HHS Public Access

Author manuscript

Reprod Toxicol. Author manuscript; available in PMC 2025 October 01.

Published in final edited form as:

Reprod Toxicol. 2024 October ; 129: 108673. doi:10.1016/j.reprotox.2024.108673.

Perinatal Nicotine Vaping Exposure Induces Pro-myofibroblastic Phenotype in Rat Bone Marrow-derived Mesenchymal Stem Cells

Celia Yu[a,*], **Jie Liu**[a,*], **Reiko Sakurai**[a], **Ying Wang**[a], **Leela Afrose**[a], **Abhishek Gour**[b], **Abhisheak Sharma**[b], **Gourav Chandan**[a], **Virender K. Rehan**[a,**]

[a]Department of Pediatrics, The Lundquist Institute of Biomedical Innovation at Harbor-UCLA Medical Center, 1124 West Carson Street, Torrance, CA, 90502, USA

[b]Department of Pharmaceutics, University of Florida, 1345 Center Drive, Gainesville, FL, 32610, USA

Abstract

Perinatal nicotine exposure via tobacco smoking results in increased proclivity to chronic lung disease (CLD); however, the underlying molecular mechanisms remain incompletely understood. We previously demonstrated that in addition to nicotine's direct effects on the developing lung, there are also adverse molecular alterations in bone marrow-derived mesenchymal stem cells (BMSCs), which are vital to lung injury repair. Whether perinatal nicotine exposure via electronic-cigarette (e-cig) vaping also adversely affects BMSCs is unknown. This is highly relevant due to marked increase in e-cig vaping including by pregnant women. Hypothesizing that perinatal nicotine exposure via e-cig vaping predisposes BMSCs to a pro-myofibroblastic phenotype, pregnant rat dams were exposed to fresh air (control), vehicle (e-cig without nicotine), or e-cig (e-cig with nicotine) daily during pregnancy and lactation. At postnatal day 21, offspring BMSCs were isolated and studied for cell proliferation, migration, wound healing response, and expression of key Wnt and PPARγ signaling intermediates (β-catenin, LEF-1, PPARγ, ADRP and C/EBPα) and myogenic markers (fibronectin, αSMA, calponin) proteins using immunoblotting. Compared to controls, perinatal e-cig exposure resulted in significant decrease in BMSC proliferation, migration, and wound healing response. The expression of key Wnt signaling intermediates (β-catenin, LEF-1) and myogenic markers (fibronectin, αSMA, calponin)

Address for Reprint Requests and Correspondence: Virender K. Rehan, MD, Department of Pediatrics, Lundquist Institute for Biomedical Innovation at Harbor-UCLA Medical Center, David Geffen School of Medicine at UCLA, 1124 West Carson Street, Torrance, CA 90502; vrehan@lundquist.org.

Author Contributions

JL and CY performed experiments, collected tissue, conducted molecular studies, prepared figures, and first draft of the manuscript; RS and GC isolated BMSCs; YW and LA collected tissue; AG and AS measured serum cotinine levels; VKR conceived and designed research, interpreted results, and edited and revised manuscript. All authors approved final version of manuscript.

*Co-first authors

Disclosures

No perceived or potential conflict of interest, financial or otherwise, are declared by the authors.

Declaration of interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

increased significantly, while PPARγ signaling intermediates (PPARγ, ADRP, and C/EBPα) decreased significantly. Based on these data, we conclude that perinatally e-cig exposed BMSCs demonstrate pro-myofibroblastic phenotype and impaired injury-repair potential, indicating a potentially similar susceptibility to CLD following perinatal nicotine exposure via vaping as seen following parenteral perinatal nicotine exposure.

Keywords

Smoking; Pregnancy; Lung injury repair; Electronic-cigarettes; PPARγ/Wnt Signaling

1. Introduction

Electronic cigarettes (e-cigs) primarily deliver nicotine through inhalation by heating an e-liquid composed mainly of nicotine, propylene glycol (PG), vegetable glycerin (VG), and flavors. Recently, due to their perceived safety, compared to conventional tobacco cigarettes, the use of e-cigs increased markedly in adult population in general and during pregnancy in particular. Nationally, on average, 3.2% of adults use e-cigs, while its use is estimated to be ~ 1.4% during pregnancy [1]. The National Health Interview Survey data from 2014 to 2017 found that 38.9% of pregnant smokers used e-cigs compared to 13.5% of non-pregnant women smokers of reproductive age [2]. The frequently reported reasons for e-cigs use include curiosity, possible help with reduction in cigarette smoking, and their perceived but unproven safety [1]. Almost all e-cigs sold in the U.S. contain nicotine [3]. This is highly relevant since most of cigarette smoke's effects on the developing fetus, especially on the developing lung and stem cells, can be attributed to nicotine [4,5,6]. Although plasma nicotine levels are generally lower with e-cig use compared to those with conventional cig smoking, these could still reach comparable or even higher levels than those achieved with traditional cigarettes [7,8,9]. This is particularly relevant since the nicotine content of some of the currently used e-liquids is up to 5 times higher than used in conventional cigarettes [10]. Moreover, all e-cigs also include additional components such as humectants (PG and VG). Accumulating evidence has shown significant harmful effects of PG and VG on developing lungs and other systems both independent and in combination with nicotine [11,12,13].

Though the damaging effects of combustible cig smoking on offspring health are well established, the effects of e-cig exposure during pregnancy are still emerging. Studies have shown that vaping during pregnancy is associated with reduced birth weight and increased prematurity [14,15]. Additionally, various animal models of perinatal vaping have demonstrated detrimental effects on several developing organs. These effects include altered expression of central homeostatic metabolic regulators [16], lung developmental programming alterations, increased expression of pulmonary pro-inflammatory cytokines [17], increased predisposition to airway hyperresponsiveness [18,19], craniofacial abnormalities [20], cardiovascular defects [21], and altered neurodevelopment with adverse behavioral and neuroimmunological consequences [22, 23].

We previously demonstrated that perinatal parenteral nicotine exposure adversely impacts the molecular and functional phenotype of bone marrow-derived mesenchymal stem cells (BMSCs) [24]. This is highly relevant since both preclinical and clinical studies suggest a potential role for BMSCs in lung injury repair. Several preclinical studies indicate that BMSCs can migrate to injured lung tissue, where they promote injury repair and modulate the inflammatory response through paracrine factors and differentiation into lung-specific cell types [25,26,27,28]. Additionally, several clinical studies assessing the efficacy of BMSCs in treating various respiratory conditions, including acute respiratory distress syndrome, bronchopulmonary dysplasia, chronic obstructive pulmonary disease, and idiopathic pulmonary fibrosis, have yielded mixed results [28,29,30]. These mixed results highlight the need for further investigation into the biology and therapeutic potential of BMSCs in these specific lung diseases.

In our previous study [24], the nicotine exposure was parenteral, i.e., it was not subjected to vaporization to generate e-vapors, a process known to generate additional toxic chemicals and the effect of perinatal PG/VG exposure on BMSCs was not studied [31,32]. We hypothesized that like parenteral nicotine exposure, perinatal nicotine and PG/VG exposure via e-cig vaping would also alter molecular and phenotypic characteristics of BMSCs, predisposing them to their pro-myofibroblastic phenotype and interfering with their injury repair function. Using an *in vivo* rat model, here we show how perinatal PG/VG ± nicotine vaping affects rat offspring BMSC differentiation and function.

2. Materials and Methods

2.1 Animals

Pregnant rat dams (200–250-gram body weight) were exposed to filtered air (Control), vehicle [Blu, aerosolized propylene glycol/vegetable glycerin (PG/VG) with gold leaf flavoring, https://us.blu.com], or e-cig (Blu, aerosolized PG/VG with 2.4% nicotine with gold leaf flavoring) through a previously established e-cig delivery system from embryonic day 6 (E6) until postnatal day 21 (PND21) [33,34,35,36]. Mimicking real-life puffing topography, the exposure protocol included 4 second puff exposure, 26 second interval between puffs, 6 puffs per vaping episode, 2 vaping episodes per hour, 12 vaping hours per day. All dams were given free access to food and water and were maintained in a 12hr:12hr light:dark cycle. Vaping exposure was skipped on the dams' delivery date and was resumed 24 hours later. After spontaneous delivery at term, some pups were sacrificed the next day for blood collection. PND1 pups were placed on heating pads to facilitate blood circulation, 3 millimeter Goldenrod animal lancets (Medipoint, Inc.) were used to puncture the submandibular vein and blood was collected for serum cotinine level measurement. Pups were then anesthetized in an isoflurane chamber and dissected to collect tissues for other purposes. The remaining pups were breast fed ad libitum until PND21, when they were sacrificed for BMSC isolation and culture following previous methods, described briefly below [24,37,38]. The experiments (control, vehicle, and e-cig nicotine exposure) were conducted four times. BMSCs were isolated from pooled marrows of 3–4 offspring from each group at postnatal day 21. All animal procedures were performed following the guidelines of the National Institutes of Health for the care and use of laboratory animals

after approval of the Lundquist Institute Animal Care and Use Committee (protocol # 31355–03).

2.2 Measurement of Serum Cotinine Levels

As noted above, at PND1, pups were sacrificed, and blood was collected and then centrifuged at 2,500 × g at 4°C for 10 minutes to obtain serum. A 20 μL of serum was processed with 80 μL of methanol containing internal standard, vortex mixed, filtered, and centrifuged at 1500×g at room temperature for 5 min. Serum cotinine levels were measured using ultra-performance liquid chromatography-mass spectrometry (UPLC-MS/MS).

2.3 Isolation of Bone Marrow-Derived Mesenchymal Stem Cells

The medullary cavities of rat femurs were flushed with Minimal Essential Medium (MEM) Alpha (1×) + GlutaMax™−1 (Life Technologies, Catalog# 32561–037) containing 1% penicillin-streptomycin. The cells were washed once with MEM and plated at 1×10^6 cells per T75 flask (Corning, Catalog # 430641U) in the complete medium: MEM Alpha containing 10% fetal bovine serum (FBS) and 1% antibiotic and antimycotic (Gibco, catalog# 15240–062), and cultured at 37°C in 5% CO_2. Non-adherent cells were removed, and fresh media was added every 48 h. At confluence, the cells were harvested and using magnetic beads (Miltenyi Biotech, Auburn, CA), macrophages were depleted with anti-CD11b antibody, and other hematopoietic cells were removed using an anti-CD45 (both from BD Biosciences, Palo Alto, CA). Cells were collected in 1 ml of DMEM supplemented with 10% FBS, and then cultured and passaged. Due to >95% purity of cells at passage (P) 3, all experiments were conducted at P3.

2.4 Immunoblot Analysis

Protein extraction and immunoblot analysis for proliferating cell nuclear antigen (PCNA, a homotrimer, which by encircling the DNA acts as a scaffold to recruit proteins involved in DNA replication), β-catenin, lymphoid enhancer binding factor 1 (LEF-1), peroxisome proliferator-activated receptor gamma (PPARγ), adipose differentiation-related protein (ADRP), CCAAT-enhancer-binding protein alpha (C/EBPα), α-smooth muscle actin (αSMA), calponin, and fibronectin were performed as previously described [39,40,41]. Briefly, cells were homogenized in 10 mM Tris (pH 7.5), 0.25 M sucrose, 1 mM EDTA, 5 mM benzamidine, 2 mM phenylmethylsulfonyl fluoride, and 10 μg/ml each of pepstatin A, aprotinin, and leupeptin, and centrifuged at 16000×g for 10 min at 4°C. Equal amounts of protein from the supernatant were dissolved in electrophoresis sample buffer, loaded to SDS polyacrylamide (4–12% gradient) gel and separated, followed by electrophoretic transfer to a nitrocellulose membrane. The membrane was stained with 0.1% Ponceau S in 5% acetic acid (Sigma-Aldrich, Catalog# P-7170) and then cut into several strips according to each marker's molecular weight. The membrane strips were blocked with 5% non-fat dry milk in 1× Tris-buffered saline containing 0.1% Tween 20 for 1 h, and then incubated with the corresponding primary antibody from Proteintech Group Inc. including fibronectin (1:1000, Catalog# 15613–1-AP), LEF-1 (1:500, Catalog# 14972–1-AP), PPARγ (1:500, Catalog# 16643–1-AP), ADRP (1:800, Catalog# 15294–1-AP)), and C/EBPα (1:800, Catalog# 18311–1-AP) except αSMA (1:20,000, Sigma-Aldrich, Catalog# 2547), Calponin (1:8,000, Sigma-Aldrich, Catalog# C2687), β-Catenin (1:800, Santa Cruz,

Catalog# sc-7963), PCNA (1:2,000, NOVUS, Catalog# NB500–106), and β-actin (1:2,000, Cell Signaling, Catalog# 13E5) overnight at 4°C. Subsequently, the membrane strips were washed with 1× Tris-buffered saline + 0.1% Tween 20 and incubated with the appropriate secondary antibody for 1 h at room temperature, washed again, and developed with SuperSignal West Pico chemiluminescent substrate (Pierce Biotechnology, Rockford IL) following the manufacturer's protocol. Membrane strips corresponding to the molecular weight of β-actin were stripped with Restore™ Western Blot Stripping Buffer (Pierce, Catalog# 21059), blocked and re-probed with the appropriate primary and secondary antibodies. The densities of the PCNA, β-catenin, LEF-1, PPARγ, ADRP, C/EBPα, αSMA, calponin, and fibronectin bands were quantitated using ImageJ software (National Institutes of Health) and normalized to β-actin.

2.5 Cell Proliferation

Cell proliferation was assessed via 1) colorimetric tetrazolium-formazan assay using CellTiter 96® Aqueous One Solution Cell Proliferation Assay (Promega, Catalog #G3580) and 2) Western blot analysis for PCNA. For tetrazolium-formazan assay, BMSCs were plated at 5×10^4 cells per well and 100 μl complete medium: MEM Alpha containing 10% fetal bovine serum (FBS) and 1% antibiotic-antimycotic (Gibco, Life Technologies, Grand Island, NY, catalog# 15240–062) and cultured at 37°C in 5% CO_2 for 72 hours, with fresh media added every 24 hours. Following 72 hours, 20 μl/well of Cell Titer 96 Aqueous One Solution Reagent was added, and plate was incubated at 37°C in 5% CO_2 for 3 hours. Absorbance at 490 nm was measured using a 96-well plate reader. Western blotting for PCNA was performed as outlined above.

2.6 Cell Migration

To assess cell migration, Boyden transwell cell migration assay using 24-Well Colorimetric Cell Migration Assay (Millipore, Catalog # ECM 508) was used. BMSCs were suspended at 5×10^5 cells/well in 10% FBS, 300 μl cell suspension was added to each insert, and 500 μl serum free media (MEM w/Glutamax + 5% BSA) was added to the lower/outer chamber. Plate was incubated for 24 hours at 37°C in 5% CO_2. 400 μl Cell Stain provided by the kit was added, and non-migratory cells were removed. Extraction buffer was added, and after 15 minutes, optical density was measured at 560 nm. The cell migration assay was performed under both normoxia (21% O_2) and hyperoxia (95% O_2). Cells were exposed to hyperoxia in Billups-Rothenberg chambers (Del Mar, CA) as described previously [42,43].

2.7 Wound Healing

To assess wound healing, the wound healing assay kit (Abcam, Catalog # ab242285) was used. Briefly, ensuring the alignment of wound fields and firm contact of inserts to the bottom of the plate wells, specific volumes of cell suspension according to cell concentration [5×10^5 cells/ml in medium containing 10% fetal bovine serum (FBS)] were added to each well. For optimal cell dispersion, half of the specific volumes of cell suspension were added to either side of the open ends at the top of the insert. After incubating for 48 hours, inserts were slowly removed from all wells using sterile forceps. For wound field baseline cells, wells were washed 3 times with PBS containing calcium and magnesium, stained with 400 μl of cell stain solution provided by the manufacturer and allowed to incubate for 15 mins at

room temperature. Cell stain was aspirated, wells washed carefully 3 times using deionized water and allowed to dry at room temperature before imaging. For wound healing cells, media were slowly aspirated, and wells were washed with media to remove dead cells and debris before adding fresh media with FBS for continued culture. Closure was monitored under a light microscope. 24 hours after inserts were removed, wells were washed, stained, and imaged following the protocol for wound field baseline cells.

2.8 Statistical Data Analysis

The data were analyzed, using either one way ANOVA with Tukey's post-hoc analysis for multiple comparisons or student's t-test, as appropriate. The results are based on four independent experiments and the values are expressed as mean ± SE. A P value of <0.05 is considered to represent statistically significant difference between the experimental groups.

3. RESULTS

3.1 Serum cotinine levels of control and perinatal e-cigarette exposed offspring

Compared to Fresh air (FA) controls, serum cotinine levels were significantly increased in e-cig with nicotine exposed group (Fig. 1).

3.2 Effect of perinatal e-cigarette exposure on BMSC proliferation

Cell proliferation, as determined by tetrazolium-formazan assay and Western blotting for PCNA (proliferating cell nuclear antigen), a key nucleic marker that plays an essential role in nucleic acid metabolism as a component of cell replication and repair machinery, was significantly inhibited in e-cig exposed cells compared to controls ($p<0.01$), while there was no significant difference in cell proliferation between the control and vehicle exposed BMSCs (Fig. 2).

3.3 Effect of perinatal e-cigarette exposure on BMSC migration

In normoxia, BMSCs from control and vehicle exposed cells showed similar migration, while BMSCs from e-cig exposed cells demonstrated only 50% migration vs. control and vehicle exposed cells ($p < 0.01$). In hyperoxia, BMSCs from all groups demonstrated higher migration vs. the corresponding normoxia groups; however, even in hyperoxia, BMSCs from the e-cig exposed group demonstrated markedly reduced migration vs. the corresponding control and vehicle exposed groups which showed similar migrations (Fig. 3).

3.4 Effect of perinatal e-cigarette exposure on BMSC wound healing activity

Complementing the BMSC proliferation and migration data, at 24h time point, BMSCs from control and vehicle exposed group demonstrated similar, i.e., 94–95% wound closure, while e-cig exposed BMSCs exhibited significantly reduced (43%) wound closure (Fig. 4).

3.5 Effect of perinatal e-cigarette exposure on BMSC lipogenic and myogenic protein marker levels

The protein level of PPARγ (Fig. 5a), which is a key nuclear transcription factor that determines lipofibroblastic phenotype, was significantly inhibited in nicotine-containing

e-cig BMSC exposed group versus control and vehicle exposed groups which exhibited statistically similar levels. Similar effects were seen with ADRP (Fig. 5b) and C/EBPα (Fig. 5c) protein levels, two known downstream targets of PPARγ. In contrast, the protein levels of myogenic markers fibronectin (Fig. 6a), αSMA (Fig. 6b) and calponin (Fig. 6c) were significantly higher in BMSCs from nicotine-containing e-cig exposure group vs. control and vehicle exposed groups, which exhibited similar protein levels of these myogenic markers.

3.6 Effect of perinatal nicotine e-cigarette exposure on Wnt signaling in BMSCs

Having determined upregulation of myogenic proteins in e-cig exposed BMSCs, the protein expression of key intermediates (β-catenin and LEF-1) of Wnt signaling, which is the main determinant of cellular myofibroblastic phenotype, was determined. Compared to control and vehicle exposed groups, protein levels of β-catenin (Fig. 7a) and LEF-1 (Fig. 7b) were significantly increased in the e-cig exposed BMSCs, but there were no significant differences between the control and vehicle exposed groups.

4. DISCUSSION

To prove our vaping regimen's efficiency, serum cotinine levels of PND1 offspring were measured. Although pups' blood was collected 24 hours following the dams' last exposure, cotinine levels were still significantly increased in the e-cig exposed group compared to controls, demonstrating that our vaping regimen functioned well. Consistent with our previous work [24], compared to controls, nicotine and PG/VG-containing e-cig vaping significantly decreased protein levels of PPARγ, the "master switch" for adipogenesis, while significantly increasing the protein levels of αSMA, fibronectin and calponin. This was accompanied by upregulation of Wnt signaling intermediates β-catenin and LEF-1, indicating BMSCs' pro-myofibroblastic phenotype. This changed molecular phenotype was also accompanied by decreased proliferation, migration, and wound healing potential of e-cig exposed BMSCs. Interestingly, contrary to our expectation, PG/VG vaping alone did not affect functional or molecular phenotype of offspring BMSCs. Based on the observations made in these experiments, perinatal e-cig vaping inhibited the lipogenic potential but enhanced the myogenic potential of rat offspring BMSCs.

Compelling evidence supports that most effects of perinatal cigarette smoke exposure on the developing lung are nicotine mediated [4,5,44,45]. It is especially noteworthy that pulmonary effects seen following perinatal nicotine exposure in several animal models, including a non-human primate model, are similar to those seen in human infants exposed to maternal smoking during pregnancy [44,46,47,48,49]. Though the precise cellular and molecular mechanisms involved in perinatal nicotine-driven lung phenotype remain incompletely understood, it has been suggested that in addition to nicotine's direct effects on various pulmonary cell types, it might also affect mesenchymal stem cells which are vital to lung homeostasis and injury repair. In the developing lung, nicotine specifically impacts mesenchymal Wnt and PPARγ signaling at both the alveolar and airway levels [44,50,51], leading to upregulation of Wnt and the downregulation of PPARγ signaling, which predisposes to the myogenic phenotype seen following perinatal nicotine/tobacco

exposure in both children and adults. This study confirms that following nicotine + PG/VG vaping during the perinatal period, similar effects are observed on offspring BMSCs.

It may be worth emphasizing once again that the lipogenic phenotype of lung fibroblasts (high PPARγ signaling) promotes homeostasis by enhancing the growth and differentiation of alveolar epithelial type II cells. In contrast, the myogenic phenotype (high Wnt signaling), which is pro-fibrotic, disrupts homeostasis [50,52]. Furthermore, our findings of potential negative impacts of e-cig vaping on offspring BMSC function are supported by several other studies that have shown impaired wound healing due to alterations in fibroblast differentiation and function (cell attachment and migration) caused by nicotine, cigarette smoke, and e-cigarette vaping [53, 54,55,56,57,58]. These studies have used a variety of fibroblasts including lung fibroblasts to demonstrate these effects. In line with these studies, e-cig vaping-induced pro-fibroblastic differentiation of BMSCs suggests impaired injury repair potential characterized by predisposition to fibrosis instead of a healthy wound healing.

Mesenchymal stem cells including those derived from the bone marrow are pluripotent cells that under appropriate conditions differentiate into a variety of specific cell-types. This multi-lineage and self-renewal potential allows mesenchymal stem cells to play a vital role in homeostasis and injury repair in a variety of organs [59,60]. These cells have attracted major attention in organ injury repair since they are easy to isolate, do not give rise to teratomas (as opposed to embryonic stem cells), and have immunomodulatory properties [61,62]. Bone marrow-derived mesenchymal stem cells are frequently recruited to the site of injury [63]. We have previously demonstrated the multi-lineage potential of BMSCs and have argued the pro-homeostatic potential of lipogenic and dys-homeostatic potential of myogenic BMSCs [24,37,38]. Although we did not specifically characterize polyploid nature of BMSCs studied by us, it's interesting to note that recently a key role of lipogenic (adiponectin +ve) polyploid BMSCs in tissue regeneration has been highlighted [64].

Although detrimental effects of perinatal nicotine exposure on offspring BMSCs were previously demonstrated, since vaping changes the chemical makeup of the e-cig liquids, it raises the possibility of different or additional toxicities [31,32]. Moreover, although the actual puffing topography of e-cig vapors varies significantly depending on factors such as an individual's vaping habit, device characteristics, e-liquid type and strength, and personal preferences, the vaping regimen we used is consistent with the typical behavior of adult vapers. This regimen has been utilized in several previous studies investigating the effects of perinatal exposures [36,65,66,67]. The number of puffs per day we employed (144) falls well within the range observed among vapers (163 ± 138, median=132) in a study that tracked 1 million puffs across 185 users [67]. Furthermore, to replicate real-time puffing topography, we conducted daily e-cigarette exposures over a period of 7 days per week, as opposed to the more convenient 5-day/week exposure utilized in some previous studies [36,66].

5. Conclusion

In line with our current data, previous in vitro studies have shown detrimental effects of tobacco cigarette and e-cigarette aerosol extracts on the survival, morphology, differentiation, and gap junction-mediated communication of BMSCs [68,69,70]. Given that BMSCs exposed to perinatal e-cig vaping are programmed to a pro-fibrotic phenotype and have reduced ability to migrate and hence engraft at injury sites, there are likely long-term detrimental effects on lung injury repair and function. These observations, combined with the direct effects of nicotine on lung development, provide compelling evidence to suggest an additional plausible mechanism for the increased susceptibility to CLD in the exposed offspring.

Acknowledgments

This work was supported by National Institutes of Health [grant number HL151769] and Tobacco Related Disease Research Program [grant numbers 27IP-0050, T32IP5044, T32IR5048, T32IR5365, T291R0737, T34IR8406] to VKR.

Abbreviations:

CLD	Chronic lung disease
BMSCs	Bone marrow-derived mesenchymal stem cells
e-cig	Electronic-cigarette
Wnt	Wingless/Integrated
PPARγ	Peroxisome proliferator activated receptor gamma
β-catenin	Beta-catenin
LEF-1	Lymphoid enhancer binding factor 1
ADRP	Adipose differentiation-related protein
C/EBPα	CCAAT/enhancer binding protein alpha
αSMA	Alpha smooth muscle actin
PG	Propylene glycol
VG	Vegetable glycerin
E6	Embryonic day 6
PND	Postnatal day
MEM	Minimal Essential Medium
FBS	Fetal Bovine Serum
Anti-anti	Antibiotic Antimycotic

CD45	Cluster of differentiation 45
DMEM	Dulbecco's Modified Eagle Medium
P3	Passage 3
PCNA	Proliferating cell nuclear antigen
Tris	Tris(hydroxymethyl)aminomethane
EDTA	Ethylenediaminetetraacetic acid
SDS	Sodium dodecyl sulfate
β-actin	Beta-actin
BSA	Bovine serum albumin
ANOVA	Analysis of variance
SE	Standard Error
FA	Fresh air

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Highlights

- E-cig exposure during pregnancy affects bone marrow stem cells (BMSCs) adversely.
- Exposure leads to decreased BMSC proliferation, migration, and wound healing.
- Wnt signaling and myogenic markers increase, while PPARγ signaling decreases.
- Perinatal e-cig vaping increases offspring's lung injury risk, like traditional cigs.

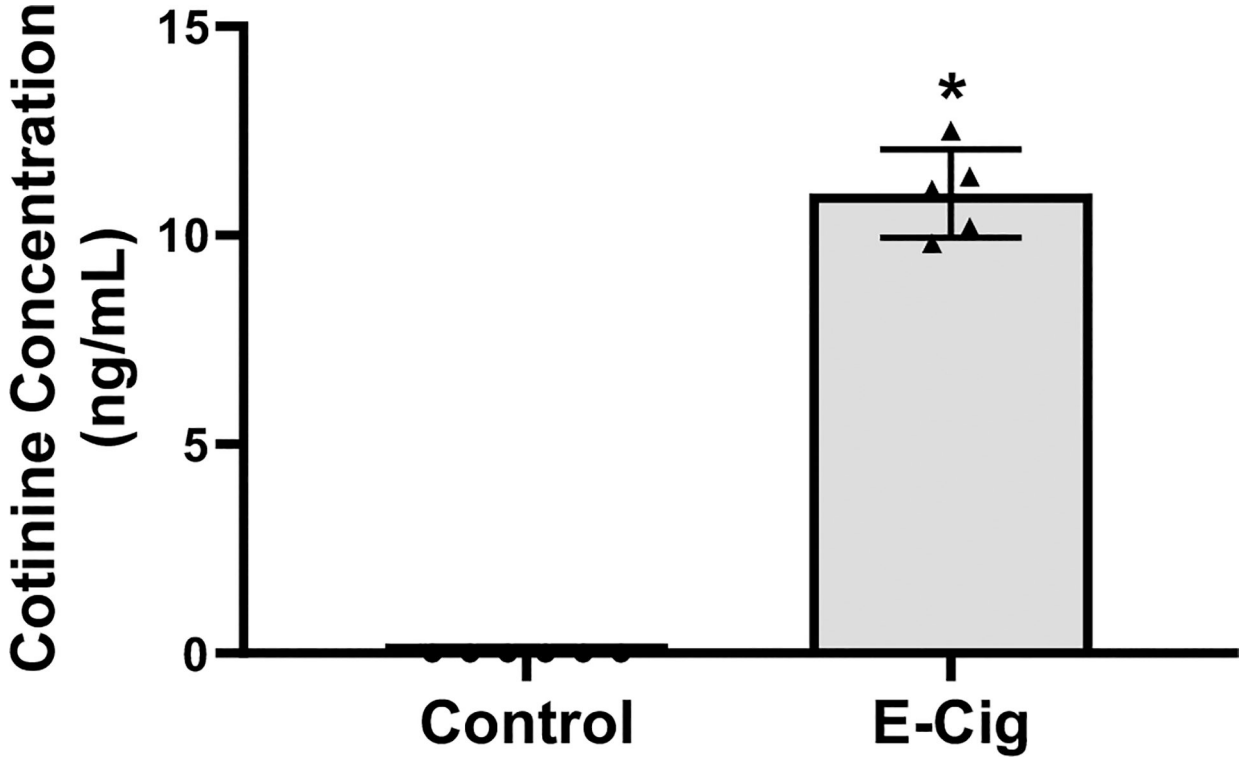

Fig. 1.

Serum Cotinine Level. Cotinine levels of serum obtained from PND1 pups of control and e-cig exposed groups were measured using UPLC-MS/MS. Compared with the control group, there was a significant increase of cotinine concentration in the e-cig exposed group. Values are means ± SE (n = 5–6 for each group). * $P < 0.05$ versus control.

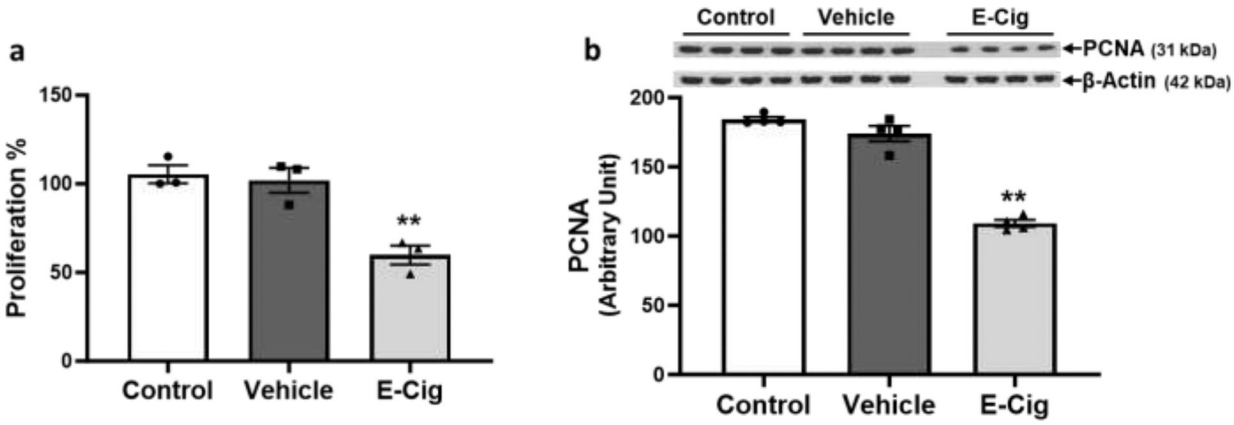

Fig. 2.

Effect of Perinatal Nicotine E-Cig Exposure on BMSC Proliferation. Passage 3 BMSCs isolated from rats under the three conditions at PND21 were evaluated and proliferation percent was determined at an absorbance of 490nm (a), and protein levels of PCNA were also determined (b). Compared with the control group, there was a significant decrease in cell proliferation in e-cig exposed BMSCs, whereas there was no significant effect on vehicle-exposed BMSCs. Values are means ± SE (n = 4 for each group). ** $P < 0.01$ versus control.

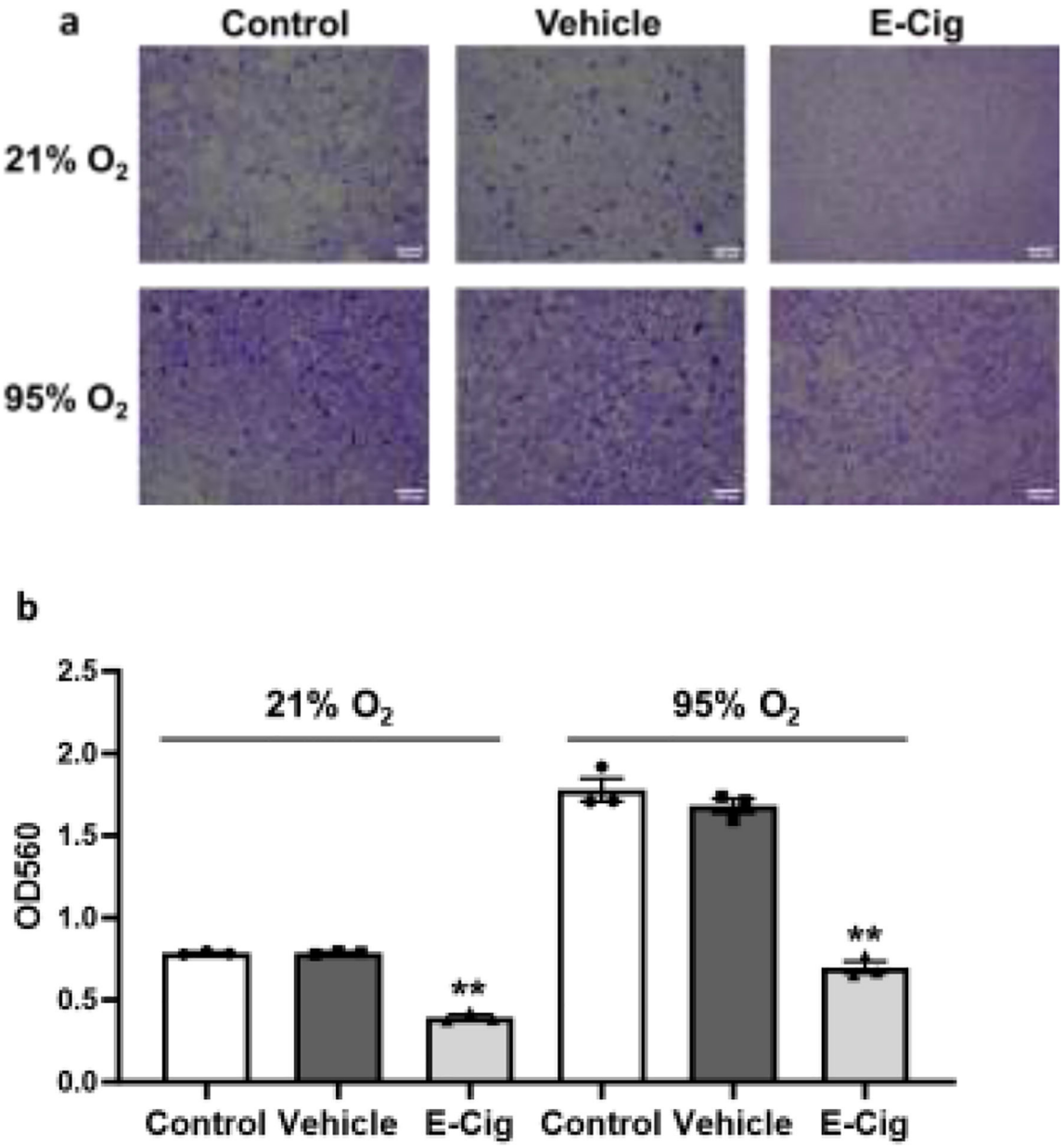

Fig. 3.

Effect of Perinatal Nicotine E-Cig Exposure on BMSC Migration. Images show cell migration of all groups in 21% O_2 and 95 % O_2 (a). Optical density of images was determined at 560nm wavelength (b). Compared to control, e-cig exposed cells showed significantly decreased levels of cell migration at normoxia 21% O_2, and at hyperoxia 95% O_2, still showed a significant decrease of cell migration when compared to control. Vehicle exposed cells showed no effect at both 21% and 95% O_2 when compared to controls. Values are means ± SE (n = 4 for each group). ** $P < 0.01$ versus control.

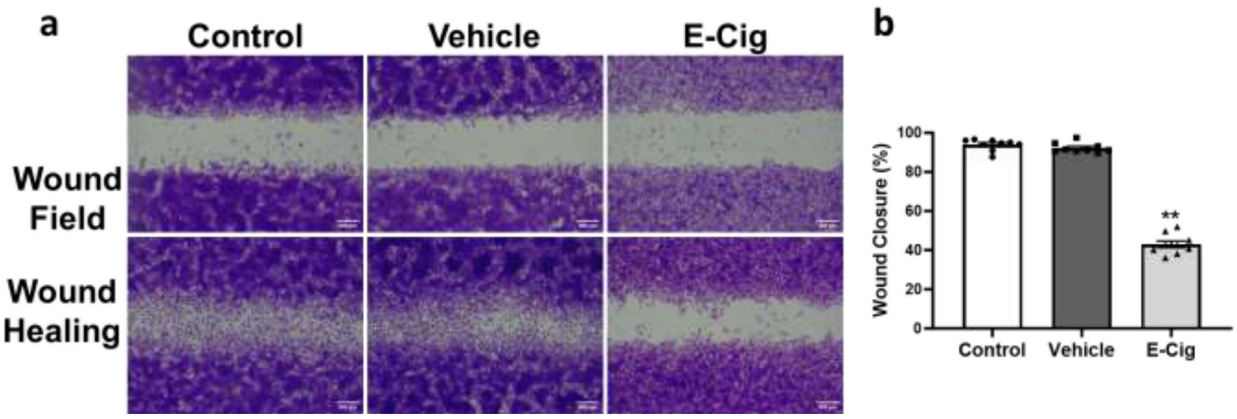

Fig. 4.

Effect of Perinatal Nicotine Exposure via Vaping on BMSC Wound Healing. 48 hours following BMSC plating serves as baseline wound field, in which images depict decreased cell proliferation in E-Cig exposed BMSCs, compared to control and vehicle cells (a). Wound closure was significantly decreased in E-Cig exposed cells (43%), compared to the control group (95%), whereas there was no effect on vehicle exposed cells (94%) (b). Values are means ± SE (n = 4 for each group). ** $P < 0.01$ versus control.

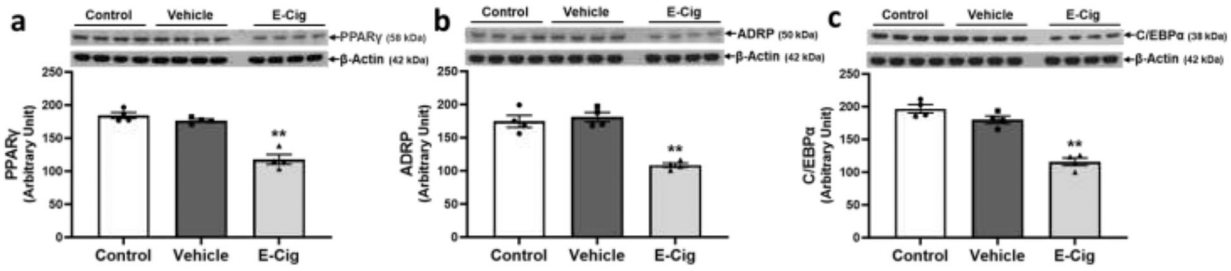

Fig. 5.

Effect of Perinatal Nicotine Exposure via Vaping on PPARγ Signaling Pathway in BMSCs. Compared to controls, the protein levels of PPARγ signaling markers PPARγ (a), ADRP (b) and C/EBPα (c) were all significantly decreased in the e-cig exposed group, whereas there was no effect on the vehicle group. Upper panels show representative Western blots for these markers and for β-actin. Lower panels show the densitometry values of the markers normalized to β-actin. Values are means ± SE (n = 4 for each group). ** $P < 0.01$ versus control.

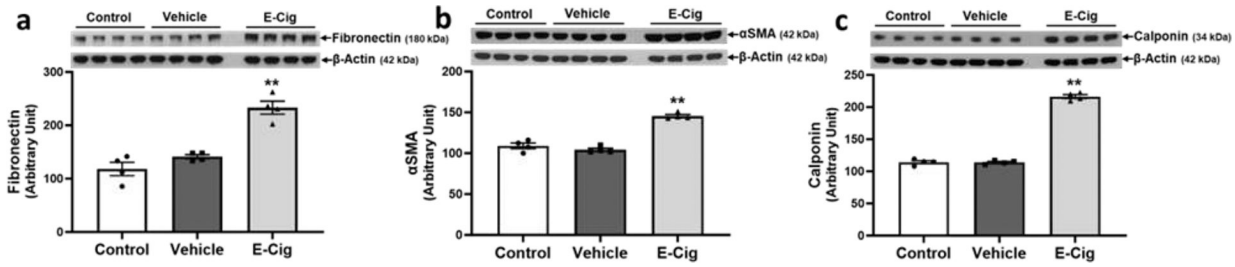

Fig. 6.

Effect of Perinatal Nicotine Exposure via Vaping on Myogenic Markers in BMSCs. Compared to controls, the protein levels of fibronectin (a), αSMA (b), and calponin (c) were all increased significantly in e-cig exposed BMSCs, whereas there was no effect in vehicle exposed cells. Upper panels show representative Western blots for these markers and for β-actin. Lower panels show the densitometry values of the markers normalized to β-actin. Values are means ± SE (n = 4 for each group). ** $P < 0.01$ versus control.

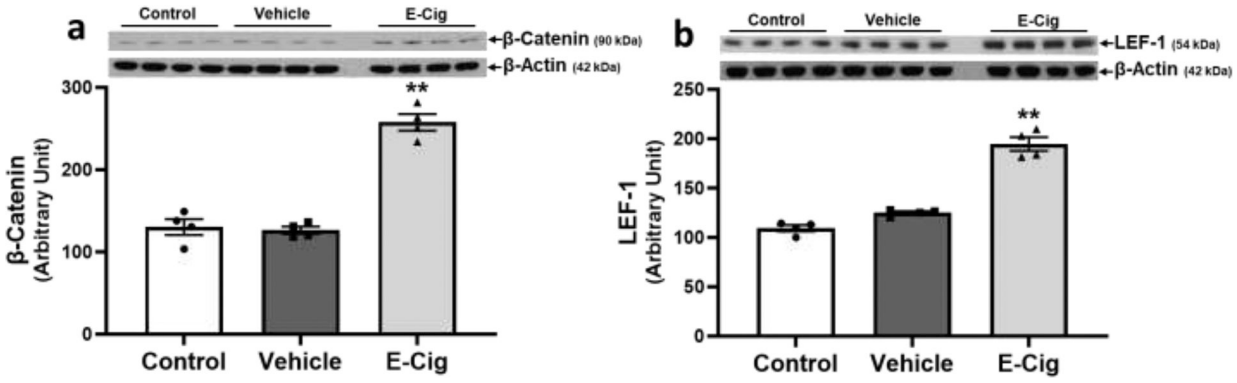

Fig. 7.

Effect of Perinatal Nicotine Exposure via Vaping on Wnt Signaling Intermediates in BMSCs. Compared to controls, the protein levels of β-catenin (a) and LEF-1 (b), central intermediates of Wnt signaling, which is a key determinant of myogenic markers fibronectin, aSMA and calponin, showed significant increase in e-cig exposed BMSCs, whereas there was no effect on the vehicle group. Upper panels show representative Western blots for these markers and for β-actin. Lower panels show the densitometry values of the markers normalized to β-actin. Values are means ± SE (n = 4 for each group). ** $P < 0.01$ versus control.