REVIEW ARTICLE

Recent updates on electronic cigarette aerosol and inhaled nicotine effects on periodontal and pulmonary tissues

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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 antiproliferative 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 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, profibrogenic 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 (Barrington-Trimis et al, 2014; Cheng, 2014). Apart from high concentration of nicotine (up to 24 mg), numerous chemicals including aldehydes (as carbonyls), heavy metals (nickel, chromium, copper-coated with silver), metal particle/ultra-fine nanoparticles, and tobacco-specific nitrosamines as well as diacetyl, 2,3-pentanedione, and acetoin (butter) are found in e-cig aerosols (Cheng, 2014; Kosmider et al, 2014). Other flavoring chemicals include ortho-vanillin (vanilla), maltol (malt), cinnamaldehyde, and coumarin, which are shown to cause pro-inflammatory response in lung cells in vitro (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 (Kosmider et al, 2014; Jensen et al, 2015). 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 (USA) and worldwide particularly among young adults (Regan et al, 2013; Krishnan-Sarin et al, 2015). In the USA, approximately 11–21% 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 (Harrison and Hicklin, 2016; Rouabhia et al, 2016; Sundar 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) (Baltacioglu et al., 2008; Pradeep et al., 2013; 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 (Brown et al., 1996; Albandar et al., 1999; Reibel, 2003; Javed et al., 2014), 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 was 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, 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 to 100 mg/ml). Nicotine is well known for its addictice 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 (Giovino et al., 2012; Regan et al., 2013). 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 (Barrington-Trimis et al., 2014; Cheng, 2014). Concentration of nicotine in e-cig varies in commercial e-fluids/e-juices (Pisinger and Dossing, 2014). There is an increasing popularity of e-cig devices among youth/adults for recreational purposes. Hence, understanding the effects of e-cig-derived inhaled nicotine on pulmonary system is important. 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 and Siegel, 2011; Cobb and Abrams, 2011). Currently, nicotine is listed as a reproductive or developmental toxicant, some studies suggest that nicotine may increase cardiovascular stress (Benowitz and Gourlay, 1997; Girdler et al., 1997), but the toxicological effects of inhaled nicotine delivered into the lung are not well known. Nicotine binds to a family of nicotinic acetylcholine receptors (nAChRs), similar to acetylcholine (ACh) (Carlisle et al., 2004; Jensen et al., 2012). nAChRs are abundantly expressed in fibroblasts and epithelial cells of the lung (Sekhon et al., 2002; Wilk et al., 2012). Moreover, these receptors trigger

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**Figure 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.
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 (Brown et al, 1996; Albandar et al, 1999; 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

### Table 1: Markers and targets for periodontal and lung diseases by inhaled e-cig aerosol containing nicotine

<table>
<thead>
<tr>
<th>Markers</th>
<th>Targets</th>
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<tbody>
<tr>
<td>Oxidative stress</td>
<td>Lipid peroxidation products, 4-hydroxy-2-nonenal, malondialdehyde, F$_2$-isoprostanes</td>
</tr>
<tr>
<td>Inflammatory responses (cytokines and prostaglandins)</td>
<td>NF-kappa B, Toll-like receptors, NLRP3 inflammasome</td>
</tr>
<tr>
<td>Antioxidants</td>
<td>Glutathione, superoxide dismutases, antioxidant enzymes, lipid peroxidation inhibitors</td>
</tr>
<tr>
<td>Innate host defense</td>
<td>RAGE receptors (S100A8 and S100A9), Advanced glycation end products, Histone deacetylases (HDACs)</td>
</tr>
<tr>
<td>Lipid mediators</td>
<td>Resolvin D2, polyunsaturated fatty acids (omega 3 fatty acids)</td>
</tr>
<tr>
<td>Proteases</td>
<td>Matrix metalloproteases (MMP-9, MMP-12)</td>
</tr>
<tr>
<td>Growth factors</td>
<td>VEGF, FGF, fibroblast growth factor (FGF), PDGF, TGF-β, PGE2, GM-CSF, prostacyclins</td>
</tr>
<tr>
<td>Myofibroblast differentiation/ wound healing</td>
<td>TGF-β, PGE2, GM-CSF, prostacyclins</td>
</tr>
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along with the destruction of connective tissue and matrix (Cesar-Neto et al., 2006; Correa et al., 2010; Giorgetti et al., 2012), 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 shown to produce harmful effects in periodontal ligament cells and gingival fibroblasts in culture (Willershansen et al., 2014; Sancilio et al., 2015). Reactive aldehydes/carbonyls derived from e-cig aerosol can cause protein carboxylation and DNA adducts/damage, and carbonyls are cleaved by aldehyde dehydrogenase (ALDH). Protein carboxylation leads to autoantibody production, which may lead to destruction of matrix and bone loss during periodontitis (Baltacioglu et al., 2008; Pradeep et al., 2013). Hence, it is possible that carbonyls/carbonyls play an important role in e-cig aerosol-induced oral toxicity. Nicotine is shown to have antiproliferative properties and affects fibroblasts in vitro (Frazer-Abel et al., 2004; Rothem et al., 2009). 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, proresolving 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 (Hasturk et al., 2007; Odusanwo et al., 2012; Mustafa et al., 2013). However, the effects of e-cig aerosols on carbonyl stress, inflammation, antioxidants, proresolving mediators, profibrogenic 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 and 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 and Sade, 2014; Raes et al., 2015; Al Amri et al., 2017). 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 downregulation 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 7–13% of patients with OSF develop 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 overexpression 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 upregulation 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 inflammation, injurious, and dysregulated repair responses via its effect on myofibroblast differentiation. This may 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 carboxylation of extracellular matrix and DNA adducts/damage, and cellular senescence. However, the association between E-cig and impaired wound 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, that is, 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.

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Author contributions
FJ, SVK, IKS, IR conceived the idea and wrote the manuscript and FJ, GER and IR edited and revised critically the manuscript.

Conflict of interests
The authors have declared that no conflict of interest exists and none to declare.

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