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Date: Wednesday, March 07, 2018 11:50PM
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Hong Kong yet again failed to increase life-saving tobacco tax this budget year. The last 'increase' was 2014.

That tiny increase was ineffectual – legal sales increased by 200m sticks since 2013-2016 here.

Effective taxation makes tobacco unaffordable and compliant with the FCTC Treaty.

When can we expect the MPFA to regulate its Trustees accordingly?

<http://www.tobaccofreeportfolios.org/news/>

<http://www.tobaccofreeportfolios.org/watch/>

<http://www.tobaccofreeportfolios.org/facts/>

Each year tobacco is responsible for an estimated 15,000 Australian deaths and 6 million deaths worldwide.

[Source](#)

2

Two out of three smoker's deaths can be attributed to smoking.

[Source](#)

3

Smokers who die from tobacco related disease are estimated to have lost between 12 and 32 years of life.

[Source](#)

4

In 2008, at least one child under 5 was treated every day at Perth's public hospital emergency departments as a result of 'passive' smoking.

Source

5

The World Health Organisation estimates that more than 10% of smoking-related deaths are due to 'passive' smoking, with children and women at greatest risk.

Source

6

Surveys show that in Australia in 2013, the average age at which young people aged 14-24 smoked their first full cigarette was 15.9 years ([source](#)). A survey of smokers in Canada, the United States, the United Kingdom and Australia shows that the proportion of smokers who agreed or agreed strongly with the statement "If you had to do it over again, you would not have started smoking" was extremely high - about 90%, and almost three quarters of current smokers have shown some interest in quitting.

Source

7

Studies from the USA Department of Health indicate that from 1997 to 2005, the nicotine yield per cigarette has increased. Nicotine is the addictive agent in cigarettes, which makes it more difficult for smokers to quit.

[Source](#)

8

The USA Department of Labor lists 15 countries that use child labour to grow tobacco leaf.

[Source](#)

9

In Australia the total social cost of smoking in 2004/2005 was over \$31 billion ([source](#)). By comparison, revenue from sales of tobacco in 2008 amounted to just \$5.6 billion.

Source

10

Globally the cost of tobacco is estimated at 2.1 trillion Euros per year, **equalling the combined expenses of war and terrorism.**

[Source](#)

Robeco latest to stub out tobacco investments

PA portfolio-adviser.com/robeco-latest-stub-tobacco-investments

By Jessica Tasman-Jones

March 7, 2018

The extension of the tobacco exclusion across its fund range includes sub-advised funds, but does not extend to client-specific mandates. The exclusion was already in place in its responsible investment funds.

The divestment will include all listed companies involved in the production of tobacco or “significant components” of cigarettes.

Divestment will be completed by Q3 2018.

No retail funds at Robeco or BNP Paribas AM hold tobacco in their top-10 holdings, according to FE. The £1bn Morgan Stanley Global Brands fund, managed by Peter Wright and William Lock, is the Investment Association fund with the largest allocation to tobacco, holding 15.3%, more than double the allocation in the PUTM UK Stock Market fund, which holds 7%.

IA funds with the highest allocation to tobacco

Morgan Stanley – Global Brands	15.3%
PUTM – UK Stock Market	7%
Schroder – Prime UK Equity	5.9%
Charles Stanley Equity	5.8%
Mitton Income	4.6%

Source: FE Analytics

Robeco said in a statement that investing in the carcinogenic product is socially disadvantageous and does not align with its commitment to responsible investing.

Seven million people die annually from tobacco-related causes and the economic cost of the industry is estimated to cost \$1trn annually, according to the World Health Organisation (WHO).

The statement said although Robeco actively engages with companies it invests in “engagement with the tobacco industry will not lead to fundamental change”.

BNP Paribas AM announced last Thursday it would divest from tobacco in all funds over which it has full discretion by the end of 2018, extending the ban it implemented in sustainable portfolios in 2002.

The WHO’s framework on tobacco control, which is signed by 180 countries, aims to reduce tobacco use by 30% by 2025 through regulation and tax increases.

BNP Paribas AM chief executive Frederic Janbon said it was one of the first global asset managers to commit to tobacco divestment.

“We recognise the important role that long-term capital plays in tackling major global issues and with an increasing number of asset owners, insurers and pension funds excluding tobacco from their investments, we are taking into account growing international concerns about the risks posed by tobacco,” Janbon said.

BNP Paribas AM said it did not have figures on the number funds that must divest from tobacco due to the divestment, but said the exclusion applied to products representing €228bn.

Michael Bloomberg launches tobacco industry watchdog

 www.theguardian.com/us-news/2018/mar/07/michael-bloomberg-launches-tobacco-industry-watchdog

Sarah Boseley

March 7, 2018

A new global watchdog agency has been launched to monitor the tobacco industry with \$20m of philanthropic funding amid fears of dirty tactics by cigarette companies hit by declining smoking rates in the west.

The funding for the agency, named Stop (Stopping Tobacco Organisations and Products), comes from Bloomberg Philanthropies, whose founder, Michael Bloomberg, a former mayor of New York, has committed almost \$1bn to the global fight against tobacco.

The agency will “aggressively monitor deceptive tobacco industry tactics and practices to undermine public health,” said Bloomberg Philanthropies. Global information and data on the behaviour of the tobacco companies, especially in low- and middle-income countries where they are seeking to grow their markets, will be collated and held on a public website.

The move follows recent uproar among anti-tobacco and public health campaigners over the investment of \$80m by the world’s biggest cigarette-maker, Philip Morris International (PMI), in a new body called the Foundation for a Smoke-Free World.

PMI, maker of Marlboro cigarettes, has said its future is in smokeless products such as new “heat not burn” tobacco-filled cigarettes that may be less harmful – although there is insufficient evidence to be sure – as well as e-cigarettes. The foundation, which PMI says is independent and is headed by a former anti-tobacco crusader from the World Health Organization, is offering funds for research projects.

The tobacco control movement has roundly denounced the foundation and accused PMI of duplicity. Michael Bloomberg, now WHO global ambassador for noncommunicable diseases, said it was “an effort by Philip Morris to confuse the public and to misinform them deliberately”.

Bloomberg Philanthropies pointed out that the industry has form. “Tobacco industry-funded research has repeatedly been a smokescreen for behaviour that has led to worse outcomes for smokers. For example, supposedly safer low-tar and filtered cigarettes led to greater numbers of smokers, deeper inhalation patterns, and higher daily consumption – all worsening public health worldwide,” it said in a statement.

At a briefing, Michael Bloomberg accused the foundation of promoting “fake science as well as fake news”, adding: “Unfortunately, I think you’ve seen this technique being used in our government to obfuscate and to confuse people.”

Enormous progress had been made in helping people stop smoking and deterring them from starting, saving 35 million lives in the last decade, he said: “I understand the tobacco companies want to protect their business, but to deliberately go out and to misinform people where lives are at stake is just something that I think we should not permit. And so my foundation has committed \$20m as a start to explain to people what’s going on.”

The Stop agency was launched in Cape Town at the 17th World Conference on Tobacco or Health, an event that is held every three years and brings together experts and campaigners from all over the world. Nobody from the Foundation for a Smoke-Free World was invited, which the organisers say is in line with the WHO's framework convention on tobacco control. That convention, signed by more than 160 countries but not yet ratified by all of them, stipulates that no negotiations must take place with the tobacco industry.

But the arrival on the scene of e-cigarettes in recent years has complicated the issues. Leading brands are made by tobacco companies. Some public health experts say they are the best hope for many long-term smokers of quitting a habit that will otherwise kill them. A report from Public Health England recently called for them to be sold in hospital shops and urged manufacturers to seek a licence for them so they can be prescribed by doctors.

Others warn that there is not enough evidence of their long-term effects. Dr Kelly Henning, director of public health programs with Bloomberg Philanthropies, said 60% of US vapers are "dual-users", so have not given up cigarettes. There are concerns that young people may get addicted to nicotine and go on to develop a smoking habit.

"The US Centers for Disease Control, the surgeon general and the Food and Drug Administration expert group very strongly said e-cigarettes are not for children," Henning said. "Governments have to have very strong regulatory frameworks to stop these products getting into the hands of children."

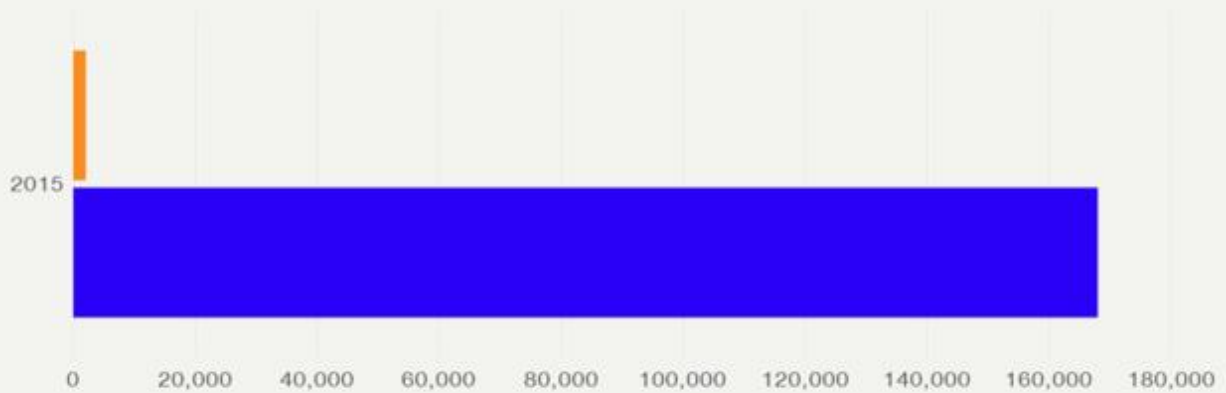
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E-Cigs Breed 81 Times as Many New Smokers as Quitters

Results based on e-cigarette use in 2014

- Adult current smokers who quit for at least 7 years after using e-cigarettes
- Adolescents and young adults who become daily smokers in their late thirties after using e-cigarettes



The Dartmouth Institute for Health Policy, Dartmouth College

Bloomberg

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E-Cigarette Study Says They Lead to More Smokers Than They Stop

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Janine Wolf

(Bloomberg) -- Electronic cigarettes have long been touted not only as a safer alternative to cigarettes but as a potential avenue by which existing smokers might quit. The industry, now worth \$11.4 billion, hasn't been hurt by this one-two pitch of safety and good public policy.

New research shows, however, that **e-cigarettes are hurting a lot more than they help.**

Researchers at Dartmouth College's Norris Cotton Cancer Center said vaping has led more people to start a real smoking habit, rather than avoid tobacco or quit in favor of e-cigarettes, according to a study published Wednesday.

Using 2014 census data, published literature and surveys on e-cigarette usage to build a model, the scientists were able to estimate that about 2,070 cigarette-smoking adults in America quit in 2015 with the help of the electronic devices. However—and perhaps more alarming—the model estimated that, at the same time, an additional 168,000 adolescents and young adults who had never smoked cigarettes began smoking and **eventually became daily cigarette smokers after first using e-cigarettes.**

The model estimates that e-cigarette use in 2014 would eventually lead to about 1,510,000 years of life lost—a figure based on an optimistic 95 percent relative harm reduction of using e-cigarettes compared to traditional cigarettes.

Samir Soneji, an associate professor of health policy at Dartmouth's Geisel School of Medicine and the paper's lead author, said that advertising e-cigarettes as a means to quit or reduce smoking has done damage, mostly to young people. E-cigarettes use cartridges of chemicals, including nicotine, that are transformed into vapor. Despite a federal requirement that purchasers be at least 18 years of age, use of the product in popular culture, combined with its fruity flavors, have proved a strong draw to younger, would-be vapers. These characteristics have been at the core of keeping youths interested in the devices, Soneji said, and should be the focus of restriction efforts by the U.S. Food and Drug Administration.

"The harms of e-cigarette use among adolescents and young adults are serious," he said. "Kids who vape are more likely to start smoking cigarettes—notably kids who were otherwise not at a high risk of starting to smoke." Currently, Soneji said, the risk of initiating cigarette smoking is three times as high for adolescents who vape than for those who do not.

In 2015, 68 percent of Americans who smoked wanted to quit, with about 55.4 percent of them doing so successfully for at least one day, according to the Centers for Disease Control and Prevention. That same year, 45.5 percent of high school-aged cigarette

smokers said they had tried to stop smoking over the previous 12 months. After first regulating the devices in 2016, the FDA embraced vaping as a way for smokers to quit.

Last July, a study published in the British Medical Journal found that e-cigarette users were indeed more likely than non-users to attempt to quit smoking—and be more successful at doing so. However, at around the same time the survey was conducted, e-cigarette use among high school students was jumping from 1.5 percent in 2011 to 16 percent in 2015, making the products the most commonly used tobacco product by young people in the U.S.

“E-cigarettes could, indeed, provide more population benefit if they were more effective as a cessation tool.”

Current research already points toward e-cigarettes being a public health risk because of the chemicals they use, making the new research even more problematic for the industry. However, the Dartmouth researchers point out that a future in which e-cigarettes do help people quit isn’t impossible—as long as they’re kept out of the hands of young people.

“E-cigarettes could indeed provide more population benefit if they were more effective as a cessation tool,” Soneji said. “For example, if smokers who used e-cigarettes to help quit were twice as likely to actually quit compared to smokers who used nicotine-replacement therapy, then the benefits of e-cigarette use would approximately balance the harms of e-cigarette use.”

Representatives from Reynolds American Inc., which owns market-leading e-cigarette Vuse, and competitor Altria Group Inc., maker of MarkTen and APEX, didn’t immediately respond to requests for comment.

Alex Clark, executive director of Consumer Advocates for Smoke-Free Alternatives Association, an e-cigarette industry lobby group, called the study’s results “surprising,” given government studies showing an overall decline in smoking. (A recent CDC study shows that while smoking has declined, vaping has increased.) Clark said his organization prefers that e-cigarette makers be truthful in advertisements by marketing products as “less risky alternatives” to smoking that have the ability to help smokers quit.

The government has made some effort to dissuade young adopters, with a new requirement for product warnings set to take effect this summer. In October, the FDA addressed youth use of e-cigarettes and other electronic nicotine-delivery systems (ENDS) through its “The Real Cost” campaign. Commissioner Scott Gottlieb said in a statement that vaping devices are by far the most common source of experimentation with tobacco products among children.

“While we continue to encourage innovation of potentially less harmful forms of nicotine delivery for currently addicted adult smokers, we can all agree no child should be using any nicotine-containing product,” he said.

Michael Bloomberg, the majority owner of Bloomberg LP, parent of Bloomberg News, provides philanthropic support to anti-smoking campaigns and other health initiatives.

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Quantifying population-level health benefits and harms of e-cigarette use in the United States

Samir S. Soneji  Hai-Yen Sung, Brian A. Primack, John P. Pierce, James D. Sargent

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Abstract

Background

Electronic cigarettes (e-cigarettes) may help cigarette smokers quit smoking, yet they may also facilitate cigarette smoking for never-smokers. We quantify the balance of health benefits and harms associated with e-cigarette use at the population level.

Methods and findings

Monte Carlo stochastic simulation model. Model parameters were drawn from census counts, national health and tobacco use surveys, and published literature. We calculate the expected years of life gained or lost from the impact of e-cigarette use on smoking cessation among current smokers and transition to long-term cigarette smoking among never smokers for the 2014 US population cohort.

Results

The model estimated that 2,070 additional current cigarette smoking adults aged 25–69 (95% CI: -42,900 to 46,200) would quit smoking in 2015 and remain continually abstinent from smoking for ≥ 7 years through the use of e-cigarettes in 2014. The model also estimated 168,000 additional never-cigarette smoking adolescents aged 12–17 and young adults aged 18–29 (95% CI: 114,000 to 229,000), would initiate cigarette smoking in 2015 and eventually become daily cigarette smokers at age 35–39 through the use of e-cigarettes in 2014. Overall, the model estimated that e-cigarette use in 2014 would lead to 1,510,000 years of life lost (95% CI: 920,000 to 2,160,000), assuming an optimistic 95% relative harm reduction of e-cigarette use compared to cigarette smoking. As the relative harm reduction decreased, the model estimated a greater number of years of life lost. For example, the model estimated 1,550,000 years of life lost (95% CI: -2,200,000 to -980,000) assuming an approximately 75% relative harm reduction and -

1,600,000 years of life lost (95% CI: -2,290,000 to -1,030,000) assuming an approximately 50% relative harm reduction.

Conclusions

Based on the existing scientific evidence related to e-cigarettes and optimistic assumptions about the relative harm of e-cigarette use compared to cigarette smoking, e-cigarette use currently represents more population-level harm than benefit. Effective national, state, and local efforts are needed to reduce e-cigarette use among youth and young adults if e-cigarettes are to confer a net population-level benefit in the future.

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Data Availability: All relevant data have been uploaded to the Harvard Dataverse and are accessible using the following DOI: 10.7910/DVN/6UNLQM .
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Introduction

The use of electronic cigarettes (e-cigarettes) has become intensely controversial since their introduction to the US in 2007 [1–7]. E-cigarettes might help the 40 million current adult cigarette smokers quit—the vast majority of whom want to stop smoking completely—by delivering nicotine with the same sensory experience as combustible, or traditional, cigarettes but without inhalation of as many toxicants [8–12]. Conversely, e-cigarettes might facilitate the transition to traditional cigarette smoking among never-smoking adolescents and young adults [13–21]. This harm is potentially substantial because youth e-cigarette use has risen rapidly over time [6,22,23]. For example, past 30-day use of e-cigarettes increased from 1.5% in 2011 to 11.3% in 2016 among high school students and exceeded their level of past 30-day use of traditional cigarettes (8.0% in 2016) [24].

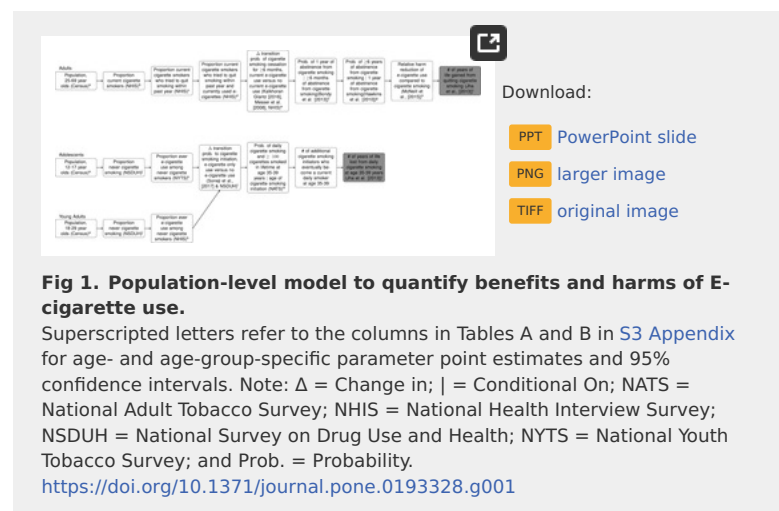
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The controversy over e-cigarettes persists because we do not yet know if e-cigarette use results in more benefit than harm at the population level [25–27]. This uncertainty creates a quandary for the US Food and Drug Administration (FDA), which recently asserted its regulatory authority over e-cigarettes and developed regulations to promote their safety and limit youth appeal [28]. Quantifying the balance of benefits and harms of e-cigarette use requires simultaneous accounting of the additional number of (1) current cigarette smokers who will quit through the use of e-cigarettes and (2) never-cigarette smokers who will initiate cigarette smoking through the use of e-cigarettes, a substantial proportion of whom may become long-term daily cigarette smokers. A recent study concluded a net population-level health benefit under a scenario in which e-cigarette use increases in the future only among cigarette smokers interested in quitting, and net harm under a scenario in which e-cigarette use increases in the future only among youth who would have never smoked [29]. A second study modeled future cigarette and e-cigarette use patterns over the next decade for young adults aged 18–24 years and concluded that e-cigarette use would have a limited impact on the prevalence of current cigarette smoking [30]. However, this study did not assess the effect of e-cigarette use among adolescents or adults aged ≥25 years. A third study estimated the population impact of e-cigarettes on smoking cessation and found e-cigarettes could increase the number of smokers who successfully quit for one year. However, this study also did not assess the effect of e-cigarette use among adolescents [31]. Thus, these last two studies could not determine the balance of benefits and harms of e-cigarette use at the population level.

In this study, we developed a Monte Carlo stochastic simulation model that extends

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Our analytic approach consists of two main steps (Fig 1). The first step estimates the number of years of life gained among the additional number of current cigarette smokers who quit smoking through the use of e-cigarettes as a cessation tool, compared to those who did not use e-cigarettes as a cessation tool, and remain continually abstinent from smoking for ≥ 7 years. We set the threshold for continual abstinence at 7 years because cohort studies found that relapse beyond this point is rare [32,33]. Additionally, the risk of death among former cigarette smokers who quit for this long begins to approximate the risk of death among never cigarette smokers [34]. We began with the US adult population of 25–69 year olds in 2014 (in five-year age groups) and multiplied these counts by the: (1) age-group-specific prevalence of current cigarette smoking, (2) age-group-specific prevalence of trying to quit smoking within the past year among current cigarette smokers, (3) age-group-specific prevalence of current e-cigarette use among current cigarette smokers who tried quitting within the past year, (4) difference in the transition probability of ≥ 6 -month cigarette smoking cessation between current smokers who used e-cigarettes as a cessation tool and current smokers who did not use e-cigarettes as a cessation tool, (5) probability of 1 year of cigarette smoking abstinence from cigarette smoking given ≥ 6 months of cigarette smoking abstinence, (6) probability of ≥ 6 years of abstinence from cigarette smoking given 1 year of cigarette smoking abstinence, and (7) age-group-specific number of years of life gained from quitting cigarette smoking. We assumed 95% relative harm reduction of e-cigarette use, compared to cigarette smoking, among current cigarette smokers who used e-cigarettes as a cessation tool and quit smoking [35]. As described below, we vary the relative harm of e-cigarette use, compared to cigarette smoking, to include the levels of relative harm inferred from in vitro and mouse model studies [36,37].



We assessed three outcomes of interest: (1) the additional number of current cigarette smokers who will quit smoking through the current use of e-cigarettes and abstain from smoking for ≥ 7 years, compared to those who do not currently

use e-cigarettes and (2) the additional number of adolescents and young adults who will initiate cigarette smoking through the ever use of e-cigarettes and eventually become daily cigarette smokers at age 35–39, compared to those who never used e-cigarettes; and (3) the total number of expected years of life gained or lost across all these population subgroups.

Table 1 describes the data source of each model parameter. S1 Appendix describes how the difference in transition probabilities of ≥6-month cigarette smoking cessation between current e-cigarette users and non-current e-cigarette users was estimated based on various parameters such as the proportion of current cigarette smokers who used pharmaceutical aids during quit attempt and the pooled odds ratio of quitting smoking among smokers interested in quitting reported by the meta-analysis of Kalkhoran & Glantz [38]. S2 Appendix describes the estimation of the difference in transition probabilities of cigarette smoking initiation between never cigarette smokers who ever used e-cigarettes compared to those who never used e-cigarettes based on the pooled odds ratio of cigarette smoking initiation reported by the meta-analysis of Soneji et al. [19]. Tables A and B in S3 Appendix show the value of each model parameter.

Parameter	Population sub-group	Source	Survey Questions & Notes
Population	All	2014 US Census	—
Current Cigarette Smoking	Current Smokers	2014 NHIS	"Have you smoked at least 100 cigarettes in your entire life?" (yes). "Do you now smoke cigarettes every day, some days or not at all?" (every day or some days).
Past Year Quit Attempt	Current Smokers	2014 NHIS	"During the past 12 months, have you stopped smoking for more than one day because you were trying to quit smoking?" (yes).
Current E-Cigarette Use	Current Smokers	2014 NHIS	"Do you now use an e-cigarette every day, some days, or not at all?" (every day or some days).
Proportion Of Current Cigarette Smokers With a Past Year Quit Attempt Who Used a Pharmaceutical Aid During Quit Attempt (%)	Current Smokers	2014 NHIS	"Thinking back to when you tried to quit smoking in the past 12 months, did you use any of the following products: a nicotine patch, a nicotine gum or dissolvable transdermal pill, a nicotine inhaler, a nicotine lozenge, a nicotine nasal spray, a nicotine transdermal system, or a nicotine injection?" (yes). "During the past 12 months, what is the length of time you stopped smoking because you were trying to quit smoking?" (2003 TCUS CPS). See (1) Appendix for calculation of e-cigarette associated transition probability of 24-months cigarette smoking cessation.
Probability of Cigarette Smoking Cessation 24 Months Among Current Cigarette Smokers Who Seriously Tried to Quit and Used a Pharmaceutical Aid During Quit Attempt (%)	Current Smokers	Meer et al. [12]	"Thinking back to the last time you tried to quit smoking in the past 12 months, did you use any of the following products: a nicotine patch, a nicotine gum or dissolvable transdermal pill, a nicotine inhaler, a nicotine lozenge, a nicotine nasal spray, a nicotine transdermal system, or a nicotine injection?" (yes). "During the past 12 months, what is the length of time you stopped smoking because you were trying to quit smoking?" (2003 TCUS CPS). See (1) Appendix for calculation of e-cigarette associated transition probability of 24-months cigarette smoking cessation.
Odds Ratio of Quitting Smoking Among Smokers with an Interest in Quitting	Current Smokers	Kalkhoran & Glantz [38]	Meta-analysis of 2 clinical trials [39,40], 4 cohort studies [41,42,43,44], and 1 case-control study [45]. See (1) Appendix for calculation of e-cigarette associated transition probability of 24-months cigarette smoking cessation.
Relative Risk Of Cigarette Smoking Cessation Among Current Cigarette Smokers Interested in Quitting, E-Cigarette Users Compared With Nicotine Patch Users	Current Smokers	Bullen et al. [19]	Primary outcome was continuous 24-month smoking abstinence self-reported abstinence over the whole follow-up period following 12 cigarettes in total and biologically verified continuous abstinence at 8 months (total breath carbon monoxide measurement <10 ppm). See (1) Appendix for calculation of e-cigarette associated transition probability of 24-months cigarette smoking cessation.
Probability of 1 Year Abstinence from Cigarette Smoking 6 Months Abstinence	Current Smokers	Beardy et al. [15]	2005–2008 Ontario Tobacco Survey
Probability of Long Term (24–36 Year) Abstinence from Cigarette Smoking 12 Year Abstinence	Current Smokers	Hawkins et al. [16]	1990–2006 British Household Panel Survey
Relative Hazard Reduction of E-Cigarette Use Compared to Cigarette Smoking	Current Smokers	McNeill et al. [11]	Continuous option
Never Cigarette Smoking	Adult & Young Adults	2014 NHIS/US	"Have you ever smoked cigarettes, even one or two puffs?" (yes).
Ever E-Cigarette Use	Adult	2014 NYTS	"Have you ever used an electronic cigarette, even just one time in your entire life?" (yes).
Ever E-Cigarette Use	Young Adults	2014 NHIS	"Have you ever used an electronic cigarette, even just one time in your entire life?" (yes).
Probability of Cigarette Smoking Initiation Among Never E-Cigarette Users	Adult & Young Adults	2012 Surgeon General's Report [19]	Initiation of cigarette smoking 15- to 17-year-olds and 18- to 21-year-olds, 2008 (2006–2010 NHIS/US). See (1) Appendix for calculation of e-cigarette associated transition probability of cigarette smoking initiation.
Adjusted Odds Ratio of Cigarette Smoking Initiation, Ever E-Cigarette Users vs. Never E-Cigarette Users	Adult & Young Adults	Soneji et al. [19]	From cohort studies pooled to random effects meta-analysis [13–15,17]. Odds ratio—adjusted for demographics, psychosocial, and behavioral risk factors—of cigarette smoking initiation between never cigarette smokers who ever used e-cigarettes and never cigarette smokers who never used e-cigarettes. See (1) Appendix for calculation of e-cigarette associated transition probability of cigarette smoking initiation.
Probability of Being a Current Daily Cigarette Smoker at Age 35–39 Age Of Cigarette Smoking Initiation	Adult & Young Adults	2009–2010 and 2012–2013 NHIS	Current daily cigarette smoker at age 35–39. "Have you smoked at least 100 cigarettes in your entire life?" (yes). "Do you now smoke cigarettes every day, some days, or not at all?" (every day or some days). Age of cigarette smoking initiation. "How old were you when you smoked a whole cigarette for the first time?"
Years of Life Gained or Lost	All	Bee et al. [19]	1967–2004 NHIS data linked to National Death Index. Years of life gained applied to current cigarette smokers who quit for 24 months. Years of life lost applied to adolescents and young adults who become current daily cigarette smokers at age 35–39.

Note: Adult = Adolescents | = Conditional On, NHIS = National Health Interview Survey, NHIS = National Health Interview Survey, NHIS/US = National Survey on Drug Use and Health, NYTS = National Youth Tobacco Survey, TCUS-CPS = Tobacco Use Supplement, Current Population Survey.

<https://doi.org/10.1371/journal.pone.0193328.t001>

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Table 1. Data Sources of model parameters.
<https://doi.org/10.1371/journal.pone.0193328.t001>

Validation of model

We validated the model against one-year intermediate outcomes (e.g., the number of adolescents and young adult cigarette smoking initiators). For current adult smokers, we applied the model to 2013 National Health Interview Survey (NHIS) data to predict the number of current cigarette smoking adults (both current and non-current e-cigarette users) who would quit in 2014 and remain continually abstinent from smoking for ≥6 months. We then compared this predicted number with the observed number in 2014, estimated from 2014 NHIS data, by identifying new ≥6-month quitters as respondents who answered six months to one year to the question: “How long has it been since you quit smoking cigarettes?”. For adolescent and young adult never smokers, we applied the model to 2013 National Survey on

Drug Use and Health (NSDUH) data to predict the number of cigarette smoking initiators in 2014 (both ever and never e-cigarette users). We then compared this predicted number with the observed number of initiators in 2014, estimated from 2014 NSDUH data, by identifying respondents who answered “yes” to the question: “Have you smoked part or all of a cigarette?” and whose current age was ≤ 1 year less than the age at which they first smoked a cigarette (“How old were you the first time you smoked part or all of a cigarette?”).

Analytic considerations and sensitivity analyses

To account for uncertainty in the prevalence and transition probability parameters, we utilized Monte Carlo simulation and independently drew from normal distributions with the means and standard deviations equal to the parameters’ means and standard errors shown in Tables A and B in [S3 Appendix](#). We repeated this process 100,000 times to create a distribution of each outcome of interest.

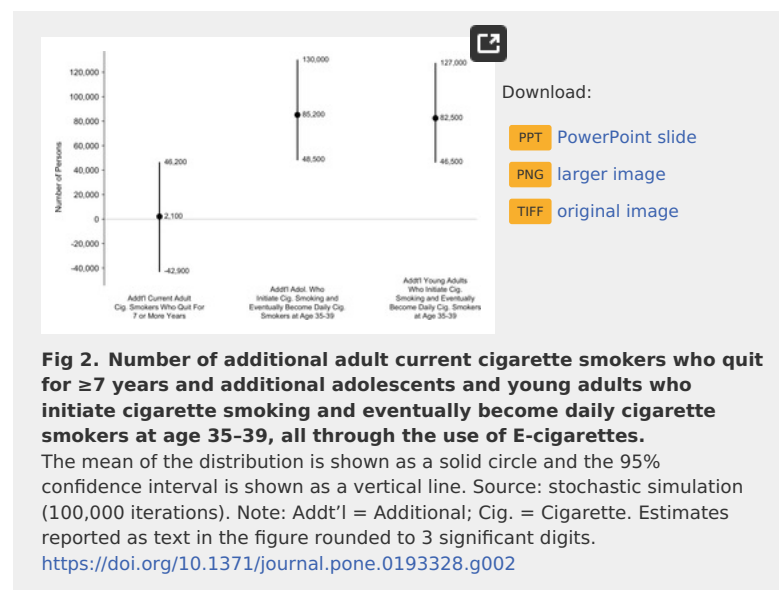
We conducted a sensitivity analysis by varying the level of four key parameters: (1) the adjusted odds ratio of smoking cessation, (2) the adjusted odds ratio of cigarette smoking initiation, (3) age-group-specific prevalence of current e-cigarette use among current cigarette smokers who tried quitting within the past year, and (4) age-specific prevalence of ever having tried e-cigarettes among never cigarette smokers. We also calculated the probability of positive total years of life gained across a wide range of possible values for these four parameters. For example, we supposed the adjusted odds ratio of smoking cessation equaled 2.5 times the baseline estimate ($2.15 = 2.5 \times 0.86$) and recalculated the years of life gained, drawing all other parameters from their baseline distributions. The probability of a positive total years of life gained under this supposition equaled the ratio of the (1) number of simulations that yielded a positive value and (2) total number of simulations (100,000). Finally, we varied from 0% to 100% the relative harm of e-cigarette use, compared to cigarette smoking, in terms of the number of years of life gained from quitting cigarette smoking. We used R, Version 3.2.3 for all analyses. Results of years of life gained were determined to be statistically significant if their 95% confidence intervals do not contain zero.

Results

Additional quitters and initiators

In 2014, 3,490,000 current adult cigarette smokers who had attempted to quit smoking in the past year had also currently used e-cigarettes. Additionally, 3,640,000 never-cigarette smoking adolescents and young adults had ever used e-cigarettes.

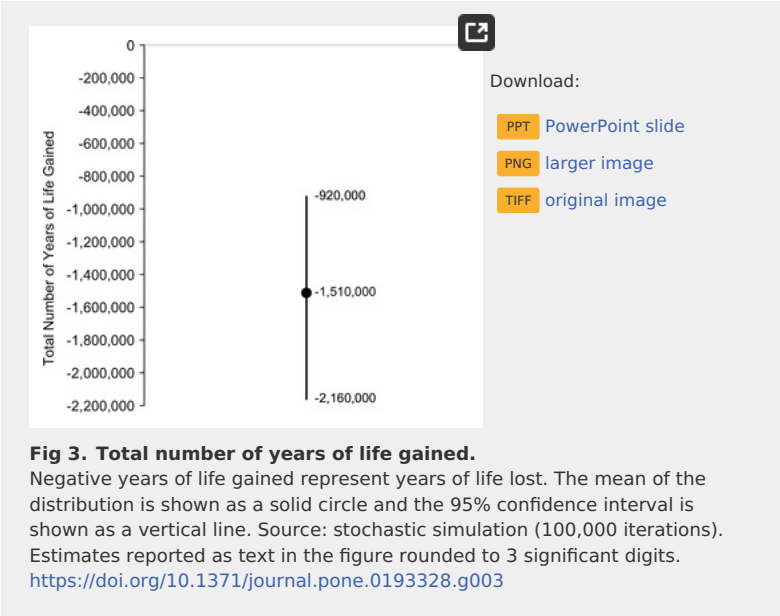
The model estimated that 2,070 additional current cigarette smoking adults (95% CI: -42,900 to 46,200) who currently used e-cigarettes in 2014 would quit smoking in 2015 and remain continually abstinent from smoking for ≥ 7 years using e-cigarettes, compared to those who did not currently use e-cigarettes ([Fig 2](#)). The model also estimated that an additional 168,000 never-cigarette smoking adolescents and young adults in 2014 (95% CI: 114,000 to 229,000) who had ever used e-cigarettes would initiate cigarette smoking in 2015 and eventually become daily cigarette smokers at age 35–39, compared to those who had never used e-cigarettes.



Years of life gained

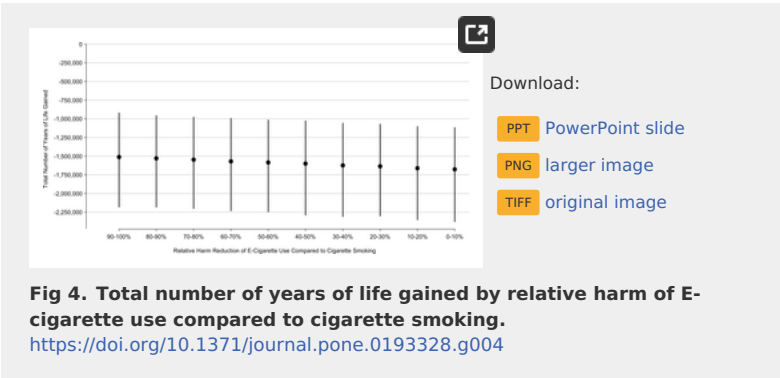
The model estimated that the 2,070 additional long-term quitters would gain -3,000 years of life (95% CI: -351,000 to 325,000). The model also estimated the

additional 168,000 adolescent and young adult cigarette smoking initiators who eventually become daily cigarette smokers at age 35-39 will lose 1,510,000 years of life (95% CI: 1,030,000 to 2,060,000). Thus, considering all population subgroups, the model estimated that e-cigarette use in 2014 would lead to 1,510,000 years of life lost (95% CI: 920,000 to 2,160,000; Fig 3) assuming an approximate 95% relative harm reduction of e-cigarette use compared to cigarette smoking.



Sensitivity analysis

Our results were sensitive to the adjusted odds ratios of cigarette smoking cessation and cigarette smoking initiation (Table 2). The model estimated that e-cigarette use in 2014 would lead to 1,150,000 years of life lost (95% CI: 2,130,000 to 242,000) under the relative risk of smoking cessation estimated by Bullen et al. (transformed to an odds ratio). The model estimated that e-cigarette use in 2014 would lead to 1,330,000 years of life lost (95% CI: 1,950,000 to 780,000) and 1,150,000 years of life lost (95% CI: 1,730,000 to 620,000) if the adjusted odds ratio of cigarette smoking initiation decreased by 10% and 20%, respectively. Our results were also sensitive to the prevalence of current e-cigarette use among current cigarette smokers who tried quitting within the past year and ever e-cigarette use and never cigarette smokers. Finally, we varied the health risks of e-cigarette use as a percentage of the risk associated with cigarette smoking. The total number of years of life lost increased as the relative harm of e-cigarette use, compared to cigarette smoking, grew (Fig 4). The model estimated that e-cigarette use in 2014 would lead to 1,530,000 years of life lost (95% CI: 2,180,000 to 960,000) and 1,580,000 years of life lost (95% CI: 2,250,000 to 1,020,000) if the health risks of e-cigarette use were 10%-20% (i.e., 80%-90% safer) and 40%-50% (i.e., 50%-60% safer) of the risks of cigarette smoking, respectively.



Parameter	Scenario	Parameter Pt. Est. (95% CI)	Years of Life Gained (95% CI)
Adjusted Odds Ratio of Cigarette Smoking Cessation	Base Case	0.80 (0.74 to 1.15)	-1,510,000 (-2,160,000 to -920,000)
	Bullen et al. ⁷	1.28 (0.42 to 2.20)	-1,530,000 (-2,180,000 to -960,000)
Adjusted Odds Ratio of Cigarette Smoking Initiation	Base Case	1.30 (1.18 to 1.50)	-1,510,000 (-2,160,000 to -920,000)
	10%	1.17 (1.04 to 1.44)	-1,330,000 (-1,950,000 to -780,000)
	20%	1.00 (0.86 to 1.13)	-1,150,000 (-1,730,000 to -620,000)
	Base Case	1.30 (1.18 to 1.50)	-1,510,000 (-2,160,000 to -920,000)
Prevalence of Current E-Cigarette Use Among Current Cigarette Smokers Who Tried to Quit Within the Past Year	Base Case	Age Group Specific	-1,510,000 (-2,160,000 to -920,000)
	10% Increase	Age Group Specific	-1,530,000 (-2,180,000 to -960,000)
	20% Increase	Age Group Specific	-1,580,000 (-2,250,000 to -1,020,000)
	Base Case	Age Group Specific	-1,510,000 (-2,160,000 to -920,000)
Prevalence of Ever E-Cigarette Use Among Never Cigarette Smokers	Base Case	Age Group Specific	-1,510,000 (-2,160,000 to -920,000)
	10% Decrease	Age Group Specific	-1,530,000 (-2,180,000 to -960,000)
	20% Decrease	Age Group Specific	-1,580,000 (-2,250,000 to -1,020,000)
	Base Case	Age Group Specific	-1,510,000 (-2,160,000 to -920,000)

Note: Pt. Est. = Point Estimate; CI = Confidence Interval.
⁷ Odds ratio and 95% CI converted from reported relative risk and probability of 16 month cessation in the nicotine patch control group (18%).
⁸ All estimates rounded to 3 significant digits.
<https://doi.org/10.1371/journal.pone.0193328.t002>

Table 2. Results of sensitivity analysis.

<https://doi.org/10.1371/journal.pone.0193328.t002>

The probability of a positive total number of years of life gained increased with the relative risk of smoking cessation: 6.7%, 44.6%, and 83.3% as the relative risk increased to 2.0, 2.5, and 3.0, respectively (Fig 5, Panel A). The probability also increased with higher prevalence of current e-cigarette use among current cigarette smokers (Fig 5, Panel B). Conversely, the probability increased to 0.0%, 0.0%, and 47.6% as the adjusted odds ratio decreased to 3.0, 2.0, and 1.0, respectively (Fig 5, Panel C). Finally, the probability increased with lower prevalence of ever e-cigarette use among never cigarette smokers (Fig 5, Panel D).

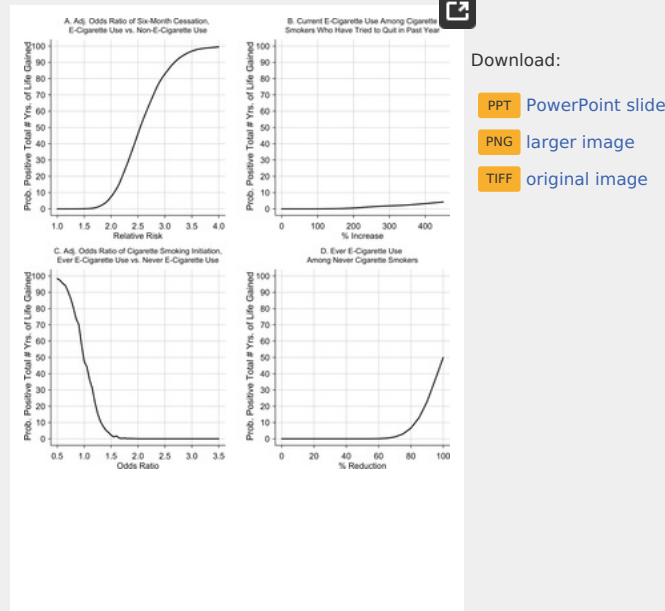


Fig 5. Probability of a positive total number of years of life gained varying the level of four key model parameters.

Note: vs. = versus; Adj. = Adjusted.

<https://doi.org/10.1371/journal.pone.0193328.g005>

Model validation

Based on 2013 NHIS data, we predicted 1.2 million current cigarette smoking adults would have quit and remained continually abstinent from smoking for ≥ 6 months in 2014 (95% CI, 1.0 to 1.4 million), which was not statistically different ($p = 0.57$) from the estimated number from the 2014 NHIS data (1.1 million, 95% CI: 0.9 to 1.3 million). Based on 2013 NSDUH data, we predicted that 5.5 million adolescents and young adults would have initiated cigarette smoking in 2014 (95% CI: 4.0 to 6.9 million), which was not statistically different ($p = 0.53$) from the observed number from 2014 NSDUH data (5.0 million, 95% CI: 4.1 to 5.9 million).

Discussion

Our study developed a Monte Carlo stochastic simulation model to assess the balance of health benefits and harms of e-cigarette use at the population level. Based on the most up-to-date published evidence, our model estimated that e-cigarette use in 2014 represents a population-level harm of about 1.6 million years of life lost over the lifetime of all adolescent and young adult never-cigarette smokers and adult current cigarette smokers in the 2014 US population. Our model also estimated even greater population-level harm if e-cigarette use confers long-term health risks.

Our study is consistent with Kalkhoran & Glantz (2015), who estimated the effects of e-cigarette use on cessation among smokers and on cigarette smoking initiation by never-smokers under various scenarios [29]. For example, their study found the largest relative health costs occurred in the scenario under which e-cigarette use increased among never-smokers because of the resulting increase in cigarette smoking initiation and the dual use of cigarettes and e-cigarettes, while e-cigarette use remained unchanged among established smokers. Our study also supports the conclusion of Cherng et al. (2016) on the relative effects of e-cigarettes on smoking initiation and cessation [39]. Our model indicates that the odds of smoking initiation among e-cigarette users would need to decrease more than the odds of smoking cessation would need to increase to achieve the same change in the total number of years of life gained.

Our conclusions differ from those of Levy et al. (2016), Levy et al. (2017), and Hill & Camacho (2017)—a tobacco industry-funded study [40–42]. Hill & Camacho found

the use of e-cigarettes would result in a decrease in smoking-related mortality in the UK from 8.4% to 8.1% in 2050 [40]. Levy et al. found that the use of vaporized nicotine products (VNPs; e.g., e-cigarettes) would lead to years of life gained for the US birth cohort of 1997 as it ages over time [41]. Hill & Camacho estimated an “overall beneficial effect from launching e-cigarettes”, in part, because they explicitly assumed the transition probability of cigarette smoking initiation among never cigarette smokers who used e-cigarettes equaled 5% [40]. Levy et al. (2016) estimated a “positive public health impact” from VNP use, in part, because they implicitly assumed the odds of cigarette smoking initiation was only marginally higher for ever e-cigarette users than never e-cigarette users (odds ratio≈1.16) among adolescents and young adults who would not have become a cigarette smoker in the absence of VNPs. Yet, both of these assumptions are substantially different from empirical estimates of these parameters from thirteen published cohort studies with a combined sample size of over 44,000 respondents [13-18,20,21,43-47]. Levy et al. (2017) estimates a substantial number of years of life gained from e-cigarette use, in part, because they explicitly assumed e-cigarette use among never cigarette smokers does not increase the rate of cigarette smoking initiation, which—again—contrasts with growing scientific evidence to the contrary. Nevertheless, these models provide useful conceptual frameworks to assess the net benefits of e-cigarette use and would likely yield substantively different conclusions under alternative—and empirically based—assumptions of e-cigarette use and cigarette smoking initiation.

E-cigarettes could, indeed, confer a positive population benefit if they were more effective as a smoking cessation device. For example, if current smokers who used e-cigarettes as a smoking cessation tool achieved six-month smoking abstinence at a rate of approximately 2.55 times greater than their counterparts who did not use e-cigarettes, then our model estimated that the probability of a positive total number of years of life gained would approach 50%. However, the estimated effectiveness of e-cigarettes for smoking cessation from all published randomized trials and nearly all cohort studies fall well below this threshold including some studies that concluded cigarette smokers who used e-cigarettes were less—not more—likely to quit than those who used standard clinic-based smoking cessation treatments [11,38,48-65]. Three cohort studies of current cigarette smokers did, indeed, estimate relative risks of smoking cessation above this threshold among intensive e-cigarette users (daily use for at least one month), daily tank e-cigarette users, and long-term (i.e., ≥2-year) e-cigarette users [59,66,67]. However, the prevalence of intensive e-cigarette use, daily e-cigarette tank use, and long-term e-cigarette use were low in these studies: only 34% of e-cigarette users were intensive users, 12% of e-cigarette users were daily e-cigarette tank users, and 14% of e-cigarette users were long-term users [59,66,67].

A decline in public acceptability of cigarette smoking has been accompanied by proscriptions on where smoking is allowed [68,69]. Nearly two-thirds of e-cigarette users reported using them when and where cigarette smoking was not allowed [70,71]. Further, an analysis of e-cigarette tweets highlighted that e-cigarette vaping was considered social acceptable by many, as opposed to cigarette smoking [72]. However, the lower level of sensation and satisfaction experienced with e-cigarettes, compared to cigarettes, may explain why some individuals who initiate with e-cigarettes then transition to cigarettes even though this transition is associated with higher nicotine ingestion [73-75].

E-cigarette use among former cigarette smokers may confer health risks. For example, e-cigarette aerosols carry high levels of aldehydes (e.g., formaldehyde) that affect cardiovascular function and high levels of fine particles that accelerate heart disease [76,77]. E-cigarette users experience equivalent reductions in vascular function (e.g., vitamin E levels and flow-mediation dilatation) as cigarette smokers. Furthermore, e-cigarette use suppresses immune and inflammatory-response genes in nasal epithelial cells and injures lung epithelial cells [78,79].

Our study has some potential limitations. First, we do not know if e-cigarette use causes cigarette-smoking initiation in adolescents and young adults. Published cohort studies have found consistent evidence of an increased risk of cigarette smoking initiation among non-smoking youth who had ever used e-cigarettes after accounting for known demographic, psychosocial, and behavioral risk factors [13-18,20,21]. We varied this longitudinal association between e-cigarette use and cigarette smoking initiation and reach similar conclusions. Perhaps more concerning that cigarette smoking initiation, e-cigarette use was independently associated with progression to heaving patterns of cigarette smoking among US adolescents [80]. Second, we do not know the type of e-cigarette currently used by cigarette-smoking adults. Second generation e-cigarettes (e.g., tank-style systems) deliver nicotine more efficiently than the first generation e-cigarettes used in Bullen et al. trial [49,81]. Third generation e-cigarettes (e.g., advanced personal vaporizers) deliver nicotine at approximately the same level and speed as traditional cigarettes [82]. However, we do not yet know the national prevalence of second and third generation e-cigarette use among current cigarette smokers who are trying to quit, and no published trials or cohort studies estimate cessation efficacy or effectiveness of third-generation e-cigarettes.

Third, in our calculation of benefit, we did not consider the possibility that e-cigarette use among current cigarette smokers leads to a reduction in the intensity of cigarettes smoked per day. A trial conducted by Caponnetto et al. found e-cigarette reduced the median number of cigarettes smoked per day among 300

Italian smokers not intending to quit [83]. Yet, similar reductions in the number of cigarettes smoked per day has not been observed in the US between dual users of e-cigarettes and cigarettes and exclusive cigarette smokers [65].

Fourth, we did not consider the potential population-level health benefit or harm of e-cigarette use among former cigarette smokers because no published trials or cohort studies assessed whether e-cigarette use among former cigarette smokers led to higher or lower rates of relapse to cigarette smoking. A recent cross-sectional study suggested long-term former cigarette smokers who use e-cigarettes may not experience any higher rate of relapse to smoking than their counterparts who do not use e-cigarettes [84].

Current public health models may yield substantively different conclusions about the net harm or benefit of e-cigarette use because there is insufficient data on the effect of e-cigarette use on cigarette smoking-related transitions and tobacco-related diseases. Conclusions may also differ because of decisions—both implicit and explicit—about the framework and underlying assumptions inherent in the model. The host of decisions required to develop a model produce structural uncertainty that may exceed parameter uncertainty [85,86]. Sensitivity analysis will not capture structural uncertainty because the model, itself, remains constant. Future work could incorporate Bayesian model averaging to account structural, or model-based, uncertainty [87]. Future work could also grade the quality of models based on published best practices [86,88].

In conclusion, based on currently available evidence on the e-cigarette associated transition probabilities of cigarette smoking cessation and initiation, our study suggests that e-cigarettes pose more harm than they confer benefit at the population level. If e-cigarettes are to confer a net population-level benefit in the future, the effectiveness of e-cigarettes as a smoking cessation tool will need to be much higher than it currently is. The US Preventive Services Task Force concludes the existing scientific evidence is insufficient to clinically recommend e-cigarettes as a smoking cessation tool [89]. In the United Kingdom, the National Institute of Clinical Excellence also notes limited evidence on the long-term health effects of e-cigarette use and does not clinically recommend e-cigarettes for smoking cessation, in contrast to Public Health England and the Royal College of Physicians [35,90,91]. Additionally, comprehensive tobacco control efforts are needed to reduce the appeal of e-cigarettes to youth.

Supporting information

S1 Appendix. E-cigarette-associated Δ transition probability of cigarette smoking cessation.

<https://doi.org/10.1371/journal.pone.0193328.s001>
(DOCX)

S2 Appendix. E-cigarette-associated Δ transition probability of cigarette smoking initiation.

<https://doi.org/10.1371/journal.pone.0193328.s002>
(DOCX)

S3 Appendix. Model parameters.

S3 Appendix including Tables A and B. Table A shows model parameters for current adult cigarette smokers. Table B shows model parameters for adolescents and young adults.

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(DOCX)

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First-ever Study Shows E-cigarettes Cause Damage to Gum Tissue

Wednesday, November 16, 2016

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Environmental Medicine

A University of Rochester Medical Center study suggests that electronic cigarettes are as equally damaging to gums and teeth as conventional cigarettes.

The study, published in *Oncotarget*, was led by Irfan Rahman, Ph.D. professor of Environmental Medicine at the UR School of Medicine and Dentistry, and is the first scientific study to address e-cigarettes and their detrimental effect on oral health on cellular and molecular levels. Electronic cigarettes continue to grow in popularity

among younger adults and current and former smokers because they are often perceived as a healthier alternative to conventional cigarettes. Previously, scientists thought that the chemicals found in cigarette smoke were the culprits behind adverse health effects, but a growing body of scientific data, including this study, suggests otherwise.

"We showed that when the vapors from an e-cigarette are burned, it causes cells to release inflammatory proteins, which in turn aggravate stress within cells, resulting in damage that could lead to various oral diseases," explained Rahman, who last year published a study about the damaging effects of e-cigarette vapors and flavorings on lung cells and an earlier study on the pollution effects. "How much and how often someone is smoking e-cigarettes will determine the extent of damage to the gums and oral cavity."

The study, which exposed 3-D human non-

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the study, which exposed 3-D human, non-smoker gum tissue to the vapors of e-cigarettes, also found that the flavoring chemicals play a role in damaging cells in the mouth.

"We learned that the flavorings--some more than others--made the damage to the cells even worse," added Fawad Javed, a post-doctoral student at Eastman Institute for Oral Health, part of the UR Medical Center, who contributed to the study. "It's important to remember that e-cigarettes contain nicotine, which is known to contribute to gum disease."



Fawad Javed, Eastman Institute for Oral Health

Most e-cigarettes contain a battery, a heating device, and a cartridge to hold liquid, which typically contains nicotine, flavorings, and other chemicals. The battery-powered device heats the liquid in the cartridge into an aerosol that the user inhales.

"More research, including long term and comparative studies, are needed to better understand the health effects of e-cigarettes," added Rahman, who would like to see manufacturers disclose all the materials and chemicals used, so consumers can become more educated about potential dangers.

This study was funded by the National Institutes of Health. Study collaborators include first author Isaac K. Sundar, UR Department of Environmental Medicine, Fawad Javed, Department of General Dentistry, Eastman Institute for Oral Health at UR, Georgios E. Romanos, Department of Periodontology, School of Dental Medicine, Stony Brook University and Irfan Rahman, Department of Environmental Medicine at UR.

###

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E-cigarettes and flavorings induce inflammatory and pro-senescence responses in oral epithelial cells and periodontal fibroblasts

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ABSTRACT

Electronic-cigarettes (e-cigs) represent a significant and increasing proportion of tobacco product consumption, which may pose an oral health concern. Oxidative/carbonyl stress via protein carbonylation is an important factor in causing inflammation and DNA damage. This results in stress-induced premature senescence (a state of irreversible growth arrest which re-enforces chronic inflammation) in gingival epithelium, which may contribute to the pathogenesis of oral diseases. We show that e-cigs with flavorings cause increased oxidative/carbonyl stress and inflammatory cytokine release in human periodontal ligament fibroblasts, Human Gingival Epithelium Progenitors pooled (HGEPP), and epigingival 3D epithelium. We further show increased levels of prostaglandin-E2 and cyclooxygenase-2 are associated with upregulation of the receptor for advanced glycation end products (RAGE) by e-cig exposure-mediated carbonyl stress in gingival epithelium/tissue. Further, e-cigs cause increased oxidative/carbonyl and inflammatory responses, and DNA damage along with histone deacetylase 2 (HDAC2) reduction via RAGE-dependent mechanisms in gingival epithelium. A greater response is elicited by flavored e-cigs. Increased oxidative stress, pro-inflammatory and pro-senescence responses (DNA damage and HDAC2 reduction) can result in dysregulated repair due to proinflammatory and pro-senescence responses in periodontal cells. These data highlight the pathologic role of e-cig aerosol and its flavoring to cells and tissues of the oral cavity in compromised oral health.

INTRODUCTION

The use of electronic-cigarettes (e-cigs) is increasing in the United States, which may pose oral health concerns [1]. E-cigs are battery operated devices, which consist of a metal heating element in a stainless steel shell, a cartridge, an atomizer and a battery. The heating element vaporizes a solution containing a mixture of chemicals including nicotine and other additives/humectants, such as base/carrying agents propylene glycol, glycerin/glycerol, and flavoring agents including fruit and candy flavors. Apart from inhaled nicotine, variable levels of aldehydes and carbonyls are detected in e-cig aerosols during vaporizations [2, 3]. Aldehyde causes carbonyl/oxidative

stress, DNA adducts/damage, as well as stress-induced cellular senescence (a state of irreversible growth arrest which re-enforces chronic inflammation) [4, 5] leading to oral health problems [6–8].

Periodontal disease is characterized by chronic inflammation of the supporting tissues of the teeth. Periodontal ligament and gingival fibroblasts as well as epithelial cells are the most abundant structural cells in periodontal tissue. Upon stimulation or stress, these cells are able to incite and maintain inflammatory responses [9]. There is an association between smoking and tooth loss, periodontal attachment level, deeper periodontal pockets, and more extensive alveolar bone loss along with the destruction of connective tissue and matrix, leading

to increased risk of periodontitis [10]. Clinical studies [11, 12] have also shown that habitual tobacco smokers exhibit a greater number of sites with plaque accumulation, clinical attachment loss and probing depth (≥ 4 mm) as compared to individuals who had never used tobacco in any form. It is important to mention that bleeding upon probing (a classical marker of periodontal disease activity) is masked in tobacco smokers than non-smokers [11, 12]. This most likely occurs as a result of the vasoconstrictive effect of nicotine (nicotine is the main component in e-cigs) on gingival blood vessels. Therefore, tobacco smokers may remain unaware of ongoing periodontal destruction until the inflammatory process reaches a stage where tooth mobility becomes evident.

We have recently shown the comparable oxidants/reactive oxygen species (ROS) reactivity in e-cig aerosols compared to conventional cigarette smoke [13, 14]. Smoking tobacco contributes to the progression of periodontal disease [10]. However, there is no information available regarding the e-cig aerosols vaping on periodontal/gingival oral health effects, especially in response to e-cig flavoring agents and nicotine. Periodontal/gingival cells in the oral cavity are the first targets by aerosols of e-cigs. Furthermore, the effects of e-cig aerosols on carbonyl stress, inflammation, and pro-senescence have not been studied on oral health. Here, we have determined the mechanism of gingival epithelial inflammation and pro-senescence by e-cig aerosols with flavorings in human oral epithelial cells and periodontal ligament fibroblasts.

RESULTS

E-cigarette exposure in human periodontal ligament fibroblasts (HPdLFs) and human gingival epithelium progenitors, pooled (HGEPP) increases protein carbonylation and pro-inflammatory responses

It is possible that ROS produced by e-cig vapors in cultured cells can augment protein carbonylation. The oxyblot assay was performed for immunodetection of the carbonyl groups that were introduced into protein side chains by e-cig-induced oxidative stress. We found that e-cig flavoring (BLU[®] Classic Tobacco, 16 mg nicotine and Magnificent Menthol, zero nicotine) had different levels of protein oxidation as confirmed by OxyBlot. E-cigarette vapors from BLU[®] Classic Tobacco showed significant increase in protein carbonylation compared to air exposed controls. E-cigarette vapors from BLU[®] Magnificent Menthol flavor also showed a trend towards increased protein carbonylation but not significant compared to the control (Figure 1A–1B).

HPdLFs and HGEPP were exposed to BLU[®] e-cig vapors (Classic Tobacco, 16 mg nicotine; and Magnificent Menthol, zero nicotine) using ALI system for 15 min. Conditioned medium was collected from

both air exposed (control) and e-cig vapor exposed cells 24 hrs post ALI exposure. IL-8 secretion in conditioned medium 24 hrs after ALI exposure was significantly increased in both BLU[®] e-cig vapors. PGE₂ secretion showed a trend of incremental increase, but was not significant compared to air exposed controls (Figure 1C). IL-8 release was significantly higher in both BLU[®] Classic Tobacco (16 mg nicotine) and Magnificent Menthol (zero nicotine) e-cig vapor exposed HPdLF cells compared to the controls. PGE₂ release was significantly higher in BLU[®] Magnificent Menthol (zero nicotine) e-cig vapor exposed cells compared to the controls (Figure 1C). Similarly, HGEPP cells also showed an increased trend in IL-8 release in BLU[®] Classic Tobacco (16 mg nicotine) e-cig vapor exposed cells compared to the controls. We did not see a significant increase in IL-8 release in HGEPP cells exposed to BLU[®] Magnificent Menthol (zero nicotine) e-cig vapor compared to control (Figure 1D). Overall, e-cig vapors with flavoring induce protein carbonylation and increase in pro-inflammatory cytokines release which are indicative of oxidative stress and inflammatory response cause by e-cig vapors in HPdLF and HGEPP.

E-cigarette exposure in human periodontal ligament fibroblasts (HPdLFs) increases inflammation and DNA damage markers

HPdLFs exposed to e-cig vapor from BLU[®] e-cig (Classic Tobacco, 16 mg nicotine; and Magnificent Menthol, zero nicotine) for 15 min using ALI system and incubated for 24 h. Then, we measured several markers of inflammation such as PGE₂-mediated COX-2 induction, HDAC2, S100A8, RAGE and phosphorylated γ H2A.X (Ser139) in whole cell lysates. We found E-cig flavoring BLU[®] Classic Tobacco (16 mg nicotine) and Magnificent Menthol (zero nicotine) showed a differential effect on levels of COX-2, HDAC2, S100A8, RAGE and γ H2A.X in HPdLFs *in vitro* (Figure 2A). HPdLFs exposed to BLU[®] e-cig vapors (Magnificent Menthol, zero nicotine) showed a significant increase in all the inflammatory markers (COX-2, S100A8, RAGE), a trend towards reduced HDAC2 and increased phosphorylated γ H2A.X (Ser139), a DNA damage marker compared to control. HPdLFs exposed to BLU[®] e-cig (Classic Tobacco, 16 mg nicotine) also showed a significant increase in S100A8 and γ H2A.X, and an increased trend in COX-2 compared to the controls. We did not observe any significant increase in RAGE or a significant decrease in HDAC2 levels in BLU[®] E-cig vapor (Classic Tobacco, 16 mg nicotine) exposed HPdLFs compared to the controls (Figure 2A). Likewise, the HPdLFs exposed to e-cig vapor from BLU[®] e-cig (Classic Tobacco, 16 mg nicotine) showed a significant increase in DNA damage as measured by the percentage of DNA in tail using the Comet assay (Figure 2B). Overall, we showed that e-cig vapors with flavoring differentially

affects inflammatory response and DNA damage markers as a result of oxidative stress and inflammatory response caused by e-cig vapors in HPdLFs.

E-cigarette exposure in human gingival epithelium progenitors, pooled (HGEpP) increases inflammation and DNA damage markers

We found E-cig flavoring BLU[®] Classic Tobacco (16 mg nicotine) and Magnificent Menthol (zero nicotine) showed differential effects on levels of COX-2, S100A8, RAGE and γ H2A.X in HGEpP cells *in vitro* (Figure 2A). HGEpP cells exposed to BLU[®] e-cig vapors (Classic Tobacco, 16 mg nicotine and Magnificent Menthol, zero nicotine) showed a significant increase in the inflammatory markers (COX-2 and RAGE), and DNA damage marker (phosphorylated γ H2A.X Ser139) compared to the controls. The effect of BLU[®] e-cig vapors (Magnificent Menthol, zero nicotine) on HGEpP cells was significantly higher compared to BLU[®] e-cig vapors Classic Tobacco (16 mg nicotine) and the controls. We

did not observe any significant increase in COX-2 and S100A8 levels in BLU[®] e-cig vapor from Classic Tobacco (16 mg nicotine) as well as S100A8 levels in Magnificent Menthol (zero nicotine) exposed HGEpP cells compared to the controls (Figure 3). Overall, we confirmed that e-cig vapors with flavoring affects inflammatory response and DNA damage markers as a result of oxidative stress and inflammatory response caused by e-cig vapors in HGEpP cells.

E-cigarette exposure in human 3D *in vitro* model of EpiGingival tissue increases inflammation and DNA damage markers

Human 3D model of EpiGingival tissues were exposed to e-cig vapor from BLU[®] (Classic Tobacco, 16 mg nicotine; and Magnificent Menthol, zero nicotine) for 15 min using the modified ALI system without culture media. The human 3D EpiGingival tissue insert was contained within 35 mm culture dishes along with 900 μ l culture medium during e-cig vapor exposure, and incubated for 24 h. Then, we measured pro-inflammatory cytokines IL-8 and PGE₂

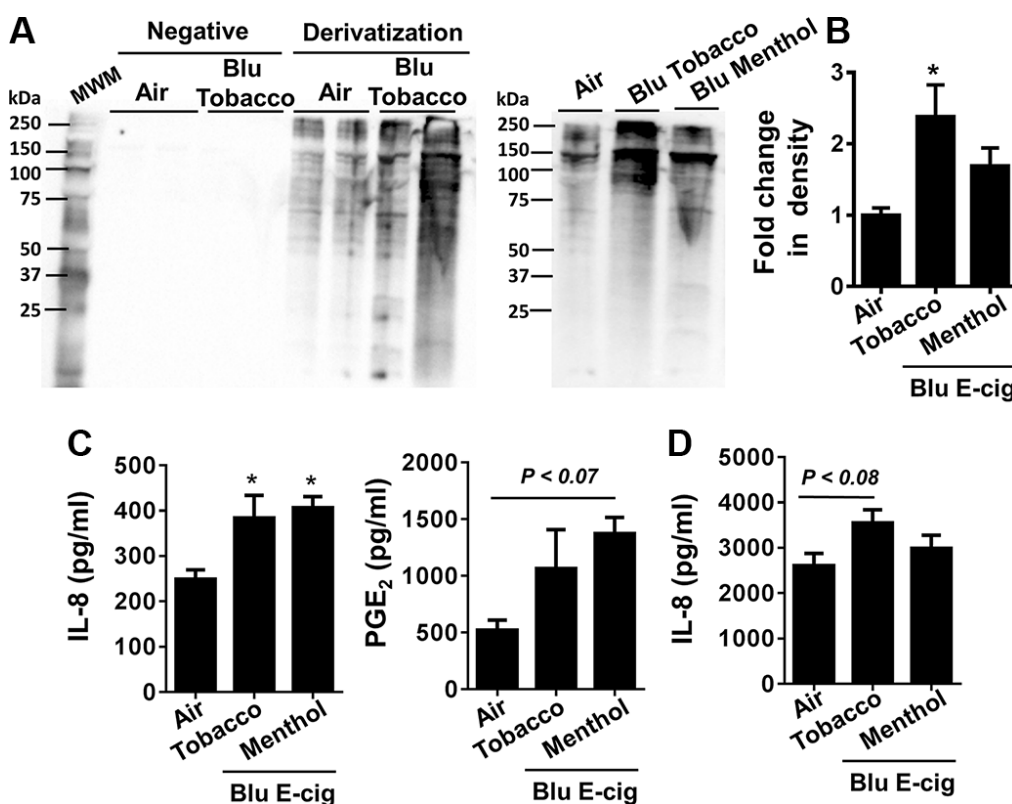


Figure 1: E-cig aerosol resulted in increased protein carbonylation and inflammatory responses in human periodontal ligament fibroblasts (HPdLFs) and human gingival epithelium progenitors, pooled (HGEpP). HPdLFs (A–C) and HGEpP (D) were exposed to aerosols from BLU[®] e-cig (Classic Tobacco, 16 mg nicotine; and Magnificent Menthol, ‘zero’ nicotine) (2 puffs/min; 4–5 sec/puff every 25 sec) using air-liquid interface system for 15 min, and then incubated at 37°C and 5% CO₂ for 24 h. (A, B) Protein carbonylation in cell lysates was determined by the oxyblots. Gel pictures shown are representative of at least 3 separate experiments for protein carbonylation. (C, D) Levels of IL-8 and PGE₂ in supernatants were determined by ELISA. Data are means \pm SE ($n = 3$ –6/group) and significance determined using 1-way ANOVA. * $P < 0.05$, vs. air. MWM: Molecular weight markers; Negative: without derivatizing reagent 2,4-dinitrophenylhydrazine (DNPH).

in conditioned medium collected 24 h post last exposure. Markers of inflammation and DNA damage, such as RAGE, PGE₂-mediated COX-2 induction and γ H2A.X were measured using whole tissue lysates from 3D EpiGingival cultures. We found e-cig flavoring BLU[®] Classic Tobacco (16 mg nicotine) and Magnificent Menthol (zero nicotine) showed an increasing trend in the levels of IL-8 release and a significant increase in the levels of PGE₂ release compared to the controls (Figure 4A). Additionally, only e-cig flavoring BLU[®] Magnificent Menthol (zero nicotine) showed an increasing trend in RAGE, COX-2, and γ H2A.X (immunoblot analysis and immunohistochemistry) in 3D EpiGingival tissues *in vitro* compared to the controls (Figure 4B–4C). The effect of BLU[®] e-cig vapors (Magnificent Menthol, zero nicotine) on 3D EpiGingival tissues was significantly higher compared to BLU[®] e-cig vapors Classic Tobacco (16 mg nicotine) and the controls. Overall, we reproduced the above findings in 3D models that e-cig vapors with flavoring differentially affects inflammatory response and DNA damage markers as a result of oxidative stress and inflammatory response caused by e-cig vapors in 3D *in vitro* model of EpiGingival tissues.

DISCUSSION

In this study, we show that e-cigs with flavorings cause increased oxidative/carbonyl stress and inflammatory cytokine release in human periodontal ligament fibroblasts,

Human Gingival Epithelium Progenitors pooled (HGEPP), and EpiGingival 3D epithelium. We further show increased levels of prostaglandin-E₂ and cyclooxygenase-2 were associated with upregulation of the receptor for advanced glycation end products (RAGE) by e-cig exposure-mediated carbonyl stress in gingival epithelium/tissue. Further, e-cigs cause increased oxidative/carbonyl stress and inflammatory responses, and DNA damage along with histone deacetylase 2 (HDAC2) reduction via RAGE-dependent mechanisms in gingival epithelium, with greater response by flavored e-cigs. Increased oxidative stress, pro-inflammatory and pro-senescence responses (DNA damage and HDAC2 reduction) can result in dysregulated repair due to proinflammatory and pro-senescence responses in periodontal cells. Our data also implicate that e-cig affects the regenerative potential of human progenitor cells due to increased inflammatory and DNA damage responses. It is well known that the mTOR pathway is activated by most oncogenes that induce cellular senescence (i.e., by Ras, Raf, MEK, and Akt). Further, the mechanistic target of rapamycin (mTOR) is generally activated in proliferating cells. During acute DNA damage, mTOR induces DNA damage response (DDR) and cell cycle arrest (induction of p21 and 16) [15]. Such arrested cells where mTOR is active play an important role in geroconversion (converts quiescence into senescence) to their pro-senescent phenotype [15, 16]. In case of oncogene-induced senescence, DDR causes cell

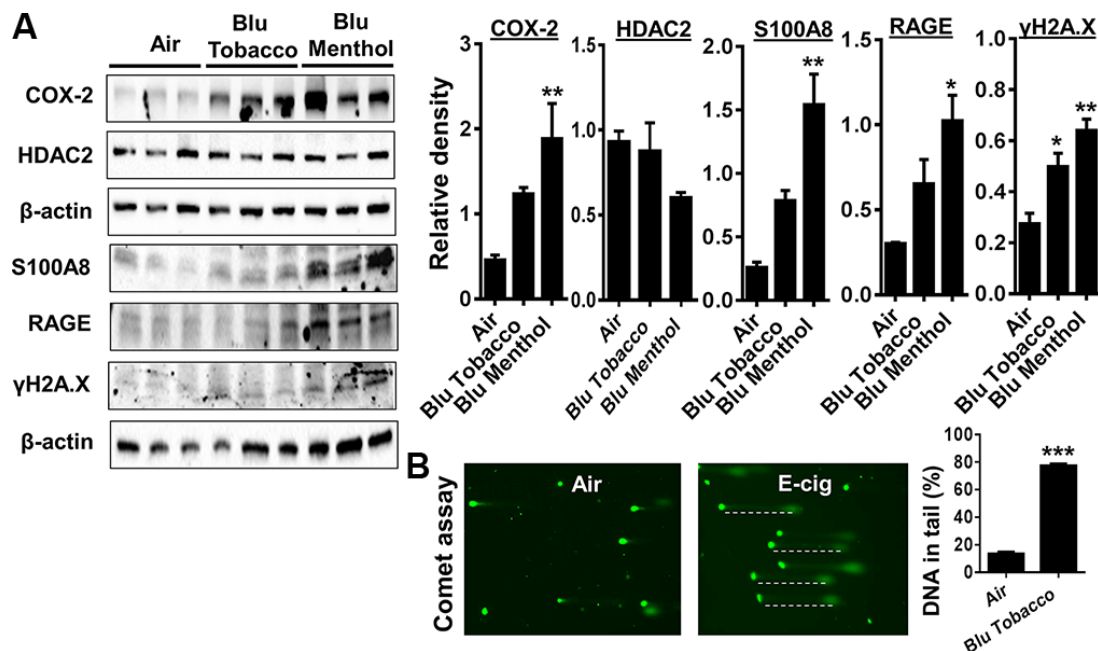


Figure 2: E-cig vapor exposure caused inflammatory responses and DNA damage in human periodontal ligament fibroblasts (HPdLFs). (A) HPdLFs were exposed to aerosols from BLU[®] e-cig (Classic Tobacco, 16 mg nicotine; and Magnificent Menthol, 'zero' nicotine) for 15 min (2 puffs/min; 4–5 sec/puff every 25 sec) using air-liquid interface system, and then incubated at 37°C and 5% CO₂ for 24 h. (A) Levels of COX-2, S100A8, RAGE, γ H2AX, and HDAC2 in cell lysates were measured by Western blotting. (B) DNA damage, indicated by an increase in fluorescent tail length, was measured by the Comet assay. Dash lines indicate DNA in tails. Data are means \pm SE ($n = 3$ –6/group) and significance determined using 1-way ANOVA or 2-tail *t*-test. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$

cycle arrest, leading to cell senescence [16]. This may be one of the mechanisms of e-cigarette induce DDR response via mTOR activation.

Conventional cigarette smoke has been shown to cause deleterious effects on oral and periodontal health [10]. However, the role of e-cig vaping and its association with carbonyl stress, inflammation, and DNA damage-triggered senescence on oral/periodontal epithelium remains unknown. Periodontal ligament and gingival fibroblasts as well as epithelial cells are the most abundant structural cells in periodontal tissue, and are the direct targets of e-cigs upon vaping. Upon stimulation or stress, these cells are able to incite and maintain inflammatory responses [9]. There is an association between smoking and tooth loss, periodontal attachment loss, deeper periodontal pockets, and more extensive alveolar bone loss along with the destruction of connective tissue and matrix, leading to an increased risk of periodontitis [10]. However, no studies are available to document the effects of e-cig vaping especially in response to e-cig flavoring agents on periodontal health in terms of oxidative/carbonyl stress and inflammation in human gingival epithelial cells *in vitro*.

We have recently shown oxidants/ROS reactivity from e-cig aerosols is comparable to conventional cigarette smoke [13, 14]. We show that e-cig flavoring caused protein oxidation as reflected in increased protein carbonylation. This was associated with increased IL-8 and PGE₂ secretion from HPdLFs and HGEPP cells upon exposure to e-cig aerosols. Direct exposure to e-liquids (this is not the case when users vape e-cigs i.e. users do not consume e-liquids) has also been shown to produce harmful effects in periodontal ligament and gingival fibroblasts in culture [17, 18]. While the

contribution of smoking tobacco to the progression of periodontal disease and other adverse oral health outcomes is well described [10, 19], no information is available regarding the impact of e-cig aerosols vaping on periodontal/gingival oral health effects, especially in response to e-cig flavoring agents. We determined the effect of flavoring on oxidative and pro-inflammatory responses, and upon exposure of periodontal ligament fibroblasts, Human Gingival Epithelium Progenitors pooled (HGEPP), and epigingival 3D epithelium to menthol flavoring agent resulted in increased oxidative/carbonyl stress, and IL-8 release. Menthol acts on transient receptor potential ankyrin 1 (TRPA1) receptors to activate inflammatory responses [20, 21]. It may be conceived that activation of TRPA receptors by e-cig aerosols will drive COX-PGE₂ mediated responses in periodontal tissues, leading to augmentation of inflammatory and pro-fibrotic and pro-carcinogenic responses. However, further studies are required to understand the augmented response by BLU[®] menthol flavoring than BLU[®] classic tobacco.

Protein carbonylation leads to autoantibody production which may lead to destruction of matrix and bone loss during periodontitis [6, 7]. Further, it is possible that carbonyls/aldehydes play an important role in e-cig aerosol-induced oral toxicity. Conventional tobacco smoke is known to cause oxidative burden leading to DNA damage and inflammatory responses [22]. The RAGE is a pattern-recognition receptor implicated in immune and inflammatory diseases including dental pulp inflammation and periodontitis [23–27]. RAGE is involved in smoking-related disorders and known to cause cellular senescence via oxidant stress [28, 29]. However, the mechanism of RAGE-mediated

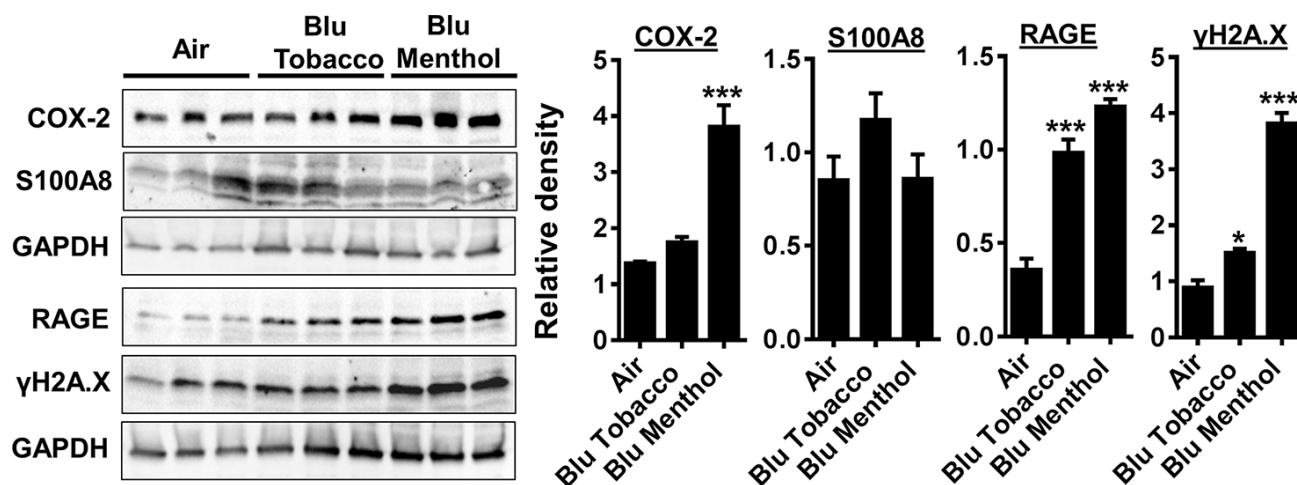


Figure 3: E-cig vapor exposure caused inflammatory responses and DNA damage in human gingival epithelium progenitors, pooled cells (HGEPP). (A) HGEPP cells were exposed to aerosols from BLU[®] e-cig (Classic Tobacco, and Magnificent Menthol) (2 puffs/min; 4–5 sec/puff every 25 sec) using air-liquid interface system for 15 min, and then incubated at 37°C and 5% CO₂ for 24 h. Levels of COX-2, S100A8, RAGE, and γH2AX in cell lysates were measured by Western blotting. Data are means ± SE (*n* = 3–6/group) and significance determined using 1-way ANOVA. **P* < 0.05, ****P* < 0.001, vs. air.

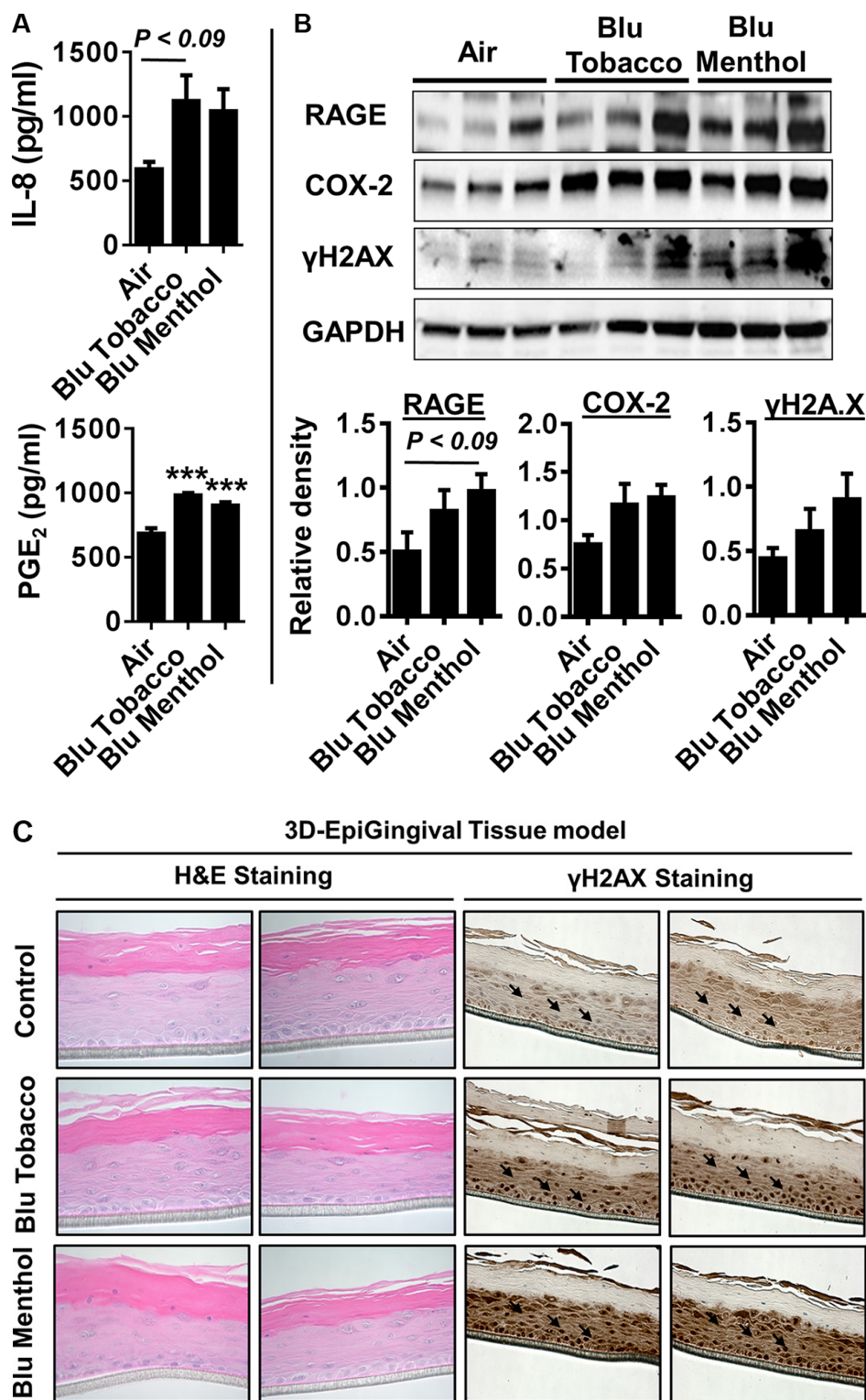


Figure 4: E-cig vapor caused inflammatory responses and DNA damage in normal human 3D *in vitro* model of EpiGingival tissues. Normal human 3D *in vitro* model of EpiGingival tissue (Cat#: GIN-100, MatTek) were exposed to aerosols from BLU® e-cig (Classic Tobacco, and Magnificent Menthol) using air-liquid interface system for 15 min, and then incubated at 37°C and 5% CO₂ for 24 h. (A) Levels of IL-8 and PGE₂ in culture media were determined by ELISA. (B) Levels of RAGE, COX-2, and γH2AX in tissue lysates were measured by Western blotting. (C) Representative images of EpiGingival tissues used for ALI exposures stained with H&E (showing histological features) and γH2AX staining 24 h post exposure to control (air) and flavored e-cig aerosols. Immunohistochemistry revealed a distinct staining in both the flavored BLU e-cig exposed EpiGingival tissues for γH2AX. Data are means ± SE ($n = 4-6$ /group) and significance determined using 1-way ANOVA. *** $P < 0.001$, vs. air.

signaling especially via its ligand S100A8 as a susceptible factor in inducing gingival epithelial inflammation and senescence by e-cigs is not known. E-cig vapor exhibited significant inflammatory response (COX2, RAGE S100A8), DNA damage as determined by the Comet assay and γ -H2AX levels (a marker of DNA damage) in gingival epithelium/tissue. Our data showing inflammatory and pro-DNA damage responses are unique in light of primary cells and 3D tissue culture models which mimic closely with users vaping of e-cigs. Our results further attest the pro-oxidant, DNA damaging and pro-inflammatory effects of e-cig vapor exposure. The increased levels of pro-inflammatory mediators IL-8 and PGE₂ would cause remodeling of the ECM during periodontitis by e-cig due to cellular senescence, where these cells have secretory phenotype to perpetuate the inflammatory responses.

It has been reported that outcomes of periodontal therapy are compromised in smokers compared with non-smokers [30, 31]. A variety of mechanisms have been proposed in this regard. For example, increased expression of RAGE occurs in gingival epithelial cells of smokers as compared to non-smokers. Furthermore, it has been reported that nornicotine (a metabolite of nicotine), upregulates RAGE expression in the gingivae of smokers and elicits a proinflammatory response by stimulating the secretion of cytokines and ROS which are involved in destruction of the periodontal tissue [32]. The vasoconstrictive effects of nicotine increase platelet adhesiveness, increases the risk of microvascular occlusion and causes tissue ischemia [32]. Furthermore, tobacco smoking is also associated with catecholamines release resulting in vasoconstriction and decreased tissue perfusion [33]. Therefore, it is hypothesized that the outcomes of periodontal surgery are compromised in e-cig users compared with non-smokers/non-users through the mechanisms comparable to those stated above. However, further studies are needed to test this contention in a clinical cohort of users and non-users of e-cigs vs conventional smokers.

In conclusion, our data showed that e-cig aerosol cause increased oxidative/carbonyl stress and inflammatory responses, and cellular senescence associated with persistent DNA damage via RAGE-HDAC2-dependent mechanisms in gingival epithelium, with greater response by flavored e-cigs. Further understanding of the chronic effect of vaping could lead to molecular mechanisms for susceptibility (inflammatory, DNA damage and senescence responses) to the development of periodontitis, and therapeutic targets or biomarkers in determining vaping-flavoring mediated oral complications in cells and tissues of the oral cavity. Our data also implicate that e-cig affects the regenerative potential of human progenitor cells due to increased inflammatory and DNA damage responses. Overall, our data suggest the pathogenic role of e-cig aerosol to cells and tissues of the oral cavity, leading to compromised periodontal health.

MATERIALS AND METHODS

E-cigarettes

BLU® rechargeable e-cigs (Lorillard Technologies, Inc.) containing two different disposable cartomizers [Flavors: classic tobacco (16 mg nicotine), and magnificent menthol (zero nicotine or 13–16 mg nicotine)] with pre-loaded e-liquid were used. The BLU® e-cigarette device and disposable cartomizer cartridges were purchased from local retailers.

Cell culture and air-liquid interface (ALI) culture/exposures

Clonetics™ Human periodontal ligament fibroblast (HPdLF, CC-7049; Lonza) were grown at 37°C in 5% CO₂ incubator to 80–90% confluence in stromal cell medium (SCGM™ BulletKit, Lonza; CC-3205) supplemented with hFGF-B (0.5 ml), insulin (0.5 ml), FBS (25 ml) and GA-1000 (0.5 ml) according to manufacturer recommendations. Human gingival epithelium progenitors, (HGEp; gingival epithelial cells) were grown in CnT-Prime medium (CnT-PR), epithelial culture medium as recommended by CELLnTEC Advanced Cell Systems. Transwell cultures were then placed into air-liquid interface (ALI) exposure chamber [14, 34]. BLU® e-cigarette vapor (Classic Tobacco containing 16 mg nicotine or Magnificent Menthol flavor containing zero or 13–16 mg nicotine) was drawn into the ALI exposure chamber (2 puffs every 1 min), 4–5 second puff followed by 25 second pause for different time durations 5, 10, and 15 minutes respectively.

Human EpiGingival tissue model

Human gingival tissues (EpiGingival, GIN-100) were obtained from MatTek Corporation (Ashland, MA). The 3D tissue model is a reconstructed oral epithelial tissue that are derived from human primary oral keratinocytes. This model and allowed to differentiate to a structure characteristic to that of *in vivo*. EpiGingival tissues were exposed to air (control) or BLU® e-cigarette vapors (Classic Tobacco and Magnificent Menthol). After 15 min exposure, the conditioned medium was collected to measure pro-inflammatory cytokines and tissues harvested for Western blotting and immunohistochemistry according to the recommendations of the manufacturer. The protein levels were measured using a BCA kit (Pierce, IL, USA).

Comet assay

Comet assay was performed as per the instructions of the manufacturer (Trevigen, Gaithersburg, MD) [34]. Comet images were captured using a Nikon ECLIPSE Ni fluorescent microscope. The images were analyzed using OpenComet software. The extent of DNA damage was expressed as a measure of percentage of DNA in tail.

Protein carbonylation/oxyblot

Protein oxidation was determined using the OxyBlot protein oxidation detection kit following the manufacturer's instruction (Millipore, S7150). Equal amount of protein was loaded for oxyblot analysis, and the results were quantified by densitometry using Image J.

Pro-inflammatory cytokine analysis

Following 24 hrs after BLU[®] e-cigarette vapor exposure using air-liquid interface approach, conditioned media was collected and stored at -80°C for measuring pro-inflammatory mediators. IL-8 (Life Technologies, Carlsbad, CA) and PGE₂ (Cayman Chemical, Ann Arbor, MI) levels were measured by enzyme-linked immunosorbent assay (ELISA) according to manufacturer's instructions.

Western blotting

For Western blots, 25 µg protein samples were separated on a 4–15% gradient and 7.5% SDS-PAGE gels. Then probed with specific primary antibodies (1:1000 dilution in 5% milk in PBS containing 0.1% Tween 20 (v/v), such as anti-COX2 (Cayman chemical; #160112), HDAC2 (ab32117), S100A8 (ab92331), RAGE (ab37647) and γH2A.X phospho S139 (ab2893) from Abcam at 4°C overnight. The bound complexes were detected using ECL method with the ChemiDoc[™] MP Imaging System (Bio-Rad). Equal loading of the samples was determined by quantitation of proteins and by stripping and re-probing membranes for β-actin or GAPDH (Santa Cruz Biotechnology, sc-1616 and sc-365062) and the results were quantified by densitometry using Image J.

Statistical analysis

Statistical analysis of significance was calculated using unpaired Student's *t*-test for comparison between two groups control vs. e-cigarette. Probability of significance compared to control for more than two treatment groups (different e-cigarette flavors) was analyzed by 1-way ANOVA (Tukey's multiple comparisons test) using GraphPad Prism 6 as indicated in figure legends. The results are shown as the mean ± SEM unless otherwise indicated. *P* < 0.05 is considered as statistically significant.

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CONFLICTS OF INTEREST

The authors have declared that no conflicts of interests exists.

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Authors' contributions

IKS, IR: Conceived and designed the experiments; IKS: Performed the experiments; IKS: Analyzed the data; FJ, GER: Provided inputs and thoughtful discussion as well as edited the manuscript; IKS, IR: Wrote and edited the manuscript.

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