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Recent Updates on Electronic Cigarette Aerosol and Inhaled Nicotine Effects on Periodontal and Pulmonary Tissues

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Short running title: E-cig oral and pulmonary effects

Abstract

E-cigarette derived inhaled nicotine may contribute to the pathogenesis of periodontal and pulmonary diseases in particular via lung inflammation, injurious and dysregulated repair responses. Nicotine is shown to have anti-proliferative properties and affects fibroblasts *in vitro,* which may interfere in tissue myofibroblast differentiation in e-cig users. This will affect the ability to heal wounds by decreasing wound contraction. In periodontics, direct exposure to e-vapor has been shown to produce harmful effects in periodontal ligament and gingival fibroblasts in culture. This is due to the generation of reactive oxygen species/aldehydes/carbonyls from e-cig aerosol, leading to protein carbonylation of extracellular matrix and DNA adducts/damage. A limited number of studies regarding the effects of e-cig in oral and lung health are available. However, no reports are available to directly link the deleterious effects on e-cigs, inhaled nicotine, and flavorings aerosol on oral periodontal and pulmonary health in particular to identify the risk of oral diseases by e-cigarettes and nicotine aerosols. This mini-review summarizes the recent perspectives on e-cigarettes including inhaled nicotine effects on several pathophysiological

events, such as oxidative stress, DNA damage, innate host response, inflammation, cellular senescence, pro-fibrogenic and dysregulated repair, leading to lung remodeling, oral submucous fibrosis and periodontal diseases.

Keywords*:* e-cigarettes; fibrosis; inflammation, lung; oxidative stress; periodontium

Introduction

Electronic cigarettes (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 hundreds of flavoring agents including fruit and candy flavors (Cheng, 2014, Barrington-Trimis et al., 2014). Apart of high concentration of nicotine (up to 24 mg), numerous chemicals including aldehydes (as carbonyls), heavy metals (nickel, chromium, copper-coated with silver), metal particle/ultrafine/nano-particles, and tobacco specific nitrosamines as well as diacetyl, 2,3-pentanedione, and acetoin (buttery) are found in e-cig aerosols (Kosmider et al., 2014, Cheng, 2014). Other flavoring chemicals include ortho-vanilin (vanilla), maltol (malt), cinnamaldehyde and coumarin (Gerloff et al., 2017). Variable levels of carbonyls (e.g., up to 380 μg formaldehyde/10 puffs) have been detected in e-cig aerosols during vaporizations (Jensen et al., 2015, Kosmider et al., 2014). Moreover, a general lack of oversight in manufacturing and marketing of e-liquid/e-juices has been reported (Lisko et al., 2015). Therefore, significant concerns exist regarding the purity and variety (e.g., flavor additives) of ingredients employed.

The use of e-cigs has increased in the United States (U.S.) and worldwide particularly among young adults (Regan et al., 2013, Krishnan-Sarin et al., 2014). In the U.S., approximately 11% to 21% of adult smokers have reported to have ever used E-cigs (Varlet et al., 2015). Despite rising e-cig use, only a limited number of studies have addressed the potential toxicological effect of e-cig smoking on oral health (Sundar et al., 2016, Harrison & Hicklin, 2016, Rouabhia et al., 2016). Exposure to e-cig aerosol mixtures with flavorings may increase oxidative/carbonyl stress and inflammatory cytokine release in human periodontal ligament fibroblasts, human gingival epithelium progenitors, pooled cells (HGEPp), and 3D EpiGingival tissues (Sundar et al., 2016). Various aldehydes including acrolein and formaldehyde are found in the aerosols from e-cigs (Cheng, 2014, Kosmider et al., 2014). E-cig-derived aldehydes cause carbonyl/oxidative stress and DNA adducts/damage, which may lead to dysregulated repair and impaired wound healing, in particular in smokers (**Figure 1**) (Pradeep et al., 2013, Baltacioglu et al., 2008, Lei et al, 2017).

While the contribution of smoking tobacco to the progression of periodontal diseases and other adverse oral health outcomes is well described (Reibel, 2003, Javed et al., 2014, Brown et al., 1996, Albandar et al., 1999); there is currently no information available regarding the impact of e-cig aerosols vaping on oral and systemic health. The aim of the present review is to briefly review and summarize the available evidence about the effects of e-cig aerosols on periodontal and pulmonary health.

PubMed (National Library of Medicine), Google-Scholar, Scopus, EMBASE, MEDLINE (OVID) and Web of Knowledge databases were searched to identify articles that assessed the effects of e-cig on periodontal and pulmonary health. All levels of available evidence (including in vitro studies, studies in animal models, case reports and case series) were included. Commentaries and letters to the editor were however not sought.

Electronic nicotine delivery system and inhaled nicotine

Nicotine is a main bioactive component of tobacco-derived products, including conventional cigarettes, cigars, cigarillos, e-cigarettes, and waterpipes (ranges from 0 mg-100 mg/ml). Nicotine is well known for its addictive properties. Nicotine delivery systems (electronic nicotine-delivery systems [ENDS]) have recently emerged. These ENDS are proposed to reduce craving for conventional cigarettes, but are not regulated like tobacco (Regan et al., 2013, Giovino et al., 2012). Recently, a rapid growth has taken place in both marketing and consumption of e-cigs (Regan et al., 2013). With each "puff," the heating element vaporizes a small amount of liquid. In this format, the ENDS user is not inhaling smoke, but an aerosol/vapor of nicotine (up to 24-100 mg) as mist/vapor (Jorenby et al., 2016, Ruhe et al., 2017). Hence, ENDS will deliver a significant amount of nicotine compared to tobacco cessation devices available commercially.

E-cig aerosols and respiratory system

ENDS are unique in their ability to deliver a nicotine laden aerosol to the lung by inhalation (Cheng, 2014, Barrington-Trimis et al., 2014). Concentration of nicotine varies in commercial e-fluids/e-juices (Pisinger & Dossing, 2014). With the recent emergence and increasing popularity among youth/adults of multiple devices for the recreational inhalation of non-combustible nicotine, e.g., e-cigarettes, the importance of understanding effects of inhaled nicotine is needed. There are increasing numbers of reports regarding the direct effect of ENDS aerosol on health in recent years (Lerner et al., 2015b, Schweitzer et al., 2015). Although carcinogens appear to be reduced or eliminated in e-cigs, health concerns surrounding nicotine have been raised (Cahn & Siegel, 2011, Cobb & Abrams, 2011). Currently, listed as a reproductive or developmental toxicant, some studies suggest that nicotine may increase cardiovascular stress (Benowitz & Gourlay, 1997, Girdler et al., 1997), but the toxicological effects of inhaled nicotine delivered in to the lung are not well known. Nicotine binds to a family of nicotinic acetylcholine receptors (nAChRs), similar to acetylcholine (ACh) (Jensen et al., 2012, Carlisle et al., 2004). nAChRs are abundantly expressed in fibroblasts and epithelial cells of the lung

(Wilk et al., 2012, Sekhon et al., 2002). Moreover, these receptors trigger protease expression (Li & Dai, 2012), mucin production (Fu et al., 2011) and smooth muscle contraction (Hahn et al., 1992), which mediate airway obstruction in chronic obstructive pulmonary disease (COPD). Short-term use of ENDS causes an increase in impedance, peripheral airway flow resistance, and oxidative stress among healthy smokers (Vardavas et al., 2012, Flouris et al., 2013). ENDS inhalation increases allergen-induced airway hyper-responsiveness, alters innate immunity/host response, and increases virulence of colonizing bacteria and virus infection (Wu et al., 2014), hence affecting local microbiota/microbiome. Nicotine acts via α7nAChR to induce MUC5AC-expression and increases mucus production (Gundavarapu et al., 2012, Chen et al., 2014). This supports the notion that nicotine imparts its effect via the nicotinic receptors in downstream signaling pathways.

Inhaled nicotine effects on airway remodeling, pro-fibrogenic response and dysregulated repair

Tobacco smoking is associated with chronic airways disease and lung remodeling (Ji et al., 2016); however, the role of nicotine (particularly inhaled nicotine) in this regard remains unclear. Nicotine can also promote airway remodeling via its receptor in airway smooth muscle (Hahn et al., 1992). Small airway remodeling is a key feature in the development of COPD (Hogg et al., 2004, McDonough et al., 2011). These studies suggest that ENDS aerosol causes adverse health effects in users, but there are no long-term studies of ENDS use are available especially in airway obstruction/emphysematous and pro-fibrogenic remodeling/dysregulated repair responses. Thus, it is possible that inhaled nicotine can have repercussions on cellular homeostasis and the pulmonary system. Further, inhaled nicotine can lead to airway remodeling, pro-fibrogenic and dysregulated repair by e-cig aerosols (Lei et al., 2017). Inhaled nicotine may affect the ability of mesenchymal stromal/stem cells (MSCs) for their ability to heal the wounds or in general repair processes.

Inhaled nicotine, oxidative stress and DNA damage

Recent studies have indicated lung cellular toxicity by e-cig products (Shimosato et al., 2012, Cahn & Siegel, 2011, Wu et al., 2014, Cervellati et al., 2014). Lerner et al. reported increased mitochondrial ROS, DNA nuclear fragmentation and impaired stability of electron transport chain complex IV subunit on human lung fibroblasts exposed to e-cig and end-products (copper nanoparticles) (Lerner et al., 2016). Likewise, Schweitzer et al. reported that components of e-cig (acrolein, propylene glycol, glycerol, and nicotine) produced a dose-dependent loss of lung endothelial barrier function and inflammation associated with increased intracellular ceramides and myosin light chain phosphorylation (Schweitzer et al., 2015). Gerloff and co-workers have recently shown that e-cig and various flavoring agents can trigger inflammatory response and barrier dysfunction in human lung epithelial cells (Gerloff et al., 2017). Therefore, it is feasible that the exposure to e-cig and its products might lead to augmented oxidative stress and inflammatory responses in lung cells and tissues in chronic exposure conditions.

E-cig aerosols and oral health effects: impact on cellular senescence

Carbonyl/oxidative stress lead to stress-induced cellular senescence (a state of irreversible growth arrest which re-enforces chronic inflammation) and impaired myofibroblast differentiation and epithelial mesenchymal transition (**Figure 1**). E-cig aerosols upregulate the receptors for advanced glycation end-products (RAGE) in human oral fibroblasts and gingival epithelial cells, which is regulated by histone deacetylase 2 (HDAC2) (Sundar *et al*., 2016). Both RAGE and HDAC2 are implicated in regulation of inflammation and cellular senescence. However, no information is available regarding the role of RAGE and HDAC2 in regulating cellular senescence and inflammatory responses by e-cig aerosol in oral tissues (**Table 1**). E-cig aerosols may affect cellular signaling in periodontal ligament fibroblasts and MSCs.

E-cigarette aerosol, inhaled nicotine, and periodontal complications

Periodontal disease is characterized by chronic inflammation of the supporting tissues of the teeth (Albandar et al., 1999, Brown et al., 1996, Hajishengallis, 2015). Periodontal ligament cells and gingival fibroblasts as well as epithelial cells are the most abundant structural cells in periodontal tissues playing a fundamental role in periodontal regeneration. Upon stimulation or stress, these cells are able to incite and maintain inflammatory responses (Ara et al., 2009). 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 (Giorgetti et al., 2012, Correa et al., 2010, Cesar-Neto et al., 2006), leading to increased risk of periodontitis (Reibel, 2003, Javed et al., 2014). Oxidants/reactive oxygen species reactivity from e-cig aerosols is comparable to conventional cigarette smoke (Lerner et al., 2015a). Moreover, direct exposure to e-liquids has also been shown to produce harmful effects in periodontal ligament cells and gingival fibroblasts in culture (Willershausen et al., 2014, Sancilio et al., 2015). Reactive aldehydes/carbonyls derived from e-cig aerosol can cause protein carbonylation and DNA adducts/damage, and carbonyls are cleaved by aldehyde dehydrogenase (ALDH). Protein carbonylation leads to autoantibody production, which may lead to destruction of matrix and bone loss during periodontitis (Pradeep et al., 2013, Baltacioglu et al., 2008). Hence, it is possible that carbonyls/aldehydes play an important role in e-cig aerosol-induced oral toxicity. Nicotine is shown to have anti-proliferative properties and affects fibroblasts *in vitro* (Rothem et al., 2009, Frazer-Abel et al., 2004). This implicates that E-cig containing nicotine affects oral myofibroblast differentiation in e-cig users; and hence may affect their ability to heal wounds by decreasing wound contraction by myofibroblasts (Lei et al., 2017). This may be due to the release of prostaglandins (PGE2) and matrix metalloproteases (MMP-9, MMP-12) as well as their effects on MSCs. Likewise, Holliday et al. reported that e-cigarette-exposed cells presented reduced viability and clonogenic survival, along with increased rates of apoptosis and necrosis *in vitro* (Holliday et al., 2016). Further, the nicotine exposed cells presented significantly increased comet tail length and accumulation of γ-H2AX foci, demonstrating increased DNA strand breaks (Sundar *et al*., 2016).

Resolvins, pro-resolving lipid mediators including resolvin D1 (derivatives of omega-3 polyunsaturated fatty acids ω-3-PUFAs), are shown to resolve inflammation in periodontitis *in vivo* and *in vitro* models including animal model of periodontitis (Odusanwo et al., 2012, Mustafa et al., 2013, Hasturk et al., 2007). However, the effects of e-cig aerosols on carbonyl stress, inflammation, antioxidants, pro-resolving mediators, pro-fibrogenic response, and cellular senescence have not been mechanistically studied (**Table 1**).

E-cigarette devices or ENDS deliver nicotine at varying concentrations. Nicotine has been associated with impaired leukocyte activity and healing by inhibiting neovascularization and osteoblastic differentiation (Levin & Schwartz-Arad, 2005). Similarly, tobacco smoking including nicotine is associated with an increased risk of implant failure, impaired healing, poor papilla regeneration, and increased bone loss (Twito & Sade, 2014, Raes et al., 2015, Al Amri et al., 2016). Therefore, it is likely that nicotine derived from e-cig may impair healing potential at the bone/implant interface. This may also be due to impair functions of MSCs or resident stem cells by nicotine. Berley et al. reported decreased bone to implant contact after 4 weeks of implant placement in rats'femur receiving subcutaneous nicotine (Berley et al., 2010). Likewise, Yamano et al. reported a down-regulation in the expression of bone matrix-related genes around implants in rats receiving nicotine for 8 weeks (Yamano et al., 2010). However, the effects of nicotine delivery by e-cig on peri-implant soft and hard tissues as well as other periodontal complications have not been studied.

E-cig aerosols and oral submucous fibrosis

Oral submucous fibrosis (OSF) is a chronic potentially malignant disorder, characterized by progressive submucosal fibrosis of the oral tissues and the oropharynx. Approximately 7% to 13% of patients with OSF develop in oral squamous cell carcinoma (Liu et al., 2015). Tobacco smoking has been associated with higher risks of OSF. Furthermore, the risk increases among smokers consuming chewable tobacco (Liu et al., 2015). It has been suggested that nicotine and arecoline might induce the

over-expression of human telomerase reverse transcriptase (hTERT) mRNA in oral keratinocytes (affecting cellular senescence due to telomerase and telomere length), which may lead to the malignancy of OSF (Gao et al., 2007). Arecoline has also shown to induce fibroblast proliferation by the up-regulation of growth factors expression and endothelial necrosis (Ullah et al., 2015). It is hypothesized that e-cig and end-products might play a role in the manifestation, progression and malignancy of OSF via cellular senescence. However, no information is available regarding the e-cig effects on OSF.

Conclusion

E-cigs and/or inhaled nicotine along with various flavoring chemicals may contribute to the pathogenesis of periodontal and pulmonary diseases in particular via lung inflammation, injurious, and dysregulated repair responses via its effect on oral myofibroblast differentiation. This may have an affect their ability to heal wounds by decreasing wound contraction by release of various pro-inflammatory mediators. E-cig and its flavoring agents along with their chemical interactions with nicotine may produce harmful effects in periodontal ligament, stem cells, and gingival fibroblasts in cultures due generation of aldehydes/carbonyls from e-cig aerosol, leading to protein carbonylation of extracellular matrix and DNA adducts/damage, and cellular senescence. However, the association between E-cig and impaired would healing, oral fibrosis and bronchiolitis obliterans (popcorn lung) remains unknown. The research findings discussed in this review will not only provide information for further research on e-cigs and inhaled nicotine, but also for other tobacco products including conventional tobacco and waterpipe/hookah smoking alone or in combinations i.e. poly-use of these products. Further research is required to establish the risk of using e-cig on oral, systemic and pulmonary responses, and could help the public health community to identify and deliver appropriate messages about e-cigarettes' (inhaled nicotine) safety and promote future product regulation.

Author contributions:

FJ, SVK, ISK, IR: Conceived the idea and wrote the manuscript; FJ, GER and IR: Edited revised critically the manuscript.

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Figure legends

Fig. 1

Possible mechanism of e-cigarette induced inflammatory and dysregulated repair responses to inhaled nicotine.

Inhaled nicotine impact effects on lung and systemic inflammatory mediators in oral fluids and causes dysregulated repair responses via its receptor α7 nicotinic acetylcholine receptor (α7nAChR). Nicotine also causes impaired wound healing due to inhibition of myofibroblasts differentiation and/or epithelial-mesenchymal transition. Oxidative stress and vascular remodeling by inhaled nicotine may trigger inflammatory responses in periodontal tissues. Oxidative stress can lead to carbonylation of extracellular matrix and further deposition of modified matrix. All these responses are associated with initiation of oral submucosal fibrosis.

Table 1

Markers and targets for periodontal and lung diseases by inhaled e-cig aerosol containing nicotine.

Table 1

Markers and targets for periodontal and lung diseases by inhaled e-cig aerosol containing nicotine

Markers	**Targets**
Oxidative stress	Lipid peroxidation products 4-hydroxy-2-nonenal, malondialdehyde, F_2-isoprostanes
Inflammatory responses (cytokines and prostaglandins)	NF-kappa B, Toll like receptors, NLRP3 inflammasome
Antioxidants	Glutathione, superoxide dismutases, antioxidant enzymes, lipid peroxidation inhibitors
Innate host defense	RAGE receptors (S100A8 and S100A9) Advanced glycation end products Histone deacetylases (HDACs)
Lipid mediators	Resolvins, polyunsaturated fatty acids (omega 3 fatty acids)

Proteases	Matrix metalloproteases (MMP-9, MMP-12)
Growth factors	VEGF, FGF, fibroblast growth factor (FGF), PDGF, TGF-β,
Myofibroblast differentiation/wound healing	TGF-β, PGE2, GM-CSF, prostacyclins

Fig. 1