TurnitinComparisonofinVitroVasculogenesisPotentialbetweenASCswithBMMSCs

by Ani Oranda Panjaitan

Submission date: 31-May-2021 03:00PM (UTC+0700)
Submission ID: 1597679036
File name: parisonofinVitroVasculogenesisPotentialbetweenASCswithBMMSCs.pdf (811.56K)
Word count: 8642
Character count: 45766

International Journal of Health Sciences and Research
DOI: https://doi.org/10.52403/ijhsr.20210557
Vol.11; Issue: 5; May 2021
Website: www.ijhsr.org
ISSN: 2249-9571
Original Research Article

Comparison of in vitro Vasculogenesis Potential between ASCs with BM-MSCs

Ani Oranda Panjaitan[1], Dewi Sukmawati[2], Ria Anggraeni[3], Jeanne Adiwinata Pawitan[4]

[1]Departement of Anatomy and Histology Faculty of Medicine, Universitas Kristen Indonesia, Jakarta
[2]Departement of Histology, Faculty of Medicine, Universitas Indonesia
[3]Stem Cell and Tissue Engineering Research Center, Indonesia Medical Education and Research Institute (IMERI), Faculty of Medicine Universitas Indonesia, Indonesia
[4]Stem Cell Medical Technology Integrated Service Unit, Dr Cipto Mangunkusumo General Hospital/Faculty of Medicine Universitas Indonesia, Indonesia

Corresponding Author: Ani Oranda Panjaitan

ABSTRACT

Tube formation assay is the most widely used method as a vasculogenesis/ angiogenesis test in vitro. Mesenchymal stem cells (MSCs) are multipotent adult cells. The paracrine effect of MSCs on neovascularization is well known. In general, MSCs do not express CD34 hematopoietic surface marker, but according to some experts, bone marrow mesenchymal stem cells (BM-MSCs) express CD34 in vivo and lose their expression when they are cultured in vitro, while adipose-derived stem cells (ASCs) still have CD34 expression in the early passages when cultured in vitro. BM-MSCs are the most widely used MSC, but ASCs are also used in stem cell therapy and tissue engineering for angiogenesis purposes. Until now, the potential of vasculogenesis between ASCs and BM-MSCs is still unclear. Expression of CD34 is also unknown whether affecting the quality of tube formation. This study wanted to compare the potential of vasculogenesis between ASC and BM-MSCs through tube formation test and CD34 expression.Measurements of vasculogenesis quality showed higher tube length, number of loopsand mean number of branch points on BM-MSC. Both BM-MSCs and ASCs showed low CD34 levels.BM-MSCs showed better tube formation ability compared with ASCs. No association was found between CD34 levels and MSC vasculogenesis capability.

Keywords: ASCs, BM-MSCs, CD34, matrigel, tube formation

INTRODUCTION

Neovascularization plays a significant role in healing tissue that is injured/damaged or ischemic. For the first time endothelial progenitor cell was identified (EPC) originating from bone marrow in 1997 [1], many studies have reported that bone marrow EPC functionally contributes to neovascularization in several models of tissue damage and remodelling, including wound healing, myocardial ischemia, retinopathy, stroke and so on. In contrast to angiogenesis, which is a process of growing new blood capillaries from existing blood vessels [2], the process contributed by EPC in forming blood vessels de novo postnatally is called postnatal vasculogenesis [3]. Artificial basement membrane matrices are commercially available or can be made in-house. Although it does not contain all the components of the endothelial basement membrane wholly and entirely, this matrix model can activate endothelial cells to form tube formation in vitro [4]. There are two types of artificial basement membranes, namely ordinary matrigel and GFR matrigel (growth factor reduced matrigel) or matrigel reduced by growth factor in it. GFR matrigel is used to observe a more rapid

formation of tube formation by adding an external angiogenic factor [2]. Each cell type has different requirements in forming tube formations in the matrigel. Some need serum, and some do not. If serum is needed, an optimization test should be performed first because serum can trigger proliferation and inhibit tube formation.

Mesenchymal stem cell (MSC) is a multipotent adult stem cell that is embryonically derived from the mesoderm, which can be found in various body tissues, including bone marrow and fat tissue and can differentiate into many cell types (multipotent) [4; 5]. Its paracrine effect on neovascularization is well known [6; 7]. MSCs of fatty origin and bone marrow origin are known to stimulate endothelial cells to form blood vessels in vitro and support endothelial cells, similar to pericytes [8]. MSCs derived from lipoaspirate secrete angiogenic cytokines, increasing in a hypoxic state [9]. In haemopoietic stem cells (HSC), CD34 appears to act as an anti-adhesion molecule associated with HSC movement as well as a progression molecule for HSC interactions with the stromal environment [10]. The hematopoietic stem cell population expressing CD34 is known to consist of cells that can differentiate into EPC and endothelial cells. It suggests the potential of a CD34-rich cell population as a vasculogenesis therapy. Various investigators report that administering human leukocytes with high CD34 levels can effectively improve neovascularization in animal models.

MSC does not express hematopoietic surface markers or endothelial cell surface markers such as CD34. However, it is argued that MSCs that do not express CD34 are MSCs cultured in vitro rather than tissue-resident MSCs. MSCs originating from bone marrow express CD34 in vivo and begin to lose expression once MSC adheres to plastics when cultured in vitro. Unlike the case with MSCs from the fat origin, which still has CD34 expression in vitro culture up to a particular passage. In 2006 Mitchell et al. obtained positive CD34 expression by MSC from lipoaspirate culture in a medium containing FBS (fetal bovine serum) ranging from P0 to P5. In 2012, Pawitan who cultured MSC from lipoaspirate using the various xeno-free medium, obtained a mean CD34 positive, ranging from P0 to P5 [11]. In vitro culture modification influenced MSC so that it can transdifferentiate into endothelial cells in vitro and in vivo by expressing CD31 +. In 2004MSCs was differentiated from bone marrow to endothelial cells. The results of flow cytometry showed a substantial increase in the expression of specific endothelial markers such as insert domain receptor kinase and FLT-1, and the results of immunofluorescent staining analysis showed the expression of von Willebrand factor.

In research conducted by experts so far, MSCs from bone marrow can form tube formations in a matrix in a hypoxic or normoxic state but with certain angiogenic supplements. In 2003 discovered that MSCs from bone marrow can form tube formations in vitro under hypoxic conditions. Then in 2004, Iwase et al. stated that MSC from mouse bone marrow was better than mouse bone marrow mononuclear cells in the formation of tube formation in matrigel with hypoxic conditions. The results of human umbilical vein endothelial cells (HUVEC) tube formation test results and MSC was compared to bone marrow implanted on matrigel in four types of medium, namely without serum, added with vascular endothelial growth factor (VEGF), added hypoxia-regulated factor erythropoietin (EPO) and added serum, all of which were normoxic. The tube formation formed best on medium with serum. In 2013, tube formation was tested from MSC from bone marrow in normoxic conditions using a kit containing growth factors such as TGF-β, fibroblast growth factor (FGF) and proteolytic enzymes such as plasminogen, tissue plasminogen activator (tPA), and matrix metalloproteinase. MMP in medium without serum in research on oxytocin can

restore the angiogenic ability of MSCs originating from bone marrow in DM-model mice in vitro [12].

So far, the MSCs most widely used in test tube formation are bone marrow-derived MSCs [24; 33; 31; 32], whereas apart from the bone marrow, MSCs have been found in many body tissues. Fat tissue is an abundant source of stem cells and is particularly suitable for tissue engineering and regenerative medical applications [13]. This tissue can be obtained by tissue excision methods, such as a tummy tuck or liposuction techniques. The low morbidity rate of both tummy tuck and liposuction today and the large number of cells obtained make human fat a potential source for stem cell therapy and engineering. In addition, MSCs from fat tissue can also be stored in tissue banks, thus opening up opportunities for everyone to have stem cell savings that can be used in the future.

In 2008 Fischer et al. stated that MSCs originating from fat tissue could not form tube formation in matrigel directly without being differentiated into endothelial cells by incubating in a medium supplemented with endothelial cell growth supplement (ECGS) [14]. A vasculogenesis study using MSC from lipoaspirate was carried out again in 2013 by Perez et al., who wanted to compare vasculogenesis between MSCs from lipoaspirate in obese patients with healthy ones in mice and humans by conducting tube formation tests [15]. In this study, MSC from lipoaspirate was found to form tube formations in matrigel in a normoxic state, which is different from the results obtained by Fischer. We have conducted preliminary research (optimization) by implanting MSCs from bone marrow and lipoaspirate origin in the matrigel with various dilutions and using different cell densities. From the results of these preliminary studies, it was found that MSC from bone marrow and lipoaspirate origin can form tube formation in matrigel in a normoxic state with medium with.

Tube formation test results can represent the ability of in vitro vasculogenesis. To date, the potential for vasculogenesis between MSCs of bone marrow origin and lipoaspirate is unclear which one is better and whether CD34 expression influences this. This study wanted to compare the potential of vasculogenesis between MSC from lipoaspirate and MSC from bone marrow through tube formation tests and CD34 expression. Based on this background, the formulation of the problem to be answered in this study is "Which has the better ability to form tube formation between mesenchymal stem cells from lipoaspirate and bone marrow origin? With the aim of research to determine the in vitro ability of mesenchymal stem cell tube formation from lipoaspirate and bone marrow.

LITERATURE REVIEW

Vasculogenesis - Blood vessels play an important role in delivering oxygen and nutrients to body tissues and attracting metabolic waste from body tissues. Vasculogenesis and angiogenesis are the fundamental processes by which blood vessels form. Vasculogenesis is a process of differentiation of angioblasts into endothelial cells and de novo forming blood vessels, while angiogenesis is a process of remodelling the growth of new blood vessels originating from existing endothelial cells [3; 4]. In the embryo, blood vessel formation occurs actively through vasculogenesis and angiogenesis. In the postnatal period, typically, new blood vessel formation is found only in certain physiological conditions, for example, in the ovarian cycle, in the placenta during pregnancy or during wound healing, in a state of tissue hypoxia/ischemia and tumour formation.

The invasion and motility of endothelial cells in forming the tubular structure of blood vessels and the formation of the lumen in different areas in the formation of blood vessels run simultaneously at the same time so that it can be said that the morphogenesis of blood vessels is a very dynamic process. Each

endothelial cell that combines itself to form blood vessels individually will produce many vacuoles through the process of pinocytosis. Some of these vacuoles will then unite to form lumen spaces connected to other lumens formed in adjacent cells [16; 17]. The process of vasculogenesis is mediated via the VEGF / VEGFR signal pathway. After the blood vessels are formed, the endothelial cells secrete platelet-derived growth factor (PDGF), which activates the surrounding MSCs to release angiopoietin 1 (ANG1). The release of ANG1 stimulates the mural cells to cover the blood vessels and then deposits the basal membrane via the ANG1 / TIE2 system pathway (endothelial cell tyrosine kinase receptor). The interaction of endothelial cells and MSC causes transforming growth factor β (TGFβ) to be activated, which causes mural cells to differentiate and at the same time stops the proliferation of endothelial cells. Finally, the blood vessels are covered by perivascular cells, and the vasculogenesis process is completed [18].

There are several things that affect vasculogenesis such as: a) endothelial progenitor cells (precursor) cells (epc); b) Extra Cellular Matrix (ECM) [19; 20; 21]; c) Vascular Endothelial Growth Factor (VEGF) [22]; d) Matrix metalloproteinase (MMP). MT1-MMP in the form of an active enzyme on the cell surface directly acts against different ECM proteins and can activate pro-MMP on the cell surface, localizing activity in the pericellular area. MMP can cleave proteins that bind to growth factor or latent growth factors and regulate their synthesis by releasing them from within cells; e) Desmoplakin.

Stem Cells - Stem cells have self-renewing characteristics and can differentiate into other, more mature cells in multi-line pathways. It allows the stem cells to maintain tissue development and maintenance [23]. Globally, these cells are divided into two types, namely embryonal and non-embryonal stem cells. Embryonic stem cells originate from the inner cell mass of the blastocyst and can differentiate in cell groups in the three embryonic germ layer pathways or are called pluripotent. Meanwhile, non-embryonal stem cells, mostly adult stem cells, have limited differentiation potential only into cells in a few more specific lines or lines when replacing damaged cells in maintaining tissue integrity and cellular homeostasis [24]. Apart from the two types of stem cells available naturally above, there is another type of stem cell called induced pluripotent stem cell (iPSC). These cells are derived from somatic cells that are "programmed" to return to their stem cell-like state through certain transcription factors. Several problems still hamper the development of treatment research using embryonal stem cells and iPSCs, such as ethics for embryonal stem cells at risk of tumorigenesis [25; 26] and immunological rejection reactions for both types of stem cells [27].

Adult stem cells are undifferentiated cells found among differentiated cells in the body's tissues or organs. Adult stem cells or somatic stem cells can be found in all human body tissues. Adult stem cells consist of mesenchymal stem cells (MSC), which produce several tissue-forming cells and hematopoietic stem cells (HSC), which produce blood cells. Ideally, stem cells used for regenerative medicine applications should meet the following criteria, namely: can be found in abundance (millions to billions of cells) and can be harvested by minimally invasive procedures, can be differentiated into cells along multi-line pathways regularly. It is reproducible and can be safely and effectively transplanted to the host tautologically or allogeneically and produced according to Good Manufacturing Practice (GMP) guidelines.

Mesenchymal Stem Cell (MSC) - Mesenchymal stem cell (MSC) is a non-haemapoietic stem cell with multipotent properties that can differentiate into cells in the mesodermal line [28]. The therapeutic potential is quite significant from MSC increased the interest of researchers from various biomedical disciplines. However,

investigators report MSC studies using different isolation and culture methods and different cell characterization approaches. Bone Marrow Mesenchymal Stem Cell (BM-MSC) - The first adult MSC discovered more than 40 years ago was bone marrow mesenchymal stem cell (BM-MSC) and is still the most prevalent MSC. is studied and is often regarded as the type of gold standard (gold standard) of MSC [29]. Bone marrow is considered to be the primary source of MSC in organisms. MSC is obtained from bone marrow aspiration by surgery, which can cause pain and infection [30]. MSCs from Adipose-derived Stem Cell (ASC) - There are many different names given to the cell population isolated from this adipose tissue. These names are Adipose-derived Stem / Stromal Cell (ASC), Adipose-Derived Adult Stem (ADAS) Cells, Adipose-Derived Adult Stromal Cells, Adipose-Derived Stromal Cells (ADSC), Adipose Stromal Cells (ASC), Adipose Mesenchymal Stem Cells (MSC), Lipoblasts, Pre-Adipocytes. These different nomenclatures create confusion in the literature. To solve this problem, the International Fat Applied Technology Society (IFATS) made a consensus to use the term Adipose-derived Stem Cell (ASC) to identify cell populations isolated from adipose tissue attached to plastic and are multipotent [31].

Effect of MSC on Vasculogenesis - MSC can increase endothelial cell growth and increase the formation of new blood vessels in vitro and in vivo due to the paracrine effect [32; 33]. MSCs can produce various angiogenic factors, including VEGF and bFGF [34]. Transplantation of MSC was stated to be more effective in inducing angiogenesis compared to MNC because the release of angiogenic factors, namely VEGF and bFGF by MSC, was more than by MNC. MSCs not only can produce angiogenic factors but can also differentiate into vascular endothelial cells. In an undifferentiated state, MSCs do not express the surface markers of hematopoietic cells or endothelial cells such as CD11, CD14, CD31, CD34, or CD45, but according to some experts changing the culture conditions may allow these cells to differentiate into endothelial cells. It suggests the potential for MSC differentiation in neovascularization [3; 23]. Studies show that MSCs are capable of differentiating into CD31 + endothelial cells in vivo [28; 29]. Direct involvement of MSCs in neovascularization is possible because their contribution to tumour angiogenesis in vivo has been investigated [35] MSC responses to tumour angiogenic factors impact tumour cell growth. There is a selective interaction of MSCs against epithelial tumour cells and on the growth and survival of myeloma plasma cells, but MSCs participate in migration, proliferation if under normal conditions, and capillary formation by differentiating into internal or external blood vessels the bone marrow environment is still being studied.

RESEARCH METHOD

This study was an in vitro experimental study using two types of cells, namely MSC from bone and MSC from lipoaspirate. The research was conducted at the UPT Stem Cells RSCM-FKUI. This research was conducted for 16 months, from August 2016 - October 2017. The samples of this study were mesenchymal stem cells from bone marrow isolated by the simple method by Pawitan et al. [36] and mesenchymal stem cells from fat tissue isolated by the simple method belonging to the RSCM-FKUI UPT Stem Cell Laboratory [37]. All cells were cryopreserved in the first phase using the xeno-free cryopreservation method. The Research Ethics Committee approved the Faculty of Medicine study design, University of Indonesia (No.703 / UN2.FI / ETIK / IX / 2016). All data and research results are kept confidential. In this study, a Tube Formation Test and an Inspection of the Tube Formation Results will be carried out. The data obtained from this study were analyzed using Statistical Product and Service Solution (SPSS) version 17.0. To analyze

the comparison of the mean tube length, the mean number of branching points and the mean number of loops produced by mesenchymal stem cells from lipoaspirate and bone marrow origin, the Student t-test was used. To see the relationship between CD34 levels and each tube formation parameter calculated from passages 3, 5 and 7, Pearson's correlation test was used.

RESULT AND DISCUSSION

This section displays an overview of BM-MSC and ASC cultures, test results for tube formation and cell characteristics of CD34 obtained during the study. The results of this data were analyzed to meet the objectives and answer the research hypothesis. In this study, the data obtained were in the form of a histological picture of the morphological morphology of BM-MSC and ASC cultures. After the tube formation test was carried out, the data obtained were in the form of the total length of the tubes formed, the number of branching points formed and the number of loops formed in one field of view of the 40x magnification inversion microscope produced by BM-MSC and ASC. The morphology of BM-MSC and ASC Pasase 2 - BM-MSC and ASC used in this study have undergone a cryopreservation process at a temperature of -180oC during passage one at the Stem Cell Unit RSCM-FKUI. After thawing and replanting, the cells are still in good condition.

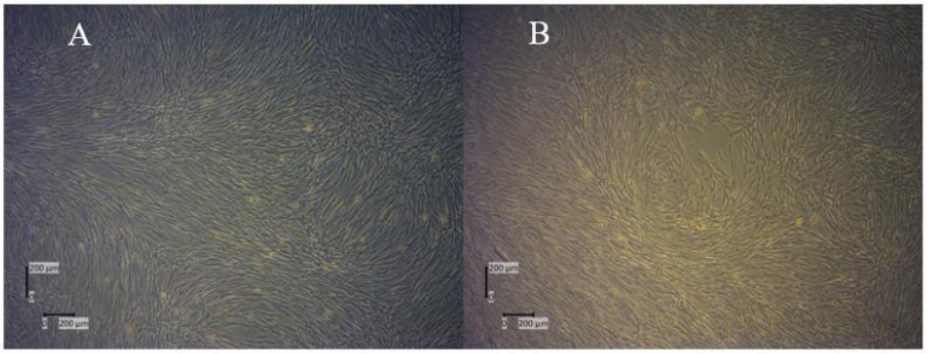


Figure 1. Picture of MSC morphology in passage two culture. (A) BM-MSC culture after two days 6. (B) Passage ASC culture 2. 40x enlargement

Morphology of BM-MSC and ASC Passage 3 - After passage 3, cells began to be used for this study. In Figure 19, the passage three culture, which will be used for the first time for the tube formation test, still shows a homogeneous picture of fibroblastic cell morphology.

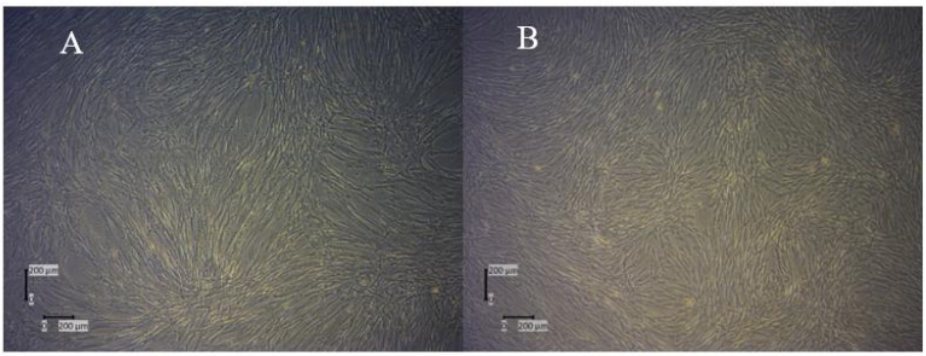


Figure 2.Passage 3 MSC morphological images (A) Passage 3 BM-MSC culture. (B) Passage 3 ASC culture. 40x enlargement

The morphology of BM-MSC and ASC Pasase 7 - Morphology of BM-MSC and ASC is still maintained following the optimal culture conditions until passage 7, which is used for the final tube formation test as shown in Figure 20.

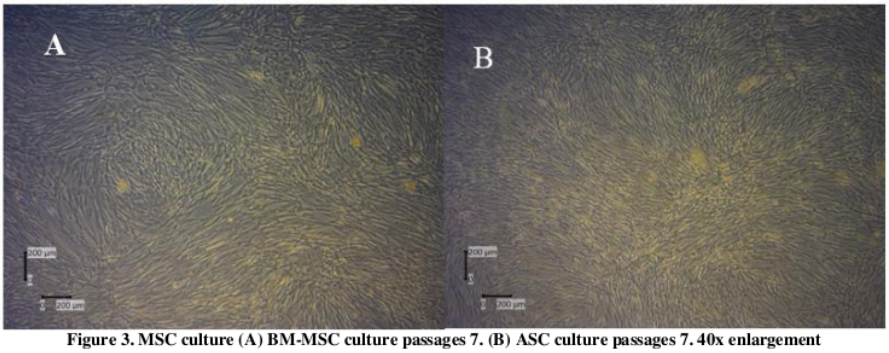
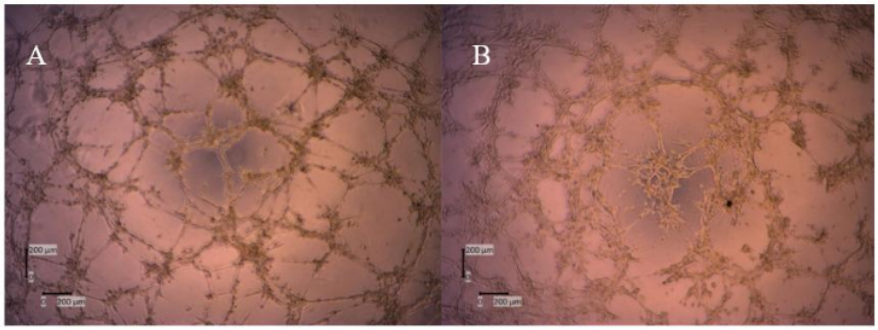


Figure 3. MSC culture (A) BM-MSC culture passages 7. (B) ASC culture passages 7. 40x enlargement

Tube Formation formed on the Matrigel Description - The cells are placed on the matrigel, and the well plate is put into a CO2 incubator. Then observations are made after 3.5 hours, 6 hours and 8 hours later. Tubes of BM-MSC formed well after 6 hours or 8 hours, whereas ASC were seen to form well after 3.5 hours.



Picture 4. Picture of BM-MSC and ASC tube formation on a matrigel with 40x magnification. (A) The optimal BM-MSC tube formation test results from passage three culture. (B) The optimal ASC tube formation test results from passage four culture. It can be seen that both tubes come out of a collection of cells clustered to form a net—40x magnification.

The tube formations formed by BM-MSC looked rougher than those formed by ASC. There are still many areas of the tube covered by cells that have migrated, but it seems that they are not yet wholly differentiated to form tubules in the BM-MSC tube formation.

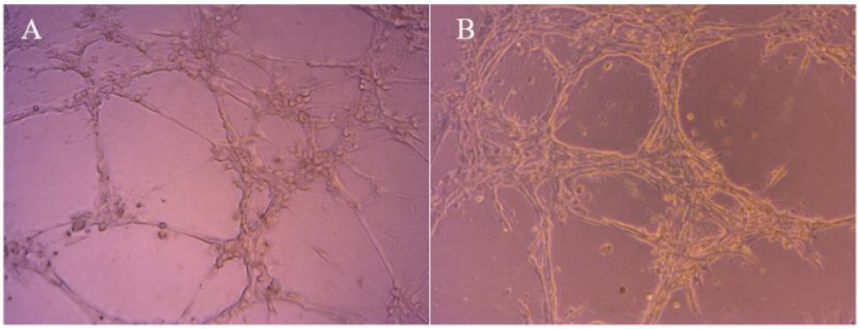


Figure 5. Picture of tube formation BM-MSC and ASC with a magnification of 100x. (A) The results of the BM-MSC passage three tube formation test. (B) The optimal ASC tube formation test results from the passage four culture

Results of Tube Formation Analysis Using the Image Raster 3 Application - Tube formations formed by BM-MSC and ASC are measured one by one in length manually using the image raster three applications. The lines formed are measured in micrometres, then using the excel application, the total length of the tube is calculated, in one field of view, a 40x magnification microscope.

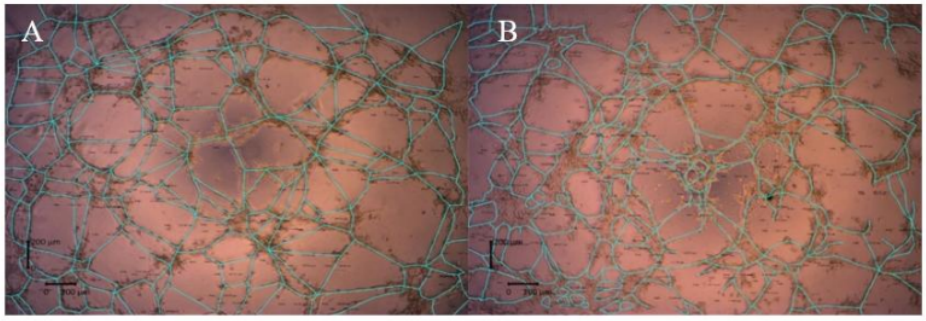


Figure 6. Image of the long analysis result of tube formation using (A) BM-MSC and (B) ASC. 40x magnification

Number of Loops (Circles) - All loops formed on tube formation from BM-MSC and ASC are manually marked one by one and then labelled with a serial number using the image raster three applications.

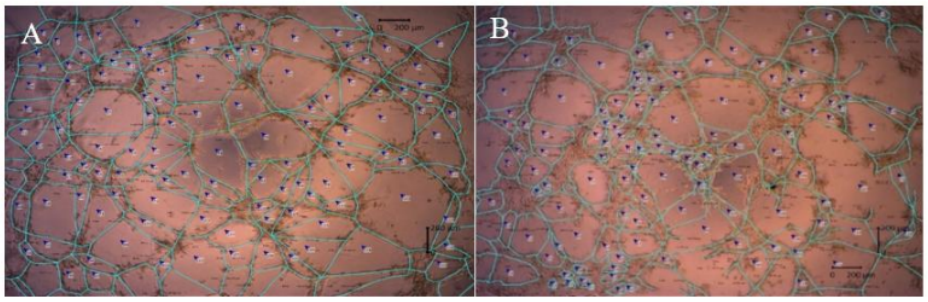


Figure 7. Figure analysis results of the number of loops from the tube formation using(A) BM-MSC and (B) ASC. 40x magnification

Branching Points - All tube formation junction points from BM-MSC and ASC are marked one by one, then recorded and accumulated automatically using the image raster three applications.

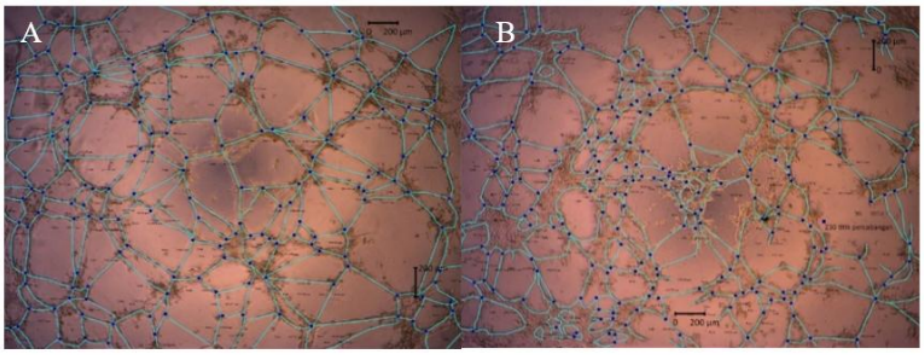


Figure 8. Image of the analysis result of tube formation branching point using BM-MSC (top) and ASC (bottom). 40x magnification

Flow cytometry examination CD34 ASC and BM-MSC levels - The results of checking the CD34 levels of each type of MSC in each passage were obtained from flow cytometric examinations. The CD34 levels of ASC passages 3, 4, 5 and 7 were 0.03%, 0.02% respectively, 0.1% and 0.1%. CD34 levels of BM-MSC passages 3, 4, 5 and 7 were 0.07%, 0.02%, 0.02%, 0.1%, respectively.

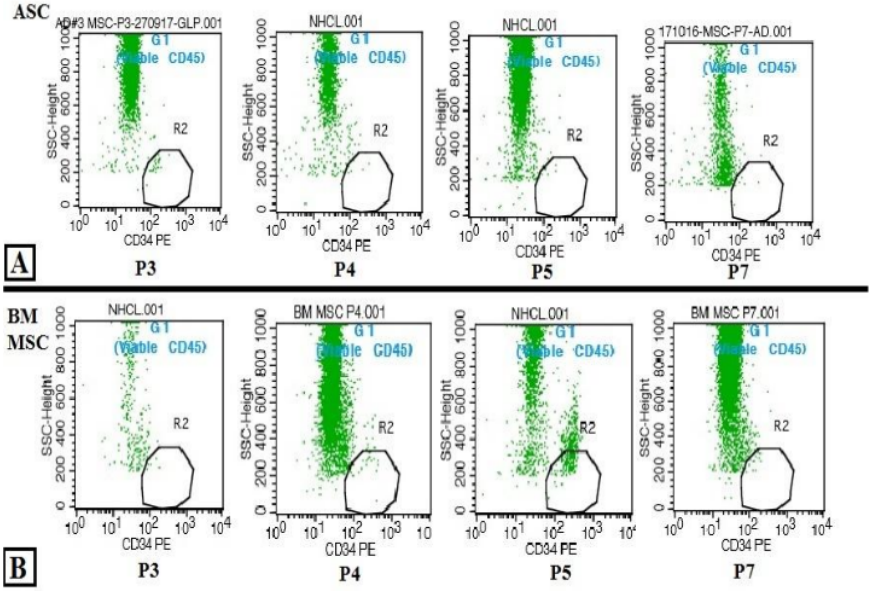


Figure 9. Figure analysis of flow cytometry of CD34 expression on (A) ASC and (B) BM-MSC

The statistical analysis of the BM-MSC and ASC Tube Formation Test with microscopic tube length measurements - The calculation of tube length from 5 wells using image raster three then calculated the mean value and analyzed according to the distribution value. Data on the mean length of BM-MSC and ASC tubes in passage three and passage five are typically distributed ($p > 0.05$) to use independent t-test analysis. Data on the mean tube length in passage four and passage seven were not normally distributed ($p < 0.05$), so that the Mann Whitney U test analysis was used. The independent t-test analysis results showed a significant difference between the mean length of ASC and BM-MSC tubes in passage three and passage 5, where the mean length of BM-MSC tubes was longer than that of ASC. The Mann Whitney U test analysis results showed a significant difference between the mean length of ASC tubes and BM-MSC in passage 7, where the mean length of BM-MSC tubes was longer than the ASC. There was no significant difference between the mean length of ASC tubes and BM-MSC in passage 4.

Table 1. Descriptive data of mean tube length

	Cell Type	N	Mean	Std. Deviation	Std. Error Mean
P3	ASC	5	18752.7780	2926.73724	1308.87668
	BM-MSC	5	104351.9700	61371.85928	27446.32985
P4	ASC	4	65079.5100	9180.07059	4590.03529
	BM-MSC	5	62661.2300	5690.78095	2544.99461
P5	ASC	4	13694.1675	802.59814	401.29907
	BM-MSC	5	32504.3420	3793.63240	1696.56398
P7	ASC	5	11960.6860	3290.84861	1471.71224
	BM-MSC	5	30149.4820	7948.70276	3554.76794

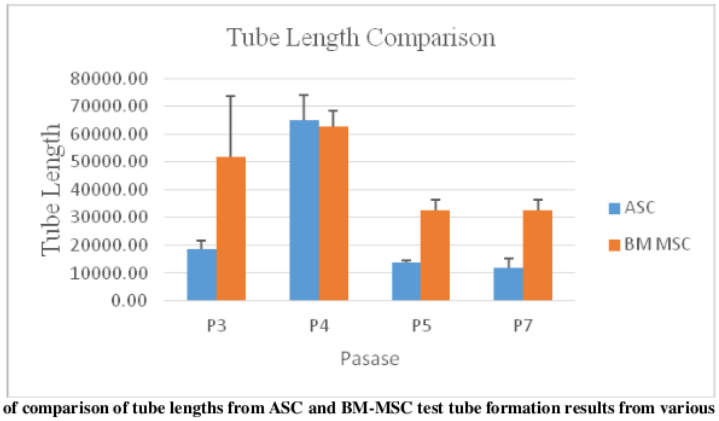


Figure 10. Graph of comparison of tube lengths from ASC and BM-MSC test tube formation results from various passages. There is a significant difference between the mean tube lengths in passages 3, 5 and 7, while in passage four, the comparison is not significant.

Microscopic Calculation of the Number of Loops - The result of calculating the number of loops from the tube in 5 wells using the image raster three then calculates the mean value and analyzes it according to the distribution value. Data on the average number of loops of BM-MSC and ASC in passages 3, 4. 5 and 7 were usually distributed (p> 0.05), so independent t-test analysis was used. The analysis results with the independent t-test showed a significant difference between the mean number of ASC loops and BM-MSC only in passage 3, where the average number of BM-MSC loops was greater than that of ASC. There is no significant difference between the mean number of ASC loops and BM-MSC in passages 4, 5 and 7.

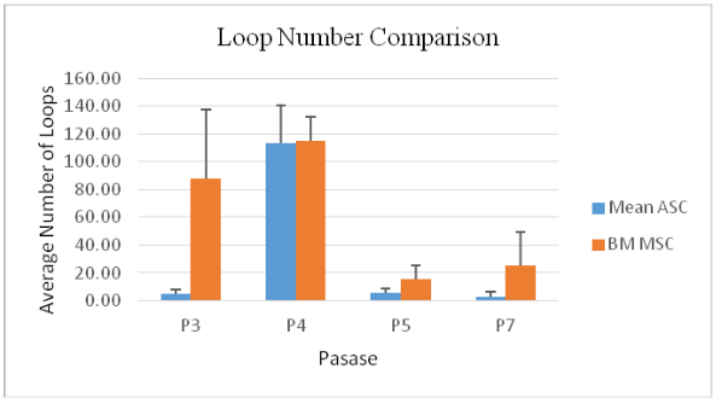


Figure 11. Comparison of the number of loops from the test results of ASC tube formation with BM-MSC from various passages

Table 2. Descriptive data of the number of loops

	Cell Type	N	Mean	Std. Deviation	Std. Error Mean
P3	ASC	5	4.8000	3.34664	1.49666
	BM-MSC	5	87.6000	50.16772	22.43569
P4	ASC	4	113.5000	27.16002	13.58001
	BM-MSC	5	115.0000	17.64936	7.89303
P5	ASC	4	5.5000	3.10913	1.55456
	BM-MSC	5	15.6000	9.98999	4.46766
P7	ASC	5	2.6000	3.43511	1.53623
	BM-MSC	5	25.6000	23.94368	10.70794

Microscopic calculation of the number of branching points - The result of calculating the number of branching points from 5 wells using the image raster three then calculates the mean value and analyzes it according to the distribution value. The mean data of BM-MSC and ASC points at passages 3,4,5, and 7 were normally distributed (p> 0.05), so independent t-test analysis was used. The results of the analysis using the independent t-test show that there is a significant difference between

the mean number of ASC and BM-MSC branching points in passages 3, 5 and 7, where the average number of BM-MSC branching points is higher than the ASC. There is no significant difference between the mean number of ASC and BM-MSC branching points in passage 4.

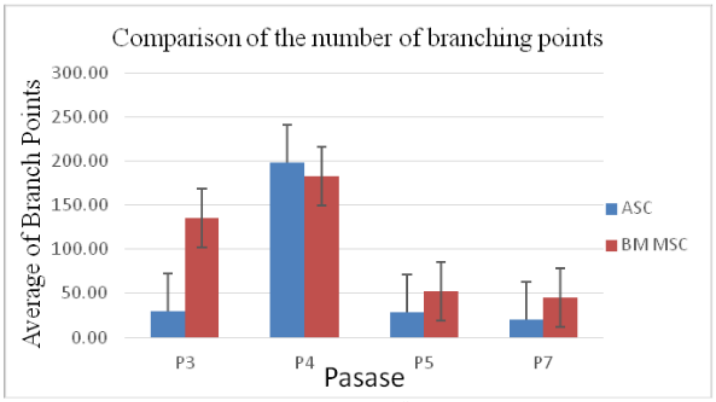


Figure 12. Comparison of the number of branching points from the ASC tube formation test results with BM-MSC from various passages

Table 3. Descriptive data of the number of branching points

	Cell Type	N	Mean	Std. Deviation	Std. Error Mean
P3	ASC	5	29.2000	9.06642	4.05463
	BM-MSC	5	135.8000	73.93037	33.06267
P4	ASC	4	198.0000	57.63679	28.81840
	BM-MSC	5	182.4000	17.15517	7.67203
P5	ASC	4	28.0000	7.70281	3.85141
	BM-MSC	5	52.6000	3.84708	1.72047
P7	ASC	5	20.0000	12.74755	5.70088
	BM-MSC	5	60.8000	25.05394	11.20446

Looking for the Relationship between CD34 Levels and Tube Length, Number of Loops and Number of Branching Points - After a statistical test was carried out, it was found that there was no relationship between CD34 levels and tube lengths from ASC and BM-MSC because the p value> 0.05 was 0.191 and 0.636.0, respectively. There was no relationship between CD34 levels and the number of loops from ASC and BM-MSC because the p value> 0.05 was 0.310 and 0.737, respectively. There is no relationship between CD34 levels and the number of branching points from ASC and BM-MSC because the p value> 0.05 is 0.285 and 0.605, respectively

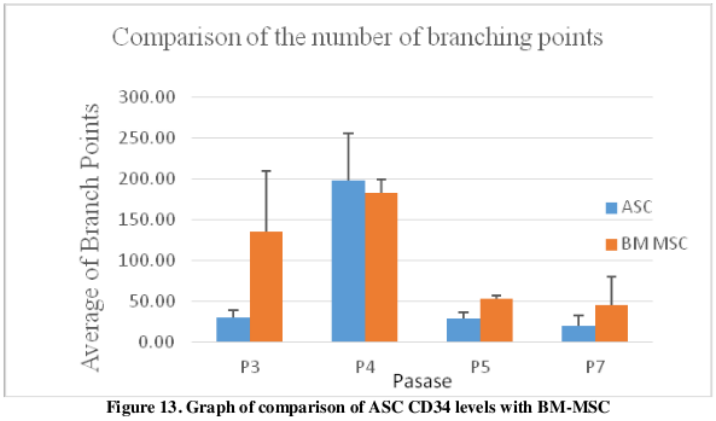


Figure 13. Graph of comparison of ASC CD34 levels with BM-MSC

Microscopic Observation of BM-MSC and ASC Cultures - There are three types of population in human MSC cultures. The first, called rapidly self-renewing cells (RS), was the most pluripotent population and expressed the best or pure surface markers for MSC (CD105, CD90 and CD73). The second type, called spindle-shaped cells (SS), is a viable population of MSC cells with only a small percentage of specific surface markers for MSC are missing. SS type is also said to be the type that has the most significant potential to differentiate into cartilage. The last type is called flattened cuboidal cells (FC), which are the most mature type of MSC with specific surface markers for MSCs mainly being lost, especially significant CD73. RS-type cells are triangular or star-shaped with a prominent membrane section, SS-type cells are elongated fibroblastic or spindle-like, and FC-type cells are large and flattened with a prominent nucleus [38]. At a glance, it appears that the dominant cell population type in the culture of the two MSC types from passage 3 to passage 7 is SS type.

Description of the Tube Formation formed on Matrigel - The number of cells plated in each well significantly affects the test results. If the number of cells is minimal, it will fail to form the expected network, whereas most cells will cause the formation of prominent cell groups or a monolayer cell morphology so that the test results are invalid [39]. Manual cell count using a hematocytometer and trypan blue stain as in this study requires a good and consistent concentration in determining cell viability. Manual cell counting at concentration settings is subjective and varies from individual to individual.

If cell counting is vital and influential in a study, an image-based cell counter with an automatic focus feature can be used, which is more accurate and eliminates the subjectivity of the calculation results. It allows for better consistency of counting between samples [40].

The rate at which individual cells migrate varies depending on the culture conditions (ECM type and presence of growth factors), around 10-50μm / hr. The individual endothelial cell motility is referred to as a persistent random walk. In a brief period, the cell moves along a straight line. Over a more extended period, the movements become random/diffusive. VEGF induces cell motility and chemotactic response in culture. Cell movement is guided by ECM, VEGF signalling and cell-to-cell contact [41].

In this study, tube formation began optimally after the cells were incubated on the matrigel for about 3.5 hours for ASC and 6 hours for BM-MSC, and after one day, the tubes were damaged. In 2012 tube formation on BM-MSC was tested, which had been differentiated into endothelial-like cells using endothelial growth medium-2 (EGM-2) differentiation medium for ten days and then for one day of culture followed by shear force culture using the XYZ machine 3D shaker with a rotational speed of 20 rpm. These cells are called pre-differentiated MSCs, which are believed to have changed their phenotypes into endothelial cell-like cells. The pre-differentiated tube formation ability of MSC is then compared to the naïve BM-MSC or simply called MSC. After the tests were carried out, it appeared that the MSCs formed the tube formation in a much shorter time than the pre-differentiated MSCs. The pre-differentiated MSC did not appear to form the tubes 20 hours after the MSC formed the tubes. However, within 24 hours, the tube from MSC seemed to have started to degrade, while the tube from the pre-differentiated MSC persisted and was getting thicker and formed a more complex network that lasted seven days [42].

The entire tube formation test usually has to be completed within 24 hours as there can be disruption of the tubular structure caused by cell death. Cell migration and adhesion between cells occur during tubule formation, but after 24 hours of apoptosis, the cells will take a role in line

with that many tubular networks are severed [43]. From the results of tube formation tests on specially treated MSCs such as in the research of Fischer et al. [14], Vishnubalaji et al. was carried out, whereas, in this study, the newly harvested MSCs after being cultured in a complete medium with a concentration of 60,000 cells per well from 96-well plates can directly differentiate into endothelial cell-like cells capable of forming blood capillary-like structures in vitro in a normoxic state after being transferred to in a medium containing 2% PRP and placed on the GFR matrigel.

PRP as xeno-free serum affects the occurrence of tube formation. In vitro studies show that PRP contains various growth factors that can induce endothelial cells to be activated and proliferate and form tube formation. In vivo PRP, released continuously, can increase the perfusion of ischemic tissue, blood capillary density, mature blood vessel density and increase CD34 + cells in ischemic areas of transgenic mice. Platelets (platelets) in PRP can modulate angiogenesis by releasing VEGF, bFGF, epidermal growth factor (EGF), PDGF and MMP. MSC itself can secrete pro-angiogenic cytokines. Dmitrieva et al. in 2012 examined the profile of cytokines secreted by BM-MSC and ASC and found that BM-MSC secreted significantly more VEGF than ASC [44].

Flow cytometry examination of CD34 ASC and BM-MSC levels - In a study conducted by Iroda et al., it was seen that the cryopreservation process could reduce CD34 levels, although it was not statistically significant [45]. ASC and BM-MSC used in this study are those that have undergone a cryopreservation process. After planting and subculture, the results are used. The flow cytometry results of the two types of MSC were not statistically significant. Namely, the results were very low or below 2%. The exact role of CD34 for hematopoietic cells is not clear. In general, most researchers found that CD34 acts as an anti-cell adhesion and a role in the cell adhesion process. In mature hematopoietic cells or blood vessel endothelial cells, CD34 inhibits cell interactions and prevents unwanted adhesions by blocking adhesion molecular bonds nearby through extracellular domains that are negatively charged. In the hematopoietic progenitor cells, it is thought that CD34 provides a means for cells to leave the micro-environment in the bone marrow and migrate to a new environment for maturation or in other more extreme situations, such as during embryogenesis, CD34 helps cells enter peripheral blood and form new tissue [46]. Drew et al. found that CD34 played a role in preventing homotypic aggregation on mast cells compared with mast cells that inhibited CD34 expression. Various other studies have concluded the role of CD34 in leukocyte adhesion in high endothelial venule (HEV). Leucocytes express L-selectin, which will bind to specific glycoforms of CD34 and MEP21 (Myb-Ets transformed progenitors-21) expressed by HEV. HEV is a specialized post-capillary venule that occurs in secondary lymphoid organs and areas of chronic inflammation. The process of sticking and rolling (rolling) of the leukocytes (which are selectin dependent on endothelial cells) indicates the start of extravasation of the leucocytes from the blood to the secondary lymphoid tissue. It relies on the CD34 and MEP21 glycosylation processes found only in HEV and not found in most other vascular cells and hematopoietic stem cells.

In this study, there was no relationship between CD34 and the ability of ASC and BM-MSC vasculogenesis. The CD34 level obtained from the ASC was also not higher than the ISCT standard. It is not consistent with previous investigators who found sufficiently high CD34 levels in ASC since early passage [26; 27; 47]. Various things can influence this difference, for example, differences in the origin of the sample, culture method, culture medium and others. The ASC and BM-MSC used in this study did not come from the same patient.

Results of statistical analysis of BM-MSC and ASC Tube formation Test - The average tube length, number of loops and number of branching points in this study were only partially statistically significant, but of all the significant differences, the more excellent value was BM-MSC. From the three parameters measured in the tube formation test, it was found that the best tube formation ability for the second MSC was at P4 then decreased at P5 and P7. The cryopreservation process of MSC can affect the results of tube formation, so that it seems that the ability of the P4 tube formation is better than that of the P3. In the study of Marquez-Curtis et al., It was found that the quality of tube formation was significantly lower in post-cryopreservation cells [48].

In vivo, human ASC was better at recovering rat hind leg ischemia than BM-MSC, which was applied by injecting each type of MSC intramuscularly to three different points (in the gastrocnemius, gracilis and quadriceps muscles) in the ischemic leg in the study. Kim et al. Apart from differentiating into endothelial cells, MSCs are also said to produce secretions that stimulate endogenous EPC. The angiogenic factors produced by ASC and BM-MSC include those such as VEGF and bFGF.

In carrying out this research, the researcher realized that there were limitations, one of which was that the ASC and BM-MSC samples did not come from the same source and had undergone a cryopreservation process. Another limitation is when removing the wall plate from the CO2 incubator and shooting the well using a microscope and computer applications that must be adjusted beforehand until getting the best image, which takes more than 1 hour due to many wells. It turns out that the observation of tube formation under a microscope should not exceed 1 hour due to room temperature, which can affect tube formation.

ASC rats/mice were reported to secrete higher VEGF and HGF secretions than the BM-MSC mice/mice [49], whereas, in human MSC studies, it was reported that ASC VEGF mRNA levels were in balance with BM-MSC. Other researchers found different things, such as in a study conducted by Wen et al. It was stated that levels of angiogenic cytokines such as VEGF-A. VEGF-C, HGF, bFGF, NGF, angiotensin (ANG), TGF-β, IL-6, IL-8, IL-1α, IL-1β and cyclooxygenase-2 (Cox-2) produced by several types of MSC including ASC and BM-MSC showed that overall, most of the pro-angiogenic cytokines produced by BM-MSC were higher than that of ASC.

The relationship between cells, ECM, growth factors and their receptors, proteases and intracellular signalling pathways when blood vessel formation is tightly regulated by dynamic macro and micro environmental factors. Therefore, differences in laboratories and equipment, culture methods may affect the results of the study. For further research, it is necessary to research the effects of paracrine ASC on endothelial cells or EPC, which can be investigated by coculture of the two types of cells using xeno-free medium or by administering conditioned medium ASC to endothelial cell culture / EPC.

CONCLUSION

From the results of this study, the mean length of the BM-MSC tube is longer than ASC, the mean of BM-MSC branching points is more than ASC, and the average number of BM-MSC loops is higher than ASC. Neither CD34 BM-MSC nor ASC expression reached a value of> 2%, and there was no significant difference between the two. The ability of ASC tube formation is better than BM-MSC has not been proven. Therefore, it is necessary to examine further the MSCs that have been incubated on the matrigel by examining the presence of von Willebrand factor and the ability of cells to absorb acetylated low-density lipoprotein (LDL) to determine whether MSCs have completely transdifferentiated into endothelial cells. It

is necessary to study the effect of MSCs that are cultured with endothelial cells or EPC on the effect of conditioned medium MSC on EPC, and further research is needed to confirm the comparison of the vasculogenesis ability of mesenchymal stem cells from fat tissue with bone marrow origin in vivo such as with the matrigel plug angiogenesis assay in experimental animals.

Acknowledgement: None

Conflict of Interest: None

Source of Funding: None

Ethical Approval: Approved

REFERENCES

1. Balaji S, Crombleholme TM, King A. The role of endothelial progenitor cells in postnatal vasculogenesis : implications for therapeutic neovascularization and wound healing. *Adv wound care*. 2013;2(6):283-295. doi:10.1089/wound.2012.0398.
2. Arnaoutova I, George ÆJ, Kleinman HK, Benton ÆG. The endothelial cell tube formation assay on basement membrane turns 20: state of the science and the art. 2009:267-274. doi:10.1007/s10456-009-9146-4.
3. Decicco-skinner KL, Henry GH, Cataisson C, et al. Endothelial cell tube formation assay for the in vitro study of angiogenesis. 2014;10(September):1-8. doi:10.3791/51312.
4. Allameh A. Stem cells, a reservoir for life. *Iran J Biotechnol*. 2013;11(4):205-206. doi:10.5812/ijb.13719.
5. Abdeen A, Jared B, Weiss B, Lee BJ, Kilian KA. Matrix composition and mechanics direct proangiogenic signaling from mesenchymal stem cells. 2014;00(00):1-9. doi:10.1089/ten.tea.2013.0661.
6. Boomsma RA, Geenen DL. Mesenchymal stem cells secrete multiple cytokines that promote angiogenesis and have contrasting effects on chemotaxis and apoptosis. *PLoS One*. 2012;7(4):2-9. doi:10.1371/journal.pone.0035685.
7. Timmers L, Kiang S, Hoefer IE, Fatih Arslan, Chai R. Human mesenchymal stem cell-conditioned medium improves cardiac function following myocardial infarction. *Stem Cell Res*. 2011;6(3):206-214. doi:10.1016/j.scr.2011.01.001.
8. Gu C, Huang S, Gao D, et al. Angiogenic effect of mesenchymal stem cells as a therapeutic target for enhancing diabetic wound healing. *Int J Low Extrem Wounds*. 2014;13(2):88-93. doi:10.1177/1534734614534977.
9. Schlosser S, Dennler C, Schweizer R, et al. Paracrine effects of mesenchymal stem cells enhance vascular regeneration in ischemic murine skin. *Microvasc Res*. 2012;83(3):267-275. doi:10.1016/j.mvr.2012.02.011.
10. Lin C-S, Ning H, Lin G, Lue TF. Is CD34 truly a negative marker for mesenchymal stem cells? *Cytotherapy*. 2013;14(10). doi:10.3109/14653249.2012.729817.Is.
11. Pawitan JA, Wulandari D, Suryani D, Damayanti L, Purwoko RY, Liem IK. Flow cytometry analysis of adipose tissue-derived stem cells that were cultured in various media. *PharmTech*. 2013;5(3):1301-1306.
12. Kim YS, Kwon JS, Hong MH, Kang WS, Jeong H, Kang H. Restoration of angiogenic capacity of diabetes-insulted mesenchymal stem cells by oxytocin. *BMC Cell Biol*. 2013;14(38).
13. Pawitan JA. Prospect of adipose tissue-derived mesenchymal stem cells in regenerative medicine. *Cell Tissue Transplant Ther*. 2009;2:0-2.
14. Fischer LJ, Mcilhenny S, Tulenko T, et al. Endothelial differentiation of adipose-derived stem cells :eEffects of endothelial cell growth supplement and shear force. *YJSRE*. 2009;152(1):157-166. doi:10.1016/j.jss.2008.06.029.
15. Perez LM, Bernal A, Martın NS, Galvez BG. Obese-derived ASCs show impaired migration and angiogenesis properties. *Informa*. 2013;3455(5):195-201. doi:10.3109/13813455.2013.784339.
16. Lubarsky B, Krasnow MA. Tube Morphogenesis : Making and Shaping Biological Tubes Many organs are composed of epithelial tubes that. 2003;112:19-28.
17. Iruela-arispe ML, Davis GE. Review Cellular and Molecular Mechanisms of Vascular Lumen Formation. *Dev Cell*. 2009;16(2):222-231. doi:10.1016/j.devcel.2009.01.013.

18. Park KM, Gerecht S. Harnessing developmental processes for vascular engineering and regeneration. 2014:2760-2769. doi:10.1242/dev.102194.
19. Hanjaya-Putra D, Yee J, Ceci D, et al. Vascular endothelial growth factor and substrate mechanics regulate in vitro tubulogenesis of endothelial progenitor cells. 2010;14(10):2436-2447. doi:10.1111/j.1582-4934.2009.00981.x.
20. Kilarski WW, Samolov B, Petersson L, Kvanta A. Biomechanical regulation of blood vessel growth during tissue vascularization. 2009;15(6):657-665. doi:10.1038/nm.1985.
21. Mammoto A, Connor KM, Mammoto T, et al. NIH Public Access. 2009;457(7233):1103-1108. doi:10.1038/nature07765.A.
22. Aviv-Yafo T. Shweiki , D , Itin , A , Soffer , D & Keshet , vascular endothelial growth factor-induced by hypoxia may mediate ... 2014;(May):7-10. doi:10.1038/359843a0.
23. Nombela-arrieta C, Ritz J, Silberstein LE. The elusive nature and function of mesenchymal stem cells. *Nat Publ Gr*. 2011;12(2):126-131. doi:10.1038/nrm3049.
24. Zhao RC. *Essentials of Mesenchymal Stem Cell Biology and Its Clinical Translation*. (Zhao RC, ed.). Dordrecht: Springer; 2013. doi:10.1007/978-94-007-6716-4.
25. Zheng YL. Some ethical concerns about human induced pluripotent stem cells. *Sci Eng Ethics*. 2015. doi:10.1007/s11948-015-9693-6.
26. Ben-David U, Benvenisty N. The tumorigenicity of human embryonic and induced pluripotent stem cells. *Nat Publ Gr*. 2011;11(4):268-277. doi:10.1038/nrc3034.
27. Kim EM, Stultz R, Bonde S. Embryonic stem cell-derived T cells induce lethal graft-versus-host disease and reject allogeneic skin grafts upon thymic selection. 2012;(10):600-609. doi:10.1111/j.1600-6143.2011.03845.x.
28. Ullah I, Subbarao RB, Rho GJ. Human mesenchymal stem cells - current trends and future prospective Bioscience Reports. 2015. doi:10.1042/BSR20150025.
29. Klingemann H. Mesenchymal Stem Cells – Sources and. 2008:272-277. doi:10.1159/000142333.
30. Levi B, Longaker MT. Adipose-derived stromal cells for skeletal regenerative medicine. *Stem Cells*. 2011;29(4):576-582. doi:10.1002/stem.612.Adipose.
31. Bunnell BA, Flaat M, Gagliardi C, Patel B, Ripoll C. Adipose-derived stem cells: isolation, expansion and differentiation. *Methods*. 2008;45(2):115-120. doi:10.1016/j.ymeth.2008.03.006.Adipose-derived.
32. Rahbarghazi R, Mahdi S, Hossein S, et al. Dynamic induction of pro-angiogenic milieu after transplantation of marrow-derived mesenchymal stem cells in experimental myocardial infarction. *Int J Cardiol*. 2014;173(3):453-466. doi:10.1016/j.ijcard.2014.03.008.
33. Gong M, Yu B, Wang J, et al. Mesenchymal stem cells release exosomes that transfer miRNAs to endothelial cells and promote angiogenesis. 2017;8(28):45200-45212.
34. Daley GQ, Scadden DT. Essay Prospects for Stem Cell-Based Therapy. 2008;1:544-548. doi:10.1016/j.cell.2008.02.009.
35. Kumar N, Kishor V, Kumar Y, Gupta P, Sharma S. Mesenchymal stem cell-based therapy : 2009;13(11):4385-4402. doi:10.1111/j.1582-4934.2009.00857.x.
36. Pawitan JA, Mediana D. Effect of platelet-rich plasma on post cryopreservation viability , morphology and proliferation of human umbilical. 2015. doi:10.3844/ojbsci.2015.42.48.
37. Diogo IH. Simple Method to Isolate Mesenchymal Stem Cells from Bone Marrow using Xeno-Free Material : A Preliminary Study Simple Method to Isolate Mesenchymal Stem Cells from Bone Marrow using Xeno-Free Material : A Preliminary Study. 2015;(April).
38. Hamsters F, Prall WC, Anz D, et al. Morphological and immunocytochemical characteristics indicate the yield of early progenitors and represent a quality control for human mesenchymal stem cell culturing. 2009:759-767. doi:10.1111/j.1469-7580.2009.01065.x.
39. Xie D, Ju D, Speyer C, Gorski D, Kosir MA. Strategic endothelial cell tube formation assay : comparing extracellular matrix and growth factor reduced extracellular matrix. 2016;(August):1-6. doi:10.3791/54074.
40. Lapenta F, Gradišar H, Drobnak I, et al. The four pillars of achieving accurate and precise cell counts. *Nat Commun*.

2017;8(1):81-88. doi:10.1016/j.cub.2017.11.050.
41. Czirok A. Endothelial cell motility, coordination and pattern formation during vasculogenesis. *Wiley Interdiscip Rev Syst Biol Med*. 2013;5(5):587-602. doi:10.1002/wsbm.1233.Endothelial.
42. Janeczek Portalska K, Leferink A, Groen N, et al. Endothelial differentiation of mesenchymal stromal cells. *PLoS One*. 2012;7(10). doi:10.1371/journal.pone.0046842
43. Iii RAF, Faibish M, Shao R. A matrigel-based tube formation assay to assess the vasculogenic activity of tumor cells. 2011;(September):2-5. doi:10.3791/3040.
44. Dmitrieva RI, Minullina IR, Bilibina AA, Tarasova O V., Anisimov S V., Zaritskey AY. Bone marrow and subcutaneous adipose tissue-derived mesenchymal stem cells: differences and similarities. *Cell Cycle*. 2012;11(2):377-383. doi:10.4161/cc.11.2.18858.
45. Irioda AC, Cassilha R, Zocche L, et al. Human Adipose-Derived Mesenchymal Stem Cells Cryopreservation and Thawing Decrease ? 4-Integrin Expression. 2016;2016. doi:10.1155/2016/2562718.
46. Ohnishi H, Sasaki H, Nakamura Y, et al. Regulation of cell shape and adhesion by CD34. 2017;6918(November). doi:10.4161/cam.25957.
47. Castilla L, Maumus M, Peyrafitte J, et al. Native human adipose stromal cells : localization , morphology and phenotype. 2011:1141-1153. doi:10.1038/ijo.2010.269.
48. Marquez-Curtis LA, Sultani AB, McGann LE, Elliott JAW. Beyond membrane integrity: assessing the functionality of human umbilical vein endothelial cells after cryopreservation. *Cryobiology*. 2016;72(3):183-190. doi:10.1016/j.cryobiol.2016.05.005.
49. Ikegami Y, Yamashita K, Hayashi S-I, et al. comparison of mesenchymal stem cells from adipose tissue and bone marrow for ischemic stroke therapy. *Cytotherapy*. 2011;13(6):675-685. doi:10.3109/14653249.2010.549122.

How to cite this article: Panjaitan AO, Sukmawati D, Anggraeni R et.al. Comparison of in vitro vasculogenesis potential between ASCs with BM-MSCs. *Int J Health Sci Res*. 2021; 11(5): 362-378. DOI: *https://doi.org/10.52403/ijhsr.20210557*

******

TurnitinComparisonofinVitroVasculogenesisPotentialbetwee...

ORIGINALITY REPORT

8%	6%	8%	4%
SIMILARITY INDEX	INTERNET SOURCES	PUBLICATIONS	STUDENT PAPERS

PRIMARY SOURCES

1	Submitted to Institute of Technology, Sligo Student Paper	2%
2	Ahmed El-Badawy, Marwa Amer, Reda Abdelbaset, Sameh N. Sherif et al. "Adipose Stem Cells Display Higher Regenerative Capacities and More Adaptable Electro-Kinetic Properties Compared to Bone Marrow-Derived Mesenchymal Stromal Cells", Scientific Reports, 2016 Publication	2%
3	Regenerative Medicine, 2015. Publication	1%
4	Bunnell, B.A.. "Adipose-derived stem cells: Isolation, expansion and differentiation", Methods, 200806 Publication	1%
5	Submitted to Federal University of Technology Student Paper	1%
6	eprints.qut.edu.au Internet Source	1%

7	umconference.um.edu.my Internet Source	1 %
8	www.ijhsr.org Internet Source	1 %
9	Pauline Gilson, Morgane Couvet, Laetitia Vanwonterghem, Maxime Henry et al. "The pyrrolopyrimidine colchicine-binding site agent PP-13 reduces the metastatic dissemination of invasive cancer cells in vitro and in vivo", Biochemical Pharmacology, 2019 Publication	1 %

Exclude quotes	On	Exclude matches	< 1%
Exclude bibliography	On		