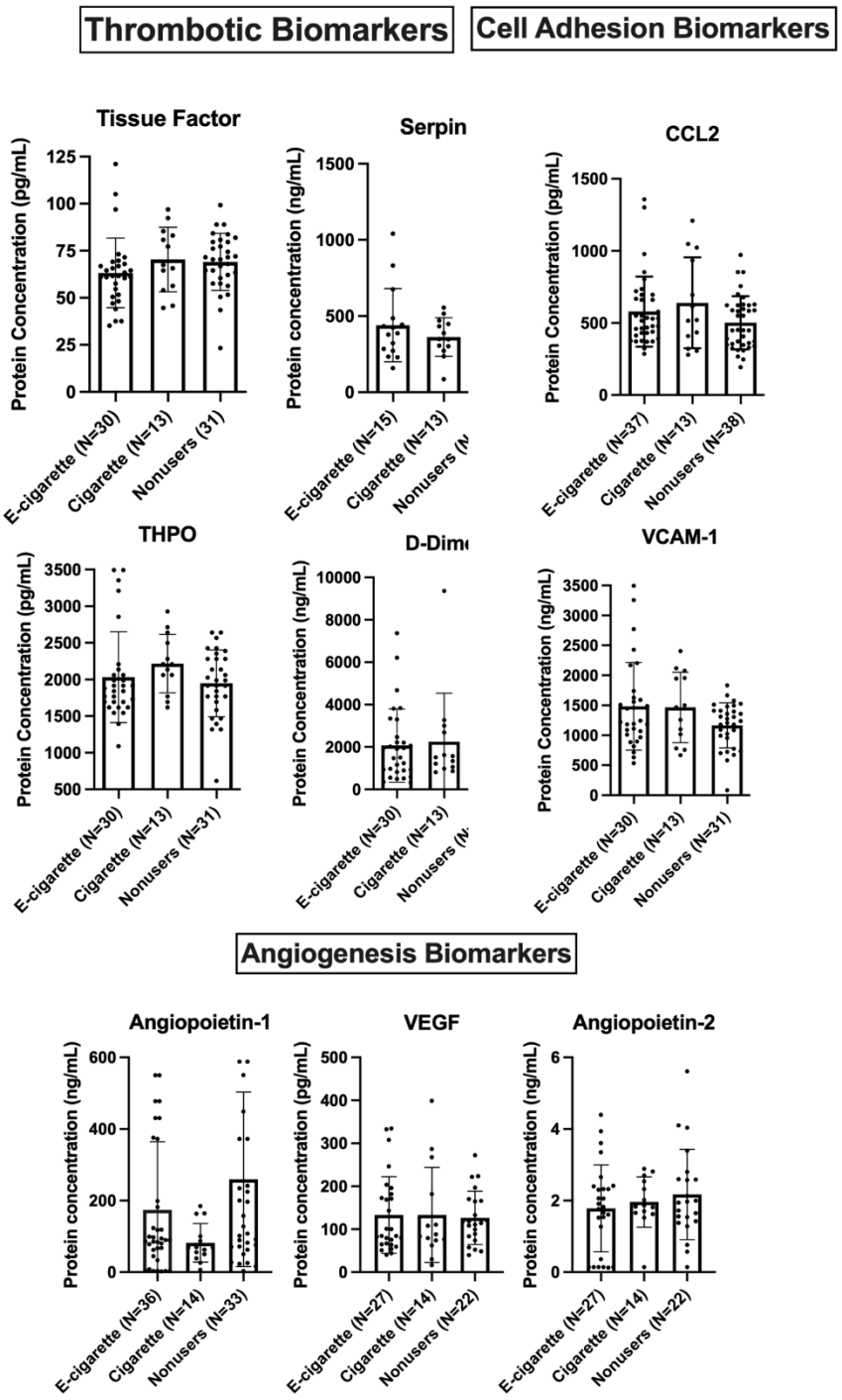

Figure S9. **No significant changes were observed in the above angiogenic, thrombotic, and cell adhesion biomarkers between all groups.** p<.05 required for significance; all p values were ≥0.1. Group means were compared by Kruskal-Wallis test with Dunn's post-hoc adjustment. Bars=SD.

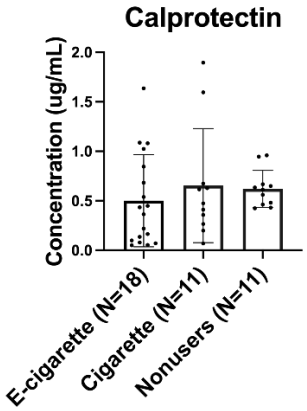

Figure S10. **Serum calprotectin levels.** There were no significant changes in the level of calprotectin in sera from e-cigarette users and cigarette smokers compared to nonusers ($p<.05$ required for significance; all p values were >0.1). Group means were compared by Kruskal-Wallis test with Dunn's post-hoc adjustment. Bars=SD.

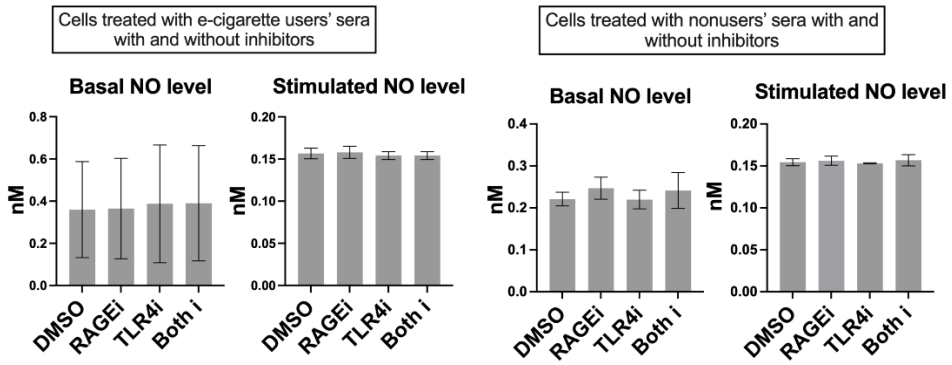

Figure S11. RAGE and TLR4 inhibition do not affect NO production in e-cigarette users and nonusers. There were no significant differences between the groups in all graphs ($p<.05$ required for significance; all p values were >0.1). Group means were compared by Kruskal-Wallis test with Dunn's post-hoc adjustment. Bars=SD.

Major Resources Table

In order to allow validation and replication of experiments, all essential research materials listed in the Methods should be included in the Major Resources Table below. Authors are encouraged to use public repositories for protocols, data, code, and other materials and provide persistent identifiers and/or links to repositories when available. Authors may add or delete rows as needed.

Cultured Cells

Name	Vendor or Source	Sex (F, M, or unknown)	Persistent ID / URL
HUVEC	Lonza, Basel, Switzerland	Unknown	https://bioscience.lonza.com/lonza_bs/US/en/Primary-and-Stem-Cells/p/000000000000184665/HUVEC---Human-Umbilical-Vein-Endothelial-Cells%2C-Pooled%2C-in-EGM-2
HMVEC-L	PromoCell,Heidelberg, Germany	Male	https://promocell.com/product/human-pulmonary-microvascular-endothelial-cells-hpmec/

Other

Description	Source / Repository	Persistent ID / URL
Human Fibronectin	Sigma-Aldrich	https://www.sigmaaldrich.com/US/en/product/sigma/ecm001
EGM-2 Endothelial Cell Growth Medium-2 BulletKit	Lonza	https://bioscience.lonza.com/lonza_bs/US/en/Primary-and-Stem-Cells/p/000000000000185303/EGM™-2-Endothelial-Cell-Growth-Medium-2-BulletKit™
Human Recombinant VEGF	Sigma-Aldrich	https://www.sigmaaldrich.com/US/en/product/mm/gf315
Recombinant Human VEGF	Sigma-Aldrich	https://www.sigmaaldrich.com/US/en/product/mm/01185
RNeasy Kits	Qiagen	https://www.qiagen.com/us/product-categories/discovery-and-translational-research/dna-rna-purification/rna-purification/total-rna/
High Capacity cDNA Reverse Transcription Kit	Applied Biosystems	https://www.fishersci.com/shop/products/applied-biosystems-high-capacity-cdna-reverse-transcription-kit-4/4368814
TaqMan Gene Expression Master Mix	Applied Biosystems	https://www.fishersci.com/shop/products/applied-biosystems-taqman-gene-expression-master-mix-6/p-4919401
Sample diluent	R&D Systems	https://www.rndsystems.com/products/sample-diluent-concentrate-1-5x_dyc001
Human eNOS DuoSet ELISA	R&D Systems	https://www.rndsystems.com/products/human-enos-duoset-elisa_dy950-05

DuoSet ELISA Ancillary Reagent Kit 2	R&D Systems	https://www.rndsystems.com/products/duoset-elisa-ancillary-reagent-kit-2_dy008b
Amplex™ Red Hydrogen Peroxide/Peroxidase Assay Kit	Thermo Fisher Scientific	https://www.thermofisher.com/order/catalog/product/A22188
ECIS ZTheta	Applied Biosystems	https://www.biophysics.com/ztheta.php
96 well Array	Applied Biosystems	https://applied-biophysics-inc.myshopify.com/products/96w20idf-pet
EGM-MV Endothelial Cell Growth Medium Bullet Kit	Lonza	https://bioscience.lonza.com/lonza_bs/US/en/Primary-and-Stem-Cells/p/000000000000185285/EGM™-MV-Microvascular-Endothelial-Cell-Growth-Medium-BulletKit™
RAGE inhibitor FPS-ZM1	Millipore Sigma	https://www.emdmillipore.com/US/en/product/RAGE-Antagonist-FPS-ZM1-Calbiochem,EMD_BIO-553030
TLR4 inhibitor TAK-242	Cayman Chemical	https://www.caymanchem.com/product/13871/tak-242
Recombinant Human S100A8/S100A9	R&D Systems	https://www.rndsystems.com/products/recombinant-human-s100a8-s100a9-heterodimer-protein-cf_8226-s8
Human Magnetic Luminex Assay	R&D Systems	https://www.rndsystems.com/products/human-luminex-discovery-assay_lxsahm
Gram Universal Vaping Machine	Gram research	https://www.gramresearch.com