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Role of MicroRNA in Smoking–Induced Periodontitis

Herman S. Cheung, Carlos Carballosa and Jordan Greenberg

Abstract

Stem cells participate in tissue restoration and therapies. The oral cavity, the site of smoking exposure, contains stem cells which are involved in the development, maintenance, and repair of oral tissues. By residing in the oral cavity, stem cells are exposed to and susceptible to the effects of smoking. Periodontitis has been associated with increased incidences of other illnesses such as cardiovascular diseases. We hypothesize that smoking suppression of stem cell potentials by miRNAs is a separate and independent pathway than the pathway via the nicotine receptor activation. Smoking perturbs miRNA expression, resulting in decreased stem cell regeneration potential that delays healing of periodontitis.

Keywords: periodontal ligament stem cells, cigarette smoking, e-cigarette vapor, regenerative potential

1. Introduction

Cigarette smoking (CS) is one of the leading causes of preventable death in the world. Despite the smoking cessation attempts in the **United States (US)**, 15.1% of US adults are still active smokers [1]. Cigarette smoke exposure increases the risk of various cancers and systemic diseases [2] in smokers; however, smoking is a difficult habit to quit due to the addictive property of nicotine. Nicotine replacement therapies (NRT) help smokers overcome their nicotine addiction through gradually decreasing nicotine dose delivery regimens. **Electronic cigarettes (ECs)**, marketed as a “safer alternative” to cigarette smoking, have not been fully regulated by the FDA and are still believed to cause major health concerns due to the concentrated delivery of toxic chemicals like nicotine, among others [3, 4]. Additionally, due to a lack of regulation, labeling and dosage of nicotine can vary widely among EC products. In the United States alone, EC use has doubled among US adults and tripled among adolescents within the last 5 years [5, 6].

Internalization of smoking- and vaping-related toxins occurs primarily through the respiratory system. For specific compounds like nicotine, the subsequent absorption into specific tissues is largely dependent on tissue pH [7]. Nicotine is a weak base (pKa of 8.0) and is more readily absorbed in slightly basic conditions where it is less “ionized” [7–9]. The oral cavity, the initial site of smoke exposure, is a slightly acidic environment. Cigarette smoke is more alkaline than originally thought [7], thereby improving oral nicotine absorption. Electronic cigarette (EC) liquids, on the other hand, are a slightly more basic pH [8]. Therefore, nicotine delivered from these devices is more readily absorbed in the mouth. Nicotine in

the smoker's saliva, which traps ionized nicotine and maintains elevated levels of exposure in the mouth, is almost 87 times higher than in the blood plasma [10–12].

1.1 Periodontal ligament stem cells (PDLSCs)

Periodontal ligament stem cells (PDLSCs) are an adult stem cell population that reside in the periodontal ligament and give rise to tooth-supporting structures such as the alveolar bone, periodontal ligament, and cementum [13, 14]. In addition to their regenerative capabilities, PDLSCs can be easily isolated following natural tooth loss or routine dental procedures [13]. A recent clinical trial using autologous PDLSCs as a therapeutic approach for the regeneration of periodontal bone defects confirmed the safety of this approach for human use [15]. Stem cells have proven to be effective for the treatment of a variety of injuries and diseases. However, compromising stem cell function, through infectious or genetic diseases, can lead to ineffective clinical outcomes; therefore these conditions are included as exclusion criteria in patient recruitment for stem cell-based therapies. Environmental risk factors such as cigarette smoking and nicotine use can also compromise stem cell function leading to inefficacious outcomes [16–19]. This suggests that they deserve consideration as exclusion criteria [18, 19].

1.2 Nicotinic acetylcholine receptor

Although the effects of nicotine exposure on human health are known, its implication in regard to stem cell biology and function remains greatly unknown. Nicotinic acetylcholine receptor (nAChRs) are one of the mediators of the nicotinic effect. Once internalized and in the blood stream, nicotine is free to complex with nAChRs. These transmembrane, ligand-gated ion channels are the main mediators behind nicotine's cellular effects [20]. Nicotine, a nAChR agonist, activates the receptor, which causes an opening of the transmembrane ionic channel and a subsequent influx of extracellular ions [22, 23]. Although all nAChRs serve the same basic purpose (i.e., transmembrane ion flux), the downstream implications initiated by receptor activation are dependent on the specific combination of subunits used to compose each nAChR. To date, 16 unique subunit varieties have been identified in the mammalian species [20–22]. Functional nAChRs are composed of five subunits and classified as either homopentameric, if subunits are identical, or heteropentameric, if subunits are nonidentical [20]. The homopentameric $\alpha 7$ nAChR has recently been identified on PDLSCs. In the presence of nicotine, these cells experience an increase in apoptosis and a decrease in migratory and osteogenic potential [23–27]. Interestingly, these effects were only partially inhibited (~40–50%) in cells pretreated with mecamylamine, a non-specific nAChR antagonist, and alpha bungarotoxin (aBTX), an $\alpha 7$ -specific nAChR antagonist [26, 27]. Others have shown that similar nicotinic effects of PDLSC regeneration potential by decreasing proliferation, migration, and osteogenic differentiation capabilities in vitro are mediated through agonist-induced activation of $\alpha 7$ nicotinic acetylcholine receptors (nAChRs) present on PDLSCs [26, 27]. However, the detrimental effects of nicotine were still partially observed even after the deactivation of these receptors [27], suggesting that there may be additional mediators behind these effects. This data suggests that additional mechanisms are likely to exist as evident by the only partial reversal of nicotinic effects in the presence of nAChR antagonists.

1.3 MicroRNAs (miRNAs)

miRNAs are 22-nucleotide-long small, noncoding RNAs which may alter gene expression by base pairing with complementary mRNA [28, 29]. These

miRNAs are expressed throughout the body, including in muscular and skeletal tissues, and have been shown to affect cell viability, cell differentiation, and even organ development by downregulating the genes associated with these biological processes. Each miRNA can target several genes, and therefore upregulation of a single strand can affect various biological processes.

2. Biological effect of nicotine

We have published studies regarding the *in vitro* and *in vivo* biological and physiological effects of nicotine and CS on mesenchymal stem cells (MSCs) and PDLSCs. While investigating the effects of nicotine on the mechanical properties of MSCs, we concluded that nicotine significantly increases the stem cell membrane of Young's modulus (i.e., stiffness) in a dose-dependent manner [30]. We predicted that this increase in stem cell stiffness would impair the ability of the stem cells to respond to mechanical stimuli, therefore hindering mechano-induction and ultimately stem cell differentiation potential. In addition, we predicted that the loss of membrane compliance would delay MSC migration from stem cell niches.

To investigate these consequences, we evaluated the effects of nicotine exposure on the three processes critical for effective stem cell regeneration potential: proliferation, migration, and differentiation [20]. *In vitro* exposure to nicotine significantly reduced the proliferation and migration potential of both MSC and PDLSCs [20]. MSC and PDLSC proliferation decreased significantly when culturing cells in media containing 1 μ M of nicotine. In fact, by day 5, both nicotine-exposed groups showed greater than a twofold decrease in the total number of cells, indicating the loss of proliferative potential while exposed to nicotine. Mesenchymal stem cells (MSC) and PDLSCs exposed to nicotine also traveled shorter distances compared to non-treated cells. The distance traveled by the nicotine-treated MSC was significantly shorter than that by the control MSC. The average speed of MSC migration was also slower in the nicotine-treated group than in the control group. Analogously, the nicotine-treated PDLSC migrated less than the control PDLSC. Furthermore, the nicotine-treated PDLSC also moved slower than the control PDLSC. Nicotine critically affected the differentiation potential of MSCs and PDLSCs [18, 19]. Under conditioned media stimulation, MSC and PDLSC differentiated into osteoblasts, and their calcium deposition increased. However, the osteogenic differentiation medium supplemented with nicotine reduced calcium deposition as evident by a decrease in MSC and PDLSC alkaline phosphatase activity. Altogether, these findings suggest that nicotine can inhibit the differentiation of osteoblasts from MSC or PDLSC.

To understand the molecular mechanism of the nicotine-induced effect, we also analyzed the global miRNA expression patterns of nicotine-treated PDLSC by microarray. A total of 225 miRNAs were differentially expressed at a twofold difference between nicotine-treated PDLSC when compared with the control [20]. The miRNA expression profiles of the control and 1.0 μ M nicotine-treated groups were differentially clustered and separated from each other by either principle component analysis or hierarchical clustering. Compared to the control group, 16 miRNAs were differentially expressed in the 1.0 μ M nicotine group ($P_{\text{corr}} < 0.05$ and fold change > 2). All of these miRNAs showed dose-dependent changes from 0.5 to 1.0 μ M nicotine. The top 10 highly expressed miRNAs were selected for validation and showed a similar expression change to the microarray results. Interestingly, five miRNAs related to osteogenesis (hsa-miR-29b, hsa-miR-30d, hsa-miR-137, hsa-miR-424, and hsa-miR-1274a) showed $P_{\text{corr}} < 0.05$.

We confirmed these results also occur *in vivo* and showed that PDLSCs isolated from cigarette smokers showed similar trends in proliferation, migration, and

differentiation potentials and miRNA expression [21]. The proliferation of PDLSC from smokers was significantly slower than those of non-smokers by 2.53-fold at day 5 ($p < 0.01$) and 2.88-fold at day 7 ($p < 0.05$). In cell migration analysis, conducted by scratch wound assay, smoker PDLSCs moved slower than the non-smoker PDLSCs. The reduction of scratch wound area by smoker PDLSC was smaller than that by non-smoker PDLSC at 12 hours ($20.07 \pm 3.78\%$ versus $25.92 \pm 4.00\%$, $p < 0.05$) and at 24 hours ($60.10 \pm 8.55\%$ versus $72.27 \pm 5.90\%$, $p < 0.05$), suggesting that CS reduces the migration ability of PDLSC. Cigarette smoking (CS) also inhibited osteogenic differentiation as seen by a reduction in alkaline phosphatase and Alizarin S Red and chondrocyte differentiation as indicated by the blue stain of acidic polysaccharides [21].

We also examined the expression of the top 10 differentially expressed miRNAs identified in our previous report. Interestingly, the miRNA profile of cigarette smokers showed similar trends in expression for the majority of the miRNAs. A miRNA-gene target list was subsequently generated for two of the similarly upregulated miRNAs, hsa-miR-1305 (629 target genes) and hsa-miR-18b (202 target genes), using TargetScan in the GeneSpring software (Agilent). **We used the Database for Annotation, Visualization, and Integrated Discovery (DAVID) to perform gene ontology analysis of the gene lists. We identified smoking-associated miRNAs (hsa-miR-1305 and hsa-miR-18b) might target the genes involved in cell cycle, cell projection, cell junction, and cytoskeleton. This suggested that cigarette smoking would account for the negative influence on PDLSC proliferation and migration potential [21].**

It is important to note that these effects persisted given that PDLSCs from smokers underwent numerous cell passages after isolation (i.e., four and eight passages, splitting cells 1:3) and that all experiments using these cells were conducted in the absence of exogenous nicotine [21]. Accordingly, we concluded that these miRNAs, and not the nicotine receptors, play an important role in an inhibitory effect on stem cell regenerative potential and the long-term deteriorative effects of CS on stem cells.

Others have shown that similar nicotinic effects of PDLSC regeneration potential by decreasing proliferation, migration, and osteogenic differentiation capabilities in vitro are mediated through agonist-induced activation of $\alpha 7$ nAChRs present on PDLSCs [26, 27]. However, the detrimental effects of nicotine were still partially observed even after the deactivation of these receptors [27], suggesting that there may be additional mediators behind these effects. These results hint at a possible mechanism for the nicotine-induced effects on PDLSC regeneration potential that involves both nAChRs and miRNA. A link between the nicotinic effect and the miRNA expression has yet to be fully determined; however, there does appear to be a correlation between the two.

Electronic cigarettes (ECs) are marketed as a “safer” alternative to cigarette smoking due to the significant reduction in toxic chemical exposure. Experienced EC users, however, are still capable of achieving cigarette-like levels of exposure to vapor compounds like nicotine, especially in the oral cavity—the initial site of exposure. Nicotine alone has been shown to significantly impact the osteogenic differentiation potential of PDLSCs [16, 17], but its effect in tandem with additional EC vapor (ECV) compounds remains unknown. PDLSCs were cultured in osteogenic media supplemented with EC vapor extract. EC vapor (ECV) was generated by VitroCell’s VC1 automated vaping robot using an eVic EC filled with 50%/50% (w/v) PG/VG, 0 or 36 mg/ml nicotine, and non-flavored e-liquid. Nicotine vapor content was determined to a final, physiological concentration of 1 or 10 μM nicotine.

EC condition media containing 10 μM reduced osteogenic differentiation. In comparison, CS blocks mineralization significantly more than EC in a

dose—response fashion. The greatly enhanced inhibitory effect of CS may be due to the 7000 other chemicals besides nicotine. miRNA microarray analysis showed that 1 μ M nicotine ECV treatments upregulated miRNA (i.e., hsa-miR-29b (4.15-fold, $p < 0.05$), hsa-miR-424 (2.10-fold, $p < 0.05$), and hsa-miR-30d (3.18-fold, $p < 0.05$)) targeted genes related to osteogenesis. After 7 days of induction, high concentrations of nicotine ECV suppressed the gene expression of early osteogenic differentiation markers RUNX2, ALPL, and COL1A1 by $>25\%$. Nicotine ECV also suppressed mineralized nodule formation after 21 days in a dose-dependent manner. In comparison, cigarette smoking is tenfold inhibitory than ESC. This may be attributed to over 7000 other toxic chemicals in CS. Our pilot study is the first to investigate the potential effects of ECV by influencing the osteogenic differentiation potential of PDLSCs. A further understanding of the consequences of ECV on adult stem cell health is imperative given the rapid rise of EC use and routine use of postnatal stem cells in regenerative applications [31–33].

3. Conclusion

In conclusion, stem cells are effective for the treatment of a variety of injuries and diseases. However, compromising stem cell function, through infectious or genetic diseases, can lead to ineffective clinical outcomes. Literature reports suggested that smoking effects of stem cell regeneration potential by decreasing proliferation, migration, and osteogenic differentiation capabilities in vitro are mediated through agonist-induced activation of $\alpha 7$ nAChRs present on PDLSCs [26, 27].

Our work focuses on the determination of the potential harmful effect of smoking on the regenerative capabilities of stem cells and the mediators behind these effects. We showed that smoking suppression of stem cell potentials by miRNAs is a separate and independent pathway than the pathway via the $\alpha 7$ nAChR activation. Smoking perturbs miRNA expression, resulting in decreased PDLSC regeneration potential that delays healing of smoking-induced periodontitis.

This contribution will be significant because it will provide much needed data regarding smoking as well as consequences of vaping on oral cell/tissue health. Moreover, the data gathered as part of this work has the potential to be used in future commercial applications such as the development of a prescreening method or an early detection system for destructive oral diseases such as periodontitis.

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References

- [1] US Department of Health and Human Services. The health consequences of smoking—50 years of progress: A report of the surgeon general. In: US Department of Health and Human Services CFDCAP. Atlanta, GA: National Center for Chronic Disease Prevention and Health Promotion; 2014
- [2] Benowitz NL, Porchet H, Sheiner L, Jacob P III. Nicotine absorption and cardiovascular effects with smokeless tobacco use: Comparison with cigarettes and nicotine gum. *Clinical Pharmacology and Therapeutics*. 1988;**44**(1):23-28
- [3] Goniewicz ML, Hajek P, McRobbie H. Nicotine content of electronic cigarettes, its release in vapor and its consistency across batches: Regulatory implications. *Addiction*. 2014;**109**:500-507
- [4] Goniewicz ML, Knysak J, Gawron M, et al. Levels of selected carcinogens and toxicants in vapour from electronic cigarettes. *Tobacco Control*. 2014;**23**:133-139
- [5] King BA, Alam S, Promoff G, et al. Awareness and ever-use of electronic cigarettes among U.S. adults, 2010-2011. *Nicotine & Tobacco Research*. 2013;**15**:1623-1627
- [6] CDC, C.F.D.C.A.P. E-cigarette use triples among middle and high school students in just one year. 2015
- [7] Benowitz NL, Hukkanen J, Jacob P 3rd. Nicotine chemistry, metabolism, kinetics and biomarkers. *Handbook of Experimental Pharmacology*. 2009:29-60
- [8] Stepanov I, Fujioka N. Bringing attention to e-cigarette pH as an important element for research and regulation. *Tobacco Control*. 2015;**24**:413-414
- [9] Lindell G, Lunell E, Graffner H. Transdermally administered nicotine accumulates in gastric juice. *European Journal of Clinical Pharmacology*. 1996;**51**:315-318
- [10] Robson N, Bond AJ, Wolff K. Salivary nicotine and cotinine concentrations in unstimulated and stimulated saliva. *African Journal of Pharmacy and Pharmacology*. 2010;**4**:61-65
- [11] Wall MA, Johnson J, Jacob P, Benowitz NL. Cotinine in the serum, saliva, and urine of nonsmokers, passive smokers, and active smokers. *American Journal of Public Health*. 1988;**78**(6):699-701
- [12] Etter JF, Bullen C. Saliva cotinine levels in users of electronic cigarettes. *The European Respiratory Journal*. 2011;**38**(5):1219-1220
- [13] Liu J, Yu F, Sun Y, Jiang B, et al. Concise reviews: Characteristics and potential applications of human dental tissue-derived mesenchymal stem cells. *Stem Cells*. 2015;**33**:627-638
- [14] Chen FM, Gao LN, Tian BM, et al. Treatment of periodontal intrabony defects using autologous periodontal ligament stem cells: A randomized clinical trial. *Stem Cell Research & Therapy*. 2016;**7**:33
- [15] Mesa F, Magan-Fernandez A, Castellino G, Chianetta R, Nibali L, Rizzo M. Periodontitis and mechanisms of cardiometabolic risk: Novel insights and future perspectives. *Biochimica et Biophysica Acta (BBA) - Molecular Basis of Disease*. 1 Feb 2019;**1865**(2):476-484
- [16] Eke PI, Zhang X, Lu H, Wei L, Thornton-Evans G, Greenlund KJ, et al. Predicting periodontitis at state and local levels in the United States. *Journal of Dental Research*. 2016;**95**(5):515-522

- [17] Ojima M, Hanioka T, Tanaka K, Inoshita E, Aoyama H. Relationship between smoking status and periodontal conditions: Findings from national databases in Japan. *Journal of Periodontal Research*. 2006;**41**(6):573-579
- [18] Greenberg JM, Carballosa CM, Cheung HS. Concise review: The deleterious effects of cigarette smoking and nicotine usage and mesenchymal stem cell function and implications for cell-based therapies. *Stem Cells Translational Medicine*. 2017;**6**(9):1815-1821
- [19] Ng TK, Carballosa CM, Pelaez D, Wong HK, Choy KW, Pang CP, et al. Nicotine alters miRNA expression and hinders human adult stem cell regenerative potential. *Stem Cells and Development*. 2013;**22**(5):781-790
- [20] Ng TK, Huang L, Cao D, Pang CP, Cheung HS, et al. Cigarette smoking hinders human periodontal ligament-derived stem cell proliferation, migration and differentiation potentials. *Scientific Reports*. 2015;**5**:7828
- [21] Albuquerque EX, Pereira EF, Alkondon M, et al. Mammalian nicotinic acetylcholine receptors: From structure to function. *Physiological Reviews*. 2009;**89**:73-120
- [22] Hoogduijn MJ, Cheng A, Genever PG. Functional nicotinic and muscarinic receptors on mesenchymal stem cells. *Stem Cells and Development*. 2009;**18**:103-112
- [23] Olale F, Gerzanich V, Kuryatov A, Wang F, Lindstrom J. Chronic nicotine exposure differentially affects the function of human alpha3, alpha4, and alpha7 neuronal nicotinic receptor subtypes. *The Journal of Pharmacology and Experimental Therapeutics*. 1997;**283**(2):675-683
- [24] Schraufstatter IU, DiScipio RG, Khaldoyanidi SK. Alpha 7 subunit of nAChR regulates migration of human mesenchymal stem cells. *Journal of Stem Cells*. 2009;**4**:203-215
- [25] Zhou Z, Li B, Dong Z, Liu F, Zhang Y, Yu Y, et al. Nicotine deteriorates the osteogenic differentiation of periodontal ligament stem cells through alpha7 nicotinic acetylcholine receptor regulating Wnt pathway. *PLoS ONE*. 2013;**8**(12):e83102
- [26] Wu L, Zhou Y, Zhou Z, Liu Y, Bai Y, Xing X, et al. Nicotine induces the production of IL-1beta and IL-8 via the alpha7 nAChR/NF-kappaB pathway in human periodontal ligament cells: An in vitro study. *Cellular Physiology and Biochemistry*. 2014;**34**(2):423-431
- [27] Kim SY, Kang KL, Lee JC, Heo JS. Nicotinic acetylcholine receptor alpha7 and beta4 subunits contribute nicotine-induced apoptosis in periodontal ligament stem cells. *Molecules and Cells*. 2012;**33**(4):343-350
- [28] Wang XJ, Liu YF, Wang QY, Tsuruoka M, Ohta K, Wu SX, et al. Functional expression of alpha7 nicotinic acetylcholine receptors in human periodontal ligament fibroblasts and rat periodontal tissues. *Cell and Tissue Research*. 2010;**340**(2):347-355
- [29] Bartel DP. MicroRNAs: Genomics, biogenesis, mechanism, and function. *Cell*. 2004;**116**(2):281-297
- [30] Seeliger C, Balmayor ER, van Griensven M. miRNAs related to skeletal diseases. *Stem Cells and Development*. 1 Sep 2016;**25**(17):1261-1281
- [31] Ruiz JP, Pelaez D, Dias J, Ziebarth NM, Cheung HS. The effect of nicotine on the mechanical properties of mesenchymal stem cells. *Cell Health and Cytoskeleton*. 2012;**4**:29-35
- [32] Lee HJ, Pi SH, Kim Y, Kim HS, Kim SJ, Kim YS, et al. Effects of nicotine on antioxidant defense enzymes

and RANKL expression in human periodontal ligament cells. *Journal of Periodontology*. 2009;**80**(8):1281-1288

[33] Carballosa CN, Baumlin N, Salathe MA, Cheung HS. E-cigarette vapor extracts reduce human periodontal ligament derived stem cell osteogenic differentiation potential and alter microRNA expression. *Scientific Report*. (In Press)

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