

ORIGINAL ARTICLE

Comparison of the In Vitro Angiogenic Potential of Mesenchymal Stem Cells Derived from Human Dental Pulp and Human Bone Marrow through the Expression of Endothelial Growth Factor Receptor-2 (VEGFR2)

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ABSTRACT

Introduction: Mesenchymal stem cells have two essential properties, namely, high self-renewal capability and multilineage differentiation potential, including angiogenic differentiation. Angiogenesis, also known as the formation of new blood vessels, is a focal point in tissue engineering. The regulation of angiogenesis is part of regenerative therapeutics by promoting neovascularisation, where blood vessels are required to provide nutrients and facilitate the removal of waste products. However, the comparison of the angiogenic potential of mesenchymal stem cells derived from human dental pulp (DP-MSCs) and human bone marrow (BM-MSCs) has not been adequately described. Thus, this project is aimed to analyse the angiogenic potential of DP-MSC and BM-MSC in vitro by measuring the expression of vascular endothelial growth factor receptor-2 (VEGFR2). **Material and Methods:** Mesenchymal stem cells were isolated from dental pulp tissue and tibial bone marrow aspirates; cultured in MSC alpha-MEM basal medium. The cells were characterised by immunophenotyping using flow cytometer. The MSCs were subjected to angiogenic differentiation for 3 weeks, and angiogenic marker VEGFR2 was detected using immunohistochemistry staining. **Results:** Both MSCs expressed the positive marker of MSCs, CD105 and the absence of haematopoietic markers, CD45 and CD34. When both sources of MSCs cultured in a standard and angiogenic media, DP-MSCs demonstrated an inherent expression of VEGFR2 at the basal level but approximately increased 60% with angiogenesis induction media. On the other hand, BM-MSCs did not display VEGFR2 expression at the basal level yet showed ~20% of expression upon induction with angiogenic media. **Conclusion:** The angiogenic differentiation potential of human dental pulp stem cells (DP-MSCs) was significantly higher than that of human bone marrow stem cells (BM-MSCs), as evidenced by VEGFR2 expression. These findings underline the potential application of DP-MSCs in tissue regeneration and dental matrix reconstitution. Further studies should explore scalability, investigate long-term clinical outcomes, and assess integration in advanced regenerative therapies.

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INTRODUCTION

Mesenchymal stem cells are characterised as cells with multilineage differentiation capability; hence considered promising candidates for regenerative medicine (1, 2). Apart from mesodermal differentiation, they have demonstrated differentiation towards various lineages,

such as odontoblasts, osteoblasts, adipocytes, myoblasts, cartilage cells, endothelial cells and nerve cells in vitro and in vivo (3, 4). MSCs distributed to a wide variety of tissues and can induce tissue repair in both models preclinical and clinical (5, 6). During tissue injury, tissue-resident MSCs or from nearby organs will migrate to injury sites to perform tissue repair at the site and endothelial-like cells, which promote angiogenesis and neovascularization (7, 8). Angiogenesis is a biological process that leads to the formation of blood vessels, which is the focal point in tissue engineering by stimulating the development of capillary-like structures to

form new blood vessels (9, 10). A good network of vessels is needed to support the metabolism of cells around the injury site by providing nutrients and a drainage channel for unnecessary waste products (11).

Mesenchymal stem cells can be isolated from various tissues, such as dental pulp, bone marrow, skin, adipose, blood and delivery wastes (12). Amongst all sources, the human dental pulp is obtained without causing additional injury since it is collected from third molars and first premolars extracted from patients with prophylactic indications for orthodontic treatment (13, 14). However, the collection of bone marrow samples is involved invasive methods, painful and often complicated with infection and hospitalisation. In general, research on human dental pulp mesenchymal stem cells (DP-MSCs) is still at the infant stage; moreover, it is very new for a country like Indonesia. Although the dental pulp is from the human tissue, most research only employs MSCs derived from other residual tissues such as adipocytes, amnion and umbilical cord (15). Therefore it is necessary to explore the potential of DP-MSCs, especially on angiogenic differentiation potential and compare it with a standard source of MSCs, which is the human bone marrow.

MATERIALS & METHODS

Samples

All samples, including human bone marrow and dental pulp tissues, were collected after obtaining written consent from the donors. The project was approved by the Health Research Ethics Committee (KEPK) Dr Soetomo Hospital Surabaya [Reference No:1928/KEPK/IV/2020] and Health Research Ethical Clearance Commission Universitas Airlangga of Dental Medicine [Reference No. 062/HRECC.FODM/II/2020]. The bone-marrow aspirate was aspirated from the tibial bone as much as 20 ml, which would produce 300 µL of bone marrow (16), collected into a heparin-coated tube and filled with transport medium mixed and homogenised to prevent clotting. Human dental pulp tissue was obtained from two donors; the first was a 34-year-old woman who donated 38 tooth, the second was a 15-year-old girl who donated both teeth 14 and 24, as prophylactic orthodontic treatment. All teeth were divided sagittally, and the dental pulp was removed and placed in the transport medium. The samples were brought to the network bank installation of Dr Soetomo Hospital for isolation, culture and differentiation processes.

Cell Isolation and Culture

The isolation of human bone marrow, due to its liquid phase, was carried out using direct and indirect techniques. The direct technique was employed by directly culturing the bone marrow fraction masses and clots (17). The indirect technique was performed on homogeneous bone marrow suspensions with transport medium and heparin. The solid tissue was chopped finely with sterile surgical scissors for human dental pulp tissue and

cultured using enzymatic and explant techniques (18). Cells were cultured using the alpha-MEM basal medium (series 12000-014, Gibco, USA), supplemented with 1% antibiotic and antimycotic supplement (series 15240062, Gibco, USA), 10% fetal bovine serum (FBS) (Gibco, USA), 1% L-Glutamine (Gibco, USA), incubated at 37°C in 5% CO₂ humidity.

Immunophenotyping

The cell surface markers of MSCs were detected using flow cytometry (BD FACS Calibur, USA). Fibroblast-like cells from human bone marrow and dental pulp were harvested after 80% confluency by trypsinisation with 0.05% trypsin-EDTA. One million cells were labelled with 1 µL of respective fluorochrome-conjugated antibodies (CD105-FITC, CD34- and CD45-) (BD Bioscience, USA) in a dark chamber at 4°C for 40 minutes and fixed with 10% formalin buffer. The data was analysed using the Cell Quest Software (Becton Dickinson).

Differentiation assays

To compare the angiogenic potential of DP-MSCs and BM-MSCs, after being cultured for a month, cells were seeded at 5 x 10⁴ cells/well in a 24-well plate coated with 0.1% fibronectin. Subsequently, cultures were stimulated with the angiogenic medium, which consists of 50 ng/ml vascular endothelial growth factor (VEGF) (Peprose Technohouse, Israel) in the standard MSCs basal culture medium.

Immunocytochemical staining

The immunoreactive score of Remmele & Stegner (IRS) was used to measure and visualise positive the somatostatin receptor (SSTR) on the mesenchymal stem cells' surface with VEGFR primer antibody. The purpose of using this IRS score is to semi-quantitatively analyse sections of MSCs stained with primary antibodies in the immunocytochemical assay using a light microscope (17).

Statistical Analysis

Data were expressed by mean standard errors, and statistics were analysed using SPSS 10.0 for Windows (SPSS Inc., USA). Independent T-test statistical analysis was used for normal & homogenous data distribution. Mann-Whitney test was used if the distribution was not normal and not homogeneous, with a significant value of $p < 0.05$.

RESULTS

MSCs Culture Results

Primary culture treatment process for both MSCs source cells, human dental pulp and human bone marrow are the same. Figure 1 shows the results of both cultures in MSC basal medium: Medium change is done every 5–7 days. After 1 month of culture there are adhering cells and began to form a colony of healthy cells (monolayer cells) and carried out passage 1 (p1).

Flowcytometry and Immunocytochemical Results

In Figures 1A and B, at the ends of the arrows are monolayer DP-MSCs that grow with migration away from the dental pulp tissue fragments, and the growing monolayer of BM-MSCs migrate away from the growth center of bone marrow cells. The two grow together with other cells with strong bonds to form a monolayer. In Figure C, DP-MSCs and BM-MSCs have separated from cell colonies. In terms of monolayer, DP-MSCs and BM-MSCs are similar, so it is hoped that the potential for differentiation of DP-MSCs will also have resemblance to BM-MSCs. In Figure D, monolayer cells after trypsinisation with TrypLE Express, cells will separate into single cells (as shown by arrowhead). Figure E shows overview of mature DP-MSCs and BM-MSCs, which have been proliferating and reached 80% confluency. In such cir-

cumstances, these two MSCs can be passed and used for propagation/cloning of MSCs.

Flowcytometry Results

Flow cytometry analysis of DP-MSC (Figure 2) and BM-MSC (Figure 3) cultures showed strong expression of CD105 and negative expression of CD34.

Immunocytochemical Test Results

Figure 4 shows the immunocytochemistry test on MSCs in α -MEM basal medium. In the DP-MSC group (Figure 4A), yellow arrow shows immunoreactive expression of vascular endothelial growth factor receptor-2 (VEGFR-2) with 3D lateral solid and nodular fold formation. In the BM-MSC group (Figure 4B), there is no expression of immunoreactive staining against VEGFR2.

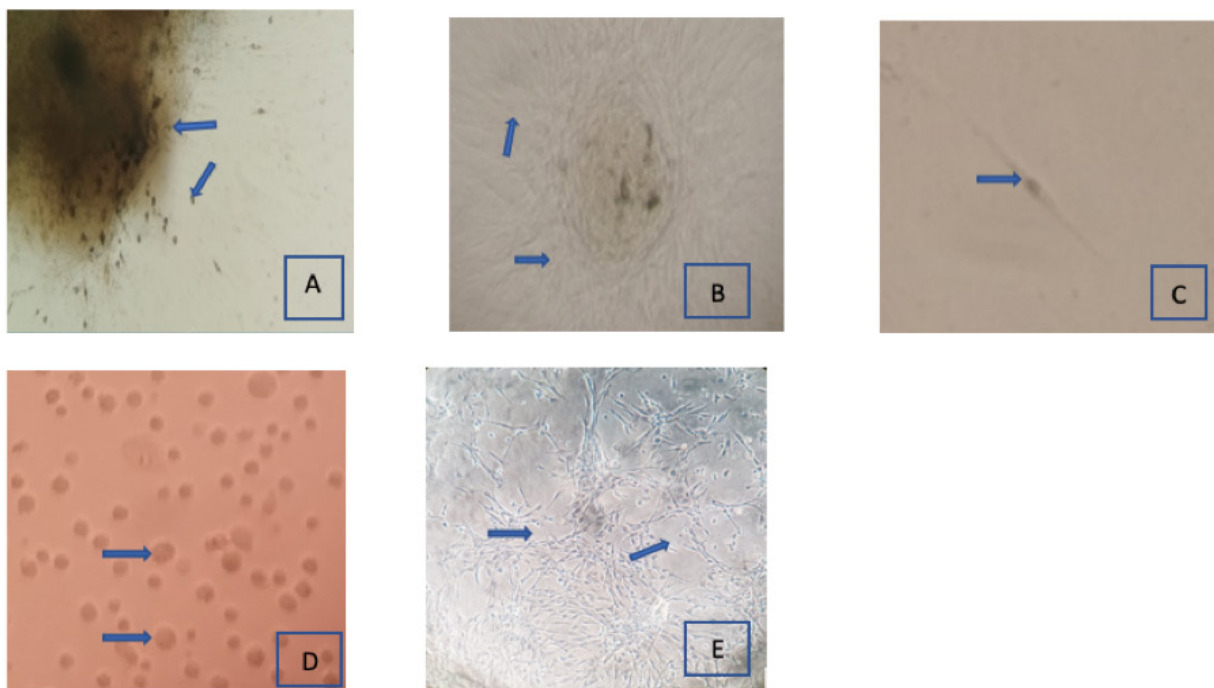


Fig. 1: Cell Outgrowth Process A. Sprouting dental pulp cells, B. Sprouting bone marrow cells, C. Overview spindle-like cells from BM-MSC & DP-MSC, D. Overview of single cell MSCs, E. Overview of MSCs that has been mature, proliferating and confluent 100%.

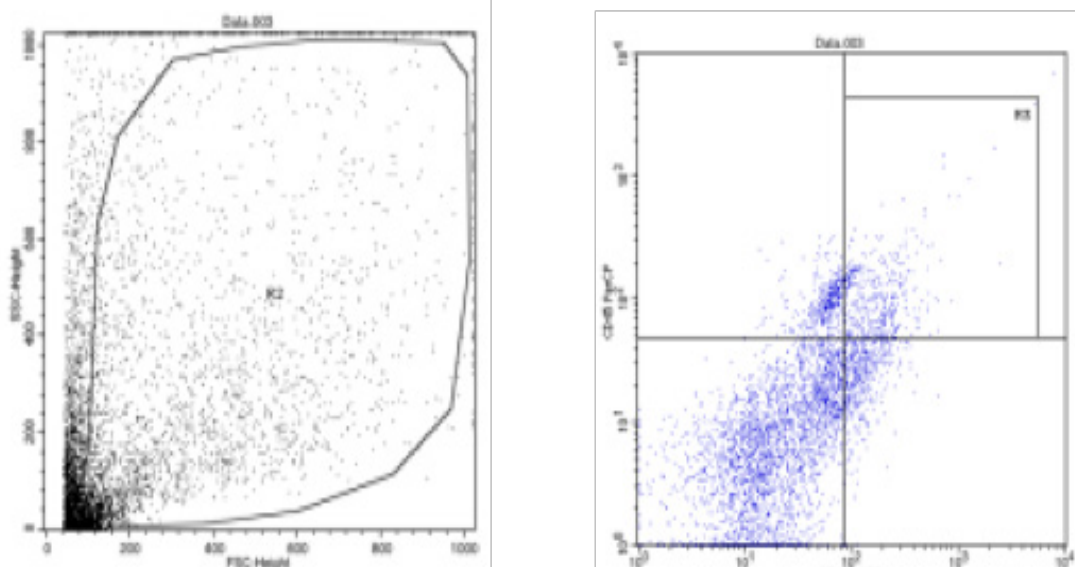


Fig. 2: Flow cytometry test results on DP-MSC

Results from the angiogenic medium (with VEGFR2 primer antibody) are shown in Figure 5. DP-MSCs showed golden yellow staining (red arrow) for VEGFR2 (Figure 5A) with lateral fold formation and 3D vascular-like structure with high cell density. BM-MSC group (Figure 5B) showed less expression of VEGFR2.

Table I presents statistical results of immunocytochemical tests comparing DP-MSC and BM-MSC in both α -MEM and angiogenic medium. In α -MEM, DP-MSC VEGFR2 expression was higher (not statistically significant, $p = 0.056$). In angiogenic medium, VEGFR2 expression in DP-MSCs was significantly higher than BM-MSCs ($p = 0.007$).

DISCUSSION

Mesenchymal stem cells (MSCs) are known for wound healing and regenerative potential in tissue engineering due to their self-renewal and plasticity. In this study, MSCs derived from human bone marrow (BM-MSCs) and human dental pulp (DP-MSCs) were used to analyse angiogenic differentiation.

Human bone marrow and dental pulp share similarities in isolation, both using direct and indirect techniques. DP-MSCs were cultured in α -MEM, which showed higher proliferation than DMEM. This medium, supplemented with L-Glutamine and serum, accelerates MSC prolif-

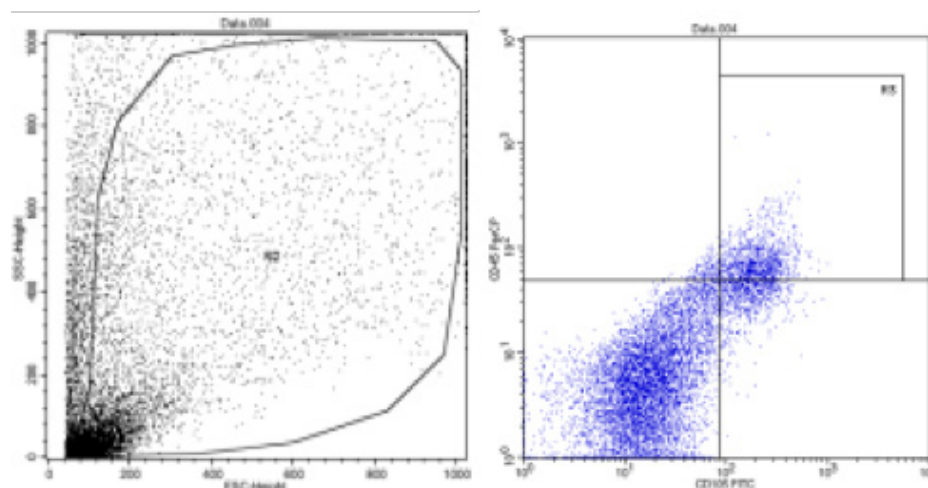


Fig. 3: Flowcytometry test results on BM-MSC

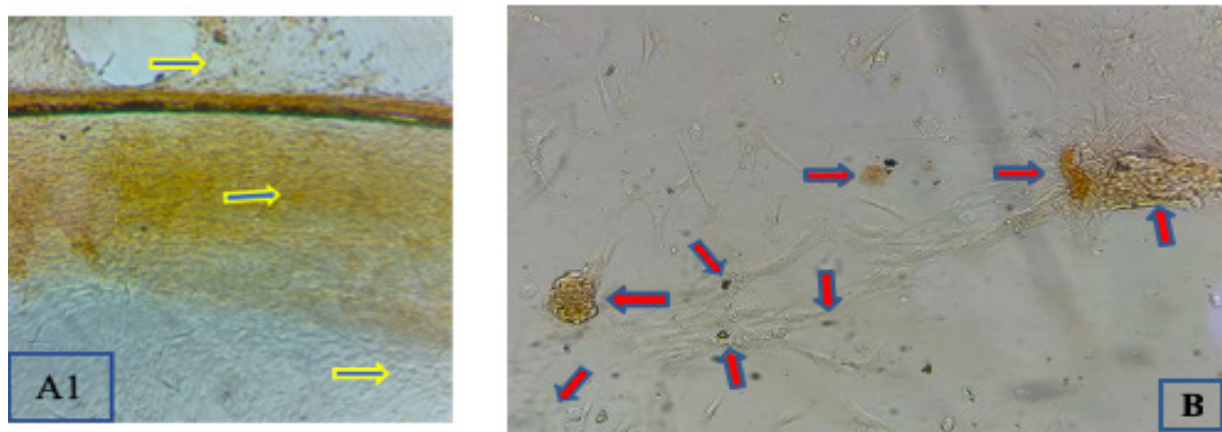


Fig. 4: Immunocytochemical test results in α -MEM medium with VEGFR primer antibody (A1: DP-MSC; B: BM-MSC).

Table I: Statistical result of VEGFR expression in DP-MSC and BM-MSC culture in α -MEM medium & angiogenic medium

Medium	VEGFR Expression		P = (P<0,05)
	DP-MSCs Mean $\pm$ Std Deviation	BM-MSCs Mean $\pm$ Std Deviation	
α -MEM	0,50 $\pm$ 0,548	0,00 $\pm$ 0,000	0,056
Angiogenic	3,3333 $\pm$ 1,506	0,8333 $\pm$ 0,983	0,007*

*significant

eration and stimulation of angiogenic potential.

BM-MSC culture includes hematopoietic and stromal cells, requiring frequent medium changes. DP-MSCs dominate with fibroblast-like cells. The characterisation confirmed expression of CD105 and absence of haematopoietic markers (CD45 and CD34). Variation in marker expression may result from mixed or early passage cells.

VEGF is a selective mitogen promoting angiogenesis via endothelial cell stimulation, permeability increase, and capillary formation. VEGFR2 expression in DP-MSCs was higher than in BM-MSCs, forming 3D vascular-like structures in vitro.

CONCLUSION

The angiogenic differentiation potential of human dental pulp stem cells (DP-MSCs) is significantly higher than human bone marrow stem cells (BM-MSCs), evidenced by VEGFR2 expression.

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