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# The effects of cigarette smoking and nicotine on the therapeutic potential of mesenchymal stem cells

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## Abstract

Due to their immunoregulatory properties and capacity for multi-lineage differentiation, mesenchymal stem cells (MSCs) have been used as new therapeutic agents in regenerative medicine. Numerous lifestyle habits and behavioral risk factors may modulate metabolic and cell growth signaling pathways in MSCs, affecting their phenotype and function. Accordingly, identification of these factors and minimization of their influence on viability and function of transplanted MSCs may greatly contribute to their better therapeutic efficacy. A large number of experimental and clinical studies have demonstrated the detrimental effects of cigarette smoke and nicotine on proliferation, homing, chondrogenic and osteogenic differentiation of MSCs. Cigarette smoke down-regulates expression of chemokine receptors and modulates activity of anti-oxidative enzymes in MSCs, while nicotine impairs synthesis of transcriptional factors that regulate the cell cycle, metabolism, migration, chondrogenesis and osteogenesis. In this review article, we summarize current knowledge about molecular mechanisms that are responsible for cigarette smoke and nicotine-dependent modulation of MSCs' therapeutic potential.

**Key words**: cigarette smoking; nicotine; mesenchymal stem cells; therapeutic potential; tissue regeneration

## List of abbreviations

Embryonic stem cells (ESCs); mesenchymal stem cells (MSCs); adipose tissue (AT); dental pulp (DP); amniotic fluid (AF); umbilical cord (UC); transforming growth factor-β (TGF-β); hepatic growth factor (HGF); nitric oxide (NO); indolamine 2,3-dioxygenase (IDO); interleukin (IL); receptor antagonist (IL-1Ra), hemeoxygenase-1 (HO-1), prostaglandin E2 (PGE2); vascular endothelial growth factor (VEGF); placental growth factor (PGF); basic fibroblast growth factor (bFGF); platelet-derived growth factor (PDGF); major histocompatibility complex (MHC); cigarette smoke extract (CSE); chronic obstructive pulmonary disease (COPD); AT-derived MSCs (AT-MSCs); tobacco-specific nitrosamines (TSNAs); polyaromatic hydrocarbons (PAHs); volatile organic compounds (VOCs); N'-nitrosonornicotine (NNN); 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK); nicotinic acetylcholine (nAChRs); β-adrenergic (β-ARs); BM-derived MSCs (BM-MSCs); stromal cell-derived factor (SDF)-1; endothelial cells (ECs); periodontal ligament-derived MSCs (PL-MSCs); endometrial epithelial cells (EECs); Runt-related transcription factor 2 (RUNX2); Collagen Type I Alpha 1 Chain (COL1A1), Collagen Type I Alpha 2 Chain (COL1A2), osteocalcin (OCN); cartilage-specific proteoglycan core protein (CSPCP); Wharton's jelly-derived MSCs (WJ-MSCs); natural killer (NK), natural killer T (NKT) cells; cytotoxic T lymphocytes (CTLs); tumor necrosis factor alpha (TNF-α); interferon gamma (IFN-γ); lung-resident MSCs (LR-MSCs); reactive oxygen species (ROS); electronic cigarettes (e-cigarettes); heated tobacco products (HTPs).

## Introduction

The discovery of self-renewable, pluripotent, embryonic stem cells (ESCs) with the capacity for differentiation into cells of ectodermal, mesodermal, and endodermal germ layers raised tremendous expectations in scientific community, that ESC-based therapy could provide enhanced regeneration of injured tissues and an efficient treatment of un-curable degenerative diseases (Martin, 1981). However, transplanted ESCs generated teratomas in experimental animals which raised serious safety concerns about their potential clinical use (Volarevic et al., 2018). Additionally, isolation of ESCs requires the destruction of human embryos, and ethical issues were the main obstacles for their use *in vitro* (Volarevic et al., 2018). Therefore, during the past three decades, many research groups focused their attention on identification of alternative, multipotent adult tissue-derived stem cells which would circumvent ESC-related ethical and safety issues.

Immediately after Friedenstein and colleagues discovered spindle-shaped, fibroblast-like, self-renewable, bone-marrow-derived mesenchymal stem cells (MSCs) with multi-lineage differentiation potential (Friedenstein et al., 1970), many other researchers identified and isolated MSCs from various adult and fetal tissues (adipose tissue (AT), dental pulp (DP), synovium, amniotic fluid (AF), umbilical cord (UC), blood, bone, muscle, skin, tonsil) (Álvarez-Viejo, 2020). MSCs spontaneously differentiate into the cells of mesodermal origin (adipocytes, chondrocytes and osteocytes) and, under specific culture conditions, may generate cells of neuro-ectodermal (neurons, astrocytes, and oligodendrocytes) or endodermal origin (hepatocytes) (Fan et al., 2020). In addition to their great differentiation potential, MSCs possess potent immuno- and angio-modulatory characteristics (Harrell et al., 2019). By producing large numbers of immunomodulatory (transforming growth factor-β (TGF-β), hepatic growth factor (HGF), nitric oxide (NO), indolamine 2,3-dioxygenase (IDO), interleukin (IL)-10, IL-6, IL-1 receptor antagonist (IL-1Ra), hemeoxygenase-1 (HO-1), prostaglandin E2 (PGE2)) and pro-angiogenic factors (vascular endothelial growth factor (VEGF), angiopoietin-1, placental growth factor (PGF), HGF, basic fibroblast growth factor (bFGF), TGF-β, platelet-derived growth factor (PDGF), IL-6), MSCs regulate immune response and vasculogenesis, crucially contributing to the enhanced repair and regeneration of injured tissues (Harrell et al., 2021).

Among stem cells, MSCs possess several unique properties which enable their clinical use (Harrell et al.,

2021). MSCs are characterized by easy acquisition, rapid growth rate, maintenance of differentiation potential after repeated passages *in vitro*, homing to the sites of injury, efficient engraftment in inflamed tissues after systemic administration, minor immunological rejection due to the low surface expression of major histocompatibility complex (MHC) antigens, and long-term survival in MHC-mismatched recipients (Harrell et al., 2021). Accordingly, the interest in MSC-based therapy of inflammatory and degenerative diseases has been progressively growing in the past two decades. An outstanding scientific production resulted in a large amount of experimental and clinical evidence demonstrating the beneficial effects of MSCs in suppression of detrimental immune response, attenuation of on-going inflammation and enhanced tissue regeneration (Harrell et al., 2021).

However, it should be noted that many factors (donor age, habits, medical history, tissue source, culture conditions) may modulate phenotype and function of MSCs, affecting their therapeutic efficacy (Song et al., 2020). Identification of these factors and minimization of their influence on fate and function of transplanted MSCs may crucially contribute to their better therapeutic efficacy (Song et al., 2020). Accordingly, prior to MSC isolation, donors of MSCs are screened for genetic and/or infectious agents that could alter biological function and therapeutic potential of MSCs (Harrell et al., 2019). Nevertheless, several other, usually overlooked, risk factors may also contribute to less efficacious clinical outcomes in MSC-treated patients. One of them is exposure to cigarette smoke, or one of its primary components, nicotine. Several lines of evidence indicate that whole cigarette smoke extract (CSE) and nicotine negatively impact differentiation potential and regenerative capacity of MSCs. In this review article, we summarize current knowledge about molecular mechanisms that are responsible for CSE and nicotine-dependent modulation of MSC characteristics (proliferation, migration, and differentiation). An extensive literature review was carried out in July 2021 across several databases (MEDLINE, EMBASE, Google Scholar, ClinicalTrials.gov). Keywords used in the selection were: “mesenchymal stem cells”, “differentiation”, “immunomodulation”, “migration”, “therapeutic potential”, “tissue repair and regeneration”, “cigarette smoke”, “cigarette smoke extract”, “nicotine”. All journals were considered, and an initial search retrieved 78 articles. The abstracts of all these articles were subsequently reviewed by two of the authors (CRH and VV) to check their relevance to the

subject of this manuscript. Eligible studies had to delineate the effects of CSE and nicotine on phenotype and function of MSCs, and their findings were analyzed in this review.

## The impact of cigarette smoke and nicotine on viability and proliferative capacity of MSCs

There are two main routes of exposure to cigarette smoke (Schaal and Chellappan, 2014). Inhalation of mainstream or "first-hand" smoke is the dominant form of exposure for active smokers where nicotine and smoke-related toxic chemicals are deposited in the oral cavity or inhaled in the lungs, from where, after adsorption, they are carried via the bloodstream throughout the body (Schaal and Chellappan, 2014). Smoke produced from the lit end of the cigarette is considered sidestream or "second-hand" smoke and has detrimental effects on lung function of both active smokers and innocent bystanders (Schaal and Chellappan, 2014). Accordingly, epidemiological studies indicated that exposure to "second-hand" smoke significantly increases incidence of malignant and cardiovascular diseases in adults and may be the main risk factor for the development of sudden infant death syndrome, asthma and chronic obstructive pulmonary disease (COPD) in children (Schaal and Chellappan, 2014; Hajdusianek et al., 2021).

Additionally, cigarette smoking impairs metabolic and cell growth signaling pathways in CSE-exposed cells, including MSCs (Wahl et al., 2016). Wahl and colleagues used a metabolic activity assay to determine the viability of AT-derived MSCs (AT-MSCs) after exposure to the different concentrations of CSE (Wahl et al., 2016). Changes in metabolism or cell viability were not observed in AT-MSCs which were 48 hours exposed to low (<1%) concentrations of CSE. However, almost all AT-MSCs exposed to 5% or 10% of CSE were metabolically inactive or non-viable (Wahl et al., 2016). Despite the fact that results obtained by Wahl and coworkers strongly suggested that cigarette smoking, under specific circumstances, negatively affected metabolism and viability of MSCs, the impact of specific compounds of CSE on growth and proliferation of MSCs was not analyzed in that study.

Cigarette smoke contains more than 4000 chemicals grouped in 17 classes (amides, imides, lactams, carboxylic acids, lactones, esters, aldehydes, ketones, alcohols, phenols, amines, N-heterocycles,

hydrocarbons, nitriles, anhydrides, carbohydrates, and ethers) (Richter et al., 2008). During the curing, processing, fermentation, and combustion of tobacco, nearly 60 carcinogens are being formed, including tobacco-specific nitrosamines (TSNAs), polyaromatic hydrocarbons (PAHs), and volatile organic compounds (VOCs). Among them, TSNAs (N'-nitrosonornicotine (NNN) and 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK) are considered as the most hazardous, life-threatening carcinogens due to their detrimental effects on DNA structure (Richter et al., 2008). By forming DNA adducts and by generating reactive oxygen species (ROS) in target cells, these compounds cause mutations in tumor-suppressor or proto-oncogenes, affecting cell cycle and proliferation [9]. Inhalation of cigarette smoke-related VOCs (benzene, toluene, styrene, 2-butanone, etc.) is associated with a variety of short- and long-term health effects (irritation and malignant transformation of target cells in the eyes and respiratory tract) (Schaal and Chellappan, 2014). Tobacco-specific PAHs (naphthalene, acenaphthylene, benzo[e]pyrene, etc.) usually act as co-carcinogens that strengthen the carcinogenicity of TSNAs and VOCs by enhancing their detrimental effects on parenchymal and stem cells within the oral cavity and the lungs (Schaal and Chellappan, 2014).

The main reason for the carcinogenic effects of TSNAs, PAHs and VOCs are related to their detrimental effects on signaling pathways which regulate cell growth and proliferation [13]. Tobacco-specific nitrosamines diffuse through the cell membrane in a receptor-independent manner, while nicotine (the addictive component of tobacco smoke) and its oncogenic derivatives (NNK and NNN) activate many intracellular signaling pathways through the binding to the nicotinic acetylcholine (nAChRs) and β-adrenergic (β-ARs) cell surface receptors (Alkam and Nabeshima, 2019). The homomeric α7 and heteromeric α4β2 subunits of nAChR have been considered as the primary receptors that enable nicotine-based effects on the cell cycle (Alkam and Nabeshima, 2019). Interestingly, expression of both subunits significantly increases in response to tobacco smoke-specific nitrosamine or nicotine (Alkam and Nabeshima, 2019).

Since MSCs express the functional α7 subunit of nAChR, exposure to nicotine significantly increases intracellular calcium levels in MSCs, leading to the activation of calcium-dependent intracellular pathways and alteration of the cell cycle in nicotine-exposed MSCs (Schraufstatter et al., 2009). Nitrosamine/nicotine: nAChRs interaction activates MAPK/ERK, PI3K/AKT and JAK/STAT signaling cascades, facilitating

increased growth and proliferation of MSCs (Schraufstatter et al., 2009). Additionally, through binding to nAChRs, nicotine activates Src kinase in a β-arrestin-1 dependent manner and inactivates Rb protein, resulting in the enhanced expression of E2F1-regulated proliferative genes (Hoogduijn et al., 2009). Thus, by affecting MAPK/ERK, PI3K/AKT, JAK/STAT and Src/E2F1 signaling pathways, the nicotine/nAChR axis regulates growth and proliferation of MSCs (Li et al., 2017). Shen and colleagues noticed a significantly increased proliferation rate of BM-derived MSCs (BM-MSCs) which were exposed to 50–100 nM nicotine for 7 days (Shen et al., 2013). Kim and coworkers showed that 1μM to 100μM nicotine was not sufficient to induce proliferation of alveolar BM-MSCs and demonstrated that only nicotine in concentration between 1 and 2 mM may increase their proliferation, while higher concentrations of nicotine (>5mM) were not able to induce optimal proliferation of BM-MSCs (Kim et al., 2012). Similar findings were obtained by Zeng et al. (Zeng et al., 2014) who observed a dose-dependent decrease in proliferation and increased apoptosis of human UC-derived MSCs previously exposed to 0.5-1.5 mg/mL nicotine (3–9 mM). The detrimental effects of nicotine on proliferation of MSCs could be explained by the fact that nicotine at high concentration (>3mM) induced oxidative stress and caused G0 cell cycle arrest in rapid proliferative cells (Li et al.,. 2017). Accordingly, a significantly increased rate of proliferation was noticed in nicotine-exposed MSCs which were cultured in growth medium supplemented with antioxidant vitamin C (Shen et al., 2013).

Importantly, a significant 2.5-fold decrease in proliferation rate has been observed in MSCs isolated from chronic smokers compared with non-smoker derived MSCs. Furthermore, decreased capacity for proliferation was noticed in MSCs after 3–5 passages, suggesting that the negative impact of chronic nicotine exposure was permanent and lasted for several generations (Ng et al., 2015). These findings strongly suggest that a full history of nicotine usage should be precisely determined for all potential donors of MSCs in order to avoid transplantation of MSCs with attenuated proliferative capacity.

## The effects of cigarette smoking and nicotine on migratory properties of MSCs

MSCs constitutively express several chemokine receptors (CXCR4, CXCR7, CCR1, CCR4, CCR7, CCR9, CCR10, CXCR5, and CXCR6) that enable their homing to injured and inflamed tissues (Ullah et al., 2019). CXCR4 and CXCR7, which bind to the stromal cell-derived factor (SDF)-1 on endothelial cells (ECs) and CD44, which catches onto the selectins, are largely responsible for MSC rolling along the vasculature wall (Ullah et al., 2019). CXCR4 and CXCR7 in SDF-1-dependent manner, enhance affinity of MSC-expressing VLA-4 to EC-expressing VCAM1, enabling firm adhesion of MSCs to ECs. Precisely, CXCR4/CXCR7-dependent conformational changes of MSC-expressing SDF-1 recruit signaling molecules talin and kindlin to the cytoplasmic domain of VLA-4 and cause conformational changes that increase the affinity of VLA-4 for VCAM1 integrin (Ullah et al., 2019).

MSCs that do not optimally express chemokine receptors have attenuated functional properties and should not be used in clinical settings (Ullah et al., 2019). Therefore, all hazards which impair expression of chemokine receptors and negatively affect the homing and therapeutic potential of MSCs should be precisely determined in order to avoid clinical use of MSCs which were obtained from donors exposed to these hazards. Cigarette smoking negatively affects homing capacity of MSCs and attenuates their regenerative and therapeutic potential (Wahl et al., 2016). CSE at concentration above 5% significantly attenuated expression of chemokine receptors on AT-MSCs and impaired their homing properties (Wahl et al., 2016). Interestingly, migratory properties of AT-MSCs were not altered when AT-MSCs were exposed to the low concentration (less than 1%) of CSE, suggesting that CSE affects the homing capacity of MSCs in a dose-dependent manner (Wahl et al., 2016).

In a similar manner to CSE, nicotine is also able to modify the migratory properties of MSCs (Schraufstatter et al., 2009). Nicotine-based modulation of MSC homing is dependent on activation of the α7 subunit of nAChR [14, 23]. Schraufstatter and colleagues noticed remarkably improved migratory potential of BM-MSCs when these cells were cultured in nicotine (1mM)-enriched chemokine free medium (Schraufstatter et al., 2009). By binding to the α7 subunit of nAChR, nicotine, even at low concentration, efficiently activated calcium-dependent intracellular pathways in MSCs and enhanced their homing characteristics (Schraufstatter

et al., 2009). However, in chemokine-supplemented MSC cultures, nicotine, at the same concentration, decreased migration of MSCs. These detrimental effects were completely reversed by bungarotoxin (α7 homopolymer antagonist), confirming the importance of α7 nAChR for nicotine-based effects on MSC migration (Schraufstatter et al., 2009).

Similar results were obtained by Ng and colleagues who showed that nicotine (at a concentration of 1mM) retarded the locomotion of periodontal ligament-derived MSCs (PL-MSCs) (Ng et al., 2015). The average speed and the travelled distance of nicotine-treated PL-MSCs were significantly lower compared to nicotine-untreated PL-MSCs (Ng et al., 2013). Based on these findings, Ng and colleagues hypothesized that increased incidence of destructive periodontal diseases and delayed healing of periodontal tissue in cigarette smokers could be a consequence of altered migratory and regenerative properties of smoker PL-MSCs (Ng et al., 2015). The results obtained in a wound closure assay confirmed this hypothesis. Smoker PL-MSCs migrated 12% slower than those isolated from non-smokers and wound closure potential of PL-MSCs was significantly reduced by cigarette smoking (Ng et al., 2015). An impaired migration of smoker PL-MSCs was based on the irreversible nicotine-induced down-regulation of focal adhesion kinase in PL-MSCs (Ng et al., 2015).

In line with these findings, increased infertility among female smokers might be explained by the detrimental effects of cigarette smoking on homing of uterine MSCs (Zhou et al., 2011). Under physiological conditions, endometrial epithelial cells (EECs) produce growth factors and immunoregulatory molecules which provide an optimal environment for implantation and early gestation (Yoshinaga, 2018). Accordingly, maintenance of optimal number of EECs is vitally important for female fertility (Saha et al., 2021). Upon uterine injury or inflammation, chemokines, released by injured EECs, attract endogenous MSCs which, through the secretion of immunomodulatory factors or through the transdifferentiation in EECs, contribute to the enhanced repair and regeneration of injured uterine tissue (Calle et al., 2021; Saha et al., 2021). As was recently documented by Zhou and colleagues (Zhou et al., 2011), exposure to cigarette smoke significantly reduced recruitment of intravenously injected BM-MSCs in the endometrium of experimental rats. The total number of exogenously administered MSCs decreased by 68% in the smoke exposed animals compared to those that were exposed to smoke free air (Zhou et al., 2011). In addition, cigarette smoke completely diminished MSC-dependent

generation of prolactin-producing cells *in vitro* and attenuated capacity of MSCs to generate functional EECs *in vivo*. The overall number of cytokeratin-expressing MSC-derived EECs decreased by 84% in the mice exposed to cigarette smoke compared to the mice of the control group (Zhou et al., 2011).

## The effects of cigarette smoke and nicotine on differentiation potential of MSCs

Due to their ability for spontaneous differentiation into osteocytes and chondrocytes MSCs have been explored as new therapeutic agents in regenerative medicine, particularly in the therapy of metabolic bone diseases (Murena et al., 2014) and in the cell-based healing of severe fractures (Huang et al., 2015).

It is well known that cigarette smoking impairs osteogenesis and bone remodeling (Al-Bashaireh, 2018). Accordingly, smokers are more prone to osteoporosis and bone fractures than non-smokers (Ward and Klesges, 2001). Several research groups demonstrated that the detrimental effects of cigarette smoke and nicotine on osteogenic differentiation of MSCs were mainly responsible for impaired osteogenesis, observed in cigarette consumers (Ng et al., 2013; Zhou et al., 2013; Ng et al., 2015; Aspera-Werz et al., 2018). Ng and colleagues observed significantly lower expression of alkaline phosphatase, a decrease in calcium accumulation and an altered expression of miRNA-1305 in MSCs obtained from smokers' PL-MSCs (Ng et al., 2013). Since miRNA-1305 controls activity of Runt-related transcription factor 2 (RUNX2) that regulates osteoblast differentiation, Ng et al. hypothesized that an impaired osteogenic potential of smokers' MSCs was a consequence of detrimental effects of cigarettes smoke on the miR-1305/RUNX2 axis (Ng et al., 2013). Similarly to cigarette smoke, nicotine also altered activity of miR-1305 and negatively affected osteogenic differentiation of MSCs (Ng et al., 2015). Significantly down-regulated expression of RUNX2 and several other osteogenesis-related genes (Collagen Type I Alpha 1 Chain (COL1A1), Collagen Type I Alpha 2 Chain (COL1A2), osteocalcin (OCN)) was observed in BM-MSCs and PDL-MSCs after exposure to 1mM nicotine (Ng et al., 2015). Zhou et al. revealed that the effects of nicotine on osteogenic differentiation of MSCs were dose-dependent and were elicited by the binding of nicotine to the α7 subunit of nAChRs (Zhou et al., 2013). Since nicotine:nAChRs signaling suppressed activity of catalase and glutathione reductase in MSCs, Aspera-Werz and colleagues suggested that disruption of the anti-oxidative system in nicotine-exposed MSCs was

largely responsible for their reduced osteogenic potential (Aspera-Werz et al., 2018).

It is well known that the structure and strength of cartilage is weakened after continuous and long-term exposure to cigarette smoke (Abate et al., 2013). Cigarette smoke inhibits proliferation of chondrocytes and negatively affects cartilage metabolism by down-regulating expression of COL1A1 (Chen et al., 2020). COL1A1 is highly expressed in MSC-derived chondrocytes, playing important roles in MSC-based repair and regeneration of injured cartilage (Le et al., 2020). Wahl and coworkers demonstrated the detrimental effects of cigarette smoke on chondrogenic differentiation of MSCs (Wahl et al., 2016). An exposure to 0.5% of CSE significantly altered expression of cartilage-specific proteoglycan core protein (CSPCP) and chondrogenic transcription factor SOX9 in MSCs (Wahl et al., 2016). Since CSE-exposed MSCs were characterized by down-regulated expression of COL1A1, yhe deleterious effects of cigarette smoke on chondrogenic potential of MSCs might be the main reason for greater cartilage loss and delayed healing of injured cartilage, which were frequently observed in cigarette smokers (Abate et al., 2013; Wahl et al., 2016).

In line with these findings are findings obtained by Deng and colleagues (Deng et al., 2012) and Yang and coworkers (Yang et al., 2017) who found that nicotine suppressed the chondrogenic differentiation potential of BM-MSCs and Wharton's jelly-derived MSCs (WJ-MSCs). Nicotine (5 μM) down-regulated expression of chondrogenic-related genes (SOX9, COL2A1, aggrecan) and impaired the synthesis of proteoglycans in WJ-MSCs (Yang et al., 2017). Yang and colleagues suggested that the deleterious effect of nicotine on chondrogenic potential of WJ-MSCs was a consequence of nicotine-induced suppression of SOX9-driven chondrogenesis (Yang et al., 2017). SOX9 is the transcription factor which controls the synthesis of type II collagen and aggrecan (Shi et al., 2015). The binding of nicotine to the α7 subunit of nAChR induced activation of voltage-dependent Ca2+ channels and altered intracellular concentration of Ca2+ in MSCs (Wahl et al., 2016). As suggested by Yang and colleagues, changes in Ca2+ levels led to the down-regulated expression of SOX9 and impaired chondrogenic differentiation of WJ-MSCs (Yang et al., 2017).

Opposite to these findings were results obtained by Roux and colleagues who noticed dose-dependent positive effects of nicotine on chondrogenic potential of AT-MSCs (Roux et al., 2013). Significantly increased synthesis of type II collagen and elevated aggrecan mRNA levels observed in nicotine-exposed AT-MSCs

suggested that local application of nicotine (at a concentration of $10^{-5}$M) might represent a promising approach for enhancing chondrogenic differentiation capacity of MSCs in cell-based cartilage tissue engineering (Roux et al., 2013). The high variability of findings obtained by different research groups could be explained by the diverse origin of MSCs and by the different concentrations of nicotine to which MSCs were exposed (Deng et al., 2012; Roux et al., 2013; Yang et al., 2017). MSCs obtained from different tissues (BM, AT and WJ) shared many morphological and phenotypic characteristics but differ in their capacity for proliferation and differentiation (Hass et al., 2011). Accordingly, up-coming studies should define the exact molecular mechanisms which are responsible for nicotine-induced effects on chondrogenic differentiation of tissue-specific MSCs. For this purpose, the exact concentration of nicotine to which MSCs are exposed in particular tissues should be determined and afterwards used *in vitro* for the treatment of tissue-specific MSCs. Only in this manner, will the molecular mechanisms responsible for nicotine-induced effects on chondrogenic differentiation of MSCs be completely understood.

Despite the fact that cigarette smoke significantly altered MSCs' capacity for osteogenic and chondrogenic differentiation (Ng et al., 2013, Roux et al., 2013), CSE-exposed MSCs did not show variations in adipogenic differentiation (Wahl et al., 2016), indicating that signaling pathways which governed adipogenic differentiation of MSCs were not significantly affected by cigarette smoke.

## Detrimental effects of cigarette smoke on immunomodulatory capacity of MSCs

Upon engraftment in an inflammatory microenvironment, MSCs obtain an immunosuppressive phenotype and, in juxtacrine and paracrine manner, attenuate antigen-presenting function of inflammatory dendritic cells (DCs) and macrophages, suppress cytotoxic properties of natural killer (NK), natural killer T (NKT) cells and cytotoxic T lymphocytes (CTLs), reduce proliferation of autoreactive T and B cells, inhibit production of pro-inflammatory cytokines (tumor necrosis factor alpha (TNF-α), IL-1β, IL-18, interferon gamma (IFN-γ), IL-17, IL-22, IL-23) in neutrophils, monocytes and lymphocytes, induce expansion of immunosuppressive T regulatory cells and enhance secretion of anti-inflammatory cytokines (IL-10, TGF-β and IL-35) in tolerogenic DCs, alternatively activated macrophages and Tregs (Gazdic et al., 2015). Due to their

immunomodulatory capacity, MSCs have been used as new therapeutic agents in the therapy of acute and chronic inflammatory diseases (Volarevic et al., 2017). Recently, Cruz and colleagues identified cigarette smoking as an important behavioral risk factor which significantly attenuated immunomodulatory and therapeutic potential of MSCs (Cruz et al., 2019). By analyzing phenotype and function of lung-resident MSCs (LR-MSCs) which were obtained from current and former smokers suffering from COPD, Cruz and colleagues demonstrated that cigarette smoking significantly attenuates immunomodulatory capacity of LR-MSCs in COPD patients (Cruz et al., 2019). Since transplantation of MSCs and their secretome efficiently alleviates COPD in mice by suppressing detrimental T cell-driven lung inflammation (Harrell et al., 2020), the effects of cigarette smoke on LR-MSC-based inhibition of T cells were analyzed. CSE-exposed LR-MSCs were not capable of optimally suppressing proliferation of CD4+T helper and CD8+ CTLs and failed to inhibit production of inflammatory cytokines in activated T lymphocytes *in vitro* (Cruz et al., 2019). As hypothesized by Cruz and colleagues, long-term exposure to tobacco smoke induces increased synthesis of reactive oxygen species (ROS) and reduced activity of anti-oxidative enzymes in LR-MSCs (Cruz et al., 2019). CSE-induced oxidative stress enhances expression of inflammation-related genes and induce generation of pro-inflammatory phenotype in MSCs (Wahl et al., 2016). Pro-inflammatory MSCs are characterized by enhanced production of inflammatory cytokines (TNF-α, IL-1β, IFN-γ), by reduced synthesis of immunosuppressive IL-10, IL-35 and TGF-β and by reduced capacity for suppression of activated T cells (Gazdic et al., 2015). Interestingly, immunomodulatory properties of COPD patients' LR-MSCs were only impaired in current smokers while immunoregulatory function of former smokers' LR-MSCs were similar to those observed in healthy controls (Cruz et al., 2019). These findings suggest that the detrimental effects of cigarette smoke on immunosuppressive function of LR-MSCs were not irreversible and that the immunosuppressive potential of LR-MSCs could be almost completely restored after smoking cessation (Cruz et al., 2019).

## Conclusions and future directions

Cigarette smoking negatively affects proliferation, migratory properties, chondrogenic and osteogenic differentiation of MSCs by down-regulating expression of chemokine receptors and by modulating the activity of anti-oxidative enzymes, metabolic and cell growth signaling pathways in MSCs (Ng et al., 2013; Wahl et al., 2016). Through the binding to the α7 subunit of nAChR, nicotine activates calcium-dependent intracellular pathways in MSCs and impairs synthesis of transcriptional factors that regulate cell cycle, metabolism, migration, chondrogenesis and osteogenesis (Schraufstatter et al., 2009; Shen et al., 2013; Yang et al., 2017). Although the therapeutic potential of MSCs in the treatment of inflammatory diseases mainly relies on their immunomodulatory characteristics (Volarevic et al., 2017), the effects of cigarette smoking and nicotine on the immunoregulatory properties of MSCs are not completely understood. Until now, only one study has described the effects of CSE on MSC-dependent suppression of T cells (Cruz et al., 2019), while cigarette smoke-induced changes in MSCs' capacity for modulation of innate immune cells and B cells are still unrevealed. Also, forthcoming studies should reveal the effects of nicotine on MSC-dependent regulation of cellular and humoral immunity, since the impact of the nicotine: nAChR axis on the immunosuppressive properties of MSCsis completely unknown.

Finally, despite the fact that a large number of people, particularly ex-smokers, frequently use electronic cigarettes (e-cigarettes) and heated tobacco products (HTPs) (Akiyama and Sherwood, 2021), the effects of these nicotine delivery systems on the viability and functional properties of MSCs have not been investigated yet. Accordingly, future studies should analyze changes in intracellular pathways of e-cigarettes and HTPs-exposed MSCs to provide evidence about their impact on the therapeutic potential of MSCs.

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## References


Abate M., Vanni D., Pantalone A. and Salini V. (2013). Cigarette smoking and musculoskeletal disorders. Muscles Ligaments Tendons J. 3, 63-69.

Akiyama Y and Sherwood N. (2021). Systematic review of biomarker findings from clinical studies of electronic cigarettes and heated tobacco products. Toxicol. Rep. 8, 282-294.

Al-Bashaireh A.M., Haddad L.G., Weaver M., Chengguo X., Kelly D.L. and Yoon S. (2018). The Effect of Tobacco Smoking on Bone Mass: An Overview of Pathophysiologic Mechanisms. J. Osteoporos. 2018, 1206235.

Alkam T and Nabeshima T. (2019). Molecular mechanisms for nicotine intoxication. Neurochem. Int. 125, 117-126.

Álvarez-Viejo M. (2020). Mesenchymal stem cells from different sources and their derived exosomes: A pre-clinical perspective. World J. Stem Cells 12, 100-109.

Aspera-Werz RH., Ehnert S., Heid D., Zhu S., Chen T., Braun B., Sreekumar V., Arnscheidt C., Nussler AK. (2018). Nicotine and Cotinine Inhibit Catalase and Glutathione Reductase Activity Contributing to the Impaired Osteogenesis of SCP-1 Cells Exposed to Cigarette Smoke. Oxid. Med. Cell Longev. 2018, 3172480.

Calle A., Toribio V., Yáñez-Mó M. and Ramírez M.Á. (2021). Embryonic Trophectoderm Secretomics Reveals Chemotactic Migration and Intercellular Communication of Endometrial and Circulating MSCs in Embryonic Implantation. Int. J. Mol Sci. 22, 5638.

Chen T., Ehnert S., Tendulkar G., Zhu S., Arnscheidt C., Aspera-Werz R.H. and Nussler A.K. (2020). Primary Human Chondrocytes Affected by Cigarette Smoke-Therapeutic Challenges. Int. J. Mol. Sci. 21, 1901.

Cruz T., López-Giraldo A., Noell G., Guirao A., Casas-Recasens S., Garcia T., Saco A., Sellares J., Agustí A., Faner R. (2019). Smoking Impairs the Immunomodulatory Capacity of Lung-Resident Mesenchymal Stem Cells in Chronic Obstructive Pulmonary Disease. Am. J. Respir. Cell Mol. Biol. 61, 575-583.

Deng Y., Li T.Q., Yan Y.E., Magdalou J., Wang H. and Chen L.B. (2012). Effect of nicotine on chondrogenic differentiation of rat bone marrow mesenchymal stem cells in alginate bead culture. Biomed. Mater Eng. 22, 81-87.

Fan X.L., Zhang Y., Li X. and Fu Q.L. (2020). Mechanisms underlying the protective effects of mesenchymal stem cell-based therapy. Cell Mol. Life Sci. 1, 1-24.

Friedenstein A.J., Chailakhjan R.K. and Lalykina K.S. (1970). The development of fibroblast colonies in monolayer cultures of guinea-pig bone marrow and spleen cells Cell Tissue Kinet. 3, 393-403.

Gazdic M., Volarevic V., Arsenijevic N. and Stojkovic M. (2015). Mesenchymal stem cells: a friend or foe in immune-mediated diseases. Stem Cell Rev. Rep. 11, 280-287.

Hajdusianek W., Żórawik A., Waliszewska-Prosół M., Poręba R. and Gać P. (2021) Tobacco and Nervous System Development and Function-New Findings 2015-2020. Brain Sci. 11, 797.

Harrell C.R., Djonov V. and Volarevic V. (2021). The Cross-Talk between Mesenchymal Stem Cells and Immune Cells in Tissue Repair and Regeneration. Int. J. Mol. Sci. 22, 2472.

Harrell C.R., Fellabaum C., Jovicic N., Djonov V., Arsenijevic N. and Volarevic V. (2019). Molecular Mechanisms Responsible for Therapeutic Potential of Mesenchymal Stem Cell-Derived Secretome. Cells, 8, 467.

Harrell C.R., Miloradovic D., Sadikot R., Fellabaum C., Markovic BS., Miloradovic D., Acovic A., Djonov V., Arsenijevic N. and Volarevic V. (2020). Molecular and Cellular Mechanisms Responsible for Beneficial Effects of Mesenchymal Stem Cell-Derived Product "Exo-d-MAPPS" in Attenuation of Chronic Airway Inflammation. Anal. Cell Pathol. (Amst), 2020, 3153891.

Hass R., Kasper C., Böhm S. and Jacobs R. (2011). Different populations and sources of human mesenchymal stem cells (MSC): A comparison of adult and neonatal tissue-derived MSC. Cell Commun. Signal 9,12.

Hoogduijn M.J., Cheng A. and Genever P.G. (2009). Functional nicotinic and muscarinic receptors on mesenchymal stem cells. Stem Cells Dev. 18, 103-112.

Huang S., Xu L., Zhang Y., Sun Y. and Li G. (2015). Systemic and Local Administration of Allogeneic Bone Marrow-Derived Mesenchymal Stem Cells Promotes Fracture Healing in Rats. Cell Transplant. 24,

2643-2655.

Kim B.S., Kim S.J., Kim H.J., Lee S.J., Park Y.J., Lee J. and You H.K. (2012). Effects of nicotine on proliferation and osteoblast differentiation in human alveolar bone marrow-derived mesenchymal stem cells. Life Sci. 90, 109-115.

Le H., Xu W., Zhuang X., Chang F., Wang Y. and Ding J. (2020). Mesenchymal stem cells for cartilage regeneration. J. Tissue Eng. 11, 2041731420943839.

Li T., Zhang J., Zhang J., Zhang N., Zeng Y., Tang S., Tao Z., Qu X., Jia J., Zhu W., Sun X. and Chen H. (2017). Nicotine-enhanced stemness and epithelial-mesenchymal transition of human umbilical cord mesenchymal stem cells promote tumor formation and growth in nude mice. Oncotarget, 9, 591-606.

Martin GR. (1981). Isolation of a pluripotent cell line from early mouse embryos cultured in medium conditioned by teratocarcinoma stem cells. PNAS, 78, 7634-7638.

Murena L., Canton G., Vulcano E., Surace M.F. and Cherubino P. (2014). Treatment of humeral shaft aseptic nonunions in elderly patients with opposite structural allograft, BMP-7, and mesenchymal stem cells. Orthopedics 37, e201-206.

Ng T.K., Carballosa C.M., Pelaez D., Wong H.K., Choy K.W., Pang C.P. and Cheung H.S. (2013). Nicotine alters MicroRNA expression and hinders human adult stem cell regenerative potential. Stem Cells Dev. 22, 781-790.

Ng T.K., Huang L., Cao D., Yip Y.W., Tsang W.M., Yam G.H., Pang C.P. and Cheung H.S. (2015). Cigarette smoking hinders human periodontal ligament-derived stem cell proliferation, migration and differentiation potentials. Sci. Rep. 5, 7828.

Richter P., Pechacek T., Swahn M. and Wagman V. (2008). Reducing levels of toxic chemicals in cigarette smoke: a new Healthy People 2010 objective. Public Health Rep. 123, 30-38.

Roux C., Pisani D.F., Yahia H.B., Djedaini M., Beranger G.E., Chambard J.C., Ambrosetti D., Michiels J.F., Breuil V., Ailhaud G., Euller-Ziegler L. and Amri E.Z. (2013). Chondrogenic potential of stem cells derived from adipose tissue: a powerful pharmacological tool. Biochem Biophys. Res. Commun. 440, 786-791.

Saha S., Roy P., Corbitt C. and Kakar S.S. (2021). Application of Stem Cell Therapy for Infertility. Cells, 10, 1613.

Schaal C. and Chellappan S.P. (2014). Nicotine-mediated cell proliferation and tumor progression in smoking-related cancers. Mol. Cancer Res. 12, 14-23.

Schraufstatter I.U., DiScipio R.G., Khaldoyanidi S.K. (2009). Alpha 7 subunit of nAChR regulates migration of human mesenchymal stem cells. J. Stem Cells. 4, 203-215.

Shen Y., Liu H.X., Ying X.Z., Yang S.Z., Nie P.F., Cheng S.W., Wang W., Cheng X.J., Peng L. and Xu H.Z. (2013). Dose-dependent effects of nicotine on proliferation and differentiation of human bone marrow stromal cells and the antagonistic action of vitamin C. J. Cell Biochem. 114, 1720-1728.

Shi S., Wang C., Acton A.J., Eckert G.J and Trippel S.B. (2015). Role of sox9 in growth factor regulation of articular chondrocytes. J Cell Biochem, 116:1391-1400.

Song N., Scholtemeijer M., Shah K. (2020). Mesenchymal Stem Cell Immunomodulation: Mechanisms and Therapeutic Potential. Trends Pharmacol Sci, 41:653-664.

Ullah M., Liu DD., Thakor AS. (2019) Mesenchymal Stromal Cell Homing: Mechanisms and Strategies for Improvement. iScience, 15, 421-438.

Volarevic V., Gazdic M., Simovic Markovic B., Jovicic N., Djonov V. and Arsenijevic N. (2017). Mesenchymal stem cell-derived factors: Immuno-modulatory effects and therapeutic potential. Biofactors, 43, 633-644.

Volarevic V., Markovic B.S., Gazdic M., Volarevic A., Jovicic N., Arsenijevic N., Armstrong L., Djonov V., Lako M. and Stojkovic M. (2018). Ethical and safety issues of stem cell-based therapy. Int. J. Med. Sci. 15, 36-45.

Wahl E.A., Schenck T.L., Machens H.G. and Egaña J.T. (2016). Acute stimulation of mesenchymal stem cells with cigarette smoke extract affects their migration, differentiation, and paracrine potential. Sci. Rep. 6, 22957.

Ward K.D. and Klesges R.C. (2001). A meta-analysis of the effects of cigarette smoking on bone mineral density. Calcif. Tissue Int. 68, 259-270.

Yoshinaga K. (2018). A historical review of blastocyst implantation research. Biol. Reprod. 99, 175-195.

Zeng H.L., Qin Y.L., Chen H.Z., Bu Q.Q., Li Y., Zhong Q., Han X.A., Chen J., Yu P.X. and Liu GX. (2014). Effects of nicotine on proliferation and survival in human umbilical cord mesenchymal stem cells. J. Biochem. Mol. Toxicol. 28, 181-189.

Zhou Y., Gan Y. and Taylor H.S. (2011). Cigarette smoke inhibits recruitment of bone-marrow-derived stem cells to the uterus. Reprod. Toxicol. 31, 123-127.

Zhou Z., Li B., Dong Z., Liu F., Zhang Y., Yu Y., Shang F., Wu L., Wang X. and Jin Y. (2013). Nicotine deteriorates the osteogenic differentiation of periodontal ligament stem cells through α7 nicotinic acetylcholine receptor regulating Wnt pathway. PLoS One, 8:e83102.