Food and Chemical Toxicology 118 (2018) 390–398

Contents lists available at ScienceDirect

Food and Chemical Toxicology

journal homepage: www.elsevier.com/locate/foodchemtox

Comparative study of the effects of cigarette smoke and electronic cigarettes on human gingival fibroblast proliferation, migration and apoptosis

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ARTICLE INFO

Keywords:
E-cigarette
Nicotine
Gingival fibroblasts
Apoptosis
Cell
Migration
Wound healing

ABSTRACT

In an effort to reduce smoking-related diseases, alternative products such as e-cigarettes have been proposed. However, despite their growing popularity, the potential toxicity of e-cigarettes remains largely unknown. In this study, human gingival fibroblasts were repeatedly exposed to cigarette smoke condensate (CSC) and to nicotine-rich (NR) or nicotine-free (NF) e-vapor condensates for 60 min once a day for various time periods. They were then used to perform different analyses. Results indicate that cells exposed to CSC or NR condensates showed an altered morphology and a reduced proliferation rate, as ascertained by MTT and BrdU assays. Fibroblast cultures exposed to either CSC or e-vapor condensates also showed increased levels of TUNEL-positive apoptotic cells, compared to that recorded in the control. Furthermore, the cell scratch test revealed that repeated exposures to CSC or to e-vapor condensates delayed both fibroblast migration and wound healing. It should be noted that CSC was much more damageable to gingival fibroblasts than were the NR and NF e-vapor condensates. The representative chain of damage thus translates to CSC > NR e-vapor condensate > NF e-vapor condensate.

1. Introduction

Electronic cigarettes are now being proposed with the goal of preventing the adverse effects of combustible cigarettes and eventually encouraging smokers to quit smoking (McRobbie et al., 2014; Jo et al., 2018), because combustible cigarettes contain hundreds of harmful chemicals, including several carcinogens (Baker et al., 2004) incriminated in smoker-related health problems (Curry et al., 2009). The e-cigarette device combines a plastic tube, an electronic heating component, and a cartridge serving as a reservoir to hold the e-liquid solutions. The liquid solution is heated and vaporized to produce an aerosol that is then inhaled by smokers through their airways (Mikheev et al., 2016), thus in initial contact with the oral mucosa. The electronic cigarette (e-cigarette) is being marketed as a “safe alternative” because it does not require combustion (Wackowski et al., 2016; Cheney et al., 2016).

Many e-liquid contains a mixture of propylene glycol, glycerin, nicotine, and flavorings (Uchiyama et al., 2016); however, following vaporization, studies have reported that not only glycerol, propylene glycol, nicotine, and flavors are present in the e-vapors, but also trace amounts of carcinogens, and heavy metals (Mikheev et al., 2016). The heated humectants (propylene glycol and glycerol) thus release various aldehydes, such as formaldehyde, acetaldehyde, and acrolein in the e-vapor (Jensen et al., 2015; Gillman et al., 2016; Farsalinos et al., 2015). Nickel content, notably, has been found to be much higher in e-cigarette vapor than in standard cigarette smoke (Williams et al., 2013), and airborne aluminum levels have been reported to be high following e-cigarette vaping (Schober et al., 2014). These chemicals may have serious adverse effects on human health.

E-cigarette vapor emissions have been shown to create harmful free radicals and inflammation leading to tissue damage (Lerner et al., 2015; Rouabhia et al., 2017). After reaching the oral mucosa, the e-vapor may harm the oral tissue, as does combustible cigarette smoke. Indeed, it is well recognized that combustible cigarette smoke can alter cell function and promote periodontal disease development and severity (Vogtmann et al., 2017). Periodontitis severity has been shown to increase with smoking intensity and duration (Lallier et al., 2017). Furthermore, cigarette smoke also reduces the host response to periodontopathic bacteria, resulting in a more aggressive periodontal breakdown (Goh et al., 2017). This situation may also occur with e-cigarettes. Upon entering the oral cavity, e-vapor comes in direct contact with the oral mucosa where epithelial cells and fibroblasts interact to maintain tissue integrity and function (McCulloch, 1995).

Gingival fibroblasts, the predominant cell type inhabiting gingival

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https://doi.org/10.1016/j.fct.2018.05.049
Received 15 January 2018; Received in revised form 8 April 2018; Accepted 19 May 2018
Available online 22 May 2018

connective tissue, play a critical role in remodeling and maintaining gingival structure and extracellular matrix (Cáceres et al., 2014). These fibroblasts are also key players in tissue repair and wound healing through their adhesion, proliferation, and migration. Exposure to e-vapor of gingival mucosa may result in impairment of the gingival fibroblast function. E-cigarette products, such as nicotine, may affect periodontal cells by inhibiting the growth/proliferation of human periodontal ligament fibroblasts through apoptotic mechanisms. The aim of this study was to investigate the effects of e-vapor condensate with or without nicotine on normal human gingival fibroblast adhesion, viability/proliferation, apoptotic process, and migration following insult. The effects of combustible cigarette smoke condensate and e-vapor condensates were also compared and analyzed.

2. Materials and methods

2.1. Gingival fibroblast isolation and culture

Biopsies of lamina propria tissue (gingival connective tissue) were collected from healthy, never smoked donors (18–25 years of age, n = 10) following their informed consent. Before tissue collection, patient were screened by the dentist to ensure the absence of inflammatory sates or periodontal diseases. This protocol was approved by the Université Laval Ethics Committee. To isolate the primary gingival fibroblasts, the connective tissue was placed in a collagenase P solution (0.125 U/mL; Boehringer Mannheim, Laval, QC, Canada) for 45 min at 37 °C under agitation. The isolated cells (2×10^5) were seeded in 75-cm^2 cell culture flasks and grown in Dulbecco's modified Eagle's (DME) medium containing 10% fetal calf serum (Invitrogen Canada Inc., Burlington, ON, Canada). Once the cells reached 90% confluence, they were subcultured and used between passages 4 and 5 in this study. Cells were used individually to perform the experiments.

2.2. Preparation of cigarette smoke condensate solution

1R3F cigarettes were purchased from the Kentucky Tobacco Research & Development Center (Orlando, FL, USA) and used to prepare the cigarette smoke condensate (CSC) solution. The preparation of the CSC was made as previously described (Yadav et al., 2016) with some modifications. Briefly, each cigarette was placed into one end of a silicone tube linked to an Erlenmeyer flask containing 20 mL of culture medium. On the other end, a second silicone tube linked to the Erlenmeyer was connected to a standard vacuum. The cigarette was attached to the cigarette holder and lit and the smoke was extracted by applying the vacuum which pulled the smoke directly into the culture medium. The procedure was repeated with a total of two whole cigarettes. The resulting CSC solution was then sterilized by filtration through a 0.22-µm filter and considered as a 100% stock solution (n = 6). It was aliquoted and stored at −20 °C until use.

2.3. Preparation of the e-vapor condensate solutions

EMOW electronic cigarette devices were chosen to deliver the e-vapor (Kanger Tech Brand, Shenzhen, China (www.kangeronline.com). The disposable e-cigarette liquid (Flavor: Smooth Canadian tobacco; http://shop.juicyejuice.com/juicy-canadian-tobacco-e-liquid.ejuice) was selected for this study. Nicotine concentration in the e-liquid was 12 mg/mL. The e-cigarette device and e-liquid were chosen because they were advertised as a “starter” e-cigarette vaping kit. The EMOW e-cigarette and disposable cartomizer cartridges were purchased from local retailers. To prepare the e-vapor condensate, 500 µl of the e-liquid containing nicotine were introduced into the EMOW electronic cigarette reservoir. Two different EMOW e-cigarette devices were used, one for NR and one for NF e-vapor condensate preparations. Thereafter, the e-cigarette device was placed into one end of a silicone tube while the other end of the tube was linked to an Erlenmeyer flask containing

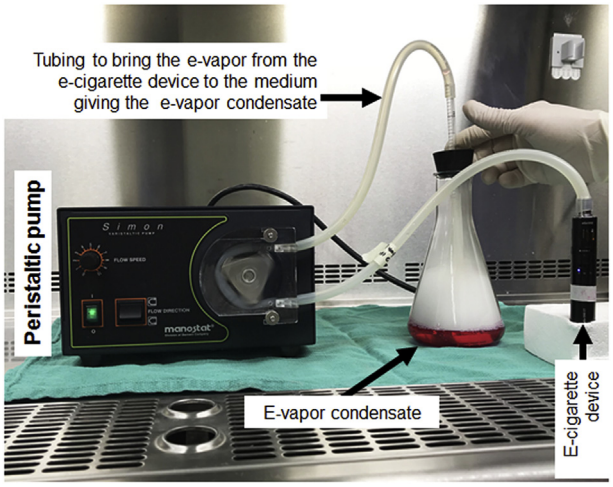

Fig. 1. Schema showing the system used to generate the e-vapor condensates.

20 mL of culture medium prior to activating the peristaltic pump which activated the e-cigarette device system to produce the e-vapor through the silicone tube (see Fig. 1). The e- vapor was drawn into the Erlenmeyer flask, and dissolves into the culture medium; this referees to e-vapor condensate. The vapor was drawn into the exposure chamber with a regime of 2 puffs every 60 s: a 10-sec puff followed by a 20-sec pause, as previously described (Lerner et al., 2016). The vaping procedure stopped when the total volume (500 µl) of e-liquid was vaped. The same procedure was used to prepare nicotine-free e-vapor condensate using a separate e-cigarette device. Collected e-vapor condensate solutions were sterilized by filtration through a 0.22-µm filter and considered as 100% stock solutions (n = 6). They were aliquoted and stored at −20 °C until use.

2.4. Effect of cigarette smoke and e-vapor condensates on human gingival fibroblast adhesion and morphology

Prior to cell seeding, five sterile glass slides (Bellco Glass Inc., Vineland, NJ, USA) (0.05 mm in diameter) were inserted into each well of a non-adherent 6-well plate (Sarstedt, Nümbrecht, Germany). Primary human gingival fibroblasts were then seeded at 10^5 cells/well in DMEM supplemented with 10% FBS. Immediately after seeding, the cells were incubated with different concentrations (0, 1, 5, or 10%) of either cigarette smoke or e-vapor condensate in triplicate at 37 °C in a 5% CO_2 incubator for 24 h. Following incubation, the cells were fixed with methanol and glacial acetic acid (75/25, v/v) for 15 min, followed by three washes with PBS. The fixed cells were then incubated with 1 µg/mL of Hoechst 33342 (H42) (Riedel de Haen, Seele, Germany) in PBS for 15 min at room temperature in the dark. After three washes with PBS, the samples were observed under an epifluorescence light microscope (Axiophot, Zeiss, Oberkochen, Germany) and photographed. In a second set of experiments, fibroblasts were cultured for 24 h without smoke products then exposed or not for 60 min once a day for three days to either CSC or e-vapor condensates. At the end of the 3-day treatment regime, cells were observed under an inverted microscope and photographed (n = 6).

2.5. Effect of cigarette smoke and e-vapor condensates on cell growth

Fibroblasts were seeded (10^4 cells/well) in 6-well plates and cultured for 24 h prior to exposure to smoke products, after which time the culture medium was refreshed and supplemented with various concentrations (0, 1, 5, or 10%) of CSC, NF e-vapor condensate, or NR e-vapor condensate. Contact of the cells with the smoke condensate was maintained for 60 min at 37 °C in a 5% CO_2 humid atmosphere.