

# We are IntechOpen, the world's leading publisher of Open Access books Built by scientists, for scientists

6,900

Open access books available

185,000

International authors and editors

200M

Downloads

Our authors are among the

154

Countries delivered to

TOP 1%

most cited scientists

12.2%

Contributors from top 500 universities



WEB OF SCIENCE™

Selection of our books indexed in the Book Citation Index  
in Web of Science™ Core Collection (BKCI)

Interested in publishing with us?  
Contact [book.department@intechopen.com](mailto:book.department@intechopen.com)

Numbers displayed above are based on latest data collected.  
For more information visit [www.intechopen.com](http://www.intechopen.com)



# Hypoxic Preconditioning as a Strategy to Maintain the Regenerative Potential of Mesenchymal Stem Cells

*Bushra Bashir, Mahmood S. Choudhery and Ijaz Hussain*

## Abstract

Mesenchymal stem cells (MSCs) are non-hematopoietic cells with high proliferative potential and multi-lineage differentiation capacity. MSCs are promising therapeutic candidates for cell-based therapies, and hundreds of clinical trials have been registered using these cells. Potential of stem cells is compromised with the factors such as disease condition and age of donor. Therefore, taking the cells from such patients for autologous use may compromise the benefits of cell-based therapies. It is therefore required to enhance the potential of these cells before use in stem cell-based therapies. Optimization of culture conditions is preferred strategies to enhance the regenerative potential of cells before use. This chapter briefly overviews the benefits of hypoxic preconditioning of stem cells to enhance the regenerative potential of cells in terms of their survival, proliferation, and differentiation.

**Keywords:** mesenchymal stem cells, hypoxic preconditioning, regenerative potentials

## 1. Introduction

Clinical use of stem cells is rapidly growing in recent years because of their capabilities to repair and regenerate tissues and organs of body [1]. Stem cells have self-renewal potential and can differentiate into cells of multiple lineages under appropriate conditions. They can secrete a large number of bioactive molecules that are involved in repair and regeneration of damaged tissues and organs. Based on their potential, stem cells are classified as totipotent, pluripotent, multipotent and unipotent. Fertilized eggs (zygote) are totipotent as they can make cells of all three lineages and extra embryonic tissue such as placenta. Embryonic stem cells (ESC) are derived from inner cell mass of 5 days old embryos and are pluripotent as they have potential to differentiate into all cells and tissues of the body except placenta. MSC are the most widely used cells and they can be isolated from various adult body tissues (bone marrow, adipose tissue, articular cartilage, synovium, synovial fluid, dental pulp, etc.) as well as from neonatal stem cell sources (cord blood, cord tissue, placenta) [2]. Currently, hundreds of clinical trials have been registered using MSCs ([www.clinicaltrials.org](http://www.clinicaltrials.org)) for various conditions such as degenerative brain disorders, stroke, cardiac dysfunctions, myocardial ischemia, renal disorders, wound healing, diabetes etc. [3]. MSCs exert their effect either by

transdifferentiation into respective tissues and/or through their paracrine effects by releasing different cytokines and growth factors [4].

The potential of adult stem cells such as MSCs is severely compromised in vitro by culture conditions and by number of passages of the cells [5]. In addition, “disease conditions” and “age” of the donor also reduces regenerative functionality of MSCs and their clinical use for repair and regeneration of damaged and lost tissues [6]. It is pertinent to note that elderly population is the main portion of population for potential stem cell-based regenerative therapies. However, autologous use of cells from aged individuals seems not to provide the expected benefits of stem cell-based therapies due to age depleted function of stem cells from such patients [7]. It is therefore required to enhance the potential of stem cells before clinical use. Different strategies have been employed for this purpose such as growth factors preconditioning [8], mild heat shock [9], and glucose depletion [10].

Different pretreatment strategies have been employed to enhance the regenerative potential of stem cells, however; hypoxic preconditioning seems more effective for enhancing stem cell function because relatively low oxygen concentrations prevail in stem cells niches as compared to normoxic conditions. Hypoxia can be an effective strategy for enhancing the cells function because it can make the cells adapt external microenvironment, reduce oxidative stress, shift metabolism towards glycolysis, enhance proliferation, differentiation and maintain stemness, and improve their motility to tolerate the hypoxic preconditioning after transplantation [11].

## **2. Regenerative potential of stem cells is compromised with age**

### **2.1 Effect of donor age on regenerative potential of stem cells**

Autologous stem cell-based therapies seem promising for several diseases. As humans get older, stem cell function deteriorate like other cells of the body. Diseases, especially degenerative diseases generally affect elderly people and therefore using autologous cells for such patients may have practical concerns [12]. With aging, superoxide dismutase activity (SOD) declines [13]. Studies indicate that differentiation potential of stem cells is negatively related with age of donor [12, 14–16], and therefore cell potential to form osteoblasts [12, 15–17], cartilage [12, 14–16] and other cell types is compromised. Another important aspect for cell-based therapies, that is, proliferation is also adversely effected with increasing donor age. Choudhery et al. [15] indicated that the number of population doublings decreased while the time of population doublings increased for cells obtained from aged donors as compared to cells from the young donors. Similarly, number of the colony forming units, size of colonies and plating efficiency of aged cells decreases in vitro [13–17]. Overall, growth kinetics and differentiation potential of the cells are inversely proportion to the donor age [15–18].

### **2.2 Effect of in vitro passaging**

Cell-based therapies require large number of cells to get the favorable results in patients. For this purpose cells are expanded in vitro before use in most of clinical applications [12]. Leonard Hayflick in 1960 described that after a limited number of cell divisions the cells stop dividing. Cell morphology changes and they become enlarged and irregular in shape. The cells undergo a replicative senescence and this limited life span of cells is called as Hayflick's limit [19]. In this way replicative senescence limits the therapeutic potential of stem cells. The differentiation

potential of cells decreases with increasing number of in vitro passages. For example, bone marrow derived MSC showed decreased differentiation towards adipogenesis, osteogenesis and chondrogenesis at late passages as compared to initial passages [20]. In addition, the proliferative potential of MSCs decreases after long term passages [21]. Human Wharton's jelly-derived mesenchymal stem cells showed significant decrease in growth kinetics and differentiation when cultured for longer time as compared to the cells in initial passages [22]. Feline adipose tissue derived MSC showed a progressive decrease in pluripotency and proliferation over continuous passaging [23].

### 3. Oxygen levels vary in tissues

The structural and functional microenvironment in tissues where stem cells reside is known as stem cell niche described for the first time by Schofield [24]. A cell niche maintains the identity and functional characteristics of resident cells [25]. Important identified stem cell niches are in bone marrow [26], vascular vessels [27], liver [28] adult kidneys [29] intestine [30] endometrium [31], oral tissue [32], skin [33] and adipose tissue [34]. **Table 1** shows variable oxygen concentrations in some important stem cell niches.

Organ/tissues	PO2 values	References
Lungs (tracheal, bronchial, bronchiolar and alveolar epithelial cells)	13–14%	[35]
Subcutaneous	3–8%	[36]
Adipose tissue	3–10%	[37, 38]
Heart	2–6%	[39]
Brain (superficial cortex to deep white matter)	3–5%	[40]
Brain (hypothalamus, hippocampus, midbrain)	0.5%	[40]
Liver (parenchyma)	4–7%	[41]
Kidney (renal cortex)	4–9.5%	[42]
Kidney (medulla)	2%	[42]
Pancreas (exocrine)	2.7–4.6%	[43]
Pancreas (endogenous beta cell)	5–6%	[43]
Stomach	6–10%	[44]
Small intestine	2–5%	[45]
Lumen	3–6%	
Mucosa	5–9%	
Serosa		
Large intestine lumen and mucosa	0–2%	[46]
Serosa	4–6%	
Uterus	2.5%	[47]
Bone marrow	1–7%	[48]
Umbilical vein and arteries	2.4–3.8%	[35]
Blood	5–13%	[35]

**Table 1.**  
*Oxygen levels in different tissue in-vivo.*

It indicates that oxygen levels significantly vary in various tissues of the body and are significantly low as compared to normoxic oxygen concentration. Initially, oxygen level of 21% were adopted for in vitro culturing the cells based on normal oxygen conditions in environment, however, latter, it was realized that the cells grow better in vitro when cultured in those oxygen conditions which are representative of their respective niche.

Similarly, when cells are transplanted in the body, they face hypoxic in vivo environmental conditions [49, 50]. A large number of grafted cells die due to harsh in vivo environmental conditions (such as hypoxia) at transplanted site. The cell death due to hypoxic microenvironment is especially considerable for tissues that are not vascularized and or already injured or wounded [51].

4. How hypoxic preconditioning enhance stem cell function

4.1 Effect of hypoxia on gene transcription

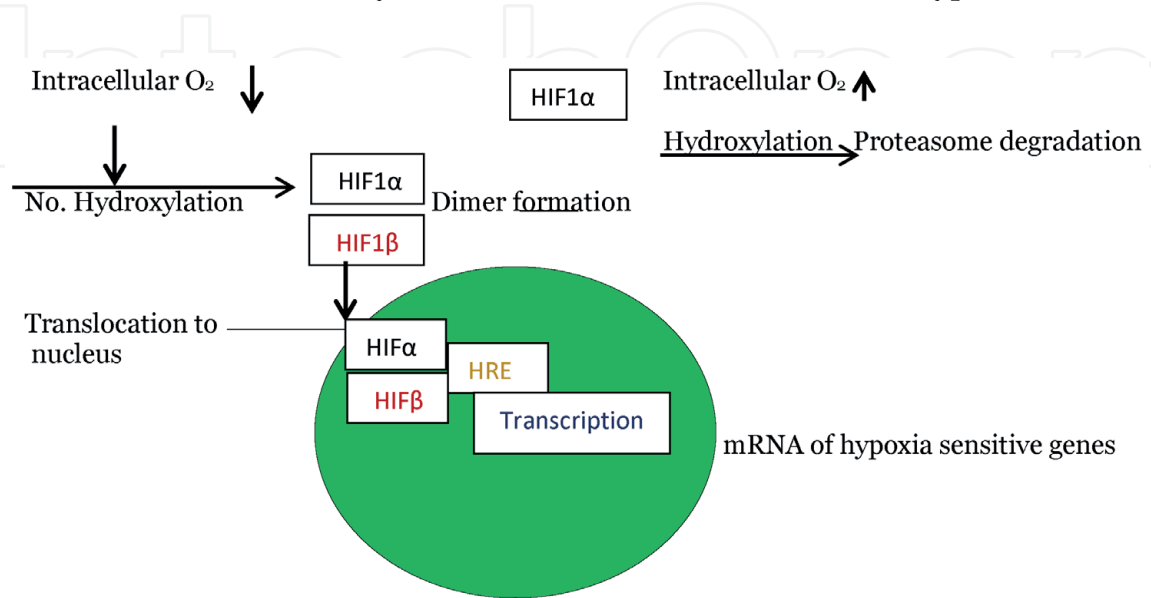
The survival and functioning of stem cells in hypoxic environment depends upon their metabolic switch controlled by hypoxia inducible factors (HIFs). HIFs are transcription factors that are present in eukaryotes. They have two subunits, that is, alpha( $\alpha$ ) and beta( $\beta$ ). Their  $\alpha$  subunit has three isoforms (HIF1-3). The post translational modification of  $\alpha$  subunit depends on hydroxylation which is oxygen dependent. When intracellular oxygen falls,  $\alpha$  subunit forms a stable  $\alpha/\beta$  dimer because hydroxylation did not occur. This dimer is transcriptionally active; it enters into the nucleus, binds to hypoxia response elements and initiates transcription of hypoxia sensitive genes [52, 53]. HIF3- $\alpha$  is the negative regulator of HIF1 and HIF2 (Figure 1).

Effect of hypoxia on HIF-  $\alpha$  is different for different types of stem cells

GRP78-Akt axis induced by HIF1 $\alpha$  is important in augmenting functions like proliferation and survival of MSCs under hypoxia [54].

4.2 Reactive oxygen species (ROS) and hypoxia

Reactive oxygen species are oxygen containing substances that are produced in cellular metabolism. They are detrimental to cellular functions. Hypoxic



**Figure 1.** Mechanism of HIF1 formation and transcription of hypoxia sensitive genes. HIF (hypoxia inducible factor), HRE (hypoxia response element).



preconditioning results in up regulation of Nuclear factor erythroid 2 related factor 2 (NRF2), which is a redox sensitive transcription factor involved in regulation of antioxidant genes [55]. Hypoxic preconditioning results in increased glycolytic metabolism and decreased tricarboxylic acid (TCA) cycle and oxidative phosphorylation. This mechanism leads to decreased mitochondrial ROS production and increase in the levels of antioxidant enzymes [52, 56]. Unbalanced redox homeostasis can cause stem cells aging and decreased proliferation. Hypoxic preconditioning augments redox metabolism [54] ROS acts paradoxically, at higher levels its cause's damage, and at lower levels it plays a role of signaling molecule. ROS also controls the hydroxylation of HIF1 $\alpha$ , it causes inactivation of prolyl-hydroxylase enzymes (PHD), as a result degradation of  $\alpha$  subunit does not occur and HIF1 formation occurs [57].

## **5. Hypoxic preconditioning improves regenerative potential of cells**

### **5.1 Effect of hypoxia on stemness and survival of stem cells**

Hypoxic preconditioning improves survival and stemness of cells and has been investigated in a number studies. SOX2, OCT4, NANOG and c-Myc are the markers that show stemness of cells. It has been found that stem cells grown in hypoxia are more viable, have decreased apoptosis through effects on HIF1 $\alpha$  and p53 pathways [58].

When cultured under hypoxic (3 or 5% oxygen) condition for 5 days, stem cell markers were found to be statistically higher in dental pulp MSC [59].

PI3K/Akt signaling pathway get activated in cells exposed to hypoxia which in turn regulates many genes of cell cycle and CDK2 resulting in increased self-renewal and decreased apoptosis. Under hypoxic conditions cells switch their metabolism more towards glycolytic pathways and less towards oxidative phosphorylation resulting in less reactive oxygen species production (ROS) and more production of antioxidant enzymes [49, 60].

Hypoxia (1%) results in decreased senescence, increased lifespan of mesenchymal stem cells and were able to maintain proliferation rate, morphology and genetic stability [61]. Cryopreserved adipose derived stem cells cultured at 2 and 5% oxygen tension resulted in increased number and viability as compared to counterparts grown at 21% oxygen. In addition, all stemness related gene expression NANOG, SOX-2, REX-1, and OCT-4 were much higher in hypoxia group than in normoxia group. Another group demonstrated the upregulation of stemness related genes OCT4, SOX2 and NANOG in MSC grown in 3% oxygen culture conditions [62, 63]. Stemness of MSCs remains preserved in hypoxic cultures. Hypoxia results in decreased expression of apoptotic BCL-2 and CASP3 and increased expression of anti-apoptotic genes [64]. Stem cells derived from apical papilla of wisdom teeth also showed increased proliferation and upregulation of SSEA4 which is an embryonic stem cell marker surface antigen. Human umbilical cord derived MSC grown in a culture under 5% oxygen showed better proliferation and maintenance of stemness [65]. MSC derived from different sources showed increased expression of pluripotency markers Oct4, C-Myc and Nanog when cultured at 5% oxygen concentration levels [66]. Low oxygen tension also helped iPSCs from liver cells to preserve their stemness and decrease the time to switch from G1 phase to S phase, increase proliferation but the physiological oxygen level of tissue of origin should be kept in mind because these cells were grown in 10% oxygen level. The same group showed that culturing these cells at very low level result in loss of stemness [67].

## 5.2 Hypoxia improves differentiation potential of cells

Most of the studies use stem cells for regenerative purposes. MSC are multipotent cells that can differentiate into adipocytes, osteoblasts, chondrocytes and neurons under appropriate conditions [68]. The main advantage of stem cell therapy is living biological replacement rather than palliation through drugs. The use of stem cells to replace functional loss of specific tissue is determined by effective differentiation [69].

The results of differentiation are controversial; this variability may be due to:

1. Use of stem cells from different sources [70].
2. Due to heterogenous population of cells with similar morphology [71].
3. Designing of delivery system for successful transplantation of stem cells [72].

Biomaterials can be designed to act as carriers for the local delivery of stem cells, support cells or molecular niche cues [73]. Basal nutrients, cell density, spatial organization, mechanical forces, growth factors and cytokines have a profound influence on hMSC differentiation [71]. The role of hypoxia preconditioning in differentiation of stem cells into other lineages seems controversial due to inconsistent results of various studies [74]. Different studies have used different hypoxia percentages with variable times. Previously, cells were usually insulted with hypoxia using hydrogen peroxide, however, new tri-gas incubators have been developed recently that creates the precise hypoxic conditions even for longer period of times [59].

It has been shown that placenta derived MSC showed up regulation of osteogenic genes including osteopontin (OPN), osteocalcin (OCN), and alkaline phosphatase (ALP) as well as increased mineralization at 5% oxygen levels [75]. Another study found an increased osteogenic differentiation of human MSCs cultured at 5% oxygen for 5 days [66]. Contrary to this, multilineage differentiation potential including osteogenic differentiation of tendon derived MSC was compromised in hypoxia cultures [76]. Minsheng Yang and colleagues found that 9% hypoxia increased osteogenesis whereas 1% results in decreased osteogenic potentials due to upregulation of Notch 1 expression [77]. Cells cultured throughout in hypoxic culture (5%) showed less osteogenic potential, less mineralization as compared to cells primed with hypoxia (5% for 7 days). These results also emphasize that appropriate time for hypoxia is important to maneuver the different potentials [78].

Gale et al. analyzed the chondrogenic differentiation potential of equine synovial membrane and bone marrow derived MSCs and found no appreciable difference between cells cultured either at 5% oxygen or in normoxic conditions for 28 days. The results of expression of chondrogenic genes SOX9, ACAN, and COL2b were also variable between the groups [79]. Similar to this Li J and Pei M found no significant differences in chondrogenic index between normoxic and 5% hypoxic culturing for 7 days in synovium derived MSCs [80]. On the contrary adipose derived MSC showed better chondrogenesis and upregulation of several chondrogenic specific genes when grown in 2% oxygen cultures [81]. Bae et al. found increased expression of COL2A1, ACAN, and the transcription factor SOX9 in synovium derived MSC cultured at 5% oxygen levels. They also observed increased proteoglycan, glycosaminoglycans and collagen II contents from pellets in hypoxic condition [82]. Henrionnet et al. observed the effects of 5% oxygen conditions on bone marrow derived MSC cultures for their chondrogenic potential and resulted

in efficient and strong overexpression of chondrogenic genes COL2A1, ACAN, SOX9, and COMP along with down regulation of osteogenic genes ALP, and RUNX2 [83].

Valorani et al. found that pre-exposure hypoxia of 2% oxygen level results in increased expression of adipogenic genes including peroxisome proliferator activated receptor  $\gamma$  (Ppar $\gamma$ ), lipoprotein lipase (Lpl) and fatty acid binding protein 4 (Fabp4) and adipogenesis in MSC derived from murine adipose tissue [84]. Another research found enhanced adipogenesis of human adipose tissue mesenchymal stem-cell (hAT-MSC) exposed to 2% hypoxic conditions for 7 days before shifting to normoxia during differentiation [85]. 2% hypoxia resulted in increased adipogenic differentiation of dental pulp and periodontal ligament derived stem cells [86]. Choi JR et al. found decreased adipogenesis and decreased expression of adipogenic genes including LPL, PPARc and FABP4 under hypoxia (2%) as compared to normoxia cultures [87]. Another research also found a decreased differentiation of stem cells under hypoxia [88].

### **5.3 Effect of hypoxia on proliferative potential of cells**

Hypoxic preconditioning results in enhanced proliferation and increased colony forming units as compared to mesenchymal stem cells cultured in normoxia. Higher oxygen tensions increase oxidative stress to cells and activate apoptosis [55]. Zhang et al. explored the effects of 1 and 5% oxygen culture conditions on rat bone marrow derived MSC and compared them with their counter parts at 18% oxygen level cultures. They found significant increase in proliferation along with upregulation of BCL2 (antiapoptotic gene) and down regulation of BAX (apoptotic gene) [89]. About 5% oxygen tension resulted in greater size, cell number and cell density of MSC colonies [90]. Antebi et al. evaluated the potential of cells at different oxygen concentrations and found that proliferative potentials of porcine MSC was higher at 1, 2, and 5% oxygen tensions as compared to normoxic conditions. They also found that 48 hour hypoxia in their study resulted in more proliferation as compared to proliferative potential of cells when cultured for longer times (10 days) [64]. Elabd et al. suggested that hypoxic preconditioning should be used as a strategy for in vitro expansion of MSC before their clinical use. They cultured human bone marrow MSCs in 5 and 20% oxygen and observed greater effects of hypoxia not on the regenerative potentials but also on the gene expressions of hypoxia exposed MSCs [91]. Notch2-c-Myc signaling cause's proliferation under hypoxia and inhibits apoptosis. Hypoxia can have a great effect on proliferation of MSCs [92]. Hypoxia (1%) increases the proliferation not only in early passages but also in late passages as compared to normoxic cultures and extends the lifespan of MSCs [61]. Significantly higher number of cells as well as increased viability of ADSC occurs in hypoxic conditions [93]. Rat bone marrow MSC cultured at 5% oxygen levels exhibited increased number of colonies and shorter population doubling time as compared to normoxic cultured cells [94]. Asadpoor Dezaki et al. showed increased expansion, population doublings, viability and colony forming unit fibroblasts in a group cultured in 2.5% oxygen tension than normoxia group [95].

### **Acknowledgements**

I acknowledge the valuable suggestions and critical analysis of my colleagues at the King Edward Medical University, Lahore.



Acronyms and abbreviation

MSCs	mesenchymal stem cells
ESC	embryonic stem cells
iPSC	induced pluripotent stem cells
SOD	superoxide dismutase activity
HIFs	hypoxia inducible factors
HRE	hypoxia response element
ROS	reactive oxygen species
PHD	prolyl-hydroxylase enzymes
OPN	osteopontin
OCN	osteocalcin
ALP	alkaline phosphatase
Ppar $\gamma$	peroxisome proliferator activated receptor $\gamma$
Lpl	lipoprotein lipase
Fabp4	fatty acid binding protein 4
NRF2	nuclear factor erythroid 2 related factor 2

Author details

Bushra Bashir<sup>1\*</sup>, Mahmood S. Choudhery<sup>2</sup> and Ijaz Hussain<sup>1</sup>

1 Department of Dermatology Unit-I, KEMU, Pakistan

2 Tissue Engineering and Regenerative Medicine Laboratory, Department of Biomedical Sciences, King Edward Medical University, Lahore, Pakistan

\*Address all correspondence to: bushra.b27@gmail.com

IntechOpen

© 2020 The Author(s). Licensee IntechOpen. This chapter is distributed under the terms of the Creative Commons Attribution License (<http://creativecommons.org/licenses/by/3.0>), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited. 

## References

- [1] Seetharaman R, Mahmood A, Kshatriya P, Patel D, Srivastava A. An overview on stem cells in tissue regeneration. *Current Pharmaceutical Design*. 2019;**25**(18):2086-2098
- [2] George J, Manjusha WA, Jegan SR, Mahija SP, Josphin JS. A review of stem cells in regenerative medicine. *International Journal of Scientific Research in Science and Technology*. 2017;**8**(3):806-815
- [3] Sousa BR, Parreira RC, Fonseca EA, Amaya MJ, Tonelli FM, Lacerda SM, et al. Human adult stem cells from diverse origins: An overview from multiparametric immunophenotyping to clinical applications. *Cytometry Part A*. 2014;**85**(1):43-77
- [4] Fu Y, Karbaat L, Wu L, Leijten J, Both SK, Karperien M. Trophic effects of mesenchymal stem cells in tissue regeneration. *Tissue Engineering Part B: Reviews*. 2017;**23**(6):515-528
- [5] Yang YH, Ogando CR, See CW, Chang TY, Barabino GA. Changes in phenotype and differentiation potential of human mesenchymal stem cells aging in vitro. *Stem Cell Research & Therapy*. 2018;**9**(1):131
- [6] Neves J, Sousa-Victor P, Jasper H. Rejuvenating strategies for stem cell-based therapies in aging. *Cell Stem Cell*. 2017;**20**(2):161-175
- [7] Brooks RW, Robbins PD. Treating age-related diseases with somatic stem cells. In: *Exosomes, Stem Cells and MicroRNA*. Cham: Springer; 2018. pp. 29-45
- [8] Lu G, Ashraf M, Haider K. Insulin-like growth factor-1 preconditioning accentuates intrinsic survival mechanism in stem cells to resist ischemic injury by orchestrating protein kinase  $\text{C}\alpha$ -Erk1/2 activation. *Antioxidants & Redox Signaling*. 2012;**16**(3):217-227
- [9] Choudhery M, Badowski M, Muise A, Harris D. Effect of mild heat stress on the proliferative and differentiative ability of human mesenchymal stromal cells. *Cytotherapy*. 2015;**17**(4):359-368
- [10] Choudhery M, Khan M, Mahmood R, Mohsin S, Akhtar S, Ali F, et al. Mesenchymal stem cells conditioned with glucose depletion augments their ability to repair-infarcted myocardium. *Journal of Cellular and Molecular Medicine*. 2012;**16**(10):2518-2529
- [11] Hu C, Li L. Preconditioning influences mesenchymal stem cell properties in vitro and in vivo. *Journal of Cellular and Molecular Medicine*. 2018;**22**(3):1428-1442
- [12] Kretlow JD, Jin YQ, Liu W, Zhang WJ, Hong TH, Zhou G, et al. Donor age and cell passage affects differentiation potential of murine bone marrow-derived stem cells. *BMC Cell Biology*. 2008;**9**(1):60
- [13] Stolzing A, Jones E, McGonagle D, Scutt A. Age-related changes in human bone marrow-derived mesenchymal stem cells: consequences for cell therapies. *Mechanisms of Ageing and Development*. 2008;**129**(3):163-173
- [14] Lee J, Lee KS, Kim CL, Byeon JS, Gu NY, Cho IS, et al. Effect of donor age on the proliferation and multipotency of canine adipose-derived mesenchymal stem cells. *Journal of Veterinary Science*. 2017;**18**(2):141-148
- [15] Choudhery MS, Badowski M, Muise A, Pierce J, Harris DT. Donor age negatively impacts adipose tissue-derived mesenchymal stem cell expansion and differentiation. *Journal of Translational Medicine*. 2014;**12**(1):8

- [16] Gao P, Han P, Jiang D, Yang S, Cui Q, Li Z. Effects of the donor age on proliferation, senescence and osteogenic capacity of human urine-derived stem cells. *Cytotechnology*. 2017;**69**(5):751-763
- [17] Zaim M, Karaman S, Cetin G, Isik S. Donor age and long-term culture affect differentiation and proliferation of human bone marrow mesenchymal stem cells. *Annals of Hematology*. 2012;**91**(8):1175-1186
- [18] Zhang M, Wang Z, Zhao Y, Zhang L, Xu L, Cao L, et al. The effect of age on the regenerative potential of human eyelid adipose-derived stem cells. *Stem Cells International*. 2018;**2018**
- [19] Wagner W, Horn P, Castoldi M, Diehlmann A, Bork S, Saffrich R, et al. Replicative senescence of mesenchymal stem cells: A continuous and organized process. *PLoS One*. 2008;**3**(5):e2213
- [20] Banfi A, Muraglia A, Dozin B, Mastrogiacomo M, Cancedda R, Quarto R. Proliferation kinetics and differentiation potential of ex vivo expanded human bone marrow stromal cells: Implications for their use in cell therapy. *Experimental Hematology*. 2000;**28**(6):707-715
- [21] Choi JS, Lee BJ, Park HY, Song JS, Shin SC, Lee JC, et al. Effects of donor age, long-term passage culture, and cryopreservation on tonsil-derived mesenchymal stem cells. *Cellular Physiology and Biochemistry*. 2015;**36**(1):85-99
- [22] Rizal R, Widodo WS, Wibowo S, Munshy UZ. Effect of serial passage on growth kinetics, biological properties, and differentiation into adipocytes of human Wharton's jelly-derived mesenchymal stem Cells. *Majalah Kedokteran Bandung*. 2019;**51**(3):127-133
- [23] Lee BY, Li Q, Song WJ, Chae HK, Kweon K, Ahn JO, et al. Altered properties of feline adipose-derived mesenchymal stem cells during continuous in vitro cultivation. *The Journal of Veterinary Medical Science*. 2018;**80**(6):930-938
- [24] Schofield R. The relationship between the spleen colony-forming cell and the haemopoietic stem cell. *Blood Cells*. 1978;**4**(1-2):7-25
- [25] Pennings S, Liu KJ, Qian H. The stem cell niche: Interactions between stem cells and their environment. *Stem Cells International*. 1 January 2018;**2018**
- [26] Zhang J, Niu C, Ye L, Huang H, He X, Tong WG, et al. Identification of the haematopoietic stem cell niche and control of the niche size. *Nature*. 2003;**425**(6960):836-841
- [27] Tang Z, Wang A, Yuan F, Yan Z, Liu B, Chu JS, et al. Differentiation of multipotent vascular stem cells contributes to vascular diseases. *Nature Communications*. 2012;**3**(1):1-3
- [28] Kordes C, Häussinger D. Hepatic stem cell niches. *The Journal of clinical investigation*. 2013;**123**(5):1874-1880
- [29] Ronconi E, Sagrinati C, Angelotti ML, Lazzeri E, Mazzinghi B, Ballerini L, et al. Regeneration of glomerular podocytes by human renal progenitors. *Journal of the American Society of Nephrology*. 2009;**20**(2):322-332
- [30] Barker N, Van Es JH, Kuipers J, Kujala P, Van Den Born M, Cozijnsen M, et al. Identification of stem cells in small intestine and colon by marker gene Lgr5. *Nature*. 2007;**449**(7165):1003-1007
- [31] Darzi S, Werkmeister JA, Deane JA, Gargett CE. Identification and characterization of human endometrial mesenchymal stem/stromal cells and their potential for cellular therapy. *Stem Cells Translational Medicine*. 2016;**5**(9):1127-1132

- [32] Gorski B. Gingiva as a new and the most accessible source of mesenchymal stem cells from the oral cavity to be used in regenerative therapies. *Postępy Higieny i Medycyny Doświadczalnej (Online)*. 2016;**70**:858-871
- [33] Cotsarelis G, Sun TT, Lavker RM. Label-retaining cells reside in the bulge area of pilosebaceous unit: Implications for follicular stem cells, hair cycle, and skin carcinogenesis. *Cell*. 1990;**61**(7):1329-1337
- [34] Berry R, Rodeheffer MS. Characterization of the adipocyte cellular lineage in vivo. *Nature Cell Biology*. 2013;**15**(3):302-308
- [35] Keeley TP, Mann GE. Defining physiological normoxia for improved translation of cell physiology to animal models and humans. *Physiological Reviews*. 2019;**99**(1):161-234
- [36] Spence VA, Walker WF. Measurement of oxygen tension in human skin. *Medical & Biological Engineering*. 1976;**14**(2):159-165
- [37] Pittman RN. Oxygen gradients in the microcirculation. *Acta Physiologica*. 2011;**202**(3):311-322
- [38] Saltzman DJ, Toth A, Tsai AG, Intaglietta M, Johnson PC. Oxygen tension distribution in postcapillary venules in resting skeletal muscle. *American Journal of Physiology. Heart and Circulatory Physiology*. 2003;**285**(5):H1980-H1985
- [39] Rivera BK, Naidu SK, Subramanian K, Joseph M, Hou H, Khan N, et al. Real-time, in vivo determination of dynamic changes in lung and heart tissue oxygenation using EPR oximetry. In: *Oxygen Transport to Tissue XXXVI*. New York, NY: Springer; 2014. pp. 81-86
- [40] Sakadžić S, Roussakis E, Yaseen MA, Mandeville ET, Srinivasan VJ, Arai K, et al. Cerebral blood oxygenation measurement based on oxygen-dependent quenching of phosphorescence. *JoVE (Journal of Visualized Experiments)*. 2011;**51**:e1694
- [41] Brooks AJ, Eastwood J, Beckingham IJ, Girling KJ. Liver tissue partial pressure of oxygen and carbon dioxide during partial hepatectomy. *British Journal of Anaesthesia*. 2004;**92**(5):735-737
- [42] Zhang JL, Morrell G, Rusinek H, Warner L, Vivier PH, Cheung AK, et al. Measurement of renal tissue oxygenation with blood oxygen level-dependent MRI and oxygen transit modeling. *American Journal of Physiology. Renal Physiology*. 2014;**306**(6):F579-F587
- [43] Carlsson PO, Palm F, Andersson A, Liss P. Markedly decreased oxygen tension in transplanted rat pancreatic islets irrespective of the implantation site. *Diabetes*. 2001;**50**(3):489-495
- [44] Cooper GJ, Sherry KM, Thorpe JA. Changes in gastric tissue oxygenation during mobilisation for oesophageal replacement. *European Journal of Cardio-Thoracic Surgery*. 1995;**9**(3):158-159
- [45] Korsbäck C, Höckerstedt K. Small bowel and liver pO<sub>2</sub> during vasopressin infusion into the superior mesenteric artery. In: *Annales chirurgiae et gynaecologiae*. Vol. 73. No. 1. 1984. pp. 50-53
- [46] Lind Due V, Bonde J, Kann T, Perner A. Extremely low oxygen tension in the rectal lumen of human subjects. *Acta Anaesthesiologica Scandinavica*. 2003;**47**(3):372
- [47] Ottosen LD, Hindkjær J, Husth M, Petersen DE, Kirk J, Ingerslev HJ. Observations on intrauterine oxygen tension measured by fibre-optic microsensors.



Reproductive Biomedicine Online. 2006;**13**(3):380-385

[48] Spencer JA, Ferraro F, Roussakis E, Klein A, Wu J, Runnels JM, et al. Direct measurement of local oxygen concentration in the bone marrow of live animals. *Nature*. 2014;**508**(7495):269-273

[49] Abdelwahid E, Kalvelyte A, Stulpinas A, De Carvalho KA, Guarita-Souza LC, Foldes G. Stem cell death and survival in heart regeneration and repair. *Apoptosis*. 2016;**21**(3):252-268

[50] Krishnan R, Ko D, Tucker T, Opara E. Strategies to combat hypoxia in encapsulated islet transplantation. *Surgery: Current Research*. 2016;**6**:259

[51] Ezquer FE, Ezquer ME, Vicencio JM, Calligaris SD. Two complementary strategies to improve cell engraftment in mesenchymal stem cell-based therapy: Increasing transplanted cell resistance and increasing tissue receptivity. *Cell Adhesion & Migration*. 2017;**11**(1):110-119

[52] Lee HJ, Jung YH, Choi GE, Kim JS, Chae CW, Han HJ. Role of HIF1 $\alpha$  regulatory factors in stem cells. *International Journal of Stem Cells*. 2019;**12**(1):8

[53] Suda T, Takubo K, Semenza GL. Metabolic regulation of hematopoietic stem cells in the hypoxic niche. *Cell Stem Cell*. 4 October 2011;**9**(4):298-310

[54] Lee JH, Yoon YM, Lee SH. Hypoxic preconditioning promotes the bioactivities of mesenchymal stem cells via the HIF-1 $\alpha$ -GRP78-Akt axis. *International Journal of Molecular Sciences*. 2017;**18**(6):1320

[55] Dai X, Yan X, Wintergerst KA, Cai L, Keller BB, Tan Y. Nrf2: Redox and metabolic regulator of stem cell

state and function. *Trends in Molecular Medicine*. 1 Feb 2020;**26**(2):185-200

[56] Salazar-Noratto GE, Luo G, Denoeud C, Padrona M, Moya A, Bensidhoum M, et al. Concise review: Understanding and leveraging cell metabolism to enhance mesenchymal stem cell transplantation survival in tissue engineering and regenerative medicine applications. *Stem Cells*. January 2020;**38**(1):22-33

[57] Cerychova R, Pavlinkova G. HIF-1, metabolism, and diabetes in the embryonic and adult heart. *Frontiers in Endocrinology*. 2018;**9**:460

[58] Lv B, Li F, Fang J, Xu L, Sun C, Han J, et al. Hypoxia inducible factor 1 $\alpha$  promotes survival of mesenchymal stem cells under hypoxia. *American Journal of Translational Research*. 2017;**9**(3):1521

[59] Ahmed NE, Murakami M, Kaneko S, Nakashima M. The effects of hypoxia on the stemness properties of human dental pulp stem cells (DPSCs). *Scientific Reports*. 2016;**6**:35476

[60] Liu F, Huang X, Luo Z, He J, Haider F, Song C, et al. Hypoxia-activated PI3K/Akt inhibits oxidative stress via the regulation of reactive oxygen species in human dental pulp cells. *Oxidative Medicine and Cellular Longevity*. 9 January 2019;**2019**

[61] Lee CW, Kang D, Kim AK, Kim DY, Kim DI. Improvement of cell cycle lifespan and genetic damage susceptibility of human mesenchymal stem cells by hypoxic priming. *International Journal of Stem Cells*. 2018;**11**(1):61

[62] Safwani WK, Choi JR, Yong KW, Ting I, Adenan NA, Pingguan-Murphy B. Hypoxia enhances the viability, growth and chondrogenic potential of cryopreserved human

adipose-derived stem cells. *Cryobiology*. 2017;**75**:91-99

[63] Werle SB, Chagastelles P, Pranke P, Casagrande L. Hypoxia upregulates the expression of the pluripotency markers in the stem cells from human deciduous teeth. *Clinical Oral Investigations*. 2019;**23**(1):199-207

[64] Antebi B, Rodriguez LA, Walker KP, Asher AM, Kamucheka RM, Alvarado L, et al. Short-term physiological hypoxia potentiates the therapeutic function of mesenchymal stem cells. *Stem Cell Research & Therapy*. 2018;**9**(1):265

[65] Yustianingsih V, Sumarawati T, Putra A. Hypoxia enhances self-renewal properties and markers of mesenchymal stem cells. *Universa Medicina*. 2019;**38**(3):164-171

[66] Kwon SY, Chun SY, Ha YS, Kim DH, Kim J, Song PH, et al. Hypoxia enhances cell properties of human mesenchymal stem cells. *Tissue Engineering and Regenerative Medicine*. 2017;**14**(5):595-604

[67] Zhi X, Xiong J, Wang M, Zhang H, Huang G, Zhao J, et al. Physiological hypoxia enhances stemness preservation, proliferation, and bidifferentiation of induced hepatic stem cells. *Oxidative Medicine and Cellular Longevity*. 1 January 2018;**2018**

[68] Shende P, Gupta H, Gaud RS. Cytotherapy using stromal cells: Current and advance multi-treatment approaches. *Biomedicine & Pharmacotherapy*. 2018;**97**:38-44

[69] Hwang NS, Varghese S, Elisseeff J. Controlled differentiation of stem cells. *Advanced Drug Delivery Reviews*. 2008;**60**(2):199-214

[70] Rebelatto CK, Aguiar AM, Moretao MP, Senegaglia AC, Hansen P, Barchiki F, et al. Dissimilar

differentiation of mesenchymal stem cells from bone marrow, umbilical cord blood, and adipose tissue. *Experimental Biology and Medicine*. 2008;**233**(7):901-913

[71] Pittenger MF, Mackay AM, Beck SC, Jaiswal RK, Douglas R, Mosca JD, et al. Multilineage potential of adult human mesenchymal stem cells. *Science*. 1999;**284**(5411):143-147

[72] Friedenstein AJ, Chailakhyan RK, Gerasimov UV. Bone marrow osteogenic stem cells: In vitro cultivation and transplantation in diffusion chambers. *Cell Proliferation*. 1987;**20**(3):263-272

[73] Lutolf MP, Gilbert PM, Blau HM. Designing materials to direct stem-cell fate. *Nature*. 2009;**462**(7272):433-441

[74] Wu R, Hu X, Wang JA. Concise review: Optimized strategies for stem cell-based therapy in myocardial repair: Clinical translatability and potential limitation. *Stem Cells*. 2018;**36**(4):482-500

[75] Camacho-Cardenosa M, Camacho-Cardenosa A, Timón R, Olcina G, Tomas-Carus P, Brazo-Sayavera J. Can hypoxic conditioning improve bone metabolism? a systematic review. *International Journal of Environmental Research and Public Health*. 2019;**16**(10):1799

[76] Yu Y, Lin L, Zhou Y, Lu X, Shao X, Lin C, et al. Effect of hypoxia on self-renewal capacity and differentiation in human tendon-derived stem cells. *Medical Science Monitor: International Medical Journal of Experimental and Clinical Research*. 2017;**23**:1334

[77] Yang M, Liu H, Wang Y, Wu G, Qiu S, Liu C, et al. Hypoxia reduces the osteogenic differentiation of peripheral blood mesenchymal stem cells by upregulating Notch-1 expression. *Connective Tissue Research*. 2019;**60**(6):583-596

- [78] Inagaki Y, Akahane M, Shimizu T, Inoue K, Egawa T, Kira T, et al. Modifying oxygen tension affects bone marrow stromal cell osteogenesis for regenerative medicine. *World Journal of Stem Cells*. 26 July 2017;**9**(7):98
- [79] Gale AL, Mammone RM, Dodson ME, Linardi RL, Ortved KF. The effect of hypoxia on chondrogenesis of equine synovial membrane-derived and bone marrow-derived mesenchymal stem cells. *BMC Veterinary Research*. 2019;**15**(1):201
- [80] Li J, Pei M. Optimization of an in vitro three-dimensional microenvironment to reprogram synovium-derived stem cells for cartilage tissue engineering. *Tissue Engineering Parts A*. 2010;**17**(5-6):703-712
- [81] Tabatabaei FS, Ai J. Mesenchymal endometrial stem/stromal cells for hard tissue engineering: A review of in vitro and in vivo evidence. *Regenerative Medicine*. 2017;**12**(8):983-995
- [82] Bae HC, Park HJ, Wang SY, Yang HR, Lee MC, Han HS. Hypoxic condition enhances chondrogenesis in synovium-derived mesenchymal stem cells. *Biomaterials Research*. 2018;**22**(1):1-8
- [83] Henrionnet C, Liang G, Roeder E, Dossot M, Wang H, Magdalou J, et al. Hypoxia for mesenchymal stem cell expansion and differentiation: The best way for enhancing TGF $\beta$ -induced chondrogenesis and preventing calcifications in alginate beads. *Tissue Engineering Parts A*. 2017;**23**(17-18):913-922
- [84] Valorani MG, Germani A, Otto WR, Harper L, Biddle A, Khoo CP, et al. Hypoxia increases Sca-1/CD44 co-expression in murine mesenchymal stem cells and enhances their adipogenic differentiation potential. *Cell and Tissue Research*. 2010;**341**(1):111-120
- [85] Valorani MG, Montelatici E, Germani A, Biddle A, D'Alessandro D, Strollo R, et al. Pre-culturing human adipose tissue mesenchymal stem cells under hypoxia increases their adipogenic and osteogenic differentiation potentials. *Cell Proliferation*. 2012;**45**(3):225-238
- [86] Zhou Y, Fan W, Xiao Y. The effect of hypoxia on the stemness and differentiation capacity of PDLC and DPC. *BioMed Research International*. 1 January 2014;**2014**:890675
- [87] Choi JR, Pingguan-Murphy B, Abas WA, Azmi MA, Omar SZ, Chua KH, et al. Impact of low oxygen tension on stemness, proliferation and differentiation potential of human adipose-derived stem cells. *Biochemical and Biophysical Research Communications*. 2014;**448**(2):218-224
- [88] Lin Q, Lee YJ, Yun Z. Differentiation arrest by hypoxia. *The Journal of Biological Chemistry*. 2006;**281**(41):30678-30683
- [89] Zhang J, Xiong L, Tang W, Tang L, Wang B. Hypoxic culture enhances the expansion of rat bone marrow-derived mesenchymal stem cells via the regulatory pathways of cell division and apoptosis. *In Vitro Cellular & Developmental Biology. Animal*. 2018;**54**(9):666-676
- [90] Caroti CM, Ahn H, Salazar HF, Joseph G, Sankar SB, Willett NJ, et al. A novel technique for accelerated culture of murine mesenchymal stem cells that allows for sustained multipotency. *Scientific Reports*. 2017;**7**(1):1-4
- [91] Elabd C, Ichim TE, Miller K, Anneling A, Grinstein V, Vargas V, et al. Comparing atmospheric and hypoxic cultured mesenchymal stem cell transcriptome: Implication for stem cell therapies targeting intervertebral discs. *Journal of Translational Medicine*. 2018;**16**(1):222

[92] Sato Y, Mabuchi Y, Miyamoto K, Araki D, Niibe K, Houlihan DD, et al. Notch2 signaling regulates the proliferation of murine bone marrow-derived mesenchymal stem/stromal cells via c-Myc expression. *PLoS One*. 2016;**11**(11):e0165946

[93] Adolfsson E, Helenius G, Friberg Ö, Samano N, Frøbert O, Johansson K. Bone marrow- and adipose tissue-derived mesenchymal stem cells from donors with coronary artery disease; growth, yield, gene expression and the effect of oxygen concentration. *Scandinavian Journal of Clinical and Laboratory Investigation*. 2020;**18**:1-9

[94] Liu Y, Yang X, Maureira P, Falanga A, Marie V, Gauchotte G, et al. Permanently hypoxic cell culture yields rat bone marrow mesenchymal cells with higher therapeutic potential in the treatment of chronic myocardial infarction. *Cellular Physiology and Biochemistry*. 2017;**44**(3):1064-1077

[95] Asadpoor Dezaki Z, Kheirandish M. Hypoxia preconditioning promotes survival and clonogenic capacity of human umbilical cord blood mesenchymal stem cells. *Iranian Journal of Blood and Cancer*. 2018;**10**(2):43-49