UNIVERSA MEDICINA

pISSN: 1907-3062 / eISSN: 2407-2230

Univ Med 2025;44:65-72

DOI: https://doi.org/10.18051/UnivMed.2025.v44.65-72

ORIGINAL ARTICLE

Rapid isolation and characterization of Wharton's jelly-derived mesenchymal stem cells maintained in fresh-prepared human AB-serum

Sianty Dewi[1], Yudy Tjahjono[2]*, Bernadette Dian Novita[1], Hendy Wijaya[1], Brilliant Dwi Putra[2], Teguh Widodo[2], Lucia Hendriati[2], Himawan Haryanto Jong[1], Franklin Malonda[1], and Suryo Kuncorojakti[3,4]

[1]Faculty of Medicine, Widya Mandala Catholic University Surabaya, East Java, Indonesia
[2]Faculty of Pharmacy, Widya Mandala Catholic University Surabaya, East Java, Indonesia
[3]Division of Veterinary Anatomy, Department of Veterinary Science, Faculty of Veterinary Medicine, Universitas Airlangga, Surabaya, Indonesia
[4]Research Centre for Vaccine Technology and Development, Institute of Tropical Disease, Universitas Airlangga, Surabaya, Indonesia

*** Correspondence Author:**
yudy.tjahjono@ukwms.ac.id

Date of first submission, December 5, 2024
Date of final revised submission, March 27, 2025
Date of acceptance, April 10, 2025

Cite this article as: Dewi S, Tjahjono Y, Novita BD, Wijaya H, Putra BD, Widodo T, et al isolation and characterization of Wharton's jelly-derived mesenchymal stem cells maintained in fresh-prepared human AB-serum. Univ Med 2025;44:65-72

ABSTRACT

BACKGROUND
Mesenchymal stem cells (MSCs) are valued in regenerative medicine for their multipotency, proliferative capacity, and immunomodulatory properties. Wharton's jelly-derived MSCs (WJ-MSCs) from the umbilical cord offer a non-invasive, promising source for clinical applications, because easy isolation, lack of ethical concerns, and the presence of both embryonic and adult stem cells have made them a valuable source for use in therapeutic applications and regenerative medicine. This study aimed to optimize WJ-MSC isolation and characterization methods.

METHODS
Human umbilical cords from three healthy donors were collected post-cesarean under strict inclusion criteria. WJ-MSCs were isolated using the explant culture method, with cells adhering to T75 flasks pre-coated with 2% gelatin. Cultures were maintained in Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% freshly prepared Human AB serum and monitored for 21 days. Flow cytometry (BD FACSAria) was performed at passages 1 and 5 to assess MSC markers CD105, CD73, CD90, and CD44, alongside the exclusion marker CD45.

RESULTS
WJ-MSCs exhibited fibroblast-like morphology by passage 1 and showed robust proliferation. Flow cytometry revealed high CD44 expression (~60%) at passage 1, while CD105, CD73, and CD90 became prominent by passage 5. CD45 remained low, suggesting minimal hematopoietic contamination.

CONCLUSIONS
This study confirms the feasibility of isolating and expanding WJ-MSCs using DMEM with 10% human AB serum. While consistent cell growth was achieved, the 21-day culture period may require optimization for

scalability, including serum concentration, substrate coatings, and oxygen levels. CPJ-MSCs may be preferable for applications demanding rapid expansion and early marker expression.

Keywords: Wharton's jelly, mesenchymal stem cells, isolation, flow cytometry, regenerative medicine

INTRODUCTION

Mesenchymal stem cells (MSCs) are multipotent stromal cells capable of differentiating into various cell types, such as osteocytes, chondrocytes, and adipocytes, making them valuable for regenerative medicine. Mesenchymal stem cells are characterized by a specific set of surface markers that distinguish them from other cell types, particularly hematopoietic cells.[1] According to the International Society for Cellular Therapy (ISCT), MSCs must express CD105, CD73, and CD90, which are markers associated with cell adhesion, growth, and differentiation potential, respectively.[2] CD105, or endoglin, plays a role in cell proliferation and vascular development,[3] while CD73 and CD90 contribute to immunomodulation and cell-cell interactions, respectively.[4,5] Additionally, CD44, a receptor for hyaluronic acid, is also commonly expressed on MSCs and is involved in cell adhesion, migration, and homing, which are essential for therapeutic applications.[6] Importantly, MSCs are negative for CD45, a pan-leukocyte marker, which ensures the exclusion of hematopoietic lineage cells.[7] This unique combination of surface markers—CD105, CD73, CD90, and CD44, with the absence of CD45—confirms the purity and identity of MSCs, supporting their use in clinical and research settings.[8]

The potential of MSCs for regenerative medicine has led to extensive research on various sources of MSCs, including those derived from Wharton's jelly of the human umbilical cord (WJ-MSCs). The use of Wharton's jelly of the human umbilical cord as a source of MSCs offers unique advantages, including non-invasive collection methods, high proliferation rates, and immunomodulatory properties, making them a promising cell type for therapeutic applications.[9] However, isolation and expansion methods must be refined to ensure the viability and consistency of WJ-MSCs.

Previous works on the isolation and characterization of Wharton's jelly derived mesenchymal stem cells (WJ-MSCs) have produced different yields, markers and abilities to differentiate. For instance, Ranjbaran et al.[10] were able to isolate WJ-MSCs in 10cm^2 plates containing high glucose-Dulbecco`s Modified Eagle Medium-F12 (HG-DMEM-F12), supplemented with 15% fetal bovine serum (FBS), and to separate the MSCs efficiently by placing pieces of Wharton's jelly at the bottom of the plates. Similarly, Cardoso et al.[11] cultured bovine WJ-MSCs in a serum-free condition and the cells displayed the typical mesenchymal stem cell phenotype up to 60 passages without having any genetic abnormalities. Abouelnaga et al.[12] also reported the successful isolation of umbilical cord Wharton's jelly mesenchymal stem cells in primary cultures, after which the cells maintained their tri-lineage differentiation potential, phenotypes, and karyotype characteristics on further passage and expansion.

The originality of our work is based on the approach, which aims at improving the isolation and growth of WJ-MSCs in a 2% gelatine coated flask and a culture medium containing 10% human AB serum. Previous studies used fetal bovine serum (FBS)[13,14], but we used human serum in our experiment to avoid immune reactions in future clinical use. Furthermore, the current study also revealed the distinct pattern of surface markers (CD44, CD105, CD73, and CD90) during the passage, with CD44 being most expressive at passage 1 and the other markers at passage 5.

The primary objective of this study was to establish a standardized protocol for the isolation and characterization of WJ-MSCs from human umbilical cords. The study aimed to optimize the culture conditions for WJ-MSCs, including the use of 2% gelatin-coated flasks and a culture medium supplemented with 10% human AB serum, to ensure high cell viability, proliferation, and purity. Additionally, the study sought to characterize the phenotypic profile of WJ-MSCs using flow cytometry to assess the expression of key surface markers (CD105, CD73, CD90, and CD44) and the absence of hematopoietic markers (CD45).

METHODS

Research design

This research was conducted in Gotong Royong Hospital in Surabaya in collaboration with the Faculty of Medicine and Faculty of Pharmacy of Widya Mandala Catholic University Surabaya. The research period was from June 2024 to December 2024.

Isolation of human umbilical cord-derived mesenchymal stem cells (WJ-MSCs)

Human umbilical cords were collected from 3 patients in Gotong Royong Hospital, immediately following cesarean section, within 5 minutes of placental removal to ensure optimal cell viability. Patient selection included strict criteria: only umbilical cords from healthy pregnancies without maternal or fetal complications were used. Specifically, cases of preeclampsia, metabolic disorders, infections, or abnormal umbilical cord coiling were excluded. Upon retrieval, each umbilical cord was placed in a sterile container with Dulbecco's Modified Eagle Medium (DMEM, Gibco, USA) containing 1% penicillin/streptomycin/amphotericin B and transported to the laboratory under aseptic conditions. In addition to morphological assessment, flow cytometry analysis was employed to confirm the MSC phenotype, assessing the expression of surface markers CD105, CD73, CD90, and CD44, with CD45 as a hematopoietic exclusion marker. This phenotypic profile aligns with the International Society for Cellular Therapy (ISCT) guidelines for MSC characterization, confirming the cells' stem cell identity and purity across passages.

Preparation and culture of WJ-MSCs

In a laminar flow cell culture hood to maintain aseptic technique, each umbilical cord was first washed thoroughly with Hank's Balanced Salt Solution (HBSS) containing 1% penicillin/ streptomycin/amphotericin B (Service Bio, China). Blood vessels were carefully removed from the Wharton's Jelly tissue to isolate the mesenchymal-rich regions, minimizing potential contamination, and promoting a higher yield of mesenchymal stem cells (MSCs). The remaining Wharton's Jelly tissue was cut into approximately 2 mm^2 pieces, which were then placed in T75 culture flasks precoated with 2% gelatin to enhance cellular adherence.[14] The tissue explants were left undisturbed for 7 days to allow cells to attach to the surface. [15] The culture medium, composed of DMEM supplemented with 10% human AB serum and 0.1% penicillin/streptomycin/amphotericin B, was carefully added to prevent dislodging the tissue fragments. Medium was replaced every 3 days, and cell outgrowth was monitored microscopically. After 21 days, successful outgrowth and proliferation of passage 1 (P1) cells were observed, characterized by the spindle-shaped, fibroblast-like morphology typical of MSCs.

Flow cytometry analysis

Flow cytometry was employed to determine the phenotype of WJ-MSCs isolated at passages 2 and 6 using a BD FACSAria flow cytometer (BD Biosciences). To assess the expression of key mesenchymal and hematopoietic markers, three antibody panels were prepared with mouse monoclonal antibodies specific to human antigens. Panel 1 included anti-human CD105 (clone 43A3, Alexa Fluor 488, BioLegend) and anti-human CD45 (clone H130, FITC, BioLegend); Panel 2 included anti-human CD73 (clone AD2, PE, BioLegend) and anti-human CD90 (clone 5E10, PE/Cy7, BioLegend); and Panel 3 included anti-human CD44 (clone IM7, PE, Elabscience). For each panel, 10^6 cells in 100 µL were aliquoted into individual tubes, and 2.5 µL of each antibody was added to each sample. The cells were incubated for 1 hour at 4°C in the dark to optimize antibody binding and prevent photobleaching. After incubation, cells were washed twice with staining buffer (PBS with 1% BSA) to remove unbound antibodies, and were resuspended in 500 µL of buffer for analysis. Gating parameters were set to exclude debris and non-viable cells based on forward and side scatter properties. Unstained controls were applied to correct for spectral overlap among fluorophores. Expression of CD105, CD73, CD90, and CD44 was assessed to confirm MSC phenotype, while CD45 was used to exclude hematopoietic contamination.[16] Data were analyzed to determine the percentage of cells expressing each marker, providing a comprehensive phenotypic profile of WJ-MSCs across passages.

Ethical clearance

The ethical clearance for collecting the human tissue was obtained from Medical Faculty, Universitas Hang Tuah (E/006/UHT.KEPK.03/II/2024).

Statistical analysis

GraphPad Prism version 8.0.1 was used for data presentation. The data were analyzed using one-way analysis of variance (ANOVA) followed by Tukey's honestly significant difference (HSD) when the data followed normality and homogeneity assumption of parametric analysis (otherwise using nonparametric Kruskal-Wallis followed by Dunn's test). Normality and homogeneity were assessed using the Shapiro-Wilk and Brown-Forsythe tests, respectively. A p-value <0.05 was considered significant. All data were presented as mean ± standard deviation (SD).

RESULTS

Isolation and culture of Wharton's jelly-derived MSCs

Our results show that the WJ-MSCs isolated through this protocol display a fibroblast-like morphology characteristic of MSCs, with consistent morphology observed through the first passage. The isolation of WJ-MSCs was achieved through the explant culture method, allowing MSCs to be successfully obtained from human umbilical cord tissue. Following isolation, WJ-MSCs adhered to the culture surface within the first few days, and after 21 days in culture, passage 1 (P1) showed significant cell proliferation. The MSCs exhibited a characteristic fibroblast-like morphology (Figure 1), typical of mesenchymal cells, with elongated, spindle-shaped bodies and a uniform, flattened appearance. Cells were tightly adherent, forming a monolayer with parallel, aligned arrangements. The morphology of the cells remained stable through passage 14 (P14), showing no signs of senescence or morphological abnormalities. This consistent fibroblast-like morphology, with the presence of prominent cell projections and well-defined cellular boundaries, suggests that the WJ-MSCs maintained their mesenchymal characteristics as evidenced by their elongated spindle-shaped morphology and high proliferation rate, and were successfully expanded in primary culture. Such morphological consistency is essential for the continued use of these cells in therapeutic research, where cell integrity and stability are paramount.

Phenotypic characterization of WJ-MSCs by flow cytometry

To confirm the MSC phenotype, we performed flow cytometric analysis on WJ-MSCs passage 5 (P5), assessing the expression of key surface markers (Figure 2). The gating strategy effectively isolated MSC populations, and the analysis revealed high expression levels of CD73 and CD90, alongside positive populations for CD105. Additionally, the cells were negative for CD45, confirming the absence of hematopoietic contamination (Figure 2). Positive CD44 expression further validated the MSC phenotype, affirming the cells' stem cell characteristics and purity.

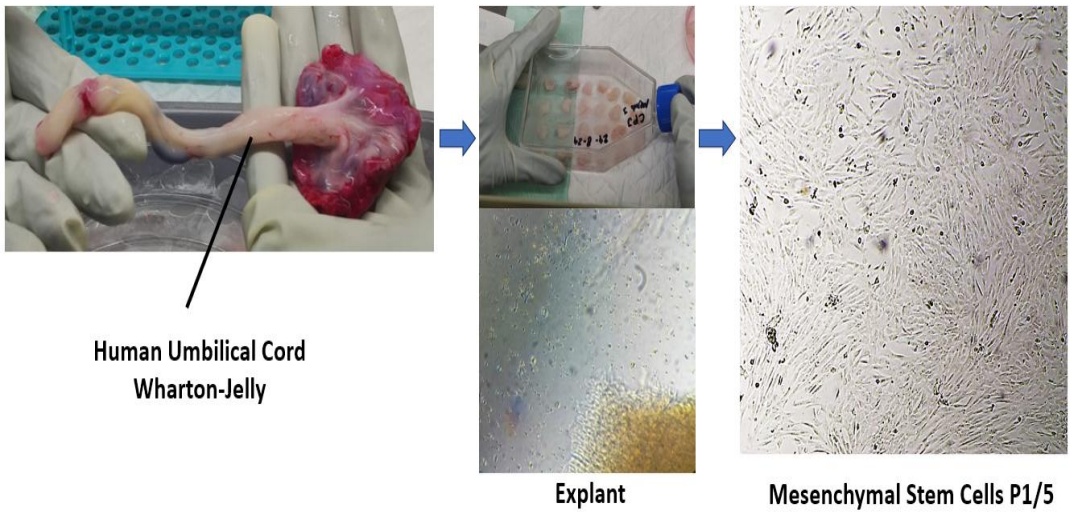

Figure 1. Explant culture of Wharton's jelly-derived MSCs
The initial explant culture of Wharton's jelly-derived mesenchymal stem cells (WJ-MSCs) was isolated from human umbilical cords. The explant technique and subsequent cell morphology are shown, with primary cultures demonstrating successful MSC adherence and proliferation.

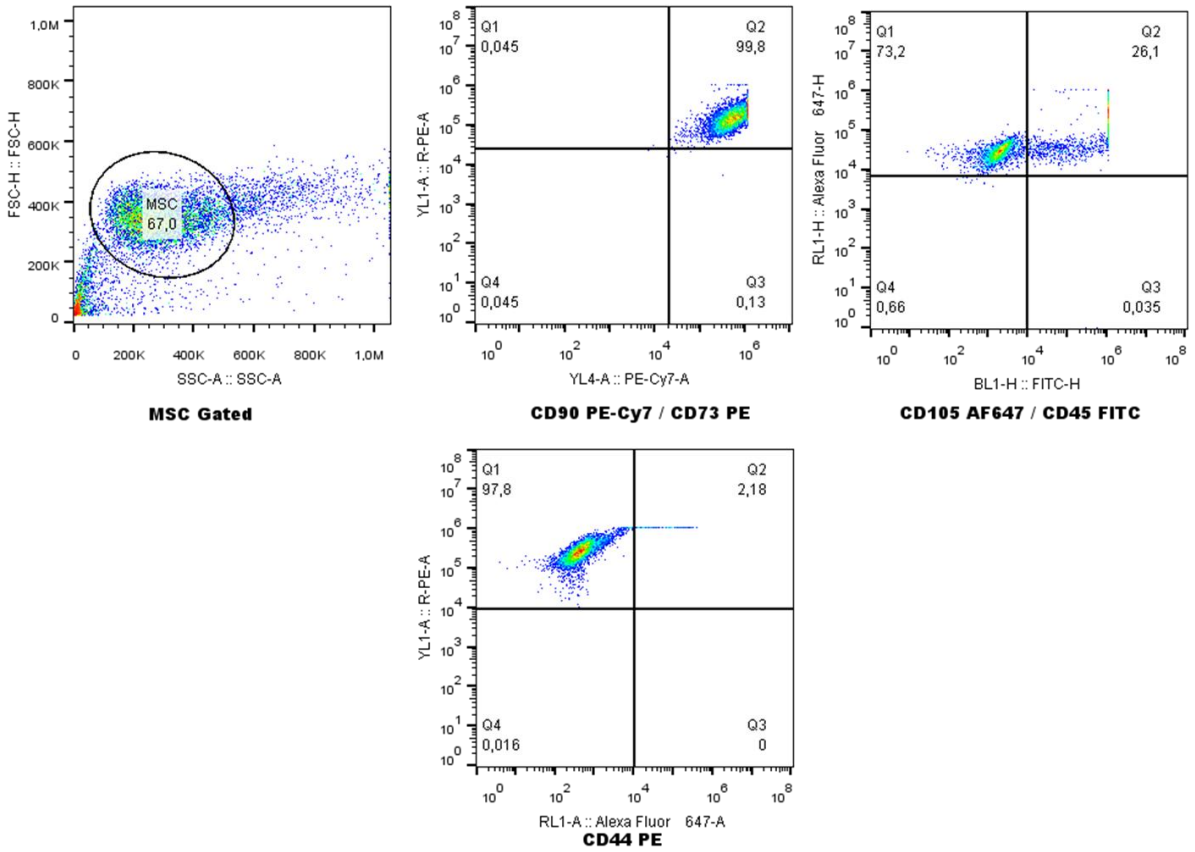

Figure 2. Representative flow cytometric analysis of WJ-MSC surface markers
The flow cytometric gating strategy assesses key surface markers on WJ-MSCs, confirming their stem cell characteristics (upper left graph). The expression levels of positive markers are shown in the upper middle graph (CD73, CD90), with CD105-positive and CD45-negative populations (Q1 for CD105; Q2 for CD45; upper right), as well as CD44-positive populations (lower middle graph), verifying the MSC phenotype.
Note: Figures with decimal commas should be read as having decimal points.

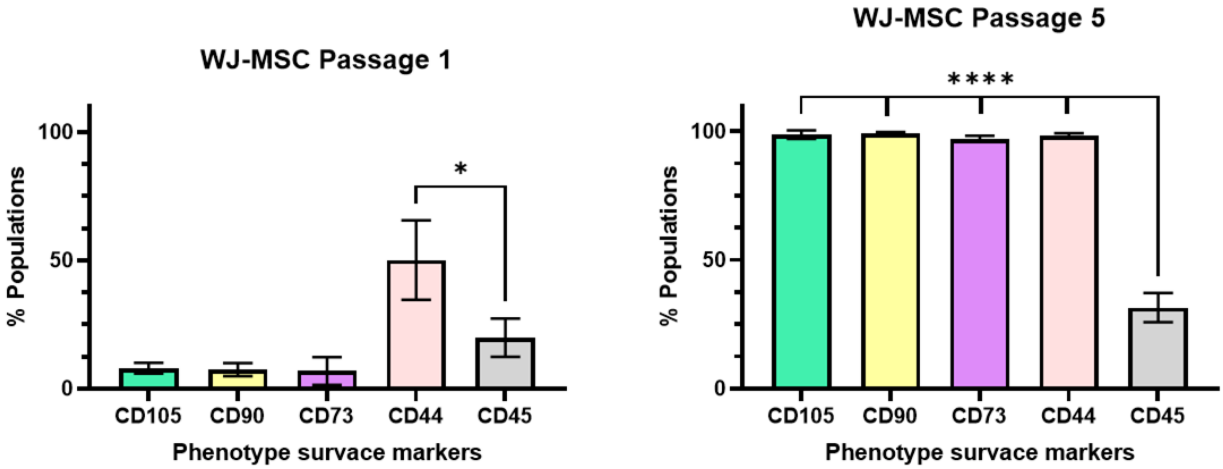

Figure 3. **Comparative cell surface markers of WJ-MSCs at passages 1 and 5**
Compared to the negative phenotype (CD45), WJ-MSCs demonstrate a significant increase in surface markers at passage 1 (p=0.010). Additionally, a dramatic increase was observed in CD105, CD90, CD73, and CD44 surface markers at passage 5 ($p < 0.000$), indicating successful MSC proliferation and phenotype stability across passages. Statistical analysis was conducted using one-way ANOVA with post-hoc tests to determine significance between groups. p-values were calculated using GraphPad Prism, with significance levels denoted as follows: $p<0.05$ (*), $p<0.010$ (**), $p<0.001$ (***), and $p<0.000$ (****)

Comparative analysis of surface markers at different passages

Comparisons of WJ-MSC surface markers between P1 and P5 revealed significant differences in marker expression (Figure 3). On passage 1, the MSCs demonstrated a statistically significant increase in pluripotency surface markers compared to the CD45-negative phenotype ($p = 0.010$). By P5, a dramatic increase in CD105, CD90, CD73, and CD44 expression was observed, with statistical significance ($p < 0.001$) (Figure 3). These findings indicate robust MSC proliferation and stable expression of essential stem cell markers across passages, supporting the successful expansion and maintenance of MSC characteristics through successive passages.

DISCUSSION

This study successfully demonstrates the rapid isolation and characterization of WJ-MSCs from human umbilical cords using freshly prepared human AB-serum. These cells exhibited typical MSC morphology and a stable phenotype through early passages, supporting their potential for future research and clinical applications.[17]. However, despite overall success, the detection of low levels of CD45 expression indicates potential hematopoietic contamination.[16] This may be attributed to residual blood cells within the Wharton's Jelly, as complete removal of all blood vessels can be challenging in clinical settings.[18] Future refinements could include additional washes or enzymatic treatments to further minimize CD45-positive cells, improving the MSC purity and strengthening the clinical potential of these cells.[19]

The MSCs present in Wharton's jelly tend to have similar characteristics, irrespective of umbilical cord regions (maternal, fetal, or central attachment).[20] Interestingly, a recent study has highlighted the potential of MSCs derived specifically from the cord-placental junction, which may offer advantages over WJ-MSCs in terms of cellular yield, differentiation potential, and immunomodulatory properties. These cord-placental junction MSCs have shown enhanced proliferation rates and greater multilineage differentiation capabilities compared to WJ-MSCs, likely due to their proximity to the placenta and the presence of rich microenvironmental cues.[13]

Our analysis of MSC surface marker expression showed unique patterns across different passages, specifically with P1 exhibiting only high CD44 expression (approximately 60%) while other markers, including CD105, CD73, and CD90, were undetectable. This selective expression at P1 could reflect an early stage in MSC culture, where cells prioritize adhesion and migration functions, as CD44 is a receptor for hyaluronic acid involved in cell adhesion and motility.[13,21] Figure 3 demonstrates a successive passage, notably by P5, when all MSC markers (CD105, CD73, CD90, and CD44) reached high expression levels. This finding suggests that these cells may require additional time or specific culture conditions to fully upregulate markers associated with mesenchymal identity and functionality. CD45, as expected, remained low across passage 5, although its continued detectability indicates potential residual hematopoietic contamination, which aligns with reports from another study.[16] Interestingly, this marker pattern differs from MSCs derived from the cord-placental junction (CPJ-MSCs), which tend to express high levels of all MSC markers, including CD105, CD73, and CD90, and low CD45 levels. These differences may arise from microenvironmental factors unique to CPJ-MSCs that show marker expression and MSC commitment earlier in culture, highlighting the potential value of CPJ as an alternative MSC source for applications requiring rapid cell expansion and stable marker profiles.[14]

Another area for improvement in our protocol is the lengthy outgrowth period, as cell attachment and expansion required 21 days before reaching significant confluence. Long outgrowth times can limit scalability and delay potential downstream applications.[22] Optimizing the initial explant culture conditions could reduce this outgrowth period, thereby increasing efficiency. This might include using higher serum concentrations, pre-coating culture surfaces with adhesion-promoting molecules such as fibronectin, or adjusting oxygen levels to more closely mimic the native environment of umbilical cord MSCs.[23,24] Reducing the outgrowth time will not only streamline the MSC isolation process but also decrease the potential for cell senescence and culture-induced variations, ultimately improving

the consistency and reliability of the cells obtained for research and therapeutic use.

The limitations of this study include, first, the contamination with CD45, which is still detected in antibody expressions. This may result from incomplete removal of blood vessels during the isolation process, potentially affecting the purity of WJ-MSCs and necessitating further refinement of the isolation protocol. Second, the prolonged outgrowth period limits the scalability and efficiency of the protocol for large-scale applications.

Despite these limitations, this study successfully isolated and characterized WJ-MSCs, providing a foundation for future clinical applications in regenerative medicine, including tissue repair, immunomodulation, and cell-based therapies. The use of human AB serum instead of fetal bovine serum (FBS) reduces the risk of immune reactions, making the protocol more suitable for clinical use.

Looking ahead, several future directions could be built on this research. First, optimizing the isolation process by carefully extracting arteries and veins could minimize CD45 contamination and improve MSC purity. Second, reducing the outgrowth period by optimizing culture conditions—such as using higher serum concentrations, adhesion-promoting molecules like fibronectin, or adjusting oxygen levels to better mimic the native environment of umbilical cord MSCs—could enhance efficiency and applicability.

CONCLUSION

This study successfully demonstrates the isolation and characterization of Wharton's jelly derived MSCs (WJ-MSC) using a culture medium supplemented with 10% human AB serum and high glucose, combined with a 2% gelatine-coated flask. This protocol achieved high cell viability and proliferation, with WJ-MSCs exhibiting fibroblast-like morphology and stable expression of MSC key markers (CD105, CD44, CD90 and CD73) by passage 5, as well as low levels of CD45 expression indicating minimal hematopoietic contamination and confirming the purity of the isolated cells.

The results highlight the feasibility of this protocol for obtaining viable WJ-MSCs, providing a path for future research and clinical applications in regenerative medicine. However, the 21-day outgrowth period and the presence of residual CD45 positivity suggest further optimization, such as refining the isolation techniques, reducing culture times, and exploring alternative MSC sources.

Authors Contribution

SD, YT, and BDN designed the experiments, carried out the experiments, and prepared the manuscript. BDP, TW, LH, HW, HHJ, FM, and SK carried out the experiments and analyzed the data. YT, SD and BDP revised the manuscript. All authors have read and approved the final manuscript.

Conflict of Interest

All authors declare no conflict of interest.

Acknowledgement

We would like to thank the Department of Obstetrics and Gynacology of Gotong Royong Hospital, Surabaya-Indonesia for the support.

Funding

This study received funding from the Research and Service Institute of Widya Mandala Surabaya Catholic University awarded to Sianty Dewi (3172/WM01/N/2024).

Data Availability Statement

The authors confirm that the data supporting the findings of this study are available within the article.

Declaration the Use of AI in Scientific Writing

During the preparation of this work, the authors used ChatGPT (OpenAI) to assist in language refinement, grammar correction, and improving technical clarity. After using this tool, the authors reviewed and edited the content as needed and take full responsibility for the content of the publication.

REFERENCES

1. Rajabzadeh N, Fathi E, Farahzadi R. Stem cell-based regenerative medicine. Stem Cell Investig 2019;6:19. doi: 10.21037/sci.2019.06.04.
2. Huynh PD, Tran QX, Nguyen ST, Nguyen VQ, Vu NB. Mesenchymal stem cell therapy for wound healing: an update to 2022. Biomed Res Ther 2022;9:5437–49. https://doi.org/10.15419/bmrat.v9i12.782.
3. Kauer J, Schwartz K, Tandler C, et al. CD105 (endoglin) as negative prognostic factor in AML. Sci Rep 2019;9:1–11. https://doi.org/10.1038/

s41598-019-54767-x.
4. Xia C, Yin S, To KKW, Fu L. CD39/CD73/A2AR pathway and cancer immunotherapy. Mol Cancer 2023;22:44. https://doi.org/10.1186/s12943-023-01733-x.
5. Chen S, Wainwright DA, Wu JD, et al. CD73: An emerging checkpoint for cancer immunotherapy. Immunotherapy 2019;11:983–97. https://doi.org/10.2217/imt-2018-0200.
6. Mattheolabakis G, Milane L, Singh A, Amiji MM. Hyaluronic acid targeting of CD44 for cancer therapy: from receptor biology to nanomedicine. J Drug Target 2015;23:605–18. https://doi.org/10.3109/1061186X.2015.105207.
7. Ghaneialvar H, Soltani L, Rahmani HR, Lotfi AS, Soleimani M. Characterization and classification of mesenchymal stem cells in several species using surface markers for cell therapy purposes. Indian J Clin Biochem 2018;33:46–52. https://doi.org/10.1007/s12291-017-0641-x.
8. He H, Nagamura-Inoue T, Takahashi A, et al. Immunosuppressive properties of Wharton's jelly-derived mesenchymal stromal cells in vitro. Int J Hematol 2015;102:368–78. https://doi.org/10.1007/s12185-015-1844-7.
9. Kamal MM, Kassem DH. Therapeutic potential of Wharton's jelly mesenchymal stem cells for diabetes: achievements and challenges. Front Cell Dev Biol 2020;8:16. https://doi.org/10.3389/fcell.2020.00016.
10. Ranjbaran H, Abediankenari S, Mohammadi M, et al. Wharton's jelly derived-mesenchymal stem cells: Isolation and characterization. Acta Med Iran 2018;56:28–33. https://doi.org/10.1111/cpr.21904.
11. Cardoso TC, Ferrari HF, Garcia AF, et al. Isolation and characterization of Wharton's jelly-derived multipotent mesenchymal stromal cells obtained from bovine umbilical cord and maintained in a defined serum-free three-dimensional system. BMC Biotechnol 2012;12:1–11. https://doi.org/10.1186/1472-6750-12-18.
12. Abouelnaga H, El-Khateeb D, Moemen Y, El-Fert A, Elgazzar M, Khal A. Characterization of mesenchymal stem cells isolated from Wharton's jelly of the human umbilical cord. Egypt Liver J 2022;12:2. https://doi.org/10.1186/s43066-021-00165-w.
13. Beeravolu N, McKee C, Alamri A, et al. Isolation and characterization of mesenchymal stromal cells from human umbilical cord and fetal placenta. J Vis Exp 2017;2017:1–13. https://doi.org/10.3791/55224.
14. Todtenhaupt P, Franken LA, Groene SG, et al. A robust and standardized method to isolate and expand mesenchymal stromal cells from human umbilical cord. Cytotherapy 2023;25:1057–68. https://doi.org/10.1016/j.jcyt.2023.07.004.
15. Hendijani F. Explant culture: An advantageous method for isolation of mesenchymal stem cells from human tissues. Cell Prolif 2017;50:1–14. https://doi.org/10.1111/cpr.12334.
16. Pham H, Tonai R, Wu M, Birtolo C, Chen M. CD73, CD90, CD105 and cadherin-11 RT-PCR screening for mesenchymal stem cells from cryopreserved human cord tissue. Int J Stem Cells 2018;11:26–38. https://doi.org/10.15283/ijsc17015.
17. Suyama T, Takemoto Y, Miyauchi H, Kato Y, Matsuzaki Y, Kato R. Morphology-based noninvasive early prediction of serial-passage potency enhances the selection of clone-derived high-potency cell bank from mesenchymal stem cells. Inflamm Regen 2022;42:1–13. https://doi.org/10.1186/s41232-022-00214-w.
18. Niknam B, Azizsoltani A, Heidari N, et al. A simple high yield technique for isolation of Wharton's jelly-derived mesenchymal stem cell. Avicenna J Med Biotechnol 2024;16:95–103. https://doi.org/10.18502/ajmb.v16i2.14860.
19. Zheng S, Gao Y, Chen K, Liu Y, Xia N, Fang F. A robust and highly efficient approach for isolation of mesenchymal stem cells from Wharton's jelly for tissue repair. Cell Transplant 2022;31:9636897221084354. doi: 10.1177/09636897221084354.
20. Bharti D, Shivakumar SB, Park JK, et al. Comparative analysis of human Wharton's jelly mesenchymal stem cells derived from different parts of the same umbilical cord. Cell Tissue Res 2018;372:51–65. https://doi.org/10.1007/s00441-017-2699-4.
21. Cao Y, Boss AL, Bolam SM, et al. In vitro cell surface marker expression on mesenchymal stem cell cultures does not reflect their ex vivo phenotype. Stem Cell Rev Reports 2024;20:1656–66. https://doi.org/10.1007/s12015-024-10743-1.
22. Bodiou V, Kumar AA, Massarelli E, van Haaften T, Post MJ, Mousatsou P. Attachment promoting compounds significantly enhance cell proliferation and purity of bovine satellite cells grown on microcarriers in the absence of serum. Front Bioeng Biotechnol 2024;12:1443914.. https://doi.org/10.3389/fbioe.2024.1443914.
23. Moniz I, Ramalho-Santos J, Branco AF. Differential oxygen exposure modulates mesenchymal stem cell metabolism and proliferation through mTOR signaling. Int J Mol Sci 2022;23:3749. doi: 10.3390/ijms23073749.
24. Kasten A, Naser T, Brüllhoff K, et al. Guidance of mesenchymal stem cells on fibronectin structured hydrogel films. PLoS One 2014;9:e109411. doi: 10.1371/journal.pone.0109411.