Current Stem Cell Research & Therapy, 2023, *18*, 440-444

PERSPECTIVE

Modified Risk Tobacco Products and Cardiovascular Repair: Still Very "Smoky"

Claudia Cozzolino[1], Vittorio Picchio[1], Erica Floris[1], Francesca Pagano[2], Wael Saade[3], Mariangela Peruzzi[3,4], Giacomo Frati[1,5] and Isotta Chimenti[1,4,*]

[1]*Department of Medical Surgical Sciences and Biotechnologies, Sapienza University of Rome, 04100 Latina, Italy;* [2]*Institute of Biochemistry and Cell Biology, National Council of Research (IBBC-CNR), Monterotondo (RM), Italy;* [3]*Department of Clinical Internal, Anesthesiologic and Cardiovascular Sciences, Sapienza University of Rome, 00185 Rome, Italy;* [4]*Mediterranea Cardiocentro, 80133 Napoli, Italy;* [5]*IRCCS NeuroMed, Pozzilli (IS), Italy*

ARTICLE HISTORY

Received: May 18, 2022
Revised: June 01, 2022
Accepted: June 01, 2022

DOI:
10.2174/1574888X17666220802142532

Abstract: Smoking habits represent a cardiovascular risk factor with a tremendous impact on health. Other than damaging differentiated and functional cells of the cardiovascular system, they also negatively affect reparative mechanisms, such as those involved in cardiac fibrosis and in endothelial progenitor cell (EPC) activation. In recent years, alternative smoking devices, dubbed modified tobacco risk products (MRPs), have been introduced, but their precise impact on human health is still under evaluation. Also, they have not been characterized yet about the possible negative effects on cardiovascular reparative and regenerative cells, such as EPCs or pluripotent stem cells. In this perspective, we critically review the still scarce available data on the effects of MRPs on molecular and cellular mechanisms of cardiovascular repair and regeneration.

Keywords: Endothelial progenitor cells, pluripotent stem cells, electronic cigarettes, modified risk products, cardiac fibrosis, cardiovascular regeneration.

1. INTRODUCTION

Fibrosis is a reparative response contributing to wound healing processes, leading to the formation of a permanent fibrotic scar. Fibrosis can be aberrantly and chronically activated in response to persistent injury, resulting in excessive accumulation of extracellular matrix components, such as collagen produced by activated fibroblasts. The fibrogenic process has unique features related to the function of each specific organ involved, but common steps are cell damage and death, recruitment of inflammatory cells, production of reactive oxygen species (ROS), the release of transforming growth factor-β1 (TGF-β1), and activation of collagen-producing cells [1, 2]. Other fundamental responses in tissue repair are neo-angiogenesis and vessel repair. Circulating endothelial progenitor cells (EPCs) are key players in this response, and they serve the purpose of both repairing damaged vessels and forming novel capillary structures [3]. Their abundance and functional phenotype are features that may be affected by multiple pathological conditions, thus impairing their capacity to preserve healthy vessels and generate new capillaries [4].

Multiple studies have provided compelling evidence that smoke can impair cardiovascular repair responses at multiple levels [5]. Indeed, due to chronic low-grade insults, smoking can sustain continuing mild repair activation in the myocardium, leading to cardiac fibrosis, by directly altering the functions of fibroblasts. Moreover, chronic tobacco smoking is indeed associated with impaired numbers and functions of circulating EPCs [6], although many aspects of the underlying mechanisms remain unclear. Furthermore, research in cardiac regenerative medicine strongly relies on adult or pluripotent stem cells as potent tools to obtain cardiovascular repair and regeneration [7]. Given the extent of smoking habits, understanding the effects of smoking also on the efficacy of applicative protocols (*e.g.*, cell therapy) is of great translational interest (Fig. **1**).

Modified risk tobacco products (MRPs) are novel smoking devices introduced in recent years, including vaping electronic cigarettes (ECIG) and, more recently, heat not burn cigarettes (HNBC) [8]. Most preclinical and *in vitro* studies performed so far have been focused on ECIG liquids or aerosols, and have mainly employed lung cells and inflammatory cells as targets [9]. Some early studies have shown that these products release significantly reduced levels of harmful chemicals. However, most of these studies were funded by tobacco companies, thus caution is needed when interpreting this data. Overall, there is still a significant gap in knowledge

*Address correspondence to this author at the Department of Medical Surgical Sciences and Biotechnologies, Sapienza University of Rome, 04100 Latina, Italy; Tel: +3907731757234; E-mail: isotta.chimenti@uniroma1.it

2212-3946/23 $65.00+.00

concerning how MRPs can affect cardiovascular reparative cells and their functions, particularly for HNBCs. Thus, to what extent MRPs can impair cardiovascular repair mechanisms and stem/progenitor cells for cardiac regenerative medicine, and how similar to traditional cigarettes, remain to be elucidated. Since many types of circulating molecules (*e.g.*, microRNAs, binding proteins, cytokines) have been identified as modulators of cardiovascular reparative cells [10], it is worth speculating that the alleged effects of MRPs (as for traditional smoke) may be direct, as well as indirect through the altered composition of the circulating molecular profile in chronic smokers [11-14].

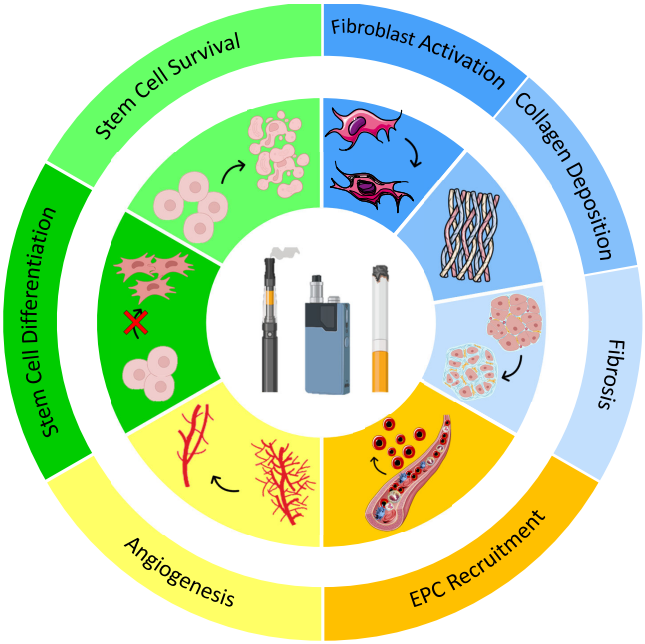

Fig. (1). A schematic representation of the mechanisms of cardiovascular repair and regeneration considered in this perspective that can be affected by traditional tobacco smoking, as well as by modified risk tobacco products. The three cell types considered are cardiac stromal cells, endothelial progenitor cells (EPCs), and pluripotent stem cells. (*A higher resolution / colour version of this figure is available in the electronic copy of the article*).

In this perspective review, we will briefly discuss a selection of key molecular and cellular mechanisms of impaired cardiovascular repair and/or regeneration due to tobacco smoke, with a comparative and critical perspective on the still very little available evidence on the parallel effects of MRPs.

2. DETRIMENTAL ACTIVATION OF MYOCARDIAL REPAIR

The main smoke-derived molecule known to be involved in the fibrogenic process is nicotine, which has been studied by multiple approaches. In a canine model, it was demonstrated that nicotine stimulates collagen production and atrial fibrosis, both *in vitro* in atrial fibroblasts, and *in vivo* in atrial tissue, through the upregulation of TGF-β1 and TGF-βRII. In particular, it has been proposed that nicotine exposure activates the α7-nAChRs receptors (ligand-gated ion channels), that regulate Ca^{2+} influx in response to acetylcholine, as well as nicotine [15, 16] in atrial fibroblasts, leading to the down-regulation of protective microRNAs (such as miR-133 and miR-590), and causing TGF-β1 and TGF-βRII activation [17].

The fibrogenic effect mediated by nicotine has also been demonstrated in rat models. Among others, Vang *et al*. have studied how nicotine can affect cellular proliferation and collagen secretion in cardiac fibroblasts isolated from right ventricular tissue. These effects are confirmed to be mediated by α7-nAChR, as demonstrated by pretreatment with specific α7-nAChR antagonists [15, 16, 18]. Moreover, the role of specific PKC isoforms has been described, that are activated by α7-nAChR signaling, and required for nicotine-induced proliferation. The proliferation and collagen production of cardiac fibroblasts are also associated with the decrease of p38-MAPK phosphorylation, which is PKC dependent [18].

The detrimental effect of nicotine on myocardial fibrosis has also been evidenced during perinatal exposure, that is, during a developmental stage with maximum regenerative capacity in the mammalian myocardium [19]. Studies have shown significant alterations in ECM protein accumulation, such as COL1A1 and COL3A1, accompanied with altered epigenetic profiles in non-coding RNAs [20, 21]. This data further stresses the potency of smoke-related myocardial injury, and its ability to impair cardiovascular reparative processes.

In addition, it has been described that the myocardium contains a resident population of primitive non-activated stromal/mesenchymal cells [22, 23]. These cells contribute to homeostatic functions of ECM deposition and paracrine support to parenchymal cells. Moreover, they are strongly activated after injury, giving rise to a proliferating activated pool, and then to different subpopulations with multiple phenotypes, including pro-repair and pro-inflammatory phenotypes, and myofibroblasts [1]. Interestingly, it has been recently reported that the levels of the primitive marker stem cell antigen 1 (Sca-1) are reduced in the heart in a mouse model of condensed smoke extract (CSE)-induced emphysema [24]. This could be interpreted as a sign of accelerated senescence of the cardiac primitive compartment, as well as the progressive depletion of the resident pool of non-activated mesenchymal primitive cells in the myocardium [1, 25].

Besides traditional combusting cigarettes, also electronic cigarettes can play a role in altered cardiac repair. Mayyas *et al*. have used rats exposed to tobacco cigarette smoke and ECIG aerosol, observing that cardiac fibrosis correlated with the increase of TGF-β1 protein. Also, levels of the cardiac remodeling marker MMP-2 were significantly increased in both groups in parallel to changes in oxidant and inflammatory biomarkers, indicating that both kinds of exposure can promote impairment of reparative signaling ending in fibrosis [26]. In another study on C57BL/6 mice exposed to ECIG vaping for up to 6 months, increased myocardial fibrosis by histological examination, associated with increased collagen expression levels, with a size effect comparable between ECIG and traditional cigarette smoke, was detected [27].

Overall, evidence on this topic is still too little to draw firm conclusions, although data seems to suggest a specific negative association between detrimental activation of repair myocardial mechanisms and MRPs, in particular, ECIGs (Fig. **1**). More importantly, though, insights on the effects mediated by HNBCs are still completely lacking.

3. ALTERED VESSEL REPAIR AND ANGIOGENESIS

Endothelial progenitor cells (EPCs) serve the purpose of repairing damaged capillaries and vessels and stimulating angiogenesis in response to different stimuli. As mentioned above, cigarette smoking is associated with diminished numbers and functions of EPCs (Fig. **1**) [6]. It has been shown that CSE causes decreased differentiation capacity to form capillary-like tubes, as well as reduced migration ability and eNOS expression in EPCs [28, 29]. Moreover, CSE treatment impairs EPCs at multiple levels, including cytoprotective mechanisms, such as autophagy and the mTOR pathway [30]. The impairment of the EPC compartment after CSE exposure, in terms of decreased proliferation and paracrine activity, has also been associated with the reduced expression of the common marker for tissue resident stem/progenitor cells, that is Sca-1 [31]. Interestingly, we already mentioned this marker in the previous paragraph as it is also reduced in the resident mesenchymal compartment of the myocardium in response to CSE exposure.

The only available study on this issue has reported that circulating EPCs acutely increase within 24 hours after short-term exposure to inhalation of ECIG in healthy young volunteers. This was also associated with increased circulating levels of microvesicles of endothelial origin [32], and similar effects were previously observed in studies on single cigarette smoke [33]. These results have been interpreted as a vascular damage compensatory response, albeit this remains purely speculative until data on long exposure times is collected. In fact, data about EPCs and chronic MRP use (including HNBC) is still missing, thus making it hard so far to draw final conclusions about endothelial repair impairment in MRP smokers (Fig. **1**).

4. EFFECTS ON STEM CELLS FOR CARDIOVASCULAR REPAIR AND REGENERATION

An interesting research question is whether chronic smoke of MRPs may alter the success of regenerative medicine protocols, such as those involving cell therapy for cardiovascular repair [7, 34]. As an example, it has been quite thoroughly assessed that traditional cigarette smoking may have a significant negative impact on mesenchymal stem cells (MSCs) for cell therapy applications, in terms of decreased proliferation, migration, and differentiation capacity [35]. Therefore, the potential impact of MRPs consumption on stem/progenitor cells of interest for cardiovascular applications in cell therapy needs great attention, both from the perspective of the microenvironment in the cell donor as well as that of the recipient.

It has been shown that adipose stem/stromal cells (ASCs) derived from chronic smokers have a lower therapeutic capacity to improve perfusion in a hindlimb ischemia mouse model, compared to ASCs from non-smokers [36]. In fact, they showed reduced support to endothelial cells, associated to reduced release of beneficial paracrine factors, such as HGF and SDF1, with parallel increased release of pro-inflammatory cytokines.

From a different perspective, cell therapy itself has been proposed as a possible direct strategy against the detrimental effects of smoking on the heart. In fact, it has been shown that induced pluripotent stem cell (iPS)-derived MSCs can be effective in curing cardiac dysfunction due to passive smoking exposure in rats. In fact, intra-venous administration of iPS-MSCs was able to attenuate indexes of cardiac inflammation and oxidative stress, as well as ameliorate cardiac remodeling parameters [37]. How these possible beneficial effects, though, could be hampered in chronic smoker recipients (of both tobacco cigarettes and MRPs) remains to be elucidated.

Pluripotent stem cells hold great promise for cardiovascular regenerative medicine applications [38]. Interestingly, it has been shown that exposure to smoke extracts from tobacco and ECIG can significantly delay mesodermal transition and cardiac differentiation of human embryonic stem cells (hESCs), albeit TCC extract exerts a slightly higher cytotoxic effect [39]. Another study has explored the cytotoxicity of ECIG refill fluids (both liquid and aerosol converted) on different cell types, and the authors reported higher sensitivity of hESC to these treatments [40]. Overall, stem/progenitor cells appear highly sensitive to the insult associated with smoking habits, including ECIGs (Fig. **1**), consistently with their higher sensitivity to many other kinds of insults [41-43].

CONCLUSION

The number of MRP smokers is increasing exponentially worldwide, but the impact of MRPs on health is still debated. More independent research studies are needed in order to characterize the harmful chemicals released by ECIG and HNBC, and to define the exact extent of their "modified risk" at medical and epidemiological levels. Moreover, studies are needed on the molecular and cellular mechanisms of damage to the cardiovascular system, including effects on repair and regeneration. In fact, the number of studies on the effects of MRP-derived molecules and MRP consumption on cardiovascular reparative cells is still very limited. Albeit no firm conclusions can be drawn so far, it is worth reasoning that the available data seems to suggest a negative association between mechanisms of cardiovascular repair/regeneration and MRPs, in particular concerning ECIGs (Fig. **1**). On the other side, insights on the effects mediated by HNBCs on the different cell types are still completely lacking, thus giving us hope that the number of investigations in this regard will grow rapidly in the near future.

LIST OF ABBREVIATIONS

EPCs	=	Endothelial Progenitor Cells
HNBC	=	Heat Not Burn Cigarettes
MRPs	=	Modified Risk Tobacco Products
ROS	=	Reactive Oxygen Species
TGF-β1	=	Transforming Growth Factor-β1

CONFLICT OF INTEREST

Isotta Chimenti is on the Editorial Advisory Board of Current Stem Cell Research & Therapy.

ACKNOWLEDGEMENTS

Isotta Chimenti is supported by Sapienza University, grant numbers RG11916B85CDBF76 and RM12117A805ED2FD.

REFERENCES

[1] Picchio V, Bordin A, Floris E, Cozzolino C, Dhori X, Peruzzi M, *et al.* The dynamic facets of the cardiac stroma: From classical markers to omics and translational perspectives 2022; 14.

[2] Kisseleva T, Brenner DA. Mechanisms of fibrogenesis. Exp Biol Med (Maywood) 2008; 233(2): 109-22. http://dx.doi.org/10.3181/0707-MR-190 PMID: 18222966

[3] Kawasaki T, Nishiwaki T, Sekine A, *et al.* Vascular repair by tissue-resident endothelial progenitor cells in endotoxin-induced lung injury. Am J Respir Cell Mol Biol 2015; 53(4): 500-12. http://dx.doi.org/10.1165/rcmb.2014-0185OC PMID: 25719275

[4] Regueiro A, Cuadrado-Godia E, Bueno-Betí C, *et al.* Mobilization of endothelial progenitor cells in acute cardiovascular events in the PROCELL study: Time-course after acute myocardial infarction and stroke. J Mol Cell Cardiol 2015; 80: 146-55. http://dx.doi.org/10.1016/j.yjmcc.2015.01.005 PMID: 25619946

[5] Salahuddin S, Prabhakaran D, Roy A. Pathophysiological mechanisms of tobacco-related CVD. Glob Heart 2012; 7(2): 113-20. http://dx.doi.org/10.1016/j.gheart.2012.05.003 PMID: 25691307

[6] Kondo T, Hayashi M, Takeshita K, *et al.* Smoking cessation rapidly increases circulating progenitor cells in peripheral blood in chronic smokers. Arterioscler Thromb Vasc Biol 2004; 24(8): 1442-7. http://dx.doi.org/10.1161/01.ATV.0000135655.52088.c5 PMID: 15191940

[7] Madonna R, Van Laake LW, Davidson SM, *et al.* Position paper of the European society of cardiology working group cellular biology of the heart: Cell-based therapies for myocardial repair and regeneration in ischemic heart disease and heart failure. Eur Heart J 2016; 37(23): 1789-98. http://dx.doi.org/10.1093/eurheartj/ehw113 PMID: 27055812

[8] Biondi Zoccai G, Carnevale R, Sciarretta S, Frati G. Electronic cigarette. Eur Heart J Suppl 2020; 22(Suppl. E): E25-9. http://dx.doi.org/10.1093/eurheartj/suaa053 PMID: 32523433

[9] Merecz-Sadowska A, Sitarek P, Zielinska-Blizniewska H, *et al.* A summary of *in vitro* and *in vivo* studies evaluating the impact of E-cigarette exposure on living organisms and the environment. Int J Mol Sci 2020; 21(2): E652. http://dx.doi.org/10.3390/ijms21020652 PMID: 31963832

[10] Angelini F, Pagano F, Bordin A, Picchio V, De Falco E, Chimenti I. Getting old through the blood: Circulating molecules in aging and senescence of cardiovascular regenerative cells. Front Cardiovasc Med 2017; 4: 62. http://dx.doi.org/10.3389/fcvm.2017.00062 PMID: 29057227

[11] Ramírez-Salazar EG, Gayosso-Gómez LV, Baez-Saldaña R, *et al.* Cigarette smoking alters the expression of circulating micrornas and its potential diagnostic value in female lung cancer patients. Biology (Basel) 2021; 10(8): 793. http://dx.doi.org/10.3390/biology10080793 PMID: 34440025

[12] Solleti SK, Bhattacharya S, Ahmad A, *et al.* MicroRNA expression profiling defines the impact of electronic cigarettes on human airway epithelial cells. Sci Rep 2017; 7(1): 1081. http://dx.doi.org/10.1038/s41598-017-01167-8 PMID: 28439113

[13] Singh KP, Maremanda KP, Li D, Rahman I. Exosomal microRNAs are novel circulating biomarkers in cigarette, waterpipe smokers, E-cigarette users and dual smokers. BMC Med Genomics 2020; 13(1): 128. http://dx.doi.org/10.1186/s12920-020-00748-3 PMID: 32912198

[14] Frati G, Forte M, di Nonno F, *et al.* Inhibition of miR-155 attenuates detrimental vascular effects of tobacco cigarette smoking. J Am Heart Assoc 2020; 9(24): e017000. http://dx.doi.org/10.1161/JAHA.120.017000 PMID: 33317369

[15] Albuquerque EX, Pereira EFR, Alkondon M, Rogers SW. Mammalian nicotinic acetylcholine receptors: From structure to function. Physiol Rev 2009; 89(1): 73-120. http://dx.doi.org/10.1152/physrev.00015.2008 PMID: 19126755

[16] Jensen K, Nizamutdinov D, Guerrier M, Afroze S, Dostal D, Glaser S. General mechanisms of nicotine-induced fibrogenesis. FASEB J 2012; 26(12): 4778-87. http://dx.doi.org/10.1096/fj.12-206458 PMID: 22906950

[17] Shan H, Zhang Y, Lu Y, *et al.* Downregulation of miR-133 and miR-590 contributes to nicotine-induced atrial remodelling in canines. Cardiovasc Res 2009; 83(3): 465-72. http://dx.doi.org/10.1093/cvr/cvp130 PMID: 19398468

[18] Vang A, Clements RT, Chichger H, *et al.* Effect of α7 nicotinic acetylcholine receptor activation on cardiac fibroblasts: A mechanism underlying RV fibrosis associated with cigarette smoke exposure. Am J Physiol Lung Cell Mol Physiol 2017; 312(5): L748-59. http://dx.doi.org/10.1152/ajplung.00393.2016 PMID: 28258105

[19] Porrello ER, Mahmoud AI, Simpson E, *et al.* Transient regenerative potential of the neonatal mouse heart. Science 2011; 331(6020): 1078-80. http://dx.doi.org/10.1126/science.1200708 PMID: 21350179

[20] Chuang TD, Ansari A, Yu C, *et al.* Mechanism underlying increased cardiac extracellular matrix deposition in perinatal nicotine-exposed offspring. Am J Physiol Heart Circ Physiol 2020; 319(3): H651-60. http://dx.doi.org/10.1152/ajpheart.00021.2020 PMID: 32795172

[21] van Rooij E, Sutherland LB, Thatcher JE, *et al.* Dysregulation of microRNAs after myocardial infarction reveals a role of miR-29 in cardiac fibrosis. Proc Natl Acad Sci USA 2008; 105(35): 13027-32. http://dx.doi.org/10.1073/pnas.0805038105 PMID: 18723672

[22] Farbehi N, Patrick R, Dorison A, *et al.* Single-cell expression profiling reveals dynamic flux of cardiac stromal, vascular and immune cells in health and injury. eLife 2019; 8: e43882. http://dx.doi.org/10.7554/eLife.43882 PMID: 30912746

[23] White AJ, Smith RR, Matsushita S, *et al.* Intrinsic cardiac origin of human cardiosphere-derived cells. Eur Heart J 2013; 34(1): 68-75. http://dx.doi.org/10.1093/eurheartj/ehr172 PMID: 21659438

[24] Liang G, He Z, Chen Y, *et al.* Existence of multiple organ aging in animal model of emphysema induced by cigarette smoke extract. Tob Induc Dis 2022; 20: 02. http://dx.doi.org/10.18332/tid/143853 PMID: 35087358

[25] Plikus M v, Wang X, Sinha S, Forte E, Thompson SM, Herzog EL, *et al.* Fibroblasts: Origins, definitions, and functions in health and disease Cell 2021; 184: 3852-72. http://dx.doi.org/10.1016/j.cell.2021.06.024

[26] Mayyas F, Aldawod H, Alzoubi KH, Khabour O, Shihadeh A, Eissenberg T. Comparison of the cardiac effects of electronic cigarette aerosol exposure with waterpipe and combustible cigarette smoke exposure in rats. Life Sci 2020; 251: 117644. http://dx.doi.org/10.1016/j.lfs.2020.117644 PMID: 32259604

[27] Crotty Alexander LE, Drummond CA, Hepokoski M, *et al.* Chronic inhalation of e-cigarette vapor containing nicotine disrupts airway barrier function and induces systemic inflammation and multiorgan fibrosis in mice. Am J Physiol Regul Integr Comp Physiol 2018; 314(6): R834-47. http://dx.doi.org/10.1152/ajpregu.00270.2017 PMID: 29384700

[28] Rammah M, Dandachi F, Salman R, Shihadeh A, El-Sabban M. *In vitro* effects of waterpipe smoke condensate on endothelial cell function: A potential risk factor for vascular disease. Toxicol Lett 2013; 219(2): 133-42. http://dx.doi.org/10.1016/j.toxlet.2013.02.015 PMID: 23454654

[29] Ejaz S, Insan-ud-din , Ashraf M, Nawaz M, Lim CW, Kim B. Cigarette smoke condensate and total particulate matter severely disrupts physiological angiogenesis. Food Chem Toxicol 2009; 47(3): 601-14. http://dx.doi.org/10.1016/j.fct.2008.12.018 PMID: 19138718

[30] Pei C, Wang X, Lin Y, Fang L, Meng S. Inhibition of galectin-3 alleviates cigarette smoke extract-induced autophagy and dysfunction in endothelial progenitor cells. Oxid Med Cell Longev 2019; 2019: 7252943. http://dx.doi.org/10.1155/2019/7252943 PMID: 31737173

[31] He ZH, Chen Y, Chen P, *et al.* Cigarette smoke extract affects methylation status and attenuates Sca-1 expression of mouse endothelial progenitor cell *in vitro*. Tob Induc Dis 2021; 19: 08. http://dx.doi.org/10.18332/tid/131625 PMID: 33542680

[32] Antoniewicz L, Bosson JA, Kuhl J, *et al.* Electronic cigarettes increase endothelial progenitor cells in the blood of healthy volunteers. Atherosclerosis 2016; 255: 179-85. http://dx.doi.org/10.1016/j.atherosclerosis.2016.09.064 PMID: 27693003

[33] Mobarrez F, Antoniewicz L, Bosson JA, Kuhl J, Pisetsky DS, Lundbäck M. The effects of smoking on levels of endothelial progenitor cells and microparticles in the blood of healthy volunteers. PLoS One 2014; 9(2): e90314.

http://dx.doi.org/10.1371/journal.pone.0090314 PMID: 24587320

[34] Pagano F, Picchio V, Chimenti I, *et al.* On the road to regeneration: "Tools" and "routes" towards efficient cardiac cell therapy for ischemic cardiomyopathy. Curr Cardiol Rep 2019; 21(11): 133. http://dx.doi.org/10.1007/s11886-019-1226-5 PMID: 31673821

[35] 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 Transl Med 2017; 6(9): 1815-21. http://dx.doi.org/10.1002/sctm.17-0060 PMID: 28696009

[36] Barwinska D, Traktuev DO, Merfeld-Clauss S, *et al.* Cigarette smoking impairs adipose stromal cell vasculogenic activity and abrogates potency to ameliorate ischemia. Stem Cells 2018; 36(6): 856-67. http://dx.doi.org/10.1002/stem.2813 PMID: 29589872

[37] Liang Y, Li X, Zhang Y, *et al.* Induced pluripotent stem cells-derived mesenchymal stem cells attenuate cigarette smoke-induced cardiac remodeling and dysfunction. Front Pharmacol 2017; 8: 501. http://dx.doi.org/10.3389/fphar.2017.00501 PMID: 28804458

[38] Yeung E, Fukunishi T, Bai Y, *et al.* Cardiac regeneration using human-induced pluripotent stem cell-derived biomaterial-free 3D-bioprinted cardiac patch *in vivo*. J Tissue Eng Regen Med 2019; 13(11): 2031-9. http://dx.doi.org/10.1002/term.2954 PMID: 31408915

[39] Palpant NJ, Hofsteen P, Pabon L, Reinecke H, Murry CE. Cardiac development in zebrafish and human embryonic stem cells is inhibited by exposure to tobacco cigarettes and e-cigarettes. PLoS One 2015; 10(5): e0126259. http://dx.doi.org/10.1371/journal.pone.0126259 PMID: 25978043

[40] Behar RZ, Wang Y, Talbot P. Comparing the cytotoxicity of electronic cigarette fluids, aerosols and solvents. Tob Control 2018; 27(3): 325-33. http://dx.doi.org/10.1136/tobaccocontrol-2016-053472 PMID: 28596276

[41] Sumanasekera WK, Tran DM, Sumanasekera TU, Le N, Dao HT, Rokosh GD. Cigarette smoke adversely affects functions and cell membrane integrity in c-kit+ cardiac stem cells. Cell Biol Toxicol 2014; 30(2): 113-25. http://dx.doi.org/10.1007/s10565-014-9273-6 PMID: 24633465

[42] Sumanasekera WK, Dao HT, Shekhovtsova V, *et al.* The mechanistic role of oxidative stress in cigarette smoke-induced cardiac stem cell dysfunction and prevention by ascorbic acid. Cell Biol Toxicol 2019; 35(2): 111-27. http://dx.doi.org/10.1007/s10565-018-9437-x PMID: 30006751

[43] Navas-Acien A, Martinez-Morata I, Hilpert M, Rule A, Shimbo D, LoIacono NJ. Early cardiovascular risk in E-cigarette users: The potential role of metals. Curr Environ Health Rep 2020; 7(4): 353-61. http://dx.doi.org/10.1007/s40572-020-00297-y PMID: 33242201