Adipose-Derived Stem Cells (ADSCs) in preconditioning of hypoxic cultures compared to normoxic cultures to prevent the formation of fibrous tissue: a review article

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ABSTRACT

Background: Stem cells are a key element in regenerative medicine for their ability to differentiate into various cell phenotypes. According to the literature review, one process that might improve the functionality of ADSCs is hypoxic culture conditions. Hypoxic oxygen culture conditions at 5% concentration are said to increase stem cell proliferation and viability after transplantation.

Methods: A comprehensive literature search was conducted by the author to obtain relevant studies from the PubMed, MEDLINE, Embase, PreMEDLINE, Embase, PsycINFO, Scopus, and Cochrane databases for the last twenty years (January 2002 – July 2022). The author used a search strategy with the following keywords: Adipose-Derived Stem Cells, hypoxic culture, normoxic culture, or pre-conditioned stem cells.

Discussion: Hypoxic culture preconditioning of ADSCs with an oxygen concentration of 5% can improve proliferation capabilities and stem cell viability post-transplantation. Hypoxic preconditioning will enhance the expression of HIF-1α, which plays an important role in stimulating cells for angiogenesis, migration and metabolism through stimulation and activation of various growth factor receptors and cytokines.

Conclusion: In hypoxic cultures, pro-inflammatory cytokines such as TNF-α decrease and anti-inflammatory cytokines such as IL-10 and FGF-2 increase. This, in turn, will suppress fibrosis.

Keywords: Adipose-Derived Stem Cells, hypoxic culture, normoxic culture, pre-conditioned stem cells.

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INTRODUCTION

Stem cells are known as a key element in regenerative medicine for their ability to differentiate into various cell phenotypes. Initially, stem cells were thought to restore functionality to tissue by differentiating into that very tissue. However, they also possess the ability to trans-differentiate, which is an ability to become cells outside of their differentiation paths. They contribute actively to their surrounding environment by secreting cytokines, growth factors and extracellular matrix molecules that act both on themselves (on an autocrine basis) and on surrounding cells (on a paracrine basis).

Based on their origins, stem cells are divided into 4 types: embryonic (embryonic stem cells), adult stem cells, fetal stem cells, and infantile stem cells. Adult stem cells are widely used nowadays for clinical applications. Adult stem cells can have Hematopoietic, Mesenchymal, Epidermal, Hepatic, Neuronal, Optical, Intestinal and Pancreatic origins. Adipose-derived stem cells (ADSCs) have been proven effective in preventing fibrous tissue formation in the urethra. The previous study showed that ADSC administration could prevent the formation of urethral strictures. Mesenchymal stem cells are capable of multipotent differentiation. Theoretically, mesenchymal stem cells are present in all human body organs, to be precise, in the part of the cell population found in the perivascular area. Based on ease and accessibility studies, there are 3 common sources of mesenchymal stem cells: bone marrow, cord blood and adipose tissue.

The normoxic culture with high oxygen (O₂) tension (> 21%), known as normoxia, commonly worked with today, causes a decrease in stem cell viability prior to transplantation. According to the literature review, one process that might improve the functionality of ADSCs is hypoxic culture conditions. In ADSCs, hypoxic oxygen culture conditions at 5% concentration are said to increase stem cell proliferation and viability after transplantation. The main purpose of this study was to compare the effectiveness of the hypoxic culture of ADSC compared to the normoxic culture of ADSC.

METHODS

A comprehensive literature search was conducted by the author to obtain relevant studies from PubMed, MEDLINE Embase, PsycINFO, Scopus, and Cochrane...
Adipose-Derived Stem Cells (ADSCs)

Stem cells have unlimited capabilities in tissue formation as cell therapy because they have the self-renewal ability and can differentiate into cell lineages, making them a viable solution for various diseases.6 Mesenchymal stem cells (MSCs) sourced from bone marrow provide sub-optimal outcomes due to the invasive procedure to retrieve the specimen. Stem cells derived from bone marrow will see a decrease in their ability to differentiate and proliferate, especially in specimens that have been stored for a long time. ADSCs are multipotent cells that can differentiate into osteocytes, adiposity, neural cells, vascular endothelial cells, cardiomyocytes, pancreatic cells, and hepatocytes. ADSCs are said to have differentiation ability similar to MSCs derived from bone marrow, and in vivo studies, ADSCs have the expression characteristics of stem cells. Easy specimen collection with minimally invasive methods, simple isolation procedures, stem cell quality, and proliferative ability that does not diminish with age make ADSCs the best alternative when compared to bone marrow MSCs.7 Almost identical to bone marrow MSCs, ADSCs have a combination of surface markers that are specific for stem cells, namely CD90, CD105, CD73, CD44, and CD166, and lower expression for hematopoietic markers such as CD45 and CD34. Some literature states that collecting specimens from different locations does not affect the viable cells obtained. ADSCs are said to be more morphologically and genetically stable in long-term cultures. Stem cells are beneficial by restoring cellular restoration and can also influence them in a paracrine manner. Several studies have demonstrated the effects of ADSCs as anti-apoptotic, anti-inflammatory, pro-angiogenic, immunomodulatory, and anti-scarring.8 To date, 130 clinical trial studies in America show the effectiveness of ADSCs in regeneration from soft tissue, regeneration from skeletal tissue, ischemic injury, myocardial infarction, and autoimmune diseases (such as Lupus, arthritis, Chron’s diseases, multiple sclerosis, diabetes mellitus, graft versus host diseases).14

ADSCs are an effective therapy for atrophy, fibrosis, retraction, and ulcer caused by radiation therapy. In addition, ADSCs are also effective in abnormal wound healing, acute graft disease vs host diseases and hematologic and immunological disorders such as idiopathic thrombocytopenic purpura. ADSCs also have an immunomodulatory function.9 Stem cells can be administered to a location where tissue damage occurs. They have unique characteristics such as being easy to collect in large quantities, high proliferative ability, ability to differentiate into the desired cell phenotypes, and ability to assist the vascularization process of the wound healing framework. In advanced regenerative therapy; stem cells are used to repair damaged tissue and even to replace organs.10

Stem cells can be divided based on their origins. Embryonic stem cells derived from embryonic tissues can induce pluripotent stem cells and reprogrammed differentiation from somatic cells. Stem cells in more mature tissues can be taken from adipose tissue, skin, bone marrow, blood, or skeletal muscle. MSCs were identified in the bone marrow. MSCs can be taken from various tissues in the body, including adipose tissue, trabecular bone, skin, skeletal muscle, pericytes, umbilical blood, periosteum, peripheral blood, synovial membrane, dermis, dental pulp, periodontal ligament, and even tumors.11 However, even though stem cells can be derived from various sources, the amount that can be taken from each source is limited. That said, adipose tissue is a potential source of stem cells because it is found in many body parts and can be taken in large quantities with lower donor morbidity. Subcutaneous fat tissue can be derived from the abdomen, thighs and arms.6,12

ADSCs derived from abdominal superficial fat are less likely to undergo apoptosis compared to fatty tissue taken from the upper arms, inner thighs and deep abdomen. Different isolation sites, cell types, species, and collection procedures affect ADSCs’ quality, function and plasticity. There are two types of ADSCs: white adipose tissue and brown adipose tissue.13 White adipose tissue is found subcutaneously and visceraally, which functions to store energy in the form of triglycerides. ADSCs are most commonly found in subcutaneous fat compared to visceral fat. The highest concentration of ADSCs was mostly found in the arms, and the one with the highest plasticity was the adipose tissue in the inguinal region. Brown adipose tissue is less in amount than white adipose tissue.14 Brown adipose tissue can be found in the mediastinum, neck and interscapular area in neonates, which function as thermogenesis. ADSCs derived from brown adipose tissue have different characteristics than white adipose tissue, where brown adipose tissue has better myogenic differentiation capability.6

ADSCs possess better proliferation capabilities than bone marrow mesenchymal stem cells (BMSCs). ADSCs can maintain normal diploid karyotype conditions up to 100 times culture and produce 40 times more cells than BMSCs.3 ADSCs also have better epithelial regeneration and collagen-forming capabilities than BMSCs. Research also shows that ADSCs can regulate the
proliferation of T lymphocytes by reducing the histocompatibility of antigens on their surface, thereby inhibiting mixed lymphocyte reaction (MLR). This gives ADSCs an immune-compatibility advantage and is highly suitable for autotransplantation compared to other stem cell sources.15

Pre-Conditioned Hypoxia and Normoxia in ADSC Cultures

Hypoxic cultures of ADSCs with low oxygen levels can increase stem cell proliferation and stemness, thereby increasing the potential of stem cells for multipotent differentiation and better long-term expansion.16 In the last few years, hypoxic cultures have been developed in many in vitro studies, and it was shown that hypoxic conditions could stimulate the proliferation of ADSCs without modifying the phenotype of ADSCs. Hypoxic cultures prevent senescence as a genomic stability inducer and increase viability, motility and tropism.17 This is due to stimulation from hypoxia-inducible factor 1-α (HIF-1 α), formation of reactive oxygen species (ROS) and decreased phosphorylation of platelet-derived growth factor receptor β (PDGFR β) as well as extracellular signal-regulated kinases ½ (ERK1/2) and Akt. This study also showed a significant increase in VEGF expression in ADSCs cultured under hypoxic conditions compared to ADSCs cultured under normoxic conditions.17,18

Conventional normoxic cultures of bone marrow mesenchymal stem cells are reported to cause cell senescence, apoptosis and gene mutations, causing a decrease in stem cell viability before transplantation. Before transplantation, 93-99% of stem cells will die on the 3rd to 7th day after transplantation, even reaching 99% on the first day of transplantation. Normoxic conditions refer to normal oxygen levels in MSCs in vitro medium at an oxygen concentration of 20%. Culture conditions with low O₂ tension (hypoxia) aim to support the microenvironment during in vitro culture to remain viable when transplanted. Hypoxic conditions cause stem cells to develop long-term maintenance (LTM) properties. LTM can be achieved when stem cells are in the G0 phase but can still proliferate and not differentiate.19 G0 means that stem cells do not go through cycling states (G1/S/G2/M) but still proliferate and do not differentiate. This condition in vivo is known as cell quiescence. Several studies regarding hypoxic preconditions to support in vitro microenvironment (niche) on several stem cell sources have been carried out, including on hematopoietic stem cells (HSCs) with an O₂ concentration of 0-5%, on adipose-derived stem cells with a concentration of 5%, on neural stem cells (NSCs) with a concentration of 1-5%, and on Human Cord Blood with a concentration of 3% for 7 days.20 Hypoxic conditions during stem cell culture became a niche factor in vitro that controlled the proliferation of stem cells that remained viable and undifferentiated without experiencing dysfunction.

Figure 1. HIF-1α plays an important role in maintaining a balance between pro-apoptosis and anti-apoptosis.21

Figure 2. Transcription regulation of HIF-1 under ambient oxygen (normoxia) and hypoxia conditions.23
apoptosis and controlled the formation of senescence cells and gene mutations. It has been agreed in the joint consensus Acta-Bionergetics Biochemistry and Biophysics in 2008 that hypoxia is achieved when oxygen levels are 3%-5% or 30-50 µM. MSCs grown at oxygen levels of 0.4% to 2.3% increased the level of apoptosis. The best oxygen level to increase the paracrine effect of VEGF and angiogenesis is 5%.

Hypoxic preconditioning greatly affects cells, especially on hypoxia-inducible factor (HIF) 1α. In the study of bone marrow cells with hypoxic preconditioning, the expression of HIF-1α increased, thereby reducing apoptosis and minimizing the loss of the ability of mitochondria. Hypoxic preconditioning has a protective effect on BM-MSCs, enabling them to survive under ischemic and hypoxic conditions. HIF-1α is a subunit of the heterodimeric transcription factor HIF-1α encoded by the HIF-1α gene. PAS helix-loop-helix HIF-1α contains protein, regulates the primary transcription of cellular responses, and allows cellular responses to thrive under hypoxic conditions. The exposure of MSCs under hypoxic conditions will improve immunomodulator and regenerative capability by increasing the expression of cytoprotective and secretory factor genes. HIF-1α has an important role in enhancing this gene. It can improve the migration of MSCs to ischemic and hypoxic areas by regulating the molecule’s expression, one of which is stromal cell-derived factor 1 (SDF1) (Figure 1). Stem cells express SDF1 receptors C-XC CXCR4 and CXCR7 and play an important role in tissue repair. HIF-1α has an anti-apoptotic function because cells containing high levels of HIF-1α are more resistant to hypoxia-induced apoptosis.

In normoxic conditions, the resident proline of the HIF-1α subunit undergoes hydroxylation by oxygen-dependent prolyl-4-hydroxylase (PHD). The Von Hippel-Lindau protein (pVHL), a ubiquitin E3 Ligase, binds to hydroxylated HIF-α and is recognized as a component of the Ubiquitin E3 ligase complex, which in turn causes degradation of the HIF protein via the 26S proteasome system. The asparagine residue of the HIF-α subunit will also be hydroxylated by the HIF inhibiting factor (HIF), which inhibits the binding of HIF to p300/CREB, a binding protein coactivator. Meanwhile, under hypoxic conditions, HIF is stabilized and translocated to the nucleus, where HIF1α binds to HIF1β and enhances the transcription of HIF target genes (Figure 1).

Under hypoxic conditions, HIF-1α is translocated into the nucleus and binds to HIF-1β to form a heterodimer which will then attach to the target gene-specific HREs, which will then undergo transcription and activate the genes encoding glucose transporters (GLUT), glycolytic enzymes and lactate dehydrogenation (LDH-A) which facilitate anaerobic respiration (Figure 2).

The expression of chemokine receptors such as CXCR4, CXCR7 and CX3CR1 will increase in MSCs with hypoxic culture. These chemokines play an important role in repairing damaged tissues and improving the potency of stem cells to go to the site of tissue damage. This shows that HIF-1α gains stability under hypoxic conditions, where it enters the nucleus where it binds to HIF-1β, which will then form a heterodimer. Subsequently, the heterodimer will bind to specific HRE genes associated with coactivators such as CBP/p300, further increasing the expression of chemokine receptors, namely CXCR4, CXCR7 and CX3CR1.

The Mechanism of ADSCs with Hypoxic Preconditioning in Preventing Fibrosis
ADSC hypoxic cultures can regulate anti-inflammation cytokines (IL-10 and FGF 2) and pro-inflammation cytokines (TNF-α) to form less fibrous tissue. ADSC hypoxic cultures are better than normoxic cultures at regulating anti-inflammation cytokines (IL-10 and FGF-2) and pro-inflammation cytokines (TNF-α). In addition, ADSC hypoxic cultures are better at decreasing the expression of myofibroblasts and fibrous tissue. This is because ADSC hypoxic cultures increase the capabilities of HIF-1α. Under hypoxic conditions, HIF-1α is translocated into the nucleus and binds to HIF-1β to form a heterodimer which will then bind to the target gene-specific HREs, which will then undergo transcription and activate the genes encoding glucose transporters (GLUT), glycolytic enzymes and lactate dehydrogenation (LDH-A) which facilitate anaerobic respiration. In addition to repressing mitochondrial respiration, HIF-1α increases the expression of pyruvate dehydrogenase kinase (PDK), which prevents the conversion of pyruvate to acetyl CoA, which further inhibits the enzymatic activity of pyruvate dehydrogenase (PDH). This will increase O2 consumption in mitochondria, further reducing the production of reactive oxygen species (ROS). HIF-1α under hypoxic conditions promotes the production of cytochrome C, thus optimizing the production of ATP and cell integrity by minimizing ROS.

ADSCs can increase IL-10 because ADSCs express TLR 2 and TLR 4 receptors, the stimulation of these receptors will increase the production of IL-10 through MYD88 and TRIF-dependent mechanism, or if TLR 4 is stimulated, it will cause transcription of type I IFNs—subsequently, the expression of IL-10 increases by activating transcription factors STAT1 and STAT3. ADSCs can decrease TNF-α through the following mechanism. Activation of receptor TLR4 will stimulate NF-κB to promote the formation of TNF-α. MSCs work by reducing the translocation of NF-κB, thus limiting the formation of TNF-α. MSCs use immune-suppressive effects by inhibiting the expression of Th1 pro-inflammatory factors (such as IFN-α, TNF-α and IL-1α). MSCs also inhibit the activation of Th1 indirectly by suppressing DC and NK cells. Thus, by inhibiting Th1, pro-inflammatory factors such as TNF-α production will be suppressed.

The higher FGF2 levels compared to the ADSC group with hypoxic culture could be due to mesenchymal stem cells excreting various growth factors, including FGF-2. ADSCs can reduce the expression of myofibroblasts because ADSCs can increase FGF-2, which functions to increase apoptosis of myofibroblasts and decrease differentiation of myofibroblasts. In addition, FGF-2 inhibits TGF-β1, thus decreasing the expression of α-SMA, reducing the formation of myofibroblasts.

ADSCs with hypoxic cultures showed better results in preventing fibrous tissue
formation than ADSCs with normoxic cultures. This could be because, as described previously, hypoxic cultures express higher HIF-1α. The limitation of this study is that no studies directly compared hypoxic cultures of ADSC with normoxic cultures of ADSC. Articles published to explain the molecular biologic pathophysiology of ADSC in preventing fibrosis are still rare.

CONCLUSIONS

Hypoxic culture preconditioning of ADSCs with an oxygen concentration of 5% can improve proliferation capabilities and stem cell viability post-transplantation. Hypoxic preconditioning will improve the expression of HIF-1α, which plays an important role in stimulating cells for angiogenesis, migration and metabolism through stimulation and activation of various growth factor receptors and cytokines. In hypoxic cultures, pro-inflammatory cytokines such as TNF-α decrease, and anti-inflammatory cytokines such as IL-10 and FGF-2 increase. This, in turn, will suppress fibrosis.

CONFLICT OF INTEREST

There was no conflict of interest. There was no financial support or relationships between the authors and any organization or professional.

ETHICAL CONSIDERATION

None.

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AUTHOR’S CONTRIBUTION


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