### Title

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### Authors

Chitteti, R
Zuniga-Hertz, JP
Masso-Silva, JA
et al.



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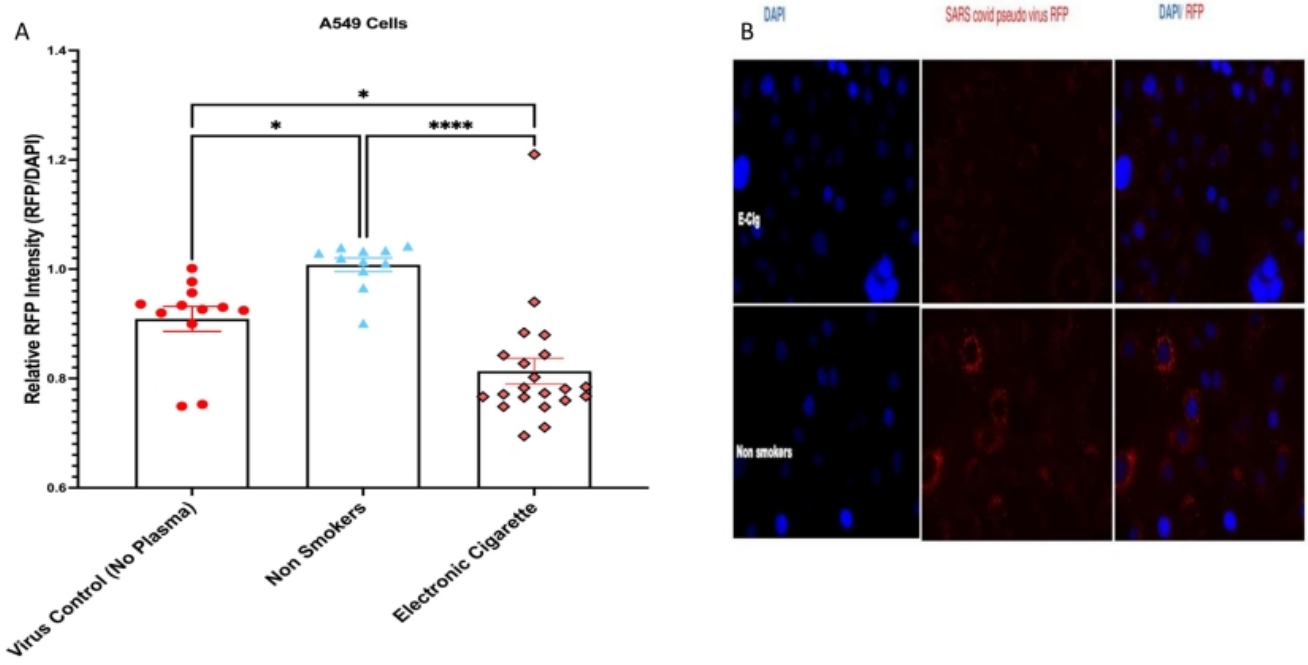
# Plasma Mediators in E Cigarette Users Inhibit SARS-CoV-2 Pseudovirus Infection

R. Chitteti[1], J. P. Zuniga-Hertz[1], J. A. Masso-Silva[2], L. E. Crotty Alexander[2], J. Shin[2], H. H. Patel[1], D. M. Roth[1]; [1]Department of Anesthesiology, VA San Diego Healthcare System, San Diego, Univesity of California, San Diego, CA, United States, [2]Pulmonary and Critical Care Section, VA San Diego Healthcare System, San Diego, Univesity of California, San Diego, CA, United States.

**Corresponding author's email: ramamurthy.pcu@gmail.com**

Rationale: Severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2), is the virus responsible for the COVID-19 pandemic. The association of smoking with susceptibility to and severity of infection is controversial in COVID-19, with some reports suggesting decreased infection rates in smoking populations. The effects of E-Cigarette (E-cig) use on COVID-19 infections have not been established. The objective of this study was to investigate plasma from E-cig users and controls for potential plasma mediators that could affect SARS-CoV-2 infection rates. Methods: A longitudinal cohort study was conducted on subjects who exclusively used E-cigarettes (n=21). Inclusion criteria were active E-cig use without known lung disease and with normal lung function, between the ages of 18-30 years. E-cig use was confirmed with in-person interviews and cotinine levels. Active vaping was defined as use of >0.5-1 mL e-liquid/day or 3.5-7 mL/week for >6 months. Twenty one active E-cig users and 10 non-smoking non-vaping controls underwent blood collection. Plasma was isolated and stored at -70°C until exosome profiles were conducted. The pseudovirus construct was prepared with an HIV1-lentivirus vector cloned with the SARS-CoV-2 spike protein and RFP (Red fluorescent protein) reporter. For adoptive transfer experiments, one hour before pseudovirus infection, A549 lung epithelial cells were conditioned with plasma from E-cig users and controls. Cells were imaged for RFP expression as a measure of viral infectivity at 24 hours post-pseudovirus treatment. Results: Nanosight analysis of plasma particles showed that total particles (per ml) were different (P<0.040) between E-cig and control groups. Exosome (20-120nm) counts were increased in E-cig plasma compared to controls (P<0.05). Importantly, our results showed a significant inhibition of SARS-CoV-2 pseudovirus infection in A549 lung epithelial cells primed with E-cig plasma compared to non-smoker controls (P<0.001) as measured by ELISA RFP intensity as well as microscoping imaging in Fig 1A &B. Conclusions: To our knowledge, our results are the first to suggest that factors in the plasma of E-cig users reduce infection rates of a SARS-CoV-2 spike protein pseudovirus in lung cells in vitro. These results may have important implications regarding health recommendations and smoking/vaping reduction strategies during the COVID-19 pandemic. We are currently investigating the underlying mechanisms of action of E-cig plasma components on SARS-CoV-2 infections. BA Fig:1 E-cig plasma inhibits SARS-CoV-2 pseudo virus infection (A) ELISA RFP intensity quantification (B) RFP fluorescence imaging of A549 cells treated with pseudovirus and E cig and control plasma. (E-cig plasma (n=21) compared to Non-smoker controls (n=10).

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