

Comparison of five growth factors in the secretomes of hypoxic bone marrow mesenchymal stem cells and hypoxic adipose mesenchymal stem cells



Zainurrahman Kurnia Putra¹, Ferdiansyah Mahyudin^{1*}, Heri Suroto¹

ABSTRACT

Introduction: The Secretome consists of proteins secreted by human cells, containing various serum proteins and growth factors (GFs) that can aid in tissue repair and regeneration. The two most commonly utilized tissues for producing secretomes are Adipose-Derived Mesenchymal Stem Cells (AdMSCs) and Bone Marrow Mesenchymal Stem Cells (BM-MSCs). Hypoxic conditions are also one of the critical preconditioning factors that enhance the efficacy of the secretome. By analyzing and comparing the growth factor profiles between these two cellular sources, we aim to elucidate their potential therapeutic applications and further our understanding of the underlying mechanisms driving their regenerative abilities.

Methods: A randomized controlled post-test-only group design study was conducted at the Cell and Tissue Bank of Dr. Soetomo Hospital. Secretome was derived from AdMSCs and BM-MSCs cultured under hypoxic conditions (5% O₂ level). VEGF, PDGF, IGF-1, TGF- β , and bFGF from each group were analyzed by ELISA and compared.

Results: Each group consisted of 7 samples. PDGF levels were significantly higher in the AdMSCs group (7.99 ± 0.644) compared to the BM-MSC group (7.21 ± 0.46); ($p = 0.013$; $p < 0.05$). Meanwhile, IGF-1 levels were significantly higher in the BM-MSCs group ($16.42 \pm 1,878$) compared to the AdMSCs group (14.2 ± 0.988); ($p = 0.021$; $p < 0.05$). There were no significant differences in other growth factors between the two groups, including bFGF (0.57 ± 0.05 vs. 0.61 ± 0.07 ; $p = 0.207$), VEGF ($1,644.00 \pm 123.65$ vs. $1,588.26 \pm 117.80$; $p = 0.405$), and TGF- β ($1,323.29 \pm 145.36$ vs. $1,264.06 \pm 121.60$; $p = 0.425$).

Conclusion: No significant differences in VEGF, TGF- β , and bFGF between the two groups. Therefore, AdMSC can be a better alternative to BM-MSC as it is easier to obtain, readily available, and has higher PDGF content.

Keywords: stem cell, secretome, growth factor, bone marrow stem cell, adipose-derived stem cell.

Cite This Article: Putra, Z.K., Mahyudin, F., Suroto, H. 2024. Comparison of five growth factors in the secretomes of hypoxic bone marrow mesenchymal stem cells and hypoxic adipose mesenchymal stem cells. *Bali Medical Journal* 13(1): 577-581. DOI: 10.15562/bmj.v13i1.5155

¹Department of Orthopaedic and Traumatology, Faculty of Medicine, Universitas Airlangga, Surabaya, Indonesia

*Corresponding to:
Ferdiansyah Mahyudin. ¹Department of Orthopaedic and Traumatology, Faculty of Medicine, Universitas Airlangga, Surabaya, Indonesia;
ferdiansyah@fk.unair.ac.id

Received: 2023-10-30
Accepted: 2023-12-22
Published: 2024-01-23

INTRODUCTION

Secretomes of stem cells are one of the most promising regenerative therapies with the potential for future development.¹ Secretomes are defined as the collection of substances secreted by an organism, tissue, or cell into the extracellular space at specific times and locations. Secretomes contain various molecules, including growth factors (GFs), that can promote tissue regeneration.²

The use of secretomes as a cell-free therapy offers several advantages. Secretomes can address immunological incompatibility, tumorigenicity, and infection transmission, which are safety concerns associated with stem cell transplantation. Secretomes can be

manufactured in large quantities and kept for extended periods without losing their effectiveness, eliminating the need for harmful cryopreservatives. Secretomes can also be preconditioned to achieve specific outcomes, reducing production costs and time.³

One approach to preconditioning stem cells is by growing them in an environment with low oxygen levels. Hypoxic preconditioning involves culturing cells at oxygen levels of 1-7 percent, which mimics the physiological oxygen levels found in various tissues: 1 to 3 percent in bone marrow, around 10 to 15 percent in adipose tissue, and 2 to 9 percent in nearly every other tissue in the body, instead of the standard culture oxygen concentration of 21 percent.⁴

Hypoxic preconditioning enhances the activity of growth factors such as IGF-1, TGF- β , VEGF, and bFGF by increasing their paracrine activity⁵ through the HIF-1 α activation.⁴ By preconditioning stem cells in a hypoxic environment, we aim to maximize the potential of therapeutics of the secretome.

Adipose-Derived Mesenchymal Stem Cells (AdMSC) and Bone Marrow Mesenchymal Stem Cells (BM-MSCs) are the most common sources of mesenchymal stem cell secretome. BM-MSCs have fewer available sources in the human body than AdMSCs. Obtaining AdMSCs is less invasive and time-consuming compared to BM-MSCs.⁶ We aim to investigate whether there are differences in growth factor content between AdMSCs' and BM-

MCSS' secretomes cultured in the same hypoxic oxygen environment (5%).

METHODS

This is a Randomized Controlled Post-Test Only Group Design experimental *in vitro* study using secretomes derived from AdMSCs and BM-MSCs cultured under hypoxic conditions collected using from the Cell and Tissue Bank RS Dr. Soetomo products with each group consisting of 7 samples.

AdMSCs Processing

1 cm³ fat tissue devoid of any connective tissue or blood clots was procured by the Cell and Tissue Bank and sent to the stem cell laboratory. The adipose tissue was extricated from the transport medium and rinsed thoroughly with a PBS solution to eliminate any adhering red blood cells. The tissue was coarsely minced and combined with collagenase enzyme before being transferred to a container equipped with a magnetic stirring technique. The mixture of tissue and enzyme was then placed in a container set to 37 degrees Celsius for thirty minutes. This allowed for the complete dissolution of the fat tissue. A stopper medium was introduced post-dissolution, and the solution was further incubated for 10 minutes to ensure uniform mixing. The resulting solution was filtered through sterile gauze into a 50 ml container to remove any remaining undissolved adipose tissue. The filtered solution underwent centrifugation at 3,000 revolutions per minute for 5 minutes, resulting in the formation of solid masses or pellets. These pellets were subsequently resuspended in MEM alpha medium until a uniform solution was achieved. The resuspended solution was then transferred to a 10 cm petri dish and placed in a carbon dioxide incubator for twenty-four hours to allow cell adherence to the dish's surface. The adherent cells were subsequently refreshed with fresh medium every 2 days until colonies formed and attained 80% confluency.^{7,8}

Colony-forming mesenchymal stem cells were expanded to achieve the required dosage for clinical applications. Once the cells in a monolayer reached 80% confluency, they underwent a rejuvenation process known as passaging.

To passage the cells, the medium was removed from the petri dish and the monolayer was washed with a Phosphate Buffered Saline solution. Subsequently, a triple express enzyme was incorporated into the dish, and the mixture was incubated for a duration of five minutes. This process facilitated the detachment of the monolayer from the surface of the petri dish. Following detachment, a stopper medium was introduced, and gentle agitation was applied to the cells until they were dispersed into individual entities. The cell suspension was then transferred to a conical tube and subjected to centrifugation until a pellet formed. The pellet was resuspended in Alpha MEM medium and agitated until a uniform solution was obtained, which was then transferred to a fresh petri dish.^{7,8}

BM-MSCs processing

The bone marrow tissue is extracted from the transport medium which was taken previously from the samples of bone marrow tissue and subsequently rinsed using a PBS solution. It is then combined with histopaque enzymes and transferred into a container with a magnetic stirring technique. The bone marrow sample within the container was placed upon a hot plate for a period of thirty minutes at a constant temperature of 37°C to facilitate the complete dissolution of the bone marrow. Once dissolved, a medium stopper is added, and the mixture is incubated for an additional 10 minutes until a uniform solution is achieved. The solution is then transferred to a 50 ml conic tube and filtered under sterile conditions to remove any remaining insoluble bone marrow tissue. The resulting solution is centrifuged at 3,000 rpm for 5 minutes until a buffy coat is formed. The pellet is then resuspended in an alpha MEM medium to create a uniform mixture. This mixture is then transferred to a ten-centimeter petri dish and placed in a Carbon dioxide incubator for twenty-four hours, allowing the cells to adhere to the base of the dish. The attached cells are then refreshed every two days until colonies form and reach approximately 80% confluency.

Passaging is needed in the cells that have formed a monolayer with a confluency of up to 80%. Passaging involves removing the medium from the petri dish and

rinsing the monolayer with PBS solution. Subsequently, the triple-express enzyme is added, and the mixture is incubated for 5 minutes until the monolayer detaches from the petri dish's base. After detachment, a medium stopper is added, and the solution is resuspended until it becomes a single-cell suspension. The solution containing the single cells is transferred to a conical tube and centrifuged until a pellet forms. The pellet is then supplemented with an alpha MEM medium and resuspended. This solution is then seeded in a new petri dish.

The Cell and Tissue Bank established a hypoxic condition for culturing at a 5% oxygen level for several days until the 4th and 8th passages. Cells from MSC obtained from the study's 1st stage are expanded up to the 4th and 8th passages. The cells are aseptically collected and seeded onto a culture dish at a density of 2x10⁷ cells/cm² in 10 dishes of 5 cm with an Iscove's Modified Dulbecco's Medium containing 15% Fetal bovine serum, Stem Cell stimulator supplement, and antibiotics (100 U penicillin/100µg/mL streptomycin) at a temperature of 37°C, using five percent of carbon dioxide, and ninety-five percent air.⁹

Hypoxic conditions are crucial for maintaining the quiescence of Mesenchymal Stem Cells (MSCs). These conditions can be achieved by placing the culture flask in a dedicated hypoxic incubator (e.g., Modular Incubator Chamber) and administering a precise dose of O₂ at a concentration of 5%. This controlled environment is essential during the early passage of MSCs to ensure the proper maintenance and preservation of their unique properties. The collected medium is then transferred into a 50 ml dialysis membrane tubing. The dialysis tubing is sealed at both ends and placed in a 500 ml beaker containing a cold PBS solution. A magnetic bar is inserted, and the beaker is placed on top of a hot plate magnetic stirrer. The solution is stirred at 500 rpm overnight until the color of the conditioned medium in the dialysis tube fades. After fading, the tubing is removed from the PBS solution, cut using sterile scissors, and the contents are poured into a 250 ml cup. The metabolite product is then filtered using a 0.22-micron filter and aliquoted into a 50 ml conical tube.

The tubes are placed in a sterile medipack, sealed, and stored at -20°C .

All reagents, standard solutions, and samples were prepared at room temperature. Standard solutions were serially diluted in the standard diluent. 50 μL of diluted standards were added to standard wells, with one well reserved as a blank. 40 μL of samples were added to sample wells, followed by 10 μL of anti-IGF-1 antibody, anti-VEGF antibody, anti-PDGF antibody, anti-TGF- β antibody, or anti-FGF antibody. 50 μL of streptavidin-HRP was added to both the standard wells and the sample wells. The plates were covered with plastic wrap and incubated at 37°C for one hour. After incubation, each well in the plate was washed five times with 350 μL of 5x wash buffer. A 50 μL substrate solution was then added to each well, followed by 50 μL of substrate solution B. The plate was covered with aluminum foil and incubated at 37°C for 10 minutes. Following incubation, 50 μL of stop solution was added, and the plate was incubated at room temperature for 10 minutes. Finally, 150 μL of standard diluent was added to the blank wells, and the OD of each well was measured using an ELISA reader at a wavelength of 450 nm.

Data Analyzes

Data collected were analyzed statistically using the SPSS 23 program. In this study, quantitative data were obtained as independent samples. The normality test was performed using Saphiro-Wilk. If the data is normally distributed, an unpaired t-test is performed, and if the data is not normally distributed, the Mann-Whitney Test is used.

RESULTS

First, we have to conduct a normality test using the Shapiro-Wilk Test. From the normality test, it is found that the only PDGF in hypoxic AdMSCs isn't normally distributed. While the other parameters (TGF- β , VEGF, IGF-1, and bFGF) in hypoxic AdMSCs and BM-MSCs are normally distributed with $p > 0.005$ (as shown in [Table 1](#)). Hence, we could do a T-independent test for all parameters except hypoxic AdMSCs using PDGF.

Due to the normal distribution data

Table 1. Normality test using Saphiro Wilk Test.

Parameter	Hypoxic AdMSCs	Hypoxic BM-MSCs
PDGF	0.004*	0.673
TGF- β	0.702	0.090
VEGF	0.362	0.531
IGF1	0.721	0.388
bFGF	0.165	0.563

Table 2. Independent T-Test between AdMSCs hypoxic and BM-MSCs hypoxic.

Parameters	n	Mean Growth Factor Content Numbers	Independent T-test results (p)
TGF- β			0.425
AdMSC hypoxic	7	1,323.29 \pm 145,357	
BM-MSC hypoxic	7	1,264.06 \pm 121,598	
VEGF			0.405
AdMSC hypoxic	7	1,644 \pm 123,651	
BM-MSC hypoxic	7	1,588,26 \pm 117,801	
IGF1			0.021*
AdMSC hypoxic	7	14.2 \pm 0.988	
BM-MSC hypoxic	7	16.42 \pm 1,878	
bFGF			0.207
AdMSC hypoxic	7	0.57 \pm 0.045	
BM-MSC hypoxic	7	0.61 \pm 0.07	

Table 3. Mann-Whitney Test for PDGF in both groups.

Parameters	n	Mean Growth Factor Content Numbers	Mann Whitney test results (p)
PDGF			0.013*
AdMSC hypoxic	7	7.99 \pm 0.644	
BM-MSC hypoxic	7	7.21 \pm 0.46	

of all parameters of the growth factor contents except for PDGF (as shown in [Table 1](#)), an Independent T Test could be used for all growth factors except for PDGF. Mann-Whitney test could be performed for the PDGF test.

The mean numbers of the growth factors content, the independent T-test, and the Mann-Whitney Results are shown in [Table 2](#) and [Table 3](#), and statistically, IGF-1 has a significant Independent T Test ($p=0.021$; $p<0.05$). It is shown in [Table 2](#) that the other parameters such as TGF- β , VEGF, and bFGF ($p=0.425$; $p=0.405$; and $p=0.207$) compared using Independent T-test aren't statistically different between AdMSCs and BM-MSCs. The Mann-Whitney test of PDGF in both groups also showed there is a statistical difference ($p=0.013$; $p<0.05$) as shown in [Table 3](#).

DISCUSSION

The application of the secretome in cell-free therapy provides distinct benefits compared to conventional cell-based approaches. In stem cell-based therapy, only a small percentage (<1%) of transplanted MSCs survive after one week, leading to limited therapeutic efficacy.³ The secretome, on the other hand, can bypass this issue, as it does not rely on cell survival for its therapeutic effects. The secretome also has a lower immunogenicity compared to living cells and proliferative cells. This reduced immunogenicity is attributed to the lower protein expression secretion of the secretome, making it less likely to trigger an immune response.¹⁰ Additionally, the secretome provides a practical and cost-effective approach for clinical applications. It eliminates the need for invasive cell

collection procedures and allows for large-scale production under controlled laboratory conditions.¹¹ The secretome can be tailored and modified to meet specific therapeutic requirements, offering greater flexibility in treatment strategies.³ The development and validation of the secretome can be streamlined, reducing the time and cost associated with stem cell development and characterization.⁶

The microenvironment is also important for culturing stem cells and secretomes, such as preconditioning and pH. During the culture of the secretome in the Cell and Tissue Bank, we found the pH in both groups is approximately 7. Based on Fliefel's research, it was found that pH has a role in culturing Mesenchymal Stem Cells. If the pH was acidic (around 6.3 and 6.7), the senescence would highly occur and affect the proliferation of the Mesenchymal Stem Cell. While some studies found that an alkaline pH delayed MSC development and lowered Alkaline Phosphatase activity, others found that lowering the extracellular pH decreased collagen levels and Alkaline Phosphatase activity in mesenchymal stem cells.¹²

Normal physiological oxygen pressure levels range from 1 to 3 percent in bone marrow, around 10 to 15 percent in adipose tissue, and 2 to 9 percent in nearly every other tissue in the body.⁴ When conditioned with hypoxia (oxygen pressure levels ranging from 0 to 10%), it is expected that there will be an increase in regeneration, cytoprotective molecules, multipotency, proliferation of MSCs, as well as an improvement in MSC's ability to survive in less conducive environments.^{13,14} Although in hypoxia, the proliferation is slower than in normoxia, the formation of MSCs in hypocyanic cultures is faster than in normoxia.⁴ This condition also increases the paracrine activity of MSCs.^{15,5} Paracrine activity is expected to be an increase in growth factors such as VEGF¹⁴ through the activation of HIF-1 α ,⁴ as well as increases in FGF, IGF-1, TGF- β , EGF, HGF.²

In this research, we found that the significant differences were between IGF-1 and PDGF. AdMSCs had a statistically higher PDGF content than BM-MSCs. Yet, the IGF-1 in BMSCs was statistically higher than in AdMSCs. While other growth factors (TGF- β , VEGF, and bFGF)

are not statistically different. Several previous researches had different results. In vivo studies have demonstrated that ASCs exhibit superior secretion of several growth factors compared to bone marrow (BM)-MSCs.¹⁶ In Dmitrieva's research, the level of VEGF, TGF β 1, and other growth factors secretion at early passages in BM-MSC was significantly higher than in AdMSC.¹⁷ AdMSCs produce growth factors affecting regeneration of the tissue, nerve, new blood vessel growth also differentiation and prevent apoptosis of the cells such as VEGF, IGF 1, TGF β , and HGF. AdMSCs also secrete platelet-derived growth factor (PDGF), which is crucial for angiogenesis. Research has demonstrated that stimulating cells with PDGF increases the production of extracellular vesicles, hence enhancing their proangiogenic qualities. AdMSCs produce IGF-1 which enhances the proliferating process of the cell, regeneration, and differentiation and the level of IGF decreases as the donor ages.¹⁶

Shin stated in their research that AdMSCs exhibited a higher abundance of proteins associated with cytoplasmic structure and development, whereas BM-MSCs showed a greater presence of proteins. Those proteins are not only related to chemotaxis and the process by which cells grow and divide to form new cells but also the process by which epithelial cells lose their epithelial characteristics and gain mesenchymal characteristics, becoming more mobile and invasive.¹⁸ Pires discovered that the BM-MSCs may be most effective in lowering oxidative stress. On the other hand, the secretome of AdMSCs is more advantageous in reducing excitotoxicity.¹⁹ MSCs derived from various cellular sources will demonstrate variations in their differentiation potential as a result of disparities in protein expression patterns, which are the primary determinants of differentiation.

AdMSCs have several benefits over BM-MSCs, mostly due to their reasonably simple collecting process via liposuction for example. These cells can maintain their long-term phenotypic and flexibility in an in vitro experiment. Additionally, these cells exhibit a lesser level of immunogenicity.⁶ AdMSC contains a greater quantity of stem cells compared

to BM-MSC. One gram of adipose tissue produces around 3.5×10^5 to 1×10^6 AdMSC, but one gram of bone marrow aspiration only provides 5×10^2 to 5×10^4 BM-MSC.²⁰ Furthermore, AdMSCs possess notable benefits in terms of their ability to multiply and differentiate. Additionally, the therapeutic impact of these stem cells produced from adipose tissue is not significantly influenced by factors such as age or region of origin. Consequently, AdMSC has successfully garnered significant attention. AdMSCs have emerged as a leading choice for cell-free therapies in the field of tissue engineering and regenerative medicine, owing to their unique properties and diverse array of applications.⁶

The AdMSC secretome is quite intricate. It has the capacity to release proteins that are important in the regenerative process of tissues and cells, new blood vessel formation, differentiation, and also wound healing. AdMSCs possess significant regeneration capabilities and are highly desirable for applications in tissue engineering and regenerative therapy across various medical and surgical fields.²¹

One limitation of this study is that it solely relied on a process to quantify a specific Growth Factor using an ELISA kit. This approach may not provide a comprehensive understanding of the complex interplay of various growth factors and other microenvironments such as microvesicle, extravesicle, and exosome levels involved in the biological process being studied. Further research on microvesicle, extravesicle, and exosome levels is required to gain more insight into the effect and mechanism of secretome for regenerative therapy.

CONCLUSION

In conclusion, the secretome of both AdMSCs and BMSCs encompasses a diverse array of bioactive molecules that actively contribute to tissue regeneration and the regulation of immune responses. While the concentrations of VEGF, TGF- β , and bFGF were not significantly different between the two groups in hypoxic conditions, the secretome of AdMSCs had higher levels of PDGF, whereas the secretome of BMSCs

had higher levels of IGF-1 in hypoxic conditions. These variations could have significant implications when selecting MSCs secretome for regenerative medicine applications. Therefore, AdMSC can be a better alternative to BM-MSC as it is easier to obtain, readily available, and has higher PDGF content. Further research is necessary to fully elucidate the mechanisms underlying the therapeutic effects of the secretome and optimize its clinical use.

AUTHOR'S CONTRIBUTION

All authors made substantial contributions to the conception and design of the study, the collection of the data, the analysis and interpretation of data, the drafting of the article, the critical revision of the article for important intellectual content, and the final approval of the version to be published.

FUNDING

The authors did not receive any grants or funding for this study.

ETHICAL CLEARANCE

The research was approved by the Ethical Committee (REC.0222/KEPK/VII/2021) of Dr. Soetomo Hospital Surabaya, Indonesia

CONFLICT OF INTEREST

There's no conflict of interest to inform

REFERENCES

- Beer L, Mildner M, Ankersmit HJ. Cell Secretome Based Drug Substances in Regenerative Medicine: When Regulatory Affairs Meet Basic Science. *Ann Transl Med*. 2017;5(7):170. doi: <https://doi.org/10.21037/atm.2017.03.50>.
- Daneshmandi L, Shah S, Jafari T, Bhattacharjee M, Momah D, Saveh-Shemshaki N, Lo KW, Laurencin CT. Emergence of the Stem Cell Secretome in Regenerative Engineering. *Trends Biotechnol*. 2020;38(12):1373-1384. doi: <https://doi.org/10.1016/j.tibtech.2020.04.013>.
- Vizoso FJ, Eiro N, Cid S, Schneider J, Perez-Fernandez R. Mesenchymal Stem Cell Secretome: Toward Cell-Free Therapeutic Strategies in Regenerative Medicine. *Int J Mol Sci*. 2017;18(9):1852. doi: <https://doi.org/10.3390/ijms18091852>.
- Mahyudin F, Setyowardoyo N, Sedar J, Hernugrahanto K, Utomo D. The Effect of Low Oxygen Level on Pluripotency and Proliferation Capability in Bone Marrow Mesenchymal Stem Cell Culture. *Eurasian Journal of Biosciences*. 2020;14(2): 3441-46.
- Xia X, Yan Chiu PW, Lam PK, Chin WC, Kwok Wai Ng E, Wong Lau JY. Secretome from Hypoxia-Conditioned Adipose-Derived Mesenchymal Stem Cells Promotes the Healing of Gastric Mucosal Injury in a Rodent Model. *Biochimica et Biophysica Acta - Molecular Basis of Disease* 2018;1864(1):178-88. doi: <https://doi.org/10.1016/j.bbadis.2017.10.009>.
- Sumarwoto T, Suroto H, Mahyudin F, Utomo DN, Romaniyanto R, Prijosedjati A, Utomo P, Prakoeswa CRS, Rantam FA, Tinduh D, Notobroto HB, Rhatomy S. Preconditioning of Hypoxic Culture Increases The Therapeutic Potential of Adipose Derived Mesenchymal Stem Cells. *Open Access Maced J Med*. 2021;9(F), 505-515. doi: <https://doi.org/10.3889/oamjms.2021.5870>.
- Putra RA, Suroto H. Evaluation of Secretome Tenogenic Potential from Adipose Stem Cells (ACS) in Hypoxic Condition with Fresh Frozen Tendon Scaffold Using Scleraxis (Scx), Insulin-Like Growth Factor 1 (IGF-1) and Collagen Type 1. *Journal of Biomimetics, Biomaterials and Biomedical Engineering*. 2021;49:111-8. doi: <https://doi.org/10.4028/www.scientific.net/jbbbe.49.111>.
- Suryawan IGR, Andrianto, Anggaraeni AD, Agita A, Nugraha RA. The Role of Human Platelet-rich Plasma to Enhance the Differentiation of Adipose-derived Mesenchymal Stem Cells into Cardiomyocyte: An Experimental Study. *Cardiovasc Hematol Agents Med Chem*. 2023;21(3):156-166. doi: <https://doi.org/10.2174/1871525720666220510211116>.
- Prajasari T, Zaim CM, Suroto H. Characterization of Scleraxis and SRY-Box 9 from Adipose-Derived Stem Cells Culture Seeded with Enthesis Scaffold in Hypoxic Condition. *Journal of Biomimetics, Biomaterials and Biomedical Engineering*. 2021;52:76-85. doi: <https://doi.org/10.4028/www.scientific.net/jbbbe.52.76>.
- Lukomska B, Stanaszek L, Zuba-Surma E, Legosz P, Sarzynska S, Drela K. Challenges and Controversies in Human Mesenchymal Stem Cell Therapy. *Stem Cells Int*. 2019;9628536. doi: <https://doi.org/10.1155/2019/9628536>.
- L PK, Kandoi S, Misra R, S V, K R, Verma RS. The Mesenchymal Stem Cell Secretome: A New Paradigm towards Cell-Free Therapeutic Mode in Regenerative Medicine. *Cytokine Growth Factor Rev*. 2019;46:1-9. doi: <https://doi.org/10.1016/j.cytogfr.2019.04.002>.
- Fliefel R, Popov C, Tröltzsch M, Kühnisch J, Ehrenfeld M, Otto S. Mesenchymal Stem Cell Proliferation and Mineralization but Not Osteogenic Differentiation Are Strongly Affected by Extracellular PH. *J Craniomaxillofac Surg*. 2016;44(6):715-24. doi: <https://doi.org/10.1016/j.jcms.2016.03.003>.
- Ejtehadifar M, Shamsasjan K, Movassaghpour A, Akbarzadehlaleh P, Dehdilani N, Abbasi P, Molaeipour Z, Saleh M. The Effect of Hypoxia on Mesenchymal Stem Cell Biology. *Adv Pharm Bull*. 2015;5(2):141-9. doi: <https://doi.org/10.15171/apb.2015.021>.
- 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 Dec 4;9:2837. doi: <https://doi.org/10.3389/fimmu.2018.02837>.
- Romaniyanto, Mahyudin F, Prakoeswa CRS, Notobroto HB, Tinduh D, Ausrin R, Rantam FA, Suroto H, Utomo DN, Rhatomy S. Hypoxia Effects in Intervertebral Disc-Derived Stem Cells and Discus Secretomes: An in vitro Study. *Stem Cells Cloning*. 2022;15:21-28. doi: <https://doi.org/10.2147/SCCAA.S363951>.
- Trzyna, Anna, and Agnieszka Banaś-Ząbczyk. 2021. "Adipose-Derived Stem Cells Secretome and Its Potential Application in 'Stem Cell-Free Therapy.'" *Biomolecules* 11(6).
- Dmitrieva RI, Minullina IR, Bilibina AA, Tarasova OV, Anisimov SV, Zaritskey AY. Bone Marrow- and Subcutaneous Adipose Tissue-Derived Mesenchymal Stem Cells: Differences and Similarities. *Cell Cycle*. 2012;11(2):377-83. doi: <https://doi.org/10.4161/cc.11.2.18858>.
- Shin S, Lee J, Kwon Y, Park K-S, Jeong J-H, Choi S-J, Bang SI, Chang JW, Lee C. Comparative Proteomic Analysis of the Mesenchymal Stem Cells Secretome from Adipose, Bone Marrow, Placenta and Wharton's Jelly. *International Journal of Molecular Sciences*. 2021;22(2):845. doi: <https://doi.org/10.3390/ijms22020845>.
- Pires AO, Mendes-Pinheiro B, Teixeira FG, Anjo SI, Ribeiro-Samy S, Gomes ED, Serra SC, Silva NA, Manadas B, Sousa N, Salgado AJ. Unveiling the Differences of Secretome of Human Bone Marrow Mesenchymal Stem Cells, Adipose Tissue-Derived Stem Cells, and Human Umbilical Cord Perivascular Cells: A Proteomic Analysis. *Stem Cells Dev*. 2016;25(14):1073-83. doi: <https://doi.org/10.1089/scd.2016.0048>.
- Tsuji W, Rubin JP, Marra KG. Adipose-Derived Stem Cells: Implications in Tissue Regeneration. *World J Stem Cells*. 2014 Jul 26;6(3):312-21. doi: [10.4252/wjcs.v6.i3.312](https://doi.org/10.4252/wjcs.v6.i3.312).
- Pinheiro-Machado E, Getova VE, Harmsen MC, Burgess JK, Smink AM. Towards Standardization of Human Adipose-Derived Stromal Cells Secretomes. *Stem Cell Rev Rep*. 2023;19(7):2131-2140. doi: <https://doi.org/10.1007/s12015-023-10567-5>.



This work is licensed under a Creative Commons Attribution