Role of Preconditioning Adipose Mesenchymal Stem Cells in Regeneration Disk: a Review Article

Romaniyanto[1], Hari Basuki Notobroto[2], Cita Rosita Sigit Prakoeswa[3], Fedik Abdul Rantam[4], Dwikora Novembri Utomo[5], Heri Suroto[6], Ferdiansyah[6]

[1]Doctoral Program, Faculty of Medicine, Airlangga University, Indonesia, Department of Orthopedic and Traumatology, Faculty of Medicine, Prof. Dr. R. Soerharso Orthopaedic Hospital, Universitas Sebelas Maret, Solo, Indonesia, [2]Associate Professor, Faculty of Public Health, Universitas Airlangga, Surabaya, Indonesia, [3]Professor, Department of Dermatology and Venereology, Faculty of Medicine, Airlangga University / Dr. Soetomo General Academic Hospital, Surabaya, Indonesia, [4]Professor, Department of Virology and Immunology, Faculty of Veterinary Medicine / Stem Cell Research and Development Center, Universitas Airlangga, Surabaya, Indonesia, [5]Professor, Department of Orthopedic and Traumatology, Faculty of Medicine, Dr. Soetomo General Academic Hospital, Universitas Airlangga, Surabaya, Indonesia, [6]Associate Professor, Department of Orthopedic and Traumatology, Faculty of Medicine, Dr. Soetomo General Academic Hospital, Universitas Airlangga, Surabaya, Indonesia

Abstract

Intervertebral disk (IVD) degeneration (IDD) is a degenerative disease and often found in daily practice. The managements that were used so far are only symptomatic and have not been able to solve the disease completely. The use of mesenchymal stem cells (MSCs) has been widely tested as a solution for the regeneration of IVD in IDD. Adipose-derived mesenchymal stem cells (ASCs) are one of the sources of MSCs which were widely studied as an alternative. One of the factors that can influence the efficiency and capacity of ASCs in IDD cases is preconditioning. Generally, there were three pre-conditioning strategies of ASCs before being used as therapy, (1) modifying the environment, (2) using Growth Factors, Cytokines, Chemokines, Hormones, (3) using pharmacological and chemical agents. Preconditioning on ASCs can change its function in various ways. We tried to link all these things together so that we could know the role of pre-conditioning ASCs in the regeneration disk.

Key words: *Adipose-Derived Mesenchymal Stem Cells, Disk Degeneration, Intervertebral Disk, Preconditioning, Regeneration, Stem Cells*

Introduction

Back pain is one of the main complaints experienced by many populations in the world[1]. In daily practice, complaints of back pain are one of the causes for patients to come to health facilities[2]. This complaint has a major impact on the social and economic fields, thereby reducing someone productivity and their quality of life[1,2]. Back pain that is not handled properly, can lead to chronic pain. The estimated prevalence rate of chronic low back pain (LBP) in adults in the world reaches 23%. The etiology of back pain, in general, can be divided into five categories, mechanical, degenerative, inflammatory, oncologic, and infectious[2]. One of the common causes of back pain is intervertebral disk (IVD) degeneration (IDD)[1].

One of the important functions of IVD is to support the body's weight[3]. If the IVD loses this function, the

Corresponding Author:
Romaniyanto, MD.
Rumah Sakit Ortopedi Prof. Dr. R. Soeharso Surakarta
Jalan Jenderal Ahmad Yani No. 157, Pabelan, Kartasura, Kabupaten Sukoharjo, Surakarta, Jawa Tengah, Indonesia
(Postal Code: 57162)
Email: dr.roman.spot@gmail.com

vertebral structure will lose its flexibility[4]. The typical symptom of IDD is back pain which is facet-mediated pain[5]. There were variations in the management of IDD, especially in relieving the main complaints of back pain experienced by patients. The initial approaches commonly used are physical therapy, education, and pain medication[1,2]. Furthermore, surgical treatments may be performed to treat IDD cases[1]. However, the management of IDD is only symptomatic and has not been able to efficiently solve the problem of IDD[1,3].

Other methods were used in the management of IDD is by triggering the regeneration of the degenerated IVD[4]. This regeneration therapy uses mesenchymal stem cells (MSCs)[6]. MSCs can be used to treat degenerative diseases in orthopedic surgery, such as osteoarthritis and IDD. The use of MSCs has become a new trend in therapeutic developments in regenerative medicine. Although their use is still in the experimental stage, the use of MSCs has provided new avenues for the management of degenerative diseases, which so far have not completely resolved the problem[7]. One of the MSCs that can be used is adipose tissue-derived stem cells (ASCs)[8].

When compared to Bone Marrow-Derived Mesenchymal Stem Cells (BMSCs), ASCs have their own advantages over BMSCs. These advantages include more sources, lower risk of taking, and more efficient yields than BMSC[6,9,10]. The requirement for ASCs to be used as therapy is to be able to survive for a long time in order to migrate effectively into the injury site and function properly. Research for the use of ASCs in regenerative medicine is kept developing. Various studies were conducted to make the most efficient use of ASCs as possible. One way to increase the therapeutic abilities of ASCs is by using pre-conditioning[8]. We try to find out the role of preconditioning ASCs in the regeneration disk.

Intervertebral Disk Degeneration

IVD is the amphiarthrotic joints and connects the vertebrae of the spinal column tightly[4,11]. Anatomically, IVD is a combined nucleus pulposus (NP) surrounded by annulus fibrosus (AF) between the cartilage that lines the subchondral part of the vertebrae[12]. The IVD extracellular matrix consists of proteoglycans, elastin, and collagen types I and II. Type I collagen is a constituent component of AF, while collagen type II is a component of NP. The assemblies work on the principle of hydrostatic pressure, so they can withstand compression loads and create flexibility[3,4].

Clinically, IDD can be described as a decreased ability of the disk to maintain stability and mobility of the vertebral structures[4]. In IDD, the main structures that are affected are NP and AF. Degeneration of the intervertebral disk is characterized by the onset of an inflammatory reaction accompanied by increased levels of inflammatory cytokines and a decrease in inhibitors. These reactions become cyclic resulting in tissue damage and inflammation. The resulting cycle is the death of NP cells and chondrocytes, an increase in shear stress, and a decrease in proteoglycans and type II collagen[3].

Symptoms resulting from the IDD sequence are pain, dysfunction, and stiffness, especially in the morning[13]. IDD can cause facet-mediated pain[5]. If the patient presents with these complaints and does not respond to initial therapy in four-six weeks, an X-ray imaging examination may be performed. However, to diagnose disk abnormalities or the presence of stenosis, an MRI imaging examination is required[2]. An MRI examination is commonly used to find disk degeneration[5]. Complications that can arise from IDD that are not handled properly are disk herniation, spinal stenosis, and instability[1]. In addition to physically affecting, untreated IDD will also affect someone social aspects, such as decreased productivity[2].

Current Disk Regeneration Therapy

Disruption in IVD will reduce the effectiveness of its function and require improvement because it can affect a person's quality of life[3,13]. Commonly used conservative management is physical therapy, education, and using a pharmacological agent like pain relief[1–3]. Pain medication that is usually used varies depending on the severity of the complaint. Pain medication includes non-steroidal anti-inflammatory drugs (NSAIDs), muscle relaxers, gabapentin, topical analgesics, and opioids. The usual non-pharmacological treatment to restore LBP is activity modification, rest, ice, and heat. The education that can be given is a decrease in BMI of less than 25 and limit activities that can worsen patient complaints[2].

These conservative treatments are usually limited to symptom relief and tend to be less effective[1,3]. Generally, surgical treatments will be used when conservative therapy fails, which is characterized by complaints that have affected a person's quality of life for more than one year[1,2]. This consideration was taken because surgical treatments are invasive and have a risk of complications. Even so, treatment with surgical treatments is more effective in treating pain than conservative therapy[1]. Spinal fusions are a surgical approach to treating discogenic back pain[14]. Management of surgical treatments can also be taken in certain cases by regarding the advantages and disadvantages. Apart from surgical treatment, chronic back pain can be treated with psychological therapy in the form of cognitive-behavioral therapy (CBT), progressive relaxation, and biofeedback[5].

Therapy aimed at regenerating from IVD is still under development. Cell-based therapy using MSCs is an alternative in disk regeneration. This therapy is different from therapy that is conservative and palliative, with the aim of returning the patient productivity[12]. The use of MSCs in IDD regeneration must have the ability to adjust to the cells to the microenvironment of the host[8]. The important thing that needs to be considered in the development of regenerative medicine using MSCs in IVD is the role of IVD as a weight-bearing organ. The development of regenerative medicine must pay attention to the regeneration of the IVD function as a mechanical support and load distribution[1]. The use of ASCs can have an effect on the management of IDD. This effect is measured based on the increase in the Oswestry disability index (ODI) and visual analog scale (VAS)[15].

ASCs have an anabolic effect on NP and AF. The use of ASCs on NP cells can reduce apoptosis and inhibit the expression of proinflammatory cytokines (interleukin-1b and TNF-a) and catabolic factors (matrix metalloproteinases (MMPs) and a disintegrin and metalloproteinase with thrombospondin motifs). The use of NP cells will also increase type II collagen, aggrecan, and tissue inhibitors of metalloproteinases (TIMPs). While the use of ASCs on AF cells will increase proliferation, increase anabolic action, and decrease apoptosis and expression of catabolic factors and proinflammatory cytokines[1,15].

Adiposed-derived Mesenchymal Stem Cells

Technological and scientific developments have helped in the development of MSCs. MSCs are adult stem cells that can self-regenerate and can develop widely[16,17]. MSCs can be obtained from various tissues, both postnatal and prenatal organs[7]. Bone marrow, peripheral blood, dental pulp, lungs, umbilical cord blood, muscle, placenta, and fat are sources that can be used for MSCs[16]. The ability of the multipotentiality of MSCs has now also expanded widely, from in vitro and in vivo, to epithelial and neural cell types[7,17]. MSCs tend to be non-immunogenic and have immunomodulatory properties. These natures make MSCs important in regenerative medicine and are widely used as potential therapeutic alternatives for treatment[17]. Adipose mesenchymal stem cells are multipotent stem / stromal cells derived from fat tissue and are known as adipose-derived mesenchymal stem cells (ASCs). Fat tissue is a loose connective tissue in the subcutaneous or visceral part[8]. There are two kinds of adipose tissue, namely white and brown. White adipose tissue is the source of ASCs[8,18].

To get functional stem cells, it takes several steps. These stages include harvest, breakdown, purification, and plastic adherence. After going through these stages, the calculation, identification of cells, and their potential stemness were carried out through adequate analysis. Advances in technology have helped insulate ASCs so that it is possible to obtain them within 85 minutes[9]. In 2013, the International Federation for Adipose Therapeutics and Science (IFATS) and the International Society for Cellular Therapy (ISCT) issued standards for the identification of ASCs: (1) plastic adherence cells with self-regenerating abilities; (2) Expressing cluster of differentiation 90 (CD 90), CD73, CD105, and CD36, and lack of CD11b, CD45, CD31, and CD106; and (3) and can differentiate into mesoderm tissue[8].

ASCs can differentiate into mesoderm tissue, such as adipocytes, osteocytes, and chondrocytes, allowing them to be used as material for disk regeneration. There are various sources and techniques for obtaining ASCs, but the largest source comes from abdominal fat which can be obtained by abdominoplasty or endoscopically[9,10]. In addition, a technique commonly used in humans is liposuction on subcutaneous fat. After taking ASCs, the

culture was carried out in the conditioned medium[10]. ASCs also can be isolated from the infrapatellar fat pad (IPFP-ASCs) which is located close to the synovial membrane. IPFP-ASCs have advantages in their proliferation capabilities and differentiation capacities[6].

Adipose-Derived Mesenchymal Stem Cells versus Bone Marrow-Derived Mesenchymal Stem Cells

There are various resources available for creating MSCs that can be used in regenerative medicine. Bone Marrow-Derived Mesenchymal Stem Cells (BMSCs) are a common source of MSCs that have been tested in clinical trials[7,9,16]. The advantage of using BMSCs is that they can be obtained from the same individual and the side effects tend to be smaller than that of pluripotent stem cells. BMSCs are believed to have a lower risk of causing tumors or malignancies and immunological reactions. In addition, from an ethical perspective, the use of BMSCs has been widely used so that it has a lower ethical risk. However, the numbers are limited and their collection through invasive aspiration is a drawback for these BMSCs[6,9]. In addition, BMSCs from younger donors were more effective than older donors. This indicates the age-dependent effect of the MSCs function[16].

In contrast to BMSCs, patients experience less discomfort in taking ASCs and the risk is lower[6]. ASCs have more sources, so they are easier to collect and tend to be less invasive. Various sources and collection techniques make it easier for ASCs to be obtained because they can adjust to the conditions of each laboratory. Techniques that can be used to obtain ASCs are liposuction and abdominoplasty[9,10,19]. When compared to BMSCs, ASCs have similar characteristics to BMSCs. The proliferation ability and marker stem cell expression level of ASCs were higher than BMSCs. In addition, ASCs are more resistant to apoptosis and have a lower risk of becoming malignant[6].

Role of Preconditioning MSCs

One of the requirements for MSCs to be used in regenerative medicine is to have a sufficient number of cells to reach the location of damage and be able to survive for a long time. The minimum number of MSCs cells required in a clinical trial for transplantation is 1 x 10^7. It takes 1-3 months for cell expansion in vitro to obtain this number of cells[16]. Various preconditioning strategies have been implemented to increase the regeneration capacity of MSCs. There were various preconditioning strategies for MSCs, such as environmental modification, use of growth factors or small molecules, and pharmacological or chemical agents[7,20].

As one of the MSCs, ASCs require a preconditioning strategy to maximize reparative capacity and regeneration. The preconditioning strategy of MSCs maximizes the potential of therapeutic efficiency. Therapy with ASCs in degenerative diseases, such as IDD, requires maximum regeneration capacity[7]. The faster growth of ASCs, the lower the costs with better results. The risk of genetic alteration and cell culture contamination in vitro can be minimized from the faster growth of ASCs, maximizing their use in regeneration[9].

1. Preconditioning using environment modification

Preconditioning with environmental modification strategies can increase the regeneration potential of MSCs. A usable condition is to create an environment with low oxygen levels[7]. This environmental modification has been shown to have a beneficial impact on ASCs. Environmental conditions with low oxygen levels multiply the multiplication and migration of ASCs by the generation of reactive oxygen species (ROS) and downstream phosphorylation of platelet-derived growth factor receptor-beta, ERK1 / 2, and Akt. If this condition is prolonged, it will increase miR-210. The effect of this series will increase the secretion of vascular endothelial growth factor thereby increasing the potential of the generational ability of ASCs[21,22]. The impact of preconditioning with low oxygen levels will increase the ability of cell proliferation and angiogenic potential so that the ability to survive MSCs will also increase[20].

Other strategies that can be used are hyperoxia and thermal preconditioning. Hyperperoxia preconditioning can reduce the number of cells undergoing apoptosis so that it can increase the potency of MSCs. MSCs incubated at 42°C for 2 hours before transplantation, can increase cell survival and reduce the oxide-induced apoptosis of MSCs. Incubation at this temperature inhibits the apoptosis pathway and prevents oxidation stress[16].

Another environmental modification that can be used is to culture MSCs in a 3-dimensional (3D) environment, known as a spheroid[7,17]. This culture associated with the production of factors that can increase the factors associated with proliferation and vascularization. These factors also influence the survival of the cells. The result of this strategy is an increase in these cells' immunomodulatory, anti-apoptotic activities, anti-fibrotic, and angiogenic. In ASCs, the use of 3D cultures can stimulate pro-angiogenic abilities[17]. The use of these 3D spheroids will produce large amounts of anti-inflammatory protein TNF-α-stimulated gene/protein 6 (TSG-6). However, cell activation in the spheroid is also very much influenced by the culture medium used[7].

2. Preconditioning using growth factors, cytokines, chemokines, hormones

Apart from environmental modification, the use of a conditioned medium derived from growth factors, cytokines, chemokines, and hormones can increase the regeneration capacity of ASCs. The use of epidermal growth factor (EGF) has been shown to increase the motility and migration capabilities of MSCs without affecting their multipotential ability. The use of EGF in preconditioning MSCs increases the expression of EGF-receptor proteins, such as Vascular endothelial growth factor (VEGF) and Hepatocyte growth factor (HGF) which play a role in accelerating wound healing[7,20,23]. The increase in early progenitor proliferation in MSC can be obtained by using soluble EGF without causing differentiation. EGF is able to increase osteogenic differentiation[23]. Besides EGF, Transforming growth factor β (TGF-β) can be used to increase VEGF formation when combined with hypoxia conditions or TNF-α[7].

Besides using EGF, there is another culture medium (CM) alternatives that can be used for pre-conditioning. Tumor necrosis factor-α (TNF-α) can also be used in preconditioning MSCs and stimulates the release of cytokines, chemokines, and proteases. The use of this CM will trigger the secretome of MSCs including inflammatory molecules. These released substances are involved in the inflammatory process, where inflammation is the key to the injury response. The cytokines and chemokines produced to play a role in the regeneration process[7,20]. Another strategy used in CM is to use hormones. The hormones melatonin and angiotensin II help increase the ability of cells to survive in oxidative stress conditions. This increase corresponds to an increase in VEGF levels[17].

3. Preconditioning using pharmacological or chemical agents

The use of pharmacological or chemical agents is another way to maximize the yield of MSCs, including ASCs. Agents that have been studied, including using atorvastatin, curcumin, diazoxide, deferoxamine, valproate, and lithium chloride[7,17]. Mostly, the use of these agents will improve the survival of the transplanted MSCs. This increase is due to an increase in VEGF, Fibroblast growth factor-2 (FGF-2), or HGF, and activation of the Akt signaling pathway. The increase in these molecules will increase neovascularization[17].

The use of atorvastatin in preconditioning MSCs can increase the expression of the CXC chemokine receptor 4 (CXCR4) and MSC migration. Other agents that can increase the expression of CXCR4 are valproate and lithium chloride[7]. CRCX4 signaling plays an important role in migrating MSCs to the damaged side of the network[24]. The use of atorvastatin can increase the potential therapeutic effects of MSCs and the regenerative efficacy of MSC transplantation[7,17,24]. Atorvastatin also has the potential to increase the immunomodulatory capacity of MSCs[17].

Curcumin is an antioxidant and anti-inflammatory agent that can prevent damage from oxidative stress. Another property possessed by curcumin is anti-apoptotic[25]. The effect of using curcumin increases the therapeutic potential of MSCs[7,25]. Another agent that can be used for preconditioning to prevent damage from oxidative stress is diazoxide. Diazoxide is a mitochondrial ATP-sensitive potassium channel opener that can upregulate HGF and FGF-2. Deferoxamine can be used as a preconditioning agent which will have the effect of increasing cell migration[7].

Role of preconditioning adipose mesenchymal stem cells in regeneration disk

Based on the summary above, the use of preconditioning strategies can improve the regenerative therapy capabilities of MSCs. The development of MSCs technology has experienced rapid development in the

field of regenerative medicine. The majority of existing studies are at the clinical trial level on animals[7]. The use of preconditioning with the above methods can increase cytoprotective factor molecules, pro-regenerative, pro-angiogenic (VEGF), and immunomodulatory cytokines[17].

ASCs are one of the most developed MSCs today. One of the advantages of ASCs is that there are fewer cells that need to be transplanted than other MSCs. The reduction in the number of transplanted cells will be followed by a decrease in the side effects of this MSCs therapy compared to others[20]. This makes it possible for ASCs to be used to treat degenerative diseases, such as osteoarthritis and IDD[7]. The use of ASCs in orthopedic surgery can provide promising results due to their ability to repair cartilage and bone[6].

As a group of MSCs, the use of ASCs can be optimized by using proper preconditioning before the ASCs are transplanted[6]. Preconditioning on ASCs can change its function in various ways, such as apoptosis, difference, migration, and regeneration. ASCs are the same as MSCs in general, which respond to changes in their environment[8] In the field of orthopedic surgery, environmentally modified preconditioning is the widely used preconditioning of ASCs[6]. The secretion of angiogenic and neurotrophic factors in the use of ASCs can be increased by using the preconditioning strategy by modifying the environment into a hypoxic environment. Angiogenic factors that can be triggered by this strategy are Angiopoietin-2 (Ang-2) and VEGF[13].

Preconditioning using cytokines in ASCs is no different from MSCs in general as described above. One that has received attention is preconditioning with pro-inflammatory cytokines or mediators because it can increase its immunomodulatory properties and therapeutic potential. An example of the use of cytokines used in ASCs preconditioning is TNF-α, especially in the orthopedic field because it can promote bone generation by increasing proliferation, mobilization, and osteogenic differentiation. The use of proinflammatory cytokines is one way to increase the effectiveness of ASCs as a therapy[8]. Even so, the use of preconditioning with proinflammatory cytokines is still developing, especially to determine the side effects that can be caused and the benefits that can be obtained.

Another example of using a preconditioning strategy in culturing ASCs is the combination of a hypoxic environment and a medium culture medium with TNF-α. This method can increase NGF. The use of ASCs has good potential to increase the expression and secretion of factors that play a role in nerve survival and vascularization under hypoxic and inflammatory conditions. This is compatible with the environment in IVD which has a hypoxic and inflammatory environment, thus requiring angiogenesis and innervation for healing[13].

The use of pharmacological agents and chemical or bioactive molecules may also be used in the preconditioning of ASCs such as MSCs. The use of curcumin improved the retention of ASCs transplanted and reducing apoptosis and increased neovascularization[8,26]. The use of curcumin in ASCs preconditioning can reduce apoptosis, increase VEGF, and increase tolerance to damage due to oxidative stress so as to increase the therapeutic potential of ASCs. These factors make curcumin a promising alternative as a strategy to increase the effectiveness of ASCs[26]. Apart from curcumin, atorvastatin, CRX4, and other agents can be used to maximize ASCs like most common MSCs[17].

Conclusion

Preconditioning ASCs have an important role in disk regeneration. The use of ASCs in regenerative medicine in the orthopedic field has enabled IDD, as a degenerative disease, to be fully managed. Treatment of IDD, which has been symptomatic only, can be curative because of its ability to regenerate disks. One of the important keys in using ASCs for disk degeneration is the preconditioning step of the ASCs. The pre-conditioning strategy chosen will determine the efficiency and capacity of these ASCs for use in regeneration disks. Preconditioning on ASCs can change its function in various ways, such as apoptosis, difference, migration, and regeneration. Preconditioning ASCs, just like MSCs in general, can increase cytoprotective factor molecules, pro-regenerative, pro-angiogenic (VEGF), and immunomodulatory cytokines which are important in disk regeneration.

Acknowledgements: Nil.

Source of Funding: Self-funding. The authors received no financial support for the research, authorship,

and publication of this article.

Conflict of Interest Statement: Nil.

References

1. Ishiguro H, Kaito T, Yarimitsu S, Hashimoto K, Okada R, Kushioka J, et al. Intervertebral disc regeneration with an adipose mesenchymal stem cell-derived tissue-engineered construct in a rat nucleotomy model. Acta Biomater. 2019 Mar 15;87:118–29.
2. Casiano VE, Dydyk AM, Varacallo M. Back Pain. StatPearls. StatPearls Publishing; 2020.
3. Rustenburg CME, Emanuel KS, Peeters M, Lems WF, Vergroesen P-PA, Smit TH. Osteoarthritis and intervertebral disc degeneration: Quite different, quite similar. JOR Spine. 2018 Dec 1;1(4):e1033.
4. Hoogendoorn RJW, Lu ZF, Kroeze RJ, Bank RA, Wuisman PI, Helder MN. Adipose stem cells for intervertebral disc regeneration: Current status and concepts for the future: Tissue Engineering Review Series. J Cell Mol Med. 2008 Dec;12(6A):2205–16.
5. Urits I, Burshtein A, Sharma M, Testa L, Gold PA, Orhurhu V, et al. Low Back Pain, a Comprehensive Review: Pathophysiology, Diagnosis, and Treatment. Curr Pain Headache Rep. 2019 Mar 1;23(3):23.
6. Huri PY, Hamsici S, Ergene E, Huri G, Doral MN. Infrapatellar Fat Pad-Derived Stem Cell-Based Regenerative Strategies in Orthopedic Surgery. Knee Surg Relat Res. 2018 Sep 1;30(3):179–86.
7. Schäfer R, Spohn G, Baer PC. Mesenchymal stem/stromal cells in regenerative medicine: Can preconditioning strategies improve therapeutic efficacy? Transfus Med Hemotherapy. 2016 Jul 1;43(4):256-67.
8. Seo Y, Shin TH, Kim HS. Current strategies to enhance adipose stem cell function: An update. Int J Mol Sci. 2019;20(15).
9. Francis SL, Duchi S, Onofrillo C, Di Bella C, Choong PFM. Adipose-derived mesenchymal stem cells in the use of cartilage tissue engineering: The need for a rapid isolation procedure. Stem Cells Int. 2018;2018.
10. Hye Kim J, Gyu Park S, Kim WK, Song SU, Sung JH. Functional regulation of adipose-derived stem cells by PDGF-D. Stem Cells. 2015 Feb 1;33(2):542–56.
11. Shapiro IM, Vresilovic EJ, Risbud M V. Is the spinal motion segment a diarthrodial polyaxial joint: What a nice nucleus like you doing in a joint like this? Bone. 2012 Mar;50(3):771–6.
12. Iatridis JC, Nicoll SB, Michalek AJ, Walter BA, Gupta MS. Role of biomechanics in intervertebral disc degeneration and regenerative therapies: What needs repairing in the disc and what are promising biomaterials for its repair? Spine J. 2013 Mar;13(3):243–62.
13. Binch ALA, Richardson SM, Hoyland JA, Barry FP. Combinatorial conditioning of adipose derived-mesenchymal stem cells enhances their neurovascular potential: Implications for intervertebral disc degeneration. JOR SPINE. 2019 Dec 19;2(4).
14. Meisel HJ, Agarwal N, Hsieh PC, Skelly A, Park JB, Brodke D, et al. Cell Therapy for Treatment of Intervertebral Disc Degeneration: A Systematic Review. Glob Spine J. 2019 May 1;9(1_suppl):39S-52S.
15. Loibl M, Wuertz-Kozak K, Vadala G, Lang S, Fairbank J, Urban JP. Controversies in regenerative medicine: Should intervertebral disc degeneration be treated with mesenchymal stem cells? JOR SPINE. 2019 Mar 1;2(1):e1043.
16. Chen Z, Chen L, Zeng C, Wang WE. Functionally improved mesenchymal stem cells to better treat myocardial infarction. Stem Cells Int. 2018;2018.
17. Ferreira JR, Teixeira GQ, Santos SG, Barbosa MA, Almeida-Porada G, Gonçalves RM. Mesenchymal stromal cell secretome: Influencing therapeutic potential by cellular pre-conditioning. Front Immunol. 2018;9.
18. Zwick RK, Guerrero-Juarez CF, Horsley V, Plikus M V. Cell Metabolism Review Anatomical, Physiological, and Functional Diversity of Adipose Tissue. 2018;
19. 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. Scand J Clin Lab Invest. 2020 Jul 1;80(4):318□26.
20. Baer PC, Overath JM, Urbschat A, Schubert R, Koch B, Bohn AA, et al. Effect of different preconditioning regimens on the expression profile of murine adipose-derived stromal/stem cells. Int J

Mol Sci. 2018 Jun 10;19(6).

21. Kakudo N, Morimoto N, Ogawa T, Taketani S, Kusumoto K. Hypoxia enhances proliferation of human adipose-derived stem cells via HIF-1α activation. PLoS One. 2015 Oct 14;10(10).
22. Kang S, Kim SM, Sung JH. Cellular and molecular stimulation of adipose-derived stem cells under hypoxia. Cell Biol Int [Internet]. 2014 [cited 2020 Nov 5];38(5):553–62. Available from: https://pubmed.ncbi.nlm.nih.gov/24446066/
23. Tamama K, Kawasaki H, Wells A. Epidermal Growth Factor (EGF) treatment on Multipotential Stromal Cells (MSCs). Possible enhancement of therapeutic potential of MSC [Internet]. Vol. 2010, Journal of Biomedicine and Biotechnology. J Biomed Biotechnol; 2010 [cited 2020 Nov 6]. Available from: https://pubmed.ncbi.nlm.nih.gov/20182548/
24. Tian XQ, Yang YJ, Li Q, Xu J, Huang P Sen, Xiong YY, et al. Combined therapy with atorvastatin and atorvastatin-pretreated mesenchymal stem cells enhances cardiac performance after acute myocardial infarction by activating SDF-1/CXCR4 axis. Am J Transl Res. 2019;11(7):4214–31.
25. Jiang S, Han J, Li T, Xin Z, Ma Z, Di W, et al. Curcumin as a potential protective compound against cardiac diseases. Pharmacol Res. 2017 May 1;119:373–83.
26. Liu J, Zhu P, Song P, Xiong W, Chen H, Peng W, et al. Pretreatment of Adipose Derived Stem Cells with Curcumin Facilitates Myocardial Recovery via Antiapoptosis and Angiogenesis. Stem Cells Int. 2015;2015.