ORIGINAL ARTICLE

Human Dental Pulp-Derived Mesenchymal Stem Cells: Unveiling Their Angiogenic and Osteogenic Capacities for Bone Tissue Engineering (An In Vitro Study)

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ABSTRACT

Introduction: Mesenchymal stem cells (MSCs) play a crucial role in regenerative medicine and bone tissue engineering. While human bone marrow-derived MSCs (hBM-MSCs) remain the gold standard, human dental pulp-derived MSCs (hDP-MSCs) represent a less invasive alternative with strong multipotent potential, essential for effective vascular and bone regeneration. **Objective:** This study directly compares the angiogenic and osteogenic differentiation capacities of hDP-MSCs and hBM-MSCs to identify the most suitable cell source for regenerative therapies. **Method:** Mesenchymal stem cells (MSCs) were isolated from human dental pulp and bone marrow, obtained from extracted teeth and os-tibia, respectively. The cells were then cultured in angiogenic and osteogenic induction media to stimulate lineage-specific differentiation. Expression of Vascular Endothelial Growth Factor Receptor 2 (VEGFR2), Bone Morphogenetic Protein 2 (BMP2), and Osteocalcin (OCN) was assessed via immunocytochemistry. Marker expression was evaluated semi-quantitatively using the Immunoreactive Score (IRS), and statistical significance was determined using the Mann–Whitney U test ($p < 0.05$). **Results:** hDP-MSCs showed significantly higher VEGFR2, BMP2, and OCN expression ($p<0.05$), indicating superior angiogenic and osteogenic/odontogenic potential compared to hBM-MSCs. Discussion: hDP-MSCs proliferate faster due to fibroblast abundance in dental pulp, unlike hBM-MSCs whose growth declines over time. This higher proliferation supports better differentiation, marked by distinct 3D-ECM folds seen only in hDP-MSCs during angiogenic and osteogenic induction. **Conclusion:** Human dental-pulp MSCs (hDP-MSCs) have remarkable dual differentiation capabilities, making them ideal candidates for simultaneous bone and vascular regeneration. These capabilities highlight potential therapeutic use of hDP-MSCs in complex tissue engineering, opening new opportunities for regenerative therapies.

Malaysian Journal of Medicine and Health Sciences (2025) 21(SUPP10):29-34. doi:10.47836/mjmhs.21.s10.7

Keywords: Human dental pulp MSCs (hDP-MSCs), osteogenic differentiation, angiogenic differentiation, bone regeneration and vessel regeneration,

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INTRODUCTION

Mandibular critical-size bone defects (MCSBD) remain a significant clinical challenge due to their inherent inability to heal spontaneously, resulting in long-

term structural deformity and functional impairment. These defects, often arising from trauma, tumours, or congenital conditions, are notoriously difficult to manage in both dental and orthopaedic reconstructive contexts. Although autogenous bone grafts remain the clinical gold standard due to their osteogenic and osteoinductive properties; however, they are often associated with complications such as donor site morbidity, limited availability, prolonged recovery time, and risk of infection. These limitations underscore the urgent need for more effective regenerative strategies. In response, bone tissue engineering (BTE) has emerged as a compelling alternative, leveraging the synergistic potential of mesenchymal stem cells (MSCs), bioactive scaffolds, and osteoinductive signals to support robust and predictable bone regeneration.(1,2)

Among the components of Bone Tissue Engineering (BTE), MSCs have attracted considerable attention due to their multipotent differentiation capacity, including osteogenic, angiogenic, chondrogenic, and adipogenic lineages. MSCs can be harvested from various tissues—such as bone marrow, adipose tissue, placenta, and dental pulp—and are typically classified by their tissue of origin (3,4). Although human bone marrow-derived mesenchymal stem cells (hBM-MSCs) have long served as the benchmark in regenerative medicine, their clinical translation is increasingly challenged by invasive harvesting procedures, age-related decline in proliferative capacity, and relatively low cellular yield.

In contrast, human dental pulp-derived MSCs (hDP-MSCs), isolated from routinely extracted premolars or third molars, offer a less invasive, ethically acceptable, and more accessible alternative. These cells have demonstrated superior clonogenicity, plasticity, and higher potential for endothelial and neurogenic differentiation. Notably, previous studies, including those by Karaıız et al. (2011), suggest that DP-MSCs exhibit greater metabolic activity and broader differentiation capabilities than BM-MSCs.(5,6).

Despite these advantages, comparative data on the dual osteogenic and angiogenic differentiation potential of both MSCs types remain limited, especially in the context of bone tissue engineering. Given the critical role of vascularization and bone regeneration in the successful treatment of critical-sized bone defects, this study aims to directly compare the osteogenic and angiogenic potential of hDP-MSCs and hBM-MSCs in vitro, to evaluate their respective suitability as cellular components for next-generation biomaterials targeting mandibular bone repair.

MATERIALS AND METHODS

Study design and ethical Approval

This analytical observational study employed an in vitro, cross-sectional design to compare mesenchymal stem cells (MSCs) derived from human dental pulp (hDP-MSCs) and bone marrow (hBM-MSCs), with six randomly allocated samples (n = 6). Ethical approval was granted by the Ethics Committees of Dr. Soetomo General Hospital (No. 1928/KEPK/IV/2020) and the Faculty of Dentistry, Universitas Airlangga (No. 062/HRECC.FODM/II/2020).

Isolation and Culture of MSCs

Human mesenchymal stem cells (MSCs) were isolated from two distinct tissue sources: bone marrow and dental pulp. Human bone marrow-derived MSCs (hBM-MSCs) were obtained from aspirates of the proximal tibial shaft of healthy adult donors undergoing elective orthopaedic procedures at Dr. Soetomo General Hospital. Immediately post-surgery, bone marrow samples were collected in sterile heparinised tubes, transported on ice, and processed within one hour at the hospital's Tissue Bank. Mononuclear cells were isolated using Ficoll-Paque density gradient centrifugation and cultured in α-MEM supplemented with 10% fetal bovine serum (FBS) and 1% penicillin-streptomycin, and 1% L-glutamine at 37°C in a 5% CO_2 humidified incubator. In parallel, human dental pulp-derived MSCs (hDP-MSCs) were isolated from healthy impacted third molars or premolars (ages 18–25) extracted for orthodontic reasons. Following a standardised disinfection protocol—triple rinsing in cold PBS, povidone-iodine immersion, and final rinse with antibiotic-supplemented PBS—teeth were stored for 4–6 hours before pulp retrieval. Access to pulp tissue was achieved using the sagittal odontotomy technique, an atraumatic method designed to preserve pulp integrity. The tissue was immersed in sterile PBS with 2% antibiotic-antimycotic solution and transported within 7–12 hours to the same laboratory (7). Upon arrival, the pulp was processed using a combination of enzymatic digestion and explant outgrowth methods with 3 mg/mL collagenase type I and 4 mg/mL dispase at 37°C for 1 hour. The dissociated cells were filtered through a 70 µm strainer and cultured under identical conditions to hBM-MSCs. Both MSCs types were expanded until Passage 3, at which point they were used for all downstream analyses. Medium changes were performed every 2–3 days to eliminate non-adherent cells and maintain cell health.

Flow Cytometry Characterisation

Flow cytometry analysis was performed to confirm the mesenchymal identity of both hDP-MSCs and hBM-MSCs following the minimal criteria set by the International Society for Cellular Therapy (ISCT). At passage 3, cells were harvested using 0.25% trypsin-EDTA, washed, and incubated for 30 minutes at 4°C in the dark with fluorochrome-conjugated monoclonal antibodies against CD105, CD90, and CD73 (positive MSCs markers), as well as CD34 and CD45 (hematopoietic lineage markers). Flow cytometric analysis was

conducted using a BD FACSCalibur (BD Biosciences, USA), and data were analysed with FlowJo software. Both hDP-MSCs and hBM-MSCs exhibited strong expression of CD105, CD90, and CD73 (≥90%) and negligible expression of CD34 and CD45 (≤2%), confirming their immunophenotypic profile as mesenchymal stem cells (8,9).

Induction of Angiogenic and Osteogenic Differentiation For both angiogenic and osteogenic differentiation, MSCs at Passage 3 were seeded at a density of 5 x 10^4 cells/well into 24-well plates pre-coated with fibronectin or gelatin. Angiogenesis was induced using endothelial growth medium (EGM-2, Lonza) supplemented with 50 ng/mL vascular endothelial growth factor (VEGF, Prospec Technogene, Israel) for 14 days. Capillary-like tube formation was assessed in Matrigel-coated wells after 6 hours of induction. In parallel, the expression of angiogenic markers, including VEGFR2 and CD31, was evaluated by immunocytochemistry (ICC). For enhanced visualisation, cells were further expanded to Passage 6 and cultured on fibronectin-coated coverslips until Passage 8 before ICC fixation and imaging.

Osteogenic differentiation was induced using odontogenic medium supplemented with potassium dihydrogen phosphate (KH_2PO_4, Cyagen, USA), or using standard osteogenic induction medium composed of α-MEM, 10% fetal bovine serum (FBS), 10 mM β-glycerophosphate, 50 µg/mL ascorbic acid, and 100 nM dexamethasone. The medium was refreshed every 2–3 days over a 21-day culture period. Cells reaching 90% confluence were dissociated with TrypLE Express (37°C, 5 min) before reseeding. Subsequent phenotypic and molecular analyses were performed to confirm lineage-specific differentiation

Immunocytochemical Analysis

Following differentiation, cells were fixed and analyzed via ICC for lineage markers: VEGFR2 (angiogenesis), BMP2 (early osteogenesis), and OCN (late osteogenesis). Expression was semiquantitatively assessed using the Immunoreactive Score (IRS) Remmele and Stegner (5,10).

RESULTS

Morphology and MSCs Identity

Human bone marrow (hBM) and dental pulp (hDP) tissues cultured in α-MEM basal MSC medium showed early sprouting and proliferation at Passage 0. By week three, confluent monolayers formed, and Passage 1 cells displayed spindle-shaped, fibroblast-like morphology (Figure 1, A1–A3 for hBM-MSCs, B1–B3 for hDP-MSCs). Flow cytometry confirmed that both cell sources fulfilled the minimal criteria for mesenchymal stem cells, demonstrating positive expression of CD105 and negative expression of hematopoietic markers CD45 and CD34. As shown in Table I, the immunophenotypic profile of hDP-MSCs and hBM-MSCs revealed consistent expression patterns typical of MSCs, with high CD105 expression (hBM-MSCs: 91.82%; hDP-MSCs: 98.74%) and low CD34/CD45 expression (hBM-MSCs: 1.37% / 1.68%; hDP-MSCs: 0.48% / 1.78%).

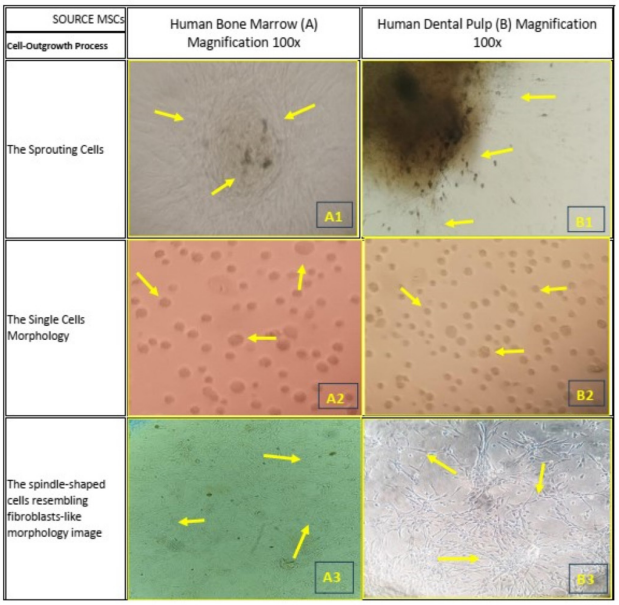

Figure 1: Culture of mesenchymal stem cells from human bone marrow and dental pulp tissues. A: hBM-MSC, B: hDP-MSC, A1, B1: Actively proliferating and sprouting cells observed during early culture., A2, B2: Isolated single-cell populations showing distinct morphology, A3, B3: Early-stage growth and confluence reveal spindle-shaped, fibroblast-like morphology typical of mesenchymal stem cells.

Table I: Flow Cytometry Analysis of MSC Identity in Human Bone Marrow and Dental Pulp Cells

Source	CD105 FITC (%)	CD45 PerCP (%)	CD34 PE (%)
Human Bone Marrow	91.82	1.68	1.37
Human Dental Pulp	98.74	1.78	0.48

Flow cytometry analysis confirming the mesenchymal stem cell (MSC) phenotype, indicated by strong positive expression of CD105 and absence of hematopoietic markers CD45 and CD34.

Lineage-Specific Marker Expression

Immunocytochemistry assessed VEGFR2 (angiogenic), BMP2 (early osteogenic), and OCN (late osteogenic) under basal and induced conditions (Figure 2). In the basal medium, both MSCs types showed negligible marker expression (Figures A1.0–A3.0, B1.0–B3.0).

Upon induction, hBM-MSCs showed weak marker expression and limited morphological changes (Figures A1–A3). In contrast, hDP-MSCs demonstrated significantly higher VEGFR2, BMP2, and OCN expression (Figures B1–B3), with structural reorganization mimicking vascular and bone-like architecture.

Semi-Quantitative Comparison

IRS analysis showed significantly greater expression of

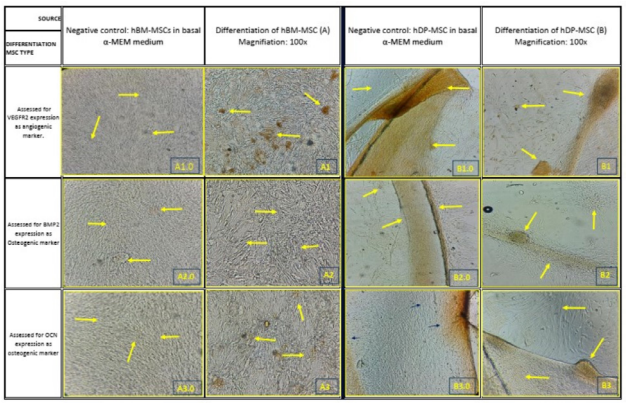

Figure 2: Angiogenic and osteogenic/odontogenic differentiation of hDP-MSCs and hBM-MSCs. Representative immunocytochemistry (ICC) images of hBM-MSCs (Panel A) and hDP-MSCs (Panel B) following angiogenic and osteogenic/odontogenic differentiation. Cells were stained using monoclonal antibodies for VEGFR2 (Panels A1, B1), BMP2 (Panels A2, B2), and OCN (Panels A3, B3). Corresponding control groups were maintained in basal medium and stained similarly (A1.0–A3.0 and B1.0–B3.0). Immunoreactivity was evaluated using the Remmele and Stegner IRS method to support a semi-quantitative analysis of marker expression.

Table II: Comparative ICC Analysis of Angiogenic and Osteogenic Differentiation in hBM-MSCs and hDP-MSCs (n=6).

Marker	DP-MSCs (Mean ± SD)	BM-MSCs (Mean ± SD)	p-value	Significance
BMP-2	1.67 ± 1.366	0.00 ± 0.000	0.007	Different
OCN	2.00 ± 1.095	0.00 ± 0.000	0.002	Different
VEGFR2	3.33 ± 1.506	0.83 ± 0.983	0.007	Different

Mean expression levels (± SD) of angiogenic and osteogenic markers in dental pulp-derived MSCs (hDP-MSCs) and bone marrow-derived MSCs (hBM-MSCs). Immunocytochemistry (ICC) analysis demonstrated significantly higher marker expression in hDP-MSCs compared to hBM-MSCs, with p-values < 0.05 indicating statistical significance.

all markers in hDP-MSCs compared to hBM-MSCs ($p \leq 0.05$) (Table II), indicating superior angiogenic and osteogenic differentiation potential.

DISCUSSION

This study offers a comparative analysis of mesenchymal stem cells (MSCs) derived from human bone marrow (hBM-MSCs) and dental pulp (hDP-MSCs), focusing on their angiogenic and osteogenic potential for bone tissue engineering. While bone marrow remains the conventional source for MSCs, its acquisition is inherently invasive, associated with donor-site morbidity, low cell yield, and patient discomfort. In contrast, dental pulp represents a minimally invasive, ethically accessible source—typically harvested from extracted third molars or premolars during routine clinical procedures—making it a compelling alternative for regenerative applications (5,11).

Upon isolation and culture under GMP-compliant conditions using α-MEM, both MSCs populations demonstrated adherence and proliferation, forming fibroblast-like monolayers. Supplementation with fetal bovine serum (FBS) supported expansion, with both cell types exhibiting similar morphological traits and viability during early passages. Notably, hDP-MSCs formed heterogeneous colonies, including rapidly proliferating small cells and larger, slower-dividing populations, suggestive of a stemness hierarchy—a trait previously associated with pluripotent-like behaviour (12,13).

Although no formal proliferation assay was conducted, visual observations indicated a decline in hBM-MSCs proliferation from passage 2 onward, while hDP-MSCs sustained or increased their proliferative activity. To standardize differentiation potential, induction protocols were initiated at Passage 2 for both cell types. Higher proliferation capacity in hDP-MSCs appeared to positively correlate with their differentiation outcomes, particularly in osteogenic and odontogenic lineages (11,13).

Immunophenotypic profiling by flow cytometry validated the mesenchymal identity of both cell sources, aligning with the WHO's minimal criteria for MSCs (14,15). Both hDP-MSCs and hBM-MSCs exhibited high CD105 expression alongside minimal CD34 and CD45 expression, indicating negligible hematopoietic contamination. Notably, hDP-MSCs showed superior mesenchymal purity, with CD105 expression reaching 98.74%, compared to 91.82% in hBM-MSCs, and lower levels of CD34 (0.48%) and CD45 (1.78%) relative to hBM-MSCs (1.37% and 1.68%, respectively) (Table I)

Beyond surface marker validation, functional assessments revealed that hDP-MSCs possess an enriched secretome profile. These cells actively secrete multiple bioactive factors including FGF2, BMPs, TGF-β superfamily members, PDGF, and IGFs (16–18). Such trophic mediators play pivotal roles in enhancing cell proliferation, migration, extracellular matrix (ECM) deposition, and lineage-specific differentiation. In particular, FGF2 modulates survival and angiogenesis, while TGF-β pathways direct osteoblastic and odontoblastic commitment.(19–21).

Differentiation induces notable shifts in MSCs morphology, size, membrane potential, and metabolic activity, driven by precise gene regulation. These changes steer multipotent cells toward lineage-specific fates, predominantly within mesodermal lineages (11,22). From passages 2 to 8, both hBM-MSCs and hDP-MSCs cultured in angiogenic and osteogenic/odontogenic media exhibited comparable spindle-shaped morphology, proliferation rates, and CFU formation. These similarities suggest maintained cytoskeletal integrity and strong cell–cell interactions, supporting their differentiation capacity (Figure 1, A3 and B3).

Upon differentiation in lineage-specific media, hDP-MSCs exhibited superior expression of VEGFR2 (angiogenic), BMP2, and OCN (osteogenic) markers compared to hBM-MSCs, as verified through

immunocytochemistry and semi-quantitative scoring ($p \leq 0.05$). Furthermore, hDP-MSCs demonstrated distinctive morphogenesis—forming lateral folds and dense nodular clusters resembling a self-assembled three-dimensional scaffold. These formations likely reflect an intrinsic ability to generate a biologically active ECM capable of enhancing spatial organization, cell-cell interaction, and microenvironmental cues essential for regenerative outcomes (Figure 2 and Table II).

These scaffold-free cellular architectures align with the findings of Dissanayaka et al. (2017), who demonstrated that hDP-MSCs micro-spheroids promote angiogenesis in vivo, even in the absence of exogenous scaffold materials. The spontaneous organisation into 3D structures underscores the adaptability of hDP-MSCs in mimicking native tissue architecture—an advantage in translational bone tissue engineering (23).

In summary, while both MSCs sources fulfil the minimal criteria for mesenchymal identity and differentiation, hDP-MSCs exhibit superior regenerative hallmarks: greater proliferative capacity, enhanced angiogenic/osteogenic marker expression, and the ability to self-organize into 3D microenvironments. These findings position dental pulp not only as a viable alternative but as a potentially superior MSCs source for therapeutic strategies targeting critical-sized bone defects.

CONCLUSIONS

Human dental pulp–derived MSCs (hDP-MSCs) exhibit robust osteogenic and angiogenic differentiation, outperforming bone marrow-derived MSCs in key marker expression and self-organized microarchitecture formation. Their superior proliferative capacity, minimal donor morbidity, and intrinsic scaffold-free organisation position hDP-MSCs as a highly versatile and accessible cell source for next-generation biomimetic scaffolds and advanced tissue-engineering applications.

ACKNOWLEDGEMENT

The authors gratefully acknowledge the invaluable support and facilities provided by the Stem Cell Laboratory team at the Tissue and Cell Bank Installation, Dr. Soetomo General Hospital, Surabaya. We also thank the Molecular Biology Laboratory team at the Faculty of Medicine, Universitas Airlangga, Surabaya, for their assistance and contributions to this study.

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