International Journal of
Sports Science & Medicine

Research Article

Thirteen Weeks of Resistance Training Exercise Increases Circulating Mesenchymal Stem Cells - ∂

Karen Ma[1], Prasad P. Devarshi[4], Tara M. Henagan[1,2] and Natalie R. Lenard[3]*

[1]*Department of Nutrition Science, Purdue University, West Lafayette, IN, USA*
[2]*School of Medicine, Louisiana State University Health Sciences Center – Shreveport, Shreveport, LA, USA*
[3]*Department of Sciences - Biology, Franciscan Missionaries of Our Lady University, Baton Rouge, LA, USA*
[4]*Science & Technology, Pharmavite LLC, West Hills, California, USA*

***Address for Correspondence:** Natalie Lenard, Department of Sciences-Biology, Franciscan Missionaries of Our Lady University, Baton Rouge, LA, USA, Tel: +1-225-526-1660; Fax: +1-225-526-1775; ORCID ID: 0000-0002-2093-4869, E-mail: natalie.lenard@franu.edu

Submitted: 06 February 2022; **Approved:** 02 March 2022; **Published:** 03 March 2022

Cite this article: Ma K, Devarshi PP, Henagan TM, Lenard NR. Thirteen Weeks of Resistance Training Exercise Increases Circulating Mesenchymal Stem Cells. Int J Sports Sci Med. 2022 March 03;6(1): 001-005. doi: 10.37871/ijssm.id55

ISSN: 2640-0936

ABSTRACT

Mesenchymal Stem Cells (MSCs) are multipotent and may be therapeutically useful to repair and regenerate tissue, to slow aging, and to modify disease processes. The developmental fate of MSCs is likely influenced by the microenvironment. For example, the ability of MSCs to proliferate and differentiate depends on the source, specifically the age and sex of the donor. Exercise creates an anti-inflammatory environment and is recommended for the prevention and treatment of several disease states associated with chronic inflammation, including Cardiovascular Disease (CVD), Type 2 Diabetes (T2D), and obesity. Limited studies show that exercise training increases the number of circulating MSCs (cMSCs). *Methods:* We determined the effects of a 13-week regimen of periodized Resistance Training (RT) on the percentage of cMSCs in whole blood from healthy young adults using FACS analysis. *Results:* The percentage of cMSCs increased in healthy young adults who performed 13 weeks of periodized resistance training. *Conclusion:* RT might increase cMSCs to support skeletal muscle tissue repair.

Keywords: Resistance training; exercise; Mesenchymal stem cells; Mesenchymal stem cells; Cardiovascular disease; Type 2 diabetes; Obesity; Inflammation; Tissue regeneration

ABBREVIATIONS

AC: Active Control; MSCs: Mesenchymal Stem Cells; RT: Resistance Training; CVD: Cardiovascular Disease; T2D: Type 2 Diabetes; CRP: C-Reactive Protein; IL6: Interleukin 6; IL10: Interleukin 10; TNFα: Tumor Necrosis Factor Alpha; cMSCs: Circulating Mesenchymal Stem Cells

INTRODUCTION

Mesenchymal Stem Cells (MSCs) are multipotent stem cells that express CD73, CD90, and/or CD105 and that lack CD11b, CD13, CD19, CD34, CD45, CD79a, or class II histocompatibility complex antigens [1] and which retain the ability to differentiate into adipogenic, osteogenic/chondrogenic, hepatic, pancreatic, and neuronal cell types [2]. Adult MSCs are found in permissive niches throughout the body that allow maintenance of the undifferentiated, multipotent state, including the bone marrow, adipose tissue, skeletal muscle, and pancreas [3]. Although MSCs may be recruited into the circulation from various tissue sources, differences in MSC proliferation and differentiation potential have been noted based on their source [4]. For example, the rate of proliferation of bone marrow-derived MSCs in vitro derived from older donors demonstrated a slower rate of proliferation, increased degree of apoptosis, and decreased differentiation potential compared to those of younger donors [5], although age did not play a role in a later study [6]. Thus, the origin of circulating MSCs (cMSCs) and microenvironment may play a critical role in determining the potential of MSCs for therapeutic purposes.

Increased physical activity and exercise training have been established as a major intervention for the prevention and treatment of Cardiovascular Disease (CVD) and Type 2 Diabetes (T2D) related to obesity, due partially to anti-inflammatory effects [7,8]. Exercise training has been found to modulate levels of circulating cytokines and pro-inflammatory markers such as C-Reactive Protein (CRP), leading to an overall anti-inflammatory state: Interleukin 6 (IL6), which can be pro-inflammatory in some cases, in the context of exercise induces anti-inflammatory cytokines like Interleukin 10 (IL10) and inhibits the pro-inflammatory Tumor Necrosis Factor Alpha (TNFα) [reviewed in [9]]. Exercise training is associated with decreases in CRP, but this is in tandem with reduction of adiposity [10]. Interestingly, a limited number of recent studies have shown that exercise may increase numbers of cMSCs, although the literature related to the modality, intensity, and duration of the exercise is still developing [11]. One of the major functions of MSCs is immunomodulation. MSCs co-cultures in vitro decrease the production of pro-inflammatory cytokines from mast cells [12] and, importantly, promote the conversion of pro-inflammatory M1 macrophages into anti-inflammatory M2 macrophages [13,14]. Exercise is also known to alter the balance of M1:M2 macrophages, favoring an anti-inflammatory environment [15]. Currently, the International Society for Cellular Therapy specifies MSCs as those that express CD73, CD90, and/or CD105 and that lack CD11b, CD13, CD19, CD34, CD45, CD79a, or class II histocompatibility complex antigens [1]. In the present study, we hypothesize that 13 weeks of periodized Resistance Training (RT) increases the percentage of cMSCs in healthy young adults.

MATERIALS AND METHODS

Subjects

Thirty-four healthy males and females aged 18-30 y were recruited from Purdue University. 20 subjects, 3 male and 17 female, were enrolled in the Resistance Training group (RT) and completed a 13-wk periodized, progressive resistance training program. Subjects in the RT received permission to participate from a state-licensed MD. Fourteen subjects, 2 male and 12 female, were enrolled in an Active Control Group (AC) and asked to maintain their regular daily activities. Both AC and RT subjects were asked to maintain their normal diets. Both groups completed weekly sickness questionnaires, activity and injury recall logs, and a three-day diet record (two weekdays and one weekend day) to estimate habitual energy and macronutrient intakes. Additionally, all subjects completed a medical history form and a PAR-Q to ensure they did not have any pre-existing conditions and to allow the researchers to become aware of any potential health issues. All subjects signed an informed consent form, and this project was approved by the Purdue University Institutional Review Board (IRB # 1304013794).

Anthropometric measurements

Before beginning (Pre) and following (Post) the intervention period, body weight, height, and body composition were measured and recorded for all subjects. Body weight and height were measured on a platform scale and stadiometer, respectively. Body composition was determined via dual-energy x-ray absorptiometry (GE/Lunar iDXA) as previously described [16].

Acclimation period

Both groups completed a 4-d exercise acclimation period to ensure proper lifting technique while performing bench press, squat, and deadlift exercises. Eight repetitions maximum, or the maximum amount of weight that can be lifted eight times, for bench press, squat, and deadlift exercises were assessed during the last 2

DOI: https://dx.doi.org/10.37871/ijssm.id56

d of the acclimation period. All acclimation and exercise sessions were preceded by 5-10 min of cycle ergometry or walking and were completed under the supervision of trained technicians.

Eight repetition maximum testing protocol

All participants in the AC and RT were shown proper lifting techniques in two separate demonstration sessions for squat, deadlift, and bench press. Following the second demonstration session, each subject estimated a resistance that would allow them to complete eight repetitions. If they could complete all 8 until failure, their resistance was recorded. If they could not perform 8 repetitions, they were asked to decrease the weight by 5-10% for bench press and 10-20% for squat and deadlift. If they could perform more than 8 repetitions, they were asked to increase the weight by 5-10% for bench press and 10-20% for squat and deadlift.

Exercise protocol

The RT completed 13 weeks of resistance training on three non-consecutive days per week, as previously described [16] with the modification of 1 week of rest added to the protocol to compensate for a national holiday. Undulating periodization progressed starting from a 2-wk adaptation period to hypertrophy lifting, power (including plyometrics), circuit/recovery period, and strength lifting (Figure 1). Compound lifts performed regularly included the bench press, push-up, bent-over row, seated row, squat, deadlift, walking lunge, and crunch. Participants were supervised to ensure proper lifting techniques, minimize the risk of injury, and ensure proper progression through the training plan.

Flow cytometry

Resting venous blood samples (20 mL per sample) were collected into EDTA vacutainer tubes (BD-Pharmingen, USA) during the acclimation period (PRE) and last week (POST) of the 13-week intervention period from all study participants. Subjects reported to the laboratory between 0700 and 1000 h following an overnight fast and having refrained from exercise from the previous 48 h. Whole blood samples were stained with CD31, CD45, and CD105 antibodies (all from eBioscience) as previously described [17]. Data was collected in the Flow Cytometry and Cell Separation Facility at Purdue University Discovery Park on the Cytomics FC 500 platform and analyzed using WinMDI software.

Statistical analysis

Two-tailed Student's t-tests were used to compare pre-post 8RM values and cMSC percentage for the AC and RT groups. Statistical significance was set with $p \leq 0.05$.

RESULTS AND DISCUSSION

The current study extends others noting that exercise increases MSC proliferation [18]. We demonstrated that 13 wks of periodized resistance training increased the percentage of cMSCs, specifically $CD105^{+}$ cMSCs (Figures 2,3, $p < 0.05$). This change occurred without any changes in body weight, BMI, or body fat percentage Pre vs Post in both AC and RT (Table 1). Likewise, there were no significant differences in body weight, BMI, or body fat percentage between AC and RT at either time point (Table 1). cMSC and 8RM measurements increased in Post *vs.* Pre, indicating that the resistance training regime utilized in the present study is effective in both improving strength and upregulating cMSCs that may be crucial for resistance training-induced skeletal muscle repair and remodeling. Indeed, many others

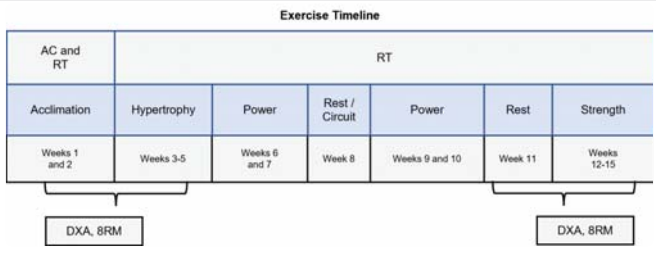

Figure 1: Adaptation period and undulating periodization schedule. AC, Active Control; RT, Resistance Training.

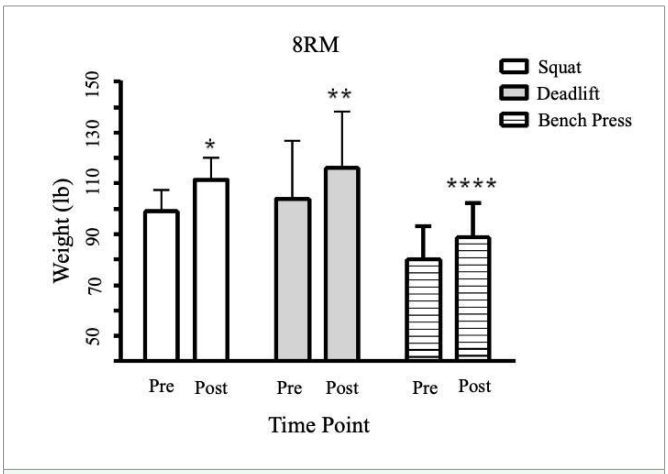

Figure 2: Effects of undulating periodized resistance training on 8RM values in squat, deadlift, and bench press exercises before (Pre) and after (Post) 13 wks of training, *$p \leq 0.05$, **$p \leq 0.01$, ****$p \leq 0.00001$.

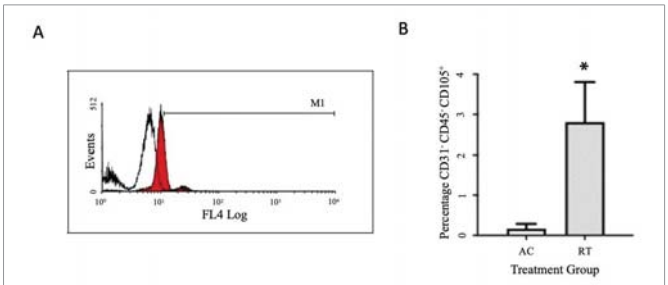

Figure 3: A) Representative picture showing FACS analysis for cMSC determination in peripheral whole blood samples. Primary gates were set on CD31-/CD45- cells, and CD105 expression (FL4 channel) was then determined in only those cells that were gated. The negative control sample (clear peak) was overlayed with the sample of interest and a marker (M1) was set to capture truly positive CD105 stained cells within the CD31-/CD45- population of gated cells in the sample of interest (red peak).
B) Percentage of circulating CD31-/CD45-/CD105+cells before (Pre) and after (Post) 13 wks in Active Control (AC) and Resistance Training (RT) groups, *$p < 0.05$.

believe that skeletal muscle repair and remodeling are thought to be major drivers in improving strength [19-22].

cMSCs arise from a wide variety of tissues throughout the body, including bone marrow, adipose tissue, and skeletal muscle. Currently, the International Society for Cellular Therapy specifies MSCs as those that express CD73, CD90, and/or CD105 and that lack CD11b, CD13, CD19, CD34, CD45, CD79a, or class II histocompatibility complex antigens [1]. Similarly, nonhematopoietic stem cells lack expression of CD13, CD34, and CD45 [23]; CD31 is expressed on hematopoietic stem cells throughout their ontogeny [24]. Importantly, those

DOI: https://dx.doi.org/10.37871/ijssm.id56

Table 1: Anthropometric measurements.

		AC	RT
Age			22.95 ± 0.79
Height (cm)		164.73 ± 1.35	168.32 ± 2.16
Body weight (kg)	Pre	60.61 ± 1.9	66.19 ± 2.93
	Post	61.04 ± 1.95	67.1 ± 2.93
Fat mass (kg)	Pre	17.62 ± 1.41	20.11 ± 1.39
	Post	17.91 ± 1.33	20.56 ± 1.4
Lean mass (kg)	Pre	40.28 ± 1.27	43.24 ± 2.28
	Post	40.42 ± 1.54	43.6 ± 2.11
Percent fat mass	Pre	24.33 ± 1.95	27.78 ± 1.92
	Post	24.53 ± 1.83	28.17 ± 1.92
Percent lean body mass	Pre	55.64 ± 1.76	59.72 ± 3.14
	Post	55.37 ± 2.11	59.72 ± 2.89

Anthropometric measurements were taken before (pre) and after (Post) 13 wks in the Active Control (AC) and Resistance Training (RT) groups. No significant differences were found in any measurements between groups or over time.

nonhematopoietic stem cells that express CD105 have been shown to improve cardiac muscle performance following myocardial infarction, an effect associated with increased angiogenesis [25].

RT is thought to increase the reservoir of cMSCs that contribute to tissue repair and regeneration [18]. We speculate that RT recruits cMSCs from the anti-inflammatory environment associated with exercise, and this recruitment influences the differentiation of cMSCs into their ultimate cell types. We further speculate that, as seen in cardiac muscle tissue [25], exercise enhances skeletal muscle tissue function through increased production of cMSCs.

CONCLUSION

CD105-expressing cMSCs may differentiate into various muscle types, including skeletal muscle, and may play an important role in skeletal muscle repair and regeneration following resistance training.

CONFLICT OF INTEREST

The authors whose names are listed immediately below certify that they have NO affiliations with or involvement in any organization or entity with any financial interest (such as honoraria; educational grants; participation in speakers' bureaus; membership, employment, consultancies, stock ownership, or other equity interest; and expert testimony or patent-licensing arrangements), or non-financial interest (such as personal or professional relationships, affiliations, knowledge or beliefs) in the subject matter or materials discussed in this manuscript.

ACKNOWLEDGEMENTS

This project was funded by Purdue University Startup funds to TMH. The article processing fees were covered by the Franciscan Missionaries of Our Lady University's Endowed Professorship program for administering grant funds from the Louisiana Board of Regents, specifically, the Sr Ann Young Endowed Professorship awarded to NRL.

REFERENCES

1. Viswanathan S, Shi Y, Galipeau J, Krampera M, Leblanc K, Martin I, Nolta J, Phinney DG, Sensebe L. Mesenchymal stem versus stromal cells: International Society for Cell & Gene Therapy (ISCT®) Mesenchymal Stromal Cell committee position statement on nomenclature. Cytotherapy. 2019 Oct;21(10):1019-1024. doi: 10.1016/j.jcyt.2019.08.002. Epub 2019 Sep 13. PMID: 31526643.
2. Uder C, Brückner S, Winkler S, Tautenhahn HM, Christ B. Mammalian MSC from selected species: Features and applications. Cytometry A. 2018 Jan;93(1):32-49. doi: 10.1002/cyto.a.23239. Epub 2017 Sep 14. PMID: 28906582.
3. da Silva Meirelles L, Chagastelles PC, Nardi NB. Mesenchymal stem cells reside in virtually all post-natal organs and tissues. J Cell Sci. 2006 Jun 1;119(Pt 11):2204-13. doi: 10.1242/jcs.02932. Epub 2006 May 9. PMID: 16684817.
4. Andrzejewska A, Lukomska B, Janowski M. Concise Review: Mesenchymal Stem Cells: From Roots to Boost. Stem Cells. 2019 Jul;37(7):855-864. doi: 10.1002/stem.3016. Epub 2019 Apr 30. PMID: 30977255; PMCID: PMC6658105.
5. Zhou S, Greenberger JS, Epperly MW, Goff JP, Adler C, Leboff MS, Glowacki J. Age-related intrinsic changes in human bone-marrow-derived mesenchymal stem cells and their differentiation to osteoblasts. Aging Cell. 2008 Jun;7(3):335-43. doi: 10.1111/j.1474-9726.2008.00377.x. Epub 2008 Jan 31. PMID: 18248663; PMCID: PMC2398731.
6. Andrzejewska A, Catar R, Schoon J, Qazi TH, Sass FA, Jacobi D, Blankenstein A, Reinke S, Krüger D, Streitz M, Schlickeiser S, Richter S, Souidi N, Beez C, Kamhieh-Milz J, Krüger U, Zemojtel T, Jürchott K, Strunk D, Reinke P, Duda G, Moll G, Geissler S. Multi-Parameter Analysis of Biobanked Human Bone Marrow Stromal Cells Shows Little Influence for Donor Age and Mild Comorbidities on Phenotypic and Functional Properties. Front Immunol. 2019 Nov 8;10:2474. doi: 10.3389/fimmu.2019.02474. PMID: 31781089; PMCID: PMC6857652.
7. Gondim OS, de Camargo VT, Gutierrez FA, Martins PF, Passos ME, Momesso CM, Santos VC, Gorjão R, Pithon-Curi TC, Cury-Boaventura MF. Benefits of Regular Exercise on Inflammatory and Cardiovascular Risk Markers in Normal Weight, Overweight and Obese Adults. PLoS One. 2015 Oct 16;10(10):e0140596. doi: 10.1371/journal.pone.0140596. PMID: 26474157; PMCID: PMC4608693.
8. Figueiredo L, Nunes RB, Marmett B, de Sá LBPC, Arbex AK. Anti-Inflammatory Effects of Physical Exercise on Obesity. Open J Endocr Metab Dis. 2017;07(01):44-51. doi: 10.4236/ojemd.2017.71005.
9. Docherty S, Harley R, McAuley JJ, Crowe LAN, Pedret C, Kirwan PD, Siebert S, Millar NL. The effect of exercise on cytokines: implications for musculoskeletal health: a narrative review. BMC Sports Sci Med Rehabil. 2022 Jan 6;14(1):5. doi: 10.1186/s13102-022-00397-2. PMID: 34991697; PMCID: PMC8740100.
10. Mendoza MVF, Kachur SM, Lavie CJ. The Effects of Exercise on Lipid Biomarkers. Methods Mol Biol. 2022;2343:93-117. doi: 10.1007/978-1-0716-1558-4_6. PMID: 34473317.
11. Schmid M, Kröpfl JM, Spengler CM. Changes in Circulating Stem and Progenitor Cell Numbers Following Acute Exercise in Healthy Human Subjects: a Systematic Review and Meta-analysis. Stem Cell Rev Rep. 2021 Aug;17(4):1091-1120. doi: 10.1007/s12015-020-10105-7. Epub 2021 Jan 2. Erratum in: Stem Cell Rev Rep. 2021 Apr 6;: PMID: 33389632; PMCID: PMC8316227.
12. Brown JM, Nemeth K, Kushnir-Sukhov NM, Metcalfe DD, Mezey E. Bone marrow stromal cells inhibit mast cell function via a COX2-dependent mechanism. Clin Exp Allergy. 2011 Apr;41(4):526-34. doi: 10.1111/j.1365-2222.2010.03685.x. Epub 2011 Jan 24. PMID: 21255158; PMCID: PMC3078050.
13. Vasandan AB, Jahnavi S, Shashank C, Prasad P, Kumar A, Prasanna SJ. Human Mesenchymal stem cells program macrophage plasticity by altering their metabolic status via a PGE_2-dependent mechanism. Sci Rep. 2016 Dec 2;6:38308. doi: 10.1038/srep38308. PMID: 27910911; PMCID: PMC5133610.
14. Gao S, Mao F, Zhang B, Zhang L, Zhang X, Wang M, Yan Y, Yang T, Zhang J, Zhu W, Qian H, Xu W. Mouse bone marrow-derived mesenchymal stem

DOI: https://dx.doi.org/10.37871/ijssm.id56

cells induce macrophage M2 polarization through the nuclear factor-κB and signal transducer and activator of transcription 3 pathways. Exp Biol Med (Maywood). 2014 Mar;239(3):366-75. doi: 10.1177/1535370213518169. Epub 2014 Feb 5. PMID: 24500984.

15. Silveira LS, Antunes Bde M, Minari AL, Dos Santos RV, Neto JC, Lira FS. Macrophage Polarization: Implications on Metabolic Diseases and the Role of Exercise. Crit Rev Eukaryot Gene Expr. 2016;26(2):115-32. doi: 10.1615/CritRevEukaryotGeneExpr.2016015920. PMID: 27480774.

16. Devarshi PP, Pereyra AS, Ellis JM, Henagan TM. A single bout of cycling exercise induces nucleosome repositioning in the skeletal muscle of lean and overweight/obese individuals. Diabetes Obes Metab. 2022 Jan;24(1):21-33. doi: 10.1111/dom.14541. Epub 2021 Sep 20. PMID: 34472674; PMCID: PMC8728694.

17. Henagan TM, Forney L, Dietrich MA, Harrell BR, Stewart LK. Melanocortin receptor expression is associated with reduced CRP in response to resistance training. J Appl Physiol (1985). 2012 Aug;113(3):393-400. doi: 10.1152/japplphysiol.00107.2012. Epub 2012 Jun 7. PMID: 22678961; PMCID: PMC4422369.

18. Bourzac C, Bensidhoum M, Pallu S, Portier H. Use of adult mesenchymal stromal cells in tissue repair: impact of physical exercise. Am J Physiol Cell Physiol. 2019 Oct 1;317(4):C642-C654. doi: 10.1152/ajpcell.00530.2018. Epub 2019 Jun 26. PMID: 31241985; PMCID: PMC6850997.

19. Cosgrove BD, Gilbert PM, Porpiglia E, Mourkioti F, Lee SP, Corbel SY, Llewellyn ME, Delp SL, Blau HM. Rejuvenation of the muscle stem cell population restores strength to injured aged muscles. Nat Med. 2014 Mar;20(3):255-64. doi: 10.1038/nm.3464. Epub 2014 Feb 16. PMID: 24531378; PMCID: PMC3949152.

20. Judson RN, Rossi FMV. Towards stem cell therapies for skeletal muscle repair. NPJ Regen Med. 2020 May 11;5:10. doi: 10.1038/s41536-020-0094-3. PMID: 32411395; PMCID: PMC7214464.

21. Ferraro E, Giammarioli AM, Chiandotto S, Spoletini I, Rosano G. Exercise-induced skeletal muscle remodeling and metabolic adaptation: redox signaling and role of autophagy. Antioxid Redox Signal. 2014 Jul 1;21(1):154-76. doi: 10.1089/ars.2013.5773. Epub 2014 Mar 6. PMID: 24450966; PMCID: PMC4048572.

22. Witard OC, Bannock L, Tipton KD. Making Sense of Muscle Protein Synthesis: A Focus on Muscle Growth During Resistance Training. Int J Sport Nutr Exerc Metab. 2022 Jan 1;32(1):49-61. doi: 10.1123/ijsnem.2021-0139. Epub 2021 Oct 25. PMID: 34697259.

23. P M, S H, R M, M G, W S K. Adult mesenchymal stem cells and cell surface characterization - a systematic review of the literature. Open Orthop J. 2011;5(Suppl 2):253-60. doi: 10.2174/1874325001105010253. Epub 2011 Jul 28. PMID: 21966340; PMCID: PMC3178966.

24. Baumann CI, Bailey AS, Li W, Ferkowicz MJ, Yoder MC, Fleming WH. PECAM-1 is expressed on hematopoietic stem cells throughout ontogeny and identifies a population of erythroid progenitors. Blood. 2004 Aug 15;104(4):1010-6. doi: 10.1182/blood-2004-03-0989. Epub 2004 May 4. PMID: 15126319.

25. Gaebel R, Furlani D, Sorg H, Polchow B, Frank J, Bieback K, Wang W, Klopsch C, Ong LL, Li W, Ma N, Steinhoff G. Cell origin of human mesenchymal stem cells determines a different healing performance in cardiac regeneration. PLoS One. 2011 Feb 10;6(2):e15652. doi: 10.1371/journal.pone.0015652. PMID: 21347366; PMCID: PMC3037376.

DOI: https://dx.doi.org/10.37871/ijssm.id56