



REVIEW ARTICLE

Hypoxia-preconditioning of human adipose-derived stem cells enhances cellular proliferation and angiogenesis: A systematic review

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ABSTRACT

Background: Human adipose-derived stem cells (hADSCs) have gained attention lately because of their ease of harvesting and ability to be substantially multiplied in laboratory cultures. Stem cells are usually cultured under atmospheric conditions; however, preconditioning stem cells under hypoxic conditions seems beneficial.

Aim: This systematic review aims to investigate the effect of hypoxia preconditioning and its impact on the proliferation and angiogenic capacity of the hADSCs.

Methods: We performed a systematic review by searching PubMed, Scopus, Embase, and Google Scholar databases from all years through March 22, 2021, following Preferred Reporting Items for Systematic Reviews and Meta-Analyses guidelines. Medical Subject Headings terms “adipose-derived stem cell,” “Hypoxia,” “cell proliferation,” and “angiogenesis” guided our search. Only articles written in English using experimental models comparing a preconditioned group against a control group of hADSCs with data on proliferation and angiogenic capacity were included.

Results: Our search yielded a total of 321 articles. 11 articles met our inclusion criteria and were ultimately included in this review. Two studies induced hypoxia using hypoxia-inducible factor-1 alpha stabilizing agents, while nine reached hypoxia by changing oxygen tension conditions around the cells. Four articles conducted *in-vivo* studies to correlate their *in-vitro* findings, which proved to be consistent. Although 1 article indicated cell proliferation inhibition with hypoxia preconditioning, the remaining 10 found enhanced proliferation in preconditioned groups compared to controls. All articles showed an enhanced angiogenic capacity of hADSCs after hypoxia preconditioning.

Conclusion: In this review, we found evidence to support hypoxia preconditioning of hADSCs before implantation. Benefits include enhanced cell proliferation with a faster population doubling rate and increased secretion of multiple angiogenic growth factors, enhancing angiogenesis capacity.

Relevance for Patients: Although regenerative therapy is a promising field of study and treatment in medicine, much is still unknown. The potential for angiogenic therapeutics with stem cells is high, but more so, if we discover ways to enhance their natural angiogenic properties. Procedures and pathologies alike require the assistance of angiogenic treatments to improve outcome, such is the case with skin grafts, muscle flaps, skin flaps, or myocardial infarction to mention a few. Enhanced angiogenic properties of stem cells may pave the way for better outcomes and results for patients.

1. Introduction

Multipotent stem cells are found in adult organs, with vast amounts residing in adipose tissue [1,2]. Multipotent mesenchymal stromal cells, or stem cells, are of great importance to researchers given their regenerative capacity and ability to be used in treatment for

various medical conditions [3,4]. Specifically, human adipose-derived stem cells (hADSCs) have garnered significant attention given their ease of harvesting and culturing with few ethical concerns [1,2,4]. hADSCs are capable of secreting a nutrient-rich secretome that contains multiple growth factors, cytokines, and chemokines, which enhance the microenvironment around them, improving the proliferation of surrounding cells [1]. Angiogenesis is upregulated by growth factors, such as vascular endothelial growth factor (VEGF), basic fibroblast growth factor (bFGF), and transforming growth factor-beta (TGF- β), all of which are known to be secreted by hADSCs [5,6].

Stem cells reside in a niche, which is defined as an anatomical area that contains a specialized microenvironment that provides the stem cells with the necessary components for self-renewal and multipotency [7-9]. The niche also serves as a regulator of stem cells through specific pathways and oxygen, the latter playing an essential part in stem cell differentiation and molecular response (Figure 1) [7,9,10]. Adaptation to low oxygen tension is mediated by multiple genes, which regulate angiogenesis, proliferation and survival, glucose, and iron metabolism (Figure 2) [11-15]. Because stem cell death on transplant into target tissue is a considerable complication of regenerative medicine, several attempts have been made to enhance cell survival and viability within the transplanted niche; scientists have recently started to manipulate cell cultures by exposing them to different oxygen tensions to improve cell survival with the intent of enhancing cell survival.

Given the clinical potential of regenerative medicine and to better understand the effect of oxygen tension on stem cells, this

study aims to report the outcome of hypoxic preconditioning on the capability of hADSC's to proliferate and produce angiogenesis. We hypothesize that adipose-derived stem cells cultured under hypoxic conditions will have increased proliferation rates and angiogenic capability as hypoxic culturing most resembles their cell niche.

2. Methods

2.1. Eligibility criteria

We searched for full-text articles of *in-vivo* and *in-vitro* experimental models comparing hypoxia versus normoxia preconditioning of hADSCs, which also included a comparison of angiogenic capacity and proliferation rate versus control groups. Articles written in a language other than English were excluded, given the risk of losing information in the translation. In addition, those that made no mention of stem cell origin or discussed cellular enhancement or mitigation by any external factor other than hypoxia were excluded, such as diabetes or other chronic conditions, given their ability to manipulate angiogenesis and proliferation.

2.2. Information sources and search strategy

A computerized search of the following databases was performed on March 22, 2021, by the first and second authors independently: PubMed (all years), Scopus (all years), Embase (all years), and Google Scholar (all years). The following Medical Subject Headings terms guided our search strategy: (“adipose-derived stem cell” OR “adipose tissue-derived mesenchymal stem cell” OR “adipose tissue-derived mesenchymal stromal cells”) AND “hypoxia” AND “cell proliferation” AND “angiogenesis.” Discordant papers were further discussed between authors before reaching a final agreement.

2.3. Study selection and data collection process

Each author independently performed the search and filtered the studies firstly by removing duplicates with the help of the computer program EndNote (Clarivate Analytics). Subsequently, the inclusion and exclusion criteria described above filtered titles and abstracts. Finally, the remaining studies were screened based on full-text readings (Figure 3).

2.4. Risk of bias assessment

The risk of bias in the included studies was assessed with the help of the Risk Of Bias in Non-randomized Studies of Interventions tool of the Cochrane Library. Illustrations of individualized bias and cross bias are shown in Figures 4 and 5.

3. Results

Of the 321 articles yielded by our search, a total of 11 studies were included in this review. Studies were found to either induce hypoxia through manipulating atmospheric conditions inside a closed chamber or using a hypoxia-inducible factor-1 alpha (HIF-1 α) stabilizer. In addition, studies assessed the proliferation

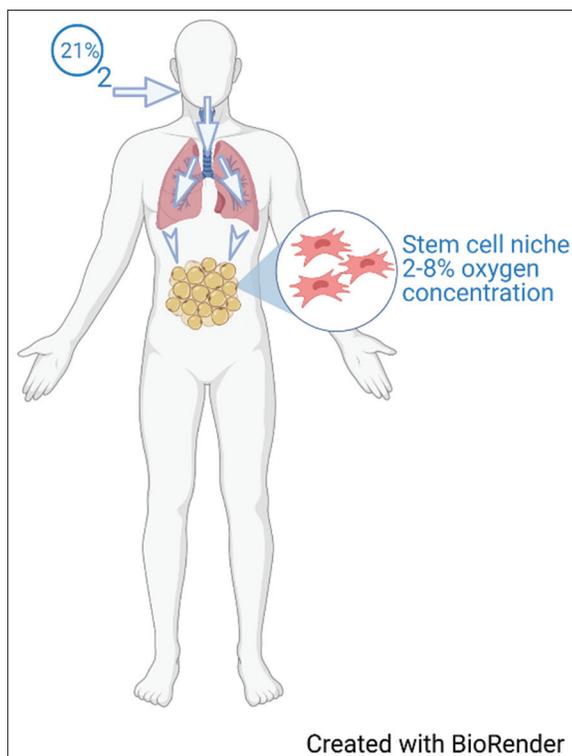


Figure 1. Oxygen concentration variability. Illustrates the difference in oxygen concentration in ambient air and as it reaches body tissues.

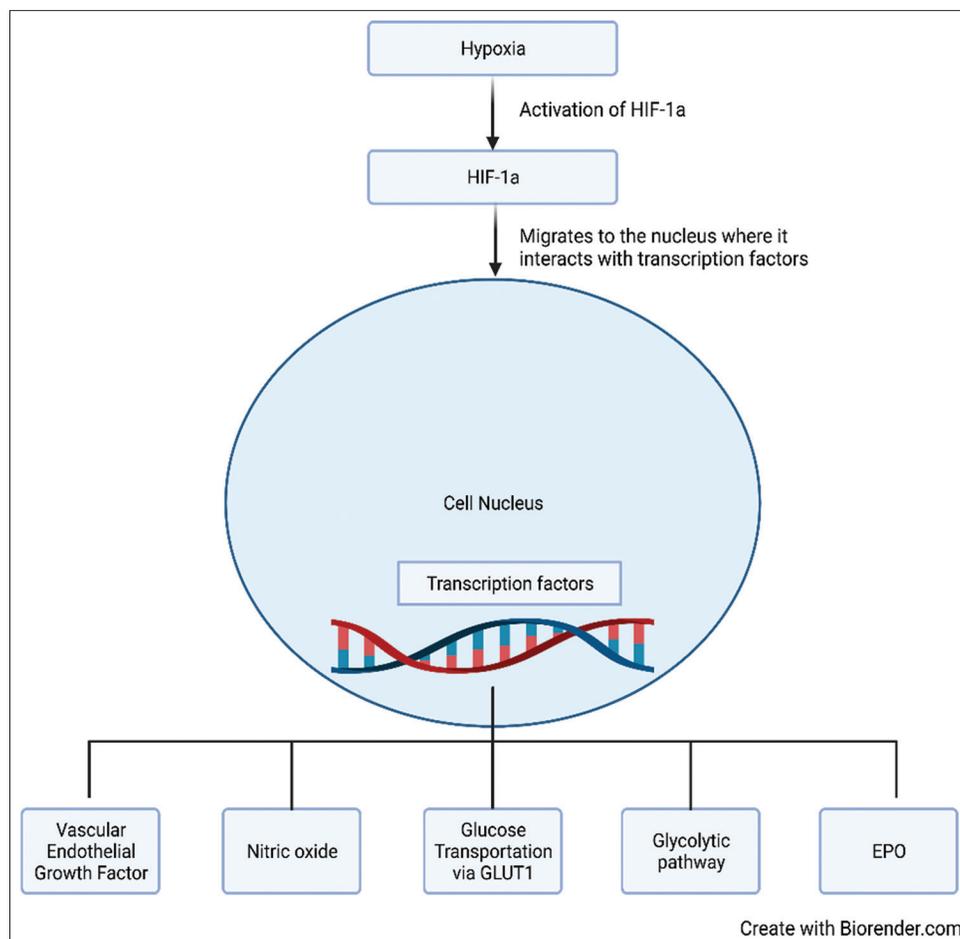


Figure 2. Hypoxia regulated genes. When a cell encounters hypoxic conditions, hypoxia inducible factor-1a is activated, this leads to its interaction with transcription factors within the nucleus which further leads to activation of downstream genes which help cells endure hypoxic conditions.

and/or angiogenic capacity of stem cells after hypoxic exposure compared to nonexposed cells. A summary of study outcomes is found in Table 1.

3.1. Proliferation

In 2009, Pilgaard *et al.* [16] found a higher apoptosis rate in hADSCs preconditioned with hypoxia; however, clonogenic precursor cells, also known as colony-forming unit fibroblasts, were preserved and displayed in higher quantity. In 2011, Weijers *et al.* [17] reported beneficial results as they found stem cell proliferation increased 1.7 fold in hypoxic cells versus normoxic cells ($P < 0.05$). Population doubling during the first passage supported this result in hypoxic versus normoxic conditions (6.3 ± 0.9 vs. 4.1 ± 0.9 , respectively; $P < 0.01$). They also reported that hypoxic preconditioning led to an increase in telomere length, with normoxic cells having telomeres that, on average, were 1.5-fold shorter ($P < 0.05$). Following these results, Valorani *et al.* [18] reported that incubation under hypoxic conditions accelerated cellular proliferation fivefold on day 14 ($P < 0.01$). They used annexin V and propidium iodide to confirm that hypoxia reduced cell death and raised stem cell viability ($P < 0.05$).

In 2013, Liu *et al.* [19] found that hypoxia exposure for 48 h led to a statistically significant higher proliferation rate from days 1 through 4 ($P < 0.05$). Chen *et al.* [20] took a different approach in 2017 by mimicking hypoxic conditions using the HIF-1a stabilizer dimethylxalylglycine (DMOG). When preconditioned cells were exposed to a hypoxic environment, these cells exhibited a higher survival rate both *in-vitro* ($P < 0.05$) and *in-vivo* ($P < 0.05$). Following these results, Almeria *et al.* [21] in 2019 cultured hypoxic cells for 6 days, reporting a higher proliferation rate. In addition, population doubling in hypoxic conditions was increased versus normoxic cells (3.0 ± 0.4 vs. 2.3 ± 0.5), respectively; ($P < 0.01$), along with cell density ($P < 0.01$). Most recently, Hwang *et al.* [22] reported increased proliferation of hADSCs after hypoxic preconditioning ($P < 0.05$).

3.2. Angiogenesis

In 2009, Pilgaard *et al.* [16] concluded that cells under hypoxic preconditioning had a higher concentration of angiogenic factors, thus crucial for treating ischemic conditions. Barros *et al.* [23] departed from their previous conclusions that indicated an inverse relationship between the age of donors and angiogenic capacity

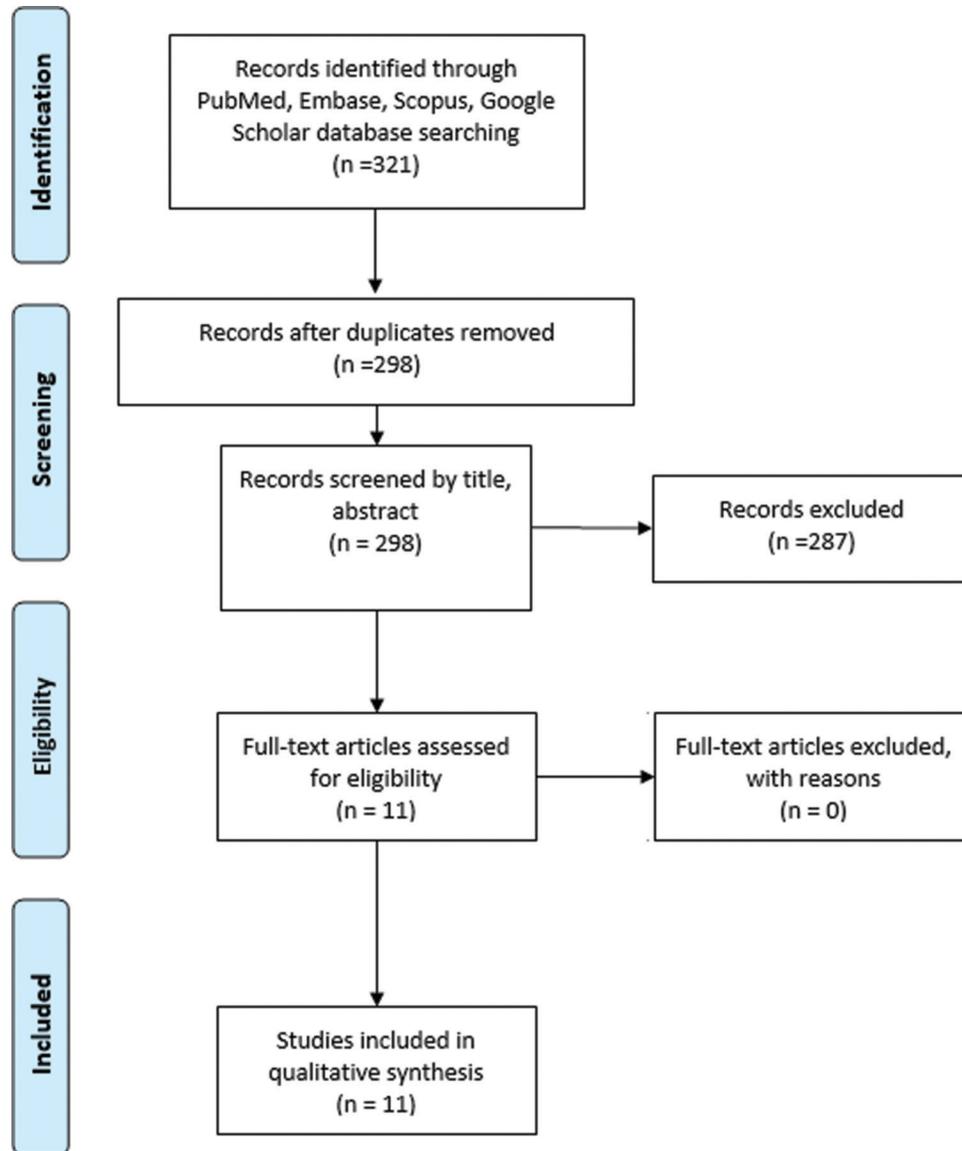


Figure 3. Study selection flow chart. Flow chart describing the study selection process according to Preferred Reporting Items for Systematic Reviews and Meta-Analyses guidelines.

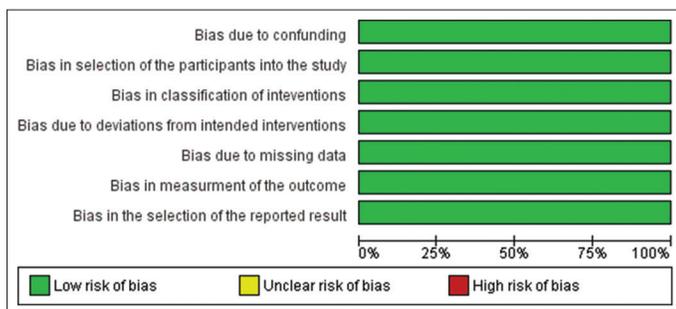


Figure 4. Risk of bias graph created with RevMan 5.3 following the Risk of Bias In Non-randomized Studies of Interventions -I guidelines of the Cochrane Library. Green indicates a low risk of bias, yellow indicates an unclear risk of bias, and red indicates a high risk of bias.

from hADSCs. They proposed that hypoxic preconditioning could reverse this age-related difference. Hypoxia increased the secretion of VEGF in cells from both young donors (20–35 years of age) and older donors (>50 years of age) ($P < 0.01$). However, cells from older donors had a more significant benefit from normoxic to hypoxic conditions ($P < 0.05$). Furthermore, Barros and colleagues tested this theory in a mouse model with an ischemic hindlimb. They concluded that hypoxic preconditioning only seemed beneficial in cells from older donors by increasing tissue perfusion, thus demonstrating that hypoxia may reverse aging impairment.

Liu *et al.* [19] in 2013 reported the upregulation of HIF-1 α with hypoxia preconditioning, which in turn stimulated the upregulation of VEGF and bFGF ($P < 0.05$). Furthermore, they said that VEGF

	Bias due to confounding	Bias in selection of the participants into the study	Bias in classification of interventions	Bias due to deviations from intended interventions	Bias due to missing data	Bias in measurement of the outcome	Bias in the selection of the reported result
Almeria, C. et al 2019	+	+	+	+	+	+	+
Barros, S.D. et al 2013	+	+	+	+	+	+	+
Chen C. et al 2017	+	+	+	+	+	+	+
Han, Y. et al 2019	+	+	+	+	+	+	+
Hwang, O.K. et al 2020	+	+	+	+	+	+	+
Liu, L. et al 2013	+	+	+	+	+	+	+
Oses, C. et al 2017	+	+	+	+	+	+	+
Pilgaard, L. et al 2009	+	+	+	+	+	+	+
Valorani, M.G. et al 2012	+	+	+	+	+	+	+
Weijers, E.M. et al 2011	+	+	+	+	+	+	+
Xue, C. et al 2018	+	+	+	+	+	+	+

Figure 5. Risk of bias summary created with RevMan 5.3 following the Risk of Bias In Non-randomized Studies of Interventions-I guidelines of the Cochrane Library. A low risk of bias is indicated by green color, yellow indicates an unclear risk of bias, and red indicates a high risk of bias.

and bFGF stimulated the formation of capillary-like structures in a Matrigel (Corning Life Sciences) assay of human umbilical vein endothelial cells (HUVECs) ($P < 0.05$). In 2017, Chen *et al.* [20] experimented with DMOG to mimic hypoxic conditions. They reported the effects of DMOG by tube formation assay in Matrigel. They concluded that the number of tubes, total tube length, and total branching points were significantly increased in the DMOG group ($P < 0.05$). In addition, a significant increase of HIF-1 α downstream factors, such as VEGF and VEGF receptor 2, was seen in preconditioned cells ($P < 0.05$).

In 2017, Oses *et al.* [24] experimented with another type of HIF-1 α stabilizer, deferoxamine (DFX). They concluded that DFX induced a dose-dependent rise of HIF-1 α ($P < 0.05$). In addition, DFX generated a significant increase in VEGF and angiopoietin 1 mRNA levels ($P < 0.05$) (Figure 6). To confirm these findings, Oses and colleagues tested their hypothesis that an increase of mRNA would translate to increased secretion of these factors; they were found to be significantly elevated ($P < 0.05$) (Figure 7).

Xue *et al.* [25] used exosomes derived from hypoxia-preconditioned cells and compared them to exosomes from nonpreconditioned cells. Matrigel tube-forming assay was conducted using HUVECs. Results showed that preconditioned exosomes significantly increased tube formation at 0.5, 2, and 4 h after administration of the exosomes ($P < 0.05$). In addition, the *Vash1* gene, an angiogenesis inhibitor, was significantly decreased

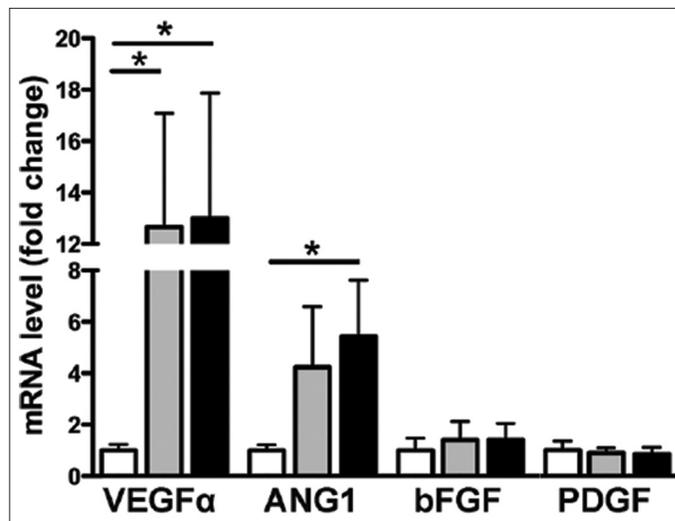


Figure 6. Deferoxamine preconditioning increases the expression levels of pro-angiogenic factors. Total RNA was obtained from MSCs exposed to 150 μ M DFX, 400 μ MDFX, or the vehicle for 48 h and subjected to quantitative reverse transcriptase \pm PCR analysis. White bars represent non-preconditioned MSCs, gray bars represent MSCs preconditioned with 150 μ M DFX, and black bars represent MSCs preconditioned with 400 μ MDFX. Data are shown as mean \pm SEM. n = 4 per experimental group (biological repeats). Experiments were repeated 3 times at the technical level. * $P < 0.05$. MSCs: Mesenchymal stem cells; DFX: Deferoxamine; PCR: Polymerase chain reaction; VEGF α : Vascular endothelial growth factor- α ; ANG-1: Angiopoietin-1; bFGF: Basic fibroblast growth factor; PDGF: Platelet-derived growth factor; SEM: Standard error of the mean.

in hypoxia-derived exosomes. Exosomes were implanted into mice subcutaneously and Matrigel to further explore these effects. Results showed that endothelial cells produced more tubular structures after 8 days when exposed to preconditioned-derived exosomes ($P < 0.05$). As seen in previous reports, VEGF expression was significantly increased ($P < 0.05$). In 2019, Almeria *et al.* [21] used a HUVEC angiogenesis assay to measure the angiogenic capacity of extracellular vehicles derived from hADSCs cultured under hypoxia compared to normoxia. Total tube length, the number of branches, branching points, and loops were significantly increased in the preconditioned group ($P < 0.01$).

In 2019, Han *et al.* [26] used exosomes secreted by hypoxia-preconditioned stem cells or normoxic cells to measure angiogenesis. Exosomes from preconditioned cells significantly increased the proliferation of HUVECs from 12 to 48 h after inoculation ($P < 0.05$). In addition, migration capacity from HUVECs was significantly increased from the preconditioned group ($P < 0.05$). Following the same pattern, the tube-like formation was increased ($P < 0.05$). The in-depth analysis revealed increased angiopoietin 1, VEGF, endothelial growth factor, and bFGF with their respective receptors ($P < 0.05$). Han and colleagues implemented a nude mice model to confirm these findings to perform fat grafting. Results demonstrated that exosomes from preconditioned stem cells dramatically increased

Table 1. Summary of studies that measured the effects of hypoxic preconditioning on cellular proliferation and angiogenic capacity

Author	Cells	Type of study	Number of cells seeded	Hypoxic model	Results
Pilgaard et al. [16], 2009 Denmark	hADSC	<i>In-vitro</i>	12 rows in a 96-well culture plate to a density of 1000 to 0.5 cells per well.	<i>XIvivo</i> hypoxic workbench/ Ambient, 15%, 10%, 5%, 1% O ₂).	<ul style="list-style-type: none"> • 1% and 5 oxygen may inhibit cell proliferation • Clonogenic precursor cells were preserved • Angiogenic factors were identified in hypoxic conditions
Weijers et al. [17], 2011 The netherlands	hADSC	<i>In-vitro</i>	3×10 ⁴ cells/cm ²	Hypoxic workstation, CO ₂ - and O ₂ -controlled, humidifier. 20% O ₂ , 5% CO ₂ , or 1% O ₂ , 5% CO ₂	<ul style="list-style-type: none"> • hADSC have increased proliferation in 1% O₂ • Reduced cell aging and preservation of stemness in low O₂%
Valorani et al. [18], 2012 United Kingdom	hADSC	<i>In-vitro</i>	1×10 ⁴ cells/cm ²	Hypoxia workstation (21% O ₂) or (2% O ₂) with 5% CO ₂	<ul style="list-style-type: none"> • Increased hADSC expansion and viability in low O₂%, • Decrease in apoptosis and necrosis in low O₂%
Barros et al. [23], 2013 France	hADSC	<i>In-vitro</i> <i>In-vivo</i>	4000 cell/cm ² 1×10 ⁶ cell/cm ²	Hypoxic incubator at 0.5% O ₂ or 21% O ₂ for 24 h	<ul style="list-style-type: none"> • hADSC enhanced <i>in-vivo</i> neovascularization • Age of the donor decreased angiogenic benefits • Hypoxic preconditioning reversed the adverse effect of aging
Liu et al. [19], 2013 China	hADSC	<i>In-vitro</i>	1500 cells were seeded in a 96-well plate	Tri-gas incubator containing 5% CO ₂ , 1% O ₂ , for 48 h.	<ul style="list-style-type: none"> • Preconditioned cells have increased proliferation • Preconditioning significantly increased mRNA levels of VEGF and bFGF • Preconditioned medium stimulated the formation of capillary-like structures
Chen et al. [20], 2017 China	hADSC	<i>In-vitro</i> <i>In-vivo</i>	1×10 ⁵ cells/well 1×10 ⁶ cells/well	DMOG at 50, 100 and 150 μmol/L and for 2, 4 and 7 days	<ul style="list-style-type: none"> • Preconditioned cells had a higher survival rate and lower death rate • 50% decrease in mitochondrial mass with reduction of ROS • Increased angiogenic capabilities via increased HIF-1α → VEGF, VEGF • Significantly promoted <i>In-vivo</i> survival of cells
Oses et al. [24], 2017 Chile	hADSC	<i>In-vitro</i>	7000 cells/cm ²	Cultured for 48 h in α-MEM without FBS with either 150 μM DFX, 400 μM DFX or double-distilled water (Control)	<ul style="list-style-type: none"> • Increased levels of HIF-1α • Upregulation of pro-angiogenic genes (VEGF-α, Angiopoietin) • Increased concentration of pro-angiogenic factors in the secretome of hADSC
Xue et al. [25], 2018 China	hADSC-derived exosomes	<i>In-vitro</i> <i>In-vivo</i>	2×10 ⁶ cells 100 mg/mL of Exosomes	1% O ₂ and 21% O ₂	<ul style="list-style-type: none"> • Preconditioned exosomes significantly improved tube forming (<i>in-vivo</i> and <i>In-vitro</i>) • Angiogenesis inhibitory gene Vash1 was significantly decreased • HIF-1α and VEGF were increased considerably 6 days after hypoxia stimulation • Angiopoietin and Flk1 were considerably increased
Almeria et al. [21], 2019 Austria	hADSC-derived extracellular vesicles	<i>In-vitro</i>	3000 cells/cm ²	21% or 5% O ₂ for 6 days	<ul style="list-style-type: none"> • Higher proliferation rate in preconditioned cells • Population doubling level and cell density were significantly higher in preconditioned cells • Total HUVECs length was significantly increased in preconditioned EV group
Han et al. [26], 2019 China	hADSC-derived exosomes	<i>In-vitro</i> <i>In-vivo</i>	5×10 ³ cells/cm ²	Tri gas incubator with O ₂ at 5% with 5% CO ₂ and balanced nitrogen.	<ul style="list-style-type: none"> • Preconditioned Exosomes enhanced proliferation, migration, and tube-forming capacity of HUVECs • VEGF, EGF, bFGF, angiopoietin-1 were significantly upregulated • Preconditioned group improved neovascularization <i>in-vivo</i> fat grafting
Hwang et al. [22], 2020 Republic of Korea	hADSC	<i>In-vitro</i>	2.5×10 ³ to 1×10 ⁴ per well.	Multi-gas incubator at 37°C, 5% CO ₂ , balanced nitrogen, and 1% O ₂	<ul style="list-style-type: none"> • Cells cultured at 1% O₂ showed significantly higher proliferation at 24 and 48 h • HIF-1α was increased by hypoxia • VEGF was expressed and secreted higher in preconditioned cells

hADSC: Human adipose-derived stem cell; mRNA: Messenger ribonucleic acid; VEGF: Vascular endothelial growth factor; bFGF: Basic fibroblast growth factor; DMOG: Dimethylxalylglycine; ROS: Reactive oxygen species; HIF-1α: Hypoxia-inducible factor -1α; A-mem: Alpha-minimum essential medium; FBS: Fetal bovine serum; DFX: Deferoxamine; HUVEC: Human umbilical vein endothelial cells; EV: Extracellular vesicles; EGF: Epidermal growth factor

neovascularization ($P < 0.05$). In addition, protein secretion of angiogenic growth factors was also significantly increased ($P < 0.05$).

Most recently, Hwang *et al.* [22] measured HIF-1 α protein secreted by preconditioned versus non preconditioned cells. HIF-1 α was increased in the preconditioned group ($P < 0.05$). In addition, upregulation of the VEGF gene and its secretion was reported with hypoxia ($P < 0.05$).

4. Discussion

Oxygen plays a crucial role in stem cells' proliferation and angiogenic capacities [7,27]. The partial pressure of oxygen decreases as it enters the body through the lungs and is transported through red blood cells. Ultimately, oxygen tension drops to 2%-8% in cellular niches [27]. Cell cultures that mimic these oxygen tensions benefit stem cells, survivability, proliferation, and angiogenic capacity.

Adaptation to low oxygen tension is mediated by multiple genes, which regulate angiogenesis, proliferation and survival, glucose, and iron metabolism [11]. Of these, HIF-1 α is the primary regulator of oxygen homeostasis and hypoxic adaptation [28]. Therefore, HIF-1 α can be described as having an inverse relationship with oxygen tension. Under normoxic

circumstances, HIF-1 α is regulated by a primary and secondary pathway (Figure 8) [28].

The primary pathway is via a family of prolyl hydroxylase enzymes, which ultimately targets HIF-1 α by ubiquitination leading to proteasomal degradation [28]. The secondary regulatory way acts by hydroxylation of asparagine residues by factor inhibiting HIF-1, preventing HIF-1 α from interacting within co-activators such as CREB-binding protein and E1A binding protein *p300* [28]. However, under hypoxic conditions, hydroxylase substrates like oxygen and iron become scarce, limiting HIF-1 α downregulation, which subsequently accumulates and translocates into the nucleus where it exerts its effects (Figure 9) [28]. Present studies have reported the contribution of hypoxia-induced HIF-1 α upregulation to angiogenesis by upregulation of genes such as VEGF and angiopoietin 1 and 2, among others [29,30]. Most cells, including hADSCs, secrete these growth factors as a response to hypoxia [31].

In this review, 9 of 11 studies reported hypoxia preconditioning by manipulating the atmospheric oxygen content in a closed chamber [16-19,21-23,25,26]. However, 1 study reported using DMOG as an alternative method [20]. DMOG is a prolyl hydroxylase inhibitor. It decreases HIF-1 α degradation, mimicking a hypoxic response (Figure 9) [32]. An additional study mimicked hypoxia using DFX [24]. DFX increases the concentration of HIF-1 α by chelating iron, which is a component necessary by hydroxylases to degrade HIF-1 α (Figure 9) [28,33].

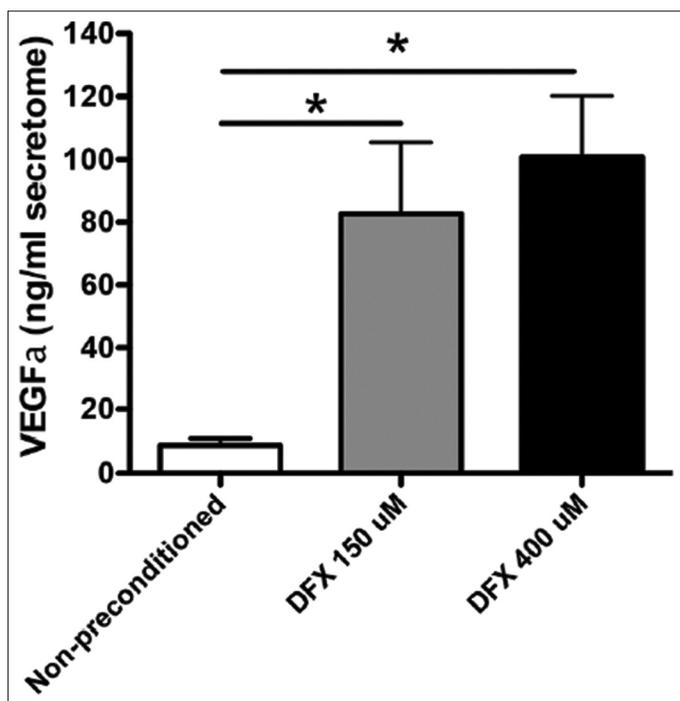


Figure 7. Deferoxamine preconditioning increases the secretion of pro-angiogenic factors. The secretomes obtained from MSCs were exposed to 150 μ MDFX, 400 μ MDFX, or the vehicle for 48 h. Quantification of VEGF α in MSC secretomes is shown. Data is presented as mean \pm SEM. N = 4 per experimental group (biological repeats). Experiments were repeated 3 times at the technical level. * $P < 0.05$. MSC: Mesenchymal stem cells; DFX: Deferoxamine; VEGF α : Vascular endothelial growth factor- α ; SEM: standard mean error.

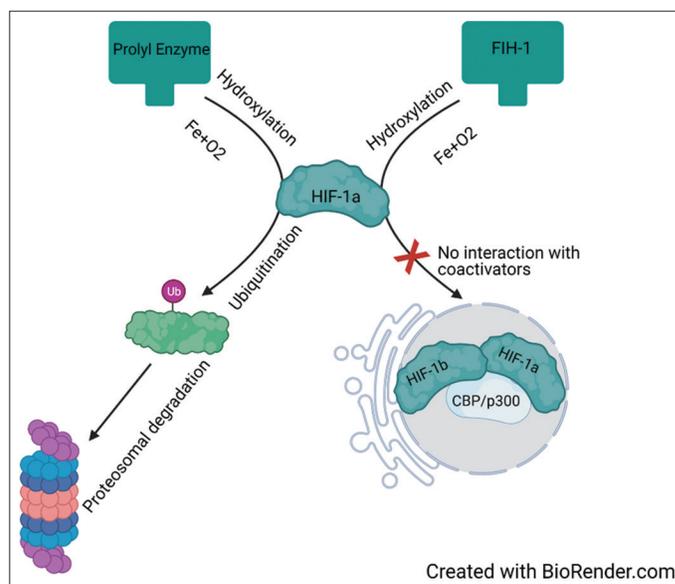


Figure 8. HIF-1 α degradation in normoxia. HIF-1 α is primarily degraded through two pathways. The first pathway utilizes prolyl hydroxylase enzymes to ubiquitinate HIF-1 α for proteasomal degradation. The second pathway acts by hydroxylation of asparagine residues by FIH-1, preventing HIF-1 α from interacting with co-activators (HIF-1b and CBP/p300) inside the nucleus. HIF-1 α , hypoxia-inducible factor-1 alpha; FIH, factor inhibiting HIF-1; HIF-1b, hypoxia-inducible factor-1; CBP, CREB-binding protein; p300, E1A binding protein *p300*.

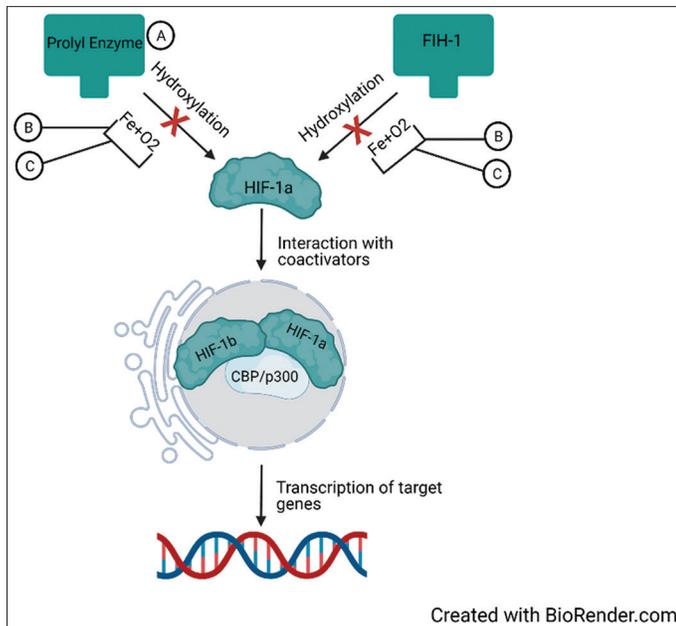


Figure 9. HIF-1a activation by hypoxia and stabilizing agents. (A) DMOG is a prolyl hydroxylase enzyme inhibitor, thus preventing ubiquitination and proteasomal degradation of HIF-1a. (B) Oxygen and Fe are required for hydroxylation. However, hypoxia decreases oxygen and Fe availability for these reactions, reducing HIF-1a degradation. (C) Deferoxamine is a Fe chelator, thus decreasing Fe availability for hydroxylation, decreasing HIF-1a degradation. As HIF-1 is accumulating, it translocates into the nucleus, where it interacts with its co-activators (HIF-1b and CBP/p300), engaging in transcriptional activity of downstream genes. HIF-1a, hypoxia-inducible factor-1 alpha; CBP, CREB-binding protein; p300, E1A binding protein *p300*; Fe, iron; DMOG, dimethylxalylglycine.

All three methods shared similar results regarding angiogenesis. All reported increased angiogenic capacity by measuring HIF-1a expression, mRNA expression of growth factors, or the quantity of secreted growth factors.

Three studies further investigated angiogenesis using HUVECs as a tube-forming assay [21,25,26]. Two of these studies used extracellular vesicles derived from hypoxia-preconditioned cells rather than actual stem cells [25,26]. Using extracellular vesicles did not affect the results as all reported increased angiogenesis.

Proliferation was also studied as an effect of hypoxic preconditioning. Although no study using DFX as a hypoxic agent reported proliferation, the other two models shared similar reports with increased proliferation. However, Pilgaard *et al.* [16] reported contrasting results, with proliferation being inhibited by hypoxia, as the 1% oxygen tension group displayed numerous detached cells along with cells with condensed nuclei indicating apoptosis. Moreover, they also found that low oxygen tension groups had a higher capacity to support the growth of cell colonies with higher colony-forming units. Although Pilgaard *et al.*'s study preserved clonogenic precursor cells and their proportions of colony-forming progenitors are within the range of previously published work, the inhibition of culture proliferation draws

questions regarding this finding. In addition, Pilgaard *et al.* did not adhere to the standard medium used in the culture of adipose-derived stem cells, which the authors mention as a possible confounding area for the proliferation of cells, as the medium has been shown to affect the proliferation rate. Hence, caution should be excised when analyzing these results. Further studies exploring the effects of different mediums on different cell lineages should be performed to standardize mediums and their consequences.

Four studies performed *in-vivo* testing to translate their *in-vitro* findings, with all four reporting increased angiogenesis [20,23,25,26]. Only Chen *et al.* [20] determined the proliferation of stem cells in an *in-vivo* model, increasing proliferation rate and survival.

Although regenerative medicine has great potential, it still takes its first steps into therapeutic means. Understanding the basic principles and effects of stem cells and regenerative medicine may catapult stem cells and their regenerative capabilities into everyday therapeutic use. This study successfully identified oxygen as an essential factor in the upregulation of angiogenic and proliferative capabilities in hADSCs. Further studies require testing hypoxic culturing on different stem cell lineages to determine the effect and compare it against the impact on adipose-derived stem cells. Gaining this knowledge may bridge the gap between low cell survival in the target tissue and increase the future therapeutic effect of stem cells.

5. Conclusion

Based on the included studies, growing cells in a hypoxic condition is a more natural process, thus culturing hADSCs under hypoxic conditions may have a beneficial role and positively impact regenerative medicine. Benefits include enhanced cell proliferation with a faster population doubling rate and increased secretion of multiple angiogenic growth factors, which improves the capacity for angiogenesis. Similar findings achieved between oxygen-dependent models and alternative models indicate that HIF-1a may have a critical role in enhancing angiogenesis and proliferation of HADSC.

6. Limitations

This study has several limitations. Since only studies published in English were included in this review, some studies may have been missed. Other limitations include the potential bias of misinterpreting data and results, and the study selection process, the latter being a potential source of bias common to systematic reviews.

7. Disclaimers

- Figures 1-5 and 8,9 were created using the BioRender.com license
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Conflict of Interest

The authors declare no conflict of interest

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