



Evaluation of Differential Oral Cell-specific Responses to the E-cigarette Component Nicotine

Ian Pearson¹, James Luke Taylor¹ and Karl Kingsley^{2*}

¹Department of Clinical Sciences, School of Dental Medicine, University of Nevada, Las Vegas, USA.

²Department of Biomedical Sciences, School of Dental Medicine, University of Nevada, Las Vegas, USA.

Authors' contributions

This work was carried out in collaboration between all authors. Author KK designed the study, performed the statistical analysis, wrote the protocol and first draft of the manuscript. Authors IP and JLT performed the experiments, managed the analyses of the study and completed the literature searches. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/IJRRD/2018/41064

Editor(s):

(1) Kanupriya Gupta, Assistant Professor, Department of Oral and Maxillofacial Pathology, Mithila Minority Dental College and Hospital, India.

Reviewers:

(1) Priyanka Agarwal, Maharashtra University of Health Sciences, India.

(2) Lazos, Jerónimo Pablo, Universidad Nacional de Córdoba, Argentina.

Complete Peer review History: <http://www.sciencedomain.org/review-history/24625>

Original Research Article

Received 23rd February 2018

Accepted 10th May 2018

Published 16th May 2018

ABSTRACT

Objectives: The recent introduction of electronic cigarettes (EC) or e-cigarettes, also known as the electronic nicotine delivery device (ENDD), has been promoted as a safer alternative to tobacco products and smoking. Many groups have advocated for the use of ECs or ENDDs as a tool to reduce carcinogenic potential, while simultaneously promoting strategies and protocols for smoking and nicotine cessation. Based upon this information, the main objective of this study was to determine the biological effects of the most basic aerosol component of all ECs and ENDDs (nicotine) on cells and tissues specifically derived from the oral cavity. The working hypothesis was that no discernable effects would be apparent at the concentrations typically associated with EC and ENDD use.

Experimental Methods: In brief, oral cell lines were obtained, which included normal, non-cancerous Human Gingival Fibroblasts (HGF-1) and two oral squamous cell carcinomas (SCC25, CAL27). Cells were exposed to nicotine at concentrations equivalent to those found in e-cigarette mixtures (5.77×10^{-5} M) for five day proliferation and viability assays.

*Corresponding author: Email: Karl.Kingsley@unlv.edu;

Results: The results of this study strongly suggest that nicotine may have negative effects on both cellular viability and cellular proliferation among cancerous and non-cancerous cells. Moreover, these effects appear to become more pronounced over time, suggesting that short-term exposure to vaping solutions comprised of water with small amounts of nicotine may be sufficient to induce these effects – at least in this experimental or *in vitro* setting.

Conclusions: In summary, these data provide further evidence that nicotine administration may present significant risks to cell viability and growth over time. In addition, this study demonstrated that these effects were evident in both cancerous and non-cancerous cells – a finding that may suggest more research in this area is needed to determine the mechanisms that might be shared between these differing cell types, which may also suggest more caution may be needed when advertising or marketing ECs or ENDDs are low- or no-risk alternatives to cigarette smoking.

Keywords: Nicotine; e-cigarette; oral response.

ABBREVIATIONS

United States (US), electronic cigarettes (EC), electronic nicotine delivery device (ENDD), polycyclic aromatic hydrocarbons (PAH), nicotine-derived nitrosamines (NDN), American Tissue Culture Collection (ATCC), human gingival fibroblast (HGF-1), Dulbecco's Modified Eagle's Medium (DMEM), fetal bovine serums (FBS), smokeless tobacco extract (STE).

1. INTRODUCTION

Tobacco use and alcohol consumption are the major contributing risk factors identified with the development and oncogenesis of oral and pharyngeal cancers in the United States (US) [1,2]. In recent years there has been an overall decline in tobacco use and smoking in the US, which has resulted in a corresponding decline the incidence of tobacco-induced diseases that include lung, oral and pharyngeal cancers [3,4]. Despite these positive advances, some evidence has demonstrated that smoking and tobacco use among specific demographic (minority) groups and those within lower socioeconomic groups may not be experiencing these same declines [5,6].

The recent introduction of electronic cigarettes (EC) or e-cigarettes, also known as the electronic nicotine delivery device (ENDD), has been promoted as a safer alternative to tobacco products and smoking [7,8]. The removal of combustion from the process of tobacco smoking is designed to reduce production, and therefore intake, of multiple carcinogenic compounds that include polycyclic aromatic hydrocarbons (PAH) and nicotine-derived nitrosamines (NDN) [9-11]. Many groups have advocated for the use of ECs or ENDDs as a tool to reduce carcinogenic potential, while simultaneously promoting strategies and protocols for smoking and nicotine cessation [12,13].

These advances have led to widespread use of ECs and ENDDs by some smokers, which have

widely become known as vaping [14,15]. These increases have led to a great expansion in the range of types and flavors of ECs, which may contain carcinogenic and deleterious compounds that may negatively impact the overall health benefits originally anticipated by this process [16-18]. In addition, the increased consumer choices have also led to uptake and use by non-smokers and younger adults and teenagers who believe that ECs and ENDDs pose only limited health risks [19,20].

These shifts in consumer perception and behavior have prompted great interest in more expansive scientific review to evaluate and determine the safety of all EC components on human cells and tissues [21,22]. Based upon this information, the main objective of this study was to determine any changes in cellular proliferation or viability to the most basic aerosol component of all ECs and ENDDs (nicotine) on cells and tissues specifically derived from the oral cavity.

2. METHODOLOGY

2.1 Cell Culture

Tissues derived from the oral cavity were obtained from American Tissue Culture Collection (ATCC). These included a normal (non-cancerous) human gingival fibroblast cell line, HGF-1 (CRL-2014), which was cultured in accordance with the recommended protocol. In brief, cells were grown in Dulbecco's Modified Eagle's Medium (DMEM) with the addition of 10% fetal bovine serum (FBS) in a humidified

tissue culture incubator that was supplemented with 5% CO₂. Other cell lines included CAL27 (CRL-2095) and SCC25 (CRL-1628), which are both squamous cell carcinomas of the tongue, which can be maintained using the same procedures as HGF-1 cells. All experimental assays were performed in 96-well tissue culture-treated plates with the addition of 1.2×10^4 cells per well.

2.2 Cell Viability

Cell viability was determined at the initiation of cell plating and during cell culture at each passage prior to experimentation. Cells were culturing until 75-85% confluence and split 1:3 to maintain appropriate levels of cell viability per the manufacturer recommendation. Baseline measurements of cell viability were taken at the start of each experiment or time point zero (T0) and then at each subsequent 24 hour time point, such as day one (T1), day two (T2), day three (T3), day four (T4) and day five (T5). Viability was assessed using the Trypan Blue exclusion assay, which was quantified using the TC20 Cell Counter from Bio-Rad (Hercules, CA) as previously described [23-26]. All measurements were done in triplicate and each individual assay involved n=8 wells – yielding n=24 replicates wells per experimental condition.

2.3 Cell Proliferation

Growth of cells was determined by two separate methods in this study. First, live cell number and total cell number were obtained from three experimental wells at each time point to determine viability. However, the Trypan Blue method is preferred for assessment of viability but may not be preferable to accurately evaluate total proliferation – in order account for all cells within each experimental well, as previously described [23-26]. To verify these results, all experimental wells for each day were then fixed with formalin and processed using Gentian Violet, as previously described [23-26]. Cell number was then obtained using a BioTek ELx808 Microplate Absorbance Reader to determine approximate cell number and cell confluence.

2.4 Materials

Purified liquid nicotine was obtained from Acros organics, L-Nicotine 99+% (CAS: 54-11-5; Geel, Belgium). Viability and proliferation assay were performed in the appropriate complete media

with (experimental) and without the addition of liquid nicotine diluted to produce an EC equivalent of 5.77×10^{-5} M in each experimental well.

2.5 Statistics

Differences between the control and experimental cell viability averages were determined using two-tailed t-tests, which is appropriate for use with parametric data with a sample size of at least twenty (n=20). Statistical significance was set at $\alpha=0.05$ and descriptive statistics were given where appropriate, along with the appropriate p-value.

3. RESULTS

In order to determine the relative change in viability in this experimental assay, baseline viability was measured at the initiation of cell culture and for five consecutive days (Fig. 1). These results demonstrated high viability for all cell lines, ranging from 86 to 94%. More specifically, viability for HGF-1 cells was initially measured as 88%, which ranged over five days between 88% - 91% resulting in an average which was consistently at or near the average of 88.8%. Similarly, SCC25 cell viability was observed at 86% and ranged between 86% and 88% - very close to the average of 86.8%. CAL27 cells exhibited an initial viability of 94%, with fluctuations observed between 93% and 95 that were near approached the overall average of 94%.

After establishment of the baseline viability, as well as the average range and consistency of these measurements, cells were plated in 96-well assay plates with and without the addition of nicotine at the concentration of 5.77×10^{-5} M (Fig. 2). These resulted revealed a slight, non-significant decline in cell viability among all cell lines. More specifically, cell viability among HGF-1 cells was reduced from 89% to 85%, while CAL27 was reduced from 94% to 88% and SCC25 declined from 87% to 82%, $p>0.1$.

Further analysis of cell viability over time revealed a more significant overall declining trend that was observed among the experimental (nicotine) wells between day two (T2) and day five (T5) (Fig. 2A). In brief, viability among HGF-1 cells was inhibited by 33.1% over the course of the experiment with the greatest declines observed between day three (T3) and day five (T5), $p<0.05$. In addition, viability among the experimental CAL27 cells declined by an

average of 49.3% with the greatest decline observed between day one (T1) and day two (T2), $p < 0.05$. Finally, viability among the experimental SCC25 cells exhibited a similar dramatic reduction in viability of 47.2% with the greatest reduction observed between day one

(T1) and day two (T2), similar to the observations with CAL27 cells. These data demonstrated a small reduction in cell viability at the onset of this assay with sustained, dramatic temporal reductions in cell viability that persisted throughout the course of this assay (Fig. 2B).

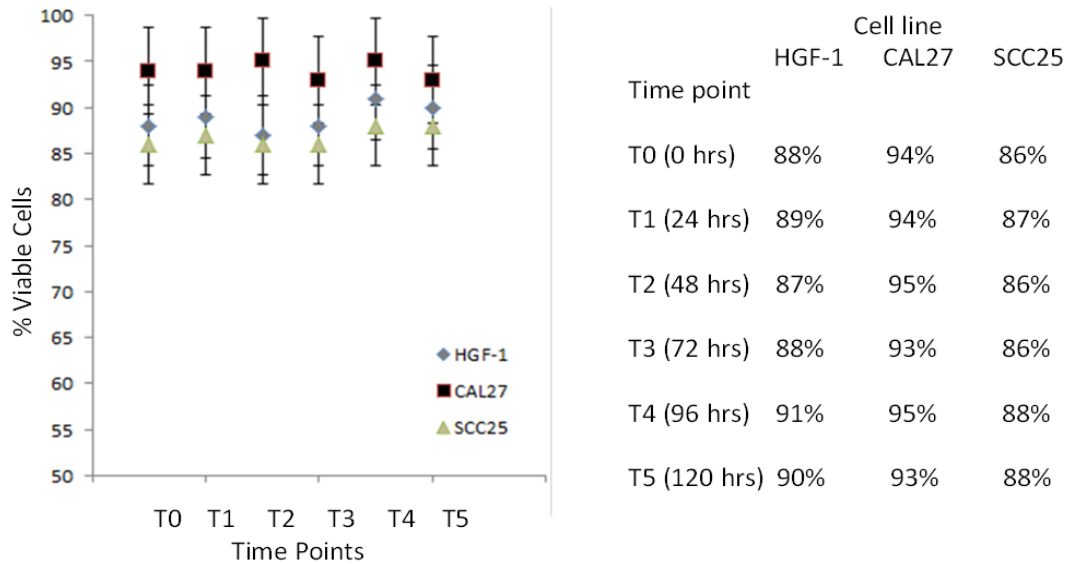


Fig. 1. Baseline measurement of cell viability prior to experimentation. Cell viability as determined by Trypan Blue exclusion assay revealed high percentages among all three cell lines; HGF-1 (88.8%), CAL27 (94%), SCC25 (86.8%), which remained relatively consistent (no significant change) over the course of several days, $p > 0.3$

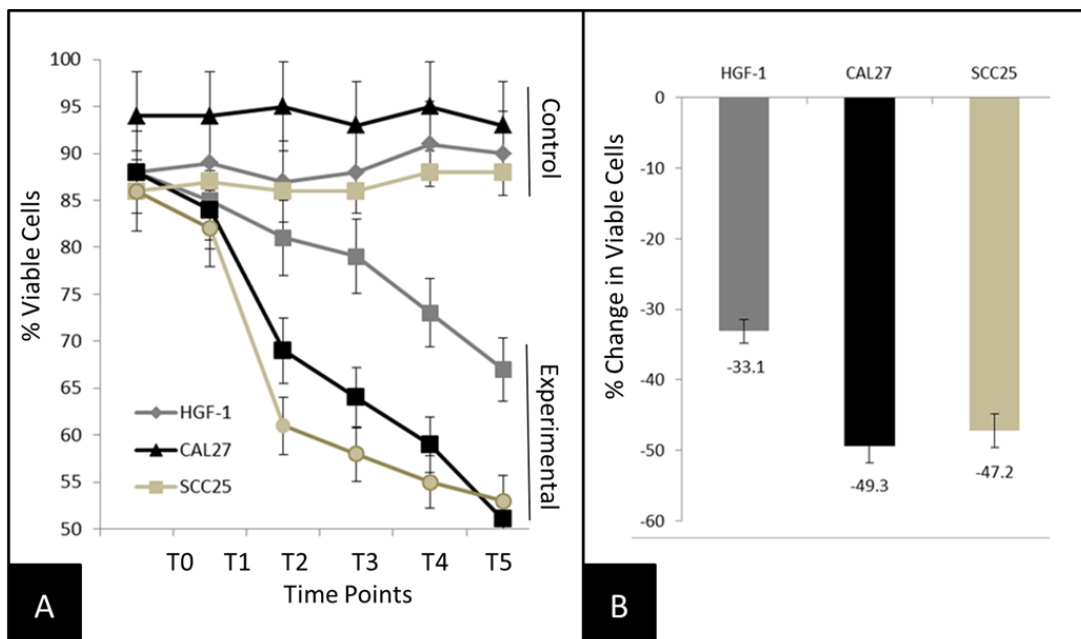


Fig. 2. Cell viability following nicotine administration. A) Results from the 96-well proliferation assays revealed differential reductions in cell viability that varied over time and between cell lines. B) Viability was significantly reduced by the assay endpoint T5 (120 hours) in all cell lines between 33.1% (HGF-1), 47.2% (SCC25), and 49.3% (CAL27), $p < 0.01$

To assess any changes in growth and proliferation in this experimental assay, baseline measurements of cell growth and proliferation were measured over five consecutive days (Fig. 3). These results demonstrated that all cell lines were found to have similar absorbance readings (approximating confluence) at the onset of this experiment (T0), evidence that cell number was equivalent at the start of each trial. Each cell line exhibited growth over the course of the five day assay – although growth rates differed significantly.

For example, HGF-1 cells exhibited a nearly three-fold increase in overall cell number over the course of five days (T0 – T5), which nearly approximates an average doubling time of 48 – 72 hours. SCC25 cells exhibited a nearly four-fold increase in overall cell number, while CAL27 exhibited a greater than four-fold increase.

The establishment of baseline growth and proliferation provided sufficient data to commence the subsequent experimental assay, with and without the addition of nicotine at the concentration of 5.77×10^{-5} M (Fig. 4). These data demonstrated that the addition of nicotine was sufficient to alter cellular growth and proliferation. In general, each cell line was capable of producing a slight increase in cell growth between plating at time point zero (T0) and the first time point at 24 hours (T1). However, none of the cell lines exhibited any further growth, while each exhibited evidence of a further decline in overall cell number.

More specifically, under nicotine administration it was noted that CAL27 proliferation was significantly lower (-14.3%) compared to the negative control cells grown without nicotine at the initial time point (T1) of 24 hours, $p < 0.05$. The inhibition of cellular proliferation was also observed on subsequent days with declines ranging from -43.9% (T3) to -90.1% (T5), $p < 0.01$. It should be noted that the absorbance at the final time point revealed fewer than half the number of cells that were originally plated.

SCC25 cells responded similarly to nicotine administration with a significantly lower amount of growth observed at day one (T1) of -32.2% compared with the negative controls. This inhibition was also observed at all subsequent time points (T2-T5), with reductions ranging from -55.6% (T3) to -86.4% (T5), $p < 0.01$. The final absorbance reading of these cells was also lower than the initial starting point of the assay.

Data generated from the normal, non-cancerous oral gingival fibroblast cell line HGF-1 revealed that administration of nicotine had similar effects on this cell line. For instance, proliferation on day one (T1) was initially reduced by 16.7%, $p < 0.05$. This inhibitory effect was sustained with significant reductions of 25.1% to 75.9% observed between T2 and T5, $p < 0.01$. Finally, it should be noted that the absorbance reading in the experimental assay for this cell line was also lower than the initial starting point of the assay – similar to the results observed with the oral cancer cell lines.

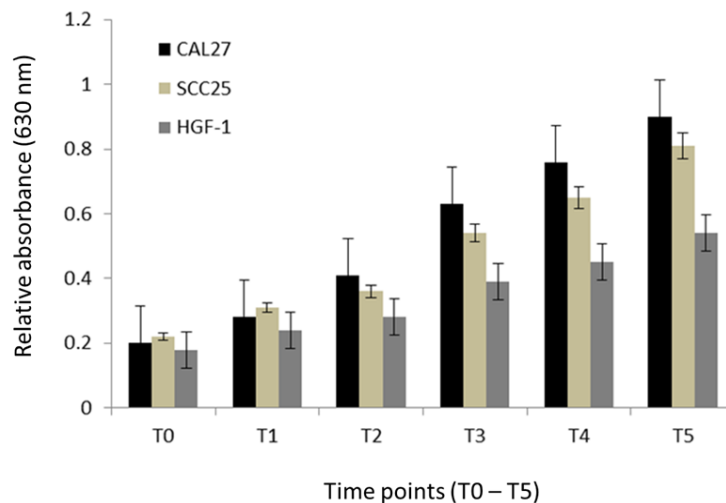


Figure 3. Baseline measurement of cell proliferation prior to experimentation. Cell proliferation (growth) as determined by fixation, staining and absorbance reading (630 nm) revealed growth among all three cell lines. Average doubling time for the normal, non-cancerous HGF-1 cells was between 48-72 hrs., while doubling time among the oral cancer cell lines CAL27 and SCC25 was between 24 – 48 hours

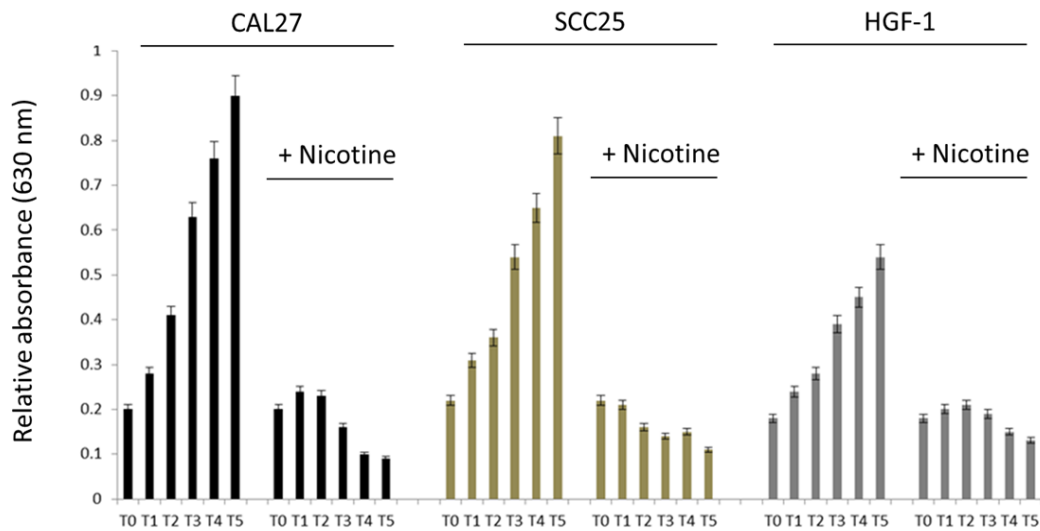


Fig. 4. Cell proliferation following nicotine administration. The addition of nicotine to the experimental wells significantly inhibited cell growth over the course of the assay. Although CAL27 cells were initially able to proliferate (T1, T2), the rate of growth was severely inhibited by nicotine administration with more significant reductions in cell numbers observed between T3 and T5. Similar results were observed with SCC25 cells and the HGF-1 normal cell controls

4. DISCUSSION

Based upon the premise that ECs and ENDDs effectively remove most of the carcinogens and other harmful products of smoking, such as PAHs and NDNs, the working hypothesis was that no discernable effects would be apparent in these experimental nicotine assays at the concentrations typically associated with vaping and electronic cigarette use. However, the results of this study strongly suggest that nicotine may have negative effects on both cellular viability and cellular proliferation among cancerous and non-cancerous cells. Moreover, these effects appear to become more pronounced over time, suggesting that short-term exposure to vaping solutions comprised of water with small amounts of nicotine may be sufficient to induce these effects – at least in this experimental or *in vitro* setting.

These data are consistent with other recently published studies that also demonstrate smokeless tobacco extract (STE) or nicotine-containing vapor is sufficient to induce cell damage and cell death at similar concentrations to those used in this study [27-29]. Moreover, these reports have revealed that some oral tissues including fibroblasts may exhibit a greater range of cellular behaviors that are negative impacted by nicotine administration, such as impaired wound healing and migration properties

[30-32]. This may suggest that nicotine administration may not be as “safe” as initially suggested by consumer marketing and advertising.

Some limitations that must be considered in this study include the route and duration of nicotine administration. Cells were not exposed directly to e-cigarette vapor, but rather had liquid nicotine added to a concentration that would be comparable to that found in an average EC or ENDD [33]. This would represent a much higher concentration than would typically be available in the blood stream and therefore bioavailable. However, it should be noted that the average EC or ENDD intake for young adults and teenagers is much higher than that of an average smoker, presumably due to the mistaken assumption that ECs remove the carcinogenic risk associated with traditional smoking and tobacco products [34-36].

Another limitation of this study was the use of only nicotine as the experimental condition. Most ECs and ENDDs include a preservative in their nicotine suspension, such as glycerol I [37,38]. Future studies will include concomitant administration of both nicotine and glycerol in order to more accurately assess the potential effects on cell growth and cell viability. Finally, the most popular ECs and ENDDs now include other additives to mask the flavor of pure nicotine

suspensions, which may introduce other harmful and damaging additives that were not part of this testing protocol.

5. CONCLUSIONS

In summary, these data provide further evidence that nicotine administration may present significant risks to cell viability and growth over time. In addition, this study demonstrated that these effects were evident in both cancerous and non-cancerous cells – a finding that may suggest more research in this area is needed to determine the mechanisms that might be shared between these differing cell types. Finally, these data also suggest the possibility that short-term use and administration of nicotine may not only inhibit growth and reduce viability but may also induce cell death – a troubling finding that appears to have a growing body of corroborating evidence that may suggest more caution may be needed when advertising or marketing ECs or ENDDs are low- or no-risk alternatives to cigarette smoking.

CONSENT

It is not applicable.

ETHICAL APPROVAL

As per international standard or university standard written ethical permission has been collected and preserved by the authors.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Kingsley K, O'Malley S, Ditmyer M, Chino M. Analysis of oral cancer epidemiology in the US reveals state-specific trends: implications for oral cancer prevention. *BMC Medical Education* 2008;8(1):9.
2. Bunnell A, Pettit N, Reddout N, Sharma K, O'Malley S, Chino M, Kingsley K. Analysis of primary risk factors for oral cancer from select US states with increasing rates. *Tobacco Induced Diseases* 2010;8:5.
3. Chang CM, Corey CG, Rostron BL, Apelberg BJ. Systematic review of cigar smoking and all cause and smoking related mortality. *BMC Public Health*. 2015;15:390. DOI: 10.1186/s12889-015-1617-5. Review. PMID: 25907101
4. LeHew CW, Weatherspoon DJ, Peterson CE, Goben A, Reitmajer K, Sroussi H, Kaste LM. The Health System and Policy Implications of Changing Epidemiology for Oral Cavity and Oropharyngeal Cancers in the United States from 1995 to 2016. *Epidemiol Rev*. 2017;39(1):132-147. DOI: 10.1093/epirev/mxw001.Review. PMID: 28402398
5. Felicitas-Perkins JQ, Sakuma KK, Blanco L, Fagan P, Pérez-Stable EJ, Bostean G, Xie B, Trinidad DR. Smoking among Hispanic/Latino nationality groups and Whites, comparisons between California and the United States. *Nicotine Tob Res*; 2017. DOI: 10.1093/ntr/ntx191. [Epub ahead of print] PMID: 29059350.
6. Singh GK, Jemal A. Socioeconomic and racial/ethnic disparities in cancer mortality, incidence, and survival in the United States, 1950-2014: Over six decades of changing patterns and widening inequalities. *J Environ Public Health*. 2017; 2017:2819372. DOI: 10.1155/2017/2819372 Epub 2017 Mar 20. PMID: 28408935
7. Franks AS, Sando K, McBane S. Do electronic cigarettes have a role in tobacco cessation? *Pharmacotherapy*; 2018. DOI: 10.1002/phar.2103. [Epub ahead of print] PMID: 29573440
8. Popova L, Owusu D, Weaver SR, Kemp CB, Mertz CK, Pechacek TF, Slovic P. Affect, risk perception, and the use of cigarettes and e-cigarettes: A population study of U.S. adults. *BMC Public Health*. 2018 Mar 22;18(1):395. DOI: 10.1186/s12889-018-5306-z PMID: 29566752
9. Wagner KA, Flora JW, Melvin MS, Avery KC, Ballentine RM, Brown AP, McKinney WJ. An evaluation of electronic cigarette formulations and aerosols for harmful and potentially harmful constituents (HPHCs) typically derived from combustion. *Regul Toxicol Pharmacol*. 2018 Mar 19. pii: S0273-2300(18)30085-0. DOI: 10.1016/j.yrtph.2018.03.012. [Epub ahead of print] PMID: 29567331

10. Thorne D, Hollings M, Seymour A, Adamson J, Dalrymple A, Ballantyne M, Gaca M. Extreme testing of undiluted e-cigarette aerosol in vitro using an Ames air-agar-interface technique. *Mutat Res.* 2018;828:46-54.
DOI: 10.1016/j.mrgentox.2018.01.008.
Epub 2018 Feb 19. PMID: 29555064
11. Lechner WV, Murphy CM, Colby SM, Janssen T, Rogers ML, Jackson KM. Cognitive risk factors of electronic and combustible cigarette use in adolescents. *Addict Behav.* 2018;82:182-188.
DOI: 10.1016/j.addbeh.2018.03.006. [Epub ahead of print]
PMID: 29549801
12. Franks AS, Sando K, McBane S. Do Electronic Cigarettes Have a Role in Tobacco Cessation? *Pharmacotherapy*; 2018.
DOI: 10.1002/phar.2103. [Epub ahead of print]
PMID: 29573440
13. Rehan HS, Maini J, Hungin AP. Vaping versus smoking: A quest for efficacy and safety of E-cigarette. *Curr Drug Saf*; 2018.
DOI:10.2174/1574886313666180227110556. [Epub ahead of print]
PMID: 29485005
14. Yoong SL, Stockings E, Chai LK, Tzelepis F, Wiggers J, Oldmeadow C, Paul C, Peruga A, Kingsland M, Attia J, Wolfenden L. Prevalence of electronic nicotine delivery systems (ENDS) use among youth globally: A systematic review and meta-analysis of country level data. *Aust N Z J Public Health*; 2018.
DOI: 10.1111/1753-6405.12777. [Epub ahead of print]
PMID: 29528527
15. El-Shahawy O, Park SH, Duncan DT, Lee L, Tamura K, Shearston JA, Weitzman M, Sherman SE. Evaluating State-Level Differences in E-cigarette and Cigarette Use Among Adults in the United States Between 2012 and 2014: Findings From the National Adult Tobacco Survey. *Nicotine Tob Res*; 2018.
DOI: 10.1093/ntr/nty013. [Epub ahead of print]
PMID: 29490078
16. Bonhomme MG, Holder-Hayes E, Ambrose BK, Tworek C, Feirman SP, King BA, Apelberg BJ. Flavoured non-cigarette tobacco product use among US adults: 2013-2014. *Tob Control.* 2016;25(Suppl 2):ii4-ii13.
DOI:10.1136/tobaccocontrol-2016-053373. Epub 2016 Oct 28.
PMID: 27794065
17. Smith DM, Bansal-Travers M, Huang J, Barker D, Hyland AJ, Chaloupka F. Association between use of flavoured tobacco products and quit behaviours: Findings from a cross-sectional survey of US adult tobacco users. *Tob Control.* 2016;25(Suppl 2):ii73-ii80.
DOI: 10.1136/tobaccocontrol-2016-053313. Epub 2016 Oct 5.
PMID: 27708123
18. Pepper JK, Ribisl KM, Brewer NT. Adolescents' interest in trying flavoured e-cigarettes. *Tob Control.* 2016 Nov;25(Suppl 2):ii62-ii66.
DOI: 10.1136/tobaccocontrol-2016-053174. Epub 2016 Sep 15
PMID: 27633762
19. Shang C, Weaver SR, Zahra N, Huang J, Cheng KW, Chaloupka FJ. The Association between Potential Exposure to Magazine Ads with Voluntary Health Warnings and the Perceived Harmfulness of Electronic Nicotine Delivery Systems (ENDS). *Int J Environ Res Public Health.* 2018;15(4). pii: E575.
DOI: 10.3390/ijerph15040575.
PMID: 29570638
20. Kim M, Popova L, Halpern-Felsher B, Ling PM. Effects of e-cigarette advertisements on adolescents' perceptions of cigarettes. *Health Commun.* 2017;13:1-8.
DOI: 10.1080/10410236.2017.1407230. [Epub ahead of print]
PMID: 29236550
21. Smith MR, Clark B, Lüdicke F, Schaller JP, Vanscheeuwijck P, Hoeng J, Peitsch MC. Evaluation of the tobacco heating system 2.2. Part 1: Description of the system and the scientific assessment program. *Regul Toxicol Pharmacol.* 2016;81(Suppl 2):S17-S26.
DOI: 10.1016/j.yrtph.2016.07.006. Epub 2016 Jul 19. Review.
PMID: 27450400
22. Palazzolo DL. Electronic cigarettes and vaping: A new challenge in clinical medicine and public health. A literature review. *Front Public Health.* 2013;1:56.
DOI: 10.3389/fpubh.2013.00056. Review.
PMID: 24350225
23. Osafi J, Hejazi A, Stutz DD, Keiserman MA, Bergman CJ, Kingsley K. Differential

- effects of 1,25-dihydroxyvitamin D₃ on oral squamous cell carcinomas in vitro. *J Diet Suppl.* 2014;11(2):145-54.
DOI: 10.3109/19390211.2013.859209.
PMID: 24670118
24. Moody M, Le O, Rickert M, Manuele J, Chang S, Robinson G, Hajibandeh J, Silvaroli J, Keiserman MA, Bergman CJ, Kingsley K. Folic acid supplementation increases survival and modulates high risk HPV-induced phenotypes in oral squamous cell carcinoma cells and correlates with p53 mRNA transcriptional down-regulation. *Cancer Cell Int.* 2012; 12:10.
DOI: 10.1186/1475-2867-12-10.
PMID: 22443202
 25. Kingsley K, Truong K, Low E, Hill CK, Chokshi SB, Phipps D, West MA, Keiserman MA, Bergman CJ. Soy protein extract (SPE) exhibits differential in vitro cell proliferation effects in oral cancer and normal cell lines. *J Diet Suppl.* 2011;8(2):169-88.
DOI: 10.3109/19390211.2011.571656.
PMID: 22432688
 26. McCabe J, Chang S, Hajibandeh J, Tran MD, Meeder CA, Sharma K, Nguyen DH, Moody M, Keiserman MA, Bergman CJ, Kingsley K. Folate supplementation induces differential dose-dependent modulation of proliferative phenotypes among cancerous and noncancerous oral cell lines in vitro. *J Diet Suppl.* 2010; 7(4):325-40.
DOI: 10.3109/19390211.2010.522552.
Epub 2010 Oct 18. PMID: 22432562
 27. Holliday R, Kist R, Bauld L. E-cigarette vapour is not inert and exposure can lead to cell damage. *Evid Based Dent.* 2016;17(1):2-3.
DOI: 10.1038/sj.ebd.6401143.
PMID: 27012563
 28. Willershausen I, Wolf T, Weyer V, Sader R, Ghanaati S, Willershausen B. Influence of E-smoking liquids on human periodontal ligament fibroblasts. *Head Face Med.* 2014;10:39.
DOI: 10.1186/1746-160X-10-39.
PMID: 25224853
 29. Kang SW, Park HJ, Ban JY, Chung JH, Chun GS, Cho JO. Effects of nicotine on apoptosis in human gingival fibroblasts. *Arch Oral Biol.* 2011;56(10):1091-7.
DOI: 10.1016/j.archoralbio.2011.03.016.
Epub 2011 Apr 17.
PMID: 21497792
 30. Silva D, Cáceres M, Arancibia R, Martínez C, Martínez J, Smith PC. Effects of cigarette smoke and nicotine on cell viability, migration and myofibroblastic differentiation. *J Periodontal Res.* 2012; 47(5):599-607.
PMID: 23091836
 31. Chang YC, Lii CK, Tai KW, Chou MY. Adverse effects of arecoline and nicotine on human periodontal ligament fibroblasts in vitro. *J Clin Periodontol.* 2001;28(3):277-82. PMID: 11284543
 32. Checchi L, Ciapetti G, Monaco G, Ori G. The effects of nicotine and age on replication and viability of human gingival fibroblasts *in vitro*. *J Clin Periodontol.* 1999;26(10):636-42. PMID: 10522774
 33. Raymond BH, Collette-Merrill K, Harrison RG, Jarvis S, Rasmussen RJ. The nicotine content of a sample of e-cigarette liquid manufactured in the United States. *J Addict Med.* 2018;12(2):127-131.
DOI: 10.1097/ADM.0000000000000376
PMID: 29280749
 34. Marynak K, Gentzke A, Wang TW, Neff L, King BA. Exposure to electronic cigarette advertising among middle and high school students - United States, 2014-2016. *MMWR Morb Mortal Wkly Rep.* 2018; 67(10):294-299.
DOI: 10.15585/mmwr.mm6710a3
PMID: 29543786
 35. Pepper JK, Farrelly MC, Watson KA. Adolescents' understanding and use of nicotine in e-cigarettes. *Addict Behav.* 2018;82:109-113.
DOI: 10.1016/j.addbeh.2018.02.015. [Epub ahead of print]
PMID: 29518664
 36. Rubinstein ML, Delucchi K, Benowitz NL, Ramo DE. Adolescent Exposure to Toxic Volatile Organic Chemicals From E-Cigarettes. *Pediatrics*; 2018. pii: e20173557.
DOI: 10.1542/peds.2017-3557. [Epub ahead of print]
PMID: 29507165
 37. Fagan P, Pokhrel P, Herzog TA, Moolchan ET, Cassel KD, Franke AA, Li X, Pagano I, Trinidad DR, Sakuma KK, Sterling K, Jorgensen D, Lynch T, Kawamoto C, Guy MC, Laguna I, Hanes S, Alexander LA, Clanton MS, Graham-Tutt C, Eissenberg T; Addictive carcinogens work group. Sugar and aldehyde content in flavored electronic cigarette liquids. *Nicotine Tob Res*; 2017.

- DOI: 10.1093/ntr/ntx234. [Epub ahead of print]
PMID: 29182761
38. DeVito EE, Krishnan-Sarin S. E-cigarettes: Impact of e-liquid components and device characteristics on nicotine exposure. *Curr Neuropharmacol*; 2017.
DOI:10.2174/1570159X156661710161644
30. [Epub ahead of print]
PMID: 29046158

© 2018 Pearson et al.; This is an Open Access article distributed under the terms of the Creative Commons Attribution License (<http://creativecommons.org/licenses/by/4.0>), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

Peer-review history:

The peer review history for this paper can be accessed here:
<http://www.sciencedomain.org/review-history/24625>